MINISTRY OF EDUCATION AND TRAINING
NHA TRANG UNIVERSITY

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RESSEARCH ON REPRODUCTIVE BIOLOGICAL CHARACTERISTICS AND ARTIFICIAL SEED PRODUCTION OF SHORT-NECKED CLAM *Paphia undulata* (Born, 1780)

A SUMMARY OF DOCTORAL DISSERTATION ON AQUACULTURE

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This research was performed and completed at Nha Trang University

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INTRODUCTION

Short-necked clam *Paphia undulata* belongs to the family Veneridae, and contains a high nutritional value and potential economic aquaculture species. The protein content in fresh meat of *P. undulata* was 12.8%. The total content of 18 amino acids was 46.21% of dry weight, including 8 essential amino acids accounting for 34.67%. The content of unsaturated fatty acids was 51.9%, containing 32.8% of DHA and EPA. *P. undulata* was high in taurine (3.03% of dry weight) and potassium (3.41% of dry weight). In the world, this clam was exploited in Malaysia, China, India, Thailand, Philippines….

In Vietnam, bivalvia has become popular species with high economic value and is exported as a favored seafood to many countries. In 2019, the bivalvia farming area was 41,200 hectares and the total production was 370,000 tons corresponding to the value of 93.642 million USD, in which Veneridae species dominated. In recent years, the short-necked clam was exploited mainly in the central of Vietnam from Phu Yen to Binh Thuan and the Southwest region such as Kien Giang and Ca Mau without any farming activities; therefore, the production of *P. undulata* was unstable and not enough for demand of domestic consumption and export.

In Vietnam, studies on short-necked clams have just been carried out in some fields such as distribution, growth characteristics, exploitation status, and preliminary information on the spawning season. Scientific studies on reproductive biological characteristics and suitable parameters for artificial seed production of *P. undulata* have not been performed.

The thesis: "Research on reproductive biological characteristics and artificial seed production of short-necked clam *Paphia undulata* (Born, 1780)" was carried out with the following objectives:

General objective: to determine reproductive biological characteristics and optimal technical parameters in seed production as a scientific basis for building the technical procedure of seed production of short-necked clam.

Specific objectives:

1. To determine reproductive biological characteristics of short-necked clam.

2. To build a technical procedure for artificial seed production of short-necked clam through activities as mature culture techniques, stimulated spawning method of broodstock, techniques for rearing larval stages and completed seed production.

To achieve the above objectives, the thesis was carried out with the following contents:

1. Research on reproductive biological characteristics of short-necked clam.

2. Research on techniques of seed production of short-necked clam: mature culture and stimulated spawning method; larvae rearing techniques in the veliger stage and spat stage and seed;

3. Building the technical procedure for seed production and applied in practice for seed production of short-necked clam.

Scientific and practical meaning:

The thesis was a source of documents providing a database on the reproductive biological characteristics of short-necked clams as well as an important contribution to teaching, researching, and scientific information for the construction of developing policies for protecting and exploitation of short-necked clams in the sea.

The thesis' results built a successful procedure for seed production of short-necked clam, which can be applied in practice with high-quality of seed. This will expand the nursery of short-necked clam in Khanh Hoa province and elsewhere and facilitate the massive aquaculture of the short-necked clam in Vietnam.

New contributions of the thesis: The thesis was the systematic and complete research on short-necked clam species published in Vietnam, from reproductive biological characteristics to build the technical procedure for seed production.

The results of thesis:

The reproductive biological characteristics of short-necked clam in Khanh Hoa province: the process of gonadal development of short-necked clam is divided into 5 stages: I: immature stage, II: development stage, III: sexual maturity stage, IV: reproductive stage, V: redevelopment stage. The male: female ratio was 1.00 : 1.08. The short-necked clam could be spawning year-round but performed the best in the two main seasons, from April to May

and from September to October. The size of initial sexual maturity in length was 43 mm for males and 44 mm for females. The mean of absolute fecundity was 1,137,467 ± 280,054 eggs/individual, relative fecundity was 114,195 ± 17,330 eggs/g body weight and 354,736 ± 59,766 eggs/g meat weight, respectively. The mean of real fecundity was 353,889 ± 165,205 eggs/spawning.

The environmental parameters: salinity: 30 – 31 ‰, pH: 7.5 – 8.5, DO: ≥ 5 mg/L, temperature: 28 – 29°C, the larval development of short-necked clam underwent 4 stages: Trochophora, D stage, Umbo and Spat, taken place in 25 days.

Algae of *Chlorella* sp. and *I. galbana* was the most suitable food for maturity culture with the best value of fat content, CI index, maturity rate, survival rate and biochemical composition of broodstock. The lighting conditions of 500 – 3,000 lux showed the highest survival rate, maturity rate of broodstock and fertilization rate, and hatching rate. The broodstock of short-necked clam responded to three stimulated spawning methods: temperature shock, light of ultraviolet and ammonia solution, but temperature shock was the optimal method.

In the veliger stage: larval rearing 1- 3 individual/mL at a salinity of 31‰ and algae (*N. oculata*, *Chlorella* sp. and *I. galbana*) used as live feed were the most suitable rearing conditions for the growth and survival rate of larvae.

In the spat stage and seed: salinity of 31‰ combined with algae (*N. oculata*, *Chlorella* sp. and *I. galbana*) and formulated food (Lansy and Frippak) were the most suitable condition for growth and survival rate. The stocking density of 2 individual/cm^2 combined with no bottom substance was the most suitable for growth and survival rate.

The most appropriate transportation method of seed was closed method with a density of 10,000 seed/bag, a transportation time of 6 hours, which presented the highest survival rate and lowest oxygen consumption.

The technical procedure of seed production was built and applied to produce 17.37 million of short-necked clam seeds with an average survival rate of 4.6% (size of seed from 3 to 5 mm) and a productivity of 190,000 seed/m^2.

The thesis contains four chapters: Introduction, Materials and Methodology, Result and Discussion, Conclusion and Recommendation

Chapter 1: Introduction

1.1 Research status of short-necked clam in the world

Over the world, short-necked clam and other species belong the genus of Paphia have been studied in terms of reproductive biological, ecological and distribution characteristics as well as seed production process. However, the studies on scientific technic as systematic and complete in the artificial seed production as research has been limited.

1.2 Research status of short-necked clam in Vietnam

In our country, *Meretrix meretrix* and *M. lyrata* are cultured in many areas with high economic value and became a key seafood product for export to many countries around the world; therefore, the research on seed production of clams belonging to the *Veneridae* family has been mainly done on these two species.

In recent years, studies on short-necked clams in Vietnam have been only focused on distribution characteristics, growth characteristics and preliminary information on the spawning season for exploitation purposes in Binh Thuan and Ca Mau provinces. The scientific research on reproductive biological characteristics and the artificial technic for seed production have not been done for short-necked clams. Therefore, to become the dominated species for aquaculture, it is necessary to focus on studies about reproductive biological characteristics and technical of rearing larvae from veliger larval stage to seed as well as building an artificial seed production process. On the other hand, the building of the artificial production process for this species will help to create new jobs and increase incomes for farmers in the coastal zone.

Chapter 2: Materials and Methodology

2.1 Objective, time and location

Objective: *Paphia undulata* (Born, 1780).

The scope of study: reproductive biological characteristics and artificial seed production of short-necked clam in Khanh Hoa province.

The time of study: Research on reproductive biological characteristics were carried out from January 2017 to December 2017. Research on artificial seed production from January 2018 to December 2019.

The location of study: Samples of short-necked clams were collected directly from farmers in Van Ninh District, Ninh Hoa Town and Cam Ranh City, Khanh Hoa Province. The reproductive biological characteristics of short-necked clams were analyzed at the Laboratory of the Experimental Center, Nha Trang University. The experiments on artificial seed production from veliger larval stages to spat larval and seed stage of short-necked clams were carried out at hatchery in Cat Loi Village, Vinh Luong Commune, Nha Trang City, Khanh Hoa province. The analysis of biochemical composition of clam' fresh meat was carried out at the Laboratory of Fish nutrition, Can Tho University.

2.2 Research contents

Study on reproductive biological characteristics of short-necked clam include gonadal development stages, fat index, mature index, sex ratios, spawning season, the size of first sexual maturity, fecundity, embryonic and larval development stages. Study on artificial seed production of short-necked clam include research on techniques of mature culture and stimulated spawning method of short-necked clam, research on techniques of the larvae rearing in the veliger stage, research on techniques of larvae rearing in the spat stage and seed. Seed production of short-necked clam was also done in practice.

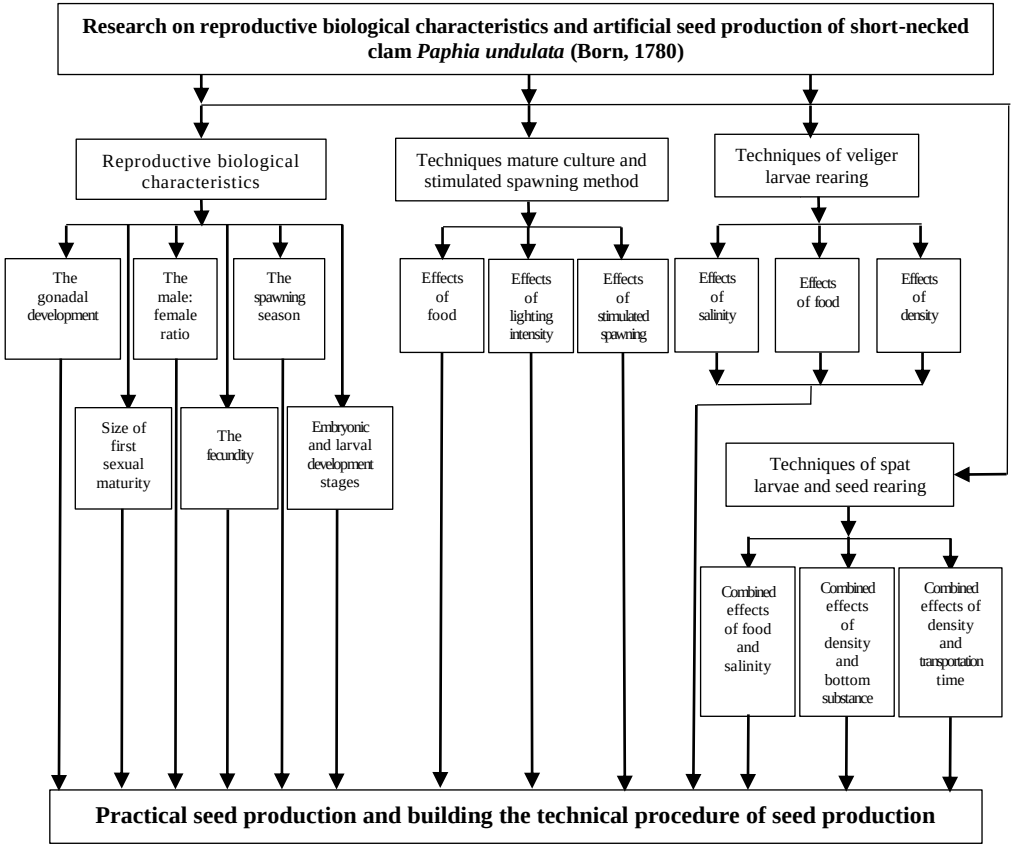

Figure 2.1: Schematic diagram of research contents

2.3 Research method

2.3.1 Sampling method

Samples of short-necked clams exploited in Khanh Hoa province were collected directly from farmers and then transported to the Laboratory of the Experimental Center, Nha Trang University for analysis. In the laboratory, samples were tested if they were alive and un-broken shells. Then, these clams were mixed and selected randomly with different sizes of 37 to 54 mm as well as a commercial size for analyzing reproductive biological characteristics. Number of samples was 120 individuals/month. Samples were continuously collected in 12 months.

2.3.2 Gonadal development stages

The gonad samples were collected directly from alive of short-necked clams and observed under microscope to determine male and female corresponding to its' products such as sperm or eggs, respectively. The gonadal development stages of short-necked clam were determined based on histological method according to Sheckan and Hrapchack (1980) method and divided into 5 stages according to method of Quayle and Newkirt (1989), Nabuab *et al.* (2010).

2.3.3 Sex ratios

The sex ratios of short-necked clam were determined based on the ratio of the number of males and females which were identified during sampling period to the total of samples.

2.3.4 Spawning season and mature index

The spawning season of short-necked clam was determined based on the numbers of sample which analyzed monthly and calculated as the percentage of mature individuals and spawning individuals to the total of samples. In the month when clams had 50% or more of mature individuals and spawning, considered as the main spawning season of this species.

The mature index was calculated monthly based on histological observation according to method of Quayle and Newkirk (1989) with value of 1 to 5, in which 1: undeveloped stage; 2: developing stage; 3: mature stage; 4: spawning stage and 5: redeveloping stage.

2.3.5 Size of first sexual maturity

The size of the first sexual maturity of short-necked clam was calculated separately for males and females. The clam shell length was graphically represented on the curve which presented a percentage of individuals that were sexually mature or breeding. The point on the curve of 50% of total sexual maturity was defined as the size of first sexual maturity.

2.3.6 Fecundity

The absolute and relative fecundity of short-necked clams were determined by the volumetric method. The gonad of female clams was dissolved in the sea water and then collected to count the number of mature oocytes using a Sedgewick rafter counting chamber. The absolute fecundity

(F_a) was determined as the total number of mature oocytes. Relative fecundity was calculated as the ratio of absolute fecundity to body weigh or to fresh meat weight. The real fecundity was determined as the number of eggs obtained from one female clam when it was spawned.

2.3.7 Embryonic and larval development stages

The broodstocks of short-necked clam were spawned for monitoring a embryo and larval development stages. Samples were collected and observed under the microscope to determine the characteristics of each stages from egg fertilization to embryonic and larval development stages. The transitional time from embryonic to larval stages was determined at the time of 50% of the total embryonic in the previous stage moved to the next stage.

2.3.8 Study on artificial seed production of short-necked clam

Study on effects of food on survival rate and mature ability of short-necked clam was done in 3 different treatments: NT1: algae (*Chlorella* sp., *I. galbana*), NT2: dry algae (Spirulina), NT3: mixed food of algae and formulated food (Lansy and Frippak). These treatments were repeated 5 times with experimental time of 21 days. At the end of the experiment, the survival rate, fat index, condition index, sexual maturity rate and biochemical composition of fresh meat were determined.

A study on the effects of lighting intensity on survival rate and mature ability of short-necked clam included 3 different treatments: NT1: light intensity ranges from 20 to 300 lux (in the hatchery), NT2: light intensity ranges from 500 to 3.000 lux (under the white plastic roof covering the black net), NT3: light intensity ranges from 5.000 to 8.000 lux (under the white plastic roof covering). These treatments were repeated 5 times with experimental time of 21 days. At the end of the experiment, the survival rate, fat index, sexual maturity rate of these clams were determined.

Study on effects of stimulated spawning method on reproductive efficiency of short-necked clam included 3 different treatments: NT1: themal shock, NT2: UV lighting, NT3: using NH_4OH solution. These treatments were repeated 5 times. The efficient time, real fecundity, spawning rate, fertilization rate, hatching rate and size of D larvae were determined.

Study on effects of salinity on growth and survival rate of short-necked clam in the veliger stages include 4 different treatments: NT1: 23‰, NT2: 27‰, NT3: 31‰ and 35‰.

Study on effects of food on growth and survival rate of short-necked clam in the veliger stages consisted 3 different treatments: NT1: algae (*N. oculata*, *Chlorella* sp., *I. galbana*), NT2: dry algae (Spirulina), NT3: mixed food of algae and formulated food (Lansy and Frippak).

Study on effects of density on growth and survival rate of short-necked clam in the veliger stages was done in 4 different treatments: NT1: 1 individual/mL, NT2: 3 individuals/mL, NT3: 5 individuals/mL and NT4: 7 individuals/mL.

Combined effects of food and salinity on growth and survival rate of short-necked clam in the spat larval stage and seed was carried out in two factors, 3 different treatments of food such as fresh algae, dry algae (Spirulina), mixed food of fresh algae and formulated food (Lansy and Frippak) at 4 different salinity levels of 23‰, 27‰, 31‰ and 35‰.

Combined effects of density and type of bottom substances on growth and survival rate of short-necked clam in the spat and seed stages were done with 4 different treatments of rearing density of 2, 4, 6 and 8 individuals/cm^2 at 3 types of bottom substances such as sand, muddy sand, and no bottom substance.

All experiments in the veliger and spat stages and the seed of short-necked clam were repeated 4 times, with a duration of 15 days in the veliger stage and 25 days in the spat and seed stages. Periodically every 5 days, 30 samples from each treatment were randomly collected to determine the criteria: average daily growth rate in length (ADG, mm/day), the specific growth rate in length (SGR, %/day), and survival rate (%).

Combined effects of density and transportation time on survival rate and dissolved oxygen consumption of short-necked clam seed were done with 3 different treatments of the density of 10.000, 15.000 and 20.000 individuals/box at 3 transportation times of 6, 12, and 18 hours. The control treatment was carried out for the seed that had the same size and nursed in

the normal condition of the hatchery. The experiment was repeated 4 times and determined separately for two sizes of small and large clams seed. The survival rate (%) and dissolved oxygen consumption (ppm/g/minute) were determined at the end of experiment.

2.3.9 Practical seed production of short-necked clam

The best results of all experiments were used to build the technical procedure of seed production and applied in practice for seed production of short-necked clams at the Mollusk hatchery in Vinh Luong Commune, Nha Trang City, Khanh Hoa Province.

2.3.10 Analysis method for biochemical composition

The broodstock of short-necked clams was randomly collected to determine length and body weight. The fresh meat of clams was removed from shells and determined weight. The fresh meat was dried at 60°C for 24 hours and then crushed and kept in a temperature condition of - 20°C for analysis.

The biochemical composition of short-necked clams such as protein, lipid, total ash was analyzed by the AOAC method (2000). Crude protein content was analyzed by the Kjeldahl method. Crude lipid were determined by sample extraction in the Soxhlet system. The ash content was determined by the burning and heating method in a furnace at 560°C for 8 hours. Moisture content was determined by drying method at 105°C for 24 h.

2.4. Data analysis

Data were collected and stored in Microsoft Excel 2013. Chi square test $\chi 2$ was used to compare the sex ratio, the ratio of gonadal development stages of short-necked clam in this study with the theory ratio of 1: 1. The mean of the experiments were compared statistically by one-way Anova and two-way Anova of variance analysis method respectively. Evaluate the difference between the mean of experiment after analysis of variance (Post Hoc Test) by Duncan test on SPSS software version 20.0. The difference between the mean values were determined at the significance level of $p < 0.05$.

Chapter 3: Results and Discussion

3.1 Reproductive biological characteristic of short-necked clam

3.1.1 Gonadal development stages

The short-necked clam separated male and female species but the sex was not distinguished even though the external characteristics observation of sexual organs were immature. The gonad of the clam was located on the visceral ridge, around the base of the leg toward the top of the shell. When mature, the gonad of the clam was swollen and covered the entire visceral mass. At this time, it was possible to distinguish the sex of these clams based on the color of the gonads. The gonads of male clam were milky white and the gonads of females were pale yellow.

The gonadal development of short-necked clam is divided into 5 stages such as I: undeveloped, II: developing, III: maturity, IV: spawning, V: redeveloping.

3.1.2 Sex ratio

In the month of April, May and September the ratio of female clams was higher than male clams and significantly different compared to the theoretical sex ratio (1.51 : 1; 1.49 : 1 and 1.53 : 1, respectively). In the other hand, in July, the ratio of male clams was higher than female (1.53 : 1). In the other months, the sex ratio of short-necked clam consisted with the theoretical ratio of 1:1. In the total of samples, the general sex ratio of short-necked clam was 1.00 : 1.09.

In the small size shell length (37 – 42 mm), the female ratio was dominant compared to males clam (1.44 : 1.00) and showed a significant difference with the theoretical sex ratio. In the bigger size shell length, the sex ratio of short-necked clams was not a significant difference between male and female clams.

3.1.3 Spawning season and mature index

In the period of sampling, there were mature and spawned clams (the gonads developed at the stage III and IV) but the ratio was different. The ratio of mature and spawned clams increased gradually from January to May as the first peak, then gradually decreased in the summertime before reaching the second peak in September and October. The mature coefficient increased gradually from January to May and then decreased from June to August before increasing again in September and reaching the maximum value in October.

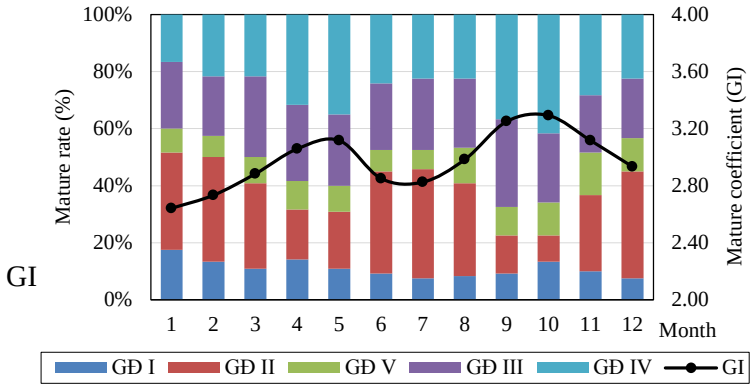

Figure 3.1: The gonadal development stages and mature coefficient of short-necked clam in Khanh Hoa province

The mature index and spawning season of short-necked clam were fluctuated and divided into two periods in the year as well as two main spawning seasons. The first season appeared from April to May and the second was from September to October.

3.1.4 Size of first sexual maturity

The size of the first sexual maturity of short-necked clam was 43 mm for males and 44 mm for females.

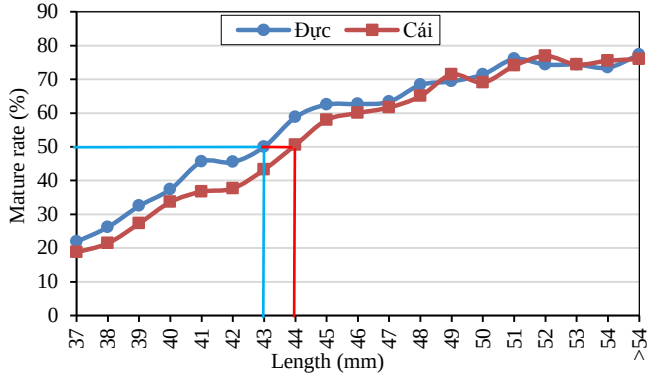

Figure 3.2: The size of first sexual maturity of short-necked clam in Khanh Hoa province

3.1.5 Fecundity

The mean absolute fecundity of short-necked clams was 1,137,467 ± 280,054 eggs/individual, the relative fecundity was 114,195 ± 17,330 eggs/g body weight and 354,736 ± 59,766 eggs/g fresh meat, respectively. The real fecundity of these clams was 353,889 ± 165,205 eggs/spawning/individual.

3.1.6 Embryonic and larval development stages

The short-necked clam is an oviparous species. The sperm and eggs from male and female clams were spawned through the two siphons. The environmental parameters of salinity was 30 – 31 ‰, pH was 7.5 – 8.5, dissolved oxygen was ≥ 5 mg/L, temperature was of 28 – 29^{o}C, the larval development stages of short-necked clam underwent 4 stages such as trochophora, D larvae, umbo larvae and spat larvae.

3.2. Techniques of mature culture and stimulated spawning method of short-necked clam

3.2.1 Effects of food on survival rate and mature ability

The fat and condition index of short-necked clam increased and reached a maximum value of 35.54 ± 0.58% and 58.34 ± 0.97%, respectively in the algae treatment.

The mature rate at the end of the experiment was highest in the algae treatment (77.2 ± 2.04%) but there was no significant difference compared to

the treatment of mixed fresh algae combined with formulated food (74.0 ± 1.92%).

The survival rate of short-necked clam decreased and showed a significant difference between treatments with a maximum value of 87.04 ± 0.99% in the algae treatment and a minimum value of 69.36 ± 1.27% in the dry algae (Spirulina).

3.2.2 Effects of food on biochemical composition

The biochemical compositions of short-necked clam were fluctuated depending on food and showed a significant difference between treatments. The biochemical composition of clams such as lipid, protein, ash increased and reached the highest value in the fresh algae treatment.

3.2.3 Effects of lighting intensity on survival rate, maturity and spawning ability

The fat index and mature ratio of short-necked clam increased and reached a peak in the treatment of lighting intensity of 500 – 3,000 lux, corresponding to 35.72 ± 0.44% and 82.67 ± 1.82%. The survival rate of this species decreased in all treatments with the lowest value of 67.80 ± 1.86% in the lighting intensity of 5,000 – 8,000 lux. In the conditions of lighting intensity of 20 – 300 lux and 500 – 3,000 lux, the survival rate was high, ranging from 84.6 to 87.0%.

Table 3.1: Results of mature culture of short-necked clam in different lighting intensity treatment

Criteria	First	Treatment		
		20 - 300 lux	**500 - 3.000 lux**	**5.000 - 8.000 lux**
L (mm)	49.10 ± 0.83^{a}	49.13 ± 0.35^{a}	49.49 ± 0.26^{a}	49.21 ± 0.22^{a}
W (g)	12.09 ± 0.69^{a}	12.33 ± 0.32^{a}	12.07 ± 0.18^{a}	11.54 ± 0.17^{a}
Fat (%)	31.77 ± 1.04^{a}	34.04 ± 0.49^{bc}	35.72 ± 0.44^{c}	32.93 ± 0.43^{ab}
Mature rate (%)	64.00 ± 2.45^{a}	76.00 ± 1.90^{b}	82.67 ± 1.82^{c}	65.33 ± 1.92^{a}
Survival rate (%)	100	87.00 ± 1.55^{b}	84.60 ± 1.86^{b}	67.80 ± 1.86^{a}

The broodstocks of clam were spawned fastest (98 minutes) in the lighting intensity treatment of 500 - 3,000 lux. The spawning ratio was high in the conditions of lighting intensity of 20 - 300 lux and 500 – 3,000 lux, 74.4% and

78.8%, respectively. The real fecundity of clam was lowest in the lighting intensity treatment of 5,000 - 8,000 lux (314.000 ± 40.570 eggs) and a highest value of 454.200 ± 64.580 eggs was recorded in the lighting intensity of 500 - 3,000 lux.

The fertilization rate and hatching rate were both highest values in the lighting intensity treatment of 500 - 3,000 lux (76.6 ± 2.25% and 78.0 ± 2.43% respectively). On the other hands, the lowest values of fertilization and hatching rate were found in the 5,000 - 8,000 lux treatment (60.4 ± 2.04% and 62.4 ± 2.50%, respectively).

Table 3.2: Results of spawning effect of short-necked clam in different lighting intensity treatment

Treament	Criteria				
	Spawning time (minute)	**Spawning rate (%)**	**Real fecundity (Number of egg)**	**Fertilization rate (%)**	**Hatching rate (%)**
20 – 300 lux	123.0±8.31^{a}	74.4 ± 3.59^{b}	442.200 ± 83.239^{a}	68.8 ± 3.68^{ab}	74.4 ± 3.14^{b}
500 - 3.000 lux	98.0±7.18^{a}	78.8 ± 2.56^{b}	454.200 ± 64.580^{a}	76.6 ± 2.25^{b}	78.0 ± 2.43^{b}
5.000 - 8.000 lux	168.0±12.41^{b}	62.4 ± 3.64^{a}	314.000 ± 40.570^{a}	60.4 ± 2.04^{a}	62.4 ± 2.50^{a}

3.2.4 Effects of stimulated spawning method on reproductive efficiency

The results showed that, all three stimulated spawning methods were affected to short-necked clam, however, the reproductive efficiency was different between treatments. The efficient time was faster in the NH_4OH solution treatment (79.0 ± 7.14 minutes) and lowest in the UV lighting treatment (141.0 ± 6.40 minutes) ($p<0.05$). The highest value of spawning rate was recorded in the NH_4OH solution treatment (82.0 ± 2.21%). The fertilization rate in the NH_4OH solution treatment was 60.4 ± 2.04% and significantly different comparing to the UV lighting treatment (76.6 ± 2.25%) ($p<0.05$).

Table 3.3: Results of spawning effect of short-necked clam in different stimulating treatment

Treatment	Criteria				
	Spawning time (minute)	**Spawning rate (%)**	**Real fecundity (Number of egg)**	**Fertilization rate (%)**	**Hatching rate (%)**
Temperature shock	102.0 ± 4.64^{b}	71.4 ± 2.44^{a}	490.200 ± 47.239^{a}	68.8 ± 3.68^{ab}	74.4 ± 3.14^{b}
Ultra violet	141.0 ± 6.40^{c}	64.4 ± 3.59^{a}	422.000 ± 42.942^{a}	76.6 ± 2.25^{b}	78.0 ± 2.43^{b}
NH_4OH	79.0 ± 7.14^{a}	82.0 ± 2.21^{b}	513.000 ± 49.285^{a}	60.4 ± 2.04^{a}	62.4 ± 2.50^{a}

The length of D larvae ranged from 140.5 – 144.3 µm and showed no significant difference between treatments ($p>0.05$). However, the coefficient of variation (CV) was 8.4% in the thermal shock treatment and significantly different comparing to the UV lighting treatment (11.73%) and NH_4OH solution treatment (13.1%) ($p<0.05$).

3.3 Techniques of larvae rearing in the veliger stage of short-necked clam

3.3.1 Effects of salinity on growth and survival rate

The length of veliger larvae was largest (473.13 ± 4.65 µm) in the treatment of 31‰ and smallest (278.49 ± 2.81 µm) in the treatment of 23‰.

Table 3.4: The growth rate of short-necked clam larvae in different salinity treatment

Salinity (‰)	L_{first} (µm)	Criteria		
		L_{end} (µm)	ADG (µm/day)	SGR (%/day)
23	110.63 ± 1.38	278.49 ± 2.81^{a}	11.19 ± 0.20^{a}	6.13 ± 0.09^{a}
27	110.63 ± 1.38	339.90 ± 2.65^{b}	15.28 ± 0.18^{b}	7.47 ± 0.07^{b}
31	110.63 ± 1.38	473.13 ± 4.65^{c}	24.17 ± 0.31^{c}	9.65 ± 0.08^{c}
35	110.63 ± 1.38	336.98 ± 2.18^{b}	15.09 ± 0.15^{b}	7.43 ± 0.06^{b}

The ADG and SGR of larvae were the highest value of 24.17 ± 0.31 µm/day and 9.65 ± 0.08%/day in the treatment of 31‰ and showed significantly higher than other treatments ($p<0.05$).

The survival rate of larvae gradually decreased and was affected by salinity. After 15 days, the survival rate of larvae was highest in the salinity treatment of 31‰ (5.09 ± 0.39%).

3.3.2 Effects of food on growth and survival rate

The result showed that the length of larvae was largest (463.1 ± 4.44 µm) in the fresh algae treatment and smallest in the dry algae of spirulina treatment (304.0 ± 3.89 µm). The ADG of larvae in the fresh algae treatment was 23.46 ± 0.30 µm/day and twice higher than those in the dry algae of spirulina treatment (12.85 ± 0.27 µm/day). The SGR of larvae also had a similar trend with the highest value of 9.48 ± 0.07 %/day recorded in the algae treatment. The lowest value of SGR (6.65 ± 0.10%/day) was found in the dry algae treatment.

Table 3.5: The growth rate of short-necked clam larvae in different food treatment

Food (‰)	L_{first} (µm)	Criteria		
		L_{end} (µm)	ADG (µm/ngày)	SGR (%/ngày)
Algae (VT)	111.3 ± 1.39	463.1 ± 4.44^{c}	23.46 ± 0.30^{c}	9.48 ± 0.07^{c}
Dry algae (TK)	111.3 ± 1.39	304.0 ± 3.89^{a}	12.85 ± 0.27^{a}	6.65 ± 0.10^{a}
VT+formulated food	111.3 ± 1.39	361.7 ± 4.46^{b}	16.69 ± 0.31^{b}	7.82 ± 0.09^{b}

The survival rate of larvae during experimental period was equal between algae treatment and mix of algae and formulated food treatment, ranged 4.15 – 5.44% and showed a significantly higher compared to the dry algae treatment (1.48%) ($p<0.05$).

3.3.3 Effects of density on growth and survival rate

The growth of larvae such as length, ADG and SGR was similar trend during the experimental period and showed an inversely proportional with increased of stocking density. The length (408.75 µm), ADG (20.15 µm/day), and SGR (8.94 %/day) of larvae in the stocking density of 1 individual/mL treatment were always the highest and most significant difference from other treatments. In the density of 3 individuals/mL, these growth parameters of larvae also had significantly higher than those in the treatment of 5 and 7 individuals/mL ($p<0.05$). The growth parameters of larvae at the density of 5 and 7 individuals/mL were very low and showed no significant difference ($p>0.05$).

Table 3.6: The growth rate of short-necked clam larvae in different density treatment

Density (individual/mL)	L_{first} (µm)	Criteria		
		L_{end} (µm)	ADG (µm/ngày)	SGR (%/ngày)
1	106.46 ± 1.60	408.75 ± 4.09^{c}	20.15 ± 0.27^{c}	8.94 ± 0.07^{c}
3	106.46 ± 1.60	365.21 ± 3.19^{b}	17.25 ± 0.21^{b}	8.21 ± 0.07^{b}
5	106.46 ± 1.60	252.03 ± 2.01^{a}	9.70 ± 0.15^{a}	5.73 ± 0.08^{a}
7	106.46 ± 1.60	245.84 ± 2.38^{a}	9.29 ± 0.17^{a}	5.56 ± 0.08^{a}

The survival rate of larvae in the density of 1 and 3 individuals/mL was equal, ranging from 6.19 – 6.43% and showed a significantly higher than other treatments ($p<0,05$). In the stocking density of 5 individuals/mL, the survival

rate of larvae was 3.12% and showed higher than those in the treatment of 7 individuals/mL (1.31%) but no significant difference was recorded ($p>0.05$).

3.4 Techniques of larvae rearing in the spat stage and seed of short-necked clam

3.4.1 Combined effects of food and salinity on growth and survival rate

The experimental result showed that food and salinity affected the growth rate of spat larvae and seeds of short-necked clam. The length of larvae always showed the highest value in the treatment group with a salinity of 31‰ and the lowest value was recorded at the treatment of 23‰. Similarly, the increase in length of larvae always reached the highest value in the treatments fed algae and the lowest value with dry algae. The combined effects of stocking density and food on the length of larvae were also found from the 5^{th} day of experimental period onwards, in which, the length of larvae was 2.20 ± 0.045 mm as well as highest value in the treatment of 31‰ combined with fresh algae.

The ADG and SGR of short-necked clams were influenced by salinity and food that the highest value was recorded in the treatment of 31‰ and food as algae. The lowest value of ADG and SGR was found in the treatment of 23‰ and dry algae. There was a combined effect of density and food on the ADG and SGR of larvae with the highest values recorded in the treatment of 31‰ combined with algae.

The survival rate of short-necked clam always reached the highest value in the treatment fed algae as food and salinity of 31‰ and lowest value was recorded in the treatment of dry algae and salinity of 23‰. During the experimental period, the combined effect of salinity and food on the survival rate was only determined after 5 days of experimental period. From the 10^{th} day onwards, the results presented no combined effect of food and salinity on survival rate of short-necked clam.

3.4.2 Combined effects of density and bottom substance on growth and survival rate

The length increase of larvae was affected of both factors of density and bottom substance. The length of larvae in the group of 2 individuals/cm^2 treatment always presented the highest value and showed a

significant difference compared to other treatments. In the high-density treatments, the length of larvae decreased and recorded the lowest value in the treatment of 8 individuals/cm^2. The results showed that combined effects of density and bottom substances on length of larvae was found with highest value in the treatment of 2 individuals/cm^2 combined no bottom substances.

The ADG of larvae was inversely proportional to density with the highest value at low stocking density (2 individuals/cm^2) and gradually decreased at higher densities. There was a combined effect of density and bottom substance on ADG of larvae, with the highest value recorded in the treatment of 2 individual/cm^2 and no bottom substance, except at day 15^{th} of the experimental period.

The SGR reached a peak value after 5 days of experiment in the treatment both of density and bottom substance. After that, the SGR gradually decreased following the experimental period. The SGR of short-necked clam showed a significantly different in the density treatments, with the highest value recorded at the density of 2 individuals/cm^2 and the lowest value at the density of 8 individuals/cm^2. The combined effect of density and bottom substance on the SGR was recorded with the highest value in the treatment of 2 individuals/cm^2 and no bottom substance.

The result showed that the survival rate of *P. undulata* in the spat larval stage and seed was always found highest at the treatment of 2 individuals/cm^2, followed by the density of 4 individuals/cm^2 and the lowest at the treatment of 8 individuals/cm^2. When nursing at the lowest density combined with no bottom substance condition, the growth and survival rates of the spat larval stage and seed were recorded as the highest value.

3.4.3 Combined effects of density and transportation time on survival rate and dissolved oxygen consumption of short-necked clam seed

For small size of the seed, the highest survival rate of 98.73% was recorded in the treatment of 6h and showed a significantly higher than the other treatments. In the density treatments, the survival rate of the seed reached a peak value at low density (10,000 individuals/bag), then the survival rate of the seed gradually decreased at higher densities.

In the large size group, the survival rate of seed in all treatments was high, but significant differences were still recorded. The survival rate in the treatment of 6h and 12h ranged from 97.75% to 98.12% and presented significantly higher than those in the treatment of 18h (96.68%). At the density treatment of 10,000 individuals/bag, the survival rate of seed was 98.04%, significantly higher than the treatment of density of 20,000 individuals/bag (97.06%).

The result of statistical analysis showed that there were no combined effects of both factors of transported time and density on the survival rate of short-necked clam seed in the small size group ($p=0.642$); Meanwhile, the combined effect of transported time and density on survival rate was recorded in the large size group ($p=0.008$) with the highest value in the treatment of lowest density and transported time (6h and 10,000 individuals/bag).

The dissolved oxygen consumption of both sizes group in all treatments was higher than those in the control treatment. In the transported time treatments, the oxygen consumption of small and large clams was 7.84 and 8.57 ppm/g/minute, respectively in the treatment of 18h and showed differently higher than treatment of 6h (2.91 and 3.37 ppm/g/minute, respectively). In the density treatments, the oxygen consumption of the small size group increased proportionally with the increase of stocking density. The oxygen consumption at the density of 20,000 individuals/bag was highest (7.87 ppm/g/minute), followed by the treatment of 15,000 individuals/bag (5.17 ppm/g/minute) and the lowest value of oxygen consumption was recorded at the treatment of 10,000 individuals/bag (3.03 ppm/g/minute).

In the group of large size, the oxygen consumption was lowest in the treatment of 10.000 individuals/bag (3.97 ppm/g/minute) and showed a significant difference compared to other treatments ($p<0.05$). The oxygen consumption in the treatment of 20.000 individuals/bag was highest (7.37 ppm/g/minute) but no significant difference was recorded compared to the treatment of 15.000 individuals/bag (6.13 ppm/g/minute) ($p<0.05$).

The analysis recorded combined effects of both factors of transported time and density on the oxygen consumption of short-necked clam seed in

the small size group (p=0.029) with the lowest oxygen consumption in the treatment of 10,000 individuals/bag and 6h of transported time; On the other hand, there were no combined effects of transported time and density on the oxygen consumption of large size group (p=0,490). Therefore, the transported time of 6h combined with a density of 10,000 seeds/bag was the best condition representing the lowest oxygen consumption.

3.5 Practical seed production and building the technical procedure of seed production of short-necked clam

3.5.1 Practical seed production of short-necked clam

The broodstocks of short-necked clam were selected with shell length of 50.7 ± 4.52 mm, weight of 12.3 ± 1.56 g. The fat index and mature rate of broodstock were 32.7 ± 3.48% and 69.4 ± 2.25%, respectively. The results of mature culture showed that all broodstocks were increased in fat index and mature rate compared to the original. In which, in the 3rd mature culture, the fat index and the mature rate of broodstocks were 34.7% and 80.6% respectively as well as highest value. The mature rates of broodstocks in three production batches were ranged from 74.1 to 80.6% and satisfied for artificial seed production.

The broodstocks of clam were stimulated and spawned by using the temperature shock method. These clams spawned in the evening, in which, the male species responded to the changed of temperature and released sperms into the environment first. Then, the sperm acted as a stimulant factor to attract the female species releasing eggs. The spawning time of clams was prolonged because they were intermittent spawning species. The results showed that the spawning rate was quite high and equal between batches with average of 68.6 ± 1.6%. The fertilization rate between batches was quite similar, ranged from 72.4% to 77.2%. Similarly, the hatching rate was also high and presented stability between batches with average of 71.2 ± 1.2%.

At the veliger larval stage, the stocking density was maintained in the range of 1 – 3 individuals/mL to improve the larval rearing efficiency. At the umbo larval stage, when larvae appeared eyes spot and foot, they were collected to transfer to short-tank for spat larval rearing stage with a

stocking density of 2 – 4 individuals/cm^2. During the veliger and spat larval rearing stages, fresh algae *(N. oculata, Chlorella* sp. and *I. galbana,* mixed ratio 1:1:1) were used to feed larvae with a time of twice a day and density ranged from 15 to 50 × 10^3 cells/mL. At the seed clams, the mixture of fresh algae and formulated food (Lansy, Frippak) with a ratio of 1:1 were used to feed with a dose of 2g/100,000 larvae/day). The larvae were fed 4 times per day.

In the spat larval stage and seed, no bottom substance was used to gain advantages in rearing larvae such as siphon, water exchange, feeding...

The results from 3 production batches were all successful and reached quite a high and stable survival rate, ranging from 4.1 to 5.37 % (average 4.6 ± 0.74%). After 55 days of rearing, 17.37 million of short-necked clam seeds (size 3.0 – 5.0 mm) were produced, corresponding to a yield of 190,000 individuals/m^2.

3.5.2 The technical procedure of seed production of short-necked clam

The technical procedure of seed production of short-necked clam in Khanh Hoa province included criteria such as: the survival rate of broodstock: 92.7 - 95.4%; the mature rate: 74.1 - 80.6%; the spawning rate: 62.2 - 76.5%; The fertilization rate: 72.4 - 77.2%; The hatching rate: 70.0 - 72.2%; The survival rate of larvae from veliger stage to spat stages: 5.2 - 6.5 %; The survival rate of larvae from spat stages to seed (size of 3 - 5mm): 79.6 - 82,7%.

The seed production season: from March to November.

The technical procedure content:

- Step 1: Technique of selecting broodstock of short-necked clam.
- Step 2: The technique of mature culture of short-necked clam.
- Step 3: Technique of stimulating method and collecting larvae.
- Step 4: The technique of rearing larvae of short-necked clam at the veliger stage to the spat stage.
- Step 5: The technique of rearing larvae of short-necked clam at the spat stage to the seed.
- Step 6: Technique of collecting and transportation of short-necked clam seed.

Chapter 4: Conclusion and Recommendation

4.1 Conclusion

4.1.1 The reproductive biological characteristic of short-necked clam in Khanh Hoa province

The gonadal development of short-necked clam is divided into 5 stages: I: undeveloped, II: developing, III: maturity, IV: spawning, V: redeveloping. The male: female ratio of short-necked clam in Khanh Hoa province was 1.00 : 1.08. These clams could be spawning year-round but dominated in two main seasons, from April to May and from September to October. The size of initial sexual maturity was 43 mm for male and 44 mm for female.

The mean of absolute fecundity was 1,137,467 ± 280,054 eggs/individual, relative fecundity was 114,195 ± 17,330 eggs/g body weight and 354,736 ± 59,766 eggs/g meat weight, respectively. The mean of real fecundity was 353,889 ± 165,205 eggs/spawning.

The environmental parameters include salinity of 30 – 31 ‰, pH of 7.5 – 8.5, DO ≥ 5 mg/L, and temperature was 28 – 29°C, the larval development of short-necked clam underwent 4 stages such as Trochophora, D stage, Umbo and Spat in 25 days.

4.1.2 The technical parameters for seed production of short-necked clam in Khanh Hoa province

Algae of *Chlorella* sp. and *I. galbana* was the most suitable food for maturity culture with the best value of fat index, CI index, mature rate, survival rate and biochemical composition of broodstock. The lighting conditions of 500 – 3,000 lux presented the highest survival rate, maturity rate of broodstock and fertilization rate and hatching rate.

This clam responded to three stimulated spawning methods (thermal shock, UV lighting and NH_4OH solution), but the thermal shock was optimal method.

The veliger larvae stage: stocking density of 1- 3 individuals/mL, the salinity of 31‰, and fresh algae (*N. oculata*, *Chlorella* sp. and *I. galbana*) as food were the most suitable rearing condition for growth larvae and survival rate.

The spat larvae stage and seed: salinity 31‰ combined with fresh algae (*N. oculata*, *Chlorella* sp. and *I. galbana*) and formulated food (Lansy and Frippak) were the most suitable condition for growth and survival rate. The stocking density of 2 individual/cm^2 combined with no bottom substance was the most suitable for growth and survival rate.

The most appropriate transportation method of seed was the closed method with a density of 10,000 seeds/bag, transportation time of 6 hours showed the highest survival rate and lowest oxygen consumption.

4.1.3 Seed production in practice and technical procedure for seed production of short-necked clam

The technical procedure for seed production of short-necked clam was built and applied in practice. After 3 batches, 17.37 million of short-necked clam seeds were produced with an average survival rate of 4.6% (shell length of seed ranged from 2 to 5 mm) and productivity of 190,000 seeds/m^2.

4.2 Recommendation

The regulation of time and size of short-necked clam harvested could be built to protect its natural resources in the sea. The harvesting size of short-necked clam should be larger than 45 mm.

Study on using algae concentrated in artificial seed production of short-necked clam to improve the growth and survival rate of larvae.

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