

Initial Attempts on Spawning and Larval Rearing of the Blood Cockle, *Tegillarca granosa* (Linnaeus, 1758), in the Philippines

DENISE VERGARA MIRANDA, VICTOR MARCO EMMANUEL NUESTRO FERRIOLS*
Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas,
Miag-ao, Iloilo, Philippines

*E-mail: vnferriols@up.edu.ph | Received: 08/11/2022; Accepted: 05/05/2023

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Abstract

Blood cockles, *Tegillarca granosa* (Linnaeus, 1758), were induced to spawn by temperature stimulation. Larval to spat rearing was accomplished in the hatchery facility of the Institute of Aquaculture of the University of the Philippines Visayas. The survival rate from day 1 larval stage to day 120 spat stage was 2.48 % (15,200) from the initial 614,100 eggs reared. The fertilised eggs (average size: 50.23 ± 5.18 μm in diameter) passed through the morula stage 5 h post-fertilisation and reached the trochophore stage after 8 h. Day 1 D-shaped larvae (average size: 84.60 ± 3.90 μm length (l) and 66.36 ± 1.92 μm height (h)) transformed into umboned larvae by day 7. The majority of the larvae reached the advanced umbo stage by day 12 (average size: 204.52 ± 21.06 μm l and 178.24 ± 17.04 μm h). By day 20, loss of velum and foot development in larvae were observed, marking the beginning of the pediveliger stage. The growth of post-set larvae starting from the initial settling stage at day 30 (average size: 360.50 ± 52.10 μm l and 309.56 ± 34.56 μm h) to day 120 (average shell length: 3.870 ± 0.400 mm) was periodically monitored. Cockle spats had squarish shells with central elevation and visible radial ribs resembling the morphological characteristics of adult *T. granosa*. Only *Isochrysis galbana* Parke, 1949, was given as food for the cockles throughout the rearing activity. Further refining of larval and post-set rearing methodology is necessary for future mass production of cockle seeds in the Philippines.

Keywords: marine bivalve, induced spawning, larval rearing, bivalve aquaculture

Introduction

Tegillarca granosa (Linnaeus, 1758), more commonly known as blood cockles or blood clams, is a marine bivalve species from the family *Arcidae* (ark clams). They are found primarily in intertidal or marginally sub-tidal regions and thrive in areas with soft substrates, such as mudflats adjacent to mangrove forests (Broom, 1985). *Tegillarca granosa* is usually identified through their thick equivalve shells, strongly protruding umbones, and 15-20 radial ribs that are stout and distinctly rugose (Afiati-Brotohadikusumo, 1994; Poutiers, 1998). Furthermore, they exhibit a characteristic reddish flesh due to the red blood pigment haemoglobin, which also aids in their survival in low-oxygen environments (Broom, 1985; Mohite and Meshram, 2015). The commercial fishery of *T. granosa*

continues to expand in countries like China, Korea, Thailand, Indonesia, and Malaysia, where cockles are a delicacy. Moreover, it has been one of the staple mollusc species produced in world aquaculture for several years, amounting to 3 % of the total molluscan fishery from 2010 to 2016 (FAO, 2018).

In the Philippines, *T. granosa* is a tasty and protein-rich shellfish usually collected by coastal inhabitants in brackish mud flats during low tides to supplement their diets or sold for cheap in the market. Although the members of the *Arcidae* family, including *T. granosa*, are an important food source, there is limited research reported in the Philippines regarding them. There is a need to develop suitable culture techniques for cockles in the country to meet the rising demand. Cockle aquaculture faces significant obstacles

worldwide due to the fluctuating seed supplies, which primarily depend on the prevailing environmental conditions in each country (Ng, 1986; Nair, 2001; Khalil et al., 2017). As a result, the production of cockle seeds in hatcheries is critical to ensure consistent availability. In other countries like Malaysia and India, the culture of cockles has been extensively studied, and several successful induced spawning methods have already been established (Wong and Lim, 1985; Muthiah et al., 1992; Mohd. Saleh et al., 2020). The most preferred seems to be the physical shocking of bivalves, specifically temperature shocking, as it is a less invasive method than other methods like chemical stimulation or gonad-stripping (Aji, 2011).

There are no known up-to-date studies of *T. granosa* production and rearing in the Philippines. On this account, the goal of the present study was to produce *T. granosa* seeds in a hatchery in the Philippines setting. Specifically, the researchers attempted to artificially induce spawning of broodstocks through temperature shock methods and establish protocols for larval and post-larval rearing of *T. granosa*.

Materials and Methods

Ethical approval

Induced spawning and larval rearing experiments with blood cockles were conducted according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines (Kilkenny et al., 2010) and were reviewed by the Institutional Animal Care and Use Committee (IACUC) of the University of the Philippines Visayas (08/08/2022).

Sample collection

Adult *T. granosa* ranging in length from 34 to 61 mm were collected from the municipality of Dumangas, Iloilo, Philippines, in November 2021 and transported to the University of the Philippines Visayas hatchery facility in Miag-ao, Iloilo, Philippines. The samples were placed inside 200 L fibreglass reinforced plastic (FRP) tanks containing filtered seawater (salinity 35 ppt). Induced spawning through temperature stimulation was attempted the following day. Twenty to thirty unsexed cockles were used for the spawning experiment following the method described by Wong and Lim (1985) with some modifications.

The samples were alternately placed in 50 L plastic tanks containing filtered chilled seawater (temperature 16–18 °C; salinity 35 ppt) for 30 min and in another tank with warm seawater (temperature 30–32 °C; salinity 35 ppt) for another 30 min. This procedure of cyclic immersion was repeated for a total of five hours. When no spawning occurred, the samples were returned back to their conditioning tanks and fed with a liberal amount of *Isochrysis galbana* Parke, 1949. Spawning occurred in the conditioning tanks 9 h after temperature stimulation. The fertilised eggs were gently siphoned from the water and thoroughly washed

with filtered seawater using 30 µm nylon mesh sieves to remove excess debris such as faecal matter, excess sperm, and other particulates. The fertilised eggs were transferred into 500 L round FRP tanks filled with 300 L filtered seawater.

Embryonic and larval development

The succeeding developmental stages of the larvae were monitored using an HD microscope camera (RS-500C, Shenzhen Hayear Electronics, China) connected to a compound microscope (Optiphot, Nikon, Japan) and analysed using S-EYE imaging software (Version 1.6.0.11; Shenzhen Hayear Electronics, China) every few minutes to observe and record the cleavage, morula, and trochophore stages. On day 1, a portion of the D-shaped larvae were strained using 30 µm sieves to estimate the total larval count using a counting chamber. Due to food and space constraints in both the outdoor and indoor set-up at the laboratory, most of the fertilised eggs were released to nearby ponds retaining only 614,100 larvae to be reared.

The rearing tanks were aerated at a prolonged bubble rate to prevent excessive water disturbance affecting the development of the larvae (Helm et al., 2004). Water was exchanged on alternate days, and the veliger larvae were fed with *I. galbana*. When introducing diets to bivalves, an important factor to consider is the food nutrient content and particle size (Knauer and Southgate, 1999). *Isochrysis galbana* (5–6 µm in size) is a golden-brown microalga that contains several essential polyunsaturated fatty acids (Liu et al., 2013) and has been proven to promote the shell growth of bivalves (Liao et al., 2017). Additionally, bivalve larvae preferentially ingest food 2–4 µm in size, while juveniles and adults readily accept food in the size range of 3–25 µm (Knauer and Southgate, 1999; Rahman et al., 2020). *Isochrysis galbana*, is a suitable food for *T. granosa* larvae and spat. Other commonly used microalgae species in bivalve hatcheries are *Chaetoceros* sp., *Thalassiosira* sp., and *Tetraselmis* sp. or a combined mixture of all species (Helm et al., 2004).

In this experiment, however, only *I. galbana* was given as cockle's food. Cell concentrations of *I. galbana* fed during each stage of development of *T. granosa* are shown in Table 1 based on feeding rates reported by Muthiah et al. (1992). The growth rate was monitored to assess larval fitness by periodically measuring the length of the larvae from anterior to the posterior axis and dorsal to the ventral axis for height (measured in µm) using an HD microscope camera and ImageJ software (Version 1.8.0_172; National Institutes of Health, USA) for accurate measurement. Water temperature and salinity were recorded in the morning and afternoon daily.

Spat rearing

Pediveliger *T. granosa* were transferred to 95 L plastic tanks, where they were held until they reached the spat stage for easy monitoring. The stocking density for each container was 527 larvae L⁻¹.

Table 1. Feeding rate of *Isochrysis galbana* given as food for *Tegillarca granosa* during larval to spat stage.

Stage of larvae	Day	Microalgal cell concentration (cells ind ⁻¹ day ⁻¹)
D-shaped	1-7	5,000
Umbo to advanced umbo	8-15	7,000
Advanced umbo to eyed	16-20	10,000
Pediveliger	21-25	12,000
Pediveliger	26-30	15,000
Newly-settled spat	31-40	20,000
Newly-settled spat	41-59	25,000
Spat	60-80	30,000
Spat	81-95	40,000
Spat	96-115	45,000
Spat	116-120	50,000

*Adapted from Muthiah et al. (1992).

In addition, complete water change was carried out every alternate day, and spats were fed *I. galbana* following feeding rates in Table 1. *Tegillarca granosa* spats were collected using 120 µm sieves during each water exchange to determine survival percentage and growth by measuring their shell axis under the microscope. The most representative morphological changes from the larval to post-larval stages were recorded. Throughout the larval to spat rearing, the salinity of the water was at 33 to 35 ppt, and the temperature ranged from 25 °C to 30 °C.

Results

Early development and larval rearing

Female *T. granosa* eggs were salmon pink, while sperm released by males had a milky appearance. Following the insemination of the sperms to the egg (Fig. 1a), the formation of the first polar body ensued within 10 min (Fig. 1b) and then succeeded by cleavage or cell division (Figs. 1c-g). After 5 h, the larvae passed through the morula stage (Fig. 1h) and reached the trochophore stage after 8 h (Fig. 1i). Fertilised eggs recovered were spherical and opaque, and their size measured 50–55 µm. D-shaped or straight-hinge larvae were observed 24 h after fertilisation.

On the first day post-fertilisation, D-shaped larvae developed shells that protected their visceral organs (Fig. 2a). The total average, minimum, and maximum size (length × height) of the D-shaped larvae were 84.60 ± 3.87 × 66.36 ± 1.92 µm, 77.68 µm × 63.9 µm and 90.44 × 67.29 µm, respectively. On day 7, a few early umbo-stage larvae were observed (Fig. 2b), but most were still at the D-shaped larval stage. It was by day 10 when the majority reached the early umbo stage while several had already transformed into the advanced umbo stage. The total average, minimum, and maximum size (length × height) of the umbo larvae were 165.29 ± 24.62 × 133.16 ± 15.05 µm, 131.94 × 113.01

µm and 194.09 × 142.35 µm, respectively. The ciliated velum used for swimming and food collection of the veliger larvae is shown in Figure 2c. Most D-shaped larvae reached the advanced umbo stage by day 12.

The relationship between the length and height of larvae during early larval development stages is described by the equation:

$$H = -5.0133 + 0.8787 L$$

where *H* and *L* represent height and length in µm, respectively. The correlation coefficient between the height and length of *T. granosa* was 0.9822 (Fig. 3).

Settlement and early nursery

On day 15, the total average, minimum, and maximum size (length × height) of the larvae were 204.52 ± 12.08 × 178.24 ± 11.41 µm, 189.59 × 164.98 µm and 231.85 × 204.95 µm, respectively. Morphological changes such as the presence of eyespot (Fig. 2d) and rudimentary foot were observed, marking the beginning of the pre-settlement or pediveliger stage. By day 20, the majority had reached the pediveliger stage. The total average, minimum, and maximum size (length × height) of larvae were 211.72 ± 14.51 × 184.13 ± 8.61 µm, 193.45 × 172.55 µm and 230.37 × 195.29 µm, respectively.

Pediveliger larvae gradually lost their velum cilia and developed a functional foot. By day 30, pediveliger started to settle on the bottom of the holding tank, signalling their metamorphosis into spats. Dissoconch secretion around the prodissoconch increased, and the cockle shell acquired an extended form. Growth lines were also more evident at this stage (Fig. 2e). Rib formation was observed, and the border of the shell formed a serrated outline (Fig. 2f). The total average, minimum, and maximum shell size (length × height) of the newly settled spats were 360.50 ± 52.10.31 × 309.56 ± 34.56 µm, 317.67 × 287.60 µm and 436.68 × 356.46 µm,

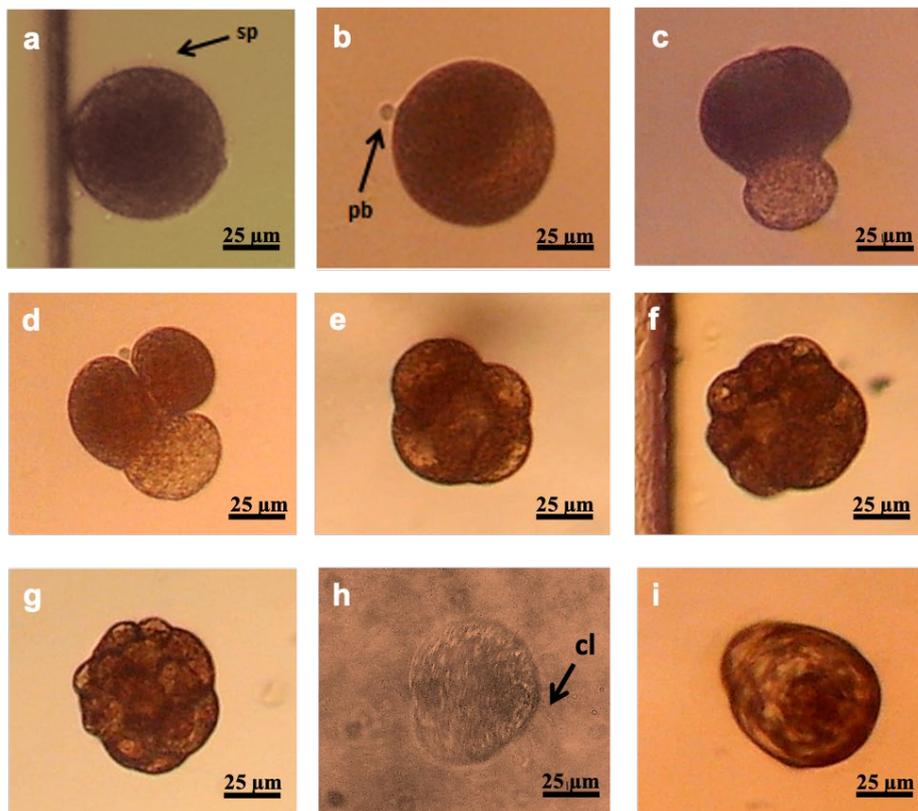


Fig. 1. Early developmental stages of *Tegillarca granosa*: (a) unfertilised eggs with attached sperms (00:00); (b) formation of the first polar body (00:08); (c) formation of first cleavage (00:18); (d) second cleavage (0:22); (e) third cleavage (00:30); (f) fourth cleavage (00:36); (g) fifth cleavage (00:45); (h) morula stage (05:40); (i) trochophore (08:00). Abbreviations: sp, sperm; pb, polar body; cl, cilia. Time stamps are in hh:mm format.

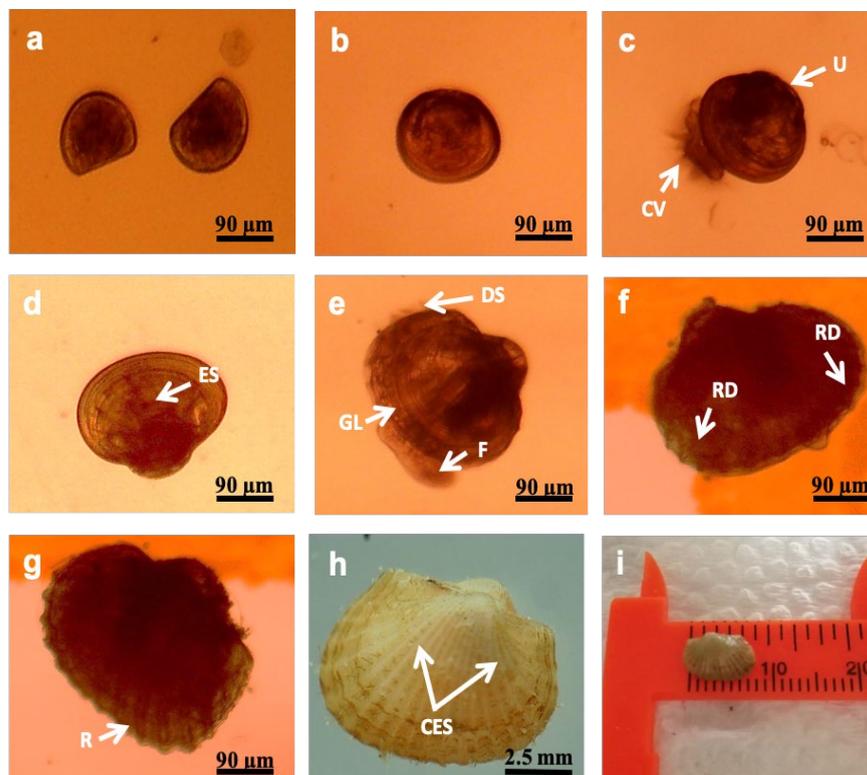


Fig. 2. Larval stages and spat *Tegillarca granosa*: (a) D-shaped larval stage (b) Early umbo stage (c) Advanced umbo stage (d) Pediveliger larval stage (e-g) Newly settled cockle spat (h-i) Spat. Abbreviations: CES, central elevation of the shell; CV, ciliated velum; DS, dissoconch; RD, developing ribs; ES, eyespot; F, foot; GL, growth lines; R, ribs; U, umbo.

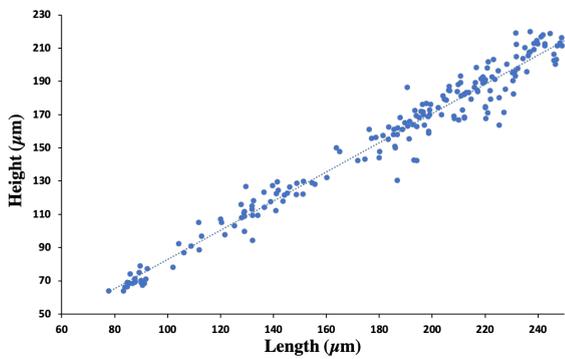


Fig. 3. Height and length correlation of *Tegillarca granosa* larvae.

respectively.

Day 40 newly settled cockle spats showed more prominent ribs separated by deep gaps in the middle and an elevated convex growth under the umbo in the centre of the shell (Fig. 2h). The total average, minimum, and maximum shell size (length × height) of spats in length were $553.15 \pm 100.72 \mu\text{m} \times 452.61 \pm 63.10 \mu\text{m}$, $389.25 \times 352.30 \mu\text{m}$ and $644.34 \times 541.92 \mu\text{m}$. From day 50 onwards, shell sizes were only measured during days of water change (every other day) to avoid damaging the shells of the spat and causing unnecessary stress.

The growth of the pediveliger to the newly-settled spat stage (day 20 to day 40) is described by the exponential equation (Fig. 4):

$$L = 78.836e^{0.048D}$$

where L is the length in μm , and D is the number of days after spawning ($r^2 = 0.96$).

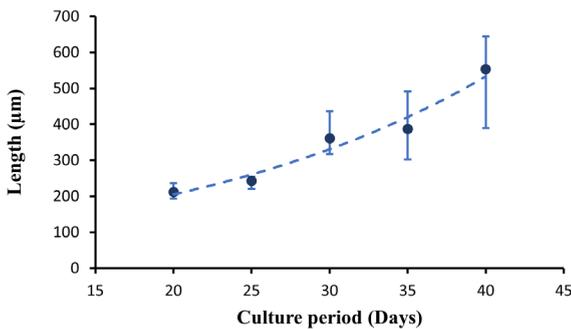


Fig. 4. Growth of *Tegillarca granosa* measured as average shell length from pediveliger larvae to newly settled cockle spat. The solid circles represent the mean and the vertical lines, the standard deviation (SD). Dashed line shows the exponential regression model and growth curve analysis.

Spat rearing

On day 60, the total average, minimum, and maximum shell size of spats (length × height) were $1.220 \pm 0.120 \times$

$0.895 \pm 0.063 \text{ mm}$, $0.958 \times 0.861 \text{ mm}$ and $1.330 \text{ mm} \times 1.010 \text{ mm}$, respectively. The morphological characteristics of spats already resembled that of an adult *T. granosa* (Figs. 2h and 2i). On day 90, the average shell length of spats was $2.960 \pm .563 \text{ mm}$, and by day 120, their average length was $3.870 \pm 0.400 \text{ mm}$. Spat growth during nursery in plastic tanks in the laboratory is shown in Figure 5.

The regression analysis fits a significant logarithmic model ($r^2 = 0.93$) and described by the equation:

$$L = 3120.8 \ln(D) - 11386$$

where L is the length in μm and D is the number of culture days during the spat stage.

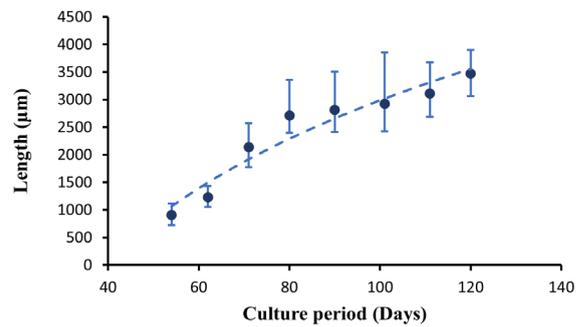


Fig. 5. Growth of *Tegillarca granosa* spat cultured under laboratory conditions at 35 ppt salinity, $28 \pm 2 \text{ }^\circ\text{C}$. The solid circles represent the mean and the vertical lines, the standard deviation (SD). Dashed line shows logarithmic regression model and growth analysis.

Tegillarca granosa production

An estimated 6,531,000 fertilised eggs were produced after spawning several *T. granosa* broodstock; however, only 614,100 day 1 D-shaped larvae were retained for larval rearing. The number of larvae that survived from day 1 to day 60 was a total of 58,200 which gave a survival rate of 9.48 %. On day 80, approximately 35,045 spat survived (5.71 %), and by the fourth month (day 120), only 15,200 remained, giving an overall survival rate of only 2.48 % from post-fertilisation (Table 2).

It is important to note that the highest mortality (lowest survival) was observed during the stages from the 1st to the 60th day of culture. This covers the period when the D-larvae transitioned through the umbo and pediveliger stages and finally metamorphosed into spats. Higher survival rates were subsequently observed from the 60th day onwards when larvae reached the spat stage.

Discussion

The importance of *T. granosa* and other anadarinids, as a food source is already established worldwide. In the

Table 2. Survival rates of *Tegillarca granosa* larvae during 120 days of culture post-fertilisation.

Day	Total larvae/Spat	Overall survival rate(%)	Survival rate from previous count (%)
0	6,531,000 [†]	Not applicable	Not applicable
1	614,100*	100	100
60	58,200	9.48	9.48
80	35,045	5.71	60.21
120	15,200	2.48	43.37

[†]total fertilised eggs from induced spawning.

*overall survival rate was based on the initial 614,100 D-larvae that were retained for further larval rearing.

Philippines, bivalve aquaculture of clam species is still limited but shows tremendous potential. In this experiment, culture and rearing of *T. granosa* in the hatchery were attempted starting by subjecting wild broodstock to a series of temperature fluctuations to induce spawning of gametes. Induced spawning through this method is the most natural and least invasive method for bivalves compared to other methods (Aji, 2011).

Clams are poikilothermic organisms; therefore, water temperature directly influences their metabolism. An increase in water temperature increases their metabolic rate; correspondingly, a decrease in water temperature decreases their metabolic rate; thus, reproduction and spawning tend to be regulated by temperature (Weber et al., 2019). The thermal shock method was first introduced by Kanno (1963), Yoo (1969), and Kim and Koo (1973) for the artificial culture of *Anadara broughtonii* (Schrenck, 1867) and by Ting et al. (1972) for *Anadara kagoshimensis* (Tokunaga, 1906). Vakily (1992) also successfully produced *Anadara tuberculosa* (Sowerby, 1833) gametes by thermal shock for a different experiment involving the genetic characterisation of larvae.

Other methods, such as the use of chemicals like serotonin and sex steroids, are rarely advised because eggs released are usually less viable (Helm et al., 2004). Physical methods such as gamete stripping are mainly done for oysters, as clams' and scallops' eggs need to undergo maturation inside the oviducts of the females (Utting and Spencer, 1991; Helm et al., 2004).

For *T. granosa*, Wong and Lim (1985) carried out induced spawning by immersing ripe cockles in warm water at 30–32 °C and cool water at 16–18 °C alternately for 1.5–2 h. The water temperature used in the study mirrored their experiment except for the immersion time. In the study by Muthiah et al. (1992), the spawning of *T. granosa* occurred when the temperature was brought down from 32 °C to 24 °C while in the study of Mohd. Saleh et al. (2020), thermally stimulated cockles spawned at temperatures of 28–29 °C to 32–35 °C. However, in the present experiment, no spawning occurred during the temperature stimulation process, and the liberation of gametes took place in the conditioning tanks 9 h after stimulation.

The fertilised eggs of *T. granosa*, as reported by Muthiah et al. (1992) measured 50–60 µm with an average of 51.9 µm. The eggs reached the trochophore stage after 5 h and finally into D-shaped larvae 20–26 h after fertilisation. Wong and Lim (1985) showed similar results wherein mature eggs measured approximately 55 µm in diameter, developed into trochophores after 4 h, and D-shaped larvae after 22 h. Finally, fertilised cockle eggs from the experiment by Mohd. Saleh et al. (2020) were 48–62 µm in diameter and became D-shaped veliger after 24 h. The results of the three experiments are comparable to the present study, albeit with slight differences in development hours.

The only food given to the larvae up until the present spat stage was *I. galbana*. According to Helm et al. (2004), a mixed algal diet of a suitably sized diatom and flagellate is recommended for improved larval development and growth rate and spat survival as opposed to a single species diet. Kim and Koo (1973) fed *Chaetoceros calcitrans* (Paulsen) Takano, 1968, *Monochrysis lutheri* Droop, 1953, *Monas* spp., and *Skeletonema costatum* (Greville) Cleve, 1873, to their D-shaped larvae. They also increased the rate of feeding as the larvae grew. Yoo (1969) recommended that optimal growth can be achieved by adjusting the quantity of food based on the algae species and the tank volume rather than the number of larvae.

During larval development, reports have shown varying times when larvae reach the umbo stage ranging from 7 to 13 days (Muthiah et al., 1992; Wong and Lim, 1985; Mohd. Saleh et al., 2020). In the present study, umbo development was observed by day 7. By day 12, the majority reached the advanced umbo stage.

Most larvae lost their velum and acquired a developed foot by day 20. The growth rates were slower than that of Muthiah et al. (1992), where the pediveliger stage was already reached by day 16 and the spat stage on day 18. In the report of Wong and Lim (1985) and Mohd. Saleh et al. (2020), settlement of the larvae occurred on day 21–22 and day 27, respectively, while in the current study, some of the larvae transformed into newly settled cockle spat by day 30 and the majority on day 40. The comparison of the days of development of *T. granosa* from larval to spat stage with other related studies is summarised in Table 3.

Table 3. Comparison of days of development of *Tegillarca granosa* from larvae to spat stage with other similar studies.

Stage of larvae	Current study	Mohd. Saleh et al. (2020)	Muthiah et al. (1992)	Wong and Lim (1985)
Fertilised egg to D-shaped	21-24 h	24 h	20-26 h	22 h
D-shaped to advanced umbo	Day 12	Day 12	Day 12	Day 13
Umbo to eyed pediveliger	Day 20	Day 19	Day 16	No data
Newly settled spat	Day 30-40	Day 27	Day 18	Day 21-22

As an initial attempt at larval rearing of *T. granosa* in the Philippines, the survival rate during the early larval development stages (from Day 1 to 60) was promising at 9.48 %. The only previous report documenting survival rates of *T. granosa* during the larval development stages showed survival rates ranging from 4.86–8.57 % (Muthiah et al., 1992). For other anadardid species, early larval survival rates until settlement have been reported to range from 5–33 % (Kanno, 1963; Kim and Koo, 1973). However, a low survival rate for post-settlement spat was recorded. High mortality is expected during this part of the production cycle because larval settlement and metamorphosis is usually a period of high stress (Sturmer et al., 2009).

Despite the promising survival rates observed in the current study, general observations suggest that the first 60 days of culture are critical in the overall survival of larvae to be used in succeeding cultures. Further studies will be necessary to improve survival rates at this stage. Aside from establishing proper management protocols, adapting other strategies, such as using upwelling systems or raceways for land-based nurseries or controlled field deployment in estuarine or coastal areas, could be explored to improve spat rearing in the Philippine setting. Reynoso-Granados et al. (2012) assessed the feasibility of using upwelling systems for the spat/juvenile nursery of *Anadara grandis* (Broderip & G.B. Sowerby, l., 1829) in a laboratory setting. They concluded that *A. grandis* juveniles cultured in upwelling units could reach acceptable growth. In the experiment of Muthiah et al. (1992), cockle spats from day 60 onwards reared in cages suspended from a rack in an enclosed bay also exhibited high survival rates.

An upwelling or downwelling system with constant flow-through of water might be necessary to reduce the accumulation of waste at the bottom of rearing vessels where cockle spats initially crawl. Additionally, water quality and feeding rations of spats are much easier to control in these systems. The present study employed relatively static systems with only aeration for water circulation. Therefore, accrued dead algae and excreta tended to accumulate at the bottom of the holding tanks even during the water change. Upon observation, it was noted that the larvae became heavily covered with epiphytic algae, which hindered their ability to filter food and caused considerable

increase in mortality rates.

Although the methods for cockle culture mentioned above are already established, Quayle and Newkirk (1989) claimed that depending on the species, most clams require substrate likened to their natural habitat. Similarly, Sturmer et al. (2009) believe that clam culture should be carried out in an environment as natural as possible. In addition, although wild *T. granosa* are commonly found in intertidal mudflats, experiment findings of Syahira et al. (2021) determined that adult *T. granosa* can also survive in sand-type substrates; thus, future studies should also consider the addition of suitable substrates when rearing *T. granosa*.

The growth of the cockle seed in the experiment was not synchronous, with some larvae developing faster than others. Moreover, there was a sharp increase in the growth of the pediveliger cockle until it reached day 80 post-set stage. Subsequently, a period of slow growth was remarked from day 80 onward. A few reasons that could be attributed to the varying growth rate of cockle are the condition of the rearing tanks or the lack of variety of food. A combination of microalgal species is often used to provide a balanced diet for larvae and spat bivalves (Mamat and Alfaro, 2014). Production of appropriate diets and a series of feeding trials should be conducted for follow-up experiments.

Aside from food, the temperature is also an essential factor affecting the clam's growth. Increasing temperature accelerates metabolic processes, and enhanced enzyme activity affects feed assimilation (Sanchez-Lazo and Martinez-Pita, 2012). Wang et al. (2017) assessed the effects of temperature and salinity on the survival and growth of juvenile ark shell *A. broughtonii*. It was found that the growth rate of *A. broughtonii* was significantly influenced by temperature and salinity. Growth of juvenile *A. broughtonii* peaked at an intermediate temperature (26 °C).

Studies regarding the effect of temperature on the growth of other bivalve species have also been documented (Sanchez-Lazo and Martinez-Pita, 2012; Teh et al., 2016; Wang and Li, 2018). In the current study, larval and spat rearing tanks' temperature conditions were not controlled. The temperature fluctuates between 25 °C to 30 °C depending on the time of the day and the current weather conditions.

Perhaps, a more temperature-controlled system in future experiments should be established in order to stabilise the growth of *T. granosa*.

Conclusion

Induced spawning of *Tegillarca granosa* by temperature shock was successful and yielded a substantial number of fertilised eggs for rearing in the hatchery. Although overall survival was considerably low at 2.48 % after 120 days of rearing, the current study also identified the first 60 days of culture as critical in improving overall survival. As such, considerable improvements in rearing techniques, such as diversity and amount of natural food and rearing systems used, are necessary to facilitate faster growth and maximise the survival rate of larvae and spat in the hatchery.

No recent documented hatchery culture of *T. granosa* from larvae to adult is available in the Philippines. There is also a scarcity in obtaining broodstock from the wild. The findings in this study may pave the way for sustainable production of *T. granosa* seeds for aquaculture production or stock enhancement purposes.

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Conflict of interest: The authors declare that they have no conflict of interest.

Author contributions: Denise Vergara Miranda: Conceptualized the study, conducted the experiments, analyzed the data, and wrote and revised the manuscript. Victor Marco Emmanuel Nuestro Ferriols: Conceptualized the study, conducted the experiments, analyzed the data, wrote and revised the manuscript and acquired the grants necessary for the study.

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