MINISTRY OF EDUCATION AND TRAINING

NHA TRANG UNIVERSITY

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NGUYEN THI HAI THANH

STUDY ON THE REPRODUCTIVE BIOLOGY AND TESTING ARTIFICIAL SEED PRODUCTION OF SADDLEBACK CLOWNFISH *Amphiprion polymnus* (LINNAEUS, 1758)

Major: Aquaculture

Major code: 9620301

SUMMARY OF DOCTORAL THESIS

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The work has been completed at Nha Trang University

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INTRODUCTION

Saddleback clownfish is a naturally distributed species in the waters of Vietnam, appearing in many coastal reefs from the North to the South including the bays of Nha Phu, Cam Ranh and Nha Trang in Khanh Hoa province. With high demand, quick adaptation to artificial habitats, the clownfish is one of the favorite marine fish groups of hobbyists so the number of wild fish caught and fully exploited by the fishermen due to higher economic value than fish used as food of the same size. However, at present, the number of new clownfish is not enough to meet the market demand due to the limited number of artificial species, the limited number of juveniles and some existing fish stocks, maternal, technical maturation maturation, techniques to take care of the fish stage. Therefore, studying the reproductive biology characteristics of the saddleback clownfish species and experimenting with artificial breeding of this species to develop local resources, diversify the species and reduce the fishing pressure to meet the export demand of ornamental fish is the necessary job.

Objective of the study:

Research some reproductive biology characteristics and identify technical solutions for artificial breeding of saddleback clownfish *Amphiprion polymnus* (Linnaeus, 1758)

Scientific and practical application of the study:

The study was performed to contribute to providing scientific information on reproductive biology of saddleback clownfish in natural condition and captive culture. This will be one of the valuable reference resources for teaching, scientific research and ornamental fish farmers. The study aimed to identify reproductive biology, technique for maturing culture, breeding and rearing larvae as a basis for building an artificially reproductive technique of saddleback clownfish. The successful study will contribute to providing the saddleback clownfish juveniles for the market, reduced pressure of natural exploitation of this fish, diversifying of the ornamental marine fish culture, one of the new and potential species for culture in Vietnam which is a long coastal and a diversity of coral reef species, including the coral reef fish.

New findings:

This is the first research works in Vietnam for studying the reproductive biology, incubated behavior, development of embryos and fish larvae, the effect of food, salinity and temperature on the efficiency of reproductive broodstock and the effect of food, salinity and density on the efficiency of rearing culture from 1 to 15 days olds of larval saddleback clownfish as a scientific and practical basis to develop the artificially productive technique of saddleback clownfish.

CHAPTER 1. LITERATURE REVIEW

1.1. Reproductive biology of saddleback clownfish

Saddleback clownfish is belongs to the Perciformes family, Pomacentridae, it is one of 29 species of anemone fish on the world and 1 in 6 species of anemone species distributed in Vietnam coastal. The saddleback clownfish is wide distribution from the Western Pacific and whole of India – Malaysia area. In Vietnam, saddleback clownfish is distributed along the coast from the southern to central, from the Con Dao to Ly Son and Bach Long Vy islands and saddleback clownfish is a common species in Khanh

Hoa according to the research of Allen's research (1972, 1980), Nguyen Huu Phung, (1995) and Astakhop DA (2002, 2008, 2012, 2015).

The saddleback clownfish is a ornamental fish with a small size as compared to coral reef fish with a maximum length around ± 14 cm. The whole body of the this fish is black or orange, the two compartments are bright white in which the first one in the gill bone and the second compartment is only a half of the body as well as the bulge in the middle of the body to form. horse saddle. The mouth of the fish is usually orange, the base of the fins are black, but the edges of the dorsal and tail fins are white is the morphological characteristics of the saddleback clownfish (Allen 1972; 1980 and Nguyen Huu Phung 1987; 1995).

Saddleback clownfish is omnivorous, mainly feeds are zooplanton, wide spectrum of food and short food chain. In captive culture and domesticated condition, the testing food for saddleback clownfish are crustaceans such as rotifers, copepoda, shrimps and sea plants as seaweeds, algae ...

The saddleback clownfish has a symbiotic life with anemones and the group fish with spawning adhesive eggs close their habitat. The natural life cycle this fish is making pairs and making nest and spawning in anemones or rock niches. Broodfish will incubate the eggs to the hatching period, the average hatching time is around 6-8 days at 25-27^{0}C, and the larval fish after hatching and living on the surface of the water and passively moving follow to the current. When they develop into the fry stage, the fish begin the symbiotic life with anemones at low depths. The period of symbiotic life depends on the fry find the appropriated anemone "nest", usually from 7-30 days. After the fish has developed to complete its digestive system and active movement. Along with the growth of size, the fish grow fastly from fry to pre-mature in the "nest", average about 2-3 months, then depending on the social position that the fish continues to develop in size and weight and making pairs and laying eggs at age 1+.

Very few of the research on productive biology of saddleback clownfish as compare to other species such as red-necked, nemo-neck and orange-neck, the research on these species are most of the breeding season, maturity, and reproductive capacity, the breeding cycle or sex for general clownfish but not for Saddleback clownfish (Nguyen Tuong Anh 1996; 2014; Luu Thi Dung 2015; Nguyen Thi Hai Thanh 2018; 2019).

The stages of development of the gonads and sex reversal from males to females of the saddleback clownfish in Thailand have been described Sukjai, R. (2005). The research on development of embryos and larvae of saddleback clownfish was reported by Rattanayuvakorn (2005) and the initially breeding research on saddleback clownfish in captive culture Museum of Marine Life Oceanography up to 4 months old (Nguyen Thi My Ngan, 2007).

1.2. The artificial reproduction of onamental marine fish

1.2.1. In the world

The thesis overviewed the studied status of artificial reproduction in the world through the documents of Allen, GR (1972, 1980, 1991, 2001, 2009, 2017), Buston, PM (1998, 2003). , 2004, 2006, 2007), Cahu, C. (2001, 2003), Calado, R. (2003, 2017), Emel'yanova, N. (2012), Fautin, DG (1986, 1991, 1992), Ghosh , S. (2011, 2012), Hoff, FH (1996) ...

To understand the growth and reproductive biology such as sexual distinction, hermaphroditic and sexual reversal of marine fish and clownfish group, the thesis explored through the research of Godwin, JR (1993), Hattori, A. (1991), Hayes, TB (1998), Henshaw, JM (2018), Heule, C. (2014), Madhu, K. (2006, 2010), Pandian, TJ (2010, 2013), Wu, GC (2005), Yamamoto, TO (1969) ...

In order to understand the required nutrition of broodstock and larvae on the artificial breeding of marine fish, the thesis explored through the research of Gordon, AK (1999, 2000, 2001), Halver. , JE (2015), Lai Van Hung (2004), Kanokrung, A. (2013), King, M. (2013), Lam, H. S. (2016), Madhu, K. (2006, 2012), Ross, M. R. (1978, 1995) ..

Understanding the active mechanism of the environmental factors as well as different hormones for the induced mature and spawning in fish is very important. It will allow us to build the seed production strategies or can improve techniques to induced spawning by different type of hormones. To understand the required nutrition of broodstock and larvae in the current of artificial reproduction, the thesis collected information through the studies of Green, B. S. (2004,2005), Gunasekaran, K. (2017), Holliday, F. G. T. (1969), Holt, G. J. (2011), Johnston, G. (2000), Kumar, T. T. A. (2010), Olivotto, I. (2003, 2008, 2011), Ye, L. (2011) ...

1.2.2. In Vietnam

The artificial reproduction and grow-out culture of marine fish species in Vietnam are very few the research in both of the number species and production scale. The information in Vietnam were collected through the published papers of Ha Le Thi Loc (2004, 2005, 2008), Truong Si Ky, 2006, Tran Thi Le Trang (2010, 2013), Tran Van Dung (2014), Tran Van Phuoc (2010), Ho Son Lam (2016), Huynh Minh Sang (2017)

1.2.3. Artificial reproduction of saddleback clownfish

Only two research on the artificial reproduction of saddleback clownfish were reported by Astakhov, D. A. (2002) and Madhu, K. (2006) in the world. There are not the artificial reproduction of saddleback clownfish research in Vietnam, the studies in Vietnam just only performed the morphological description and larval rearing trials of saddleback clownfish by Nguyen Thi My Ngan (2007), but this study nursed the larvae of saddleback clownfish from one broodfish pairs and no replication yet.

CHAPTER 2. MATERIALS AND METHODOLOGY

2.1. Subject and scope of the study

Research objects: Saddleback clownfish – *Amphiprion polymnus*

Study period and sites: Time of study is from 1/2013 to 12/2018

+ Ecology Laboratory, Coastal Branch, Vietnam - Russia Tropical Center.

+ The ecological research house at the Dam Bay Marine research station, Vietnam-Russia Tropical Center

+ Department of Aquaculture Technology, Institute of Oceanography.

+ Experimental farm producing fish seeds, 13 group, Duong De Hamlet, Vinh Hoa Ward.

Sampling location: Nha Trang, Nha Phu, Van Phong, Cam Ranh bay, Khanh Hoa Province.

2.2. Contents

1. To determine some reproductive biology of saddleback clownfish: sex and rate of hermaphrodites, stages of development of gonads and egg cells, size of maturity at the first time, the maturation coefficient and the breeding season, fecundity, the correlation between length, weight and maturation coefficient.

2. To study the reproductive behavior, hatching, embryo development and larval development of saddleback clownfish.

3. To study the effect of feed, salinity and temperature on reproductive efficiency of saddleback clownfish.

4. To study the effect of feed, salinity and density on the rearing efficiency of larval fish from 1 - 15 days old.

5. Testing artificial seed production and rearing fish larvae to 15 days old.

2.3. Methodology

2.3.1 Reproductive biology characteristics methods

2.3.1.1. Method of sampling

The total number of samples collected from 2014-2018 was 1406. The number of mackerel samples 2014 and 2015 has not been implemented in all seasons of the year for reference only. The statistics are to analyze reproductive biological characteristics from 1158 samples collected from 2016-2018, the Table 2.1.

Table 2.1. Number of fish samples used for reproductive biology analysis

Year	Month												Total
	1	2	3	4	5	6	7	8	9	10	11	12	(sample
2014			32	30	32	18		17				18	**147**
2015	30	22	24	25									**101**
2016	36	30	30	31	33	32	31	30	36	30	30	30	**379**
2017	31	32	34	30	30	31	30	34	30	30	33	31	**376**
2018	30	32	30	31	32	36	38	30	30	32	35	47	**403**
	127	**116**	**150**	**147**	**127**	**117**	**99**	**111**	**96**	**92**	**98**	**126**	**1,406**

2.3.1.2. Methods of analyzing samples of reproductive biology characteristics: Allen (1972); Nguyen Huu Phung (1995); Dao Tan Ho (2001); Astakhov D. A. (2002).

a) Determination of length and weight of fish: Body length (TL), economic length (SL), fish weight (BW), abandoned fish (BW0) and gonads (GW).

b) Analysis of gonads: Gender determination and description of the stages of the gonads and genital cells: a six-step division method of Nikolsky (1963), Xakun and Buskaia (1968) and Nguyen Tuong Anh (1996, 1999).

Modeling method on histogenesis histology: following the method of Patki et al (1989)

c) Determine the size and number of eggs (n = 48): the gonads are observed below on the Olympus CX51 microscope, using the LC30 automated imaging and measurement software connected to the stereo, a microscope with a computer (0.01µm).

2.3.1.3 Method of processing data on reproductive biology characteristics

a) Gender and ratio of male and female

Percentage of individuals with male / female sex cells = (a/b) * 100(%)

Where:

a: Number of individuals with gonads containing male / female sex cells

b: Total number of fish gonads

b) First maturity size*:* King's method (2001)

c) Coefficient of maturation and breeding season

Maturity rate per month: Percentage of stages of the gonads that are calculated.

Coefficient of maturation: Qasim's method (1973)

$$\mathbf{GSI = GW / BW_0 * 100\%}$$

Where:

GSI is the maturity coefficient (%)

GW is the gonad weight (g)

BWo is the weight of fish without intact (g)

Breeding season: is the time when fish have gonads in the maturation period (III, IV, V).

d) Fertility: The number of eggs of 117 ovaries per stage III, IV, V (ovaries the sixth stage is calculated as the thirst stage).

Absolute spawning capacity: Laurence & Briand's method (1990):

$$\mathbf{Fa = (n * GW) / GW_m \text{ (egg/sample)}}$$

Where:

Fa is the absolute spawning capacity

n is the total number of sampled eggs

GW_m is the weight of past sampled ovaries (g)

GW is the ovary weight

Relative spawning capacity:

$$\mathbf{Fr = Fa / BW_0}$$

Where:

Fr: Relative spawning capacity

Fa is the absolute spawning capacity

BWo is the body weight of fish without intact (g)

e. Correlation with length, mass: according to Pearson's coefficient and monovariate linear regression analysis.

2.3.2. Testing artificial seed production

2.3.2.1. Reproductive behavior of parental fish, embryonic development stages and larval metamorphosis

a. Materials

Fish: purposeful scuba diving for each pair of Saddleback clownfish (a pairs of fish with the largest size in the wild anemone nest) from 3 pairs of parents.

Observe the embryo development process by randomly collecting 10 eggs from fertilization to hatch.

b. Method of parental fish

Describe the process of pairing, laying eggs, hatching of parents, observing reproductive habits with the naked eye and filming, photographing by Canon Power Shot SD 960 IS.

c. Method of embryonic development stages

The division of stages of embryo and larval development is based on documents of Nguyen Tuong Anh (1996), Le Hoang My Dung, Luu Thi Dung (2005) Rattanayuvakorn (2005), Yasin (2007).

The embryos were observed with the Olympus SZ61 stereo microscope, CKX41 reverse microscope, and taken photos with Canon EOS 650D digital camera. Measure the size of the workpiece with a microscope eyepiece microscope (0.1 mm).

2.3.2.2. The effect of food, salinity and temperature on reproductive efficiency

a. The effect of foods on reproductive efficiency

Fish: 20 pairs

Experimental: The experiment was carried out for 6 months with 20 glass tanks. Each tank had 1 pair of parents. Each treatment had 4 randomly arranged repeating tanks with fish rations fed to fullness.

Foods: 5 formulas

Formula 1(NT 1)	fodder
Formula 2 (NT 2)	Shrimp, Squid, Fodder, Vitamins, Algae
Formula 3 (NT 3)	Fish meal, Scallops, shrimp, Egg yolk, Spinach
Formula 4 (NT 4)	Fish meal, Cylop - eze, Moi powder, Squid meal, Rice flour, Seaweed, Spirulina, Bread yeast
Formula 5 (NT 5)	Shrimp, Squid, Marinara, Spirulina, Multivitamins, Fish oil, Garlic

b. The effect of salinity on reproductive efficiency

Fish: similar to the experiment on food effects. Choose 16 pairs of Saddleback clownfish from July 2017.

Experimental: The experiment was carried out for 6 months with 16 glass tanks, each with 1 pair of parents. Each treatment had 4 tanks randomly arranged.

Management: Feed the fish with Formula 4 food

Salinity: 25 ‰, 29 ‰, 33 ‰, 37 ‰

c. The effect of temperature on reproductive efficiency

Fish: Selecting 16 pairs fishes from July 2017.

Experimental: The experiment was carried out for 6 months with 16 glass tanks each with 1 pair of parents. Each treatment has 4 repeating tanks, randomly arranged.

Management: Feed the fish with processed food (formula 4). The timing, feeding, care, and siphon diets were similar in the feed experiment. In which the change factor is the different temperature values.

Temprature: 24 ^{0}C, 27 ^{0}C, 30 ^{0}C, 30 ^{0}C

d. Data on reproductive efficiency indicators

Spawning frequency of fish (time/month): Number of spawning fish in the whole experimental period /months.

Actual fertility (Number of eggs / female): The total number of newly born eggs will be counted directly with the naked eye through the enlarged image of the entire egg drive with Canon powershot A2200HD camera 14.1 mega pixels.

Egg size (mm): determined by the eyepiece micrometer in Olympus CX51 microscope, x4 objective and x10 eyepiece. Egg size is an average of the 5 eggs collected.

Loss ratio (%): The number of eggs left after incubation × 100 / number of eggs laid on the first day.

Hatching rate of eggs (%): Number of hatched eggs / number of eggs before transferring to hatching tanks × 100%

Larva size (mm): is the average of 5 larvae.

Time of hatching (days): counting from the date of spawning to the fry

2.3.2.3. The effect of feed, salinity and density on nursery larvae rearing efficiency from the 1st – 15th days

a. The effect of different types of feed on nursery rearing efficiency from the 1st – 15th days

Fish: Juveniles hatch on the first day (1 day old).

Management: Food rations for fish to fill. Supplementing with microalgae *Nannochloropsis oculata, Isochrysis galbana* at the rate of 1: 1, the density of 5-6,000 cells/mL.

Experimental: The number of fish raised in each tank is 20 fish/tank. Experimental rearing tanks have a water content of 10 liters/tank, corresponding to a density of 2 individuals/L. Each treatment was performed with 5 replicates. Experimental period is 15 days.

Foods: The diets used in nursery rearing of Saddleback clownfish correspond to 5 formulas:

Formula 1 (NT 1)	Fodder INVE (200-300µm)
Formula 2 (NT 2)	Artemia nauplius 2-3 sample/L
Formula 3 (NT 3)	Copepoda 2-3 sample/L
Formula 4 (NT 4)	Rotifer 10-15 sample/L
Formula 5 (NT 5)	Artemia nauplius, Copepoda, Rotifer

b. Study the effect of salinity on nursery rearing efficiency from the 1st – 15th days

Fish, care, management: similar in the type of food experiments on nursery rearing efficiency.

Management: The number of fish raised in each tank is 20 fishes/tank. Experimental rearing tanks have a water content of 10 liters/tank, corresponding to a density of 2fishes/L. Each treatment was performed with 5 replicates. Experimental period is 15 days.

Salinity: 20 ‰, 25 ‰, 30 ‰, 35‰, 40 ‰

c. The effect of density on larval rearing efficiency

Fish, care, management: similar in the type of food experiments on nursery rearing efficiency.

Management: The number of fish raised in each tank is 20 fishes/tank. Each treatment was performed with 5 replicates. Experimental period is 15 days.

Density: 1sample/L, 3 samples/L, 5 samples/L, 7 samples/L

d. Collecting and processing data on larval rearing efficiency indicators

Using anesthesia for animals, Russian MS 22 clove oil with a concentration of 1ppm for 2-3 minutes before determining the indicators of length, weight of fish at the beginning and the end of the experiment.

Starting and ending length: The length of the fish is the value of the average length of the fish at the beginning and the end.

Starting and ending weight: Determine the fish weight of the fish put in the initial experiment (T1) and the weight of fish collected at the end of the experiment after 15 days of culture (T2). The weight value of fish is the average weight value of fish at the beginning and the end.

Specific growth length: *(SGR_L)*

$$SGR_L\ (\%/ngày) = [(LnL2 - LnL1) / (T2 - T1)] \times 100$$

Where:

L1, L2: corresponding fish length at time t1, t2.

Specific growth weight:

$$SGR_W(\%/ngày) = [(LnW2 - LnW1) / (T2 - T1)] \times 100$$

W1, W2: corresponding fish weight at time t1, t2.

Population variety *(%)*

$$SR\ (\%) = (Se / Si) \times 100$$

Where:

SR is the coefficient of variance.

Se is the standard deviations of body weight and length.

Si is the means of body weight and length

2.4. Data analysis

Excel 2010 software is used to set up the input database, draw charts, process input data as an input database for SPSS, SPSS Station 16.0 for window and R software to calculate Pearson coefficient (Pearson correlation coefficient) and Multi Linear Regression analysis determine the relationship between length, mass and gonadal maturation coefficients. Using the one-way variance analysis function (oneway - ANOVA) and Ducan test to verify the statistically significant difference ($P < 0.05$) of the one-variable parameters among the experiments in each experiment.

CHAPTER 3. RESULTS AND DISCUSSIONS

3.1. Reproductive biology

Total 1,158 saddleback clownfish in Khanh Hoa coastal collected and analyzed the reproductive biology of from 2016-2018. The average length of fish was 7.58 ± 2.89 cm in the range of min. 2.5 – max. 14.54 cm. The average weight of fish was 14.18 ± 13.97 g in the range of min. 0.72 – max. 62.59 g. The results of size and weight of samples are shown in Table 3.1

Table 3.1 Length and weight of saddleback clownfish in Khanh Hoa (n = 1158)

Value	TL (cm)	SL (cm)	BW (g)	BW_0 (g)
Medium	7.58	6.40	14.18	13.19
Average value	2.89	2.42	13.97	13.27
Max.	14.54	12.10	66.26	62.59
Min.	2.50	2.07	0.93	0.72

3.1.1. Sex and ratio of hermaphrodite saddleback clownfish

A total of 120 samples of saddleback clownfish collected and determined sexual identify through the spermatocyte male and oocyst products. The results of sexual determination were shown in Table 3.2

Table 3.2 Ratio of hermaphrodite saddleback clownfish in Khanh Hoa coastal

Phase	Month												Sample	Oocyte (%)	Sperma-tocyte (%)	Note
	1	2	3	4	5	6	7	8	9	10	11	12				
I	6	2	0	0	5	0	2	2	0	6	0	2	**25**	100	100	F. 3.2
II	0	4	2	2	0	3	2	0	0	2	6	0	**21**	100	100	F. 3.3
III	0	2	0	3	2	2	0	0	8	0	4	5	**26**	100	100	F. 3.4
IV	1	2	3	4	0	0	5	6	0	0	0	0	**21**	100	100	F. 3.5
V	2	1	3	0	1	6	1	0	0	0	4	9	**27**	100	100	F. 3.6
	9	11	8	9	8	11	10	8	8	8	14	16	**120**			

The oocyte and spermatocyte were 100% with the phase development from I to V in all the hermaphrodite saddleback clownfish in Khanh Hoa coastal. The results shown that there were not enough the information to confirm sex reversal of saddleback clownfish.

3.1.2. Different development phase of gonads and oocytes

The gonads of the saddleback clownfish consisted of two lobes and they were not the same size during development, a large lobe and a small lobe on either side of the abdominal sinus and trough up on the wall of body sinuse by the membrane. The later of the gonads was a short duct, two ducts connected together and linked to the outside through the genital hole. Figure 3.1

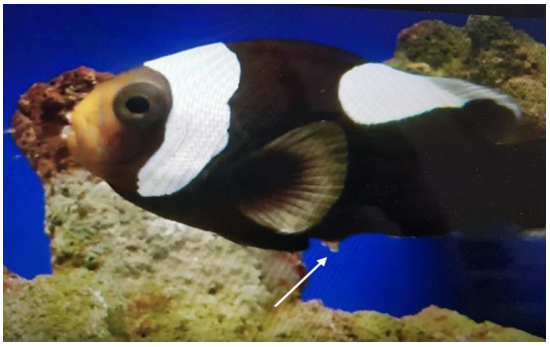

Figure 3.1. The genital hole of saddleback clownfish while spawning and fertilizing

The presence of oocytes and spermatocytes at different stages in the ovary indicated that this species was an asynchronous ovule and spawned many times per year.

3.1.2.1. Phase I

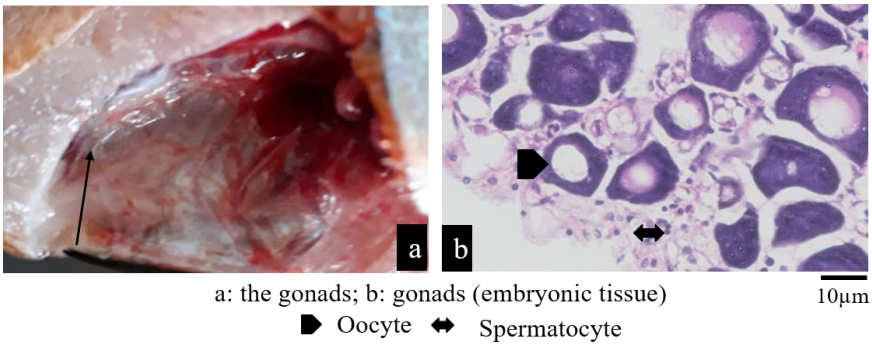

Figure 3.2. Phase I of the gonad

3.1.2.2. Phase II

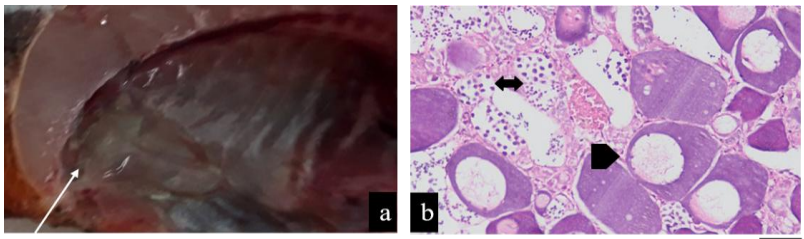

Figure 3.3. Phase II of the gonad

3.1.2.3. Phase III

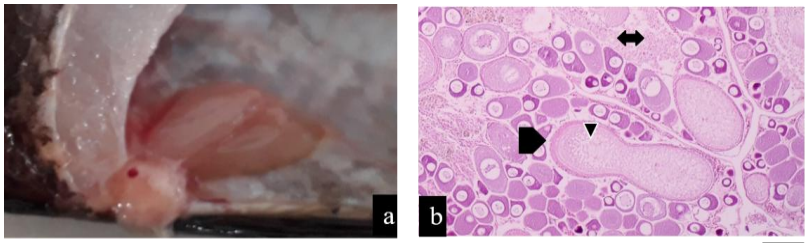

Figure 3.4. Phase III of the gonad

3.1.2.4. Phase IV

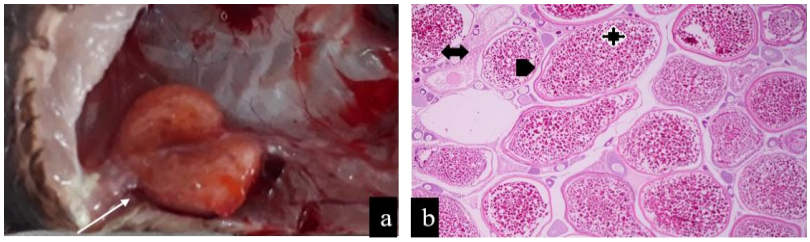

Figure 3.5. Phase IV of the gonad

3.1.2.5. Phase V

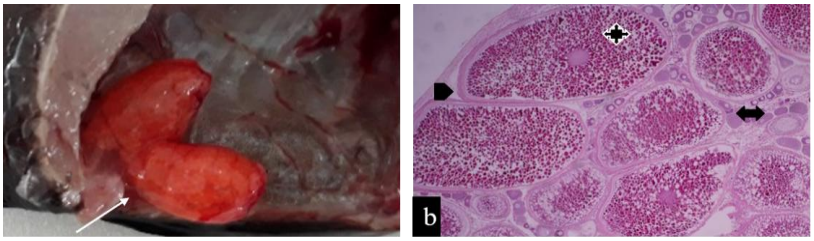

Figure 3.6. Phase V of the gonad

3.1.2.6. Phase VI

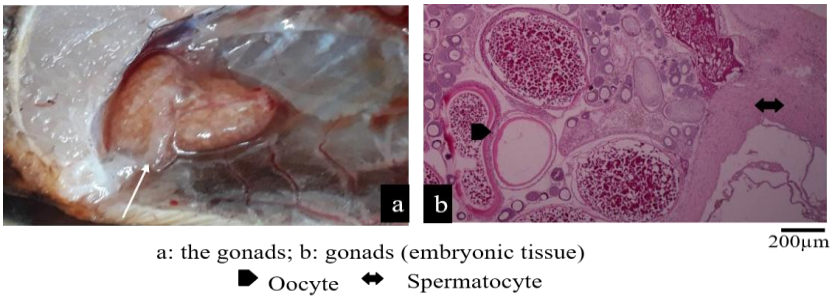

Figure 3.7. Phase VI of the gonad

3.1.3. The size of first maturation

Through analysed samples, they showed that saddleback clownfish were 100% maturation rate with the size bigger than 7.5 cm. Group of fish with the length smaller than 4.6 cm were not participated for spawning. Group of fish with the length larger than 6.5 cm were higher 50% of the maturation rate.

To determine the maturation size of the saddleback clownfish, the correlation between length and Ln ((1-P) / P) was deed. From the correlation equation (y = -1.9676 * x + 12.478), the first maturation size of fish was determined at 6.37 cm. The results were shown in Figure 3.8

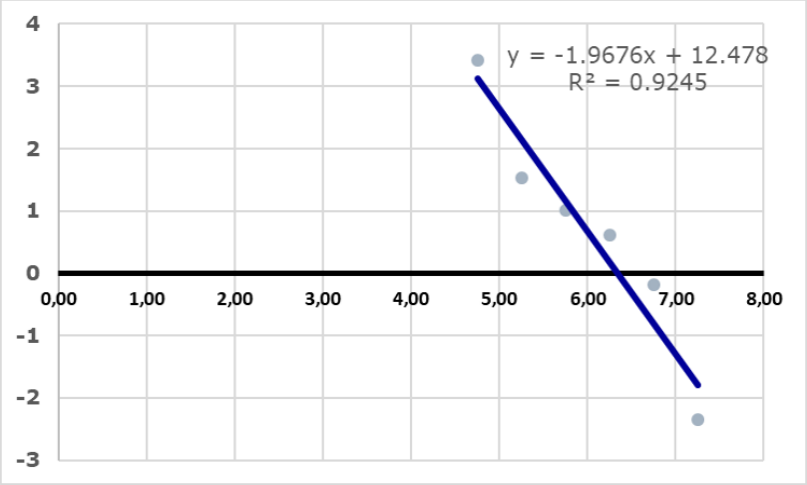

Figure 3.8 The correlation between size group of fish and Ln ((1-P) / P)

3.1.4. Maturing coefficient and breeding season

The GSI of the saddleback clownfish of the study was shown in Figure 3.9. The GSI index of saddleback clownfish in the year reached the highest value in April (1.05 ± 1.31), the lowest in November (0.33 ± 0.56) and the average was 0.57 ± 0,85. The fluctuations of the GSI index showed that the fish matured whole year, in which the mainly spawning season from February to July with a peak from March to May and the extra spawning season from November to December.

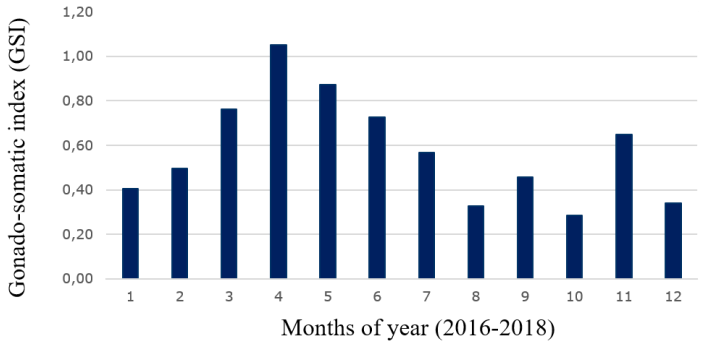

Figure 3.9. The fluctuation of maturating coefficient on saddleback clownfish

The caughting rate of matured fish with gonads at phases III, IV and V was highest in May (73%) and lowest in December (36%). The results were shown in Table 3.2

Table 3.3. The maturating rate of the saddleback clownfish

Month	1	2	3	4	5	6	7	8	9	10	11	12
Total sample	97	94	94	92	95	99	99	94	96	92	98	108
Number of maturating fish	62	61	65	60	69	59	49	47	66	46	56	39
Maturating rate (%)	64	55	70	65	73	60	50	50	69	50	57	36

The results of the breeding season shown that saddleback clownfish spawned during the year, shown in Figure 3.10.

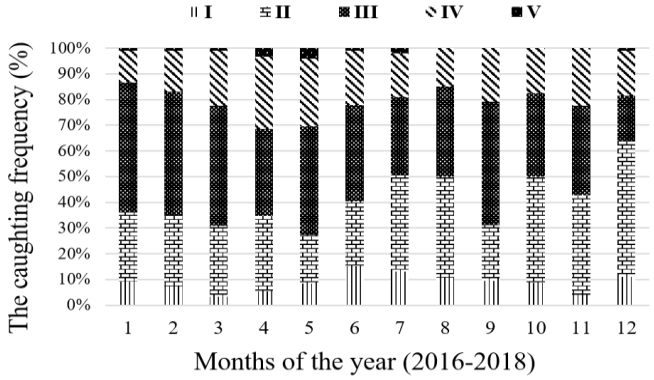

Figure 3.10. The caughting frequency in the development of gonadal phases during the year

3.1.5. Fecundity of saddleback clownfish

117 gonad samples of the fish with the phase at III, IV and V used to counting the number of eggs and measuring the weight of fish for determining the reproductive index. The analyzed saddleback clownfish for the reproductive ability were the length in range from 7.2 to 13.7 cm with the weight from 13.12 to 57.21 (g), respectively. The results were shown in Table 3.3

Table 3.4. The fecundity of saddleback clownfish in Khanh Hoa coastal

Value	TL (cm)	SL (cm)	BW (g)	BW0 (g)	GW (g)	GSI	Fa (egg/fish)	FGr (egg/g)
Average	10.61	8.95	28.85	27.00	0.41	1.48	823.59	42.91
Standard deviation	1.98	1.63	12.71	12.11	0.39	1.30	259.80	40.96
Max	13.70	11.40	57.21	54.38	1.43	6.25	1830.00	316.16
Min	7.20	5.27	13.12	2.67	0.00	0.02	306.00	10.42

The average of actual fecundity of the saddleback clownfish aranged 823.59 ± 259.80 (eggs/females), coresponding to 306-1,830 (eggs/females). The relative fecundity was 42.91 ± 40.96 eggs/g of fish, coresponding to 10.42 to 316.16 eggs/g fish.

3.1.6. The correlation between length, weight and maturating coefficient

The comparation of the correlation between length and weight of saddleback clownfish was positive correlation. In which, the correlation between length and weight of fish was positive correlation with $R^2 = 0.867$, with high value and positive value for growth. This showed that when the fish were a large length so the weight of fish increased.

The explanation for this matter may be due to a particular feature in the growth of the group of neck anemone fish in general, that the development of size and sex fish depends on the social rank of each individual. Living characteristics of anemone fish from small populations with 2 individuals up, the number of fish in the pre-maturity

stage does not depend on the size of the parents, the fish always exist in the "nest" during this stage and suddenly growth in size and weight into broodstock.

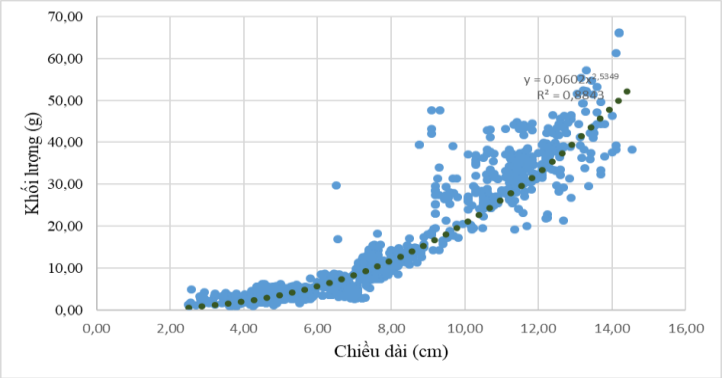

Figure 3.11 The correlation between length and weight

3.2. Reproductive behavior, hatching, embryo development and larvae to fry up to 15 days

3.2.1. Reproduction behavior and hatching

During the experiment (February - May 2015), TL and BW females ranged from 10.8 to 12.6 cm; 35-45g / fish at the first sampling and 11.2-12.9 cm; 34.5-48 g / fish at the end of the sample collection.

The courtship and mating activity usually take place a few days before spawning. The typical manifestation of this time is that the parents heads up and down repeatedly were swiming and flirted at the site where they lay eggs and constantly used their mouths to clean the spot where they would laid eggs later. In addition, there are other manifestations such as biting, chasing and spreading the wide rays, flipping back and forth over the body or aggressive actions, repelling each other with slight biting, chasing, rubbing. When approaching fish at this time, the fish often aggressively move to the opposite side of the platform.

One day before spawning, fish stop eating or eat very little compared to every day. The diameter of abdomen fish is slightly increased compared to normal. During spawning, the female swims slowly, rubbing the abdomen with the fallopian tube on the surface of substrates. The egg is ejected from the fallopian tube about 3 - 5 mm long. Two females (function) swim slowly in a zigzag manner and the fish's belly is close to the surface of the substrate in a rhythm from 30 'to 3 minutes each time they release an egg or sperm. The number of eggs per spawn varies from 300 to nearly 2,500 eggs depending on age and size of broodstock. The time for one egg laying lasts from 60 to 150 minutes. Eggs are laid on the surface of the substrate and cared for by both parents.

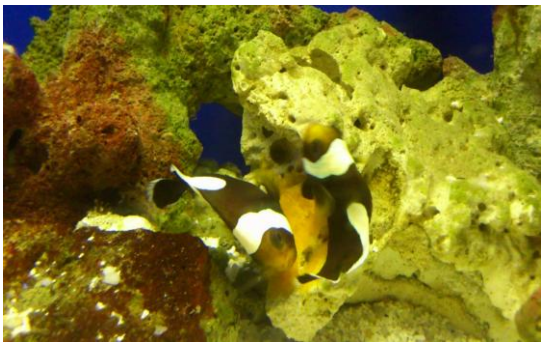

Figure 3.12 Laying eggs and fertilizing of saddleback clownfish parents

3.2.2. The development stages of embryos, larvae and fry up to 15 days

The process of developing embryos and metamorphosis of saddleback clownfish was similar to the orther fish, which was divided into different stages including: fertilization and egg activation, cleavage and blastocyst stage, embryo and differentiated organ formation.

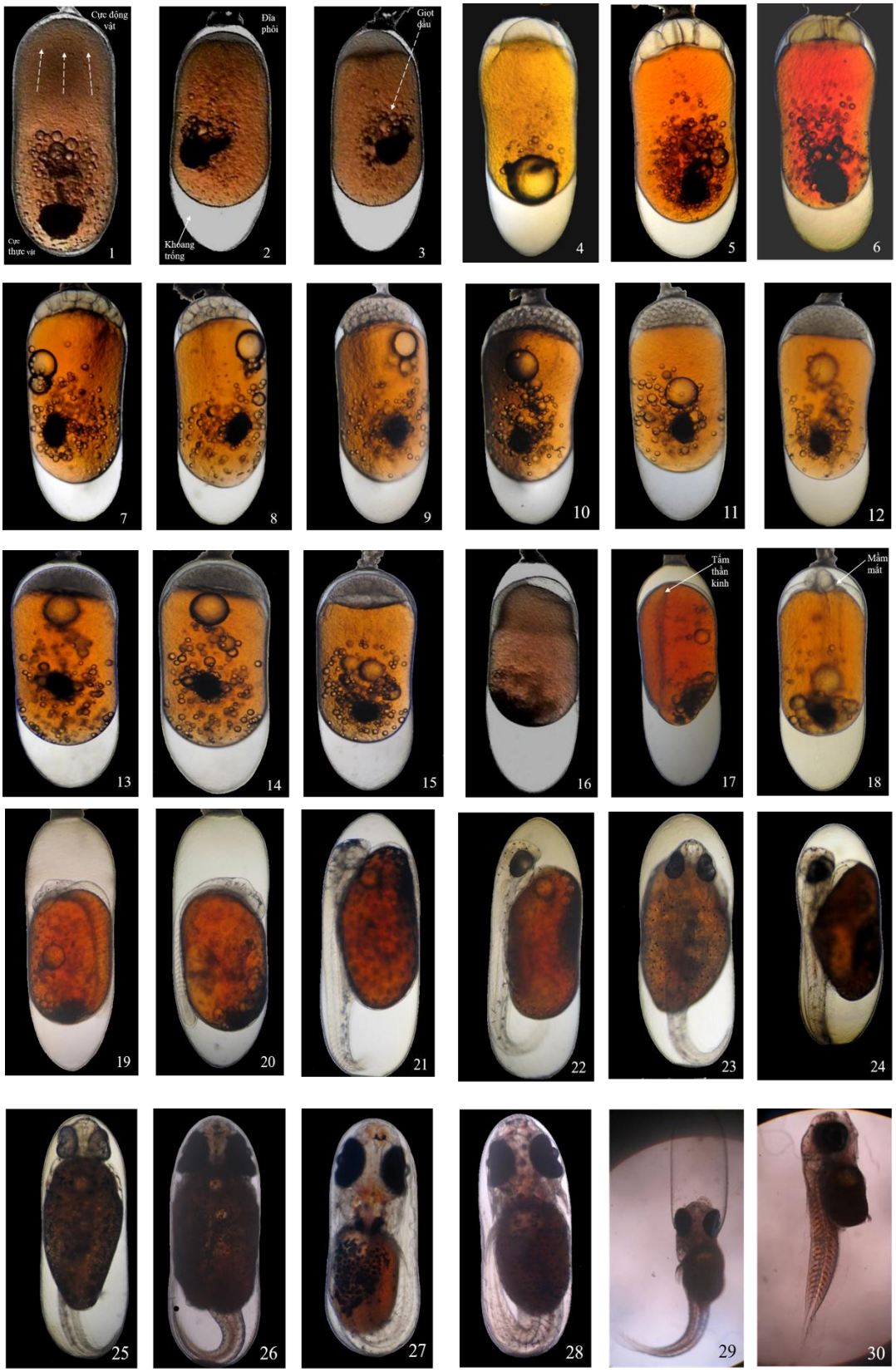

Figure 3.13. The embryo and larval development stages of saddleback clownfish

Time of development of stages was presented in Table 3.4.

Table 3.5. The period of time in embryonic development of saddleback clownfish

Stages	Time			Characteristic growth
	Day	Hour	Minute	
Fertilization and activation of eggs				
1			10	Immediately after fertilization, the cytoplasm converges on the animal pole
2		1	25	Formation of an empty space around the yolk (due to extreme penetration of plants and animals)
3		1	30	1 cell
Egg cleavage phase				
4		1	55	2 cells
5		2	5	4 cells
6		2	10	8 cells
7		3	5	16 cells
8		4	55	32 cells
9		5	20	64 cells
The blastocyst stage				
10		6	02	128 cells
11		7	25	256 cells
12		13	15	High blastocysts
13		15	25	Low blastocysts (waist tightening)
14		17	5	Low blastocysts (waist tightening disappears)
15		18	15	Late blastocysts
Embryonic stage				
16		20	10	Early stage embryos
17	1	0	15	Neural plate formation
18	1	3	20	Tthe end of the embryonic stage, the eye germ forms
Differentiate and form organs				
19	1	4	35	Formation of nerve grooves, vertebrae and spine
20	1	5	50	Forming caudal sprouts
21	1	22	10	The heart appears, the tail grows longer and begins to move slowly, the eye vein appears, three primary brain vesicles appear: the front, middle and posterior brain
22	2	8	40	The head separates gradually from the yolk, the ear stone appears
23	2	12	5	Mouth appears
24	2	15	3	The heart begins to work harder, the lungs appear
25	3	2	10	The gill covers appearence, the eyes have a lot of dark pigments
26	4	10	50	Anal appearance
27	5	14	55	The size of the yolk becomes smaller, the gill covers clearly, forming organs
28	6	1	30	The embryos move a lot, carrying their heartbeat
29	7	5	20	Embryos hatch

In our study, the embryo fully hatched after 173 ± 6 hours, which corresponds to 7 days and 6 hours after fertilization. The process of cleaving the embryos of KCLYN fish takes place only in the animal poles similar to the general rule in bone.

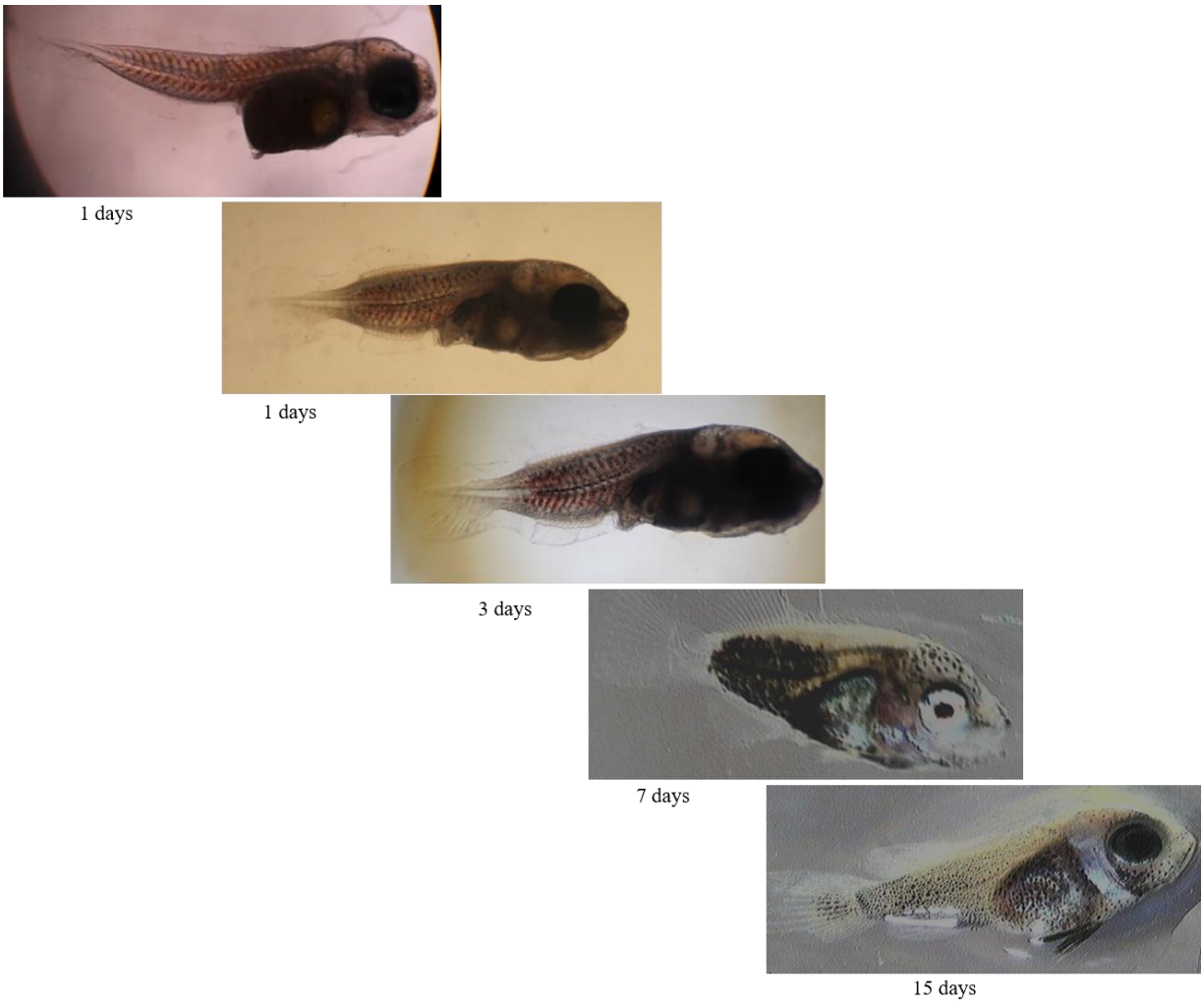

Figure 3.14. Fry development up to 15th days

3.3. The effect of food, salinity and temperature on the reproductive efficiency of saddleback clownfish

3.3.1. The effect of foods on reproductive efficiency

The study used 5 types of feed in which NT1 is a synthetic feed commonly used by farmers in rearing marine family ornamental fishes. The remaining NTs are a blended feed in which the composition consists of many different foods (shrimp, squid, fish meal, fishmeal, algae, algae ...). The results showed that the feed had a great influence on the reproductive efficiency of the parents of Saddleback clownfish in maturing and spawning. The mixed feed for neck cavity in the experiment could be used for clownfish saddleback fish however synthetic feed is not suitable for clownfish saddleback fish at this stage. Among all 4 types of mixed feed, the type of compound feed according to NT4 achieved the highest reproductive efficiency based on observed indicators ($P< 0,05$). Using NT1 synthetic feed, broodstock only give birth once but the The number of eggs, fertilization rate and hatching rate is low, the food composition does not affect the size of Saddleback clownfish larvae and there is only difference in egg size when using synthetic feed compared to other compound feed type is significantly lower ($P< 0,05$).

Table 3.6. Efficient rearing broodstock with different types of food

Targets \ NT	NT2	NT3	NT4	NT5
Frequency of calving (time / month)	0.25 ± 0.462^{a}	0.37 ± 0.079^{ab}	0.67 ± 0.067^{c}	0.47 ± 0.042^{b}
Number of eggs (eggs / nest)	396 ± 36^{a}	495 ± 54^{ab}	708 ± 71^{c}	618 ± 48^{bc}
Egg size (mm)	1.38 ± 0.047^{a}	1.49 ± 0.032^{ab}	1.50 ± 0.043^{b}	1.53 ± 0.025^{ab}
Fertilization rate (%)	31.19 ± 3.919^{a}	55.43 ± 1.864^{b}	72.45 ± 4.310^{b}	58.81 ± 9.561^{b}
Hatching rate (%)	34.17 ± 7.945^{a}	53.73 ± 5.493^{b}	81.33 ± 4.005^{c}	61.89 ± 6.676^{b}
Number of larvae (animals)	39 ± 13^{a}	150 ± 27^{b}	409 ± 21^{c}	211 ± 22^{b}
Larva size (mm)	2.39 ± 0.034^{a}	2.51 ± 0.017^{b}	2.61 ± 0.027^{b}	2.54 ± 0.041^{b}
	$9,37 \pm 0,426^{a}$	$8,5 \pm 0,353^{a}$	$7,35 \pm 0,239^{b}$	$7,63 \pm 0,375^{b}$

The figures with different alphabetic characters in the same row represent a statistically significant difference, P <0.05.

Spawning frequency and number of eggs, the number of larvae increases when feeding fish from NT2 to NT4 and decreases in NT5. However, the farrowing frequency, fertilization rate and hatching rate did not have any statistical difference between the fish fed with NT 4 and NT5 with values of 0.66 ± 0.017 times / month, 87.31 ± 2,892 respectively. %, 75,29 ± 1,724% in NT4 and 0,56 ± 0,074 times / month, 89,39 ± 0,809%, 76,02 ± 1,480% in fish fed with NT5.

Analysis of composition of experimental feeds (NT1 is data provided by the manufacturer), the results are obtained in Table 3.6.

Table 3.7. Food recipes of different types of food

Targets \ Ingredient	Protein	Lipid	Moistrute	Ash	NFI
1	38.1	9.7	2.8	8.2	41.2
2	39.95	12.95	4.96	14.43	27.70
3	45.22	11.45	14.16	15.80	13.37
4	49.83	10.54	13.36	12.75	13.53
5	55.12	9.88	13.23	10.50	11.28

3.3.2. The effect of salinity on reproductive efficiency

Salinity is one of the factors that affect fertility, hatching time and the ability of the egg to attach to the substrate. Research results show that saddleback clownfish have the ability to adapt and can reach a state of complete maturity and spawn at salinity of 25 - 37 ‰. This is of practical significance in suggesting that farmers can grow and breed at low salinity levels, in order to reduce the use of seawater in hatcheries. Results of experiments on rearing and reproducing parental saddleback clownfish at different salinity levels are presented in Table 3.7

Table 3.8. Efficient rearing broodstock at different salinity levels

Targets \ Salinity	25‰	29‰	33‰	37‰
Frequency of calving (time / month)	0.33 ± 0.067^{a}	0.75 ± 0.046^{b}	0.71 ± 0.104^{b}	0.21 ± 0.04^{a}
Number of eggs (eggs / nest)	450 ± 34^{a}	681 ± 34^{b}	683 ± 57^{b}	316 ± 51^{a}
Egg size (mm)	1.50 ± 0.048^{b}	1.54 ± 0.037^{b}	1.50 ± 0.044^{b}	1.25 ± 0.044^{a}
Fertilization rate (%)	70.75 ± 5.508^{b}	83.99 ± 3.238^{b}	78.85 ± 4.631^{b}	52.88 ± 5.559^{a}
Hatching rate (%)	60.27 ± 6.174^{b}	79.98 ± 1.657^{c}	78.45 ± 2.338^{c}	41.37 ± 3.341^{a}
Number of larvae (animals)	202 ± 53^{b}	505 ± 19^{d}	398 ± 29^{c}	69 ± 14^{a}
Larva size (mm)	2.40 ± 0.023^{ab}	2.61 ± 0.086^{c}	2.57 ± 0.061^{bc}	2.229 ± 0.051^{a}
Time to larvae (days)	$6{,}42 \pm 0{,}21^{a}$	$7{,}35 \pm 0{,}16^{b}$	$7{,}10 \pm 0{,}38^{b}$	$12{,}25 \pm 0{,}38^{c}$

The figures with different alphabetic characters in the same row represent a statistically significant difference. P <0.05.

The spawning frequency of Saddleback clownfish is affected by salinity level. at salinity level of 29 ‰ the fish has the highest spawning frequency. Then. salinity levels of 33. 25 and 37 ‰ ($P \leq 0.05$ respectively). The number of eggs. fertilization rate and larval size were statistically different in increasing order of salinity at 37 ‰. 25 ‰ and no difference at salinity level of 29 and 33 ‰. The highest number of larvae was 29 ‰ then 33 ‰. The hatching time at 25 mặn salinity level is the fastest ~ 6-7 days. At 29-33-salinity. Saddleback clownfish larvae hatch after ~ 7-8 days.

At 37 mặn salinity. the reproductive efficiency of the broodfish is lowest ($P< 0.05$) with the number of larvae only 69 ± 14. in addition to other reproductive indicators such as number of larvae. fertilization rate . the hatching rate is all the lowest compared to other salinity levels. It can be seen at this salinity. Saddleback clownfish can reproduce but the reproductive efficiency is not high.

It can be seen that the Saddleback clownfish is adapted to the salinity level of 25-37 ‰. however. the salinity affects the reproductive efficiency of the parent Saddleback clownfish in it. the salinity is suitable for breeding fish and Larvae development is 29-33 ‰. A salinity of 37 ‰ is not suitable for the breeding and breeding of Saddleback clownfish. This result shows that. in captivity. farmers should pay attention to evaporation of seawater and regular salinity checks when breeding Saddleback clownfish.

3.3.3. The effect of temperature on reproductive efficiency

In Saddleback clownfish. temperature is one of the factors affecting the reproductive efficiency of broodstock. At temperatures from 24 - 30^{0}C. the reproductive efficiency of fish is shown in Table 3.8

Because of fish is a thermoplastic animal. temperature is the most influential environmental factor in metabolism. thereby affecting the reproductive process of fish. Each fish requires a certain amount of maturity. The same species of fish living in low-temperature waters often have more maturing age and longer maturation times than fish of the same species living in warmer temperatures. Each fish only spawns at a certain temperature range [4]. In Saddleback clownfish. temperature is one of the factors affecting the reproductive efficiency of broodstock. At temperatures from 24 - 30^0C. the reproductive efficiency of fish is shown in Table 3.8

Table 3.9. Efficient rearing broodstock at different temperatures

Temperatures / Targets	24ºC	27ºC	30ºC	33ºC
Frequency of calving (time / month)	0.40 ± 0.023^b	0.732 ± 0.019^d	0.63 ± 0.018^c	0.20 ± 0.019^a
Number of eggs (eggs / nest)	633 ± 39^a	884 ± 40^b	715 ± 10^a	639 ± 16^a
Egg size (mm)	1.48 ± 0.0116^a	1.47 ± 0.017^a	1.48 ± 0.012^a	1.51 ± 0.044^a
Fertilization rate (%)	82.19 ± 2.321^b	91.29 ± 1.772^c	79.10 ± 3.248^b	57.98 ± 2.186^a
Hatching rate (%)	57.68 ± 0.994^b	79.76 ± 1.546^d	70.18 ± 0.823^c	35.06 ± 1.179^a
Number of larvae (animals)	299 ± 17^b	645 ± 41^d	399 ± 25^c	129 ± 3^a
Larva size (mm)	2.98 ± 0.032^b	2.83 ± 0.015^c	2.81 ± 0.017^{bc}	2.57 ± 0.102^a
Frequency of calving (time / month)	11.40 ± 0.50^d	8.20 ± 0.16^c	7.20 ± 0.38^b	6.42 ± 0.21^a

The figures with different alphabetic characters in the same row represent a statistically significant difference. P <0.05.

Best breeding performance of Saddleback clownfish at 27^0C. In which the observed indicators: egg frequency. number of eggs. fertilization rate. hatching rate and number. size of larvae are all higher than the remaining temperatures in the experiment and valuable indicators. respectively: 0.732 ± 0.019 times / month. 884 ± 40 eggs / nest. 91.29 ± 1.772%. 79.76 ± 1.546%. 645 ± 41 birds. 2.83 ± 0.015 mm. These observed parameters did not differ much when breeding broodstock at 24 and 30^0C. but at temperatures lower than 24^0C. some of the reproductive parameters of the fish are better than 30^0C (hatching rate. number of larvae). At a temperature of 33^0C. the reproductive parameters are lowest (P< 0.05) except the shortest hatching time. The hatching time of larvae is inversely proportional to the temperature increase. at 24^0C. the hatching time of fish lasts ~ 12 days leading to long care period. high loss rate during hatching. broodstock reproductive maturity slowly. not high economic efficiency. The suitable temperature for breeding Saddleback clownfish is from 27-30^0C in which 27^0C is the best temperature (P< 0.05).

3.4. The effect of feed. salinity and density on the nursery rearing efficiency from the 1st – 15th days

3.4.1. The effect of different foods on larval rearing efficiency

Saddleback clownfish larvae are fed with live feeds with a combination of a variety of feeds (< rotifers. *Artemia* and copepoda) for survival. growth rate of length and weight respectively: 57.03 ± 1.07%. 7.513 ± 0.062% day and 14.414 ± 0.200% day. Compared to treatments for individual larvae fed each feed. the combination of live feeds is best for Saddleback clownfish larvae aged 1-15 days of age (P< 0.05).

Copepoda of different sizes can be used in the nursery process of saddleback clownfish larvae however. the effect is not as good as using a combination of 3 types of live feed (P< 0.05). Survival rate. growth rate of length and weight were respectively 42.90 ± 0.86%. 76.236 ± 0.020% day and 14.248 ± 0.1122% day (P< 0.05).

Table 3.10. Growth and survival of larvae with different types of food

Food / Targets	Fodder	Rotifer	*Artemia*	Copepoda	Rotifer + Artemia + Copepoda
First length (mm)	2.60	2.57 ± 0.003	2.58 ± 0.003	2.59 ± 0.003	2.57 ± 0.002
End length (mm)	_	5.97 ± 0.015^{a}	6.41 ± 0.015^{b}	6.60 ± 0.023^{c}	8.00 ± 0.071^{d}
First mass (g)	0.0039	0.0038	0.0038	0.0037	0.0038
End mass (g)	_	0.0254 ± 0.0017^{a}	0.0269 ± 0.0008^{a}	0.0315 ± 0.0005^{b}	0.0337 ± 0.0011^{b}
SR (%)	_	26.03 ± 0.39^{a}	25.46 ± 0.67^{a}	42.90 ± 0.86^{b}	57.03 ± 1.07^{c}
SGRL (% day)	_	5.63±0.109^{a}	6.09 ± 0.023^{b}	6.27 ± 0.017^{c}	7.58 ± 0.006^{d}
SGRW (% day)	_	12.62 ± 0.427^{a}	13.19 ± 0.241^{a}	14.04 ± 0.170^{b}	14.41 ± 0.200^{b}

The figures with different alphabetic characters in the same row represent a statistically significant difference. P <0.05.

Artemia and rotifers are unsuitable feed for Saddleback clownfish larvae if used individually. the larvae have the lowest survival rate and volume growth with survival values of ~ 25-26% and the volume growth rate of ~ 12-13% day (P< 0.05). Unable to use synthetic feed in rearing of Saddleback clownfish larvae from 1-15 days of age. fish die after 2-3 days of rearing

3.4.2. The effect of salinity on larval rearing efficiency

Testing of larval rearing of Saddleback clownfish at salinity levels of 20-40 ‰ showed that salinity also significantly affected the growth and survival of experimental fish. After 15 days of rearing. fish reared at 30 ‰ salinity reached the survival rate. the growth rate of the length. the growth rate of the highest typical weight were 58.81 ± 0.95% and 6.484 ± 0.091. respectively. % / day and 16.048 ± 0.469% / day (P <0.05). Fish reared at 25 ‰ salinity had a higher survival rate. growth rate and specific weight

than those at 35 ‰ (P <0.05). respectively. 53.30 ± 2. . 49%. 6.212 ± 0.044% day and 16.110 ± 0.269% day at 25 ‰ and 43.41 ± 2.44%. 5.732 ± 0.063% day. 12.484 ± 0.111% day at salinity level 35 ‰.

Comparison between experimental salinity levels. 40 ‰ level is not the most suitable for the growth and development of Saddleback clownfish larvae. fish larvae have low survival rate. reaching 18.26 ± 1.63%. Experimental results studying the effect of different salinity levels on larval growth and development. Thus. it can be seen that the salinity from 25 - 35 ‰ is suitable for rearing Saddleback clownfish larvae. The results are shown in Table 3.10

Table 3.11. Larval growth and survival at different salinity levels

Salinity / Targets	20‰	25‰	30‰	35‰	40‰
First length (mm)	2.77 ± 0.006	2.77 ± 0.005	2.77 ± 0.006	2.77 ± 0.003	2.76 ± 0.004
End length (mm)	3.37 ± 0.088^{b}	7.03 ± 0.059^{d}	7.32 ± 0.241^{e}	6.56 ± 0.066^{c}	3.00 ± 0.055^{a}
First mass (g)	0.0046	0.0042	0.0044	0.0043	0.0045
End mass (g)	0.0204 ± 0.0007^{a}	0.0479 ± 0.0002^{c}	0.0488 ± 0.0030^{c}	0.0281 ± 0.0004^{b}	0.0188 ± 0.0013^{a}
SR (%)	25.99 ± 1.15^{b}	53.30 ± 2.49^{d}	58.81 ± 0.95^{e}	43.41 ± 2.44^{c}	18.26 ± 1.63^{a}
SGRL (% day)	1.34 ± 0.172^{b}	6.212 ± 0.044^{d}	6.484 ± 0.091^{d}	5.732 ± 0.063^{c}	0.556 ± 0.117^{a}
SGRW (% day)	10.266 ± 0.279^{a}	16.110 ± 0.269^{c}	16.048 ± 0.469^{c}	12.484 ± 0.111^{b}	9.674 ± 0.469^{a}

The figures with different alphabetic characters in the same row represent a statistically significant difference. P <0.05.

The energy consumption that is responsible for the reduction of fish growth in the experiment. This explains why fish cultured at salinities below 20 ‰ or above 35 ‰ have lower growth rates than those raised at salinities of 25 - 35 ‰.

It can be seen that the most suitable salinity level for larval rearing of saddleback clownfish in the period of 0 - 15 days of age is 25 - 35 ‰.

3.4.3. The effect of density on larval rearing efficiency

Different densities during the rearing process of saddleback clownfish larvae shows that the rearing density has an effect on the growth and survival rate of Saddleback clownfish larvae with the general trend that the higher the density. the the growth index and survival rate is lower. however. this trend does not have much variation in the stocking density of 3 -5 fish / L. fish has the best developmental indicators at the stocking density of 1 fish / L. At the density of 7 fish / L. the survival rate and fish quality (growth of length. weight) of larvae decreased. Specifically. the survival rate. the specific growth in length and weight of fish larvae at 3 and 5 fish / L respectively: 59.86 ± 1.43%. 5.994 ± 0.111% day. 15.492 ± 0.197% day and 62.46 ±

2.05% at stocking density of 3 animals / L. 6.196 ± 0.095% day and 15.959 ± 0.221% day at 5 heads / L (P> 0.05).

Results of stocking density effecton the growth and development of saddleback clownfish larvae were shown in Table 3.11.

Table 3.12. Growth and survival larvae at different densities

Density / Targets	1 sample/L	3 samples /L	5 samples /L	7 samples /L
First length (mm)	2.59 ± 0.002^a	2.59 ± 0.004^a	2.59 ± 0.003^a	2.59 ± 0.002^a
End length (mm)	7.99 ± 0.135^c	6.56 ± 0.098^b	6.36 ± 0.119^b	5.75 ± 0.162^a
First mass (g)	0.0043 ± 0.0000^a	0.0042 ± 0.0000^a	0.0044 ± 0.0000^a	0.0043 ± 0.0000^a
End mass (g)	0.0564 ± 0.0019^c	0.0468 ± 0.0018^b	0.0448 ± 0.0014^b	0.0345 ± 0.0008^a
SR (%)	58.70 ± 0.65^b	62.46 ± 2.05^b	59.86 ± 1.43^b	44.98 ± 1.22^a
SGRL (% day)	7.515 ± 1.117^c	6.196 ± 0.095^b	5.994 ± 0.118^b	5.317 ± 0.190^a
SGRW (% day)	17.145 ± 0.259^c	15.959 ± 0.221^b	15.492 ± 0.197^b	13.757 ± 0.168^a

The figures with different alphabetic characters in the same row represent a statistically significant difference. P <0.05.

Survival rate. specific growth in length and weight of fish larvae reared at a density of 7 sample/L were respectively 44.98 ± 1.22%. 5.317 ± 0.1190% day. 13.757 ± 0.168 % day (P< 0.05). Although there was a statistically significant difference between the density of reared larvae rearing the saddleback clownfish in the range of 1-7 sample/L. the amplitude of variation between the stocking densities at the survival rate. the growth rate. characteristic length and volume of larvae are not high. Therefore. it is possible to raise saddleback clownfish larvae at the density of ≤7 sample/L.

3.4.4. Testing artificial seed production and rearing nursery larvae

There are 4 pairs of broodstock as a result of testing different types of feed that continue to be used for artificial breeding of KCYN fishes at Duong De marine fish hatchery farm. Vinh Hoa ward. city. Nha Trang. Data on size and weight of broodstock are shown in Table 3.13

Table 3.13. Size and weight of broodstock in artificial seed production (n = 8)

Targets	TL (cm)	BW (g)
Average	12.41	48.83
Standard deviation	0.90	10.85
Max	14.12	66.07
Min	11.74	40.14

Experimental results of hatchery production at scale of hatchery production from July 2. 2017 to September 19. 2017 are shown in Table 3.14.

Table 3.14. Testing artificial seed production and rearing nursery larvae

Spawning	Time	Eggs	Fertilization rate (%)	Hatching rate (%)	Number of larvae (animals)	SR (%)	Fishes	Note
1	15/7/17	780	67.0	84.4	441	45.6	201	Pair II
2	16/7/17	650	83.8	64.0	349	60.0	209	Pair III
3	17/7/17	460	73.7	85.0	288	57.0	164	Pair IV
4	22/7/17	650	74.3	66.7	322	58.0	187	Pair I
5	8/8/17	350	81.9	75.0	215	48.0	103	Pair II
6	12/8/17	550	74.5	84.2	345	60.0	207	Pair IV
7	14/8/19	750	85.7	71.0	456	60.0	274	Pair III
8	28/8/17	560	75.0	72.6	305	58.0	177	Pair II
9	28/7/17	620	77.4	65.0	312	57.7	180	Pair I
10	17/1/00	420	93.4	65.0	255	71.0	181	Pair III
11	19/9/17	450	96.1	58.8	254	42.0	107	Pair IV
Average		567	80.2	72.0	322	56.1	181	
Standard deviation		138	8.9	9.2	74	8.1	47	
Max		780	96.1	85.0	456	71.0	274	
Min		350	67.0	58.8	215	42.0	103	
Total		**6,240**			**3,542**		**1,990**	

Used 4 pairs of saddleback clownfish fish from 11.74 to 14.12 cm length. And 40.14 to 66.07g mass. They have spawned 11 times with 6,240 eggs (average 568 eggs / nest). There were 3.542 larvae that have been hatched (average 322 larvae / nest). With a density of 3-5 fish / L. after 15 days of culture. fingerlings reach a length of 8-10 mm. Average survival rate of 56%. there were 1.990 fishes after 15 days old were produced.

CHAPTER 4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusion

The saddleback clownfish was of the hermaphrodite group. the gonad including both spermatocytes and oocytes products at the rate of 100%. There was not enough evidence to determine whether the saddleback clownfish was sex change or self-fertilization. The gonads with the fish were asynchronous and the fish spawn many times a year; The first matured size of saddleback clownfish is 6.37 cm. the maturation rate is 100% in fish ≥ 7.5 cm. group of fish smaller than 4.6 cm were not participated in spawning; The GSI index of saddleback clownfish in the year reached the highest value in April (1.05 ± 1.31). the lowest in October (0.28 ± 0.34); The main breeding season was May 3-5 and the secondary season in November 12. The encounter rate of

the most mature individuals in April, the lowest in September; The average absolute fertility of the saddleback clownfish was 823.59 ± 259.80 (eggs / females). ranging from 306-1830 (eggs / females). Relative fertility 42.91 ± 40.96 eggs / g fish; ranges from 10.42 – 316.16 eggs / g fish; The correlation between length and weight of fish and the GSI coefficients in pairs are both positively correlated. but the correlation between length and weight with R^2 = 0.867 was a stronger correlation than the correlation between length. fish weight with GSI;

2. The typical reproductive behavior of fish was to pair. nest. stick eggs. fertilize and take care of embryos during delivery to hatching larvae lasting 6-8 days.

2. Food. temperature and salinity affect reproductive efficiency of Saddleback clownfish:

3. Mixed feed NT4 (ingredients: fish meal. Cylop - eeze. moi powder. squid meal. rice flour. seaweed. Spirulina and bread yeast). Protein content ~ 50% is the best feed for feeding production of Saddleback clownfish broodstock with laying frequency 0.66 ± 0.017 times / month. number of eggs 828 ± 16 eggs / nest. fertilization rate 87.31 ± 2.892%. hatching rate 75.29 ± 1.724% ($P \leq 0.05$). Compound feed not suitable for breeding Saddleback clownfish. Can use foods for red-necked fish. nemo-neck and orange-necked cavity (ingredients: shrimp. squid. synthetic food. vitamins. algae ...) with protein content from 40- 55% of the Saddleback clownfish fed in the rearing breeding had a lower reproductive effect than the feed of marine creatures with more diverse and suitable sources ($P< 0.05$).

+ Saddleback clownfish mature and spawn at salinity 25 - 37 ‰. Salinity of 29 ‰ fish has the highest spawning frequency (0.77 ± 0.024 times / month). followed by salinity of 33. 25 and 37 ‰ ($P< 0.05$). The highest number of larvae was 29 ‰ then 33 ‰ with a value of 486 ± 19 and 435 ± 73. The hatching time at 25 mặn salinity level is the fastest ~ 6-7 days. At 29-33-salinity. Saddleback clownfish larvae hatch after ~ 7-8 days. Fish can produce 37 ‰ salinity. but the lowest reproductive efficiency ($P< 0.05$).

+ The best reproductive efficiency of fish at temperature of 27^0C. fish with spawning frequency. number of eggs. fertilization rate. hatching rate and number. larvae size are respectively 0.732 ± 0.019 times / month. . 884 ± 40 eggs / nest. 91.29 ± 1.772%. 79.76 ± 1.546%. 645 ± 41 heads. 2.83 ± 0.015 mm. There was not much difference in broodstock breeding at 24 and 300C ($P< 0.05$). At the temperature of 33^0C. the reproductive parameters are lowest ($P< 0.05$) but the hatching time is the shortest. Balancing the reproductive efficiency of fish. suitable temperature for breeding Saddleback clownfish is from 27-30^0C in which 27^0C is the best temperature ($P< 0.05$).

4. The effect of nursery of saddleback clownfish larvae in the period of 1 - 15 days old is influenced by feed. salinity and stocking density.

+ Fish larvae reared on live feeds with a combination of a variety of feeds (Rotifer. Artemia and Copepoda) for survival. growth rate of length and weight. respectively: 57.03 ± 1. 07%. 7.513 ± 0.062% day and 14.414 ± 0.200% day. Copepoda of different sizes can be used in the nursery process of Saddleback clownfish larvae however. the effect is not as good as using a combination of 3 types of live feed (P< 0.05). Artemia and Rotifer are unsuitable foods for Saddleback clownfish larvae if used individually. Could not use synthetic feed in nursery of Saddleback clownfish larvae from 1-15 days of age. fish die after 2-3 days of culture (P< 0.05)

+ Salinity of 25 - 35 ‰ is suitable for rearing of saddleback clownfish larvae. 40 ‰ salinity level is not most suitable for the growth and development of fish larvae. The larvae reached the survival rate. the growth rate of the length. the highest typical weight growth rate at 30 ‰ salinity were 58.81 ± 0.95% and 6.48 ± 0.09% / day and 16.048. respectively. ± 0.46% / day (P <0.05).

+ Saddleback clownfish larvae grow best with 1-l / l rearing density. there is not much variation in the 3 -5 / l rearing density. survival values. characteristic growth in the afternoon The length and weight of fish larvae at 3 and 5 fish / L respectively were 59.86 ± 1.43%. 5.99 ± 0.11% day. 15.492 ± 0.19% day and 62.46 ± 2.05%. Saddleback clownfish larvae can be reared at 3-5 fish / L density.

5. Used 4 pairs of saddleback clownfish fish from 11.74 to 14.12 cm length. And 40.14 to 66.07g mass. They have spawned 11 times with 6,240 eggs (average 568 eggs / nest). There were 3.542 larvae that have been hatched (average 322 larvae / nest). With a density of 3-5 fish / L. after 15 days of culture. fingerlings reach a length of 8-10 mm. Average survival rate of 56%. there were 1,990 fishes after 15 days old were produced.

4.2. Recommendations

1. There should be studies on the reproductive capacity of the parents' saddle-neck anchovies focusing on solutions to improve the nutritional quality and supplement of trace elements and at the same time take measures to overcome the phenomenon. irregular laying. loss of eggs during broodstock caring for eggs. improving egg quality and hatching rate.

2. Continuing further studies to improve the survival rate of fishes during nursing. Solutions should focus on enriching live food for Rotifer. Copepoda and Artemia nauplius. Evaluate the impact of food on a number of physiological. biochemical parameters. shock resistance of larvae. digestibility of larvae in order to improve survival. reproductive quality. growth of larvae and fish stages to form the basis for building saddleback clownfish breeding process.

KEY FINDINGS

1. Saddleback clownfish *Amphiprion polymnus* (Linnaeus, 1758) (KCYN) is a bidirectional hermaphrodites (Serial Sex-Changers) species. In gonal of fish, there are both oocytes and sperm in the same time, this is a hermaphrodite form of reproduction fish. There was no see protandrous sex ratio from male-to-female sex change as usual in among clownfishes or anemone fishes.

2. Saddleback clownfish is a spawn many times per year, asynchronously matured fish gonads. The main breeding season from March to May and the secondary breeding season in November and December. The highest rate of maturation of individuals is in May and the lowest in September. The maturity ratio of fish is highest in April and lowest in May.

3. The first matured size of Saddleback clownfish is 6.37 cm, the maturation rate is 100% in fish ≥ 7.5 cm, group of fish smaller than 4.6 cm has not participated in spawning.

4. The average absolute reproductive power of the Saddleback clownfish is 823.59 ± 259.80 (eggs/fish), ranging from 306-1830 (eggs/fish). Relative fertility 42.91 ± 40.96 eggs/g fish; ranges from 10.42 - 316.16 eggs/g fish.

5. The behavior of Saddleback clownfish is reproductive which is to pair, nest, stick eggs, fertilize and take care of embryos during delivery to hatching larvae (about 6-8 days).

6. Compound feed with ingredients: fish meal, Cylop - eze, moi powder, squid meal, rice flour, seaweed, Spirulina and bread yeast, Protein content ~ 50% is the best food for breeding. clownfish saddleback fish. Can use foods for red-necked fish, nemo-neck and orange-orange neck cavity (ingredients: shrimp, squid, synthetic feed, vitamins, algae ...) but should not use food synthetic for breeding artificial Saddleback clownfish. The salinity is suitable for artificially spawning KCYN fish farming at salinity of 27 -33 ‰ of which the best reproductive efficiency is 29 ‰. Best breeding performance of Saddleback clownfish at 27^0C.

7. Saddleback clownfish larval rearing effect in the period of 1 - 15 days old is influenced by feed, salinity and stocking density. Fish larvae are fed live foods with a combination of a variety of foods (Rotifer, Artemia and Copepoda) is best. The best salinity and density for nursing larval neck larvae to 15 days of age is 30 ‰ and 1- 3-5 fishes/L.

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LIST OF PUBLICATION AND CONFERENCE

1. Nguyen Thi Hai Thanh, Ngo Anh Tuan, Huynh Minh Sang, Nguyen Van Quang, Vo Thi Ha, 2018. Developmental stages of oocytes of Saddleback clownfish – *Amphiprion polymnus* (Linnaeus, 1758). Journal of fisheries science and technology. 3, pp. 82-88.

2. Nguyen Thi Hai Thanh, Ngo Anh Tuan, Huynh Minh Sang, Nguyen Van Quang, Vo Thi Ha, Le Thi Kieu Oanh, 2019. Embryology and early ontogeny of Saddleback clownfish – *Amphiprion polymnus* (Linnaeus, 1758) in the laboratory. Journal of fisheries science and technology. 3, pp. 57-65.