



# Effects of Foods on Maturity and Spawning Induction Methods on Ovulation of Rice Field Eel *Monopterus albus* (Zuiew, 1793) in Thua Thien Hue Province, Vietnam

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**Abstract:** Adult rice field eels with  $125.52 \pm 27.99$  g body weight corresponding to  $44.79 \pm 5.93$  cm in length were maturity cultured in a  $5 \text{ m}^2$  cement tank without mud for three months with density  $30 \text{ eels/m}^2$ . Eel broodstocks were fed with three types of food including: sea fish ( $T_1$ ), commercial pellet food ( $T_2$ ) and mixed food—50% sea fish and 50% commercial pellet food ( $T_3$ ). Result showed that the maximum gonadosomatic index (GSI) of female eels fed by sea fish ( $2.89\% \pm 0.67\%$ ) was higher than both commercial pellet food ( $1.62\% \pm 0.62\%$ ) and mixed food ( $2.03\% \pm 0.82\%$ ) ( $P < 0.05$ ), while, GSI of male eels was  $1.27\% \pm 0.31\%$ ,  $0.68\% \pm 0.23\%$  and  $1.14\% \pm 0.41\%$  ( $P > 0.05$ ). Maturity rate of female fed by sea fish ( $88.91\% \pm 9.64\%$ ) was higher than commercial pellet food ( $61.12\% \pm 9.64\%$ ) ( $P < 0.05$ ). The maturity rate of male eels was rather low and there was not significantly different among treatments ( $P > 0.05$ ). Then, the eels were induced for spawning with two kinds of hormones, including  $T_1$ : human chorionic gonadotropin (HCG) at  $1,500 \text{ IU/kg}$ ,  $T_2$ : luteinizing hormone-releasing hormone (LHRH-a) at  $150 \text{ } \mu\text{g/kg}$  and domperidon  $10 \text{ mg/kg}$ , and  $T_3$ : control without hormone. The results of spawning induction methods showed that the reproduction rate of female eels induced by LHRH-a and HCG hormones were higher than that by natural reproduction method ( $P < 0.05$ ). Others, the survival rate of fry eels after 5 d using natural reproduction method ( $92.65\% \pm 2.54\%$ ) was higher than both using LHRH-a ( $67.77\% \pm 1.91\%$ ) and HCG ( $68.65\% \pm 1.23\%$ ) hormones ( $P < 0.05$ ). The average diameter of eggs was  $3.40\text{-}3.41 \text{ mm}$  and the length of newly hatched eels was  $1.72 \pm 0.19 \text{ cm}$ .

**Key words:** Food, maturity culture, ovulation, spawning induction method, rice field eel.

## 1. Introduction

The rice field eel *Monopterus albus* is native to sub-tropical and tropical Asia, and is widely distributed in many countries from India to China, Japan, Malaysia, Indonesia and Bangladesh [1]. Reproductive biology characteristics of this species begin to be studied in the 1960s, and at the first time, it studied about respiratory astrodynamics to reproductive physiology. Natural conversion from female to male of the gonads was researched too in 1967 [2]. The natural sex of eels was influenced by mLH (mammalian luteinizing hormone) hormone of

mammals to change sex of eels; but methyltestosterone, testosterone and 11-ketotestosterone hormone did not affect the change sex of this species [3, 4]. Results show that relative length of gut index was 0.65, indicating that rice field eels are carnivorous. The body length of female rice eels was upper 30 cm, the male over 50 cm and the hermaphrodite ones from 40 cm to 50 cm [5]. However, according to Tao et al. [6], hormones can change sex of eel (*Monopterus albus*). In Southeast Asia, eels were distributed in Vietnam, Myanmar, Thailand and Cambodia. In Vietnam, eels are present in most watersheds, and they live and develop from the upper of the Hong River to the Truong Son highlands mountainous, Southeast and Mekong Delta.

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Besides, rice field eel is also found from the North to the South in Vietnam [7]. This is an economic species with a large market for both domestic consumption and export, because there is much high levels nutrient, such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA)—a group of omega-3 fatty acids, vitamins B<sub>1</sub> and B<sub>2</sub>, in its' meat, which are very good for human health [7]. Over the past few years, the eel-raising activity has developed rapidly in Thua Thien Hue province, Central Vietnam. However, seed for grow-out is a major problem, because most of farmer who collected fingerlings of rice field eels by fishing wild individuals. This species which is easy raising and these achieves are more profit than some other small size fish-culture activities [8]. In recent years, the market able rice field eel culture has increased strongly in some areas of Vietnam [9]. Several researchers have indicated that food sources for broodstocks and induction method are keys for artificial reproduction [10]. In addition, Nghia et al. [11] who reported that the gonads development of rice field eel depends on food and ecological characteristics. It is therefore, finding appropriate food to improve gonads in maturation culture and induction method for sperm and oocyte release is of importance in seed production in aquaculture [12]. The present study aimed to investigate the proper food and induction method in seed production of rice field eel.

## 2. Materials and Methods

### 2.1 Experiment 1: Effects of Foods on Maturity of Rice Field Eels

Over 10-month old of rice field eels were obtained from the commercial farming for breeder, which had  $44.79 \pm 5.93$  cm in length and  $125.52 \pm 27.99$  g in weight. The main selection criteria to identify adult eel by suitable breeders were relying upon normal body shape, color and normal behavior.

Rice field eels were checked using the selection criteria mentioned above. Selected eels were then weighed, their sexes were checked and they were

immediately transferred to reproduction tanks prepared ready. After quarantine, broodstocks (both male and female eels) were moved to a series of concrete tanks for the experiments. The tanks had an area of 5 m<sup>2</sup>, water level was 30 cm and without mud in the tanks. These tanks were equipped by using bamboo frames as shelters for broodstocks. The number of eels in each tank was 150 eels.

Rice field eel *Monopterus albus* (Zuiew, 1793) was fed with three different types of foods: sea fish (T<sub>1</sub>), commercial pellet food (T<sub>2</sub>) and mixed food (50% sea fish and 50% commercial pellet food) (T<sub>3</sub>). The commercial pellet food contains at least 35% crude protein. Broodstocks were fed once a day at 5:00 pm with a quantity of about 5%-10% body weight. About 50%-70% of water in tanks was changed daily after feeding. In addition, the tanks were periodically spray irrigation for 30 min for every 3 d. The duration of maturation experiment was taken for three months. Gonadosomatic index and maturity rate were checked monthly by collecting randomly 10 eels from each tank. Gonads weight was determined as the following: first, used a knife to operate along the abdomen from the top and down of eels to separate the gonads from the body and then the gonads were weighed by electronic scales with accurate to 0.01 g (SHIMADZU brand, Japan). Signs of the ripe eggs are round, straw yellow and sperm are whitish and dissolved in water very quickly. In addition, slices were used to determine the developmental stages of oocytes and sperm by histologic methods as described by OXakun and Buskaia with six stages [13].

The gonadosomatic index (GSI) and maturity rate were determined as the following Eqs. (1) and (2):

$$\text{GSI (\%)} = \frac{\text{weight of gonads}}{\text{weight of body}} \times 100\% \quad (1)$$

$$\text{Maturity rate (\%)} = \frac{\text{No. of eels with gonad maturation}}{\text{No. of eels in the experiment}} \times 100\% \quad (2)$$

## 2.2 Experiment 2: Effects of Different Induction Methods on Ovation of Eel

After broodstocks was matured, three other methods, i.e., T<sub>1</sub>: luteinizing hormone-releasing hormone (LHRH-a) with 150 µg/kg and domperidon 10 mg/kg, T<sub>2</sub>: HCG with 1,500 IU/kg dose and T<sub>3</sub>: natural method without hormone (control), were used to inducing spawning, and each experiment was tested three times again. The dose of LHRH-a and HCG hormones was according to the method of Huong et al. [12].

The average size of broodstocks in this experiment was 145.52 ± 32.52 g in weight and 48.13 ± 4.71 cm in length. The spawning tank had an area of 5 m<sup>2</sup> with 30-40 cm of mud at the bottom. Stocking density was five pairs of male and female per square meter. Broodstocks were induced by using only once dose of gonadotropins, and the same for both male and female. The injection site was at the back muscle and the solvent was distilled water with 2% body weight. The effective time was calculated from the beginning of the injection to the first appearing of the first egg nest.

The reproduction rate, fertilization rate and hatching rate were calculated by the following Eqs. (3)-(5):

$$\text{Reproduction rate (\%)} = \frac{\text{No. of female eels produced eggs}}{\text{Total No. of females in the experiment}} \times 100\% \quad (3)$$

$$\text{Fertilization rate (\%)} = \frac{\text{No. of eggs with embryo (fertilized)}}{\text{Total eggs tested}} \times 100\% \quad (4)$$

$$\text{Hatching rate (\%)} = \frac{\text{No. of fry eels}}{\text{Total No. of fertilized eggs}} \times 100\% \quad (5)$$

The diameter and the length of eggs were measured by ruler with a precision of 0.1 mm. Duration from hatching to finish yolk-sac was the time (days) from the fry eel born until finishing yolk-sac. Survival rate

of fry eel after 5 d was calculated by the following Eq. (6):

$$\text{Survival rate of fry eel after 5 d (\%)} = \frac{\text{No. of survival fry eel}}{\text{Total fry eels after 5 d}} \times 100\% \quad (6)$$

Length of fry rice field eel after hatching was also measured by ruler with a precision of 0.1 mm every day.

## 2.3 Statistical Analysis

All experiments were conducted with three replicates. All data on variables and testing were analyzed using Excel and SPSS version 16.0. The data were analyzed by ANOVA (multi-comparisons Tukey-Kramer LSD post-hoc test). Statistical comparison tests were conducted at a level of significance of  $P = 0.05$ .

## 3. Results

### 3.1 Effects of Foods on Gonads Development

#### 3.1.1 Gonadosomatic Index

Gonadosomatic index is an important indicator to evaluate the maturity of gonads. The results about gonadosomatic index of the female and male eels between the treatments are shown in Tables 1 and 2. It can be seen that the gonadosomatic index of female eels was increased with the time of culture, and significant difference of the gonadosomatic index between treatments were found from the second month. The gonadosomatic index after three months rearing was as follows: 2.89% ± 0.67% in T<sub>1</sub>, higher than 1.62% ± 0.62% in T<sub>2</sub> and 2.11% ± 0.82% in T<sub>3</sub> ( $P < 0.05$ ). However, there was no significant difference between T<sub>2</sub> and T<sub>3</sub> ( $P > 0.05$ ). The gonadosomatic index of male eels in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> after three months was 1.27% ± 0.31%, 0.68% ± 0.24% and 1.14% ± 0.41%, respectively ( $P > 0.05$ ). The study shows that female rice field eel fed with sea fish is better than commercial pelleted feed, which is in agreement with previous study by Huong et al. [14]

**Table 1 The gonadosomatic index of female rice field eel.**

Time	Gonadosomatic index (%)		
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
January, 2016	1.52 ± 0.49 <sup>a</sup>	1.11 ± 0.54 <sup>a</sup>	1.27 ± 0.43 <sup>a</sup>
February, 2016	2.05 ± 0.69 <sup>a</sup>	1.19 ± 0.57 <sup>b</sup>	1.76 ± 0.41 <sup>ab</sup>
March, 2016	2.89 ± 0.67 <sup>a</sup>	1.62 ± 0.62 <sup>b</sup>	2.11 ± 0.82 <sup>b</sup>

The values expressed are mean ± standard deviations. <sup>a,b</sup> Data with the same letter in row were not significantly different between treatments ( $P > 0.05$ ).

**Table 2 The gonadosomatic index of male rice field eel.**

Time	Gonadosomatic index (%)		
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
January, 2016	0.17 ± 0.01 <sup>a</sup>	0.12 ± 0.06 <sup>a</sup>	0.24 ± 0.16 <sup>a</sup>
February, 2016	0.61 ± 0.13 <sup>a</sup>	0.29 ± 0.03 <sup>a</sup>	0.65 ± 0.14 <sup>a</sup>
March, 2016	1.27 ± 0.31 <sup>a</sup>	0.68 ± 0.24 <sup>a</sup>	1.14 ± 0.41 <sup>a</sup>

The values expressed are mean ± standard deviations. <sup>a,b</sup> Data with the same letter in row were not significantly different between treatments ( $P > 0.05$ ).

**Table 3 The maturity rate of rice field eel after three months culture.**

Sex	The maturity rate (%)		
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Female	88.91 ± 9.64 <sup>a</sup>	61.12 ± 9.64 <sup>b</sup>	77.82 ± 9.58 <sup>ab</sup>
Male	66.71 ± 11.54 <sup>a</sup>	53.32 ± 11.54 <sup>a</sup>	60.21 ± 20.03 <sup>a</sup>

The values expressed are mean and standard deviations. <sup>a,b</sup> Data with the same letter in row were not significantly different between treatments ( $P > 0.05$ ).

**Table 4 Result of artificial reproduction stimulation of rice field eel.**

Induction methods	The number of eels released oocyte and sperm	Effective time (d)	Number of eggs per nest	Reproduction rate (%)
LHRH-a	25	11.67 ± 3.03 <sup>a</sup>	262.72 ± 112.32 <sup>a</sup>	58.33 ± 14.71 <sup>a</sup>
HCG	25	12.32 ± 2.72 <sup>a</sup>	244.03 ± 79.42 <sup>b</sup>	53.33 ± 12.12 <sup>ab</sup>
Naturally	25	19.67 ± 3.14 <sup>b</sup>	232.47 ± 107.63 <sup>c</sup>	43.33 ± 8.12 <sup>b</sup>

The values expressed are mean and standard deviations. <sup>a-c</sup> Data with the same letter in column were not significantly different between treatments ( $P > 0.05$ ).

who recommended that broodstocks fed with small shrimp, worm and sea fish by spraying method combined with injection showed a higher gonadosomatic index (3.1% ± 1.27%).

### 3.1.2 Maturity Rate

The maturity rate of rice field eel is described in Table 3. After three months culture, the average maturity rate of female rice field eel at T<sub>1</sub> (sea fish) reached 88.91% ± 9.64%, which was higher than that in T<sub>2</sub> (commercial pellet food) with 61.12% ± 9.64% ( $P < 0.05$ ). However, there was no significant difference ( $P > 0.05$ ) to maturity rate when compared between T<sub>1</sub> and T<sub>3</sub> (77.82% ± 9.58%). Meanwhile, the

maturity rate of male rice field eel was relatively low and no significant difference in maturity rate of male rice field eel between treatments ( $P > 0.05$ ). The maturity rates of male eels of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were 66.71% ± 11.54%, 53.32% ± 11.54% and 60.21% ± 20.03%, respectively.

## 3.2 Effects of Induction Methods on Ovation of Rice Field Eel

### 3.2.1 Effective Time

Table 4 showed that the effective time of LHRH-a and HCG on ovulation of female rice field eel was 11.67 ± 3.03 d and 12.32 ± 2.72 d, respectively ( $P >$

0.05). However, natural reproduction method showed a longer effective time ( $19.6 \pm 3.14$  d). According to Huong et al. [12], the effective time of rice field eels induced with 50, 100 and 150  $\mu\text{g}/\text{kg}$  LHRH-a hormones was two weeks and the effective time of eels induced with 1,000, 1,500 and 2,000 IU/kg HCG was 16 d. Moreover, eels were injected with 150-160  $\mu\text{g}/\text{kg}$  LHRH-a hormone which effective time was 40 h can be up to 70-75 h [7].

### 3.2.2 Number of Eggs per Nest

The egg-nest is shown in Fig. 1. The average number of eggs per nest was the highest in LHRH-a treatment and reached  $262.72 \pm 112.32$  eggs/nest, which was higher than others ( $P < 0.05$ ). In contrast, the numbers of eggs per nest in natural method (control) was the lowest, on average of  $232.47 \pm 107.63$  eggs/nest ( $P < 0.05$ ). These results can be comparable with report by Huong et al. [12] who told that there are about 18-596 eggs/nest.

### 3.2.3 Reproduction Rate

The reproduction rate of rice field eel when using LHRH-a (150  $\mu\text{g}/\text{kg}$ ) and HCG (1,000 IU/kg) was  $58.33\% \pm 14.71\%$  and  $53.33\% \pm 12.12\%$ , respectively. There was a significant difference in reproduction rate between LHRH-a and HCG hormones and natural method ( $P < 0.05$ ), however, no significantly difference was found between LHRH-a and HCG hormones ( $P > 0.05$ ). Reproduction rate by natural reproduction method was  $43.33\% \pm 8.12\%$ . These

results were much lower than the reproduction rate reported by Huong et al. [12], when using LHRH-a and HCG hormone, the reproduction rate was 50.0% and 75.0%, respectively, but natural induction method was 0% [12].

### 3.2.4 Size of Eel Eggs

Results in Table 5 indicated that the size of eel eggs ranged from 3.40 mm to 3.41 mm, in which the results was the same between LHRH-a and HCG treatment with average of  $3.41 \pm 0.14$  mm; and nature treatment reached  $3.40 \pm 0.16$  mm. However, there was no significant difference between treatments ( $P > 0.05$ ). This result was consistent with the study by Huong et al [12] who reported the diameter of eel eggs ranging from 3.17 mm to 3.58 mm with the average  $3.47 \pm 0.09$  mm.

### 3.2.5 Fertilization Rate

Embryos developed are shown in Fig. 2. The results about the fertilization rate of natural method ( $93.62\% \pm 1.03\%$ ) was much higher that of LHRH-a and HCG hormone ( $74.52\% \pm 6.12\%$  and  $74.21\% \pm 8.12\%$ , respectively) ( $P < 0.05$ ). Whereas, the fertilization rate when using by HCG (1,500 IU/kg), LHRH-a (150  $\mu\text{g}/\text{kg}$ ) hormone and natural reproduction was 73.0%, 79.0% and 96.0%, respectively [12].

### 3.2.6 Hatching Rate

The results showed that the hatching rate was the highest at natural reproduction method ( $96.15\% \pm 1.35\%$ ), which was significantly higher than that by

**Table 5 Results of Reproductive characteristics**

Reproductive characteristics	Treatments		
	LHRH-a	HCG	Nature
Fertilization rate (%)	$74.52 \pm 6.12^a$	$74.21 \pm 8.12^a$	$93.62 \pm 1.03^b$
Hatching rate (%)	$87.52 \pm 3.53^a$	$83.33 \pm 4.52^a$	$96.15 \pm 1.35^b$
Hatching time (d)	$5.60 \pm 0.71^a$	$5.62 \pm 0.82^a$	$6.02 \pm 1.03^a$
Size of eel eggs (mm)	$3.41 \pm 0.14^a$	$3.41 \pm 0.14^a$	$3.40 \pm 0.16^b$
Time to finish yolk-sac (d)	$5.11 \pm 1.32^a$	$5.02 \pm 1.63^a$	$5.83 \pm 1.24^a$
Survival rate of fry eel after 5 d (%)	$67.77 \pm 1.91^a$	$68.65 \pm 1.23^a$	$92.65 \pm 2.54^b$
Length of newly hatched eels (cm)	$1.72 \pm 0.19^a$	$1.72 \pm 0.19^a$	$1.72 \pm 0.19^a$
Length of fry rice field eel after 10 d (cm)	$4.02 \pm 0.22^a$	$4.01 \pm 0.21^a$	$4.02 \pm 0.20^a$

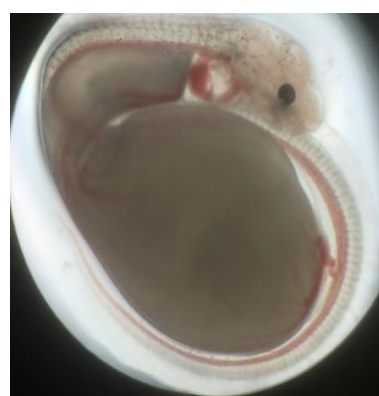
The values are expressed as mean  $\pm$  standard deviations. <sup>a, b</sup> Data with the same letter in column were not significantly different between treatments ( $P > 0.05$ ).



**Fig. 1** Egg-nest.



**Fig. 2** The embryo is developing



**Fig. 3** Fry rice field eel.

LHRH-a ( $87.52\% \pm 3.53\%$ ) and HCG hormones ( $83.33\% \pm 4.52\%$ ) ( $P < 0.05$ ). Huong et al. [12] have documented that the hatching rate when inducing rice field eel by LHRH-a ( $150 \mu\text{g}/\text{kg}$ ), HCG ( $1,500 \text{ IU}/\text{kg}$ ) and natural reproduction at a similar doses with the present study was  $81.0\%$ ,  $95.0\%$  and  $97.0\%$ , respectively. In addition, according to study by Hiep et al. [15] who reported that when using of HCG ( $2,000 \text{ IU}/\text{kg}$ ) and natural reproduction, the hatching rate reached  $98.4\%$  and  $100.0\%$  in 2013.

### 3.2.7 Time to Finish Yolk-Sac

Results showed that time to finish yolk-sac by LHRH-a, HCG hormone and natural reproduction methods was  $5.11 \pm 1.32$ ,  $5.02 \pm 1.63$  and  $5.83 \pm 1.24$  d, respectively, ( $P > 0.05$ ).

### 3.2.8 Survival Rate of Fry Eel after 5 d

The survival rate of fry after finishing yolk-sac (started feeding) was also relatively high and there was a significant difference between natural reproduction method and other treatments. After 5 d rearing, the survival rate of fry by natural reproduction method was  $92.65\% \pm 2.54\%$ , which was higher than both of LHRH-a ( $67.77\% \pm 1.91\%$ ) and HCG ( $68.65\% \pm 1.23\%$ ) hormone ( $P < 0.05$ ), respectively. In addition, fry eel was produced by natural reproduction method had a stronger behavior and better tolerance than those of hormone methods.

### 3.2.9 Length of Fry Rice Field Eel after 10 d

Fry eel was born with very small body and a large yolk-sac along belly (Fig. 3). Besides, it is moved

hardly, just lied under the bottom of the tank [12]. The result showed that the length of the eel from hatching until the eel (7 d) reached  $2.90 \text{ cm}$ . The length of the fry eel was increased by time. The length of newly hatched eels was  $1.72 \pm 0.19 \text{ cm}$ , and reached  $2.9 \pm 0.1 \text{ cm}$  and  $4.02 \pm 0.22 \text{ cm}$  after 7 d and 10 d, respectively. These data are in agreement with Huong et al. [12], who reported that the length of fry eel after 7 d was  $2.9 \pm 0.1 \text{ cm}$ .

## 4. Discussion

The rice field eel is cold-blooded animals, so the temperature of eel body often changes depending on surrounding water environment. Eels like to live in the mud and soil [7]. The problem of maturity culture and artificial reproduction of the rice field eel has been studied extensively in the world, as well as in Vietnam. According to Khanh et al. [5], the rice field eel is a species with thick gut where the correlation between gut length and total length (RLG) was  $0.65$ . On the other hand, the eel has a wide mouth, large mouth openings, sharp teeth, long tubular stomach and thickness septum which lies along the length of the body. Observation inside the eel's digestive tract shows that the most of the food in the digestive tract is fish, crab and shrimp. Combination of external characteristics, digestive tube shape, food composition in the gastrointestinal tract and RLG index, it be can made sure that rice field eels are predatory animal and they can eat large food [5]. Therefore, when studying

the feed which help broodstocks have skill of developing gonads, sea fish and commercial pellet food which has high protein and mix food were chosen. The present study showed that when eels fed with feeds which are very good for eels, maturity rate was over 60% for female and over 50% for male eel. And according to Huong et al. [12], when the eels fed with a combination diet of marine trash fish and freshwater snail, the result indicated that gonadosomatic index of females in the tanks with nylon-string substrate increased from  $0.33\% \pm 0.37\%$  to  $1.73\% \pm 1.73\%$  after two months. In addition, studies on breeding season of eels are also needed. The spawning season of eel is from March to September in Mekong Delta [5]. And the highest GSI of the female, hermaphrodite and male rice field eels was 9.12%. Mean fecundity ranged in 143-6,813 eggs/female and the diameter was 1.48 mm [5]. The highest absolute fertility in the 54-70 cm in length was 638.67 eggs/female. Relative fertility averaged in 3.54-7.89 eggs/g of body weight. The largest absolute reproduction was 750 eggs/female with 55.5 cm in length and 180 g in weight [11]. Currently yet in Vietnam, there are multiple researches about reproduction of eels. The results of these studies suggested that the most effective density for natural propagation was 4 eels/m<sup>2</sup> at the male and female ratio of 1:1, and the average spawning rate was 61.9% [9]. While the spawning experiment in this study was effectuated with 10 eels/m<sup>2</sup> density. Broodstocks often are checked after each reproduction cycle finish to determine the number of female eels which take part in reproduction cycle, exactly. The results showed that reproduction rate of the whole experiment was significantly different between treatments. Specific, the birth rate of rice field eel which was injected by LHRH-a (150 µg/kg), HCG (1,000 IU/kg) and natural reproduction (control) were  $58.33\% \pm 14.71\%$ ;  $53.33\% \pm 12.12\%$  and  $43.33\% \pm 8.12\%$ , respectively. The results of spawning induction methods showed that the reproduction rate of female eels induced by

LHRH-a and HCG hormones were higher than that by natural reproduction method ( $P < 0.05$ ). Others, the survival rate of fry eels by natural reproduction method ( $92.65\% \pm 2.54\%$ ) was higher than both using LHRH-a ( $67.77\% \pm 1.91\%$ ) and HCG ( $68.65\% \pm 1.23\%$ ) hormones after 5 d ( $P < 0.05$ ). The diameter of eggs was 3.40-3.41 mm and the length of newly hatched eels was  $1.72 \pm 0.19$  cm in length. According to Huong et al. [12], the birth rate of the eels used LHRH-a hormone (150 µg/kg) was 75.0% in average, which is higher than the result in this study. While for HCG hormone (1,500 IU/kg), the birth rate was 50.0% which is quite similar to the result in this study (53.33%). Particularly for the natural reproduction (non-hormone), the eels did not product (0%) [12].

Huong et al. [14] also described the effects of densities on spawning and conducted it with three stocking densities with triplicated for each. The highest spawning ratio was 66.7%-100% with male:female = 1:1 treatment, which was higher than that in male:female = 4:4 (8.33%) and male:female = 6:6 (0%-5.55%). The first spawning of hormone pre-injection eel was one week, which was shorter than those without hormone pre-injection (two weeks). The eel can spawn many times in a spawning cycle (from 8 d to 11 d). The embryo development period is 95 h in 30 °C water [14].

Study by Huong et al. [12] reported the eels injected with 150 µg/kg LHRH-a produced three egg-nests or 75.0% animals spawned with a highest spawning rate; the highest fertilization rate (80.0%) was found in the group injected with 2,000 IU/kg HCG, and the mean number of eggs per egg-nest was  $188.5 \pm 142.9$  and mean size of newly hatched eels was  $1.7 \pm 0.1$  cm [12].

## 5. Conclusions and Recommendation

Rice field eel broodstocks fed with sea fish showed a higher gonadosomatic index and maturity rate than commercial pellet food and mix of sea fish and pellet food. Spawning induction rice field eel by natural

method had longer effective time than that by LHRH-a and HCG hormones, and also fry rice field eels induced with natural method had stronger behavior, better endurance and higher survival rate. Thus, it is recommended to feed rice field eel broodstocks by sea fish for better gonads development and apply natural reproduction method to induce eel breeders release oocyte and sperm in reproductive processes. Moreover, using natural reproduction method for spawning induction of rice field eel had a lower cost and simple application.

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