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CONTENT

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DOUBLE EXTIRPATION OF EYESTALK IN *SPIRALOTHELPHUSA HYDRODROMA* INDUCED GONADAL MATURATION

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ABSTRACT

The suitability of bilateral eyestalk ablation as a means of induced breeding technique in *S. hydrodroma* was tested under laboratory conditions by considering growth and survival indices, reproductive indices, biochemical and histological indices. The daily growth rate (g) was higher for ablated males (0.3±0.2) against 0.16±0.09 in control males. A positive correlation with feed intake of 0.71±0.04 (g) in ablated males against 0.62±0.84 in control males and a negative correlation with Hepatosomatic Index (%) of 7.02±2.4 against 13.77±3.2 are recorded. Female crabs shows less survival rate (%) compared to males (64±12.7/88±10.33). Ovarian and testicular index in comparison to control were found to be higher (0.2±0.08/0.15±0.02 and 2.6±0.33/1.08±0.06 respectively). Nutrient mobilization was evident from decreased muscular protein and carbohydrates as of hepatopancreatic lipid after extirpation and increased gonadal proteins, carbohydrates and lipids. The double extirpation procedure increased the number of oocytes, follicle diameter and number in females and an increase spermatozoa and spermatocytes in males as reveled by histological indices.

Keywords: Freshwater crab aquaculture, *S. hydrodroma*, Bilateral eyestalk ablation, Induced breeding.

INTRODUCTION

Crab induced breeding is a technique in which the economically important crabs are artificially cultured through pituitary injection or eyestalk ablation. Eyestalk ablation is a frequently adopted procedure for induced maturation of gonads. The eyestalk factors regulate the storage and mobilization of organic reserves that are utilized for moulting and reproduction (Liu and Laufer, 1998). Since the sinus glands are the sites that release gonad inhibiting hormone (GIH), and moult inhibiting hormone (MIH), the removal of eyestalks leads to an increased rate of gonadal development (Laufer *et al.,* 1993). The increased use of eyestalk ablation

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technique in the captive breeding of crabs has brought forth both positive and negative effects on the quality of spawning and seed production (Bray and Lawrence, 1992).

The freshwater crab, *Spirlothelphusa hydrodroma*, is an important human food source in various parts of South India. Aquaculture through induced breeding in this species is scarcely reported. However, eyestalk ablation is documented in other freshwater crabs including *Potamon persicum* by Khazraeenia and Khazraiinia (2009) to study the gonadal maturity, moulting and biochemical changes in the hemolymph of females. Unilateral and bilateral eyestalk ablation can accelerate moulting and promote gonad development and maturation (Snyder and Chang, 1986). Eye stalk ablation is regarded as an alternative approach to hormone-induced-breeding technique and is reported to cause rapid ripening of ovaries and a suitable technique to enhance meat yield in aquaculture of the crab *Barytelphusa lugubri.* Therefore, the present investigation focuses on the suitability of Bilateral Eyestalk Ablation (BEA) in S. *hydrodroma.*

MATERIALS AND METHODS

Collection and Maintenance of Crabs

Crabs were collected from Chinnaputhur lake, Tirupur district of Dharapuram, Tamil Nadu, India. The crabs were transported to the laboratory using aerated plastic bag and maintained in an aquarium with continuous aeration and optimum temperature. During this period, the crabs were fed with commercial feed and water was changed daily. They were acclimatized to the laboratory condition and maintained for one week prior to experiment.

Morphometric Indices

The morphometric indices such as carapace length, carapace width, major chelate legs, minor chelate legs and weight were recorded for male and female specimens. Male of 4.37 ± 0.802 length and 28.8 ± 4.509 in weight and females of 4.63 ± 0.321 in length and 29.84 ± 2.520 in weight were used for the study.

Eyestalk Ablation

The experimental crabs were divided into four groups. Two group was used for eyestalk ablation experiment and the other two group was kept as intact control. Bilateral eyestalk ablation was performed in male and female crabs by ablated both the eyes at its base with a fine and sterilised scissor. The wound was cauterized using a hot blunt needle in order to prevent the loss of haemolymph and mortality. Eyestalk ablated and non-ablated crabs were introduced into plastic tubs and 1gm of commercial pelletized feed was given once a day as a feed for both ablated and non-ablated crabs. Experiment was carried out after 10 days of ablation.

Sample preparation

Ablated crabs were kept in laboratory condition for ten days and the tissues of muscles, hepatopancreas, heart, testis and ovary was dissected out to study growth, survival, reproductive, biochemical indices and histological indices.

Growth and survival indices

a. Feed Intake

The ablated and non-ablated crabs were fed with 1gm of fresh commercial pelletized feed per day. The unfed were collected and measured after every 24hrs of feeding. The feed consumption of ablated and non-ablated crabs were calculated by using the following formula:

Feed intake (g) = Total feed offered (g) – feed Unfed (g)

b. Survival Rate

During the experimental period the mortality of the ablated and non-ablated male and female crabs were noted. By using the following formula the survival rate was calculated.

$$\text{Survival rate \%} = \frac{\text{total number of crabs} - \text{total number of dead crabs}}{\text{total number of crabs}} \times 100$$

c. Daily Growth Rate

Before eyestalk ablation the crabs were weighed and the initial weights of the crabs were measured. After 10 days of ablation the weight of the crabs were measured and it is considered as final weight which gained by the crabs during the experimental period. The Daily growth rate was calculated by using the following formula:

$$\text{Daily growth rate (g)} = \frac{\text{Final weight} - \text{initial weight}}{\text{No. of days}} \times 100$$

d. Hepatosomatic Index (HSI)

After 10 days of experimental setup both ablated and non-ablated male and female crabs were dissected out and hepatopancreas was removed and weighed. The HSI was determined using the following formula:

$$\text{HSI (\%)} = \frac{\text{Wet weight of the hepatopancreas}}{\text{weight of the crabs}} \times 100$$

e. Coagulation Time

The coagulation time was estimated by the following method, Mattson *et al.,* (1985) and Eddy *et al.,* (2007).

Samples (0.5 ml) of hemolymph were withdrawn by puncture of the perioarthrodal membrane at the base of the fourth walking legs (Hemolymph was placed on a glass slide and shook slowly until jellification). The time between hemolymph withdrawal and jellification was considered the coagulation time in minutes.

f. Heart Indices (HI)

The hearts were removed from the crabs by cutting along the dorsal surface just below the cuticle to ensure no perforation of tissue occurred. The incision was then carefully opened and the hearts were removed. Heart indices were determined by the standard formula

$$\text{Heart index (\%)} = \frac{\text{Wet weight of the heart}}{\text{Wet weight of the crab}} \times 100$$

g. Moulting Frequency

To observe the moult events, the number of moults was recorded daily in control and ablated males and females.

Reproductive indices

The colour of the ovary was recorded in ablated and non-ablated crabs.

a. Gonadosomatic Index (GSI)

After 10 days of experimental setup both the ablated and non-ablated male and female crabs were dissected out and gonads was removed and weighed. The GSI was calculated using the following formula:

$$\text{GSI (\%)} = \frac{\text{Wet weight of the gonad}}{\text{weight of the crab}} \times 100$$

Histological Indices

After an experimental period both eyestalk ablated and non ablated male and female crabs were dissect out and tissues of gonads were taken for histological studies.

Cross section of testis and ovary using a microtome sections were stained using a differential stain (H&E). Stained slides were viewed under light microscope of 40 x illumination and photomicrograph were taken for further investigation.

Biochemical indices

Gonadal tissues were examined for protein, carbohydrate and lipid quantatively. Muscle tissues were examined for protein and carbohydrate and hepatopancreas for lipids in controls and ablated crabs. Estimations were done following the standard methods: Roe *et al.,* 1955 for carbohydrates, Lowry *et al*., 1951 for proteins and Folch *et al.,* 1957 for lipids.

Statistical Analysis

The morphometric indices, growth and survival indices, reproductive indices, biochemical indices data were analysed by using SPSS statistics version 20 package software (Zar, 2009). Data were obtained as mean ± S.D of triplicate analysis.

RESULTS

Table 1: Morphometric indices of *S. hydrodroma*

Indices	Male	Female
CL (cm)	4.36±0.80	4.63±0.32
CW (cm)	4.33±0.39	5.13±0.25
Major chela (cm)	6.7±0.82	5.86±1.16
Minor chela (cm)	5.56±1.067	4.93±1.01
Weight (g)	28.8±4.51	29.84±2.52

Data obtained as a result of triplicate analysis expressed as Mean ± S.D.
All values are significant at P±0.05.

Table 2 : Growth and survival indices of *S. hydrodroma*

Indices	Male		Female	
	Control	Ablated	Control	Ablated
Feed intake (g)	0.62±0.84	0.71±0.04	0.3±0.09	0.425±0.2
Survival rate (%)	100±0.00	88±10.33	100±0.00	64±12.7
Daily growth rate (g)	0.16±0.09	0.3±0.2	0.05±0.00	0.12±0.1
Heart index (%)	3.14±0.1	4.02±1.7*	2.95±0.43	3.88±0.93
HSI (%)	13.77±3.2	7.02±2.4	13.2±0.13	11.21±2.4
Coagulation time (minutes)	8.9±0.8	40.9±2.42	12.8±1.34	47.8±2.5

Data obtained as a result of triplicate analysis expressed as Mean±S.D. values significant at P≤0.05
*Values not significant at P≤0.05.

The histological studies showed that the increased follicle diameter, follicle number and post-vitellogenic oocytes in ablated female (fig-4). In the control female, histological sectioning showed pre –vitellogenic oocytes (fig. 3). Ablated male histological section showed developed spermatozoa and matured spermatocytes in seminiferous tubules (fig. 2). In control male histological section

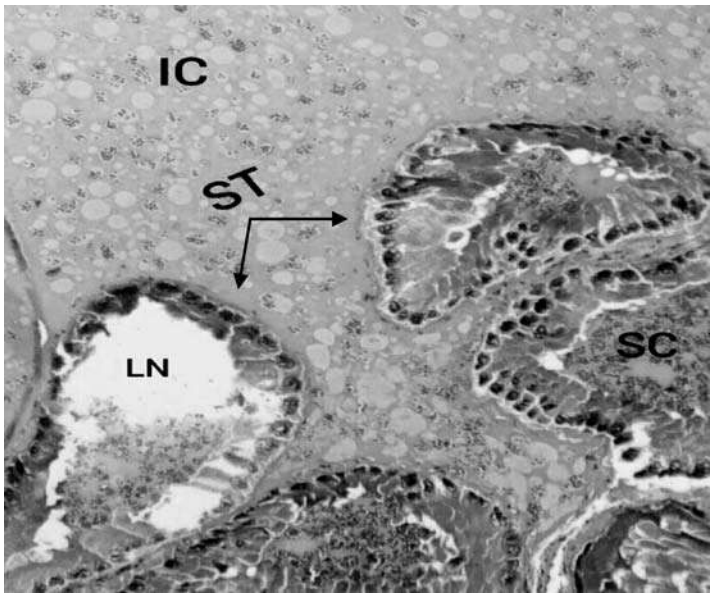

Fig. 1: Photomicrograph on cross section of control male *S. hydrodroma* showing developing spermatozoa and immatured spermatocytes in seminiferous tubules (X540). H and E stain.

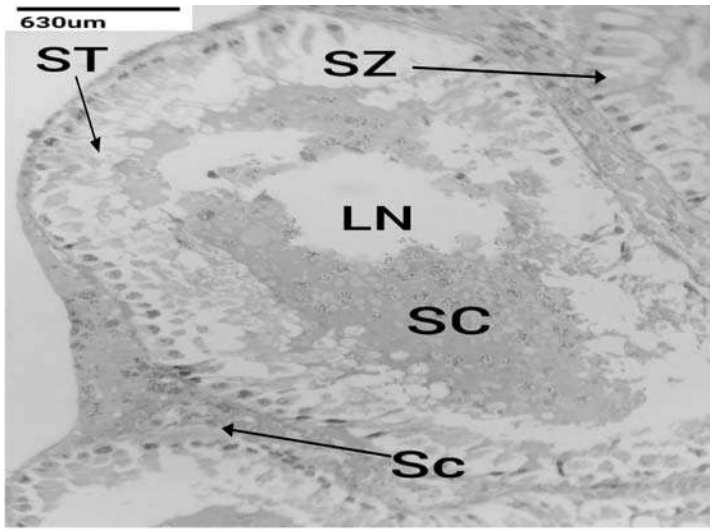

Fig. 2: Photomicrograph on cross section of ablated male *S. hydrodroma* showing developed spermatozoa and matured spermatocytes in seminiferous tubules (X340). H and E stain. IC- Interstitial Cell, ST- Seminiferous Tubules, LN- Lumen, SC- Sertoli Cells, Sz- Spermatozoa, Sc- Spermatocytes.

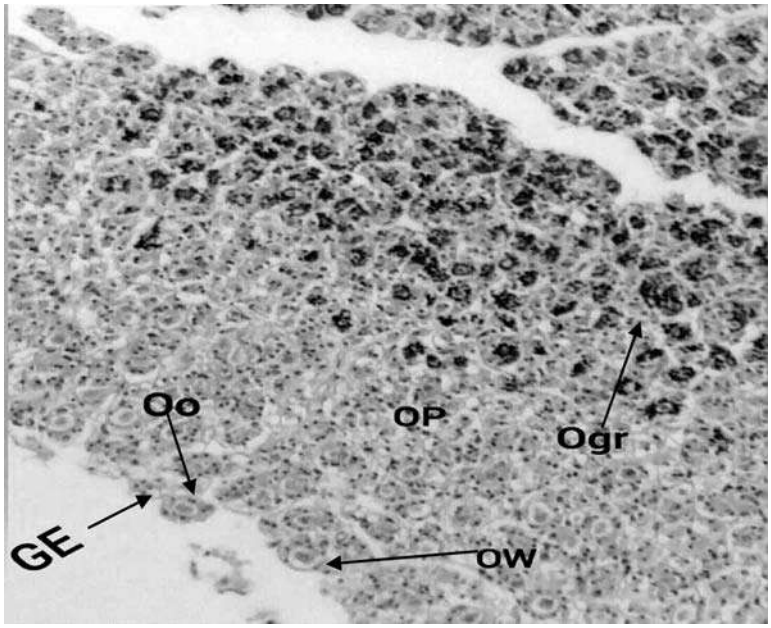

Fig. 3: Photomicrograph on cross section of control female *S. hydrodroma* showing pre-vitellogenic (primary) oocytes (X340). H and E stain.

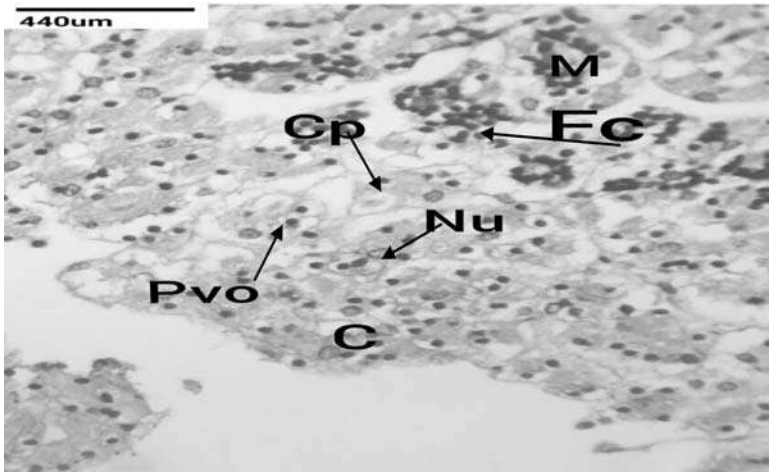

Fig. 4: Photomicrograph on cross section of ablated female *S. hydrodroma* showing increased follicle diameter, follicle number and post-vitellogenic oocytes (X340). H and E stain. Oo-Oocyte, OP- Oogenetic Pouch, Ogr-Ovarian granules, OW- Ovarian Wall, GE- Germinal Epithelium, Fo- Follicles, Cp-Cytoplasm, PVO- Post-vitellogenic oocyte, Nu-Nucleus, Ov-Ovum.

showed developing spermatozoa and immatured spermatocytes in seminiferous tubules (fig. 1). The colour of the ovary in ablated female crab was found to be dark yellow compared to the watery white colour of the ovaries in the control crabs.

Table 3: Reproductive indices of *S. hydrodroma*

Indices	Control female	Ablated female
Ovarian index	0.15±0.02	0.2±0.08*
Testicular index	Control male	Ablated female
	1.08±0.06	2.6±0.33

Data obtained as a result of triplicate analysis expressed as Mean± S.D. All values are significant at p≤0.05* values not significant at p≤0.05

Table 4: Muscle and hepatopancreatic biochemical indices of *S. hydrodroma*

Indices	Male		Female	
	Control	Ablated	Control	Ablated
Muscle carbohydrate (mg)	5.6±1.12	3.71±1.	4.43±1.2	1.6±0.71*
Muscle protein (mg)	141.2±1.2	103.2±1.2	138.3±1.1	121.3±0.8
Pancreatic lipid (mg)	87.5±1.10	81.4±10	91.52±1.2	87.6±0.9

Data obtained as a result of triplicate analysis expressed as Mean± S.D. All values are significant at P≤0.05.*Values not significant at P≤0.05

Table 5: Gonadal biochemical indices of *S. hydrodroma*

Indices	Male		Female	
	Control	Ablated	Control	Ablated
Protein (mg)	19.31±1.17	28.21±1.0	32.22±0.80	41.42±0.80
Carbohydrate (mg)	92.9±0.92	247±1.13	91.09±1.1	165±1.22
Lipid (mg)	29.21±0.87	32.4±1.04	32.09±0.9	34.15±0.90

Data obtained as a result of triplicate analysis expressed as Mean± S.D. All values are significant at P≤0.05.

Moulting Frequency

Throughout the experimental trial of 10 days, control as well as ablated male and female crabs *S. hydrodroma* were in interphase stage and no moulting stage was observed.

DISCUSSION

In the present study, the daily feed intake of bilateral eyestalk ablated male group (0.71±0.043) and female group (0.425±0.19) were significantly increased, when compared to control male group (0.62±0.84) and female group (0.30±0.01) and the results are in contrast to the reports of Venkitraman *et al*., (2004), who assumed that bilateral eyestalk ablation decreased the food consumption rate significantly when compared to the control.

It has been observed, the daily growth rate was enhanced in bilateral eyestalk ablated male group (30%) and female group (12%), when compared to control male group (16%) and control female group (5%), and the results are similar to the reports of Varalakshmi and Reddy (2010).

In bilateral eyestalk ablation, percentage survival rate was decreased in male group (88%) and female group (64%) when compared to control male group (100%) and female group (100%) in comparison to Primavera *et al.,* (1978); Radhakrishnan and Vijayakumaran, (1984); Koshio *et al.,* (1992) reporting, shrimps and other crustaceans eyestalk ablation resulted in heavy mortality. Pervaiz (2011) stated that, "mortality was directly related to the degree of ablation."

In the present investigation, the weight of the gonad increased significantly in ablated male (2.6±0.33) and female (0.2±0.8) crabs than control male (1.08±0.06) and female (0.15±0.02) crabs, in comparison to Wang *et al.,* (1995) claims that, "as organ-sinus gland complex is destroyed, gonad development inhibition is relieved, gonad nutrient accumulation is promoted and gonad maturation is accelerated."

Gonadosomatic index was enhanced in BEA crabs due to the increase in weight of the gonad after eyestalk ablation and simultaneously decreased in hepatosomatic index. The GSI were inversely proportional to the HIS. These results were similar to the reports of Khazraeenia and Khazraiini (2009) showing the indicative utilisation of these reserves in the tissue synthesis. The hepatosomatic index was decreased in BEA males (7.02±2.4) compared to control males (13.77±3.22) and also BEA females (11.21±2.4) compared to control females (13.2±0.13). A similar trend was observed by Aiken (1980) with depletion of hepatopancreas reserves due to the eyestalk ablation in *H. americanus*.

The present study resulted with large dark yellow colored ovaries with average weight of 0.16±0.0058 g in double extirpated crabs but in the controlled sets ovaries were watery white in colour with average weight of 0.017±0.003g. A similar result was found by Rana, (2018) in all crabs except controlled, moulted within 20 and 40 days and bilaterally ablated with large dark yellow colour ovaries with average weight 0.16g, and unilaterally ablated with dark creamy ovaries of average weight 0.08g and controlled sets with average weight 0.04g. A similar trend reporting that, eyestalk ablation in *Scylla serrata* helped to speed up the maturation of the ovary corroborate with present findings (John and Sivadas, 1978)

The eyestalk hormones are known to regulate the carbohydrate, protein and lipid metabolism in crustaceans (Highnam and Hill, 1979). The carbohydrate content in muscles was decreased due to BEA of both male and female crabs and increased in gonads compared to the control. There was decreased carbohydrate content in BEA males (3.71±1) and females (1.6±0.1) compared to the control males (5.6±1.17) and females (4.43±1.20). These results are contradictory to the reports of Varalakshmi and Reddy, (2010), assuring that the eyestalk ablations resulted in the reduction in the carbohydrate content of hepatopancreas and increased in the case of ovary. The carbohydrate content in gonads was increased due to eyestalk ablation. However, Soundrapandian and Ananthan (2008) reported no significant change in carbohydrate content following eyestalk ablation in *M. malcomsoni*. A similar trend was investigated by Khazraeenia and Khazraiinia, (2009), concluding that, due to direct removal of eyestalk, which is a major source of crustacean hyperglycemic hormone (CHH) resulted in lowered muscle carbohydrate. CHH is responsible for glucose metabolism by simulating the glycogenolysis in muscles and the hepatopancreas and inhibits the synthesis of glycogen (Keller and Orth, 1990).

The protein content in ablated crabs was decreased in muscles compared to the control males and females whereas increased in gonads. These trends were similar to the reports of Varalakshmi and Reddy (2010). The study confirmed their mobilisation from hepatopancreas and carcass towards faster ovarian maturation. The gonadal lipid content of ablated males (32.4±1.04) and females (34.15±0.9) was higher when compared to the control males (29.21±0.87) and females (32.09±0.9), whereas decreased in the hepatopancreas of ablated crabs. Samyappan *et al., (*2015) reported a similar result in *O. Senex senex*, where the content of various lipids (cholesterol, phospholipids, triglyceride, free fatty acids) were reduced significantly in the hepatopancreas of the treated when compared to control. Similar results were reported by Sandhiyapriya *et al*., (2018) in *B. cunicularis* affirmating, the protein quantity in various somatic tissues after an unilateral eyestalk ablation (UEA).

In the present study, heart index was significantly higher in ablated male and female crabs compared to the control male and female crabs. The result is in comparison with Allayie *et al.,* (2011). The coagulation time was recorded to be higher in the ablated crabs during experiment period compared to the control. The coagulation time of ablated male (40.9±2.42) and female (47.8±2.5) crabs was higher when compared to the control male (8.9±0.8) and female (12.8±1.34) crabs. However these data were in contrast with the results, found by Asusena *et al.,* (2012), in that the clotting mechanism of *M. americanum* females was not found to be affected by unilateral eyesatalk ablation.

The histological studies on the ovaries provide detailed information on the changes occurred due to BEA. In the histological sections of the bilateral eyestalk ablated crabs the oocytes shows significant increases in number (fig. 4) when compared to the control (fig. 6). These reports were similar to that of Chamberlain and Lawrence, (2009) stating, eyestalk ablation

increased gonad size and mating frequency in comparison to normal. The histological studies on testis shows increase in number of follicles, and matured follicles, spermatozoa, spermatocytes, spermatids, and seminiferous tubules (fig. 2) compared to the control (fig. 1). Similarly, Pervaiz *et al*., (2011), reported that the removal of eyestalk accelerated the gonad development, causing an increase in testicular index in *M*. *malcolmsonii*.

Khazraeenia, (2009) observed Precocious moulting in destalked crabs, which was probably related to the removal of MIH and consequent activation of the Y-organ, which produces the moulting hormone (MH) as discussed by Laufer, (1993). However in the present investigation, the short span of experimental trial has not sufficiently resulted in moulting. Increase in experimental span of more than 10 day is assumed to demonstrate moulting phase in *S. hydrodroma.*

CONCLUSION

The present study on BEA in male and female *S. hydrodroma* clearly demonstrated the potentiality of BEA to induce growth and gonadal development, which is evident from increased Daily growth rate, feed intake, GSI, HI, ovarian and testicular index. Furthermore, the de-stalkation procedure added to the follicle diameter and number of females; whereas, it enhanced sperm production in males. Therefore BEA in *S. hydrodroma* is highly suitable for male and female gonad maturation and growth enhancement.

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