REPRODUCTIVE BIOLOGICAL OF TROCHUS *Rochia nilotica* (Linnaeus, 1767) IN CON DAO NATIONAL PARK, BA RIA-VUNG TAU PROVINCE, VIET NAM

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Abstract: Reproductive characteristics, including spawning season, gonadal developmental stages, sex ratio, size at first maturity, gonadosomatic index (GSI), and fecundity, are essential for developing artificial breeding protocols and establishing management strategies for aquatic resources. The reproductive biology of the trochus *Rochia nilotica* in Con Dao National Park, Ba Ria-Vung Tau Province, was studied from December 2023 to November 2024, using 381 individuals. The results showed that *R. nilotica* is a gonochoric species. Male individuals have white gonads, while female gonads are green. The sex ratio was 1:1. The mean absolute fecundity was 531,557 ± 275,379 eggs per female, ranging from 244,074 to 1,405,464 eggs. The mean relative fecundity was 1,690 ± 877 eggs per gram of body weight, ranging from 877 to 6,170 eggs per gram. Ovarian development of *R. nilotica* passes through five stages. The spawning season extends from May to September, with a peak in September. The GSI of females was highest in September (2.03 ± 0.75), lowest in January (0.71 ± 0.26), and averaged 1.32 ± 0.51. For males, the GSI was highest in September (1.86 ± 0.64), was lowest in February (0.63 ± 0.32), and averaged 1.16 ± 0.49. The size at first maturity for *R. nilotica* was determined to be 65.52 mm in total length.

Keywords: *Rochia nilotica*, trochus, reproductive characteristics, fecundity.

1. INTRODUCTION

The trochus *Rochia nilotica* (commonly known as trochus) is a mollusk species of significant economic importance to fishermen in tropical and subtropical regions of the eastern Indian Ocean and western Pacific. This species is highly prized for its delicious meat, and its shells are used for handicrafts and the production of pearl buttons. However, due to overexploitation, populations of *R. nilotica* have declined drastically [1] prompting conservation measures in several countries, including Indonesia [2], Vietnam [3, 4], and India [5]. Management strategies, including artificial breeding, have been proposed to address the depletion of *R. nilotica* stocks.

The first attempts at artificial breeding and stock enhancement were conducted in the 1980s [6] and gained attention globally with the establishment of hatcheries [7]. Research efforts have included the impact of juvenile size on survival rates [8], natural habitat requirements for juveniles [9], differences in shell patterns between wild and hatchery-reared juveniles [1], habitat studies [10], and biology and reproduction [5]. However, survival hatchery rates in remain low, and post-release survival in the wild is even lower, mainly due to predation on released pre-adults, significantly reducing the success of population restoration efforts [6].

In addition to research into artificial reproduction, other measures have been implemented to prevent overexploitation and enhance *R. nilotica* stocks, such as size and seasonal harvest regulations and the establishment of marine protected areas. Despite these efforts, the depletion of natural *R. nilotica* populations continues [6].

In Vietnam, the increasing demand for *R. nilotica* has led to a dramatic decline in its abundance. Coral reef monitoring in southern Vietnam from Cu Lao Cham (Quang Nam) to Phu Quoc (Kien Giang) during 1993-2007 revealed that the average density of Trochidae species was low, with less than one individual per 100 m². *R. nilotica* was found only in Con Dao waters but at low densities [11]. Surveys of mollusc species composition on coral reefs from Con Co (Quang Tri) to Binh Thuan during 2016-2017 did not record the presence of *R. nilotica* [12]. More recent surveys of invertebrate resources on coral reefs in Con Dao revealed only 10 individuals at 15 reef sites surveyed, a decline compared to 37 individuals at 12 sites surveyed in 2005 [13].

These findings indicate that natural populations of *R. nilotica* face a severe risk of decline due to overexploitation and habitat loss, leading to an alarming depletion of *R. nilotica* stocks on coral reefs in Vietnam, particularly in Con Dao. Therefore, it is crucial to study the reproductive biology of *R. nilotica*, including parameters such as sex ratio, spawning season, size at sexual maturity, gonadal development stages, and fecundity. Such research will provide the basis for further studies on the artificial reproduction of this species.

2. MATERIALS AND METHODS

2.1. Sample collection and analysis

Specimens of *Rochia nilotica* were collected from Con Dao National Park, Ba Ria-Vung Tau Province, from December 2023 to November 2024. After collection, the trochus specimens were transported alive from Con Dao using an aerated method and immediately transferred to the laboratory of the Institute of Oceanography for analysis reproductive characteristics. A minimum of 30 trochus individuals of random size were collected each month, resulting in a total of 381 samples over 12 months.

2.2. Data collection

Measurement of external morphological charateristics

The shell height and aperture diameter were measured using a Palme caliper with an accuracy of 0.1 mm. The aperture diameter was measured from the widest part of the aperture to the opposite side, while the shell height was measured from the aperture to apex of the shell.

Measure the shell height

Measure the aperture diameter

Figure 1. *Determination of external morphological parameters*

Measuring weight

An electronic balance Sartorius BP 110S with an accuracy of 0.001 g was used to measure the total body weight (W), soft body weight (Wtm), and gonadal weight (Wtsd).

2.3. Methods for studying some reproductive biological characteristics of *Rochia nilotica*sex and sex ratio

Determining sex: In *Rochia nilotica*, sex is not determined by external morphology but through dissection, where the color of the internal gonads is observed with the naked eye and under a light microscope.

Sex ratio: All samples collected each month are dissected to extract the testes and ovaries in order to determine the sex ratio using the following formula:

Sex ratio of males = (a/c) × 100 %

Sex ratio of females = (b/c) × 100 %

Sex ratio = 1:(b/a)

where:

a = Number of male trochus specimens collected;

b = Number of female trochus specimens collected;

c = Total number of trochus samples collected.

The independence of the sex distribution was tested using the Chi-square method [14], by comparing the observed results with the theoretical 1:1 distribution.

Developmental stages of the gonads: The gonads of the trochus were dissected to distinguish between males and females. After observation, a small sample of the gonads was mixed with seawater and examined under an Olympus light microscope to preliminarily identify the developmental stages of the gonads. The sample was then fixed for histological preparation.

Determining the spawning season: The spawning season of *Rochia nilotica* was determined based on the monthly analysis of biological samples, the percentage of individuals with mature gonads actively participating in reproduction (gonads at stage IV), and individuals that had already spawned (gonads at stage V). A month in which more than 50 % of the individuals had mature gonads and were actively reproducing or had already spawned was considered the main spawning season of *R. nilotica*. The spawning season was identified based on sample analysis combined with results from surveys of the occurrence of the species at different distribution sites.

Determining the Gonadal Maturity Index (GSI): The gonadal maturity index (GSI) is calculated using the method of [15], which is the percentage ratio of gonad weight to total body weight (including gonads). The formula is as follows:

$$GSI = (Wtsd/W) \times 100\ \%$$

where:

GSI = Gonadal Maturity Index (%);

Wtsd = Gonad weight (g);

W = Total body weight (g).

Determining the fecundity of *Rochia nilotica*: Approximately 0.3 g of eggs (sampled from three positions: the beginning, middle, and end of the ovary) are mixed with seawater to create a suspension. The eggs are counted in a counting chamber to determine the number of eggs per 1 mL of the solution. The egg count is repeated three times, and the average number of eggs per 1 mL of the sample is calculated to estimate the total number of eggs in the ovary.

Absolute Fecundity (Fa): Absolute fecundity (number of eggs per individual) is determined by counting the number of eggs in the mature stage. The total number of eggs per individual is calculated using the following formula:

$$Fa = (a/n) \times Wtsd$$

where:

Fa = Absolute fecundity;

a = Number of eggs counted;

n = Weight of the portion of the ovary used for counting (g);

Wtsd = Gonad weight (g).

Relative Fecundity (Frg): The number of eggs per gram of individual is calculated as follows:

$$Frg = Fa/W$$

where:

Frg = Relative fecundity (number of eggs per gram of individual);

Fa = Absolute fecundity;

W = Total body weight of the trochus (g).

Determining the Size at First Maturity: The size at first maturity is determined using King's method (2001) [16] which is the size at which at least 50 % of the individuals are sexually mature during the spawning season.

2.4. Method for preparing histological slides of ovaries and testes

Histological slides of the ovaries and testes of *Rochia nilotica* were prepared according to the method of Patki et al. (1989) [17]. The gonads were fixed in Bouin's solution for 24 hours, then transferred to 70 % ethanol for preservation.

- Sample Processing: The samples were sequentially immersed in 95 % ethanol and 100 % ethanol, each for 4 hours, then in Methyl salicylate for 24 hours.

- Paraffin Embedding: The samples were embedded in paraffin to form blocks of approximately 2×2×1 cm.

- Sectioning: Sections were cut using a microtome, with a thickness of 5 µm. The sections were placed in warm water (40-50 °C) containing albumin for 1-2 minutes to spread the sections and prevent wrinkling.

- Staining: The slides were stained with Hematoxylin - Mayer and Eosin. Paraffin was removed, and the samples were cleared using xylene solution. The samples were dehydrated and rehydrated with different concentrations of ethanol (100 %, 95 %, 80 %, 50 %).

- Observation and Photography: The prepared slides were observed and photographed using an Olympus BX53 light microscope (Japan).

The developmental stages of the gonads of *Rochia nilotica* were described using the 5-stage scale of [18].

2.5. Data Processing Method

The data were recorded and processed using Microsoft Excel 2007. The means were calculated using the Average function, and the standard deviations were calculated using the Stdev function. The means are presented as: Mean ± Standard Deviation (SD).

3. RESULTS

3.1. Gender determination

Rochia nilotica is a sexually dimorphic species. The gender cannot be distinguished based on the external shell appearance of the trochus; but must be determined by dissection and observation of the gonad colour. The analysis revealed that the female *Rochia nilotica* has green-colored gonads, while the male has white-colored gonads. The intensity and glossiness of the gonads depends on the specific developmental stage of each individual trochus.

Female trochus

Male trochus

Figure 3. *Gonad coloration of Rochia nilotica*

The gonad colouration of *Rochia nilotica* is clearly distinguishable during gonad dissection, especially in mature individuals during the breeding season, and in individuals with gonads atstages III and IV. At these stages, the gonads are swollen, bright, and translucent, and can flow when disturbed. In the case of eggs, they can be observed with the naked eye.

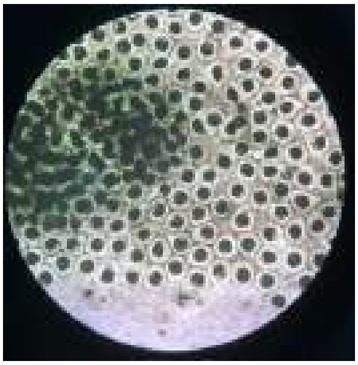
3.2. Sex ratio

The sex ratio of *Rochia nilotica* was analysed over 12 months of random sampling, with a total of 381 samples, including 178 males and 203 females. No samples were undifferentiated by sex. The male/female ratio varied irregularly over the months, as shown in Figure 5.

The analysis results indicate a sex ratio of 1:1.14. However, the chi-square test for the independence of the observed sex ratio (1:1.14) compared to the theoretical ratio (1:1) yielded a $\chi 2$ value of $1.640 < 3.841$ ($df = 1$, $P < 0.05$), indicating no significant difference between the observed and theoretical ratios. Therefore, the sex ratio of *Rochia nilotica* is 1:1.

Female gonad, stage 4

Male gonad, stage 4



Fresh eggs, magnification 40x

Sperm, magnification 40x

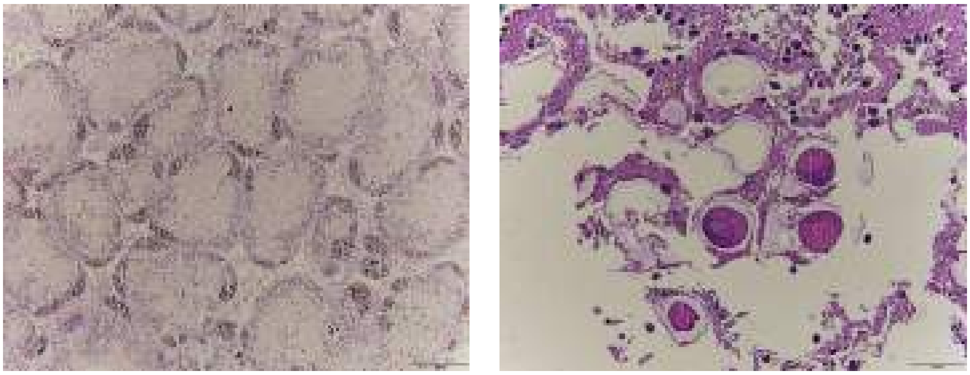
Figure 4. *Gonads and reproductive products of Rochia nilotica*

Figure 5. *Sex ratio of Rochia nilotica*

3.3. Developmental stages of the ovary and testis

Observation of fresh specimens and gonadal tissue slides of *Rochia nilotica* samples revealed that the gonads of this species undergo 5 developmental stages. Each stage is characterised by distinct features in terms of shape and size. However, during sampling, no individuals with gonads in Stage I were encountered. The developmental stages of the gonads of *Rochia nilotica* are as follows:

Stage II: Developmental stage. The testes and ovaries begin to develop at this stage. The size of the ovary increases, and the testes and ovaries can be distinguished by the colour of the gonads. The male gonads are white or pale, while the female gonads are green. Spermatocytes and oocytes are round or oval in shape.

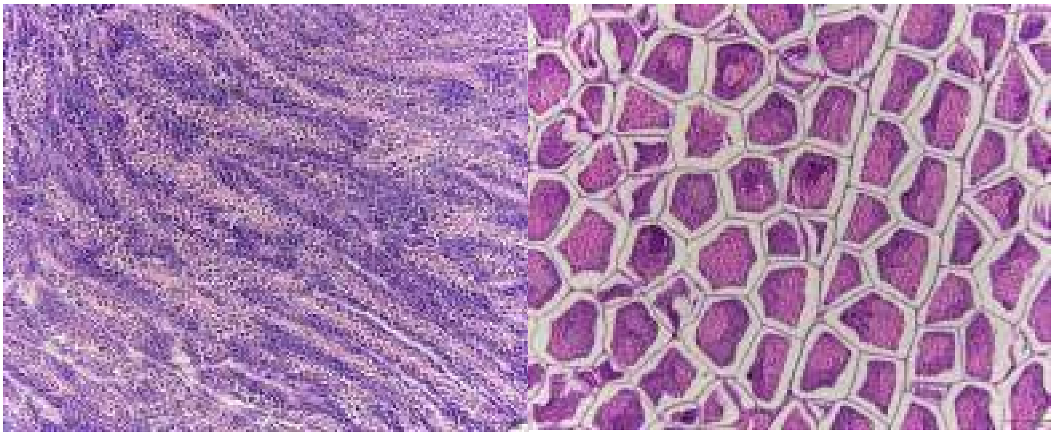


Testis *Ovary*

Figure 6. *Gonads of Rochia nilotica at stage II (magnification 100x)*

Stage III: Early maturation stage. The male gonads are milky white, with gametes forming small oval shapes clustered together in groups, and thickened follicular walls. The female gonads are light green, with ovaries containing follicles of uneven sizes. Observation of the sectioned specimens shows small spherical cells that begin to accumulate yolk.

Stage IV: Maturity stage (reproductive maturity). The gonads are swollen, larger in size, and the color of the gonads is darker compared to stage III.



Testis *Ovary*

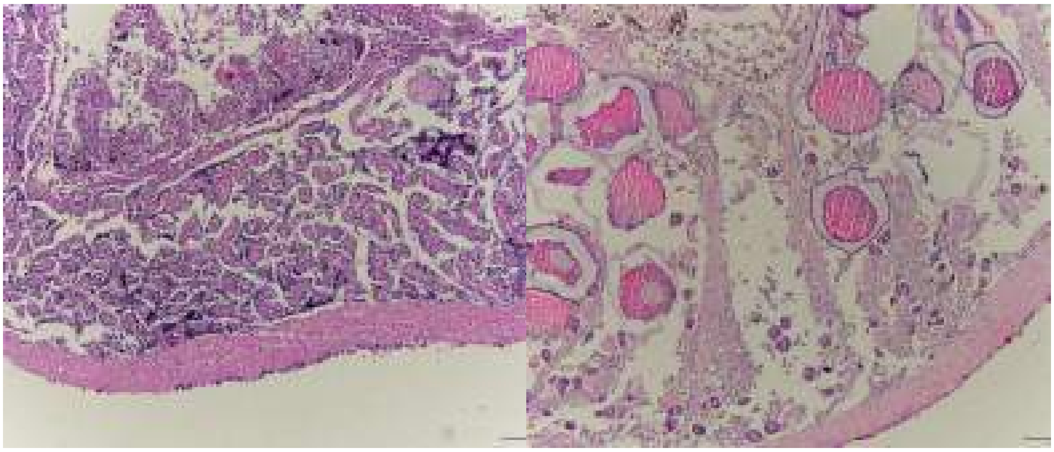
Figure 7. *Gonads of Rochia nilotica at stage III (100x magnification)*

Testis *Ovary*

Figure 8. *Gonads of Rochia nilotica at stage IV (100x magnification)*

Male germ cells are concentrated into clusters and separated from each other; sperm begins to detach from the germ cells. Female germ cells have completed the nutrient accumulation process and reached their maximum size. The egg cells are spherical, large, and separate from each other.

Stage V: This is the post-spawning stage. The gonads shrink, the volume decreases, and the colour becomes pale and blotchy.



Testis *Ovary*

Figure 9. *Gonads of Rochia nilotica at stage V (100x magnification)*

3.4. Reproductive season

Maturity Rate by Month

The maturity rate was highest in September, reaching 100 %, and lowest between December and March (0 %). The research also showed that from December to March, most individuals had gonads in stage II, with very few individuals in stage III, and there were no mature females (gonads in stages IV and V).

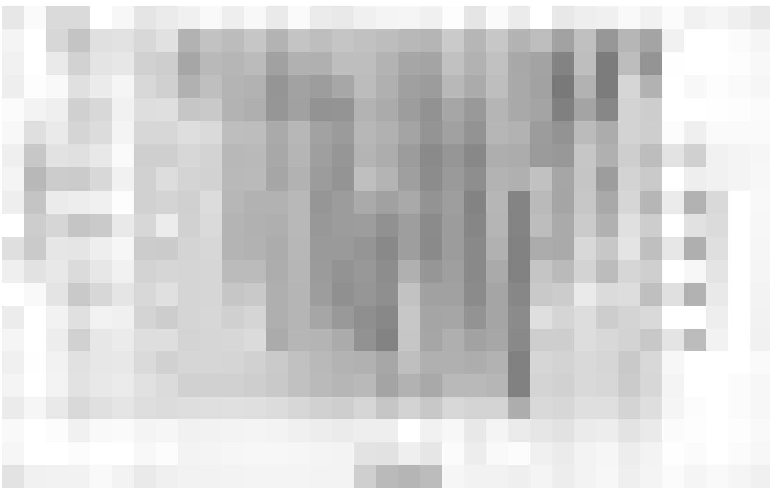


Figure 10. *Gonadal stages of Rochia nilotica*

Starting in April, mature individuals (stages IV and V) began to appear, indicating that this is the time when Rochia nilotica begins to be able to reproduce. The maturity rate remained high (ranging from 63.33 % to 100 %) from May to September of the following year (Figure 10). However, during the remaining months (April, September, and October), there were still individuals in the mature stage, with gonads in stages IV and V.

Gonadal maturity index (GSI)

The GSI of female *Rochia nilotica* was highest in September (2.03 ± 0.75), lowest in January (0.71 ± 0.26), and averaged 1.32 ± 0.51. For male Rochia nilotica, the GSI was highest in September (1.86 ± 0.64), lowest in February (0.63 ± 0.32), and averaged 1.16 ± 0.49.

Figure 11. *Gonadal maturity index and maturity rate through the study months*

From the research results on gonadal maturity rate and gonadal maturity index (Figure 11), it shows that *Rochia nilotica* in Con Dao National Park, Ba Ria-Vung Tau province, has a breeding season that lasts from May to September, with the peak reproductive period in September.

3.5. First Sexual Maturity Size

The research results show that *Rochia nilotica* with a size larger than 80 mm has a 100 % maturity rate. The group of *Rochia nilotica* smaller than

50 mm has not yet reproduced. The group of Rochia nilotica larger than 66 mm has a sexual maturity rate of over 50 %. To determine the exact size at first sexual maturity of Rochia nilotica, the relationship between length and Ln((1-P)/P) must be considered, as shown in Figure 12.

By solving the correlation equation y = -0.2205*x + 14.448, the first sexual maturity size of *Rochia nilotica* was determined to be 65.52 mm.

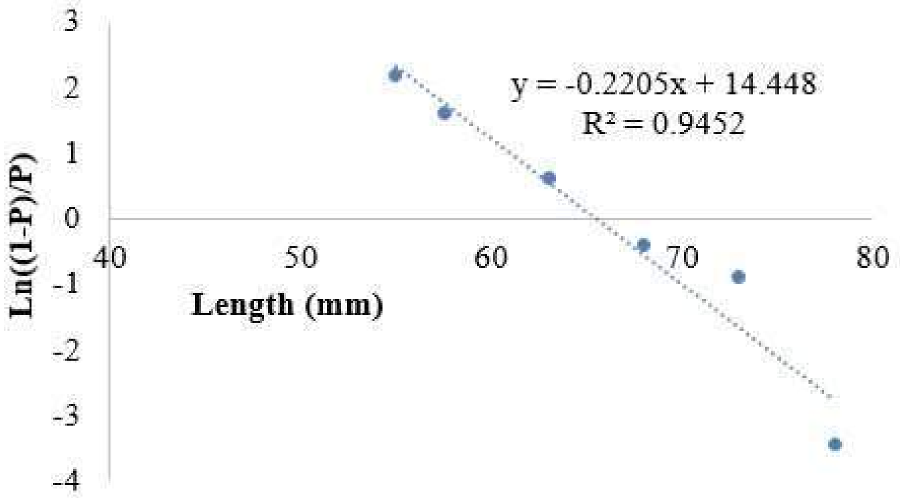


Figure 12. *Graph showing the correlation between size groups and Ln((1-P)/P)*

3.6. Fecundity

The results show that *Rochia nilotica* has an absolute fecundity of approximately 531,557 eggs per individual on average. However, fecundity varies significantly between individuals, with some individuals producing only 244,074 eggs, while others have a much higher absolute fecundity, reaching up to 1,405,464 eggs per individual. The relative fecundity of Rochia nilotica is 1,690 ± 877 eggs/g of body weight, ranging from 877 to 617,067 eggs/g.

4. DISCUSSION

The research results show that the sex ratio of *Rochia nilotica* in Con Dao National Park, Ba Ria-Vung Tau Province is distributed at a ratio of 1:1. This finding is entirely consistent with previous studies by [19-21], which reported the sex ratio of *Rochia nilotica* to be 1:1. When compared to the sex ratios of other species such as *Babylonia areolate, Strombus canarium and Nerita balteata*, there is little variation among these species. The average sex ratio for *Babylonia areolata* is 1:1.49 [22], for *Strombus canarium* is 1:1.27 [23], and for *Nerita balteata* is 1:1.34 [24]. Meanwhile, the sex ratio for the Red-lip conch (*Strombus luhuanus*) was determined to be 1.27:1 [25], which falls within the general ratio observed in most gastropod species.

The reproductive season of Trochus varies depending on the geographical region. In this study, *R. nilotica* has a reproductive season lasting from May to September. However, some published results indicate *R. nilotica* reproduces sporadically throughout the year in tropical regions [20]. In Eastern Indonesia, the main reproductive season of the species occurs from March to April and from September to December, with an additional secondary spawning season [15]. Meanwhile, the study by [26] showed that their reproductive cycle in Western Australia (King Sound) occurs year-round with two main phases: a short reproductive phase from April to August and a long reproductive phase from December to April of the following year. However, according to [27], the reproductive season of *R. nilotica* in Okinawa consists of five phases: a resting phase (from November to February), a preparation phase (from March), the onset of spawning (from April to May), a peak spawning phase (from June to September), and a contraction phase (October). On the other hand, [6] suggested that snails in tropical regions reproduce year-round. These differences highlight the complexity of the reproductive cycle of *R. nilotica* and underline the importance of conducting further studies to clarify the factors influencing the timing and frequency of reproduction in this species in different environments.

The study results indicate that the first maturation size of female Trochus is 65.52 mm. This finding is consistent with previous studies conducted on female Trochus. Specifically, females distributed in the Andaman Islands have a first maturation size ranging from 60–70 mm

[28], while those in New Caledonia exhibit a first maturation size between 65–70 mm [29]. However, the maturation size reported in this study is smaller than that documented by [21], who found that female Trochus in New Caledonia reached their first maturation size at 80 mm. Other studies have reported even smaller sizes, such as 50–60 mm for snails in the Great Barrier Reef, Australia [19], and 55–65 mm [30]. These variations highlight that the first maturation size of female Trochus is significantly influenced by geographical distribution and hydrological factors, that affect growth and nutrient accumulation for reproduction, leading to regional differences.

According to [31], the gonadal maturation index varies between species, between individuals within a population, and even within an individual during the breeding season. Typically, the index increases from stage I to stage IV and then decreases sharply at stage V. This suggests that the gonadal maturation index of female Trochus follows the general patterns observed in other aquatic species. Additionally, in artificial breeding studies, F1 generation female Trochus were found to be capable of reproduction when they reached an average size of approximately 55 mm [32, 33]. Notably, [32] analyzed the relationship between snail size and the percentage of individuals at different maturation stages. The findings revealed that approximately 50 % of individuals with a size exceeding 65 mm had reached maturity, underscoring the critical role of body size in determining the reproductive potential of this species. These insights contribute to a deeper understanding of the biology of female Trochus and support conservation and management efforts in natural populations as well as aquaculture programmes.

The study also shows that female Trochus exhibit considerable variability in fecundity, consistent with previous research. [6] reported that the fecundity of female Trochus ranged from 100,000 to 1,000,000 eggs per individual, while [30] documented a range of 300,000 to 2,000,000 eggs per female. Similarly, other gastropod species also demonstrate substantial variability in fecundity. For instance, the primary spawning season of *Tectus pyramis* is associated with fecundity levels of approximately 419,840 eggs per individual and 13,960 eggs per gram of body weight, while secondary spawning yields 145,720 eggs per individual and 2,740 eggs per gram [34]. *Strombus luhuanus* was found to have a fecundity of 251,080 ± 89,843 eggs per individual, ranging from 166,000 to 441,000 eggs. These differences likely arise from uneven gonadal maturation between individuals, with asynchronous and prolonged spawning throughout the breeding season.

5. CONCLUSION

The female Trochus snail is a sexually dimorphic species, but gender cannot be determined based on external morphology. During the spawning season, females exhibit dark green gonads, whereas males have milky white gonads. The natural male-to-female ratio in Trochus is 1:1. Gonadal development in Trochus progresses through five stages. In the waters around Con Dao, Ba Ria-Vung Tau Province, the spawning season of the female Trochus snail extends from May to September, with a peak in September. The relative fecundity of the female Trochus snail varies considerably, ranging from 244,074 to 1,405,464 eggs per individual, with an average of 531,557 eggs per individual. The relative fecundity was observed to average 1,690 ± 877 eggs per gram of body weight, with a range spanning from 877 to 6,170 eggs per gram. The first maturation size of female Trochus in the Con Dao region is 65.52 mm.

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