MINISTRY OF EDUCATION AND TRAINING

NHA TRANG UNIVERSITY

LE VAN LENH

RESEARCH ON THE SCIENCE OF MATURITY CULTURE AND ENHANCEMENT OF REPRODUCTION TECHNIQUE OF BUTTER CATFISH *Ompok bimaculatus* (Bloch, 1794) IN AN GIANG PROVINCE

Major: Aquaculture

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SUMMARY OF PHD THESIS

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The thesis can be found at: the National Library and the Library of Nha Trang University

INTRODUCTION

Butter catfish is an indigenous species in the Mekong Delta, however in recent years this species has become increasingly scarce. Therefore, butter catfish will be potential to develop farming in aquaculture systems in the Mekong Delta under the current condition. In Vietnam, there are only two research projects on butter catfish. The first study focused on some biological characteristics of butter catfish. The second study was on semi-artificial reproduction techniques and nursing butter catfish from fry to 60 days old. These studies were in some particular aspects and only mentioned some of biology and reproductive techniques related to butter catfish. Therefore, in order to contribute to the development of sustainable culture of butter catfish, it is necessary to complete a more comprehensive study focusing on thoroughly solving the problem of seed supply.

From practical and scientific needs, the PhD thesis **"Research on the scientific foundation of maturity culture and enhancement of reproduction technique of butter catfish *Ompok bimaculatus*, (Bloch 1794) in An Giang province"** was carried out.

General objective: To provide scientific data on maturity culture technique and supplement of scientific data on butter catfish reproduction, providing a scientific basis to contribute to improve and complete the butter catfish reproduction process and to proactively produce sufficient quantity and quality of butter catfish fingerlings to farmers and diversify freshwater fish species in the Mekong Delta.

Specific objectives: Research was to determine (1) the effect of the different feeds on some maturity parameters; (2) the type and dose of stimulants to stimulate fish reproduction; (3) to improve techniques of nursing butter catfish from fry to fingerlings (determining the feed selection index, the effect of feed combination and density, different protein content, nursing in the recirculating system affecting the growth and survival rate of butter catfish).

he scientific and practical significance of the research

Scientific significance: the research results of the thesis are scientific data for maturity culture butter catfish in captivity, fish reproduction and nursing from fry to fingerlings.

Practical significance: Successful research is to proactively culture butter catfish for reproduction, to create a source of new native fish species, which contributes to the diversification of species of freshwater aquaculture, then limit the exploitation of wild butter catfish to protect this fish's resources in the nature.

New points of the thesis

This is the first project in Vietnam and goes into depth on:

Research on some physiological characteristics of butter catfish reproduction.

Maturity culture of butter catfish with different feed in captivity.

Using steroid hormones (progesterone) for fish reproduction and artificial insemination.

Research on development of digestive tract, feed selection index and ability to tolerate some environmental factors of butter catfish.

Research on feed combinations, formulated feed with different protein contents, different densities and the effect of recirculating system on the growth and survival rate of butter catfish in the stage from fry to fingerlings.

After that, the production of butter catfish was completed.

CHAPTER 1. RESEARCH OVERVIEW

1.1. Research on stimulation of butter catfish reproduction

Sridhar et al. (1998) conducted the reproduction of butter catfish with the hormone Ovaprim with a dose of 0.5 ml /kg female, the male injection dose was equal to the female injection, after 5-6 hours of injecting hormone, fish spawn and after 24 hours, they hatched into fry. Average fertility was 4,012 ± 100 eggs /female, fertilization rate was about 75%, hatching rate was 55% - 60%.

Using sGnRH and antagonism dopamine to stimulate butter catfish to reproduce with a dose of 0.7 ml /kg body weight for female and 0.5 ml /kg male. The effect of time was 7-8 hours at a temperature of 27 ± 0.5 ^{0}C. Fertilization and hatching rates were 75 - 90% and 80 - 90% respectively. Eggs hatched at 21 ± 1 hour after fertilization and the yolk sac was completely absorbed within 48 hours. The survival of the larvae was significantly reduced after 5 days and to 10.4% after 10 days of culture (Sudhir et al., 2013).

According to Le Van Lenh (2012), experiments to stimulate semi-artificial reproduction of butter catfish with different stimulants and doses. Results showed that the effect of time was 7.5 - 9 hours. Realistic fecundity was 46 - 154 eggs /g female. The fertilization rate was 71 - 93%. The hatching rate was 83 - 90%. The survival rate after 3 days was 76 - 88%. The time to develop embryos was 22–24 hours.

1.2. Research on nursing butter catfish from fry to fingerlings

Sridhar et al. (1998) cultured Butter catfish in a glass container with a density of 100 individuals /liter, fed with egg yolks, Chironomus larvae, unitl 15 days old transfered to 1.5 m^3 cement tanks; fed with liver, chopped beef. According to Choltisak Chawpaknum (1999), when culturing butter catfish from 3 to 15 days old with three types of feed: processed feed, newly hatched moina and diluted egg yolks, the results showed that processed feed and newly hatched moina showed better survival and growth rate of egg yolks.

Choltisak Chawpaknum (2003) supported that the optimal protein nutritional requirements of butter catfish from 1-4 days old was 37.66%. In another study by the author also concluded that the optimal energy level in the 40% protein feed for the growth of butter catfish was about 466.40 and 489.50 kcal /100 g 40% protein feed. The experimental butter catfish with an average weight of 0.5 g and length of 3.9 cm were nursed in a cement tank with a density of 175 individuals/ m^3. Fish were fed commercial pellets with different protein contents ranging from 21.70% to 39.34% for 90 days. The experimental results showed that growth, survival rate, feed conversion rate, feed consumption, and protein efficiency were shown well in the treatment with protein content of 35.79% and 39.34%.

Pradhan and Debtanu Barman (2013) studied 2 day-old butter catfish larvae cultured under laboratory conditions for 12 days and at the end of the experiment period, their survival rate were 47% to 62%. Larvae in cement tanks for 30 days showed a survival rate of about 90%. These results showed that the ability to improve larval survival by providing safe feed and water quality conditions could be important.

Samir Malla and Banik (2015) conducted an experiment of 28-day culture to evaluate the survival and growth rate of butter catfish. When fish were 7 days old, they

are stocked in tanks with a volume of 30 liters with 5 different types of food (zooplankton, red worms, zooplankton + red worms, egg yolks, and commercial feed). Experiments showed that the specific growth rate (SGR) when feeding fish with zooplankton + red worms was only (4.79 ± 0.58), followed by only red worms (4.11 ± 0.52), zooplankton (3.94 ± 0.14), egg yolk (3.46 ± 0.31), and lowest growth rate observed with commercial feed (2.93 ± 0.24), the difference was statistically significant ($p < 0.05$). The highest weight gain (%) was when feeding fish with zooplankton + red worms (13.67 ± 1.5) and the lowest was commercial feed (8.13 ± 0.9). The highest average survival rate when feeding the zooplankton + red worms (66.50 ± 2.14%) and only red worms (61.75 ± 2.02%), the lowest survival rate (45.82 ± 1.03%) was for commercial feed on the 28th.

According to Le Van Lenh (2012), when conducting experiments on nursing Butter catfish from fry to 60 days old with different types of feed, the results of growth in weight and length of fish after 60 days with different types of feed (shrimps, Indian mackerel, red worms) all showed better growth than commercial feed (ranging from 1.3 to 2.1 g /individual). The lowest survival rate was commercial feed (48.9%) and the highest rate was Indian mackerel (64.9%).

CHAPTER 2. MATERIALS AND METHODS

2.1. Research location and time

2.1.1. Research location: Research on maturity culture, stimulation of reproduction, enhancement techniques of nursing butter catfish was conducted in An Giang province.

2.1.2. Research time: Research was conducted from 2017 to 2020.

2.2. Research Scheme

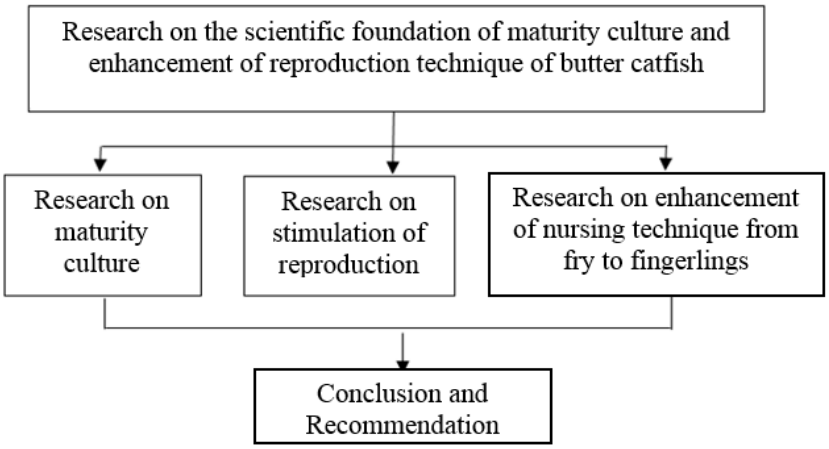


Figure 2.1: Block diagram of research contents

2.3. Materials and methods of research

2.3.1. Research on maturity culture of butter catfish

2.3.1.1. Experiment 1: Study on some reproductive physiological characteristics of Butter catfish

- Sampling method: Fish samples were collected from dry twigs put in river in An Giang province during the breeding season in the wild. A survey of 10 individuals for each stage of gonal development was conducted. Fish samples were kept alive and transported to the An Giang University's laboratory. Each individual was weighed and measured in length. After being weighed and measured, blood samples were collected

and analyzed some parameters. Fish anatomy was taken place to determine sex and the stage of gonal development (TSD).

- Analytical parameters:

+ Number of red blood cells H (106 cells / mm^3) = C x 200 x 5 x 10

C is the total number of countable erythrocytes in the 5 count regions; 200: number of dilutions; 5: to have an area of mm^2; 10: to have a volume mm^3

+ Ratio of blood cells (hematocrit), average volume of red blood cells:

Hematocrit rate (%) is determined according to the method of Larsen and Snieszko (1961) extracted in Do Thi Thanh Huong and Nguyen Van Tu (2010).

Mean RBC volume MCV (μm^3) = 10 x [HBC ratio (%) / RBC count (106 / mm^3)]

+ Muscle and liver protein were determined according to the method of Lowry et al. (1951), using Bovine serum albumin (BSA, Sigma) as a standard curve.

+ Content of vitellogenin is determined through the concentration of plasma phosphate protein.

Plasma protein phosphate = µg ALP /mL plasma : mg protein /mL plasma = µg ALP : mg protein

- Determining the TSD stages by directly observing the TSD morphology based on the 6-order scale of Xakun and Buskaia (1968) and combining with the histological template based on the method of Drury and Wallington (1967) and Kiernan (1990).

2.3.1.2. Experiment 2: Maturity culture with different types of protein in captivity

- Experimental design: The experimental system was in pond nets (3x2x2) m. Experimental fish were healthy and with relatively uniform size, weight of 40-50 g /female, caught wild. Fish feed in the experiment included small shrimp (62.4% protein) + trash fish (39% protein) and commercial feed (30% protein, 35% protein and 40% protein). The experimental arrangement was completely randomized with 4 treatments and 3 replicates. The density was 30 /m^2, the ratio of males was 1/1, the culture period was 12 months. Feeding and culturing after the fish spawns with actively 4 - 5% /weight of fish /day, the stage of maturation before spawning fish 1 month to feed 2 - 3% /weight of fish /day, feeding once a day at 17h00. Water was periodically changed 7 days /time 30%.

- Monitoring environmental parameters: Water environmental parameters including temperature, pH, DO, NH_3/NH_4^+, NO_2^- and H_2S was checked at 6:00 and 14:00, checked every 7 days. 9 males and 9 females were randomly checked for one treatment, every 1 month (30 days) including sexual maturity (maturity rate, maturity coefficient, absolute fecundity (Stage IV), egg diameter size).

2.3.2. Research on stimulation of Butter catfish reproduction

2.3.2.1. Experiment 3: Stimulating the reproduction of wild butter catfish

- Experimental design: Broodstock were caught wildly in Hau river, An Giang province. Broodstock, when captured, were cultured for 30-45 days, then selected matured fish to inject stimulants. Types of stimulants used included: LHRH-a + DOM; HCG and Progestogen (P). Females and males were injected one single dose. Injection location was the back muscle. The experiment was arranged in a completely randomized design. Natural fertilization was after injecting stimulants, fish were placed into spawning tanks with aeration, the ratio of male /female was 1/1. Artificial insemination when the female was ovulated, then striped the eggs and dissected the male sperm for artificial fertilization, the ratio of male / female was 1/1. Weys system (6 weys) made of

plastic with a volume of 7.5 liters per one was used to incubate eggs. Incubation density was 5,000 eggs /liter.

Table 2.1: Dosage and types of stimulants

Dosage and type (/kg cá)	Number of injected fish
100 µg LHRH-a + 10 mg DOM	
150 µg LHRH-a + 15 mg DOM	4 couples /dose
200 µg LHRH-a + 20 mg DOM	
2.000 UI HCG	
2.500 UI HCG	4 couples /dose
3.000 UI HCG	
10 mg P	
15 mg P	4 couples /dose
20 mg P	

- Monitoring environmental parameters: Water environmental parameters (spawning tanks and incubators) including temperature, pH, DO, NH_3/NH_4^+, NO_2^- were measured daily at 6:00 and 14:00. Reproduction indicators including effect time (hours), rate of spawning fish (%), fertilization rate (%), hatching rate (%), relative absolute fecundity (eggs /kg), egg size (mm), embryo development (hours), deformity rate (%), size of hatching fish mouth (mm).

2.3.2.2. Experiment 4: Stimulating Butter catfish reproduction from maturity culture

- Experimental design: Matured fish was selected from cultured fish (experiment 2) to stimulate reproduction. Same techniques were conducted as the experiment 3.

- Monitoring parameters: Similar to experiment 3. Comparing reproductive parameters from wild fish and cultured fish between natural fertilization and artificial insemination.

2.3.3. Research on enhancement of nursing technique of Butter catfish from fry to fingerlings

2.3.3.1. Experiment 5: Study on the developmental characteristics of digestive tract and feed selection index of butter catfish.

- Experimental system: After the yolk was absorbed, fish were transferred to nursery ponds with a small area (5x3x0.5) m, the pond bottom was covered with a layer of mud of 25 cm thick, the density of nursing was 2 fish /liter of water. Before stocking fish into the pond, water in the nursery pond was filled with plankton by dissolving concentrated powdered feed (42% protein) which was dissolved in water at a dose of 10 g /m^3 and fertilized continuously for 3 days. During nursing, 8 cloth bags containing 5 g of fish meal /m^3 /day were suspended to maintain plankton in the pond during the experiment period.

- Collecting and analyzing digestive morphological samples: Fish samples were collected on 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30 and daily collected 10 live individuals to observe and take photos of the developmental stages of the digestive tract, measure the intestinal length, body length, yolk size and mouth opening size of fish. Method of analyzing fish from 1 - 15 days old was to observe the shape of the digestive tract on a microscope to capture and measure the length. For fish from 16-30 days old, the parameters of digestive tract were seen with naked eyes and measured on a graph paper with an accuracy of 1 mm. Determination of the mouth size of fry according to Shirota (1970). Methods of histological analysis of the digestive tract according to the method of Drury and Wallington (1967) and Kiernan (1990).

- Sampling and analysis to determine feed selection index (E): Collect samples of plants, aquatic animals and fish on days 2, 3, 4, 5, 6, 8, 10, 15, 20 , 25, 30 after arranging experiments. 20 fish /sample were collected per day and stored in 10% commercial formol solution. Water samples were analyzed for qualitative phytoplankton and zooloplankton according to the classification documents of Shirota (1966), Dang Ngoc Thanh et al. (1980) and Boltovskoy (1999), Quantitative analysis by the method of Boyd and Tucker (1992). Analysis of feed in the digestive tract to determine nutritional spectra of the Butter catfish fry by the quantitative method of Biswas (1993). Feed selection index of Butter catfish E (electivity index) was according to Ivlev (1961).

2.3.3.2. Experiment 6: Study on the tolerance of Butter catfish from 1 to 30 days-old for some environmental factors

- Experimental fish: From artificially reproducted fish, fish was nursed from 1 to 30 days old and then used to determine the ability to tolerate some environmental factors (upper and lower threshold). Environmental factors included temperature, oxygen and intensity of oxygen consumption, pH, salinity for 1, 5, 10, 15, 20, 25 and 30 day-old fish.

- Determination of upper and lower lethal temperatures: In this study, the method of determining high and low temperatures causing death of Butter catfish was based on the method of Lahdes and Vainio (2003).

- Determination of threshold of oxygen and intensity of oxygen consumption: The threshold of oxygen was determined by the closed vessel method of Wokoma and Marioghae (1996). The intensity of oxygen consumption (mg O_2 /g.hour) was also determined by the closed vessel method.

- Determination of upper and lower lethal pH value: Determination of high pH and low pH causing fish death was followed by the method of Wokoma and Marioghae (1996).

- Determination of lethal salinity: Study on the effects of salinity on freshwater fish causing fish death, which is usually determined with mean lethal salinity (LC50 in ppt or ‰) according to Bringolf et al. (2005).

2.3.3.3. Experiment 7: Effects of feed combinations on the growth and survival of butter catfish at the period of 1 - 30 days old in composite tanks

- Experimental system: 0.5 m^3 composite tanks (0.35 m^3 water) were located in sheltered farm, 24/24 aeration, with nylon wire substrates, PVC pipe. Experimental fish from artificial reproduction was 1 day old, uniform size and healthy. Feed in the experiment consisted of 4 combination treatments as shown in Table 2.2

Table 2.2: Feed combinations for Butter catfish from 1 - 30 days old

Fish age (days)	Treatment of feed combinations			
	NT1	NT2	NT3	NT4
2 – 4	50% egg yolk + 50% soybean meal	Nearly hatching Artemia	Rotifers	Fripak
5 – 10	Moina	Hatching Artemia nở (biomass)	Steamed pureed trash fish	Lancy
11 – 30	50% red worms + 50% pureed trash fish	Red worms	Pureed trash fish	Commercial feed (powder and pieces)

- Experimental design: Totally randomized, 4 treatments and 4 replicates. Density was 10 individuals /liter with the experimental duration 30 days. Fish was fed on

demand, 4 times a day, after feeding on hour, excess feed was siphoned and water was supplied again as the original. Water was exchanged every two days from 30-50%.

2.3.3.4. Experiment 8: Effects of different densities on growth and survival of butter catfish at the period of 1 - 30 days old in composite tanks

- Experimental system: Similar to the experiment 7. Feed used in this experiment was the best feed combination in the experiment 7.

- Experimental design: Totally randomized, 4 treatments and 4 replicates. Density of NT1 was 10 individuals /liter, NT2 was 20 individuals /liter, NT3 was 30 individuals/ liter and NT4 was 40 individuals /liter, the experimental period was 30 days. Feeding and taking care were similar to that of the experiment 7.

2.3.3.5. Experiment 9: Effects of processed feed with different protein content on the growth and survival rate of 31 - 90 day-old butter catfish in composite tanks

- Experimental system: 0.5 m^3 composite tanks (0.35 m^3 water) were located in sheltered farm, 24/24 aeration, with PVC pipe substrates. Experimental fish were nursed to 30 days old, then relatively homogeneous and healthy fish were selected. Processed feed contained levels of 35% protein, 40% protein, 45% protein and 50% protein, and 9% lipid. Protein content in feed was analyzed by Kjeldahl method while lipid content was conducted followed the Soxhlet method at the laboratory of An Giang University.

Ingredients (g)	Processed feed			
	35% protein	40% protein	45% protein	50% protein
Fish meal (62% protein)	27.5	32.2	36.9	41.6
Bone meal (48% protein)	8.5	10	11.5	13
Soybean meal (48% protein)	22.2	26	29.8	33.6
Flour (10% protein)	36	26	16	6
Fish oil	5	5	5	5
Micronutrients	0.15	0.15	0.15	0.15
Mixed vitamins	0.15	0.15	0.15	0.15
Mono Di-Calcium phosphate (MDCP)	0.5	0.5	0.5	0.5
Total weight (g)	100	100	100	100
Analysical results				
Protein (%)	35.2	40.1	44.9	49.7
Lipid (%)	8.9	9.1	9.3	9.5

- Experimental design: Totally randomized, 4 treatments and 4 replicates. Density was 1 individual /liter, the experimental duration was 60 days. Fish was fed on demand, twice a day. Water was exchanged 30 - 50% water every two days.

2.3.3.6. Experiment 10: Effects of different densities on the growth and survival of butter catfish at the period of 31 - 90 days in composite tanks

- Experimental system: Similar to the experiment 9. Processed feed was the one with the best protein content from the the experiment 9.

- Experimental design: Totally randomized, 4 treatments and 4 replicates. Density (NT1 1 individual /liter, NT2 1.5 individual /liter, NT3 2 individuals /liter and NT4 2.5 individuals /liter), the experimental duration was 60 days. Feeding and taking cared were similar to that of the experiment 9.

2.3.3.7. Experiment 11: Effects of processed feed with different protein content on the growth and survival of butter catfish at the period of 31 - 90 days of age in the recirculating system.

- Experimental system: 0.5 m^3 composite tanks (0.35 m^3 water) were placed in sheltered farm, 24/24 aeration and with PVC pipe subtrates. Water was recirculated. Experimental fish was 30 days old, which was chosen as relatively homogeneous and was healthy fish. Processed feed contained 35% protein, 40% protein, 45% protein and 50% protein content; Lipids was 9%.

- Experimental design: Totally randomized, 4 treatments and 4 replicates. Density was 1 individual /liter, the experimental duration was 60 days. Fish was fed on demand, twice a day. One hour after feeding, waste feed was siphoned and water was refilled as before.

- Recirculating system: Water from the fish tank flowed to the sedimentation tank (10 m^3), water from the sedimentation tank flowed through a 1 µm filter bag into the storage tank (10 m^3). Water from the storage tank flowed through the 1 m^3 mechanical filter tank consisting of a 4x6 stone layer, a 2x3 stone layer, a gravel layer, a large sand layer, an activated carbon layer, a fine sand layer, a water filter cotton layer. Water from the mechanical filter tank flowed through the 1 m^3 bio-filter tank including 1/3 kaldnes material, 5 g of water treatment microorganisms (ingredients: Bacillus, Lactobacillus, Streptomyces, Saccharomyces, Nitrobacter, Nitrosomonas, ...) per day with strong aeration. Water from the bio-filter tank through the water tank was used to pump up to fish tanks again.

2.3.3.8. Experiment 12: Effects of different densities on the growth and survival of Butter fish at the period of 31 - 90 days of age in the recirculating system.

- Experimental system: Similar to the experiment 11. Processed feed was the one with the best protein content from the experiment 11.

- Experimental design: Totally randomized, 4 treatments and 4 replicates. Density (NT1 1 individual /liter, NT2 1.5 individuals /liter, NT3 2 individuals /liter and NT4 2.5 individuals /liter), the experimental duration was 60 days. Feeding and taking care were similar to that of the experiment 11.

* The monitoring parameters from the experiment 7 to 12:

Water environment: Temperature, pH, DO, NH_3/NH_4^+, NO_2^- were checked at 6:00 and 14:00 every 3 days.

Growth indicators: The weight and length of the fish. Fish samples were weighed and measured every 15 days; 30 individuals for each treatment were sampled randomly one time. Growth rate was determined by measuring total length and weight. Fish length was measured with checkerboard paper or ruler, fish weight was recorded with an electronic balance of four and two odd digits.

Coefficient of variation was determined. Feed convertion ratio (FCR) was calculated after 60 days of nursing (at the period of 31 - 90 days of age). The survival rate was determined after 30 and 90 days of nursing.

2.4. Data analysis

All data were collected, calculated average value using 2013 Excel software. At the same time, the average values were compared according to one way ANOVA analysis, comparing the mean values after analysis of variance (post hoc test) between treatments by DUNCAN test at 95% significance level ($p < 0.05$) using the SPSS 22.0 software.

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Some reproductive physiological characteristics of butter catfish

3.1.1. *Hematocrit, red blood cell number and mean red blood cell (MCV) volume according to the developmental stages of Butter catfish's gonads*

Table 3.1: Ratio of blood cells, number of red blood cells and average volume of erythrocytes in different developmental stages of butter catfish's gonads

Gonad stages	Hematocrit (%)		Number of RBC (10^6 cells /mm^3)		MCV (μm^3)	
	Female (60 samples)	Male (40 samples)	Female (60 samples)	Male (40 samples)	Female (60 samples)	Male (40 samples)
I-II	$37,76^{d}$±0,21	$25,29^{a}$±0,12	$2,09^{b}$±0,02	$2,40^{a}$±0,01	$193,08^{d}$±4,32	$105,87^{b}$±0,89
III	$35,48^{c}$±0,33	$28,93^{b}$±0,22	$2,82^{d}$±0,03	$3,36^{b}$±0,04	$125,97^{b}$±2,07	$86,12^{a}$±1,45
IV-V	$26,91^{b}$±0,27	$33,14^{c}$±0,26	$2,57^{c}$±0,02	$3,71^{c}$±0,03	$106,92^{a}$±0,77	$89,20^{a}$±0,68
VI	$16,85^{a}$±0,65		$1,21^{a}$±0,05		$139,32^{c}$±2,51	

The values shown are mean ± standard error; Mean values on the same column with identical characters have no significant differences (p> 0.05).

3.1.2. *Changes in the protein content in muscle and liver according to the gonad development stages of butter catfish*

Table 3.2: Protein content in muscle and liver at different stages of gonad development of butter catfish

Gonad stages	Protein in muscle (mg protein /g live sample)		Protein in liver (mg protein /g live sample)	
	Female (60 samples)	Male (40 samples)	Female (60 samples)	Male (40 samples)
I-II	$16,00^{d}$±0,09	$14,79^{c}$±0,11	$31,29^{d}$±0,28	$27,96^{c}$±0,06
III	$14,36^{c}$±0,21	$13,05^{b}$±0,13	$28,87^{c}$±0,15	$23,94^{b}$±0,51
IV-V	$11,98^{b}$±0,14	$11,35^{a}$±0,19	$25,97^{b}$±0,11	$19,16^{a}$±0,28
VI	$10,01^{a}$±0,13		$23,70^{a}$±0,28	

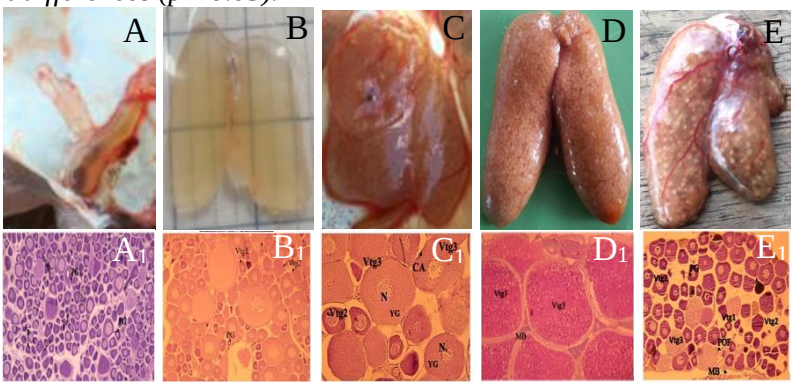
The values shown are mean ± standard error; Mean values on the same column with identical characters have no significant differences (p> 0.05).

3.1.3. Changes in plasma phosphate protein (vitellogenin) content of female butter catfish through the developmental stages of the ovaries

Table 3.3: Vitellogenin content in the stages of Butter catfish's ovary (60 samples)

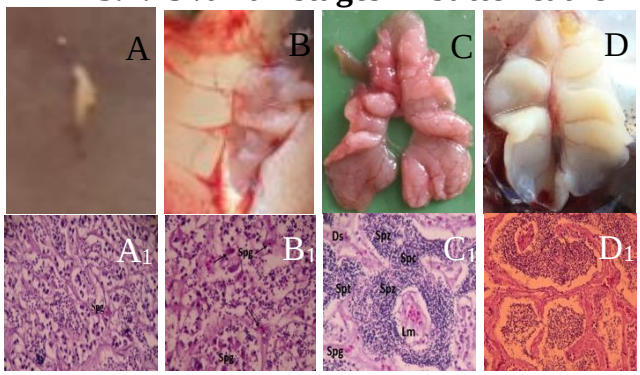
Ovary stages	Vitellogenin content (µg ALP /ml plasma)
I-II	$62,12^{b}$ ± 0,44
III	$101,59^{c}$ ± 1,02
IV-V	$121,17^{d}$± 2,70
VI	$60,78^{a}$ ± 1,07

The values shown are mean ± standard error; Mean values on the same column with identical characters have no significant differences (p> 0.05).



Note: *A, B, C, D and E are ovaries of stages I, II, III, IV and VI respectively;*
A_1, B_1, C_1, D_1 and E_1 are the ovarian histology of stages I, II, III, IV and VI respectively.

Hình 3.1: Ovarian stages in butter catfish



Note: *A, B, C and D are testine of stages I, II, III and IV respectively;*
A_1, B_1, C_1 and D_1 are histology of testine of stages I, II, III và IV respectively.

Figure 3.2: Stages of testine in butter catfish

3.2. Maturity culture of butter catfish by different types of feed with different protein contents in captivity

3.2.1. Water environmental parameters in butter catfish's pond of maturity culture

Table 3.4: Water environmental parameters in butter catfish's pond (n=10 /month)

Month	Temperature (^{0}C)	pH	DO (mg/l)	NH_3/NH_4^+ (mg/l)	NO_2^- (mg/l)	H_2S (mg/l)
01	28.5 ± 1.6	8.0 - 8.5	4.5 ± 0.3	0.03 - 0.08	no detection	no detection
02	28.6 ± 1.8	8.0 - 8.2	4.6 ± 0.3	0.03	no detection	no detection
03	28.4 ± 1.7	8.0 - 8.5	4.5 ± 0.3	0.03	no detection	no detection
04	28.5 ± 1.6	8.0 - 8.5	4.6 ± 0.2	0.03 - 0.08	no detection	no detection
05	26.9 ± 0.9	7.5 - 8.5	4.3 ± 0.2	0.009 - 0.08	no detection	no detection
06	27.1 ± 1.6	8.0 - 8.5	4.4 ± 0.2	0.03 - 0.08	no detection	no detection
07	27.8 ± 1.3	7.5 - 8.0	4.4 ± 0.2	0.009 - 0.03	no detection	no detection
08	27.1 ± 0.9	7.5 - 8.0	4.5 ± 0.2	0.009 - 0.03	no detection	no detection
09	28.6 ± 1.2	8.0 - 8.5	4.4 ± 0.2	0.03	no detection	no detection
10	27.4 ± 1.4	7.5 - 8.0	4.3 ± 0.2	0.009 - 0.03	no detection	no detection
11	29.0 ± 1.5	7.5 - 8.5	4.6 ± 0.3	0.009 - 0.03	no detection	no detection
12	28.9 ± 1.7	8.0 - 8.5	4.5 ± 0.3	0.03 ± 0.08	no detection	no detection

3.2.2. Percentage of matured butter catfish in maturity culture

Butter carfish were cultured for 12 months, the percentage of sexual maturity over the months is shown in Figure 3.3 and Figure 3.4.

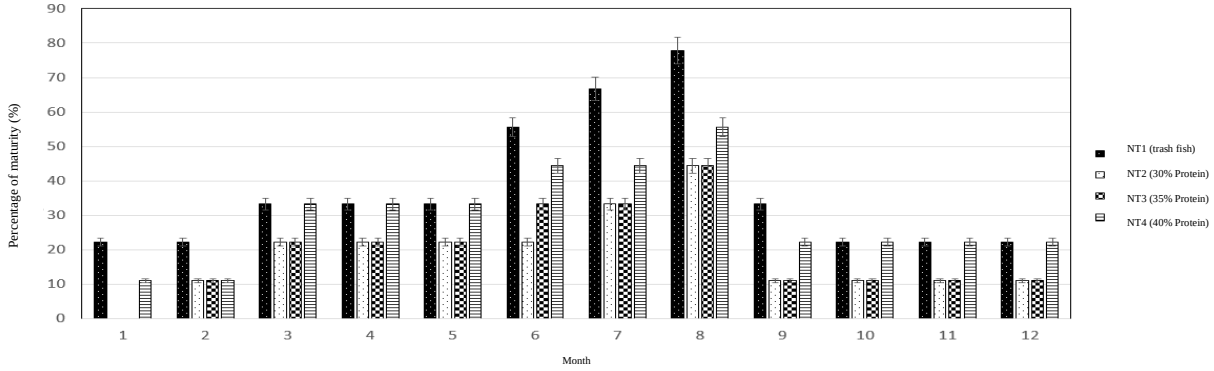


Figure 3.3: Percentage of matured female in maturity culture net (n = 9 months)

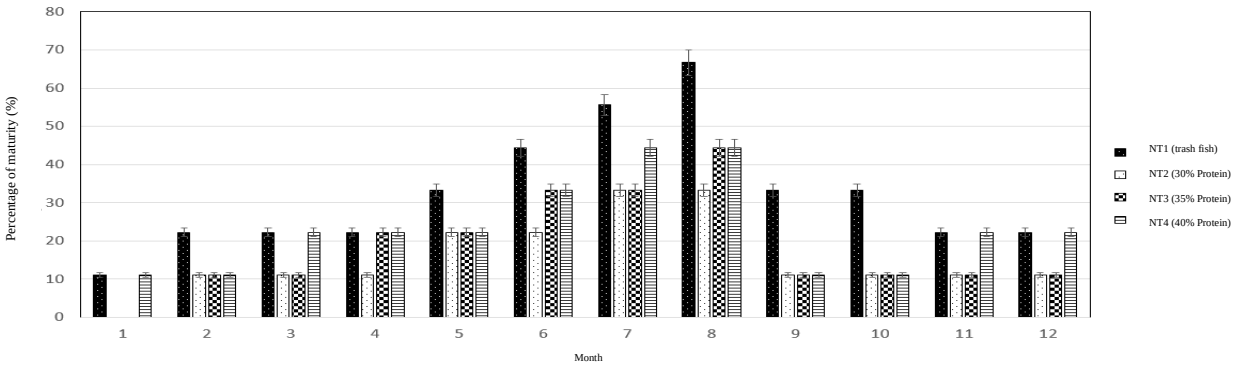


Figure 3.4: Percentage of matured male in maturity culture net (n = 9 months)

3.2.3. Gonado Somatic Index (GSI) of butter catfish during maturity culture

Table 3.5: Variation of GSI of butter catfish through months

Time (month)	Treatment	GI (%)	
		Male (n=9 /month)	Female (n=9 /month)
01	NT1 (trash fish)	$0.26^{a} \pm 0.04$	$1.33^{a} \pm 0.18$
	NT2 (30% protein)	$0.21^{a} \pm 0.02$	$1.11^{a} \pm 0.01$
	NT3 (35% protein)	$0.23^{a} \pm 0.04$	$1.16^{a} \pm 0.06$
	NT4 (40% protein)	$0.23^{a} \pm 0.00$	$1.30^{a} \pm 0.37$
02	NT1 (trash fish)	$0.39^{b} \pm 0.01$	$1.41^{a} \pm 0.53$
	NT2 (30% protein)	$0.22^{a} \pm 0.04$	$1.27^{a} \pm 0.36$
	NT3 (35% protein)	$0.23^{a} \pm 0.03$	$1.29^{a} \pm 0.40$
	NT4 (40% protein)	$0.30^{a} \pm 0.04$	$0.96^{a} \pm 0.49$
03	NT1 (trash fish)	$0.60^{a} \pm 0.07$	$2.23^{a} \pm 1.07$
	NT2 (30% protein)	$0.43^{a} \pm 0.04$	$1.81^{a} \pm 0.50$
	NT3 (35% protein)	$0.49^{a} \pm 0.03$	$1.88^{a} \pm 0.44$
	NT4 (40% protein)	$0.55^{a} \pm 0.07$	$2.04^{a} \pm 0.25$
04	NT1 (trash fish)	$0.70^{b} \pm 0.00$	$6.16^{a} \pm 0.63$
	NT2 (30% protein)	$0.53^{a} \pm 0.07$	$5.46^{a} \pm 0.16$
	NT3 (35% protein)	$0.55^{ab} \pm 0.02$	$5.52^{a} \pm 0.15$
	NT4 (40% protein)	$0.64^{ab} \pm 0.06$	$5.68^{a} \pm 0.12$
05	NT1 (trash fish)	$0.90^{c} \pm 0.05$	$6.52^{a} \pm 0.16$
	NT2 (30% protein)	$0.72^{ab} \pm 0.03$	$6.00^{a} \pm 0.36$
	NT3 (35% protein)	$0.68^{a} \pm 0.08$	$6.15^{a} \pm 0.26$
	NT4 (40% protein)	$0.85^{bc} \pm 0.03$	$6.10^{a} \pm 0.04$
06	NT1 (trash fish)	$1.03^{b} \pm 0.07$	$7.43^{a} \pm 0.29$
	NT2 (30% protein)	$0.83^{a} \pm 0.03$	$6.63^{a} \pm 0.07$
	NT3 (35% protein)	$0.84^{a} \pm 0.03$	$6.56^{a} \pm 0.36$
	NT4 (40% protein)	$0.97^{ab} \pm 0.04$	$7.08^{a} \pm 0.49$
07	NT1 (trash fish)	$1.18^{b} \pm 0.06$	$9.02^{b} \pm 0.20$
	NT2 (30% protein)	$0.89^{a} \pm 0.02$	$8.16^{a} \pm 0.18$
	NT3 (35% protein)	$0.96^{ab} \pm 0.08$	$8.14^{a} \pm 0.27$
	NT4 (40% protein)	$1.06^{ab} \pm 0.09$	$8.55^{ab} \pm 0.26$
08	NT1 (trash fish)	$1.43^{a} \pm 0.08$	$13.88^{b} \pm 0.78$
	NT2 (30% protein)	$1.19^{a} \pm 0.02$	$10.86^{a} \pm 0.64$
	NT3 (35% protein)	$1.21^{a} \pm 0.04$	$10.02^{a} \pm 0.11$
	NT4 (40% protein)	$1.32^{a} \pm 0.13$	$11.51^{ab} \pm 1.10$
09	NT1 (trash fish)	$0.82^{b} \pm 0.06$	$6.11^{a} \pm 0.49$
	NT2 (30% protein)	$0.55^{a} \pm 0.01$	$5.10^{a} \pm 0.32$
	NT3 (35% protein)	$0.55^{a} \pm 0.00$	$5.13^{a} \pm 0.29$
	NT4 (40% protein)	$0.72^{b} \pm 0.06$	$5.93^{a} \pm 0.39$
10	NT1 (trash fish)	$0.65^{b} \pm 0.03$	$5.34^{a} \pm 0.75$
	NT2 (30% protein)	$0.51^{a} \pm 0.03$	$4.23^{a} \pm 0.22$

	NT3 (35% protein)	$0.51^{a} \pm 0.04$	$4.32^{a} \pm 0.24$
	NT4 (40% protein)	$0.58^{ab} \pm 0.03$	$5.55^{a} \pm 0.70$
11	NT1 (trash fish)	$0.52^{a} \pm 0.04$	$3.39^{a} \pm 0.07$
	NT2 (30% protein)	$0.40^{a} \pm 0.07$	$2.54^{a} \pm 0.05$
	NT3 (35% protein)	$0.43^{a} \pm 0.01$	$2.72^{a} \pm 0.66$
	NT4 (40% protein)	$0.50^{a} \pm 0.10$	$3.06^{a} \pm 0.61$
12	NT1 (trash fish)	$0.32^{a} \pm 0.08$	$2.62^{a} \pm 0.52$
	NT2 (30% protein)	$0.20^{a} \pm 0.04$	$1.29^{a} \pm 0.14$
	NT3 (35% protein)	$0.22^{a} \pm 0.04$	$1.57^{a} \pm 0.61$
	NT4 (40% protein)	$0.31^{a} \pm 0.08$	$2.53^{a} \pm 0.36$

The values shown are mean ± standard error; Mean values on the same column with identical characters have no significant differences (p> 0.05).

3.2.4. Fecundity of butter catfish in maturity culture net

Table 3.6: Fecundity of butter catfish in maturity culture net put in pond

Treatment	Fecundity (n=9 /treatment)	
	Absolute fecundity (eggs /female)	Relative fecundity (eggs /kg female)
NT1 (trash fish)	$9{,}586^{b} \pm 1{,}371$	$238{,}736^{a} \pm 27{,}001$
NT2 (30% protein)	$5{,}571^{a} \pm 572$	$167{,}149^{a} \pm 16{,}919$
NT3 (35% protein)	$6{,}151^{ab} \pm 846$	$183{,}396^{a} \pm 17{,}839$
NT4 (40% protein)	$7{,}205^{ab} \pm 1{,}154$	$200{,}566^{a} \pm 24{,}523$

The values shown are mean ± standard error; Mean values on the same column with identical characters have no significant differences (p> 0.05).

3.2.5. Diameter of butter catfish's eggs in maturity culture

The average size of egg cells in stage III was 1.05 ± 0.10 mm (ranged from 0.73 to 1.25 mm) and stage IV was 1.34 ± 0.06 mm (ranged from 1.05 - 1.43 mm). The diameter of butter catfish's eggs in maturity culture tended to be larger in the wild in stage III (1.04 ± 0.10 mm) and stage IV (1.32 ± 0.12 mm) (Vo Thanh Tan, 2016). From this result, selected female, with the egg cell diameter > 1.3 mm, for reproduction can be injected with stimulants.

3.3. Reproductive stimulation of wild Butter catfish

3.3.1. Water environmental parameters

Table 3.7: Water environmental parameters in reproduction tanks and incubators

	Temperature (^{0}C)	pH	DO (mg/L)	NH_3/NH_4^+ (mg/L)	NO_2^- (mg/L)
Reproduction tanks	26 – 27.9 26.64 ± 0.47	7.5 – 7.7	5.4 – 5.7 5.54 ± 0.07	no detection	no detection
Incubators	26.5 – 26.7 26.53 ± 0.05	7.5 – 7.6	5.4 – 5.5 5.43 ± 0.05	no detection	no detection

The first row shows min. and max. The second row shows ± standard deviation

3.3.2. Reproductive stimulation of butter catfish by using LHRH-a at different doses

The weight of female was 50 - 105 g /fish (average 78.8 g /fish). The weight of male was 30-60 g /fish (average 41.9 g /fish). When using LHRH-a + DOM at three doses of 100, 150 and 200 µg /kg fish, the spawning rate was 100%, the results were shown in Table 3.8.

Table 3.8: Results of reproductive parameters of wild butter catfish using LHRH-a + DOM

Fertilization methods	Dose(µg)	Female (fish)	Effect Time (h)	Ovulation rate (%)	Reality fecundity (eggs /g)	Fertilization rate (%)	Hatching rate (%)	Survival rate of fingerlings (%)	Deformity rate (%)

Natural fertilization	100	4	8.7^{b} ± 0.2	100	114.0^{a} ± 12.7	84.5^{a} ± 5.7	87.6^{a} ± 1.3	83.1^{b} ± 2.9	10.3^{a} ± 0.7
	150	4	8.0^{ab} ± 0.3	100	132.5^{a} ± 24.1	81.4^{a} ± 6.5	84.2^{a} ± 3.2	79.5^{ab} ± 2.7	12.0^{b} ± 0.4
	200	4	7.5^{a} ± 0.3	100	159.0^{a} ± 5.4	70.8^{a} ± 2.1	81.2^{a} ± 1.7	73.2^{a} ± 1.2	13.1^{b} ± 0.3
Artificial insemination	100	4	8.5^{a} ± 0.3	100	247.3^{a} ± 6.3	47.6^{a} ± 1.5	50.4^{b} ± 2.4	84.1^{b} ± 2.2	10.2^{a} ± 0.7
	150	4	8.0^{a} ± 0.3	100	274.3^{ab} ± 8.5	45.2^{a} ± 1.7	47.6^{ab} ± 1.2	80.0^{ab} ± 1.8	11.9^{b} ± 0.3
	200	4	7.5^{a} ± 0.3	100	291.3^{b} ± 15.6	44.9^{a} ± 1.2	43.5^{a} ± 1.5	76.5^{a} ± 0.4	13.2^{b} ± 0.3

The values shown are mean ± standard error; Mean values on the same column with identical characters have no significant differences (p> 0.05) according to fertilization methods.

3.3.3. Reproductive stimulation of butter catfish by using HCG at different doses

The weight of female was 60 - 110 g /fish (average 88.1 g /fish). The weight of male was 30 - 50 g /fish (average 41.7 g /fish). When using HCG at three doses of 2,000, 2,500 and 3,000 UI /kg fish, the spawning rate was 100%, the results were shown in Table 3.9.

Table 3.9: Results of reproductive parameters of wild butter catfish using HCG

Fertilization methods	Dose(µg)	Female (fish)	Effect Time (h)	Ovulation rate (%)	Reality fecundity (eggs /g)	Fertilization rate (%)	Hatching rate (%)	Survival rate of fingerlings (%)	Deformity rate (%)
Natural fertilization	2.000	4	8.3^{a} ± 0.2	100	61.0^{a} ± 5.8	90.9^{a} ± 1.1	90.9^{b} ± 1.2	87.0^{a} ± 1.1	9.0^{b} ± 0.2
	2.500	4	8.0^{a} ± 0.3	100	66.5^{ab} ± 7.7	91.0^{a} ± 1.5	89.7^{ab} ± 0.6	87.2^{a} ± 1.6	8.3^{a} ± 0.3
	3.000	4	8.0^{a} ± 0.3	100	87.0^{b} ± 8.0	91.1^{a} ± 1.1	87.2^{a} ± 1.1	86.8^{a} ± 0.9	9.3^{b} ± 0.1
Artificial insemination	2.000	4	8.5^{a} ± 0.3	100	185.5^{a} ± 8.3	53.5^{b} ± 3.8	51.9^{b} ± 2.5	85.0^{b} ± 2.2	8.2^{a} ± 0.1
	2.500	4	8.5^{a} ± 0.3	100	197.8^{a} ± 8.2	36.8^{a} ± 1.6	48.5^{ab} ± 2.0	81.2^{ab} ± 1.0	8.7^{b} ± 0.1
	3.000	4	8.0^{a} ± 0.3	100	244.8^{b} ± 6.6	32.9^{a} ± 5.3	43.0^{a} ± 2.0	79.8^{a} ± 1.2	9.5^{c} ± 0.1

The values shown are mean ± standard error; Mean values on the same column with identical characters have no significant differences (p> 0.05) according to fertilization methods.

3.3.4. Reproductive stimulation of butter catfish by using P at different doses

The weight of female was 40-100 g /fish (average 76.6 g /fish). The weight of male was 30-50 g / fish (average 37.3 g /fish). When using P at three doses of 10, 15 and 20 mg / kg fish, the spawning rate was 100%, and the results were shown in Table 3.10.

Table 3.10: Results of reproductive parameters of wild butter catfish using P

Fertilization methods	Dose(µg)	Female (fish)	Effect Time (h)	Ovulation rate (%)	Reality fecundity (eggs /g)	Fertilization rate (%)	Hatching rate (%)	Survival rate of fingerlings (%)	Deformity rate (%)
Natural fertilization	10	4	8.7^{a} ± 0.2	100	117.5^{a} ± 7.2	89.0^{b} ± 2.0	86.9^{a} ± 2.0	86.1^{a} ± 1.5	7.5^{a} ± 0.2
	15	4	8.5^{a} ± 0.3	100	141.3^{b} ± 2.6	84.4^{a} ± 0.7	89.4^{a} ± 0.7	85.3^{a} ± 1.8	7.5^{a} ± 0.3

	20	4	$8.5^{a} \pm 0.3$	100	$142.3^{b} \pm 1.5$	$84.6^{a} \pm 1.0$	$86.4^{a} \pm 1.1$	$84.9^{a} \pm 1.3$	$8.1^{a} \pm 0.2$
Artificial insemination	10	4	$8.8^{a} \pm 0.2$	100	$232.8^{a} \pm 3.8$	$48.5^{b} \pm 1.3$	$49.4^{b} \pm 0.8$	$86.0^{b} \pm 1.3$	$7.7^{a} \pm 0.2$
	15	4	$8.5^{a} \pm 0.3$	100	$246.3^{a} \pm 6.7$	$45.9^{ab} \pm 0.9$	$47.3^{ab} \pm 1.6$	$79.0^{a} \pm 1.3$	$8.5^{b} \pm 0.2$
	20	4	$8.5^{a} \pm 0.3$	100	$262.3^{b} \pm 1.5$	$44.3^{a} \pm 0.8$	$45.4^{a} \pm 0.5$	$74.9^{a} \pm 1.3$	$9.3^{c} \pm 0.2$

The values shown are mean ± standard error; Mean values on the same column with identical characters have no significant differences (p> 0.05) according to fertilization methods.

3.3.5. Diameter of butter catfish's eggs

The size of eggs when being striped out ranged from 1.1 to 1.4 mm, average 1.35 ± 0.04 mm. After incubating for 120 minutes (swollen water), the size of eggs varied from 1.2 to 1.5 mm, average 1.41 ± 0.05 mm. This result was nearly equivalent to the diameter of butter catfish's eggs that Sridhar et al. (1998) studied in India, in which the average size was 1.3 ± 0.03 mm. Butter catfish's eggs are sinking eggs (lightly sticky).

3.3.6. The process of embryo development of butter catfish

The process of embryo development of butter catfish from fertilized eggs to hatching were shown in Table 3.11 as follows:

Table 3.11: Process of embryo development of Butter catfish

Time	Stage	Description
0 min.	Eggs fertilized	Eggs meeting sperm
After 20 min.	Germ plate	Germ plate on the yolk sac
After 40 min.	2 cells	Divided germ plate into 2 cells
After 1 hour 5 min.	4 cells	Divided germ plate into 4 cells
After 1 hour 15 min.	8 cells	Divided germ plate into 8 cells
After 1 hour 30 min.	16 cells	Divided germ plate into 16 cells
After 1 hour 45 min.	32 cells	Divided germ plate into 32 cells
After 2 hour 5 min.	Many cells	Divided germ plate into 64 cells
After 3 hour 45 min.	High blastocyst	Germ plate protruding high above the yolk sac.
After 4 hour 25 min.	Low blastocyst	Germ plate covering the yolk sac.
After 4 hour 50 min.	Early embryo	Embryo plate covering 1/3 - 1/2 the yolk sac.
After 5 hour 25 min.	Embryo	Embryo plate covering 7/8 the yolk sac.
After 6 hour 35 min.	Late embryo	Mesoderm and spinal cord separating from the inner embryo part.
After 11 hour 10 min.	Vertebral formation	Outside embryo part differientated.
After 13 hour 15 min.	Eye point formation	Two vesicles emerging on both sides of the anterior brain.
After 15 hour 25 min.	Embryo moving	The embryo moves harder, the heart beats faster and harder.
After 23 – 24 hour	Hatching	The movement of the embryo and the effect of hatching ferment, fry escapes.

Butter catfish absorbed their yolk sac for about 46 - 48 hours. The mouth size of butter catfish after absorbing the yolk sac ranged from 453 - 537 µm (average 505 ± 31 µm, equivalent to 0.5 mm).

3.4. Reproductive stimulation of butter catfish from maturity culture

3.4.1. Water environmental parameters

The water environment in spawning tanks and incubators included the temperature of 26.4 - 27.9^{0}C; pH = 7.5 - 7.8; DO = 5.4 - 5.8 mg /l; NH_3/NH_4^+ and NO_2^- not detected. The environmental parameters in this experiment were all suitable for butter catfish.

3.4.2. Reproductive stimulation of butter catfish by using LHRH-a at different doses

The weight of female was 60 - 110 g /fish (average 87.1 g /fish). The weight of male was 35-60 g /fish (average 41.3 g /fish). When using LHRH-a + DOM at three doses of 100, 150 and 200 µg /kg fish, the spawning rate was 100%, the results were shown in Table 3.12.

Table 3.12: Results of reproductive parameters of butter catfish from maturity culture using LHRH-a + DOM

Fertilization methods	Dose(µg)	Female (fish)	Effect Time (h)	Ovulation rate (%)	Reality fecundity (eggs /g)	Fertilization rate (%)	Hatching rate (%)	Survival rate of fingerlings (%)	Deformity rate (%)
Natural fertilization	100	4	$9.0^{b} \pm 0.3$	100	$128.7^{a} \pm 11.7$	$85.7^{b} \pm 2.8$	$84.1^{a} \pm 1.1$	$82.9^{b} \pm 1.9$	$9.3^{a} \pm 0.3$
	150	4	$8.0^{a} \pm 0.3$	100	$150.0^{ab} \pm 12.1$	$78.7^{ab} \pm 4.6$	$83.1^{a} \pm 2.0$	$78.5^{b} \pm 2.7$	$11.2^{b} \pm 0.3$
	200	4	$7.5^{a} \pm 0.3$	100	$174.5^{b} \pm 5.9$	$70.1^{a} \pm 1.2$	$80.3^{a} \pm 1.3$	$71.5^{a} \pm 1.6$	$12.3^{b} \pm 0.5$
Artificial insemination	100	4	$8.8^{b} \pm 0.2$	100	$255.0^{a} \pm 5.0$	$48.0^{a} \pm 1.1$	$49.6^{a} \pm 2.7$	$83.5^{b} \pm 2.6$	$10.3^{a} \pm 0.4$
	150	4	$8.0^{ab} \pm 0.3$	100	$275.3^{ab} \pm 7.2$	$46.0^{a} \pm 1.8$	$46.9^{a} \pm 0.6$	$79.3^{ab} \pm 1.3$	$11.9^{b} \pm 0.4$
	200	4	$7.5^{a} \pm 0.3$	100	$295.0^{b} \pm 14.0$	$45.1^{a} \pm 2.0$	$44.9^{a} \pm 1.4$	$77.3^{a} \pm 0.8$	$12.5^{b} \pm 0.1$

The values shown are mean ± standard error; Mean values on the same column with identical characters have no significant differences (p> 0.05) according to fertilization methods.

3.4.3. Reproductive stimulation of butter catfish by using HCG at different doses

The weight of female was 80 - 110 g /fish (average 94.2 g /fish). The weight of male was 35 - 50 g /fish (average 42.9 g /fish). When using HCG at three doses of 2,000, 2,500 and 3,000 UI /kg fish, the spawning rate was 100%, the results were shown in Table 3.13.

Table 3.13: Results of reproduction parameters of buter catfish from maturity culture using HCG

Fertilization methods	Dose (µg)	Female (fish)	Effect Time (h)	Ovulation rate (%)	Reality fecundity (eggs /g)	Fertilization rate (%)	Hatching rate (%)	Survival rate of fingerlings (%)	Deformity rate (%)
Natural fertilization	2.000	4	$8.2^{a} \pm 0.2$	100	$76.8^{a} \pm 6.3$	$89.6^{a} \pm 0.3$	$89.3^{b} \pm 0.7$	$86.4^{a} \pm 0.8$	$8.5^{a} \pm 0.2$
	2.500	4	$8.0^{a} \pm 0.3$	100	$80.5^{ab} \pm 7.4$	$87.9^{a} \pm 1.3$	$86.0^{ab} \pm 0.8$	$86.0^{a} \pm 1.8$	$8.2^{a} \pm 0.3$
	3.000	4	$8.0^{a} \pm 0.3$	100	$100.3^{b} \pm 6.6$	$88.6^{a} \pm 1.0$	$85.2^{a} \pm 1.6$	$85.9^{a} \pm 0.9$	$9.3^{b} \pm 0.1$
Artificial insemination	2.000	4	$8.5^{a} \pm 0.3$	100	$193.3^{a} \pm 8.2$	$56.9^{b} \pm 4.0$	$53.6^{b} \pm 2.0$	$84.8^{b} \pm 2.1$	$8.3^{a} \pm 0.4$
	2.500	4	$8.0^{a} \pm 0.3$	100	$200.0^{a} \pm 9.9$	$38.5^{a} \pm 1.7$	$47.6^{a} \pm 0.9$	$80.5^{ab} \pm 1.3$	$8.8^{a} \pm 0.1$
	3.000	4	$8.0^{a} \pm 0.3$	100	$254.8^{b} \pm 6.6$	$36.1^{a} \pm 3.6$	$44.4^{a} \pm 2.2$	$79.5^{a} \pm 0.9$	$9.7^{b} \pm 0.1$

The values shown are mean ± standard error; Mean values on the same column with identical characters have no significant differences (p> 0.05) according to fertilization methods.

3.4.4. Reproductive stimulation of butter catfish by using P at different doses

The weight of female was 60 - 105 g /fish (average 90.2 g /fish). The weight of male was 35 - 50 g /fish (average 41.5 g /fish). When using P at three doses of 10, 15 and 20 mg /kg fish, the spawning rate was 100%, the results were shown in Table 3.14.

Table 3.14: Results of reproductive parameters of butter catfish from maturity culture using P

Fertilization methods	Dose (µg)	Female (fish)	Effect Time (h)	Ovulation rate (%)	Reality fecundity (eggs /g)	Fertilization rate (%)	Hatching rate (%)	Survival rate of fingerlings (%)	Deformity rate (%)
Natural fertilization	10	4	$8.5^{a} \pm 0.3$	100	$129.0^{a} \pm 7.7$	$86.8^{a} \pm 1.1$	$84.1^{a} \pm 2.3$	$84.9^{a} \pm 1.3$	$7.6^{a} \pm 0.2$
	15	4	$8.0^{a} \pm 0.3$	100	$157.8^{b} \pm 4.5$	$83.2^{a} \pm 1.1$	$88.8^{a} \pm 1.1$	$83.4^{a} \pm 1.3$	$7.6^{a} \pm 0.3$
	20	4	$7.5^{a} \pm 0.3$	100	$156.5^{b} \pm 2.0$	$84.5^{a} \pm 1.3$	$86.1^{a} \pm 0.9$	$83.7^{a} \pm 1.5$	$8.2^{a} \pm 0.2$
Artificial insemination	10	4	$8.5^{b} \pm 0.3$	100	$247.5^{a} \pm 3.4$	$50.7^{b} \pm 1.3$	$53.1^{b} \pm 1.2$	$85.7^{b} \pm 1.3$	$7.8^{a} \pm 0.1$
	15	4	$7.5^{a} \pm 0.3$	100	$254.5^{a} \pm 7.6$	$45.4^{a} \pm 1.0$	$47.6^{a} \pm 1.5$	$78.3^{a} \pm 0.8$	$8.8^{b} \pm 0.2$
	20	4	$7.5^{a} \pm 0.0$	100	$271.5^{b} \pm 1.4$	$45.4^{a} \pm 0.8$	$46.9^{a} \pm 0.7$	$75.7^{a} \pm 1.0$	$9.1^{b} \pm 0.1$

The values shown are mean ± standard error; Mean values on the same column with identical characters have no significant differences (p> 0.05) according to fertilization methods.

3.5. Developmental characteristics of the digestive tract and feed selection index of butter catfish from fry to 30 days old

3.5.1. Developmental characteristics of the digestive tract

3.5.1.1. Nutritional time and size of butter catfish's yolk sac

The nutritional time of butter catfish using yolk sac in this study was 46 - 48 hours, which was supported by the study of Sudhir et al. (2013) that butter catfish completely absorbed the yolk sac in 48 hours. The mean yolk sac diameter was 0.79 ± 0.05 mm.

3.5.1.2. Mouth size of butter catfish

Table 3.15: Changes in body length and mouth size of Butter catfish

Days old	Total length (mm)	Upper jaw length (mm)	Mouth size at 90^{0} (mm)
2	3.34 ± 0.20	0.36 ± 0.02	0.50 ± 0.03
3	4.77 ± 0.56	0.42 ± 0.01	0.59 ± 0.02
4	5.37 ± 0.32	0.53 ± 0.07	0.76 ± 0.10
5	5.70 ± 0.67	0.56 ± 0.05	0.80 ± 0.08
6	6.37 ± 0.60	0.57 ± 0.07	0.81 ± 0.09
7	7.30 ± 0.82	0.62 ± 0.06	0.88 ± 0.09
8	8.20 ± 0.42	0.64 ± 0.06	0.90 ± 0.09
9	8.45 ± 0.72	0.70 ± 0.06	0.98 ± 0.09
10	9.05 ± 0.92	0.71 ± 0.09	1.00 ± 0.14
15	18.40 ± 1.83	1.40 ± 0.14	1.97 ± 0.21
20	26.10 ± 3.31	1.93 ± 0.16	2.72 ± 0.23
25	29.00 ± 3.58	2.03 ± 0.22	2.87 ± 0.32
30	34.25 ± 6.06	2.35 ± 0.40	3.32 ± 0.57

The values shown are mean ± standard deviation.

3.5.1.3. The relative gut length (RLG) of butter catfish

RLG is used to determine the diet of fish in general and butter catfish in particular, the RLG value shows the correlation between gut length and total length.

Table 3.16: The relative gut length of butter catfish from 2-30 days old

Days old	Total length (mm)	Gut length (mm)	RLG
2	3.34 ± 0.20	0.83 ± 0.24	0.249
3	4.77 ± 0.56	1.25 ± 0.23	0.262
4	5.37 ± 0.32	1.58 ± 0.09	0.294
5	5.70 ± 0.67	1.75 ± 0.25	0.307
6	6.37 ± 0.60	2.01 ± 0.24	0.315
7	7.30 ± 0.82	2.37 ± 0.17	0.324
8	8.20 ± 0.42	2.68 ± 0.14	0.327
9	8.45 ± 0.72	2.92 ± 0.24	0.345
10	9.05 ± 0.92	3.17 ± 0.23	0.350
15	18.40 ± 1.83	8.16 ± 0.92	0.443
20	26.10 ± 3.31	12.80 ± 1.98	0.490
25	29.00 ± 3.58	15.85 ± 1.98	0.546
30	34.25 ± 6.06	19.95 ± 2.42	0.582

The values shown are mean ± standard deviation.

3.5.1.4. Histological development of digestive tract

Butter catfish opened its mouth and started eating outside feed at the end of day 2 when the yolk sac was small; At this time, the digestive tract could be distinguished some components such as the oral cavity, esophagus, intestine and anus (Figure 3.5 and Figure 3.6).

Figure 3.5: Longitudinal histology of 2 day-old butter catfish (10X). *Note: (A) oral cavity, (B) esophagus, (C) intestine, (D) anus and (E) yolk sac*

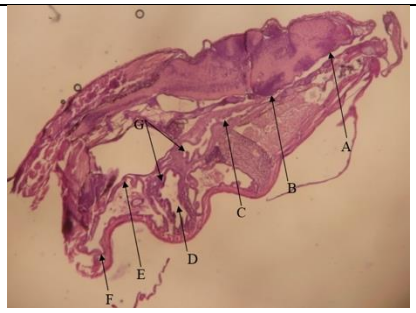


Figure 3.6: Longitudinal histology of 10 day-old butter catfish (10X). *Note: (A) oral cavity, (B) throttle, (C) esophagus, (D) stomach, (E) intestine, (F) anus và (G) folds*

a) Oral cavity

After 2 days of hatching, fish could be clearly distinguished the oral cavity from other organs in the digestive tract (Figure 3.7).

Figure 3.7: 2 day-old butter catfish's oral cavity (40X). *Note: (A) oral cavity, (B) taste buds*

b) Esophagus

Butter catfish's esophagus could be recognized when fish was 2 days old, the esophagus is a short connection between the oral cavity and the stomach. The esophagus is short and thick-walled, the muscular system intertwined with striated muscle fibers extending to the stomach, the esophagus wall forms folds and ripples. Esophagus could be distinguished by the apperance of root cells (Figure 3.8 and Figure 3.9).

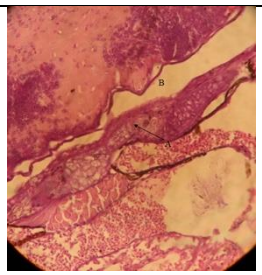


Figure 3.8: Longitudinal histology of esophagus of 3 day-old butter catfish (40X). *Note: (A) root cell, (B) esophagus cavity*

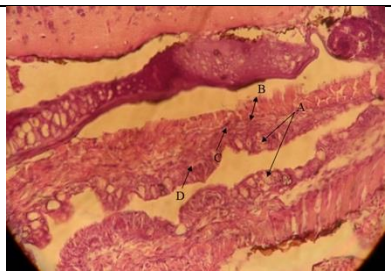


Figure 3.9: Longitudinal histology of esophagus of 15 day-old butter catfish (40X). *Note: (A) root cells, (B) muscular layer, (C) submucosa layer, (D) mucous layer*

c) Stomach

2 day-old Butter catfish started to eat outside food. At this time, the stomach has not clearly formed, just a straight tract of the digestive system. When fish is 3 to 4 days old, the stomach is the largest bulge in the digestive tract, after the esophagus and ends at the first part of the intestine. When butter catfish eats outside food, then the stomach also appears a mucous layer, high cylindrical cell layer and folds (Figure 3.10). When fish is 15 days old, the stomach structure and function are completely changed (Figure 3.11).

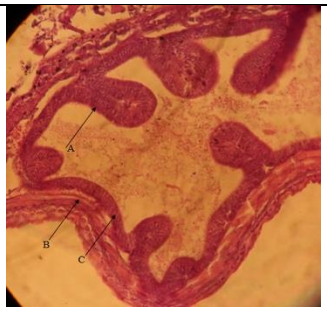


Figure 3.10: Longitudinal histology of 5 day-old butter catfish's stomach (40X). *Note: (A) folds, (B) mucous layer, (C) stomach wall*

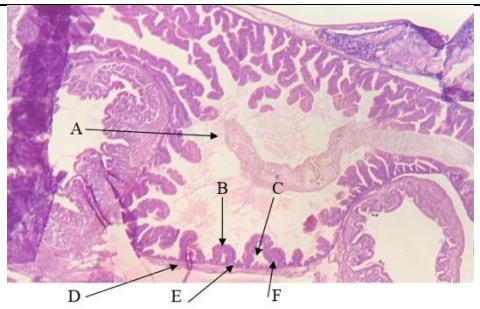


Figure 3.11: Longitudinal histology of 15 day-old butter catfish's stomach (40X). *Note: (A) stomach mucus, (B) stomach gland, (C) folds, (D) smooth muscle layer, (E) submucosa layer, (F) mucous layer*

d) Intestine

Intestine is the longest part of the digestive tract, starting from the back of the stomach and extending to the rectum and anus, its functions are to digest food and absorb nutrients to nourish the body. The 1-day-old butter catfish's intestine is a straight tube covered by a layer of microflora. When the fish is 2-3 days old, the intestine starts to have folds and the thickness of the epithelium increases with age, lipid vacuole appears when the fish is 5 days old (Figure 3.12 and Figure 3.13).

Figure 3.12: Longitudinal histology of the intestine of 5 day-old butter catfish

Figure 3.13: Longitudinal histology of 15 day-old butter catfish's intestine (40X). *Ghi chú: (A) folds, (B) intestinal*

(40X). *Note: (A) lipid vacuole, (B) intestine wall, (C) intestine cavity, (D) intestine, (E) anus*	*cells, (C) lower layer of mucosa, (D) basal layer, (E) epithelium layer, (F) mucosal layer, (G) lipid vacuole, (H) intestine wall, (I) intestinal fluids*

3.5.2. Feed selection of butter catfish

3.5.2.1. Plankton compostition in pond

a) Phytoplankton: Results of survey of phytoplankton in the nursery pond showed 4 phyla and 25 genera. The phytoplankton composition was almost stable during the experiment. The density of phytoplankton ranged from 117,172 to 1,263,636 cells /m^3.

b) Phytoplankton: There were 4 phyla and 22 genera of zooplankton in the nursery pond. The plankton composition of the zooplankton in the nursery pond was almost stable during the experiment. The density of zooplankton ranged from 33,636 to 1,430,545 individuals /m^3. They tended to decrease in density from day 1 to nearly end of the experiment. The dominant genus was Moina which was 506,364 individuals /m^3 and Brachionus 159,818 individuals /m^3.

3.5.2.2. Plankton composition in fish digestive tract

When the yolk sac was consumed, fish started to eat feed outside at the end of day 2. The live feed not found in the digestive tract was phytoplankton, Amoebozoa from day 2 to day 30. Food abundant in the digestive tract of butter catfish was zooplankton including Rotifera, Cladocera, Nauplius larvae and Copepoda, only found on day 6.

The proportion of feed ingredients in the digestive tract of butter catfish from day 2 to day 9 mostly was Rotifera, accounted for 28.23 - 60.22% and Nauplius larvae accounted for 17.52 - 70.48%. From day 10 to day 30, the Cladocera accounted for a high rate from 88.98 - 99.70%, mainly Daphnia and Moina, the Copepoda only accounted for 0.3% on day 6. This is because of the mouth size of butter catfish and the availability of food in the environment as small prey could be easier to digest.

3.5.2.3. Feed selection index (E) of butter catfish from fry to 30 days old

Table 3.17: E index of butter catfish

Zooplankton	Days old												
	2	3	4	5	6	7	8	9	10	15	20	25	30
Thermocyclops	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-	-1
Bosminopsis	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Bosmina	-1	-	-1	-	-	-1	-1	-1	-	-	-1	-0,1	-0.2
Pseudosida	-1	-	-	-1	-1	-1	-1	-1	-	-1	-1	-	-1
Macrothrix	-1	-	-1	-	-1	-	-1	-1	-0.4	-	-1	-0.1	-0.1
Daphnia	-1	-1	-0.1	-0.2	-0.2	0.10	0.14	0.33	0.61	0.61	0.45	0.50	0.40
Moina	-1	-1	-1	-1	-1	-1	0.16	0.19	0.55	0.63	0.73	0.88	0.85
Brachionus	0.49	0.55	0.46	0.42	0.41	0.29	0.36	0.29	-1	-1	-1	-1	-1
Nauplius	0.73	0.71	0.62	0.55	0.51	0.43	0.42	0.18	-1	-1	-1	-1	-1

Table 3.17 shows that butter catfish had a choice of food when the fish started to eat outside and there was a change in the development of the fish's body. In the first time of the experiment, from day 2 to day 9 live feed with small sizes such as *Brachionus sp* and Nauplius larvae were selected by fish with the index from 0.18 to 0.73. From day 10 to the end of the experiment, the fish did not choose them, which was shown through negative index. At the end of the experiment, the fish selected larger live feed such as Daphnia and Moina. Specifically, from day 7, fish started to choose Daphnia with index 0.10 - 0.61 and Moina from day 8 with index from 0.16 - 0.88. Feed selection may be related to the availability of food in the water, favorite food, feeding habits of the species, mouth size and the completeness of the digestive tract structure.

3.6. The ability to tolerate some environmental factors of butter catfish from 1 to 30 days old

3.6.1. Upper and lower lethal temperature

Table 3.18: Lethal temperature at different age of butter catfish

	Days old						
	1	5	10	15	20	25	30
Upper lethal temperature (^{0}C)	41.4±0.5	40.8±0.2	40.2±0.2	40.3±0.2	39.7±0.2	39.3±0.1	39.2±0.5
Lower lethal temperature (^{0}C)	14.3±0.5	14.0±0.5	13.8±0.2	13.7±0.5	14.0±0.5	14.3±0.5	14.2±0.3

The values shown are mean ± standard deviation.

3.6.2. Upper and lower lethal pH value

Table 3.19: Lethal pH value at different age of butter catfish

	Days old						
	1	5	10	15	20	25	30
Upper pH value	9.9±0.07	10.1±0.05	10.6±0.04	10.7±0.04	10.1±0.26	10.8±0.13	10.4±0.17
Lower pH value	3.5±0.03	3.5±0.01	3.4±0.04	3.3±0.05	3.3±0.10	3.1±0.12	3.1±0.01

The values shown are mean ± standard deviation.

3.6.3. Oxygen threshold and oxygen consumption of butter catfish

Table 3.20: Oxygen threshold (mg O_2 /L) and oxygen consumption (mg O_2 /g.hour) of butter catfish at different age

	Days old						
	1	5	10	15	20	25	30
Oxygen threshold	0.83±0.05	0.80±0.08	0.75±0.05	0.72±0.03	0.70±0.01	0.64±0.05	0.52±0.04
Oxygen consumption	2.94±0.16	2.82±0.16	2.29±0.17	1.96±0.06	1.52±0.03	0.98±0.05	0.54±0.03

The values shown are mean ± standard deviation.

3.6.4. Lethal salinity of butter catfish

Research determined that lethal salinity of butter catfish ranged from 14.3 to 16.3‰. Specifically, lethal salinity at 1 days old (14.3 ± 0.6), 5 and 10 days old (14.7 ± 0.6 ‰), 15 days old (15.0 ‰), 20 days old (15.3 ± 0.6 ‰), 25 days old (15.7 ± 0.5 ‰) and 30 days old fish (16.3 ± 0.5 ‰). Lethal salinity tended to increase with the fish's age although the variation was not large.

3.7. Effects of feed and density on the growth and survival rate of butter catfish from 1 to 30 days old in composite tanks

3.7.1. Effects of feed combinations on the growth and survival of butter catfish

3.7.1.1. Water environmental parameters

Water environment in nursery tanks included temperature 24,0 – 28,6^{0}C; pH = 7,0 – 7,8; DO = 5,4 – 6,0 mg /L; NH_3/NH_4^+ = 0,0 – 0,06 mg /L and NO_2^- = 0,0 – 0,5 mg /L. The environmental parameters in this experiment were suitable for butter catfish to grow and develop.

3.7.1.2. Weight and length gain

Table 3.21: Weight and length gain after 30 nursing days by different feed combinations

Time	Factors	NT1 (LĐT)	NT2 (Ar)	NT3 (L)	NT4 (Fri)
Initial weight	W (g)	$0.0005^{a} \pm 0.0$	$0.0005^{a} \pm 0.0$	$0.0005^{a} \pm 0.0$	$0.0005^{a} \pm 0.0$
Final weight	W_{30} (g)	$0.2539^{c} \pm 0.0111$	$0.4066^{d} \pm 0.0047$	$0.1028^{b} \pm 0.0028$	$0.0586^{a} \pm 0.0169$
	DWG (g/day)	$0.0084^{c} \pm 0.0004$	$0.0135^{d} \pm 0.0002$	$0.0034^{b} \pm 0.0001$	$0.0020^{a} \pm 0.0006$
	SGR_W (%/day)	$20.74^{c} \pm 0.1748$	$22.35^{d} \pm 0.0400$	$17.75^{b} \pm 0.0831$	$15.32^{a} \pm 0.5340$
Initial length	L (mm)	$2.2833^{a} \pm 0.0333$	$2.2500^{a} \pm 0.0500$	$2.2833^{a} \pm 0.0441$	$2.2667^{a} \pm 0.0167$
Final length	L_{30} (mm)	$32.50^{c} \pm 0.3215$	$38.72^{d} \pm 0.5600$	$22.97^{b} \pm 0.1692$	$20.82^{a} \pm 0.1878$
	DLG (mm/ngày)	$1.0072^{c} \pm 0.0099$	$1.2155^{d} \pm 0.0203$	$0.6894^{b} \pm 0.0048$	$0.6183^{a} \pm 0.0067$
	SGR_L (%/day)	$8.86^{c} \pm 0.0327$	$9.50^{d} \pm 0.1258$	$7.71^{b} \pm 0.0471$	$7.41^{a} \pm 0.0530$

The values shown are mean ± standard error; Mean values on the same row with identical characters have no significant differences (p> 0.05).

3.7.1.3. Coefficient of variation and the survival rate

Table 3.22: Coefficient of variation and the survival rate of butter catfish after 30 nursing days by different feed combinations

Factors	Time	NT1 (LĐT)	NT2 (Ar)	NT3 (L)	NT4 (Fri)
CV_W (%)	01 day	0	0	0	0
	30 days	7.60	1.99	4.77	5.0
CV_L (%)	01 day	2.52	3.85	3.34	3.27
	30 days	1.71	1.51	1.28	1.56
Survival rate (%)	30 days	$18.50^{b} \pm 1.27$	$37.50^{d} \pm 1.79$	$23.10^{c} \pm 0.56$	$12.18^{a} \pm 0.86$

The values shown are mean ± standard error; Mean values on the same row with identical characters have no significant differences (p> 0.05).

The results of this study could conclude when using live feed in combination with red worms or trash fish for nursing fish to 30 days old for the best growth and survival rate.

3.7.2. Effect of density on the growth and survival rate of butter catfish

3.7.2.1. Water environmental parameters

Water environment in nursery tanks from 1 – 30 days old with different density included temperature 25,1 – 28,6^{0}C; pH = 7,0 – 8,0; DO = 4,2 – 5,1 mg /L; NH_3/NH_4^+ = 0,0 – 0,09 mg /L and NO_2^- = 0,0 – 0,5 mg /L. The environmental parameters in this experiment were suitable for butter catfish to grow and develop.

3.7.2.2. Weight and length gain

Table 3.23: Weight and length gain after 30 nursing days by different densities

Time	Factors	NT1 (10)	NT2 (20)	NT3 (30)	NT4 (40)
Initial weight	W (g)	$0.0004^{a} \pm 0.0$	$0.0005^{a} \pm 0.0$	$0.0004^{a} \pm 0.0$	$0.0004^{a} \pm 0.0$
Final weight	W_{30} (g)	$0.3053^{a} \pm 0.0038$	$0.3606^{b} \pm 0.0165$	$0.5274^{c} \pm 0.0099$	$0.3910^{b} \pm 0.0065$
	DWG (g/day)	$0.0102^{a} \pm 0.0001$	$0.0120^{b} \pm 0.0006$	$0.0176^{c} \pm 0.0003$	$0.0130^{b} \pm 0.0002$
	SGR_W (%/day)	$21.84^{a} \pm 0.0544$	$22.29^{b} \pm 0.0923$	$23.82^{d} \pm 0.0548$	$22.93^{c} \pm 0.1139$
Initial length	L (mm)	$2.4333^{a} \pm 0.0167$	$2.3600^{a} \pm 0.0100$	$2.4000^{a} \pm 0.0289$	$2.3667^{a} \pm 0.0441$

Final length	L_{30} (mm)	$35.33^{a} \pm 0.2906$	$37.17^{b} \pm 0.3371$	$42.58^{d} \pm 0.4475$	$39.13^{c} \pm 0.1481$
	DLG (mm/day)	$1.0968^{a} \pm 0.0091$	$1.1605^{b} \pm 0.0111$	$1.3391^{d} \pm 0.0154$	$1.2260^{c} \pm 0.0046$
	SGR_L (%/day)	$8.93^{a} \pm 0.0080$	$9.21^{b} \pm 0.0233$	$9.58^{d} \pm 0.0685$	$9.38^{c} \pm 0.0586$

The values shown are mean ± standard error; Mean values on the same row with identical characters have no significant differences (p> 0.05).

3.7.2.3. Coefficient of variation and the survival rate

Table 3.24: Coefficient of variation and the survival rate of butter catfish after 30 nursing days at different densities

Factors	Thời gian	NT1 (10)	NT2 (20)	NT3 (30)	NT4 (40)
CV_W (%)	01 day	0	0	0	0
	30 days	0.02	7.93	3.26	2.86
CV_L (%)	01 day	1.19	0.73	2.08	3.23
	30 days	1.42	1.57	1.82	0.66
Survival rate (%)	30 days	$41.50^{c} \pm 2.03$	$32.28^{b} \pm 1.43$	$30.10^{b} \pm 0.75$	$25.50^{a} \pm 0.67$

The values shown are mean ± standard error; Mean values on the same row with identical characters have no significant differences (p> 0.05).

Butter catfish nursing from 1 - 30 days at different densities resulted in the highest growth rate at 30 individuals /liter, the lowest one at 10 individuals /liter and the highest survival rate at 10 individuals /liter, the lowest one at 40 individuals /liter. Therefore, nursing butter catfish from fry to 30 days old with a density of 20-30 individuals /liter would be appropriate.

3.8. Effects of processed feed with different protein contents and densities on the growth and survival rate of butter catfish in the period of 31 - 90 days old in composite tanks

3.8.1. Effects of processed feed with different protein contents on the growth and survival of butter catfish

3.8.1.1. Water environmental parameters

Table 3.25: Water environmental parameters in composite nursery tanks using processed feed with different protein contents

Parameters	NT1 (35%)	NT2 (40%)	NT3 (45%)	NT4 (50%)
Temperature (^{0}C)	24 – 27.5 25.9 ± 1.2	24 – 29 26 ± 1.4	24 – 28 25.9 ± 1.3	24 – 28 25.8 ± 1.2
pH	7 – 7.5	7 – 7.5	7 – 7.5	7 – 7.5
DO (mg/L)	5 – 6 5.5 ± 0.5	5 – 6 5.4 ± 0.5	5 – 6 5.6 ± 0.5	5 – 6 5.3 ± 0.5
NH_3/NH_4^+ (mg/L)	0 – 0.009 0.007 ± 0.0	0 – 0.009 0.007 ± 0.0	0 – 0.009 0.006 ± 0.0	0 – 0.009 0.006 ± 0.0
$N\text{-}NO_2^-$ (mg/L)	0 – 2 1.2 ± 0.7	0 – 2 1.3 ± 0.7	0 – 2 1.6 ± 0.7	0 – 2 1.6 ± 0.7

The first row shows min. and max. The second row shows ± standard deviation (n = 160 samples /parameters)

All water environmental parameters were suitble for fish growth because the experiment was arranged in sheltered farm and in tanks with full aeration.

3.8.1.2. Weight and length gain

Table 3.26: Weight and length gain after 60 nursing days using processed feed with different protein contents

Time	Factors	NT1 (35%)	NT2 (40%)	NT3 (45%)	NT4 (50%)
Initial weight	W (g)	$0.30^{a} \pm 0.01$	$0.31^{a} \pm 0.01$	$0.32^{a} \pm 0.01$	$0.32^{a} \pm 0.01$

Final weight	W_{60} (g)	$4.36^{a} \pm 0.52$	$4.93^{ab} \pm 0.51$	$5.18^{ab} \pm 0.20$	$5.77^{b} \pm 0.18$
	DWG (g/day)	$0.07^{a} \pm 0.01$	$0.07^{a} \pm 0.01$	$0.08^{a} \pm 0.0$	$0.09^{a} \pm 0.0$
	SGR_W (%/day)	$4.37^{a} \pm 0.16$	$4.57^{ab} \pm 0.12$	$4.61^{ab} \pm 0.06$	$4.78^{b} \pm 0.07$
Initial length	L (mm)	$35.28^{a} \pm 0.30$	$35.40^{a} \pm 0.43$	$35.33^{a} \pm 0.84$	$35.11^{a} \pm 0.39$
Final length	L_{60} (mm)	$83.82^{a} \pm 4.51$	$88.30^{ab} \pm 3.00$	$88.97^{ab} \pm 0.67$	$94.97^{b} \pm 0.43$
	DLG (mm/day)	$0.81^{a} \pm 0.08$	$0.88^{ab} \pm 0.05$	$0.89^{ab} \pm 0.0$	$1.0^{b} \pm 0.01$
	SGR_L (%/day)	$1.43^{a} \pm 0.11$	$1.52^{ab} \pm 0.06$	$1.54^{ab} \pm 0.02$	$1.66^{b} \pm 0.01$

The values shown are mean ± standard error; Mean values on the same row with identical characters have no significant differences (p> 0.05).

3.8.1.3. Coefficient of variation, feed conversion rate and survival rate

Table 3.27: Coefficient of variation, feed conversion rate and survival rate of butter catfish after 60 nursing days using processed feed with different protein contents

Factors	Time	NT1 (35%)	NT2 (40%)	NT3 (45%)	NT4 (50%)
CV_W (%)	01 day	6.67	6.45	6.25	6.38
	60 days	20.64	18.05	6.74	5.55
CV_L (%)	01 day	1.50	2.09	2.10	1.94
	60 days	9.32	5.89	1.30	0.79
FCR	60 day	$1.60^{c} \pm 0.06$	$1.31^{b} \pm 0.02$	$1.23^{b} \pm 0.01$	$1.12^{a} \pm 0.02$
Survival rate (%)	60 days	$39.18^{a} \pm 0.86$	$39.23^{a} \pm 0.48$	$50.05^{b} \pm 0.41$	$52.15^{b} \pm 1.27$

The values shown are mean ± standard error; Mean values on the same row with identical characters have no significant differences (p> 0.05).

When nursing butter catfish from 31 - 90 days old in composite tanks using processed feed with 4 protein levels: 35%, 40%, 45% and 50%, the 45% and 50% protein resulted in more effective growth, coefficient of variation, feed conversion rate and survival rate than the other two levels of 35% and 40% protein. However, there was no significant difference at 2 levels of protein content 45% and 50% (p> 0.05) so it would be appropriate to choose processed feed with 45% protein content for butter catfish nursing.

3.8.2. Effects of different density on growth and survival rate of butter catfish in the period of 31 - 90 days old

3.8.2.1. Water environmental parameters

Water environment in nursery tanks included temperature ranging from 22,0 – $29,0^{0}$C, pH 7,0 – 7,5, DO 5,0 – 6,0 mg /L, NH_3/NH_4^+ 0,0 – 0,09 mg /L and $N\text{-}NO_2^-$ 0,0 – 2,0 mg /L. All parameters were stable and suitable for fish growth.

3.8.2.2. Weight and length gain

Table 3.28: Weight and length gain after 60 nursing days at different densities

Time	Factors	NT1 (1)	NT2 (1,5)	NT3 (2)	NT4 (2,5)
Initial weight	W (g)	$0.32^{a} \pm 0.0$	$0.32^{a} \pm 0.01$	$0.32^{a} \pm 0.0$	$0.33^{a} \pm 0.0$
Final weight	W_{60} (g)	$4.44^{a} \pm 0.17$	$5.20^{ab} \pm 0.47$	$5.66^{b} \pm 0.20$	$5.84^{b} \pm 0.05$
	DWG (g/day)	$0.07^{a} \pm 0.0$	$0.08^{ab} \pm 0.01$	$0.09^{b} \pm 0.01$	$0.09^{b} \pm 0.0$
	SGR_W (%/day)	$4.33^{a} \pm 0.06$	$4.64^{b} \pm 0.12$	$4.77^{b} \pm 0.06$	$4.80^{b} \pm 0.01$
Initial length	L (mm)	$35.23^{a} \pm 0.41$	$35.18^{a} \pm 0.27$	$35.25^{a} \pm 0.15$	$35.18^{a} \pm 0.55$
Final length	L_{60} (mm)	$80.10^{a} \pm 1.21$	$85.73^{ab} \pm 2.50$	$91.57^{bc} \pm 2.31$	$92.15^{c} \pm 0.38$
	DLG (mm/day)	$0.75^{a} \pm 0.02$	$0.84^{ab} \pm 0.05$	$0.94^{b} \pm 0.04$	$0.95^{b} \pm 0.01$
	SGR_L (%/day)	$1.37^{a} \pm 0.03$	$1.48^{ab} \pm 0.06$	$1.59^{b} \pm 0.04$	$1.61^{b} \pm 0.02$

The values shown are mean ± standard error; Mean values on the same row with identical characters have no significant differences (p> 0.05).

3.8.2.3. Coefficient of variation, feed conversion rate and survival rate

Table 3.29: Coefficient of variation, feed conversion rate and survival rate of butter catfish after 60 nursing days at different densities

Factors	Time	NT1 (1)	NT2 (1,5)	NT3 (2)	NT4 (2,5)
CV_W (%)	01 day	3.13	6.25	3.13	3.03
	60 days	6.53	15.58	6.01	1.37
CV_L (%)	01 day	2.04	1.31	0.74	2.73
	60 days	2.62	5.06	4.37	0.71
FCR	60 day	$1.07^a \pm 0.03$	$1.17^b \pm 0.03$	$1.36^c \pm 0.02$	$1.47^d \pm 0.02$
Survival rate (%)	60 days	$49.2^d \pm 1.15$	$46.4^c \pm 0.88$	$40.5^b \pm 0.30$	$23.8^a \pm 0.20$

The values shown are mean ± standard error; Mean values on the same row with identical characters have no significant differences (p> 0.05).

Results showed that the highest growth rate at a density of 1.5-2.5 individuals /L. The lowest FCR was at 1.0 individual /L and the highest was at 2.5 individuals /L. The highest survival rate was found at 1.0 individual /L and the lowest at 2.5 individuals /L. Therefore, the density of 1.5 - 2.0 individuals /L would be the most suitable.

3.9. Effects of formulated feed with different protein contents and different densities on the growth and survival rate of butter catfish in the period of 31 - 90 days old in the recirculating system

3.9.1. Effects of formulated feed with different protein contents on the growth and survival rate of butter catfish in the recirculating system

3.9.1.1. Water environmental parameters

All water environmental parameters were shown in Table 3.30 and all suitable for fish growth.

Table 3.30: Water environmental parameters in recirculating system

Parameters	NT1 (35%)	NT2 (40%)	NT3 (45%)	NT4 (50%)
Temperature (^{0}C)	24 – 29 26.5 ± 1.4	24 – 29 26.9 ± 1.4	24 – 29 26.9 ± 1.4	24 – 29 26.9 ± 1.4
pH	7.5 – 8.0	7.5 – 8.0	7.5 – 8.0	7.5 – 8.0
DO (mg /L)	5.7 – 6.2 5.97 ± 0.1	5.8 – 6.2 5.97 ± 0.1	5.7 – 6.2 5.95 ± 0.1	5.7 – 6.2 5.97 ± 0.1
NH_3/NH_4^+ (mg /L)	0 – 0.09	0 – 0.09	0 – 0.09	0 – 0.09
$N\text{-}NO_2^-$ (mg /L)	0 – 2 1.0 ± 0.7	0 – 2 1.0 ± 0.7	0 – 2 1.0 ± 0.7	0 – 2 1.0 ± 0.7

The first row shows min. and max. The second row shows ± standard deviation (n = 160 samples /parameters)

3.9.1.2. Weight and length gain

Table 3.31: Growth rate of butter catfish in recirculating system

Time	Factors	NT1 (35%)	NT2 (40%)	NT3 (45%)	NT4 (50%)
Initial weight	W (g)	$0.27^a \pm 0.00$	$0.28^a \pm 0.00$	$0.28^a \pm 0.01$	$0.28^a \pm 0.01$
Final weight	W_{60} (g)	$4.49^a \pm 0.25$	$4.75^a \pm 0.21$	$5.66^b \pm 0.25$	$5.82^b \pm 0.29$
	DWG (g/day)	$0.07^a \pm 0.01$	$0.07^a \pm 0.00$	$0.09^b \pm 0.01$	$0.09^b \pm 0.01$
	SGR_W (%/day)	$4.65^a \pm 0.10$	$4.67^a \pm 0.05$	$5.04^b \pm 0.07$	$5.02^b \pm 0.11$
Initial length	L (mm)	$35.57^a \pm 1.09$	$34.87^a \pm 0.60$	$34.97^a \pm 0.41$	$35.01^a \pm 0.30$
Final length	L_{60} (mm)	$85.02^a \pm 0.33$	$88.35^{ab} \pm 1.65$	$91.32^b \pm 1.65$	$91.48^b \pm 1.26$
	DLG (mm/day)	$0.85^a \pm 0.01$	$0.89^{ab} \pm 0.03$	$0.94^b \pm 0.02$	$0.94^b \pm 0.02$
	SGR_L (%/day)	$1.51^a \pm 0.01$	$1.47^a \pm 0.08$	$1.60^a \pm 0.02$	$1.60^a \pm 0.02$

The values shown are mean ± standard error; Mean values on the same row with identical characters have no significant differences (p> 0.05).

3.9.1.3. Coefficient of variation, feed conversion rate and survival rate

Coefficient of variation, feed conversion rate and survival rate of butter catfish after 60 nursing days in a recirculating system using processed feed were showed in Table 3.32.

Table 3.32: Coefficient of variation, feed conversion rate and survival rate of butter catfish after 60 nursing days in a recirculating system using processed feed with different protein contents

Factors	Time	NT1 (35%)	NT2 (40%)	NT3 (45%)	NT4 (50%)
CV_W (%)	01 day	0	0	7.14	7.14
	60 days	9.56	7.58	7.77	8.67
CV_L (%)	01 day	5.34	2.98	2.06	1.46
	60 days	0.68	3.24	3.12	2.37
FCR	60 day	$1.48^{b} \pm 0.06$	$1.43^{b} \pm 0.04$	$1.11^{a} \pm 0.04$	$1.03^{a} \pm 0.01$
Survival rate (%)	60 days	$45.72^{a} \pm 1.30$	$45.07^{a} \pm 1.05$	$47.36^{ab} \pm 1.47$	$50.29^{b} \pm 0.71$

The values shown are mean ± standard error; Mean values on the same row with identical characters have no significant differences (p> 0.05).

The highest growth rate was found at protein content of 45% and 50%. The low FCR was found at protein content of 45% and 50%. Survival rate was high at 45% and 50% protein but there was no difference (p> 0.05) at these 2 levels of protein. Therefore, it would be appropriate to choose processed feed with a protein content of 45% for butter catfish nursing to fingerlings.

3.9.2. Effects of processed feed with different densities on the growth and survival rate of butter catfish in a recirculating system

3.9.2.1. Water environmental parameters

Water environmental parameters in a recirculating system included temperature ranging from 24,0 – 29,0^{0}C, pH 7,5 – 8,0, DO 5,7 – 6,0 mg /L, NH_3/NH_4^+ 0,0 – 0,09 mg /L and N-NO_2^- 0,0 – 2,0 mg /L. All parameters were stable and suitable for fish growth

3.9.2.2. Weight and length gain

Table 3.33: Growth rate of butter catfish in a recirculating system by different densities

Time	Factors	NT1 (1)	NT2 (1,5)	NT3 (2)	NT4 (2,5)
Initial weight	W (g)	$0.27^{a} \pm 0.0$	$0.28^{a} \pm 0.0$	$0.27^{a} \pm 0.0$	$0.28^{a} \pm 0.0$
Final weight	W_{60} (g)	$4.61^{a} \pm 0.11$	$5.39^{ab} \pm 0.23$	$6.00^{bc} \pm 0.21$	$6.39^{c} \pm 0.37$
	DWG (g/day)	$0.07^{a} \pm 0.00$	$0.08^{b} \pm 0.00$	$0.10^{c} \pm 0.00$	$0.10^{c} \pm 0.01$
	SGR_W (%/day)	$4.69^{a} \pm 0.05$	$4.90^{ab} \pm 0.09$	$5.11^{bc} \pm 0.07$	$5.18^{c} \pm 0.09$
Initial length	L (mm)	$35.96^{a} \pm 0.07$	$36.16^{a} \pm 0.07$	$35.98^{a} \pm 0.07$	$35.95^{a} \pm 0.10$
Final length	L_{60} (mm)	$87.00^{a} \pm 0.73$	$91.80^{ab} \pm 2.35$	$91.92^{ab} \pm 1.35$	$95.58^{b} \pm 1.98$
	DLG (mm/day)	$0.85^{a} \pm 0.01$	$0.90^{a} \pm 0.07$	$0.89^{a} \pm 0.06$	$0.99^{a} \pm 0.03$
	SGR_L (%/day)	$1.47^{a} \pm 0.02$	$1.55^{ab} \pm 0.04$	$1.55^{ab} \pm 0.06$	$1.63^{b} \pm 0.04$

The values shown are mean ± standard error; Mean values on the same row with identical characters have no significant differences (p> 0.05).

3.9.2.3. Coefficient of variation, feed conversion rate and survival rate

Bảng 3.34: Coefficient of variation, feed conversion rate and survival rate of butter catfish in a recirculating system by different densities

Factors	Time	NT1 (1)	NT2 (1,5)	NT3 (2)	NT4 (2,5)
CV_W (%)	01 day	3.70	3.57	3.70	0.0
	60 days	4.34	7.24	6.00	7.19
CV_L (%)	01 day	0.36	0.36	0.36	0.50
	60 days	1.46	4.44	2.55	3.59
FCR	60 day	$1.08^{a} \pm 0.02$	$1.17^{b} \pm 0.02$	$1.25^{c} \pm 0.01$	$1.42^{d} \pm 0.03$
Survival rate (%)	60 days	$51.65^{d} \pm 0.74$	$47.68^{c} \pm 0.88$	$44.25^{b} \pm 0.27$	$25.88^{a} \pm 0.37$

The values shown are mean ± standard error; Mean values on the same row with identical characters have no significant differences (p> 0.05).

Nursing butter catfish from 31 - 90 days old in the recirculating system at different densities from 1.0 to 2.5 individuals /L showed the growth in weight ranging from 4.61 to 6.39 g /individual. The length growth ranged from 87.00 to 95.58 mm /individual. FCR ranged from 1.08 to 1.42 and there were differences between treatments ($p < 0.05$). Survival rates ranged from 25.88 to 51.65%. High density showed low survival rate and differed ($p < 0.05$) among the 4 treatments. Therefore, the density of 1.5 - 2.0 individuals /L for butter catfish nursing in the recirculating system from fry to fingerlings would be appropriate.

CHAPTER 4. CONCLUSION AND RECOMMENDATION

4.1. Conclusion

Female butter catfish had a hematocrit (16.85 - 37.76%), the number of red blood cells (1.21 x 106 - 2.10 x 10^6 cells / mm^3), the mean volume of red blood cells (106.92 – 193.13 μm^3), muscle protein (10.01 - 16.00 mg protein /g fresh sample) and liver (23.70 - 31.29 mg protein /g fresh sample), vitellogenin content (60,78 - 121.17 µg ALP /ml plasma). Male butter catfish had a hematocrit (25.29 - 33.14%), the number of red blood cells (2.40 x 106 - 3.71 x 10^6 cells / mm^3), the mean volumn of red blood cells (86.12 - 105.87 μm^3), muscle protein (11.35 - 14.79 mg protein /g fresh sample) and liver (19.16 - 27.96 mg protein /g fresh sample).

Butter catfish are matured in ponds all year round, reaching the highest maturity rate in August of 77.8% (female) and 66.7% (male). Maturity rate peaked in August (13.88% female and 1.43% male). The relative fecundity ranged from 167,149 to 238,736 eggs /kg female.

As for butter catfish matured from the wild or culture, three stimulants for butter catfish, LHRH-a, HCG and P, stimulated fish to ovulate and reproduce. For natural fertiliztion, the effect time was 7.5 - 9.0 hours, fecundity was 61 - 1995 eggs /g female, fertilization rate was 70.8 - 91.1%, hatching rate wa 81.2 - 90.9%, survival rate of fish with no yolk sac was from 73.2 - 87.2%, deformity rate was 7.5 - 13.1% and the embryoc development time was 23 - 24 hours. For artificial insemination, the effect time was 7.5 - 9.0 hours, fecundity was 185 - 291 eggs /g female, fertilization rate was 32.9 - 53.5%, hatching rate was 43.0 - 51.9%, survival rate of fish with no yolk sac was from 74.9 - 86.0%, deformity rate was from 7.7 to 13.2%. The reproductive parameters of butter catfish from maturity culture were equivalent to those of the wild butter catfish.

After 2 days, it is possible to distinguish different organs in the digestive tract such as oral cavity, esophagus, stomach, intestines and anus. Butter catfish's digestive tract fully developed as adult fish at the 15th days old. From 2 to 30 days old, butter catfish only chose to eat zooplankton.

Butter catfish from 1 - 30 days old had the upper lethal temperature ranging from 39.2 to 41.4^{0}C and the lower lethal temperature between 13.7 and 14.3^{0}C. The high lethal pH value ranged from 9.9 to 10.8 and the lower ranged from 3.1 to 3.5. The oxygen threshold of butter catfish fluctuated from 0.52 to 0.83 mg O_2 /L and oxygen consumption was in the range of 0.54 to 2.94 mg O_2 / g.hour. Lethal salinity of butter catfish ranged from 14.3 to 16.3‰.

Zooplankton in combination with red worms or trash fish resulted in the best growth and survival rates of butter catfish when nursing from fry to 30 days old. The

best growth rate was found at a density of 30 individuals /liter, the lowest at 10 individuals/ liter and the highest survival rate was observed at 10 individuals /liter, while the lowest was at 40 individuals /liter during the period of 1 - 30 days old nursing at different densities.

Processed feed at protein content of 45% was the most effective when nursing butter catfish from 31 to 90 days old in composite tanks. The highest growth rate was observed at a density of 1.5 - 2.5 individuals /L for 31 - 90 day-old butter catfish in composite tanks while the lowest FCR was found at 1.0 individual /L and the highest at 2.5 individuals /L, the highest survival rate was at 1.0 individual /L and the lowest at 2.5 individuals /L. The result was similar in in the recirculating system.

4.2. Recommendation

Research on effects of stocking density on some sexual maturity factors such as maturity rate, maturity coefficient, absolute and relative fecundity of butter catfish.

Research to determine the time when butter catfish uses processed feed efficiently.

Research on nursing butter catfish from fry to fingerlings in earthen ponds in order to provide more seeds for commercial farmers.

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