MINISTRY OF EDUCATION AND TRAINING
NHA TRANG UNIVERSITY

PHUNG BAY

RESEARCH ON SOME REPRODUCTIVE CHARACTERISTICS AND ARTIFICIAL REPRODUCTION OF SCALY GIANT CLAM (*Tridacna squamosa* Lamarck, 1819)

SUMMARY OF DOCTORAL THESIS

(Major: Aquaculture)

KHANH HOA - 2024

The thesis is completed at the Nha Trang University

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The thesis is defended at the Nha Trang University Council, meeting at the Nha Trang University at the Hour…….Date……Month……..2024

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INTRODUCTION

Scaly giant clam (*Tridacna squamosa* Lamarck, 1819) is a species of the Tridacnidae, Bivalvia with highly economic value, although its resource has been seriously depleted. This species is in the Vietnam's Red List of Threatened Species (2000), as an endangered species which needs to be strictly protected.

Together with developing the policies for long-term exploitation of the wild scaly giant clam, research on reproductive characteristics and artificial propagation and seed production for growthout, as well as for conservation purposes are required toreduce the pressures on natural resources of this species.

In order to develop the protocols for artificial propagation and seed production, studies on reproductive characteristics, such as sex, spawning season, size at first sexual maturity, fecundity, embryonic and larval development are being encouraged. In addtion, determining technical criteria for artificial reproduction protocol, such as broodstock rearing, induced spawning, rearing laravae (floating and bottom stages) is essential for mass seed production of the scaly giant clam. Therefore, we caried out the study "Research on reproduction characteristics of and artificial reproduction of scaly giant clams (*Tridacna squamosa* Lamarck, 1819)".

Research objectives

This study aimed to determine some reproductive characteristrics of scaly giant clam and technical criteria for artificial propagation and seed production protocol for growthout and restoration resources of the species. Details of the objectives are as follows,

1. Determining data on reproductive characteristics of scaly giant clam.
2. Establishing technical criteria for artificial propatation (broodstock rearing induced spawning, and larvae and juvenile rearing) and mass seed production of giant clam.

Research contents

The contents of the study are listed as below.

1. Research on some reproductive characteristics of scaly giant clam;

2. Determine technical criteria for artificial propagation of scaly giant clam;

    2.1. Broodstock rearing

    2.2. Induced spawning

2.3. Rearing larvae at the floating stage

2.4. Rearing larvae at bottom stage and juvenile

3. Application of artificial seed production of scaly giant clam

Scientific and practical significance of the thesis

- Scientific significance: This thesis provides the scientific data on reproductive characteristics of scaly giant clam. These data will contribute the sources for training, teaching, research and policy makers in protection and sustainable exploitation of the wild scaly giant clam resources, as well as conservation of marine biodiversity in Vietnam.

- Practical significance: This study has practical significance in contributing to the socio-economic development in Vietnam. The study determined technical criteria for artificial propagation and seed production of scaly giant clam which help to develop the protocol for mass seed production for growth out and conservation of this species in practice.

CHAPTER 1: LITERATURE REVIEW

1.1. Reproductive characteristics of the giant clams (Tridacnidae)

Scaly giant clams are sequential and simultaneous hermaphrodites. During the first 3 years, most of the clam are males then their gonads will develop into two separate parts, one part containing sperm while the other part containing eggs. At spawning, sperms are always released first together with the secretion of reproductive stimulant compounds that stimulate other nearby individuals to participate in ejaculation and egg release. Absolute fecundity of giant clams is vey high which can reaches 300 million eggs per individual per spawning.

1.2. Overview on giant clam seed production in the world

Research on developing the technique in giant clam seed production in order to provide the seed for commercial farming and resource restoration has been carried out in many countries around the world, such as the US, Australia, Thailand, Indonesia, Malaysia, and Singapore. The giant clam brookstocks involved in such studies are both collected from local sources and/or imported from other areas.

The broodstocks can be reared in large cement tanks of large open surface with filtered and clean water. The scaly giant clams can be induced spawning by several different methods, such as gonad serotonin injcection method in the United States and Malaysia, and the dry and flow current stimulation method in Thailand. Temperature, diet including symbiotic algae and salinity are key factors that affect the development of scaly giant clam larvae**.**

1.3. Overview on giant clam seed production in Vietnam

From 2009 to 2011, Nguyen Quang Hung from the Research Institute for Marine Fisheries carried out the project "Research on the restoration and development of giant clam resources (family Tridacnidae) in Vietnam's seawaters". Of which the Research Institute for Aquaculture No.3 participated in the project aiming to carry out the pilot seed production and evaluating rearing models for restoration of giant clam resources in Nha Trang bay. Most of the important technique in giant clam seed production have been applied, including induced spawning, isolation and differentiate and storage of symbiotic algae, addition of symbiotic algae to clam larvae and technique for rearing juveniles reaching the size for commercially growthout. Through out the project, one hundred and thirty for (134) scaly giant juveniles of 1.5-2.0 cm in length was produced with the survival rate of 5% from the bottom larvae stage to growthout size.

CHAPTER 2: MATERIALS AND METHODS

2.1. The subject, scope, period and location of this study

2.1.1. The subject and scope of this study

Scientific name: *Tridacna squamosa* Lamarck, 1819

English name: Flute Giant Clam, Scaly Giant Clam

Scope of the research: Research on some reproductive characteristics and artificial reproduction of scaly giant clams.

2.1.2. The period of study

Research on some reproductive characteristics of scaly giant clams was carried out from January 2018 to December 2018. And study on artificial reproduction of scaly giant clams was from January 2018 to February 2021.

2.1.1. Research location

Specimens for studying the reproductive characteristics and artificial reproduction of scaly giant clams were collected in the seawaters of Quang Ngai provinces (Ly Son island), Quang Nam (Cu Lao Cham island), Khanh Hoa (Nha Trang bay) and Binh Thuan (Phu Quy island).

Research on reproductive characteristics and artificial seed production of scaly giant clams was carried out at the Research Institute for Aquaculture No3.

2.2. Research content: The thesis includes 3 contents, as follows.

2.2.1. Research on some reproductive characteristics of scaly giant clams.

2.2.2. Research on scientific rationales for artificial reproduction of scaly giant clams.

2.2.3. Seed production application of scaly giant clams.

2.3. Research Methods

2.3.1. Research on some reproductive characteristics of scaly giant clams

2.3.1.1. Sample collection and analysis method

Live giant clam samples were collected directly from seawaters, Ly Son island in Quang Ngai province, Cu Lao Cham island in Quang Nam province, Nha Trang bay in Khanh Hoa province and Phu Quy island in Binh Thuan province. The number of

samples collected was 32 individuals per month. The samples were collected continuously for 12 months, from January 2018 to December 2018. The samples were determined for morphological characteristics using specialized tools, then dissected and sliced for analysis of the gonad developmental status.

2.3.1.2. Sex

The sex ratio of scaly giant clams was determined based on the ratio bewteen the number of males, females and hermaphrodites identified and the total number of monthly samples.

2.3.1.3. Development of the gonads

The sex of the scaly giant clam was determined by anatomical method and observing genital products on an Olympus BX41 optical microscope (Japan) at 40 times resolution.

The gonad developmental stages of scaly giant clams were evaluated based on histological specimens, following the method recommended by Sheckan and Hrapchack (1980).

2.3.1.4. Spawning season

The spawning season of giant clams was determined based on the percentage of sexually mature individuals in total number of analyzed samples. The month with 50% or more of individuals reaching sexual maturity stage was considered the main spawning season.

2.3.1.5. Absolute and relative fecundity

The absolute and relative fecundity of scaly giant clams were determined by the volumetric method. Actual fecundity was determined by the total number of eggs collected by an individual female clam in one spawning session. Effective reproductive capacity was determined by the total number of healthy D-shaped larvae prodcued by the female clam in one spawning session.

2.3.1.6. Size at first maturation

The size of first maturation of scaly giant clams was calculated during the main spawning season. Determining the size of first maturity was based on the length of the clam when graphed on a line of the percentage of individuals that have reached sexual maturity or are reproducing. The line has 50% of individuals reaching maturity, corresponding to the size at first sexual maturity

2.3.1.7. Embryonic and larval development

After fertilization, eggs are filtered and placed in a 100L funnel-shaped tank with a density of 10 eggs/mL, lightly aerated to monitor the development of embryos and larvae.

Collect samples and observe on a microscope to determine the stages of development, transition time and characteristics of each stage from egg fertilization, egg cleavage, embryonic and larval development stages. The transition time between embryonic and larval development stages is determined at the time when 50% of the total number of embryos/larvae in the previous stage move to the next stage.

2.3.2. Research on the scientific rationale for artificial reproduction of scaly giant clams

2.3.2.1. Broodstock rearing

Experiment 1 (Ex1): Research on the effects of light intensity on the survival rate, fat index and sexual maturity rate of the giant clams

The experiment was arranged in square composite tanks, volume of 4,000 liters/tank, bottom area of 4 m^2. Salinity 32 ppt at temperature 28-30 ºC. The experiment was arranged with 3 treatments (NT) with different light intensities: 2,000, 4,000, 6,000 lux. The density of brood clams is 2 clams/m^2 of bottom area. The experiment was repeated 4 times. The experiment period was 45 days. Determine the survival rate, fat index, and sexual maturity rate of the clams at the end.

2.3.2.2. Induced spawning

Experiment 2 (Ex2): Research on the effects of different stimulation methods on the duration of the stimulation effect, spawning rate, fertilization, hatching and effective fecundity of scaly giant clams

The experiment was arranged into 4 treatments (NT) corresponding to 4 different fecundity stimulation methods. Each treatment was repeated 4 times.

+ NT1: Dry clams for 30 minutes. The clams are placed in a seawater tank at a temperature of 28 ºC, salinity 32ppt and created with a flow rate of 3 m^3/h for 30 minutes.

+ NT2: Dry clams for 30 minutes. The clams are placed in a seawater tank at a temperature of 28 ºC, salinity is 20 ppt, creating a flow rate of about 3 m^3/h for 30 minutes.

+ NT3: Clam are in a stimulation tank at a salinity of 32 ppt and a temperature of 28 ºC. Raise and lower the temperature from 31-21°C, flow rate of 3 m^3/h for 30 minutes.

+ NT4: Clams are placed in a stimulation tank at a salinity of 32 ppt and a temperature of 28 ºC. The pH of water is raised from 8 to 9 with 1% concentration NH4OH solution. Soak the clams in this solution for 10 minutes, flow for 30 minutes.

Evaluation criteria: effect time is the time the parents react to the stimulus, reproductive rate, fertilization rate, hatching rate and effective reproductive fecundity.

Experiment 3 (Ex3): Research on the effects of temperature on fertilization rate, hatching and effective fecundity of scaly giant clam eggs (25, 27, 29 ºC)

The experiment was arranged with 3 treatments: 25, 27, 29 ºC, arranged in 100L tanks, salinity 32 ppt. Egg density for the experiment was 10 eggs/mL. Each treatment was repeated 4 times. Evaluation criteria: fertilization rate, hatching rate and effective fecundity.

2.3.2.3. Rearing the larvae at the floating stage

Experiment 4 (Ex4): Study the effects of salinity on growth and survival rate of scaly giant clam larvae (24, 27, 30, 33 ppt).

The experiment was arranged with 4 treatments: 24 ppt, 27 ppt, 30 ppt and 33 ppt. One-day-old D-shaped larvae were put into the experiment in a 100l tank, temperature 28-30ºC. Each treatment was repeated 6 times, the experimental period was 8 days. Determine the criteria: average daily growth rate in length (ADG, µm/day), specific growth rate in length (SGR %/day) and survival rate (%) of larvae.

Experiment 5 (Ex5): Research the effects of different food combinations on growth, survival rate of scaly giant clam larvae.

The experiment was arranged in 100L tanks, salinity 32ppt and temperature 28-30ºC with 3 treatments: F1 Mixture of algae *Chaetoceros muelleri* and *Isochrysis galbana*, F2 Mixture of algae *Nannochloropsis oculata*, *Isochrysis galbana* and *Chaetoceros muelleri* and F3 control group without feeding. D-shaped larvae (1 day old) with a density of 5 larvae/mL. Algae feeding density is 15,000 cells/mL, once a day until the end of the experiment. Each treatment was repeated 6 times. Evaluation criteria: length, average growth rate in length and survival rate of scaly giant clam larvae.

Experiment 6 (Ex6): Research the effect of symbiotic algae density on the growth, survival rate and bottom rate of scaly giant clam larvae.

The experiment was arranged on D-stage giant clam larvae (4 days old) in a 100L funnel-shaped composite tank with 4 treatments of symbiotic algae density: NT1: 1,000 cells/mL, NT2: 3,000 cells/mL. mL, NT3: 5,000 cells/mL, and NT4: 7,000 cells/mL. Each treatment was repeated 6 times. Density of D-shaped larvae: 5 larvae/mL. The experimental

plots were illuminated with a light intensity of 750Lux. Experimental duration: 5 days of culture. Evaluation criteria: growth rate and survival rate, bottom rate of larvae.

Experiment 7 (Ex7): Research on the effects of stocking density on the survival rate and growth rate of scaly giant clam larvae.

The experiment was arranged in 100L composite tanks, salinity 32 ppt at temperature 28-30 ºC. Gồm with 4 treatments corresponding to 4 different larval rearing densities: 3,5,7,9 larvae/mL. Each treatment was repeated 6 times, the experimental period was 8 days. Evaluation criteria: growth rate, settle rate, and survival rate of larvae.

2.3.2.4. Rearing the settle stage larvae and juveniles

Experiment 8 (Ex8): Research on the effects of substrate on growth, settle rate, and survival rate of scaly giant clam larvae.

The experiment was set up in 200L round, flat bottom tanks, salinity 32 ppt at temperature 28-30 ºC with 4 treatments corresponding to 4 different types of substrate: Nylon filter mesh) with mesh size of 200 µm, substrate is dead coral rock, substrate is sand and composite tank bottom. Determine the criteria: height, absolute growth rate in pond depth (ADG, µm/day), settle rate and survival rate (%) of larvae.

Experiment 9 (Ex9). Research on the effects of light intensity on growth, survival rate and bottom descent of scaly giant clam larvae

The experiment was arranged in 200L round, flat bottom tanks, salinity 32 ppt at temperature 28-30 ºC with 4 treatments corresponding to 4 different light intensities: NT1 2,000 lux, NT2 4,000 lux, NT3 6,000 lux and NT4 8,000 lux. Determining criteria: growth in length, height, absolute growth rate in length, height, survival rate of bottom-dwelling larvae and seed.

Experiment 10 (Ex10): Effects of different transportation methods on the survival rate of scaly giant clams

The experiment was randomLy arranged into 3 treatments: NT1: Dry, moist and closed transport. Closed foam container, temperature 22-25 °C, NT2: Open transport, with water and aeration. Styrofoam water container, 28-30°C, NT3: Transport dry, not damp, not sealed. Styrofoam boxes do not have wet objects. Transport temperature from 28-30°C. Each foam box puts 1,000 clams in it. Evaluation criteria: survival rate after 4 days of culture.

2.3.2.5. Study one some pathogens and predators in broodstock rearing for artificial reproduction of scaly giant clams

Research on ectoparasitic agents followed the method of Ha Ky, Bui Quang Te (2007). For internal parasites, use histopathological techniques and culture methods according to OIE guidelines (2009). Methods of culturing and identifying bacteria were based on documents by Sindermann (1990), Weingarten and Elston (1990). Use API 20 bacterial identification test to identify bacteria.

Through daily observations using the senses, a magnifying glass with a magnification of 5-10 times and a microscope with objectives of 4, 10, 40, 100 and the limitations encountered during the rearing process.

2.3.3. Research on artificial seed production technique for scaly giant clams

Research on seed production technique for scaly giant clams was carried out at the mollusc breeding experimental station of the Aquaculture Research Institute III. The clam broodstocks were reared in cement tanks with a volume of 20 m3/tank. Larvae were reared in hemispherical composite tanks with a volume of 1m3/tank and cement tanks of 4m3/tank. The juvenike clams were reared in 2m3 rectangular tanks placed outdoors under shade covers.

2.4. Data analysis

Data were stored and processed using Microsoft Excel 2013 software. SPSS software version 16.0 was applied to compare the differences if any between the mean values of each biological parameters at the significance level $P<0.05$. One-way ANOVA with Duncan test was performed on data with normal distribution (length and growth rate of larvae). For survival rate, the Arcsin was applied to the data before performing ANOVA.

CHAPTER 3: RESULTS AND DISCUSSIONS

3.1. Reproductive characteristics of scaly giant clams

3.1.1. Sex

Table 3.1. Morphological and sex criteria of the scaly giant clam

Time	L (mm)	Wtt (kg)	Fatness (%)	Male ratio (%)	Female ratio (%)	Hermaphrodite ratio (%)
1/2018	223.3±77.5	1.657±0.65	14.94±5.47	53.33	3.33	43.34
2/2018	219.3±80.7	1.512±0.60	14.93±7.78	50.00	6.67	43.33
3/2018	216.5±77.5	1.616±0.59	15.81±6.59	43.33	10.00	46.67
4/2018	215.5±78.81	1.723 ±0.58	14.93± 8.26	40.00	10.00	50.00
5/2018	215.5±48.08	1.893±0.37	18.78±7.90	33.33	13.33	53.33
6/2018	226.5±48.08	2.013±0.37	17.70±6.90	30	13.33	56.67
7/2018	229.7±54.58	1.946±0.44	16.20±6.84	23.33	13.33	63.33
8/2018	218.2±61.37	1.844±0.45	17.80±7.06	26.67	16.67	56.67
9/2018	229.6±60.7	1.944±0.44	16.19±6.78	30.00	13.33	56.67
10/2018	228.3±57.44	1.820±0.48	15.35±6.33	36.67	10.00	53.33
11/2018	238.2±73.37	2.024±0.65	15.01±6.30	40.00	10.00	50.00
12/2018	228.2±63.40	1.824±0.61	15.39±6.49	43.33	10.00	46.67
Mean	**224.8±65.7**	**1.820±0.53**	**16.04±6.84**	**37.5±9.23**	**10.83±3.52**	**51.67±6.11**

The smallest average length was in April (215,5 ± 78,81mm) and the largest was in November (238,2 ± 73,37mm). The average length of the 12-month study period was 224.8±65.7mm. Whole body weight increases proportionally to length, the largest in November (2,024±0.65 kg/individual. Fatness increases during the breeding season (from May to August).

3.1.2. Stages of gonad development of scaly giant clams

Stage I: gonads are small in size and colorless. The characteristic of stage I is that male and female cannot be distinguished.

Stage II: This is the stage where the gonads are still immature. The gonads are pale white and it is difficult to distinguish male and female forms with the naked eye.

Stage III: Sexual development stage, at this time the size of the gonads has increased rapidly and is slightly milky white in color. Female gonads: gonads are quite clear, filled with oocytes. Male gonads: Sperm cells predominate, small amount of sperm in sperm follicles.

Stage IV: (Mature stage): Female gonads: ovaries are fully mature, developed to their maximum extent. Male gonads: Sperm cells mostly contain many mature sperm.

Stage V: Degeneration stage, postpartum stage. Female gonads: Egg cells have been released from the follicle. Male gonads: Sperm are released from the seminal follicles.

3.1.3 Spawning season

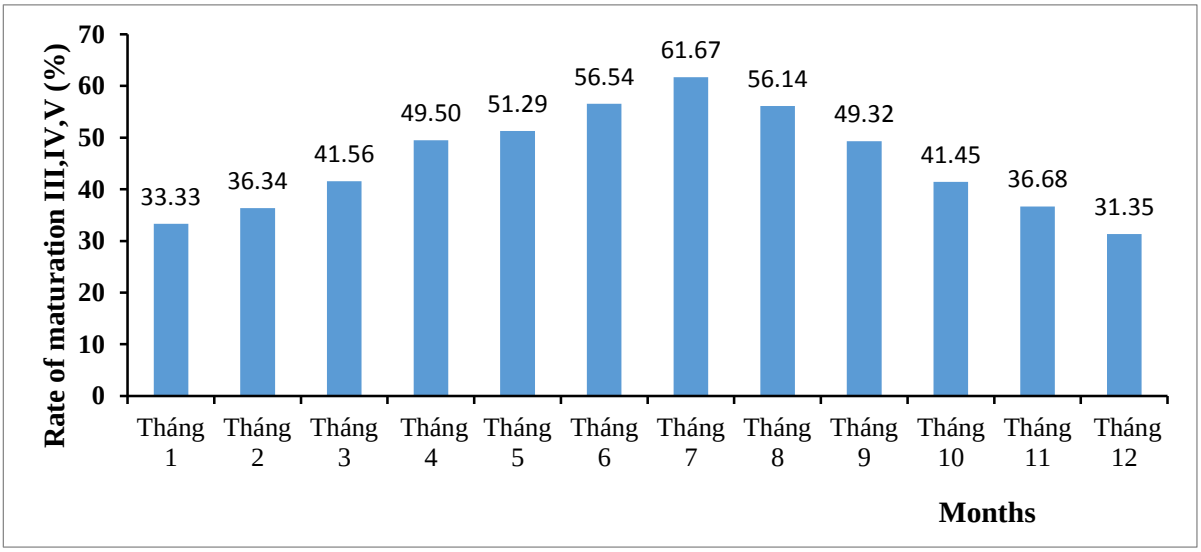

Figure 3.1 Rate of maturation in scaly giant clams

The rate of sexual maturity of scaly giant clams over the 12 months of the study was lowest in December (31.35%) and highest in July (61.67%). From May to August, the sexual maturity rate is >50%. The sexual maturity coefficient also tended to increase or decrease proportionally to the sexual maturity rate and this was due to the spawning season.

3.1.4 Absolute and relative fecundity

Table 3.2. Absolute and relative fecundity of scaly giant clams

Sample total	Size group (cm)	Absolute fecundity (eggs/individual)	Relative fecundity	
			eggs/g whole body weight	eggs/g internal soft tissue weight
5	11-15	431,000^{a} ± 24,303	344,98^{a} ±7.7	1,364^{a} ± 35.03
5	16-20	2,338,000^{b} ± 198,957	1,188.7^{b} ±52.6	5,559^{b} ± 169.4
5	21-25	4,408,000^{c} ±253,700	1,979.8 ^{c}±46.3	8,475.7^{c} ±226.8
5	26-30	7,277,500^{d} ± 116,270	2,810.6^{d} ± 31.1	12,923^{d} ± 1.038
5	31-35	11,605,000^{e} ± 243,361	3,851.2^{e}± 80.8	16,518^{e} ± 138.8
Average		**5.211.900±167.319**	**2,035±44.0**	**8,968±322.6**

The absolute fecundity of scaly giant clams increased with size group. The absolute and relative fecundity according to whole body weight and internal soft tissue weight was highest in the group of 31-35cm/individual, with an average of 11,605,000 ± 243,361 eggs/individual, 3,851.2 ± 80.8eggs/g whole body weight and 16,518 ±138.8 eggs/g internal soft tissue weight, respectively.

3.1.5 Determine size at first sexual maturity

Results from this study showed that the size at first sexual maturity of the scaly giant clam was determined by the shell length of 19.1 cm.

3.1.6 Results on the embryonic and larval development of scaly giant clams

The fertilized egg shrinks, the fertilization membrane appears, and the nucleus gradually dissolves in the protoplasm. Twenty-five (25) minutes post fertilization, the first sphere appears, then 20 minutes later, the second sphere appears. The blastocyst appears at around 12 hours and 30 minutes post fertilization, and the embryo appears after 15 hours. The diameter of the fertilized egg is about 90-100 µm.

Development of larval stages

* Trochophora larvae: Appear 10-12 hours after fertilization. The larvae are diamond-shaped and have many cilia concentrated in the cilia rim.

* D-shaped larvae (Veliger): D-shaped, with 2 transparent shell covers, the cilia are located between the 2 shell covers. The larvae move continuously.

* In the Pediveliger larval stage: the Veliger larva develops a leg muscle, transitions to the Pediveliger larval stage, and forms attached larvae.

* The larvae complete a symbiotic relationship: Around the mantle, brown streaks appear which are symbiotic cells, which can be observed with the naked eye.

* 1-2cm clam stage: larvae attach firmLy to the attachment and grow by nutrients from the photosynthesis process of symbiotic algae.

* 2-4cm seed: after about 4-6 months, the clam will reach 2-4cm in size.

3.2 Scientific basis for artificial reproduction of scaly giant clams

3.2.1 Broodstock rearing

Results of the influence of light intensity (2,000, 4,000, 6,000 lux) on survival rate, fatness and sexual maturity rate ofcultured giant clams (Ex1)

Table 3.4. Survival rate, fatness and sexual maturity rate of cultured clams under different light intensity treatments

Assessement criteria	Light intensity (lux)		
	2,000	***4,000***	***6,000***
Survival rate (%)	$63.50^{a} \pm 0.58$	$62.75^{a} \pm 0.96$	$59.25^{b} \pm 1.27$
Fatness (%)	$24.25^{b} \pm 0.50$	$20.75^{c} \pm 1.50$	$19.25^{c} \pm 0.50$
Maturity rate (%)	$52.75^{a} \pm 0.50$	$48.50^{a} \pm 1.30$	$44.25^{b} \pm 0.50$

The highest fatness of reared clams was in the light intensity treatment of 2,000 lux (24.25±0.50%). The fatness value in the 4,000 lux treatment (20.75±1.50%) was higher than the fatness value in the 6,000 lux treatment (19.25±0.50%). The survival rate of clams raised in the 2,000 lux treatment was the highest (63.50±0.58%) and the difference was not statistically significant compared to the 4,000 lux treatment (62.75±0.96) (P>0.05).

3.2.2 Spawning induction

3.2.2.1 Effects of different induction methods on induction effect duration, spawn rate, fertilization rate, hatching rate and effective fecundity (Ex2)

Table 3.5 Reproductive efficiency of scaly giant clams using different reproductive induction methods

Treatments	Assessment Criteria				
	Effect time (minute)	Spawn rate (%)	Fertilization rate (%)	Hatching rate (%)	Effective fecundity (Larvae Dx 10^3)
Drying+ Flow	136.75^{a} ± 8.84	47.50^{a} ± 7.19	64.00^{a} ± 0.87	60.50^{a} ± 1.29	$4,920^{a}$±53.54
Drying + Flow + salinity reduction	112.50^{b}±2.08	53.00^{b}±3.56	58.25^{b}±1.71	56.75^{a}±2.22	$4,582^{b}$±69.94
Temperature shock	86.75^{c} ± 1.29	59.75^{c} ± 0.82	51.50^{c} ± 3.11	51.25^{b} ± 3.59	$4,190^{c}$ ± 86.79
NH_4OH	83.50^{c} ± 1.29	67.00^{c} ± 0.81	42.00^{d} ± 3.37	43.50^{c} ± 4.20	$3,722^{d}$± 90.12

The effect time of the two treatments of soaking clams in NH_4OH solution and heat shock (83.50 ± 1.29 minutes and 86.75 ± 1.29 minutes, respectively) was much faster than the other two treatments. The spawning rate of the treatment soaking clams in NH_4OH solution was the highest (67.00 ± 0.81%). The lowest spawning rate was in the drying treatment combined with flow creation (47.50 ± 7.19%). The fertilization rate and hatching rate in the treatment: flow drying and flow drying were the highest (64.00 ± 0.87% and 60.50 ± 1,29%, respectively). The effective reproductive capacity was highest in the drying treatment to create flow (4,920,000 ±53.54 D-shaped larvae) and lowest in the treatment soaking clams in NH_4OH solution (3,722 ,000± 90.12 larvae).

3.2.2.2 Effect of temperature on fertilization and hatching rate of scaly giant clam eggs (25, 27, 29 ºC)(Ex3)

Table 3.6 Effect of temperature on fertilization, hatching rate of scaly giant clam eggs

Assessment Criteria	Temperature treatment (ºC)		
	25	***27***	***29***
Fertilization rate (%)	$56.25^{a} \pm 0.58$	$60.50^{b} \pm 1.30$	$62.50^{b} \pm 1.30$
Hatching rate (%)	$74.00^{a} \pm 0.82$	$70.00^{b} \pm 0.82$	$57.00^{c} \pm 1.83$
Hatching time (h)	$24.50^{a} \pm 1.29$	$20.00^{b} \pm 0.85$	$18.25^{b} \pm 1.26$

The fertilization rate was highest in the 29 ºC treatment (62.50± 1.30%). The highest hatching rate was in the 25 ºC treatment (74.00 ± 0.82%) and the lowest was in the 29 ºC treatment (62.50 ± 1.30%). The hatching time for D-shaped larvae was the fastest in the 29 °C treatment (18.25 ± 1.26 minutes) and the slowest was in the 25 °C treatment (24.50± 1.29 minutes).

3.2.3 Rearing larvae in the floating stage

3.2.3.1 Research on the effects of salinity on growth and survival rate of scaly giant clam larvae (24, 27, 30, 33 ppt) (Ex4).

Table 3.8. Length and growth rate of larvae when reared at different salinities

Criteria	Salinity treatments			
	24ppt	***27ppt***	***30ppt***	***33ppt***
Initial lenght (µm)	140.12 ± 1.45	140.12 ± 1.45	140.12 ± 1.45	140.12 ± 1.45
End lenght (µm)	$206.50^{a} \pm 3.27$	$211.00^{b} \pm 2.19$	$224.50^{c} \pm 1.87$	$221.83^{c} \pm 1.47$
DGR (µm/day)	$9.50^{a} \pm 0.47$	$10.14^{b} \pm 0.31$	$12.07^{c} \pm 0.26$	$11.69^{c} \pm 0.21$
SGR (%/day)	$5.60^{a} \pm 0.23$	$5.90^{b} \pm 0.15$	$6.72^{c} \pm 0.12$	$6.61^{c} \pm 0.09$

Growth in length of clam larvae at salinities 30 ppt and 33 ppt was highest, followed by salinity 27 ppt and lowest at salinity 24 ppt (Table 3.8; $P<0.05$). DGR and SGR of clam larvae in the 30 ppt salinity treatment were highest, 12.07 ± 0.26 µm/day and 6.72 ± 0.12%/day. Meanwhile, in the 24ppt treatment, clam larvae only reached 206.50 ± 3.27 µm in length; DGR was 9.50 ± 0.47 µm/day and SGR was 5.60 ± 0.23 %/day.

3.2.3.2 Effects of the combination of different foods on growth, survival rate and bottom rate of giant clam larvae (Ex5).

Table 3.9. Growth and survival rate of giant clam larvae in different food treatments

Criteria	Food treatments		
	F1	***F2***	***F3***
Lenght (µm)	$206.5^{b} \pm 2.6$	$217.5^{a} \pm 3.1$	$183.0^{c} \pm 1.3$
SGR (%/day)	$6.32^{b} \pm 1.76$	$7.18^{a} \pm 0.34$	$4.31^{c} \pm 0.31$
Survival rate (%)	$28.6^{a} \pm 2.2$	$31.5^{a} \pm 1.6$	$17.2^{b} \pm 1.4$

Larvae cultured with a combination of three microalgae species *Nannochloropsis oculata, Isochrysis galbana and Chaetoceros muelleri* (F2) had the highest shell length and SGR, respectively 217.5 ± 3.1 µm and 7.18 ± 0.34 %/ day. Larvae had the smallest length and lowest specific growth rate in treatment F3 (no microalgae supplementation).

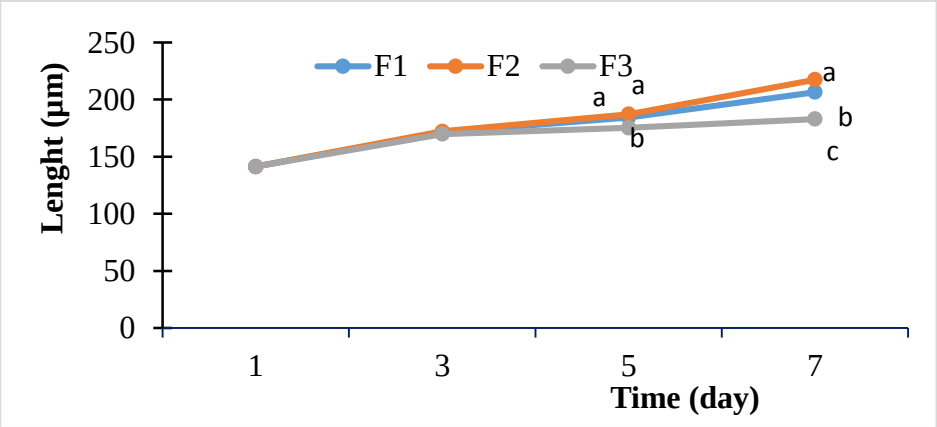

Figure 3.2. Larval length growth in different food treatments

3.2.3.3 Effects of symbiotic algae density on growth and survival rate of scaly giant clam larvae (Ex 6)

Table 3.8. Characteristic length and growth dimensions of giant clam larvae at different densities of symbiotic algae (µm)

Experiment time (day)	NT1	NT2	NT3	NT4
1	166.70±0.60	166.70±0.60	166.70 ±0.60	166.70 ±0.60
3	178.30 ± 0.49^{b}	180.80 ± 0.7^{b}	187.50 ± 0.88^{a}	186.50 ± 1.31^{a}
5	196.30 ± 1.56^{b}	198.00 ± 1.61^{b}	213.67 ± 1.2^{a}	212.50 ± 1.52^{a}
SGR (%/day)	4.09 ± 1.95^{b}	4.30 ± 0.24^{b}	6.20 ± 0.21^{a}	6.07 ± 0.16^{a}

Larvae in treatment 3 (symbiotic algae density of 5,000 cells/mL) had the highest length and characteristic growth rate (213.67±1.2µm and 6.2±0.21%/day, respectively). days), followed by larvae in NT4 (symbiotic algae density 7,000 cells/mL) with a length of 212.5±1.52µm; SGR of 6.07±0.16%/day). The length and growth of larvae in NT1 (symbiotic algae density 1,000 cells/mL) and NT2 (symbiotic algae density 3,000 cells/mL) are lower and have significant differences compared to NT3 and NT4 (P<0.05).

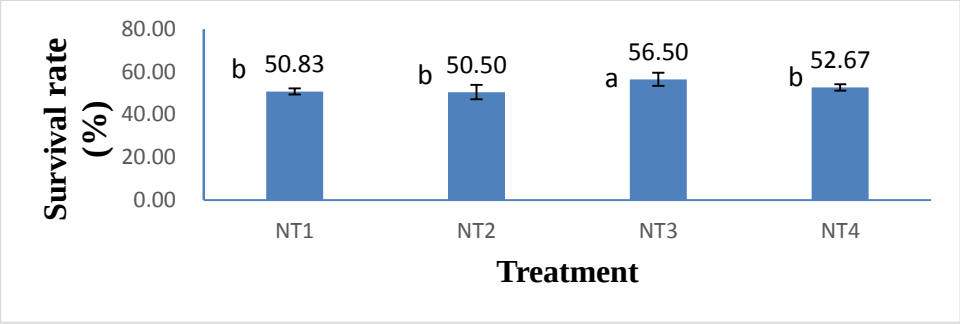

Figure 3.3 Survival rate of giant clam larvae in different treatments

The survival rate of giant clam larvae was highest in treatment NT3, the density of symbiotic algae was 5,000 cells/mL and there was a statistically significant difference compared to other treatments (P<0.05). Larvae in the remaining 3 treatments did not have statistically significant differences (P>0.05).

3.2.3.4. Effect of stocking density on growth rate and survival rate of clam larvae

Table 3.9 Growth and survival rate of clam larvae raised at different densities

Criteria	Density treatments			
	D3	D5	D7	D9
Length (µm)	$228.2^{a} \pm 2.43$	$221.8^{a} \pm 2.01$	$189.83^{b} \pm 2.38$	$186.7^{b} \pm 3.94$
SGR (%/day)	$7.99^{a} \pm 0.15$	$7.51^{a} \pm 0.16$	$4.91^{b} \pm 0.39$	$4.62^{b} \pm 0.36$
Survival rate (%)	$42.3^{a} \pm 1.15$	$39.7^{a} \pm 0.81$	$29.3^{b} \pm 07.9$	$21.67^{c} \pm 1.19$

High growth rate at density 3, 5 larvae/mL and low at density of 7, 9 larvae/mL. At the end of the experiment, the length, SGR and survival rate were highest at the density of 3 larvae/mL (228.2 ± 2.43 µm; 7.99 ± 0.15 %/day, and 42.3 ± 1.15% respectively), not different significant from the density of 5 larvae mL/1 (221.8 ± 2.01 µm; 7.51 ± 0.16%/day and 39.7 ± 0.81% respectively) (p>0.05). The length, SGR and

survival rate were lowest at the density of 9 larvae/ mL (186.7 ± 3.94 µm; 4.62 ± 0.36%/day, and 21.67±1.19% respectively). There was significantly different from the density of 7 larvae/mL and 9 larvae/mL.

3.2.4 Nursery for settlement stage larvae and juveniles

3.2.4.1 Effect of substrate on growth, survival rate and bottom rate of scaly giant clam larvae (Ex8)

Table 3.10 Average height of giant clam larvae in different substrate treatments

Culture time (day)	Average height (µm)			
	200 µm mesh bottom	Dead coral bottom	Sand bottom	Composite
1	233.33±0.46^{a}	**233.33±0.46^{a}**	233.33±0.46^{a}	233.33±0.46^{a}
6	351.06 ±0.74^{c}	**391.73±0.70^{a}**	345.57±0.10^{d}	361.57±0.41^{b}
11	487.33±0.31^{c}	**540.75±0.59^{a}**	475.15±0.17^{d}	502.73±0.35^{b}
16	588.55±0.22^{c}	**701.48±0.17^{a}**	571.29±0.27^{d}	635.13±0.70^{b}
21	657.22±2.27^{c}	**850.89±0.79^{a}**	634.33±0.37^{d}	751.33±0.18^{b}
26	722.22±0.54^{c}	**1012.8±0.54^{a}**	685.89±0.83^{d}	890.64±0.27^{b}

Table 3.11 Daily growth rate (DGR) according to the height of larvae in the treatments

Culture time (day)	DGR (µm/day)			
	200 µm mesh bottom	Dead coral bottom	Sand bottom	Composite
1	23.55±0.24^{a}	**31.62±0.15^{a}**	22.39±0.18^{d}	233.40±0.35^{b}
6	27.25 ±0.75^{c}	**29.80±0.21^{a}**	25.92±0.23^{d}	28.23±0.43^{b}
11	20.24±0.98^{c}	**32.15±0.43^{a}**	19.23±0.54^{d}	26.48±0.36^{b}
16	13.73±0.94^{c}	**29.88±1.45^{a}**	12.61±0.56^{d}	23.24±0.23^{b}
21	13.01±1.25^{c}	**38.98±0.32^{a}**	10.31±0.13^{d}	27.86±0.32^{b}
26	19.55±0.79^{c}	**31.48±0.23^{a}**	18.09±0.46^{d}	26.29±0.46^{b}

Larvae in the dead coral substrate treatment had the highest average height (1020.8 ±0.54 µm), the difference with the remaining treatments was statistically significant (P<0.05); The average daily growth rate is also the highest, 31.48±0.23 µm/day. Next are the larvae in the composite tank bottom treatment, with a height of 890.64±0.27 µm (P<0.05), and an average daily birth rate of 26.29±0.46 µm/day. Larvae in the remaining 2 substrate treatments, 200 µm mesh bottom and sandy bottom, had average height (722.22±0.54 µm and 685.89±0.83 µm) and average daily growth rate. height (19.55±0.79 µm/day and 18.09± 0.46 µm/day) was the lowest (P<0.05).

Table 3.12 Effects of substrate on the bottom rate and survival rate of scaly giant clam larvae

Time	Bottom			
	200 µm mesh bottom	**Dead coral bottom**	**Sand bottom**	**Composite**
Settle rate (%)	30.6^{c}±0.79	**55.2^{a}±0.40**	29.7^{c}±0.25	42.0^{b}±0.87
Survival rate from settle to the experiment end (%)	26.4^{c} ± 0.95	**42.8^{a}±0.29**	25.5^{c}±0.50	38.6^{b}±0.57

Dead coral substrate provides the highest growth, growth rate in height, bottom rate and survival rate of giant clam larvae.

3.2.4.2 Effects of lighting intensity on the survival rate and growth of giant clam larvae in the benthic stage (2,000, 4,000, 6,000, 8,000 lux) (TN9)

Table 3.13 Average length of giant clam larvae in different light intensity treatments

Culture time (day)	Average length (µm)			
	2,000 lux	**4,000 lux**	**6,000 lux**	**8,000 lux**
1	265.30 ± 0.29^{a}	265.30 ± 0.29^{a}	265.30 ± 0.29^{a}	265.30 ± 0.29^{a}
6	399.30 ± 1.26^{b}	424.00 ± 2.24^{a}	385.35 ± 1.73^{c}	357.00 ± 1.41^{d}
11	483.25 ± 2.03^{b}	550.00 ± 1.41^{a}	463.30 ± 1.26^{c}	431.10 ± 1.63^{d}
16	600.00 ± 1.71^{b}	697.30 ± 0.42^{a}	580.00 ± 1.71^{c}	484.00 ± 1.71^{d}
21	736.00 ± 1.91^{b}	855.00 ± 1.71^{a}	704.00 ± 2.22^{c}	565.00 ± 2.80^{d}
26	839.00 ± 0.96^{b}	$1{,}036.20 \pm 1.73^{a}$	782.10 ± 2.16^{c}	612.20 ± 1.71^{d}

Table 3.13 Average height of giant clam larvae in different light intensity treatments

Rearing day	Average height (µm)			
	2,000 lux	4,000 lux	6,000 lux	8,000 lux
1	236.20 ± 0.14[a]	236.20 ± 0.14[a]	236.20 ± 0.14[a]	236.20 ± 0.14[a]
6	369.30 ± 1.26[b]	390.10 ± 0.81[a]	352.90 ± 1.73[c]	327.20 ± 1.41[d]
11	450.80 ± 1.71[b]	520.30 ± 1.41[a]	433.90 ± 1.26[c]	401.20 ± 1.63[d]
16	572.20 ± 1.89[b]	667.40 ± 1.71[a]	550.30 ± 1.71[c]	454.80 ± 11.71[d]
21	706.10 ± 1.91[b]	825.40 ± 1.71[a]	675.50 ± 1.71[c]	535.60 ± 2.08[d]
26	831.20 ± 1.71[b]	1,032.10 ± 1.91[a]	793.20 ± 0.58[c]	611.50 ± 1.29[d]

Figure 3.3 Average daily growth rate according to length and shell height of giant clam larvae in different light intensity treatments

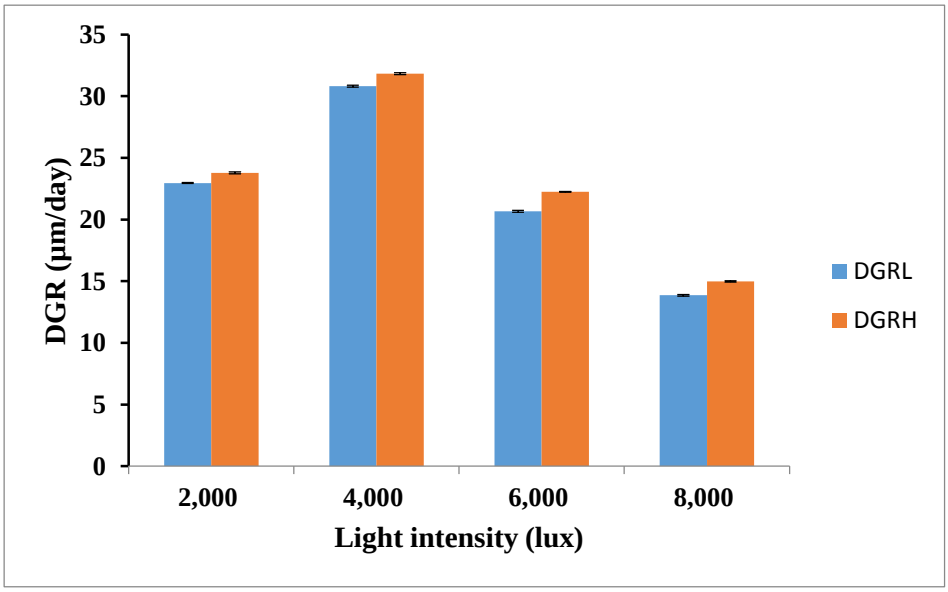

Figure 3.4. DGR in length and height of scaly larvae at different light intensities

The length and height of larvae in the 4,000 lux light intensity treatment were the highest, reaching 1,036.2 ± 1.73 µm and 1,032.1 ± 1.91 µm, respectively. The length and height of giant clam larvae were lowest in the 8,000 lux light intensity treatment, with values of 612.2 ± 1.71µm and 611.5 ± 1.29 µm, respectively.

The average daily growth rate of length and height was highest in the 4,000 lux light intensity treatment, reaching 30.81 and 31.82 µm/day, respectively, and the lowest average growth rate in the treatment of 8,000 lux, it only reaches 13.86 and 14.97 µm/day, respectively.

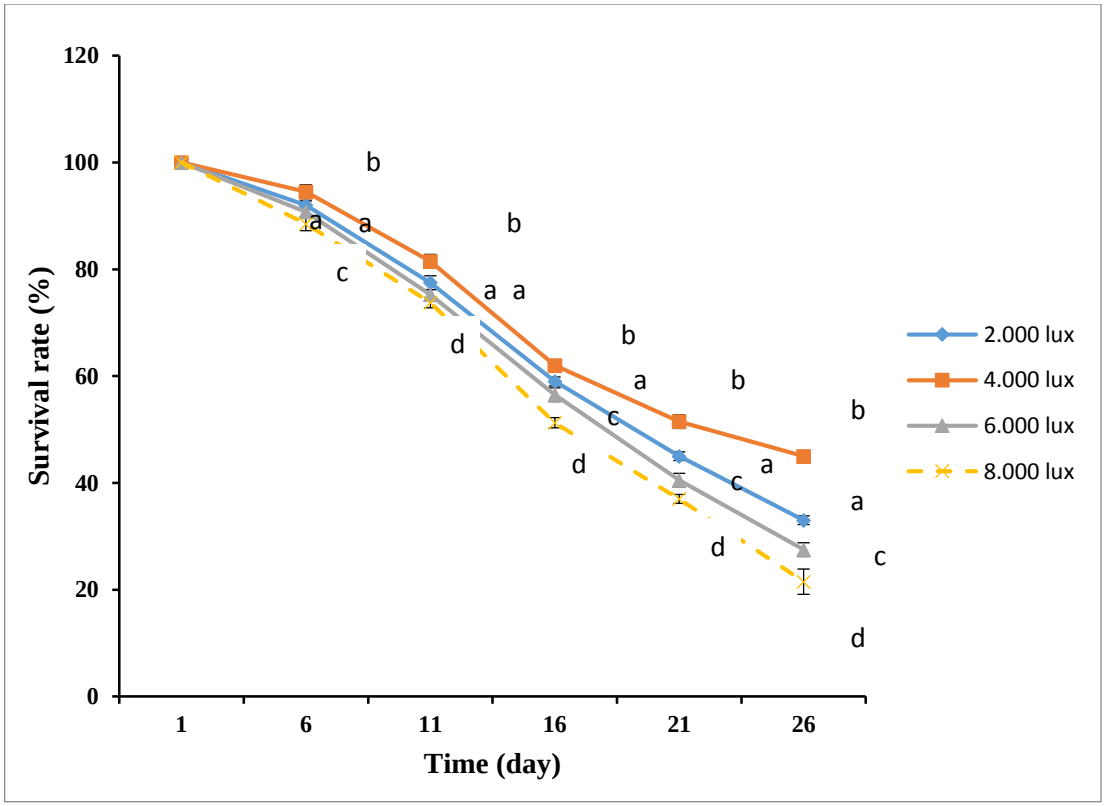

Figure 3.5 Effect of light intensity on survival rate of scaly giant clam larvae

The survival rate of giant clam larvae differed between different light intensity treatments ($p<0.05$). Light intensity of 4,000 lux gave the highest survival rate of giant clam larvae (reaching 45%) and had a statistically significant difference compared to the remaining treatments ($p < 0.05$). At 8,000 lux light intensity, the survival rate of larvae was lowest, reaching only 21.5% at the end of the experiment.

3.2.4.3 Effects of different transportation methods on the survival rate of scaly giant clams (Ex10)

Table 3.15. The survival rate of the breed after transportation within 4 hours by 3 different methods

Criteria	Transport treatment		
	Dry, moist – tight (22 -25 °C)	Tight, water, oxy (28 - 30 °C)	Open, not damp (28 - 30 °C)
Survival rate (%)	$86,33^{a} \pm 1,21$	$82,50^{b} \pm 1,47$	$63,17^{c} \pm 1,47$

Moist - sealed transfer method, survival rate reached 86.33%. Next is the closed transport method, using water with oxygen pump, the survival rate reaches 82.50%.

T.Test test shows that there is a significant difference in survival rate between these two transport methods. From there, it shows that the clam seed is most suitable for being transported by the moist-closed method.

3.2.5. Results of research on diseases and pests in raising scaly giant clams

3.2.5.1. Parasitic and bacterial diseases on farmed giant clams

No parasites were detected. Attachment organisms: sun *Balanus s*p., polychaete Polydora sp.., Isopoda, and Cirripedia.

After preliminary examination of the morphological characteristics of colonies as well as bacteria, 01 bacterial strain was selected and identified, named *Aeromonas hydrophila*.

3.2.5.2 Harmful enemies during the process of raising scaly giant clams

Snail *Cymmmatium s*p., blanket seaweed *Cladophora* sp. Seaweed is found clinging to clam shells or in the walls of tanks for raising or rearing clams.

3.3 Artificial seed production of scaly giant clams

Table 3.16 Results from the pilot seed production of scaly giant clams

Batch	Number of egg	Number of D larvae	Pediveliger	Symbiotic larvae	Number of 2cm Juvenile	Survival D- 2cm juvenile (%)
1	48.245.210	33.771.647	16.885.824	6.754.329	1.165.789	3,45
2	63.628.910	45.856.230	22.392.670	8.957.068	1.791.414	3,91
3	55.673.245	40.821.340	20.054.960	8.021.984	1.645.326	4,03
4	123.474.950	91.234.563	47.834.550	19.133.820	4.123.457	4,52
Total	**291.022.315**	**211.683.780**	**107.168.004**	**42.867.201**	**8.725.986**	**3,98**

Through 4 batches of seed production, the average survival rate from D shaped larvae to 2cm juveniles was 3.98% and 8,725,986 seed were obtained.

CHAPTER 4: CONCLUSION AND RECOMMENDATIONS

4.1. CONCLUSIONS

- **Reproductive characteristics of scaly giant clams**

The scaly giant clam is a simultaneous hermaphrodite that develops as a male, and then becomes simultaneous hermaphrodite. The gonad development is divided into 5 stages, including stage I: undeveloped stage, stage II: growth stage, stage III: development stage, stage IV: reproductive stage, and stage V: degeneration stage.

The size at first maturation is of 19.1 cm in length. The main spawning season in scaly giant clams is from May to August. Absolute fecundity is 5,211,900±167 eggs/female and relative fecundity is 2,035±44 eggs per gram of whole body weight, corresponding to 8,968±322 eggs per gram of internal soft tissue body weight. Effective reproductive capacity is 5,463,000 ± 50.13 D-larvae per female each spwaning.

- **Scientific rationale for artificial reproduction of scaly giant clams**

Light intensity of 2,000-4,000 lux is for broodstock rearing of scaly giant clam. And induced spawning can be achieved by drying the brookstock under the shade (30 °C for 30 minutes), while egg fertilization at 27-29 ºC.

At floating stage, scaly giant clam larvae should be reared at salinity 30-33ppt, with a stocking density of 3-5 D-shaped larvae/mL. The larvae are fed on a combination of microalgae, including *Nannochloropsis oculata, Chaetoceros muelleri* and *Isochrysis galbana* with a feeding density of 15,000 cells/mL, a symbiotic algae density of 5,000 cells/mL.

Techniques for rearing larvae of scaly giant clams at the settlement stage and fingerling stages: light of 2,000-4,000 lux and dead coral rock as attachment.

For seed clams, the closed transportation method at a temperature of 22 – 25 °C, density of 1,000 clams/styrofoam box (size 40 x 60 x 40cm).

Through the four batches of seed production, this study produced 8,725,986 juveniles of 2 cm in length, with a survival rate of 3.98% from the Veliger (D stage) to juveniles.

4.2. RECOMENDATIONS

In order to improve the survival rate of scaly giant clams, futher studies on the protocols for isolation, storage and biomass production of symbiotic algae, as well as the time at supplementation the symbiotic algae are recommended**.**

Research on diet supplementation during broodstock rearing in order to improve quality of sexual products, larvae and juveniles are required.

LIST OF PUBLICATIONS RELATED TO THE DOCTORAL THESIS

1. **Phung Bay, Ton Nu My Nga, Nguyen Thi Thuy Trang, 2018**. Initial results of studying the influence of substrate on the survival rate and growth rate of giant clam larvae in the benthic stage. Journal of Fisheries Science and Technology, No. 3/2018. pg. 1-9.
2. **Phung Bay, Ton Nu My Nga, Nguyen Van Minh, Ngo Anh Tuan, 2023**. Research on the effects of light intensity on the growth and survival rate of giant clam larvae (*Tridacna squamosa* Lamarck, 1819) bottom stage. Journal of Fisheries Science and Technology, No. 4/2023, pg. 87-95.
3. **Phung Bay, Nguyen Van Minh, Ngo Anh Tuan, 2023**. Effects of symbiotic algae density and salinity on growth and survival rate of giant clam larvae (*Tridacna squamosa Lamarck*, 1819). Journal of Fisheries Science and Technology, No. 4/2023, Pg. 106-115.
4. **Phung Bay, Ngo Anh Tuan and Nguyen Van Minh, 2023**. Effects of microalgae and stocking density on growth and survival rate of giant clam (*Tridacna squamosa Lamarck*,1819) larvae. International Conference on Marine Sustainable Development and Innovation 2023. IOP Conference Series: Earth and Environmental Science. doi:10.1088/1755-1315/1278/1/012002.