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Reproductive biology of lampshell, *Lingula anatina* (Lamarck, 1801), family Lingulidae in Thuy Trieu lagoon – Khanh Hoa province, Vietnam

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Abstract
Brachiopod, lampshell, *Lingula anatina* (Lamarck, 1801) is one of the economic species in the Thuy Trieu lagoon, Khanh Hoa, Vietnam. However, the reproductive biology of this species is still not understood, causing difficulties in sustainable fisheries management. Therefore, this study aimed to examine some reproductive biology characteristics of lampshell, providing baseline data to manage and exploit the resource of lampshell at the Thuy Trieu lagoon, Khanh Hoa. A total of 864 lampshells were sampled monthly for a whole year. The results show that the sex ratio of *L. anatina* was 1.02: 1. Reproduction was found year-round; however, spawning season was between July to November. The absolute fecundity was 191,430 ± 132,093 eggs per female, while the relative fecundity was 80,351 ± 44,477 eggs g^{-1} of total body weight of the female. Size at the first maturity was estimated at 28 mm of shell height. The paper provides baseline data for the management and sustainable exploitation of lampshell resources.

Keywords: Lampshell, lingula, reproductive biology

1. Introduction
Brachiopod, genus *Lingula* (lamp shell) belongs to family Lingulidae and phylum Brachiopoda, which is widely distributed in the Pacific and Indian, Atlantic oceans near the west coast of Africa [1]. Thuy Trieu Lagoon is located on the south-central coast of Vietnam, belongs to Cam Lam District, Khanh Hoa Province. The Thuy Trieu lagoon has many species of marine living resource such as crustaceans, fish and mollusks, which carries a high income for local people in the area along the lagoon. Lampshells were reported as a valuable species, which brought income for local fishermen at Thuy Trieu lagoon. They were exploited and provided feed for giant tiger shrimp or lobster aquaculture or sold as food for humans. This income has stimulated people to use exploitation of this species heavily. The lampshell exploitation of the local fishermen is unprompted; however, there is no scientific data to propose reasonable policies to protect the resource of lampshell in the Thuy Trieu lagoon.
Together with other fisheries assessment, research on the reproductive biology characteristics will provide an essential and necessary scientific basis data for proposing solutions to manage the exploitation of aquatic resources. Reproduction plays a vital role in population regeneration [2, 3]. Reproductive biological characteristics such as the ratio of males, the stages of development of the gonads, reproductive capacity, the smallest maturity size, the spawning season are considered as relevant data to manage and exploit the natural resource more sustainable [2].
Population dynamics of brachiopod *L. unguis* was reported in Korea [4] or the ecology and reproduction of *L. anatina* in Australia [5, 6] or distribution of *L. reevii* in the USA [7], population biology of lingula was done by Samanta [8]. The biological characteristics of *Lingula*, including morphology, gut content, biomass and ambient environment were reported [9]. Also, the length-weight relationship and condition factor of *Lingula* at Thuy Trieu lagoon were reported previously [10]. However, there is no information on the reproductive biology of brachiopod, *L. anatina*, in Vietnam. As the researches on lampshell are very limited, so the understanding of biological and ecological characteristics of marine species is limited, making it difficult and challenging to introduce management and management policies for sustainable utilization of clamshell in the Thuy Trieu lagoon.

Therefore, this report will provide the reproductive biology of lampshell, *Lingula anatina*, including sex ratio, fecundity, spawning season, size at first maturity. Those fundamental data would contribute an essential and necessary scientific basis for the planning of exploitation management and using lampshell resources in the Thuy Trieu lagoon.

2. Materials and methods

2.1. Study area and sampling

There were 864 lampshell samples were gathered in the Thuy Trieu lagoon, Khanh Hoa (Figure 1). Samples were fixed in 10% formalin and then analyzed in the Laboratory of the Institute of Oceanography, Nha Trang, Vietnam.

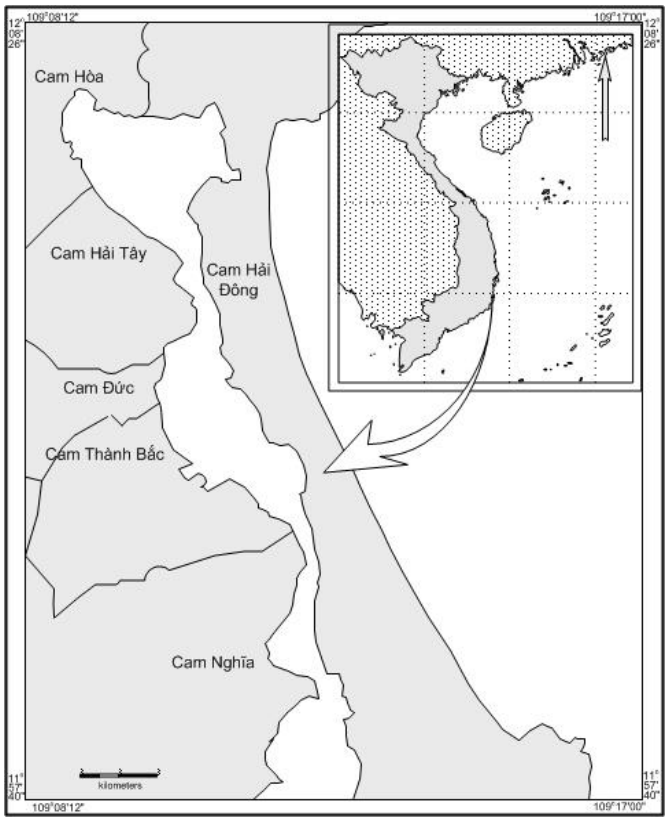

Fig 1: Sampling location (Thuy Trieu lagoon, Khanh Hoa province, Vietnam). (12º02'16N to 12º05'56"N and 109°09'25"E to 109º10'43"E)

Fig 2: Lampshell, *Lingula anatina* collected in Thuy Trieu lagoon.

2.2 Measurement and weighing the lampshell

The shell dimensions of lampshell, including length, height and thickness of the shells were measured using a caliper with 0.1 mm accuracy. The whole body weight and weight of the soft part were weighed by using electronic balance 0.01g. After measuring and weighing, samples of gonads were taken and observed under a microscope at 10×10 and 10×40 magnifications to determine the sex and the reproductive stage of each individual of lampshell.

2.3. Determination of sex, reproductive stage and spawning season

Sexual determination and stages of development of the gonads were determined following Braley [11] and Baron [12]. The estimation of the spawning season was based on the number of females with ripe gonad (stage III) was dominant [13].

2.4. Determination of fecundity

Female ovary at stage III was collected and fixed in formalin 10% to determine fecundity. The procedure was as follows: weighed the entire ovary (Wbt), then sampled at three parts of the ovary (the anterir, middle and porterior of the ovary) and then weighed every three parts (Wm). The total number of eggs (Nm) of the three parts were counted. Absolute fecundity and relative fecundity were calculated as the following: Absolute fecundity = Nm x Wbt / Wm; Relative fecundity = Absolute fecundity / total body weight (g)

2.5. Estimation of the size at first maturity

The size at first maturity was the smallest size group where at least 50% of female quantity have matured gonads, calculated by the following formula: Ln [(1-P) / P] = aL + b. Where P is the proportion of female individuals maturing in different size groups (L), a and b are coefficients of the first-order function. The smallest maturity size (L_{50}) was calculated as L_{50} = b / a (King 2001).

2.6. Data calculation and statistical analysis

Data was presented by mean ± SD. The sex ratio was compared to the natural ratio of 1:1 by using the Chi-square test (χ^2). Data calculation and analysis were performed in Excell and SPSS.

3. Results and discussion

3.1. The sex ratio of lampshell in Thuy Trieu lagoon

The result of the analysis of the sex ratio of the lampshell is shown in Figure 3. The calculated overall sex ratio was 1.02: 1. However, the sex ratio of lampshell was not statistically significantly different in comparison to the 1: 1 ratio (P = 0.238). Therefore, the sex ratio of lampshell at Thuy Trieu followed the gender rule in nature in which the male: female ratio is approximately 1: 1. The individual that was unable to identify male or female was about 3% in the total specimens, which were usually small individual or juveniles. Data analysis showed that the sex ratio of the lampshell did not show variations by seasons. No hermaphrodite individual was found in the collected samples. This is different from other findings, which reported the hermaphrodite individuals in other mollusk species [14].

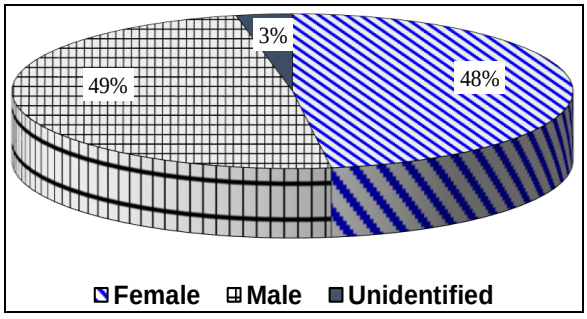

Fig 3: The sex ratio of lampshell, *Lingula* in Thuy Trieu lagoon.

3.2. Histology gonads of lampshell

The analysis of 864 specimens showed that the gonads of the lampshell were under the muscle layer, eggs and sperm were very small, the colors are quite similar, so it was only possible to distinguish the male/ female and determined the reproductive stages by taking a piece of gonad sample and observed under a microscope [12, 15]. The gonad development of the lampshell could be divided into four stages (Figure 4):

Phase I: The gonads were small and unable to distinguish between males and females (Figure 3G).

Phase II: The gonads began to develop, the size of the gonad increased and was more prominent than female at phase I. Under the microscope, the eggs and the sperms could be seen so that the sex could be determined. However, at this phase, the oocytes in the ovary were not fully developed, the nuclear membrane was quite clearly observed, eggs were stuck to each other and were difficult to separate them (Figure 4A and 4B).

Phase III: This is the ripe stage of the gonad; the gonads have enhanced the size. Eggs were easy to separate on the glass slide with a drop of water and a needle. Observed under the microscope, the size of eggs increased, being larger than eggs at Stage II and were round. Sperms were liquified (Figure 4C and 4D).

Phase IV: All eggs were released out of the ovaries, the ovaries were flat and soft. The ovary has many empty cavities and with a few eggs at phase III still existed at this stage. After this stage, the gonads return to stage I (Figure 4E and 4F).

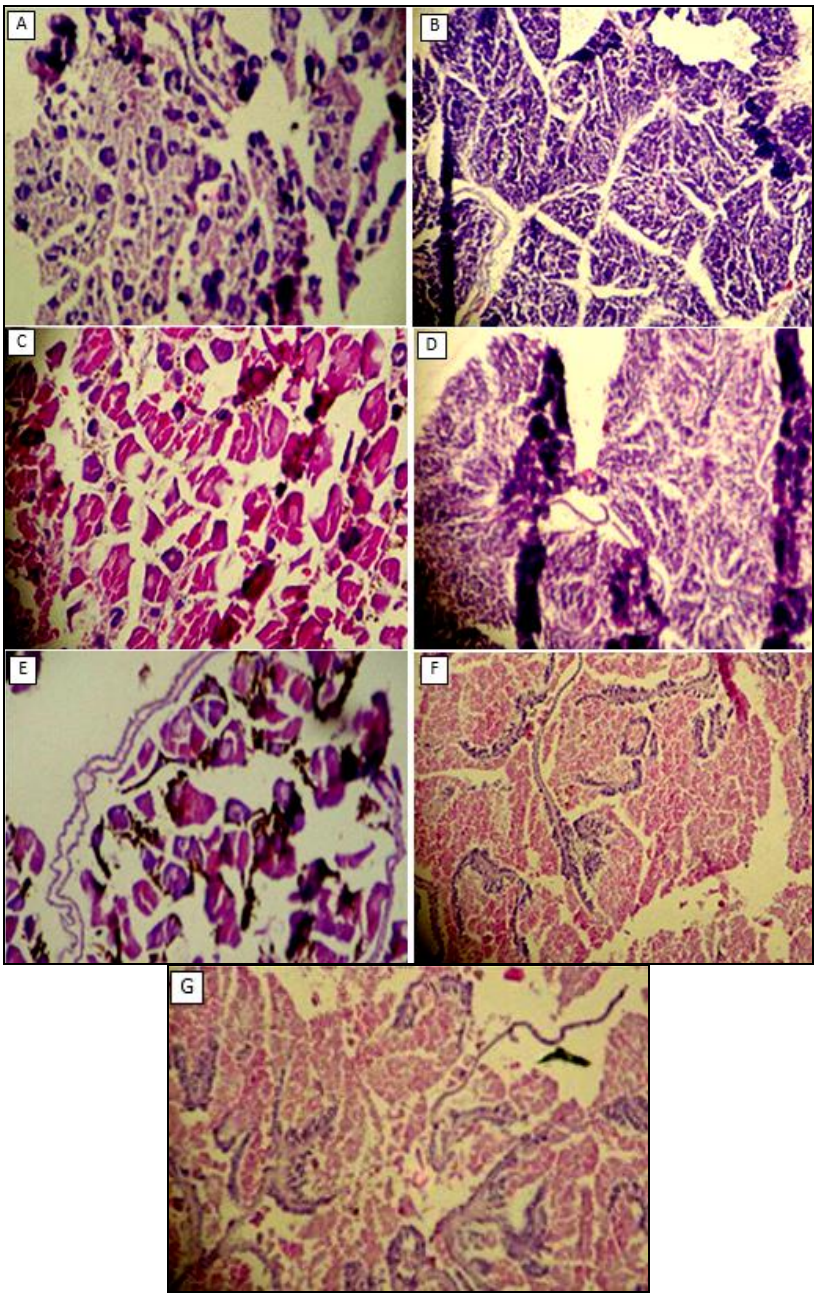

Fig 4: Histology of reproductive organs of lampshell.

Stage II: female (A) and male (B);
Stage III: female (C) and male (D);
Stage IV: female (E) and male (F); Unidentified sex (G)

3.3. The breeding season of the lampshell

The results of the analysis of the gonad development stages by month are presented in Figure 5. The results of the gonadal analysis showed that from July to November, there was a slightly higher number of mature females compared to other months of the year. However, mature females could find all year round. Similarly, breeding year-round, often occurred in many aquatic species distributed in tropical waters (Figure 5). Besides, studies by other authors suggest that mollusk breeding seasons may vary among geographic regions [16] and possibly also depend on many factors such as food, temperature, salinity and light [17].

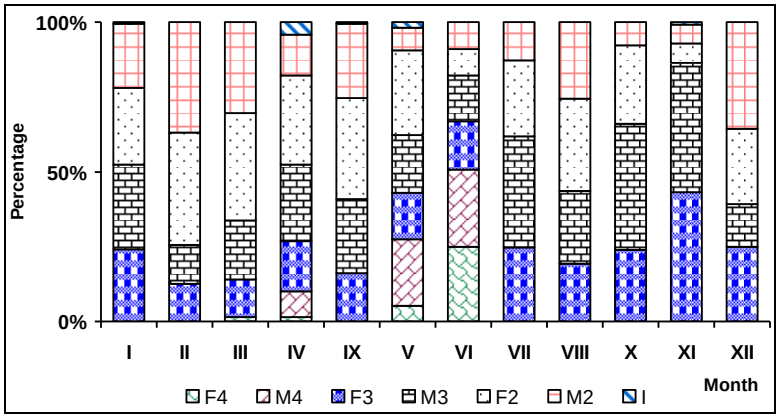

Fig 5: Percentage of gonad development stages of the lampshell (Fn: female at stage n, Mn: male at stage n)

3.4. Reproductive stages of lampshell by size class

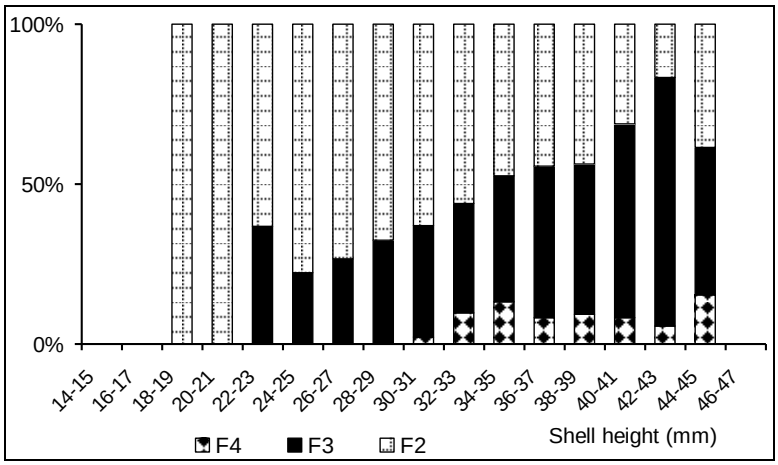

Fig 6: Percentage of female reproductive stages of the size group of the lampshell (Fn: The female gonad at stage n)

The analysis showed that the mature lampshells were more dominant at the size greater than 40 mm for females and greater than 30 mm for males. The smallest female with ripe ovary (stage III) was 22 - 23 mm. Mature individuals were dominant in lampshell sizes over 40 mm for females (Figure 6) and over 30 mm for males (Figure 7).

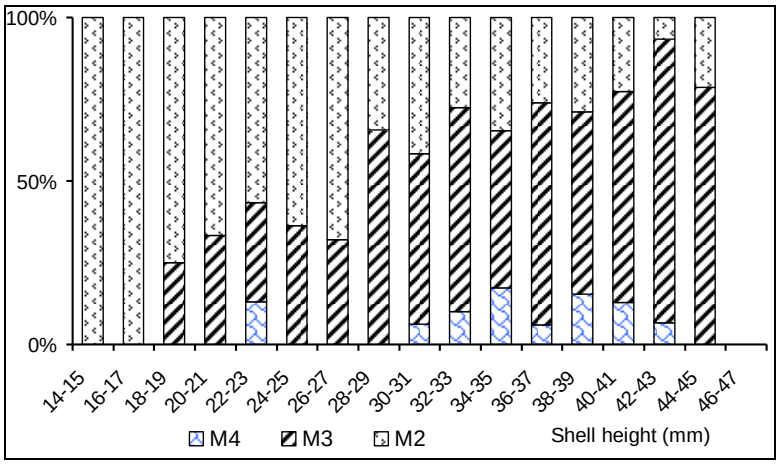

Fig 7: Percentage of male reproductive stages of the size group of the lampshell (Mn: The male at stage n)

3.5. Absolute and relative fecundity of lampshell at Thuy Trieu lagoon

Table 1: Fecundity of lampshell in Thuy Trieu lagoon

Ind.		Whole body weight (g)	Absolute fecundity (eggs/ind.)	Relative fecundity (eggs/ whole body weight)
1		1.23	123,237	100,192.63
2		1.40	43,763	31,259.10
3		1.45	69,649	48,033.56
4		1.50	148,267	98,844.44
5		1.52	152,105	100,069.02
6		1.58	217,581	137,709.71
7		1.63	58,508	35,894.37
8		1.71	134,278	78,525.35
9		1.74	109,517	62,940.90
10		1.85	182,271	98,525.09
11		1.97	104,935	53,266.28
12		2.05	285,186	139,115.30
13		2.48	530,061	213,734.29
14		2.49	98,604	39,600.16
15		2.52	105,441	41,841.76
16		2.95	133,131	45,129.07
17		3.11	205,280	66,006.55
18		3.22	233,915	72,644.38
19		3.24	30,573	9,436.12
20		3.36	312,624	93,042.86
21		3.81	360,200	94,540.64
22		3.83	290,170	75,762.47
23		4.23	473,595	111,960.96
Mean			191,430	80,351
SD			132,093	44,477

The results of the fecundity of the lampshell are shown in Table 1. The average absolute fecundity of the lampshell was 191,430 ± 132,093 eggs/ per individual and the average relative fecundity was 80,351 ± 44,477 eggs / g of whole body weight. In general, the fecundity of the lampshell was quite high and seemed not depend on body size. High fecundity is one of the indicators of high ability to add more offsprings to populations. This is a free egg-laying species, fertilized in the water and parents do not care for their offspring. The high fecundity will increase the numbers of larvae surviving in the wild, which would contribute to the regeneration of the natural population. This characteristic is also similar to other findings on the reproductive habits and fecundity of many other aquatic animals [12, 15].

3.6. Size at the first maturity

The size at the first maturity (L_{50}) was defined as the smallest size group, where 50% of the females have matured gonads. The results showed that the smallest size of sexual maturity (L_{50}) of the lampshell was approximate ~ 28 mm of the shell height (Figure 8). Therefore, it is necessary to limit the exploiting lampshell smaller than 28 mm to ensure that the lampshell has enough time to grow and give the first spawning to replenish their natural population.

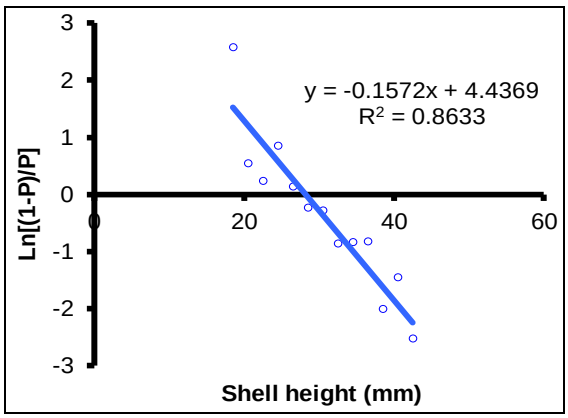

Fig 8: Correlation between size and maturity of lampshell. Represented by equation y = *a*L + *b*, where y = Ln [(1-P) / P] and P was maturation rate in different size groups (L, mm). The first size of maturity (L_{50}) was calculated as *b*/*a*.

4. Conclusions and Recommendations

Lampshell gonads developed in 4 stages. The sex ratio was 1: 1. The breeding season of the lampshell was scattered all year-round, more ripe females found in between July to November. Size at first maturity was ~28 mm. The absolute fecundity was 191,430 ± 132,093 eggs/ individual and relative fecundity was 80,351 ± 44,477 eggs / gram of body weight.

It is necessary to restrict the exploitation of lampshell between July to November and only exploits individuals larger than 28 mm. The initial results have provided some

reproductive characteristics of lampshell species in the Thuy Trieu lagoon, contribute primary data for sustainable management. However, there are still many matters that need further research, such as habitat factors, food and population dynamic of this species. Also, a survey on spawning grounds and habitats for larvae, juveniles and adults should be conducted.

5. Acknowledgments
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