MINISTRY OF EDUCATION AND TRAINING

NHA TRANG UNIVERSITY

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STUDY ON THE PLASMA STEROID HORMONE LEVELS IN THE REPRODUCTIVE CYCLE OF THE RABBITFISH *Siganus guttatus* (Bloch, 1787)

Major: Aquaculture

Major code: 9620301

SUMMARY OF DOCTORAL THESIS

Khanh Hoa - 2022

The work has been completed at Nha Trang University

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INTRODUCTION

The production of rabbitfish in Vietnam in particular and the world in general still faces various restrictions such as low spawning rate, difficult larval rearing, leading to the low survival rate of larvae, and difficulty in achieving fingerling size. Currently, research on reproductive biology, physiology, reproductive endocrinology, and reproductive stimulation in captivity has not received sufficient attention [17]. In addition, the study of changes in steroid hormone levels during the spawning cycle in rabbitfish has not been conducted [2]. In that setting, the topic **"Study on the plasma steroid hormone levels in the reproductive cycle of the rabbitfish *Siganus guttatus* (Bloch, 1787)"** was carried out, which aims at providing scientific data, contributing to perfect the process of artificial reproduction and rabbitfish hatchery.

The objective of the study

Clarifying the fluctuation of steroid hormone levels in the blood plasma of rabbitfish *Siganus guttatus* (Bloch, 1787) during the spawning cycle as a basis for studies on artificial reproduction of rabbitfish in particular and marine fish in general.

Scientific of the study

Research can be attributed to agencies, universities, and research institutes by providing methodology information, knowledge of fish reproductive endocrinology for university training activities, postgraduate courses, and training courses for aquaculture staff and students.

Practical application of the study

Research results on fluctuations in steroid hormone levels during the spawning cycle of rabbitfish can serve as a basis for future research on the artificial reproduction of marine fish.

New findings

This is the first research in Vietnam to study the fluctuations of E_2, T, and 11-KT concentrations in the spawning cycle of rabbitfish, as well as the fluctuations of E_2 under the influence of hCG and LHRH - A.

CHAPTER 1. LITERATURE REVIEW

1.1. Reproductive biology of rabbitfish

The rabbitfish has a long oval shape and is flattened on both sides, with small round scales, both sides of the head are more or less scaled, and the lateral line is complete. Each side of the snout has 2 nostrils and a small mouth. Pectoral fin round, moderately large. Pelvic fin below the chest. Caudal fin is flat or slightly lobed. I have many dots, there are some narrow oblique stripes on the side of the head, and the stripe from the edge of the mouth to below the eyes is the most obvious. The end of the dorsal fin has pale stripes. The outer color of the fish ranges from pale yellow to brown. The fish has 13 dorsal fin rays, 7 anal fin rays, and 2 pelvic fin rays [1].

Geographically, the scorpionfish is distributed in the tropics, from latitude 30o North to 30o South, from the eastern Indian Ocean to the western Pacific Ocean,

including countries such as the Andaman Islands, Australia, Indonesia, Thailand, Malaysia, Singapore, Ryukyus (Japan), south and southeast China, Taiwan, Philippines and Palau. In Vietnam, the sea bream is distributed in coastal areas from the Gulf of Tonkin to the Gulf of Thailand, which the most in the waters of Quang Thai (Thua Thien Hue), the lower Thu Bon River, and the alluvial plains of Quang Ninh province. South, downstream of Hieu and Ben Hai rivers (Quang Tri).

The distribution area of scorpion fish is greatly affected by temperature. In the wild, it is possible to catch fish in waters with temperatures ranging from 24-28^{0}C. Dumplings are generally able to tolerate a wide range of temperature and salinity variations [7]. Fish can adapt gradually when the salinity is low to 5 ‰, the temperature is 25 - 34^{0}C. The ability to tolerate low dissolved oxygen is also very good. However, fish cannot tolerate it if the dissolved oxygen content is < 2mg O_2/L [11].

Newly hatched peacock larvae have a small size of 1.5 - 1.6 mm. The larvae open their mouth 36 hours after hatching, and start to eat at 60 hours after hatching, the yolk is completely absorbed when the larvae are released. 70 hours after hatching [6]. In the first three days, larvae feed on yolk and oil drops, larvae start feeding 3 days after hatching at 28 - 30^{0}C. In the larval stage, the fish feed mainly on zooplankton, but in the juvenile and adult stage, it is completely fed by aquatic plants [14]. In the wild, scads often eat plants on the seabed, head down, and can eat day and night [9]. Juvenile and adult stages: Like other species of scorpion, during juvenile and adult stages, peacocks feed exclusively on aquatic plants [16].

Male and female sharks are difficult to distinguish based on appearance alone. However, during the breeding season, it is possible to observe the abdomen to identify the female by its round shape or visit the eggs, while the male when gently stroked will have white discharge (semen). In addition, the males are usually smaller than the females and the females are less active than males during the breeding season. Crayfish can mature in captivity if environmental conditions are favorable and provided with adequate and quality food [10, 13].

1.2. Situation of research on rabbitfish in the world and Vietnam

1.2.1. In the world

Research on the artificial reproduction of scads in captivity has been carried out for a long time [12], especially after the 1972 Hawaiian conference on barnacles. However, rearing was not successful from this stage. larval stage to the end of metamorphosis. Most of the research on larva larvae has not been successful in the early stages or if so, the survival rate is very low [18]. Some studies report survival rate until complete metamorphosis is less than 1%. The survival rate was 9% in S. vermiculatus and the most successful in S. lineatus [15]. In the years from 1981 to 1983, Juario et al. (1985) increased the survival rate when rearing larva until the end of metamorphosis from 1.9% to 12.8%, but the results were not satisfactory. determined. The author could not explain why the survival rates of 1982 and 1983 were worse than those of 1981. In 1985, the study on spawning and rearing of larva in the Southeast

Asia Fisheries Development Center (SEAFDEC) was conducted. In Indonesia, the survival rate is very low and it is not possible to develop a production process for this fish species.

1.2.2. In Viet Nam

There are relatively few studies on scorpion fish in Vietnam. Dumplings are described with taxonomic characteristics and listed in the list of marine fish species in Vietnam [6]. This fish species has been studied for reproductive biology in Thi Nai lagoon [5]. The research on scorpion fish by Le Van Dan, and Le Duc Ngoan (2006), carried out in Tam Giang lagoon - Thua Thien Hue is valuable works. Research has shown that, in captivity, the reproductive cycle of the opossum is not clear, only mature fish are found in March and May, and the maturation rate is low (8.3%); the time of maturation of the male carp is from March to July next year, the maturation rate is high in March (72.7%) and June (61.5%); The fish is unisexual, in the cytogenetic structure of the gonads, there are many sex cells that develop through different periods, the mature oocytes have different sizes, indicating that the fish spawn many times a year and prolonged calving time [6]; The age of first maturation of female fish as well as male fish is 01 year, the average mature weight of female fish is 488.57g and male fish is 432.85g, absolute fecundity of female fish weight from 386g - 820g ranged from 551,586 - 1,082,650 eggs/individual and relative fertility ranged from 1,437 - 1,862 eggs/g [4], the maturation rate in 8 months (January - August) was very high. (male >89%, female >96%); The hatched larvae only live for 3-4 days, by day 5 the survival rate is only 5% and completely dead at day 7 [3, 4].

Dumplings have long been brought into the culture by people in the lagoon area and by sea cage farmers, mainly in the form of polyculture. Within the framework of the IMOLA project, the Thua Thien - Hue Fisheries Extension Center has implemented a model of fish farming in combination with yellow seaweed and black tiger shrimp with good results, which is a model of great economic significance. - Social, and practical, helping people in polluted black tiger shrimp farming areas create an appropriate direction for economic development and recovery of farming areas. The model also contributes to diversifying farming subjects, overcoming the phenomenon of prolonged loss of shrimp farming in some localities [7].

In 2007, the Thua Thien Hue Fisheries Extension Center conducted a trial of an artificial seed-breeding model in combination with black tiger shrimp (Penaeus monodon) and achieved positive results [7]. Although there have been studies on artificial fertility of scorpion fish and evaluation of egg and sperm quality to improve the quality of larvae and seed, it is not feasible and has not had much success. From 2009 - 2013, Phan Van Ut and his colleagues studied the technical parameters, developed a technical process for artificial seed production and obtained the necessary results. The fish can be spawned by hCG or LHRHa with doses of 2,000 IU or 40 µg/kg of female fish, respectively. The effect time is from 40 - 72 hours, the average fertilization rate is over 80%. The embryo development time is from 16 to 20 hours,

and the average hatching rate is 85.9%. The total number of fish larvae through 16 spawning times reached 14.37 million. Fish were reared at a density of 50 - 150 larvae/L [8].

CHAPTER 2. MATERIALS AND METHODOLOGY

2.1. Subject and scope of the study

Research objects: Rabbitfish - *Siganus guttatus* Bloch, 1787

Study period and sites:

The study period runs from 5/2017 to 5/2021

- Institute of Aquaculture (Nha Trang University).

- Institute of Biotechnology and Environment (Nha Trang University).

- Samling location: Cam Ranh City, Khanh Hoa Province ($12^0$52'15''N, 108^0 40' 33''E).

2.2. Contents

Content 1: Study on the fluctuations of E2, T, and 11-KT concentrations in the blood plasma of Siganus guttatus (Bloch, 1787) and their relationship with the development of gonads in the reproductive cycle.

Content 2: Study on the fluctuation of E2 content under the influence of human chorionic gonadotropin hormone hCG, and LHRH – A.

Content 3: Research on the influence of hCG, and LHRH - A on reproductive physiology and biochemical composition of spermatozoa and ovary.

2.3. Methodology

2.3.1. Experimental

Experimental 1: Steroid hormones in the reproductive cycle

Every month, at least 10 male and 10 female fish samples were randomly collected of blood, gonadal, size, and weight measurements. The body length and weight for broodstock were 24 ± 2 cm and 520 ± 60 g, respectively. Blood samples were collected, then centrifuged to separate plasma, and stored at -800C until analysis for E2 in females and T,11-KT in males.

Experimental 2: E_2 and T fluctuations under the influence of hCG, LHRH – A

In this experiment, the broodstock of rabbitfish has 120 individuals aged 1+, with a total body length and weight of 30 ± 4 cm and 550 ± 80 g, respectively.

Treatment 1 (Control): 1ml saline solution/kg female fish

Treatment 2 (hCG): 1,500 IU/kg female

Treatment 3 (LHRH – A + DOM): 50 µg + 5 mg/kg female

After injection, fish were stocked into a 4 m3 tank, water temperature, salinity, pH and dissolved oxygen were 30 ± 2^0C, 32 ± 2‰, 7.8 – 8.6, and 5 ± 0.5 mg, respectively/l. Do not feed the fish during the experiment. In each treatment after injection, all fish were caught to collect blood samples at 6, 12, 24, and 48 hours. Then, the blood sample was centrifuged to separate plasma and stored at -800C until analysis for T and E_2.

Experimental 3: Effects of hCG, LHRH – A on reproductive physiology and biochemical composition of testes and ovaries

The fish used for this experiment had an average length and body weight respectively: male fish were 30.64 ± 1.03 cm and 524.55 ± 84.54 g; female fish were 31.22 ± 2.28 cm and 606.67 ± 104.04 g, natural color, normal swimming, flexible, no deformity, deformity and no disease symptoms, then were domesticated for 10 days in a 4m³ cement tank with a density of 6 fish/m³ (3kg/m³) before being injected with hormones.

The experiment was arranged into 3 treatments, each treatment consisted of 20 individuals:

Treatment 1: fish were injected with 1.500 IU hCG/kg fish

Treatment 2: fish were injected with 50 g LHRH – A + 5 mg DOM/kg fish

Treatment 3 (control): fish were injected with 1 mL physiological saline/kg fish

We randomly dissected 10 female and 10 male fish before the hormone injection to assess the maturity of the ovaries and sperms, as well as to determine some physiological and biological characteristics of reproduction.). After the fish were injected with hormones, 12 hours and 24 hours later, we conducted dissection, assessed the maturity level and analyzed the biochemical components of the ovaries and testes to compare with before injection.

2.3.2. Sample collection and analysis

2.3.2.1. Sample collection and fixation method

We collect samples once a month on average, with approximately 10 rabbitfish per month). Fish were anesthetized with ice and blood samples were taken right into the pond culture, blood samples (3ml) were stored in styrofoam containers containing ice to be transported to the laboratory. After taking blood, weigh the individual fish and measure the length to have a basis to evaluate the fluctuations of E_2, T and 11 - KT related to the length and body weight of fish or not, the Whether the maturation of fish in the spawning cycle is related to length and weight, etc., record the data collected to determine the parameters of length and weight.

The fish were dissected to remove the gonads and liver and weighed to determine the liver coefficient and maturation factor. Ovaries were fixed in 10% formol solution to conduct gonadal histology and analyze biochemical components in the eggs. All collected samples will be brought to the laboratory and placed in a freezer at -80^0C, ensuring the fastest time so as not to affect the quality of the collected samples.

2.3.2.2. Methods for determining reproductive biological indicators

Gonadosomatic index (GSI):

$$\text{GSI} = \frac{GW}{BW} \times 100\%$$

Hepatosomatic index (HSI):

$$HSI = \frac{HW}{BW} \times 100\%$$

Absolute fecundity (AF): The total number of eggs in the ovary at stage IV.

Relative fecundity (RF): The number of eggs per unit of body mass, according to the following formula:

$$RF = \frac{AF}{BW} \text{ (trứng/g)}$$

2.3.2.3. Methods of making templates to study gonads

The gonads, after being fixed in 10% formaldehyde, will be used as histological specimens. The process is carried out in 5 steps, according to Patki et al (1989).

2.3.2.4. Read the results on the microscope

At objective 10, an eyepiece-mounted eyepiece micrometer was used to measure the oocyte diameter. Oocyte size at each phase is measured by 15 oocytes, and is calculated by the formula:

$$L = 0.1 * (A/n)$$

2.3.2.5. Methods of analyzing steroid hormone levels in plasma

In this study, E_2, T, and 11-KT in the blood plasma were analyzed by enzyme-linked immunosorbent assay (*Enzyme Linked Immunosorbent Assay*: ELISA). EIA steroid hormone kit from the manufacturer (Enzyme Immuno Assay: EIA) Cayman Chemical Company (Ann Arbor, MI, USA).

2.4. Determination of biochemical components of caviar through stages

Protein composition, lipid, ash and moisture were analyzed according to the method of AOAC (2000) at the Institute of Biotechnology and Environment - Nha Trang University.

2.5. Data analysis and processing methods

Data are presented as an average value ± standard deviation (Mean ± SD). The data were preliminarily processed by Microsoft Excel 2013. The influence of hCG and LHRH-A hormones on the biochemical composition of fish was analyzed by the one-way ANOVA method and tested. Duncan with a significance level of $P < 0.05$ using the software SPSS version 20.0.

CHAPTER 3. RESULTS AND DISCUSSIONS

3.1. The development of the gonads of the fish during the reproductive cycle

3.1.1. Size of fish studied

The broodstock has a body length the total (TL) ranging from 19 to 34 cm. The largest average length is 31.33 ± 1.87 cm and the smallest is 20.86 ± 1.68 cm. The body weight (BW) of the rabbitfish ranged from 130 to 800 g. The largest average weight is 606.67 ± 104.04 g and the smallest is 154.29 ± 29.92 g. During the study period, the size of the broodstock did not change much.

3.1.2. Ovarian development during in the reproductive cycle

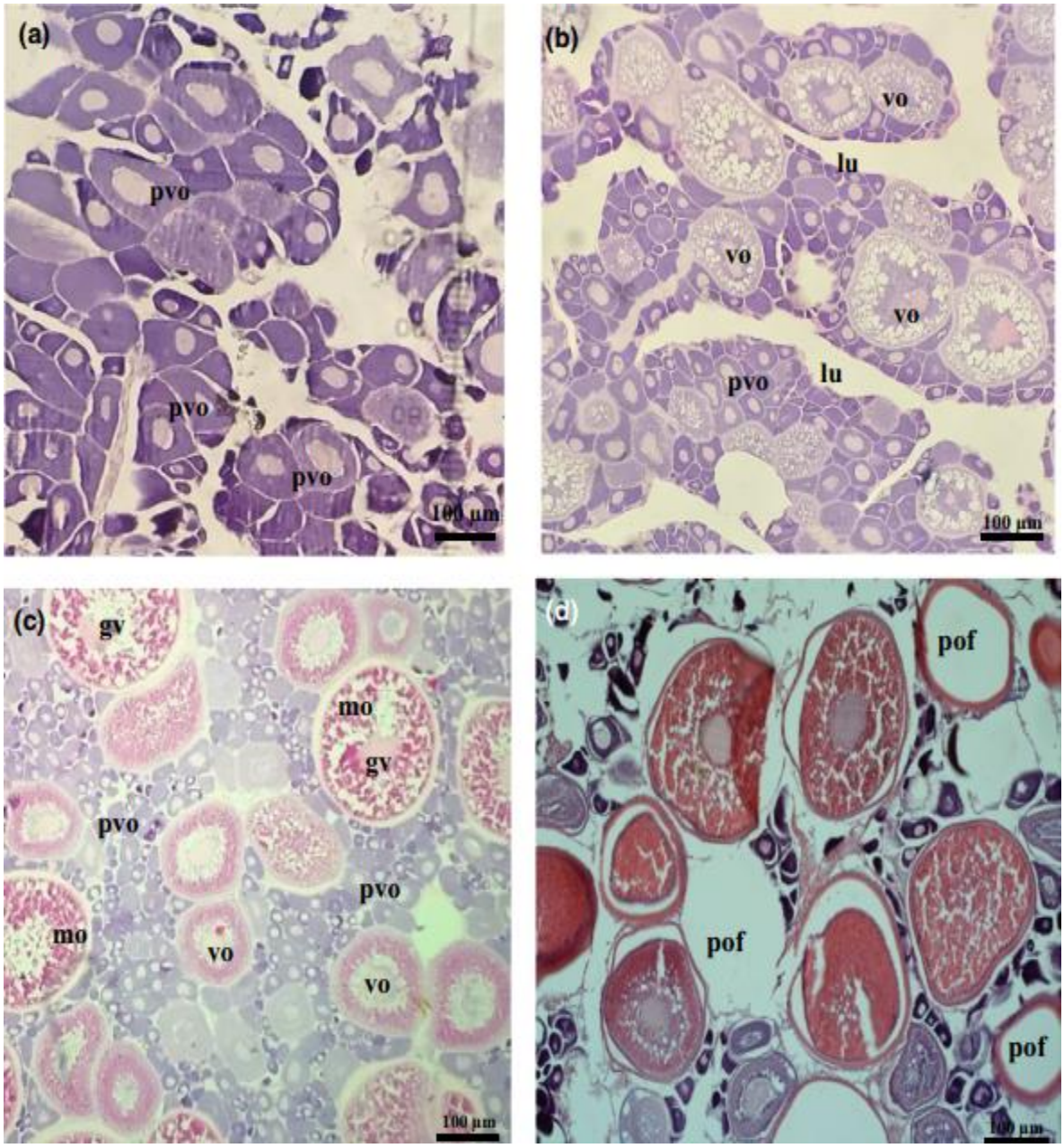

Figure 3.1. Histological sections of ovaries collected during study period of the golden rabbitfish

Figuge 3.1a: Stage II ovary; Figuge 3.1b: Stage III ovary; Figuge 3.1c: Stage IV ovary; Figuge 3.1d: Stage V ovary.

3.1.3. The development of sperm in the reproductive cycle

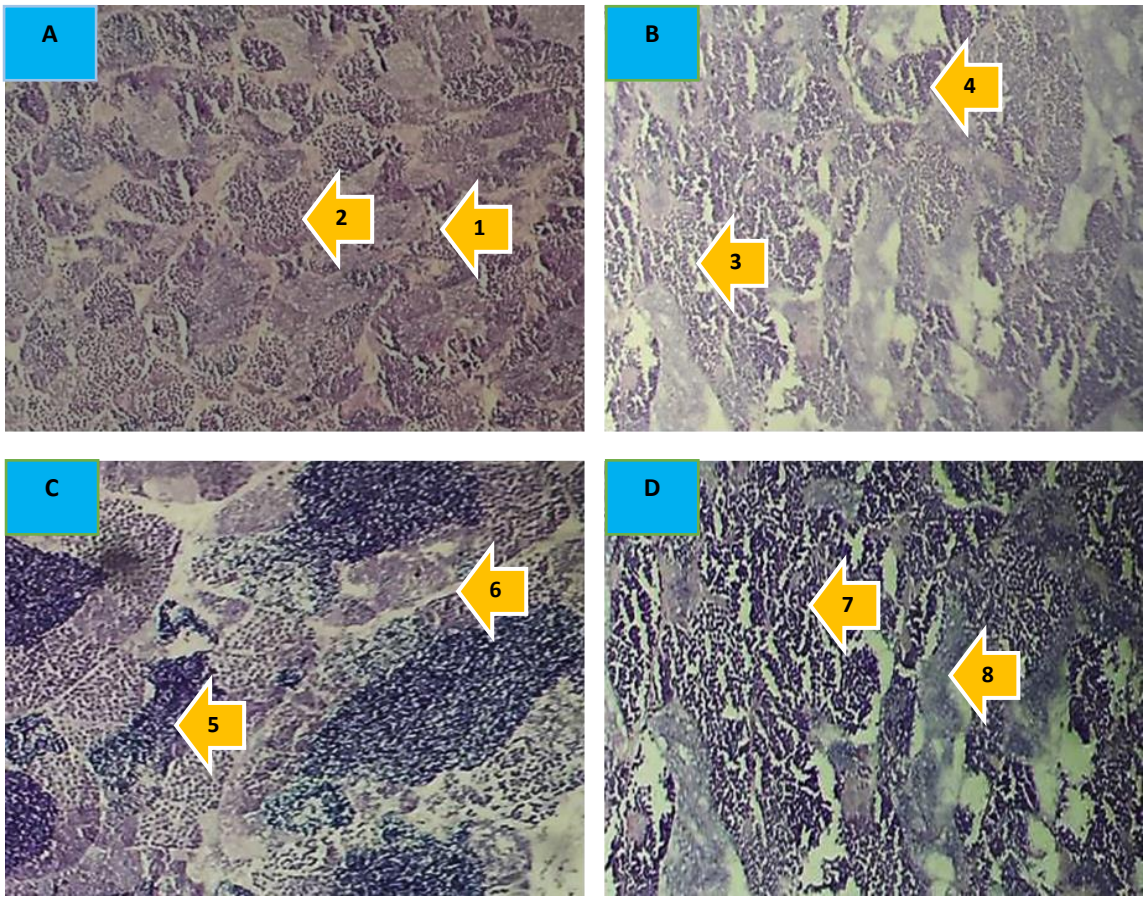

Figure 3.2. Histological sections of testes in golden rabbitfish

A: Stage II testes, B: Stage III testes, C: Stage IV testes, D: Stage V testes.

3.2. HSI

In this study, HSI in female fish changed during sampling time. Specifically, during the breeding season, HSI had a significant change ($P<0.05$) between March (1.69%) and June (1.14%), peaking in May (1.72%); During the period from March to May, the change in HSI was not statistically significant ($P>0.05$).

In males, the HSI varied significantly between sampling months. The HSI value reached the maximum in January (1.77%) and the lowest in April (1.01%).

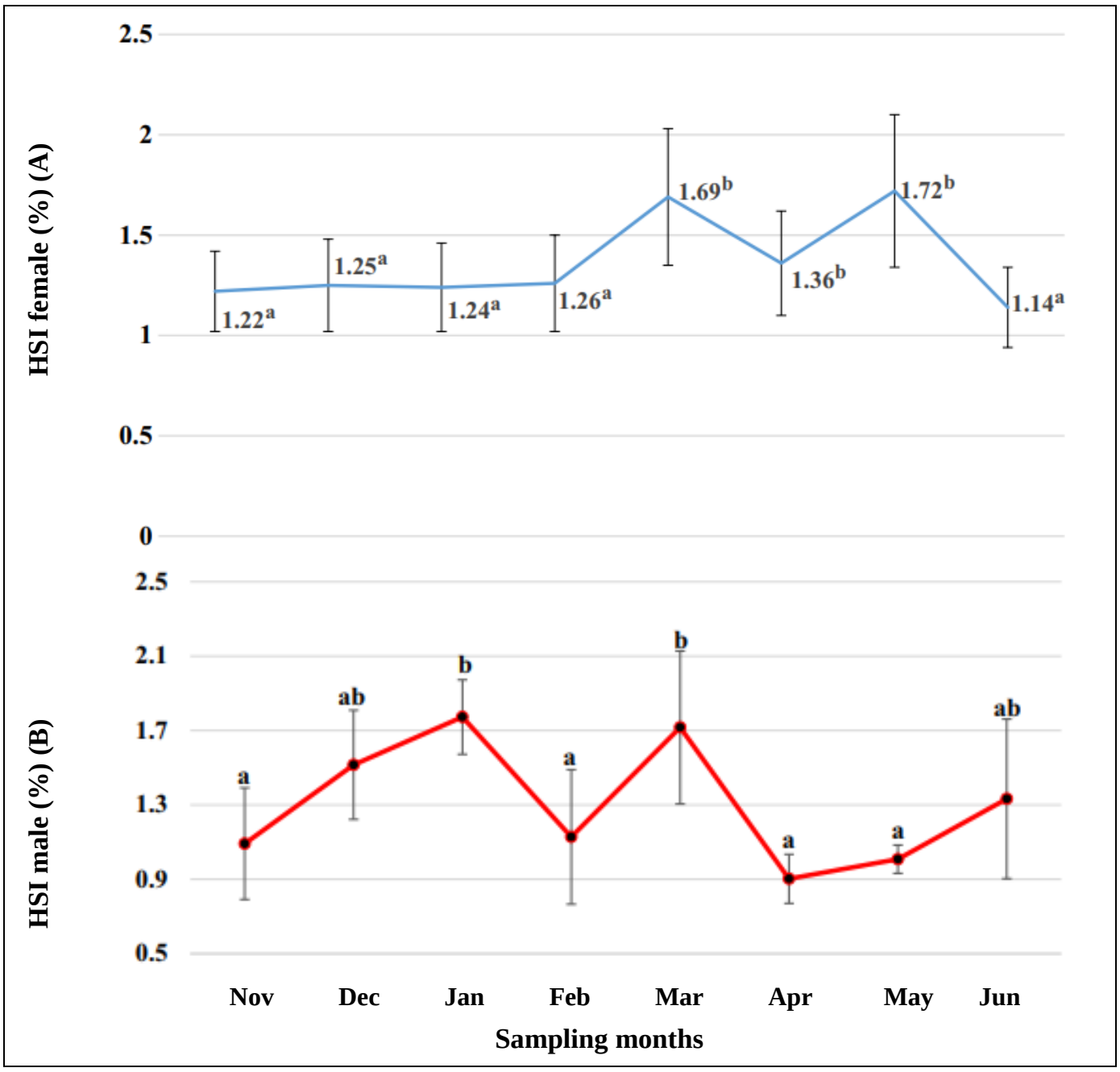

Figure 3.3. HSI in monthly changes

Different superscripts against each value (data point) indicate significant differences ($p < 0.05$)

In females, the highest HSI values (1.9%) were observed during the yolk accumulation phase (Stage III). For males, the highest HSI values (1.49%) were also observed during the spermatogenesis stage (Stage III).

In general, HSI fluctuations in female and male fish were relatively similar for each month of sampling as well as for the stages of ovarian development and spermatogenesis.

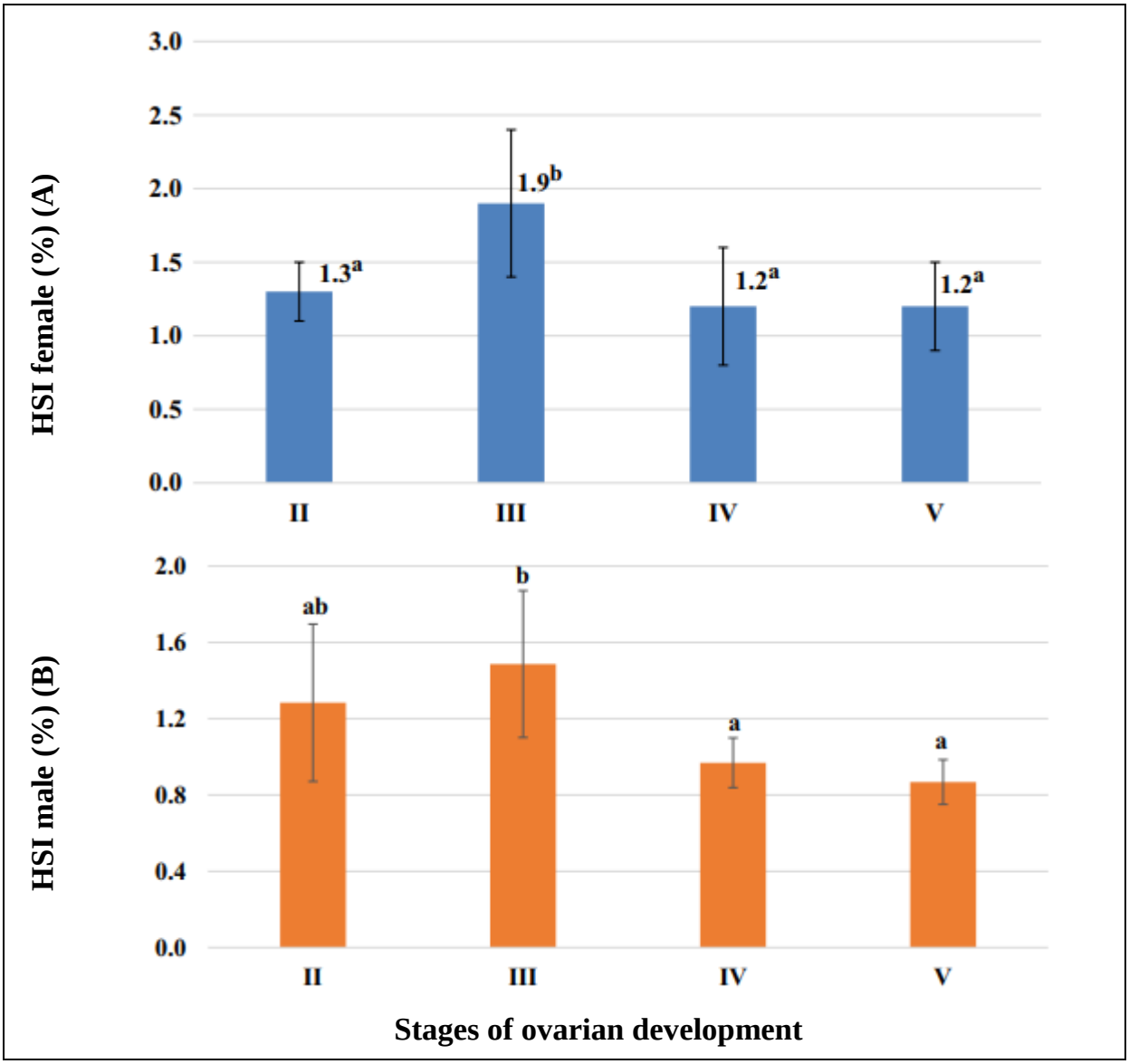

Figure 3.4. HSI in stages of ovarian development

Different superscripts against each value (data point) indicate significant differences ($p < 0.05$)

3.3. GSI

The results showed that the GSI in female barnacles fluctuated according to the calving cycle. Specifically, GSI was lowest in December (1.26%) and reached the highest value in June (3.58%). From November to March of the following year, there was no statistically significant difference (P>0,05), ranging from 1.25% to 1.49%. However, from April to June, GSI increased significantly, the highest value recorded was 3.58%.

GSI on females in the months 4, 5, and 6 was higher and showed a statistically significant difference (P<0.05) compared with the remaining months during the sampling period, because this is the spawning season. the main product of the year of scorpion fish.

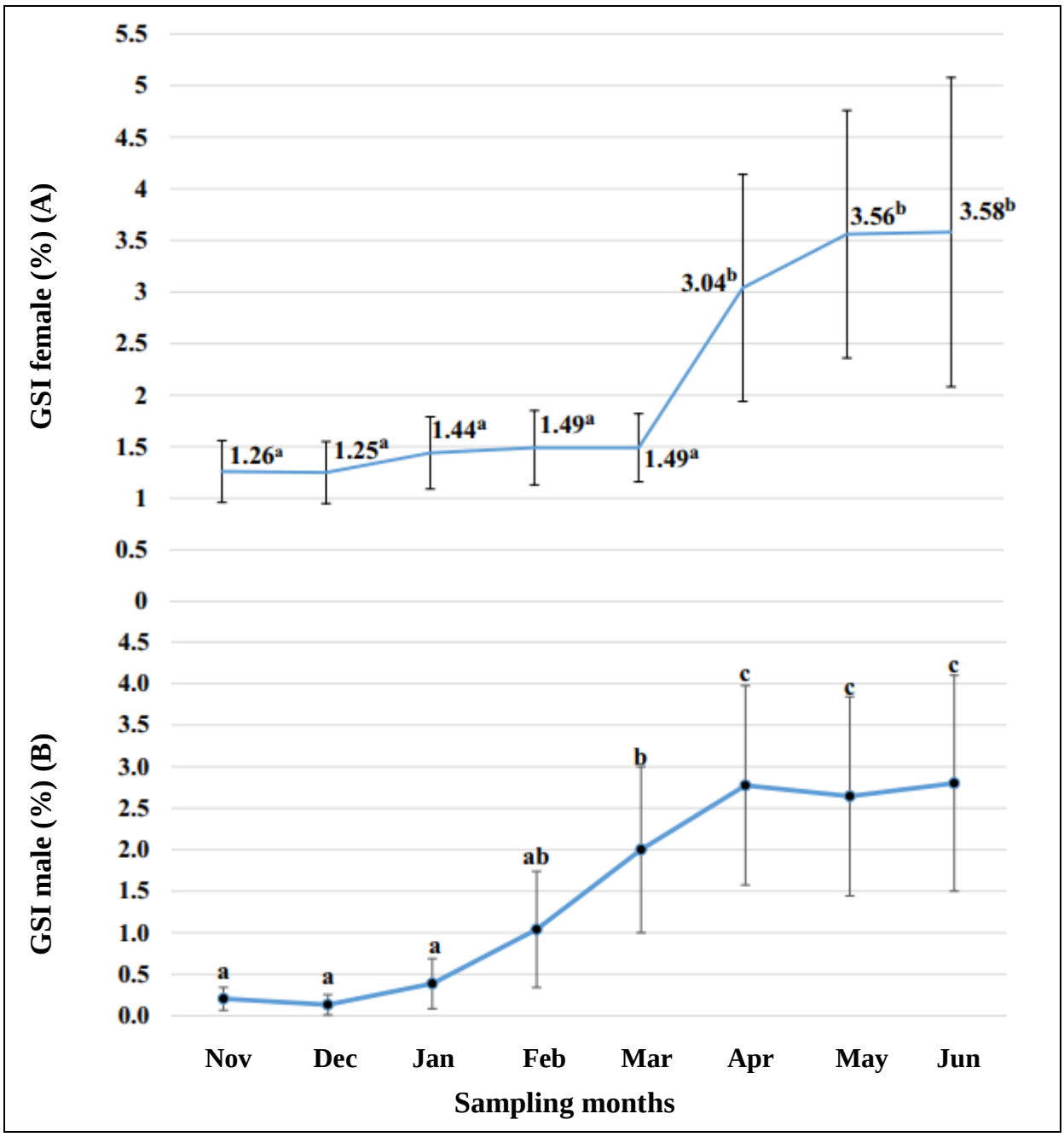

Figure 3.5. GSI in monthly changes

Different superscripts against each value (data point) indicate significant differences ($p < 0.05$)

The GSI value of male fish fluctuates with the reproductive cycle in a similar way to that of female fish. GSI increased continuously from December to April and remained at a high level until the end of June. The average GSI value of males over the period of the 8-month study ranged from 0.13% ± 0.12% to 2.77% ± 1.94% (Figure 3.5A).

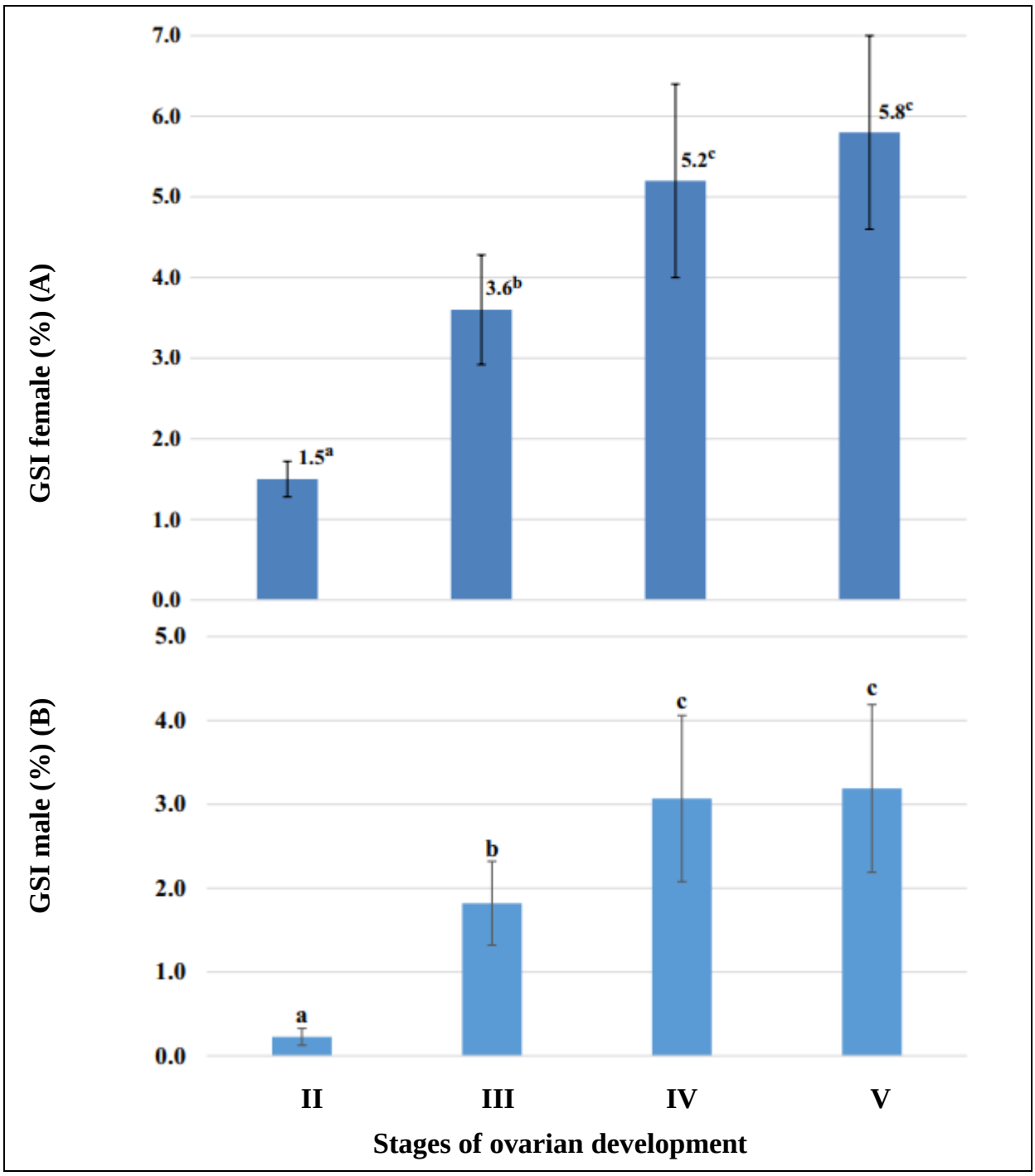

Figure 3.6. GSI in stages of ovarian development

Different superscripts against each value (data point) indicate significant differences ($p < 0.05$)

For female fish, GSI increased from stage II (growth) to stage V (ripening and ovulation) peaked at 5.89%.

In males, GSI also increased continuously from stage II (immature stage) to stage V (reproduction), reaching the highest level in stage V (3.19%) and lowest in stage II (0.23%) (Figure 3.6B).

In general, the trends of HSI and GSI fluctuations at different stages of gonadal development are opposite. Specifically, HSI values increased from stage II to stage III, then gradually decreased from stage III to stage V (Figure 3.4). Meanwhile, GSI increased continuously from stage II to stage V (Figure 3.6).

3.4. Fluctuations in concentrations of steroid hormone in plasma

3.4.1. Variation of E_2 content in female

The E_2 concentration in the blood plasma of rabbitfish) showed a statistically significant difference ($P<0.05$) between (the) months of the year (Figure 3.7). In June, the E_2 concentration in blood plasma was at its highest (1.445,62 pg/ml).

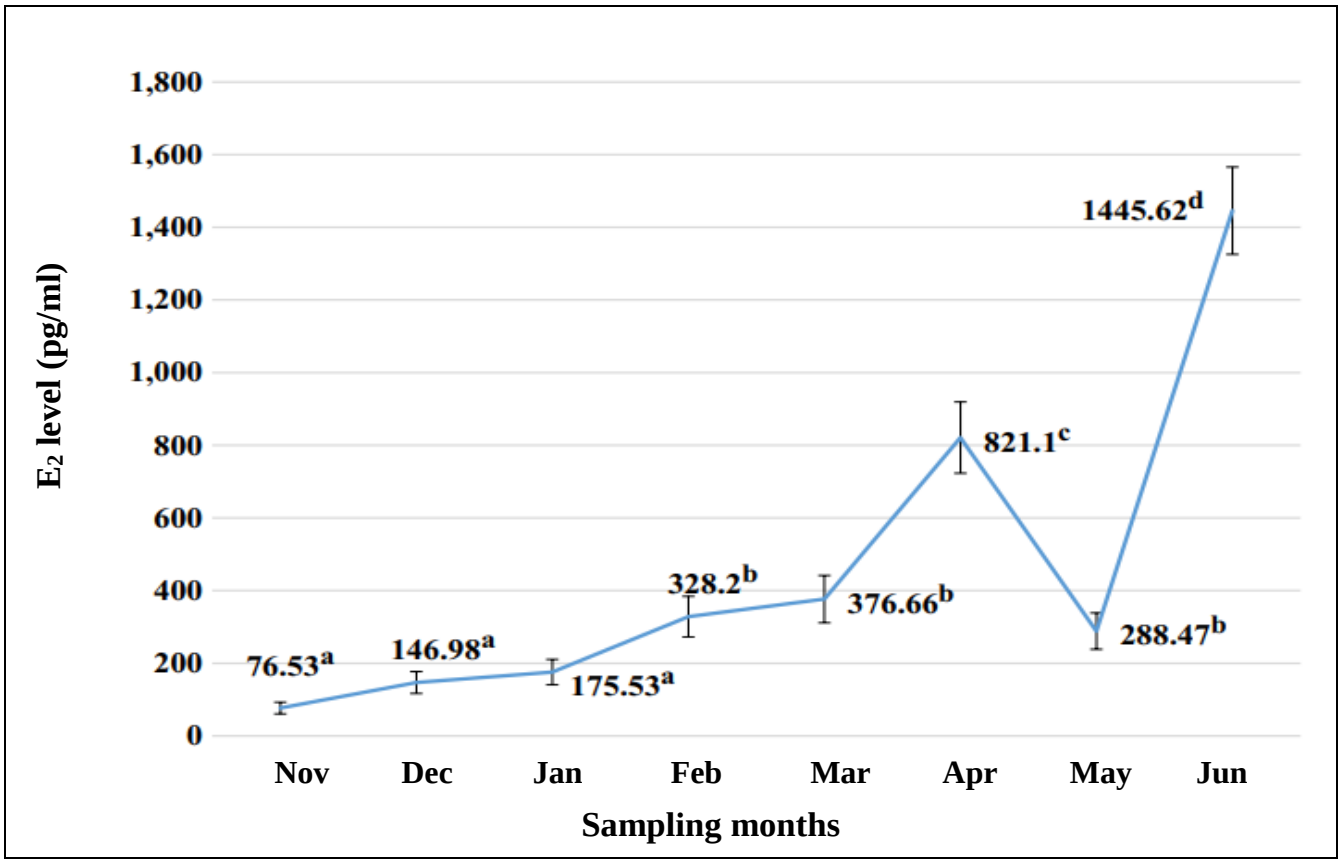

Figure 3.7. Monthly changes in plasma E_2 level (pg/ml) of female rabbitfish during the reproductive season

Different superscripts against each value (data point) indicate significant differences ($p < 0.05$)

Research showed that E_2 in the blood plasma had a fairly clear relationship with the stages of ovarian development. E_2 concentration in plasma varied with ovarian stages, and the highest E_2 levels (2.305 pg/ml) were observed in stage III.

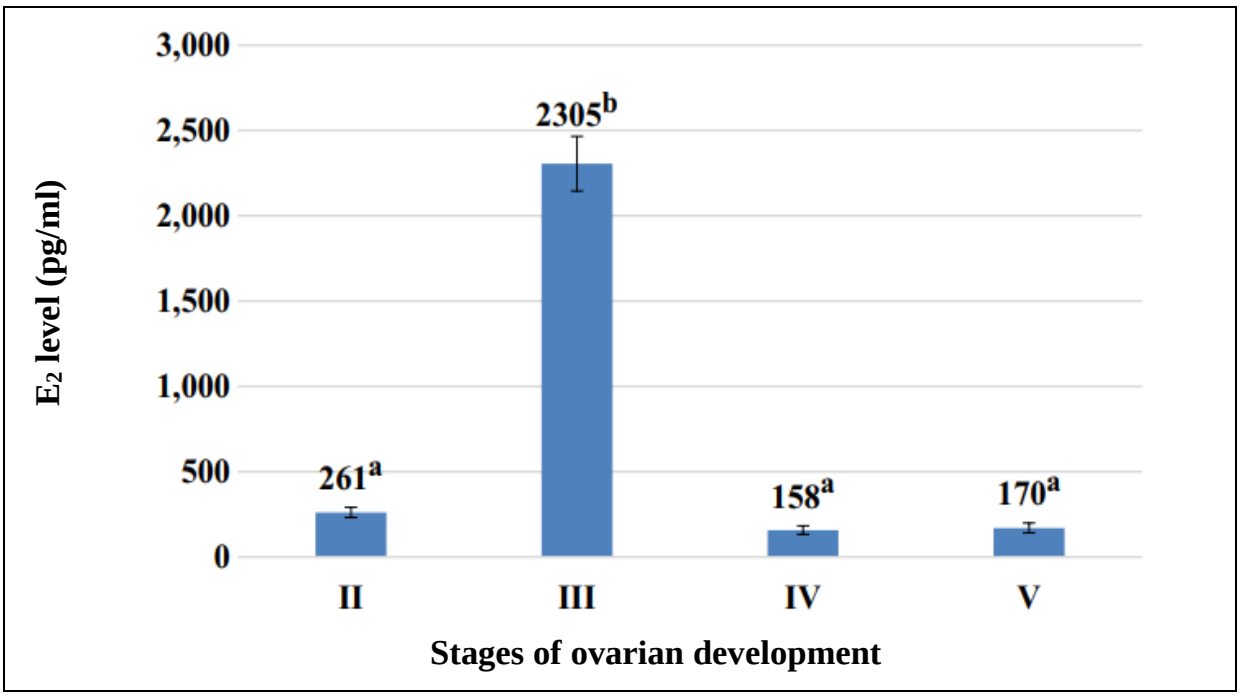

Figure 3.8. Relationships between stages of ovarian development and E_2 levels (pg/ml) in female rabbitfish

Different superscripts against each value (data point) indicate significant differences ($p < 0.05$)

3.4.2. Relationship between E_2 and HSI, GSI and ovarian development

The relationship between E_2 and HSI, GSI and ovarian development observed in this study has made a better understanding of the reproductive cycle of rabbitfish in captivity. Research results have shown that there is a relationship between plasma E_2 levels and oocyte development stages, GSI and HSI in female rabbitfish.

3.4.3. Variation of T and 11-KT concentrations in male rabbitfish

The concentration of T in the blood of redhead fish changed according to the months of the year (Figure 3.9). T values are low during spring from November to January. In summer, T concentrations increase and peak in June (301.986 pg/ml). Specifically, the T concentration gradually decreased from November (117.922 pg/ml) to January (76.907 pg/ml). Then it increased again in February and March (214.918 pg/ml, 202.050 pg/ml) peaking in June. During the period from November to January fish participated in the spawning of the spawning season. Before that, GSI decreased and started the 2nd spawning season from February to June, increased sharply in May and June, during this time fish accumulate nutrients again to mature and participate in spawning. In February and March, fish begin to participate in spawning. The T content increased, but in April the T concentration decreased.

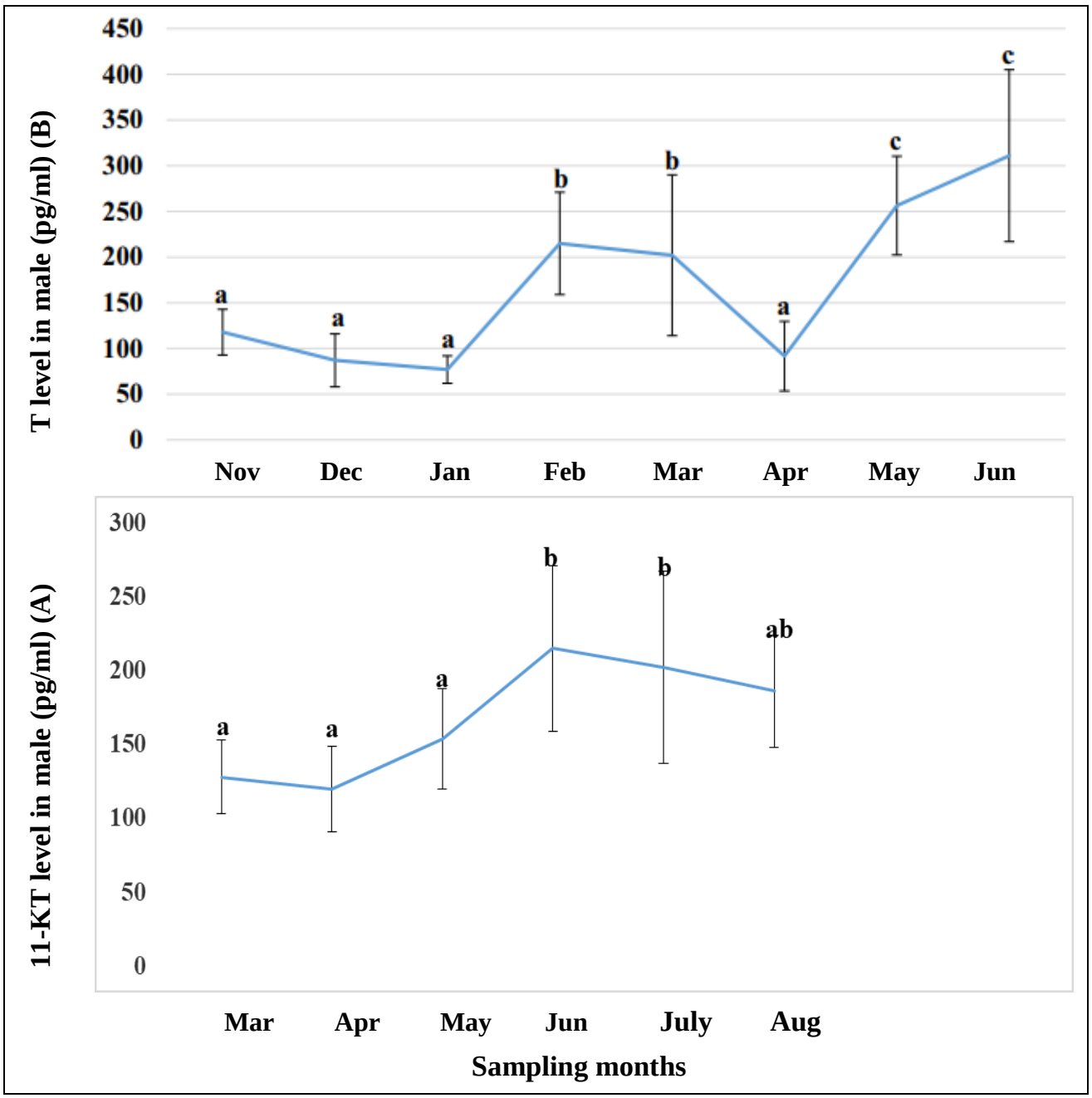

Figure 3.9. Monthly changes in plasma T and 11-KT levels of male golden rabbitfish during the reproductive season

Different superscripts against each value (data point) indicate significant differences ($p < 0.05$)

In this study, the highest plasma T concentration in stage III showed that T stimulated spermatogenesis in male rabbitfish (Figure 3.9B).

Alike T, 11-KT is a typical male steroid hormone. In this study, the fluctuations of 11-KT content during the year were quite similar to that of T. 11-KT with its highest in June (215 pg/ml) and lowest in April (120 pg/ml) (Figure 3.9A).

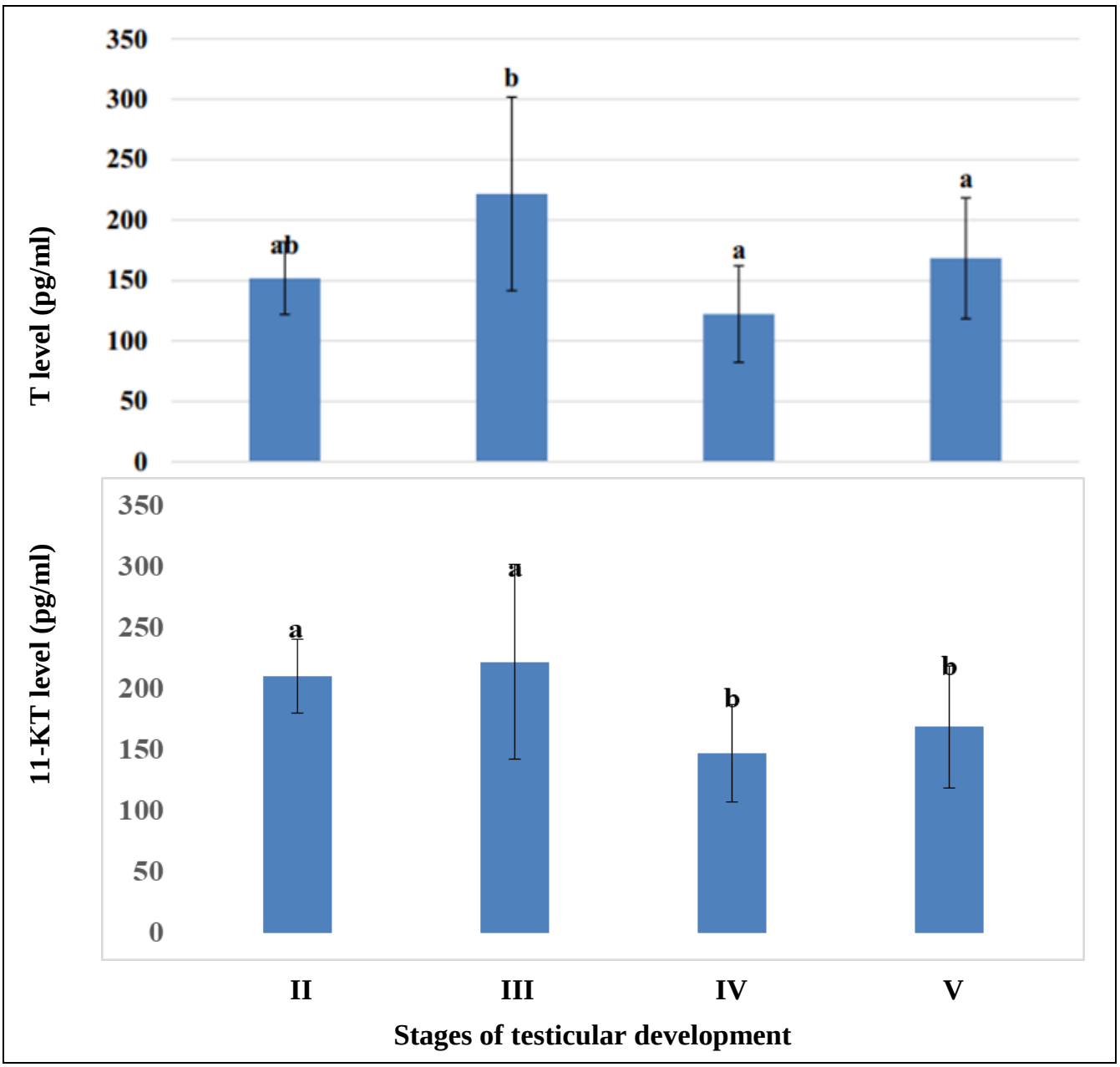

Figure 3.10. Relationship between stages of testicular development and plasma T, 11-KT levels in male golden rabbitfish

Different superscripts against each value (data point) indicate significant differences ($p < 0.05$)

The highest T and 11-KT values observed in stage III were 221.7 pg/ml and 222 pg/ml, respectively; the lowest in stage IV, with 122.3 pg/ml and 147 pg/ml.

3.4.4. Relationship between T, 11 - KT with HSI, GSI and ovarian development

The immature males observed in November to February were associated with low T levels. The concentration of T from the blood plasma of the male rabbitfish remains high in the months of March, May and June. When male rabbitfish reach the spawning and post-reproductive stages (stages IV-V), the T concentration in their blood drops dramatically and remained low. From our data, it can be concluded that there is a relationship between the plasma T content and the developmental stages of sperms, GSI and HSI in male rabbitfish.

3.5. Effects of hCG, LHRH – A on plasma E_2 and T concentrations

3.5.1. E_2 content fluctuations under the influence of hCG and LHRH-A in female rabbitfish

In the experiment using hCG and LHRH – A + DOM, we noticed significant changes in E_2 content. For 12 hours after injection, E_2 levels reached the highest, 2.567 ± 192 and 524 ± 33 pg/ml in hCG and LHRH – A + DOM treatments, respectively. (Table 3.1).

Table 3.1. Changes in plasma estradiol-17β levels (pg/ml) in female golden rabbitfish (*Siganus guttatus*) injected with 1.500 IU hCG/kg and 50 µg D-Ala^6, Pro^9- Net-mGnRH/kg plus 5 mg DOM

Time interval	Treatment group		
	Control (saline)	1.500 IU hCG	50 µg D-Ala^6, Pro^9- NetmGnRH plus 5 mg DOM
Before injection	81 ± 26^a	81 ± 26^a	81 ± 26^a
6 hr post injection	48 ± 5^a	48 ± 8^a	61,93 ± 3^a
12 hr post injection	84 ± 15^a	2.567 ± 192^c	524 ± 33^b
18 hr post injection	39 ± 11^a	354 ± 22^b	246 ± 21^b
24 hr post injection	21 ± 3^a	82 ± 13^a	28 ± 3^a

Different superscripts against each value (data point) indicate significant differences ($p < 0.05$)

3.5.2. Variation of T content under the influence of hCG and LHRH-A in male rabbitfish

In male rabbitfish, T content changed significantly after hCG and LHRH – A + DOM injection. In the hCG treatment, the plasma T concentration reached its peak 24 hours after injection (190 pg/ml). T reached the maximum value when injecting LHRH – A + DOM after 6 hours of injection (650 pg/ml).

Table 3.2. Changes in plasma Testosterone levels (pg/ml) in male golden rabbitfish (*Siganus guttatus*) injected with 1.500 IU hCG/kg and 50 µg D-Ala6, Pro9- Net-mGnRH/kg plus 5 mg DOM

Time interval	Treatment group		
	Control (saline)	1.500 IU hCG	50 µg D-Ala^6, Pro^9- NetmGnRH plus 5 mg DOM
Before injection	92 ± 39^a	92 ± 39^a	92 ± 39^a
6 hr post injection	59 ± 10^a	170 ± 12^b	650 ± 102^c

12 hr post injection	66 ± 10^{a}	135 ± 10b	648 ± 118^{c}
18 hr post injection	82 ± 6^{a}	149 ± 3^{b}	490 ± 62^{b}
24 hr post injection	37 ± 4^{a}	190 ± 5^{b}	339 ± 39^{b}

Different superscripts against each value (data point) indicate significant differences ($p < 0.05$)

3.6. Effects of hCG, LHRH - A on reproductive physiology and biochemical composition of spermatids and ovaries

3.6.1. Fertility and oocyte size of experimental fish

Fertility is one of the important reproductive biological indicators. Thereby, we can estimate the number of eggs laid each time, thereby building a breeding plan close to reality and requirements. The results of our fertility determination on female fish with weights ranging from 440 to 600 grams are shown in Table 3.3. In this study, the absolute fecundity of rabbitfish ranged from 420.632 to 732.353 eggs, corresponding to the relative fecundity ranging from 826 to 1.221 eggs/g female.

Table 3.3. The reproductive capacity of the study

Serial	Length and weight	Absolute fecundity (agg III-IV/female)	Relative fecundity (agg/gram female)
1	29 cm; 440 g	420.632	956
2	31 cm; 580 g	478.943	826
3	30 cm; 600 g	732.353	1.221
Average ± SD		**543.976 ± 165.724**	**1.001 ± 201**

3.6.2. Oocyte size of experimental fish

During the main breeding season, the size of the oocyte grows very rapidly, reaching its maximum size and reaching full maturity. In the nucleus phase and pre-peripheral chromatin (corresponding to the stage I of the ovary) the smallest size (53 µ); phase of polarization, maturation, and ovulation (corresponding to stage V of the ovary), the oocyte reaches the largest size (371 µ) (Table 3.4).

Table 3.4. The size of the oocytes of the experimental fish

Serial	Oocyte development stages	Diameter (µ)
1	Phase nucleus and pre-perinuclear chromatin	53 ± 12
2	Phase perinuclear chromatin	82 ± 18
3	Phase vacuolation	148 ± 40
4	Phase yolk accumulation	364 ± 40
5	Phase polarization, maturation and ovulation	371 ± 52

3.7. Effects of hCG, LHRH - A on the biochemical composition of sperms and ovaries

3.7.1. Effects of hCG, LHRH - A on the biochemical composition of stilts spermatids

3.7.1.1. Biochemical composition of spermatids in mature and immature stages

The results showed that the protein and ash content of spermatozoa in the mature stage was higher than that in the immature stage. In contrast, the lipid content in the mature stage was lower than that in the immature stage. Meanwhile, the moisture content does not differ much between the two stages of spermatogenesis.

Table 3.5. Biochemical composition of 1+ year-old pole bream reared in earthen ponds in Khanh Hoa

Targets	Testicular (III)	Testicular (IV-V)
Protein (%)	13.60 ± 1.30	14.80 ± 1.20
Lipid (%)	13.14 ± 0.60	11.91 ± 0.50
Tro (%)	0.90 ± 0.02	1.69 ± 0.06
Moisture (%)	75.59 ± 2.80	79.23 ± 2.40

3.7.1.2. The influence of hormones on the biochemical composition of rabbitfish

The male fish prior to injection were randomly dissected 10 fish to assess the maturity of the sperm. The results showed that the male spermatozoa were identified at stages III, IV, and V. All male fish sperm were moved to stage V after 12 and 24 hour injections. This showed that hCG and LHRH – A + DOM has an effect on the complete maturation of the spermatids after 12 hours.

Table 3.6. The level of maturity of the male shark after hormone injection

Time interval	Treatment group		
	Control (saline)	hCG	50 µg D-Ala6, Pro9-NetmGnRH plus 5 mg DOM
Before injection	Stages III, IV và V		
12 hr post injection	Stages V	Stages V	Stages V
24 hr post injection	Stages V	Stages V	Stages V

Table 3.7. Effect of hCG on the biochemical composition of sperm (Stages IV-V)

Time interval	Biochemical ingredients			
	Moisture (%)	Tro (%)	Protein (%)	Lipid (%)
Before injection	79.23 ± 0.10^{a}	1.69 ± 0.10^{a}	14.80 ± 0.05^{a}	11.91 ± 0.10^{b}
12 hr post injection	67.25 ± 0.10^{b}	1.49 ± 0.10^{a}	18.66 ± 0.05^{b}	6.96 ± 0.10^{a}
24 hr post injection	59.56 ± 0.10^{b}	1.55 ± 0.10^{a}	16.25 ± 0.05^{b}	8.27 ± 0.10^{a}

Different superscripts against each value (data point) indicate significant differences ($p < 0.05$)

Protein content was highest after 12 hours of injection (18.66%), and lipid content decreased compared to before injection. (Table 3.7).

Similarly, when analyzing the biochemical composition of the sperm of the rabbitfish in the treatment of 50 µg LHRH-A + 5 mg DOM/kg fish, it showed that the content of ash, protein and lipid were all reduced compared to before injection (Table. 3.8).

Table 3.8. Effects of LHRH-A + DOM on the biochemical composition of the sperm (Stages IV-V)

Time interval	Biochemical ingredients			
	Moisture (%)	Tro (%)	Protein (%)	Lipid (%)
Before injection	79.23 ± 0.10^{a}	1.69 ± 0.10^{a}	14.80 ± 0.05^{a}	11.91 ± 0.10^{a}
12 hr post injection	77.65 ± 0.10^{a}	1.57 ± 0.10^{a}	14.61 ± 0.05^{a}	9.22 ± 0.10^{a}
24 hr post injection	72.32 ± 0.10^{b}	1.62 ± 0.10^{a}	12.44 ± 0.05^{b}	10.26 ± 0.10^{a}

Different superscripts against each value (data point) indicate significant differences ($p < 0.05$)

3.7.2. Effects of hCG, LHRH – A on biochemical composition of the fish ovary

3.7.2.1. Biochemical composition of the ovary in mature and immature stages

Table 3.9. Biochemical composition of 1+ year old fish ovary reared in an earthen pond in Khanh Hoa before sex injection

Targets	Ovary (III)	Ovary (IV-V)
Protein (%)	21.74 ± 0.05	28.33 ± 0.05
Lipid (%)	28.83 ± 0.10	23.82 ± 0.10
Tro (%)	0.32 ± 0.10	0.9 ± 0.10
Độ ẩm (%)	67.91 ± 0.10	60.86 ± 0.10

3.7.2.2. Assess the maturity level of fish after hormone injection

The ovaries of the fish studied before hormone injection were assessed to have uneven maturity. The ovaries of the female fish were captured in stages II, III and IV.

After 12 and 24 hours of hormone injection, there was no difference in the maturity of the ovaries in all treatments.

Table 3.10. The maturity level of female fish after hormone injection

Time interval	Treatment group		
	Control (saline)	**1.500 IU hCG**	**50 µg D-Ala6, Pro9-NetmGnRH plus 5 mg DOM**
Before injection	Stages II, III và IV		
12 hr post injection	Stages III	Stages V	Stages II, III
24 hr post injection	Stages III, IV	Stages II, IV	Stages II, III

3.7.2.3. Changes in biochemical composition in the ovary of the fish (stage IV)

Table 3.11. Changes in biochemical composition in the ovary of the fish (stage IV) after injection of 1.500 IU hCG/kg female fish

Time interval	Biochemical ingredients			
	Moisture (%)	**Tro (%)**	**Protein (%)**	**Lipid (%)**
Before injection	$67,91 \pm 0,1^{a}$	$0.32 \pm 0,1^{a}$	$21,74 \pm 0,05^{a}$	$28,83 \pm 0,1^{b}$
12 hr post injection	$65,40 \pm 0,1^{a}$	$0,75 \pm 0,1^{a}$	$37,90 \pm 0,05^{b}$	$6,5 \pm 0,1^{a}$
24 hr post injection	$66,80 \pm 0,1^{a}$	$0,78 \pm 0,1^{a}$	$36,98 \pm 0,05^{b}$	$5,5 \pm 0,1^{a}$

Các ký tự khác nhau trong cùng một cột cho thấy sự khác nhau có ý nghĩa thống kê (p<0.05)

Analytical results show no difference in moisture and ash. However, there was a statistically significant difference (P< 0.05) in protein and lipid content 12 hours and 24 hours after the hormone injection (Table 3.11). Similarly, the biochemical composition of the ovary was also observed for LHRH-A + 5mg DOM injection (Table 3.12).

Table 3.12. Changes in biochemical composition in the fish ovary (Stage IV) after injection of 50 µg D-Ala6, Pro9-NetmGnRH plus 5 mg DOM female fish

Time interval	Biochemical ingredients			
	Moisture (%)	**Tro (%)**	**Protein (%)**	**Lipid (%)**
Before injection	$67,91 \pm 0,1^{a}$	$0.32 \pm 0,1^{a}$	$21,74 \pm 0,05^{a}$	$28,83 \pm 0,1^{b}$
12 hr post injection	$71,80 \pm 0,1^{a}$	$0,79 \pm 0,1^{a}$	$36,89 \pm 0,05^{b}$	$6,4 \pm 0,1^{a}$
24 hr post injection	$58,80 \pm 0,1^{a}$	$0,72 \pm 0,1^{a}$	$33,95 \pm 0,05^{b}$	$8,1 \pm 0,1^{a}$

Các ký tự khác nhau trong cùng một cột cho thấy sự khác nhau có ý nghĩa thống kê (p<0.05)

The analysis results showed that there was no difference in moisture content

and ash ($P>0.05$) before and after the female fish were injected with hCG. However, the protein and lipid content had a statistically significant difference ($P<0.05$) at 12 hours and 24 hours after the hormone injection (Table 3.11). Similarly, the biochemical composition of the fish ovary was also observed for LHRH-A + 5mg DOM injection, the results showed that the protein and lipid content had statistically significant differences (Table 3.12).

CONCLUSIONS AND RECOMMENDATIONS

1. CONCLUSION

The concentrations of E_2, T, and 11-KT in the blood plasma fluctuated with the reproductive cycle and had a relationship with the developmental stages of the gonads, HSI and GSI. In females, the E_2 concentration reached the highest value (2.305 pg/ml) during the yolk accumulation phase (stage III). In males, the highest plasma concentrations of T and 11-KT were observed during spermatogenesis (stage III) with values of 221.7 pg/ml and 222 pg/ml.

Rabbitfish is a species that spawns many times a year, the spawning season is long. The gonadal learning organization is asynchronous, with many sex cells at different stages of development at the same time. GSI and HSI fluctuate significantly during the reproductive cycle. The HSI value increased from stage II to stage III, then gradually decreased from stage III to stage V. In contrast, GSI increased continuously from stage II to stage V.

Two exogenous hormones, hCG and LHRH - A, affect the E_2 content in female fish and T in male fish. When fish were injected with two hormones, plasma E_2 and T levels increased, promoting yolk sac formation in female fish and spermatogenesis in male fish.

When injecting two hormones hCG and LHRH - A, protein, lipid and moisture content (male); Proteins and lipids (female) had significant variation. When fish were injected with these two hormones, the protein levels in the testes and ovaries increased after 12 and 24 hours. This shows that exogenous hormones affect the change in the biochemical composition of the gonads, promoting sexual maturation in the fish.

2. RECOMMENDATION

During the spawning cycle of rabbitfish, in addition to the determination of E_2, T and 11-KT, studies of P during the spawning cycle are needed. In addition, the sampling process needs to supplement the remaining months of the year. Thereby, it shows us more clearly the correlation between E_2, T, 11-KT, and P in the reproductive cycle, as well as the relationship between GSI and HSI.

In the experiment of injecting hCG and LHRH - A, it is recommended to repeat many times and test at many different hormone concentrations to compare the effect of using as well as selecting the optimal hormone and dosage in artificial reproduction of rabbitfish.

On the basis of understanding the changes of steroid hormones in the spawning

cycle of scorpion fish, it is necessary to conduct studies on nutrition for broodstock and improve the efficiency of artificial reproduction of this subject.

In addition, further studies are needed on the relationship between plasma steroid hormone levels and the biochemical composition of gonads in order to clarify the role of plasma steroid hormone in the development of gonads in this region. particular and bony fish in general.

KEY FINDINGS

1. Research has shown that E_2, T and 11-KT concentrations in fish plasma fluctuate with the reproductive cycle and have a relationship with the development stages of gonads, HSI and GSI.

2. The histological organization of the gonads of the fish is asynchronous, with many developmental stages at the same time. GSI and HSI fluctuate significantly and inversely during the reproductive period. The HSI value increased from stage II to stage III, then gradually decreased from stage III to stage V. In contrast, GSI increased continuously from stage II to stage V.

3. The two exogenous hormone hCG and LHRH – A affect the E_2 content in female fish and T in male fish, significantly increase E_2 and T in gonads, promote egg production in female fish. and spermatogenesis in male rabbitfish.

4. hCG and LHRH – A affect the protein, lipid and moisture (female); protein and lipid (male), promoting sexual maturation in fish.

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LIST OF PUBLISHED ARTICLES

1. **Nguyễn Văn An**, Nguyễn Văn Minh, Phạm Quốc Hùng (2021), "Hàm lượng 11-Keto Testosterone huyết tương trong mùa sinh sản của cá dìa đực *(Siganus guttatus)*". *Tạp chí Khoa học – Công nghệ Thủy sản*, Số 2/2021, tr 2-7.
2. Pham, H. Q., Phan, U. V., **Nguyen, A. V**., Augustine Arukwe., Le, H. M., (2020), "Effects of human chorionic gonadotropin and gonadotropin releasing hormone analogue on plasma steroid hormones and spawning performances in golden rabbitfish *Siganus guttatus". Journal of Applied Ichthyology*, 2020;36:212–218.
3. Pham, H. Q., **Nguyen, A. V**., (2019), "Seasonal changes in hepatosomatic index, gonadosomatic index and plasma estradiol-17β level in captively reared female rabbitfi sh *(Siganus guttatus)". Aquaculture Research,* 50 (8): 2191-2199.
4. **Nguyễn Văn An**, Nguyễn Văn Minh, Phạm Quốc Hùng (2019), "Ảnh hưởng của hCG và LHRH – A lên thành phần sinh hóa của tinh sào cá dìa *(Siganus guttatus)*". *Tạp chí Khoa học – Công nghệ Thủy sản*, Số 3/2019, tr 3-8.
5. Phạm Quốc Hùng, Phạm Huy Trường, **Nguyễn Văn An** (2018), "Ảnh hưởng của hCG và LHRH – A lên đặc điểm sinh lý sinh sản cá dìa *(Siganus guttatus)*". *Tạp chí Khoa học – Công nghệ Thủy sản*, Số 3/2018, tr 38-43.