

FIRST REPORT ON OPHTHALMIC DELIVERY OF A GONADOTROPHIC HORMONE FOR INDUCED BREEDING IN IUCN ENLISTED (LC) *SPIRALOTHELPHUSA HYDRODROMA*

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ABSTRACT

Intra-ocular delivery a synthetic gonadotrophic hormone (WOVA-FH) was administered to a freshwater crab for its first time and the effect of the drug was assessed based on growth, survival, reproductive and biochemical indices. 100% Survival Rate was recorded for male and female crabs after 10 days of dosage with reduced Heart Index and fairly increased Daily Growth Rate (g) compared to their controls ($0.30 \pm 0.10/0.16 \pm 0.09$ and $0.06 \pm 0.06/0.05 \pm 0.008$). Significant increase in ovarian index ($1.4 \pm 0.06/0.15 \pm 0.02$) and slight increase in testicular index ($1.29 \pm 0.10/1.08 \pm 0.06$) both positively correlated with its protein, carbohydrate and lipid contents recorded against control. The somatic biochemical indices reduced abruptly after stimulation against control i.e., $105.0 \pm 1.0/138.3 \pm 1.1$, $3.9 \pm 0.1/4.43 \pm 1.20$, $87.4 \pm 1.0/91.52 \pm 1.2$ and $129.0 \pm 1.0/141.2 \pm 1.2$, $5.1 \pm 1.0/5.6 \pm 1.12$, $83.5 \pm 1.0/87.5 \pm 1.1$ are the protein, carbohydrate and lipid vales in male and female/control.

Keywords: WOVA-FH, Induced breeding, Freshwater crab aquaculture, *Spiralothelphusa hydrodroma*, IUCN.

INTRODUCTION

Spiralothelphusa hydrodroma is an edible freshwater crab, native to India and a significant food for South Indians. These crabs belong to the order Decapoda and the sub-phylum Crustacea. Freshwater crabs are widely distributed and are cheap source of proteins, also reported to have medicinal value (Kumar et al., 2019). Crabs have high demand in export market. However, *S. hydrodroma* is Exant to India alone and is assessed by IUCN to be Least Concerned (LC) species (Cumberlidge, 2008). Due to its limited extant distribution, LC category with IUCN and important food with medicinal value, cultivation by means of rapid techniques is vital. Induced breeding is highly suitable to rapidly raise such mall population.

Hypophysation technique uses natural pituitary extract to stimulate growth, development, maturity and ovulation of eggs. However, synthetic hormones are highly effective in aquatic organisms using Human Chorionic Gonadotropin (HCG) (Adebayo and Fagbenro, 2004), ovaprim (Ghanemi and Khodadadi, 2017), ovatide (Chand et al., 2011) and WOVA-FH (Singh et al., 2015). WOVA-FH has a composition of Synthetic Gonadotropin Releasing Hormone (SGnRH) which results in high fertilization and hatching rate.

This study reports the first intra-ocular drug delivery for a crustacean species. Previous methods of drug delivery for these animals were using, intra-muscular injection in an area

between carapace and first abdominal segment (Robbins, 1959), also at the internode region of swimming leg (Farizah et al., 2017). Moreover, Induced breeding in freshwater crabs, especially with that of a synthetic gonadotrophic hormone is not yet documented. Therefore, an attempt has been made in the present investigation to deliver the drug ophthalmic via and examine the effects on reproductive as well as growth indices.

MATERIALS AND METHODS

Specimen collection, selection, transportation and maintenance

Male of 3.36 ± 0.33 cm in length and 23.4 ± 5.66 grams in weight and females of 3.43 ± 0.63 cm in length and 20.93 ± 6.87 grams in weight were used for the study. Live specimens were collected from Cauvery river basin at Bhavani in the Northern periphery of Erode district of Tamil Nadu, India. The collected specimens were transported to the laboratory in perforated plastic containers. Mature male and female crabs with measured size are taken for the induced breeding. Females with triangular flap were avoided as they were immature. The selected crabs were acclimatized for about a week in glass aquaria with moist sand and gravel. Unfed feed and excreta were removed daily. The crabs were subjected to a pool of water daily to avoid dehydration.

Intra-ocular WOVA-FH discharge and sample assessment

The breeding hormone was purchased from BIOSTADT, Dwaraka Aqua Tech, Tamil Nadu India and used at level of 1 ml/kg body weight using 2 mm Gauze needle. During the procedure crabs were held tight, wrapped with clean linen to fasten the chelipeds. Needle loaded with drug was inserted till quarter zone and gently withdrawn.

Following which, the crabs were uniformly fed with 1 gm feed and maintained for 10 days, proceeding with dissection and obtaining tissue sample including: muscle, hepatopancreas, heart, testis and ovary to study growth, survival, reproductive, biochemical and histological indices.

Growth and survival indices

a. Daily Growth Rate (DGR): Initial and final weights of crabs were measured prior and after injection respectively. The growth rate was calculated by using the following formula:

$$\text{Daily growth rate (g)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Number of days}}$$

Survival Rate (SR): During the experimental period survival rate in both control and injected crabs were noted. Survival rate was calculated using the following formula:

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

b. Heart index (HI): 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 formula:

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

c. Hepatosomatic Index (HSI): Similarly after injecting, the hepatosomatic index was calculated by removing the hepatopancreas and the weight was recorded in grams. The hepatopancreatic index can be determined by the using the following formula:

$$\text{Hepatosomatic index (\%)} = \frac{\text{Wet Weight of the hepatopancreas}}{\text{Wet Weight of the crab}} \times 100$$

Gonadosomatic Index (GSI)

A. Ovarian index: After 10 days in the experimental period, the injected crab was dissected out and the moisture of ovary is removed with the help of blotting paper and then the weight of ovary was recorded in grams. The ovarian index was determined by the using the formula:

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

B. Testicular index: After 10 days in the experimental period, the crabs were dissected out and moisture of testis is removed with the help of blotting paper and then the weight of testis was recorded in grams. The testicular index was

determined by the using the formula:

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

Biochemical analysis

Gonadal tissues were examined for protein, carbohydrate and lipid quantitatively. Muscle tissues were examined for protein and carbohydrate and hepatopancreas for lipids. Estimation for carbohydrates (Roe, 1955), proteins (Lowry et al., 1951) and lipids (Folch et al., 1957) were done following standard methods.

Behavioral change assessment

Throughout the experimental trial of 10 days, male and female crabs were monitored for notable behavior changes.

Statistical analysis

All Data obtained from growth, reproductive and biochemical indices were analyzed using SPSS statistical V-20 software. Data were obtained as mean ± standard deviation of triplicate analysis (Zar, 2009).

RESULTS

Ophthalmic delivery of WOVA-FH in male and female *S. hydrodroma* resulted in 100% SR, increased DGR (0.30 ± 0.10/0.16 ± 0.09 and 0.06 ± 0.06/0.05 ± 0.008), reduced HI (2.53 ± 1.0/3.14 ± 0.10 and 1.72 ± 0.10/2.95 ± 0.43), reduced HSI (3.99 ± 1.0/13.77 ± 3.22 and 8.31 ± 1.00/10.2 ± 0.13) / control. Data are represented in Table 1. Significant increase in ovarian index (1.4 ± 0.06/0.15 ± 0.02) and slight increase in testicular index (1.29 ± 0.10 / 1.08 ± 0.06) are also observed in experimental crabs against control. These data are represented in Table 2.

Table 1: Growth and survival indices in *S. hydrodroma* Data obtained as a result of triplicate analysis expressed as Mean ± S.D.*Values not significant at P ≤ 0.05.

Indices	Male		Female	
	Control	Induced	Control	Induced
Daily growth rate (g)	0.16 ± 0.09	0.30 ± 0.10	0.05 ± 0.008	0.06 ± 0.06*
Survival rate (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
Heart index (%)	3.14 ± 0.10	2.53 ± 1.0	2.95 ± 0.43	1.72 ± 0.10
Hepatosomatic index (%)	13.77 ± 3.22	3.99 ± 1.0	10.2 ± 0.13	8.31 ± 1.00

Table 2: Photomicrograph on cross section of induced male *S. hydrodroma* showing developed Spermatozoa, matured spermatocytes in seminiferous tubule (X470) .H and E stain. (SC-Spermatocytes; LN-Lumen; Sc -Sertoli cells; ST-Seminiferous tubules).

Indices	Control female	Induced female
Ovarian index (g)	0.15 ± 0.02	1.4 ± 0.06
Indices	Control male	Induced male
Testicular index (g)	1.08 ± 0.06	1.29 ± 0.10

Table 3 reports a decline in the level of major biochemical components present in somatic tissues of induced male and female crabs against control. Where, $105.0 \pm 1.0/138.3 \pm 1.1$, $3.9 \pm 0.1/4.43 \pm 1.20$, $87.4 \pm 1.0/91.52 \pm 1.2$ represents protein, carbohydrate and hepatopancreatic lipid levels in induced males against control. $129.0 \pm 1.0/141.2 \pm 1.2$, $5.1 \pm 1.0/5.6 \pm 1.12$, $83.5 \pm 1.0/87.5 \pm 1.1$ represents protein, carbohydrates and hepatopancreatic lipid levels in induced females against control.

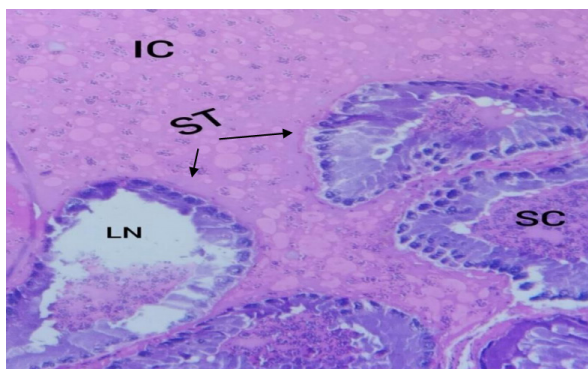
Table 3: Somatic biochemical indices in *S. hydrodroma*. Data obtained as a result of triplicate analysis expressed as Mean \pm S.D. All values are significant at $P \leq 0.05$.

Indices	Male		Female	
	Control	Induced	Control	Induced
Muscle protein (mg/g)	141.2 ± 1.2	129.0 ± 1.0	138.3 ± 1.1	105.0 ± 1.0
Muscle carbohydrate (mg/g)	5.61 ± 0.12	5.1 ± 1.0	4.43 ± 1.20	3.9 ± 0.1
Pancreatic lipid (mg/g)	87.5 ± 1.1	83.5 ± 1.0	91.52 ± 1.2	87.4 ± 1.0

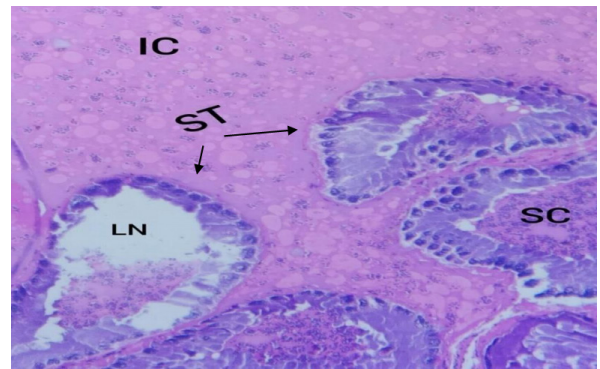
Table 4 reports an elevation in the level of major biochemical components present in gonadal tissues of induced male and female against control. Where, $23.75 \pm 1.0/19.31 \pm 1.17$, $310.0 \pm 1.0/92.9 \pm 0.92$, $33.66 \pm 1.0/29.21 \pm 0.87$ represents protein, carbohydrate and lipid levels in induced males against control. $37.45 \pm 1.0/32.22 \pm 0.8$, $102.37 \pm 1.0/91.09 \pm 1.1$, $54.8 \pm 1.0/32.0 \pm 0.9$ represents protein, carbohydrate and lipid levels in induced females against control. No observable behavioral change is recorded for females. However, males were sex-driven and showed courtship indications following female attraction, evident through grasping female crabs frequently (Figures 1-4).

Table 4: Gonadal biochemical indices in *S. hydrodroma*. Data obtained as a result of triplicate analysis expressed as Mean \pm S.D. All values are significant at $P \leq 0.05$.

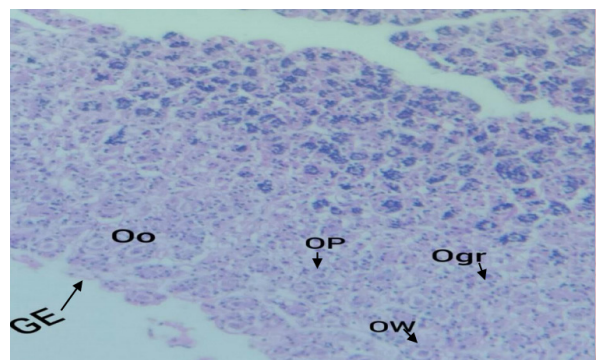
Indices	Male		Female	
	Control	Induced	Control	Induced
Protein (mg/g)	19.31 ± 1.17	23.75 ± 1.0	32.22 ± 0.8	37.45 ± 1.0
Carbohydrate (mg/g)	92.9 ± 0.92	310.0 ± 1.0	91.09 ± 1.1	102.37 ± 1.0
Lipid (mg/g)	29.21 ± 0.87	33.66 ± 1.0	32.0 ± 0.9	54.8 ± 1.0



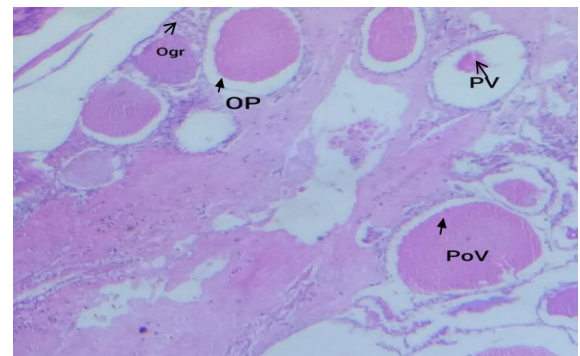
Figures 1. Photomicrograph on cross section of control male *S. hydrodroma* showing developing spermatozoa and immature spermatocytes in seminiferous tubules (X540). H and E stain.



Figures 2. Photomicrograph on cross section of induced male *S. hydrodroma* showing developed Spermatozoa, matured spermatocytes in seminiferous tubule (X470). H and E stain. (SC-Spermatocytes; LN-Lumen; Sc -Sertoli cells; ST-Seminiferous tubules).



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



Figures 3. Photomicrograph on cross section of induced female *S. hydrodroma* showing increased follicle diameter, follicle number and post-vitellogenic oocytes (X570). H and E stain. (Ogr- Ovarian granule; OP- Oogenetic pouch; PoV-Post vitellogenic oocytes; PV- Pre vitellogenic oocytes).

DISCUSSION

Importantly, the ophthalmic injection of WOVA-FH showed 100% SR in induced male and female crabs. In other terms, this drug delivery system is found to be harmless for LC category species.

DGR (g) of the induced male crabs (0.30 ± 0.10) and female crabs (0.06 ± 0.06) were found higher than the control male (0.16 ± 0.09) and female crab (0.05 ± 0.008).

DGR is recorded to have negative correlation with HSI (%) $3.99 \pm 1.0/ 13.77 \pm 3.22$ for males and $8.31 \pm 1.00/10.2 \pm 0.13$ were the HSI for male and female induced/control crabs, demonstrating the mobilization of some reserve from hepatopancreas to ovary during the ovarian development and is a costly process in females with a severe biochemical synthesis with mobilization of lipids and proteins for the oocyte's development (Sastry, 1983). Furthermore, molt and reproduction require mobilization of organic reserves from storage sites such as HP to the epidermis and gonad, a process coordinated by hormones.

The growth indices of the heart among the control and induced groups differ significantly. The heart indices of the induced male (2.53 ± 1.0) and female (1.72 ± 0.10) group were less compared to the control male (3.1 ± 0.10) and female (2.95 ± 0.43). HI is reported to show antagonistic behavior in the females in the stage III ovarian development, as observed in relationship to the males; however these variations of Heart index during the process of molt are not significant (Magalhaes et al., 2012).

The key finding of the present study has been the rapid maturation of *S. hydrodroma* of gonads after treating with WOVA-FH. The crabs treated gave satisfactory results of reproductive symptoms. $1.4 \pm 0.06/0.15 \pm 0.02$ is the ovarian index of induced females to that of their controls. $1.29 \pm 0.10/ 1.08 \pm 0.06$ is the testicular index in induced males to that of their controls. Similar results owing to the effect of bilateral eyestalk ablation on ovarian development in the freshwater prawn *Macrobrachium rosenbergii* with shortened reproductive cycles in the destalked female prawns is reported (Okumara, 2007). Few (Tsutsui et al., 2005) reported the same in immature female prawn *Marsupearneus japonicus*, stating the development and increased ovarian weight, haemolymph vitellogenin levels and vitellogenin mRNA levels in the ovary.

The somatic protein, carbohydrate and lipid contents negatively correlated with the gonadal contents after ablation. Also, the protein content in gonads were noticed higher in the induced male (23.75 ± 1.0) and female (37.45 ± 1.0) crabs, compared to their control male (19.31 ± 1.17) and female (32.22 ± 0.8) crabs. The carbohydrate content in the gonads of the induced male (310.0 ± 1.0) and female (102.37 ± 1.0) crabs were significantly higher compared to their control male (92.9 ± 0.92) and female crabs (91.09 ± 1.1). Similarly, the gonads lipid levels shows a significant rise in the induced male (33.66 ± 1.0) and female crabs (54.8 ± 1.0) compared to the control male (29.21 ± 0.87) and female crabs (32.0 ± 0.9). These values were found to be statistically significant. However, the values of hepatopancreatic lipid content showed a decreased level in induced male (83.5 ± 1.0) and female crabs (87.4 ± 1.0) compared to control male (87.5 ± 1.1) and female crabs (91.52 ± 1.2). Muscle biochemical proportions observed as antagonistic to gonad biochemical proportions is attributed to the accumulation of protein, lipid and carbohydrate in gonads due to the effective mobilization on stimulation with WOVA-FH. Similar results were reported in *B. cunicularis* (Sandhiyapriya, 2018) affirming, the

decrease in protein quantity of various somatic tissues after a Unilateral Eyestalk Ablation (UEA).

Gonadal stimulation in females is characterized by increased follicle diameter, follicle number and post-vitellogenic oocytes. And the same is characterized in males by matured spermatocytes in seminiferous tubule of testis.

CONCLUSION

Ophthalmic delivery of WOVA-FH stimulated male and female gonads of *S. hydrodroma* by causing mobilization of protein, lipid and carbohydrate from muscle and hepatopancreatic tissues thereby inducing gonadal development and maturation in the IUCN enlisted threatened species without causing mortality and therefore highly recommended for its propagation or culture.

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