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RESEARCH ARTICLE

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A trial study on on hormone induced spawning and biochemical changes in *Etroplus suratensis*

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ABSTRACT

A study on induced spawning was carried out by using synthetic hormones such as ovaprim, HCG+LHRH in the fish *Etroplus suratensis* grown in aquarium tanks of 5 tons capacity. Biochemical parameters such as triglyceride, total protein and cholesterol level in the blood, liver and gonads were estimated in hormone treatment and it was compared with the control. The length and width of the egg development stages such as oocyte, pre-vitellogenic and matured eggs were also analyzed in different hormone treatments and were compared with the control. The percentage of eggs in the ovary of control and hormonated ovary were also compared. In all these studied parameters, the combined hormone HCG+LHRH administered experimental fishes showed the highest increased level was recorded in the present study. It was suggested that the administration of the synthetic hormone HCG+LHRH to get success on induced spawning in *E. suratensis*.

Key words : Hormones, *E. suratensis*, Ovaprim, HCG+LHRH, spawning

INTRODUCTION

Etroplus suratensis belonging to the family chichilidae commonly found in the estuaries and inland waters of India and Srilanka (Talwar and Jingran, 1992; Rao, 1995; Blaber, 1997). It occurs in brackish as well as fresh water and has been observed to breed in these habitats (Rishi and Singh, 1982). It involves in commercial fisheries (Gopakumar, 1997), yet this fish is preferred as candidate species for aquarium. It breeds freely both in freshwater and brackish water environment and exhibits parental care, in which both male and female participate. Mode of reproduction is dioceses (Pethiyagoda, 1991; Arkipehuk, 1999). Among the fish species, it has low fecundity rate with about 500 eggs laid in single spawning (Jayaprakas *et al.*, 1990). The eggs are attached to submerged logs, rocks or sometimes roots and weeds. These guardian parents take care the eggs until hatching and within four days, the eggs will be hatched out. The fry shoal around their parents during the first weeks of growth in natural condition.

Almost all the fish species are spawned in the natural environment; only limited species are successfully spawned through induced breeding in laboratory condition. The success of induced spawning depends upon several factors, which was not clearly understood in most of the fishes (Stuart *et al*., 1988). During the past three to four decades, induced breeding technique has been attempted in many of the fresh water and marine fishes. For this technique, many of the alternative hormones such as human chronic Gonadotropin (HCG) (Adebayo and Fagbenro, 2004); Inyang and Hettiarachchi, 1994), luteinizing hormone – releasing hormonoeo (De Leeuw *et al*., 1985; Fermin, 1992) and ovaprim (Alok *et al*., 1993; Haniffa *et al*., 1996) were used. Treatments using the above hormones are effective in many of the fish species. But so far attempt in induced breeding of *E. suratensis* is scanty. Only very few works were carried out in the direction of larval propagation in *E. suratensis* (Eschmeyer, 1990). Few works were focused on the induced breeding by applying hormones since the attempts were not encouraging (Karnfield, 1984). The present study is an attempt to induce breeding in *E. suratensis* using synthetic hormonoes like HGG and LHRH and to document the earlier larval stages of the same species.

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MATERIALS AND METHODS

Brood stock capture and management:

E. suratensis brooders with the size of 15 – 20 cm length and weight group of approximately 110±10 g were collected from the backyard estuarine waters at Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam, Kanyakumari District, India. The collected fish were stocked in aquarium tanks (5T capacity) for 7 days to ensure the disease free status of the experimental fish. The healthy fish were transferred to the circular brood stock tanks (1T capacity). The water quality parameters such as temperature, salinity and oxygen level were maintained at 27 – 30^{0}C, 5 pit and 5 mg/l, respectively. A 100% water exchange was made daily. During this period, the fish were fed with lap-lap at the rate of 3% of fish body weight daily. After 5 days, the gonad maturity of fish was determined in both male and female be performing catheter biopsy in the gonads through the genital opening. The collected biopsy samples were observed for the gonad maturity with the parameters of eggs diameter and stage of the eggs in female, as well as sperm motility in the male.

Harmone administration:

Three groups with 12 female *E. suratensis* in each group were stocked in individual spawning tank (1.5 ton capacity) for the hormone administration. In the first group (GO), ovaprim (Syndel Co., Canada) was administered with the optimum concentration of 1 ml/kg fish. The second group (GH) was administered with HCG (Profess and SIGMA, USA) 1000 U/kg fish with the combination of LHRH (SIGMA, USA) 60 µg/kg fish. The third group was treated as control (GC) received the sterile saline injection (0.81% NaCl).

After 48 of hormonal administration the blood samples from the three groups of experimental fish were collected by using sterile syringe. Therefore, fishes were sacrificed; the liver and gonad samples were dissected carefully from each group and individually stored at –20^0 C until further use. The biochemical parameters such as triglyceride and cholesterol were estimated in all the blood, liver and gonad samples using ELISA–micro plate method (Palacious *et al*., 1998). Total protein content in the same samples were estimated by the method described by Bradford (1976).

To characteristic the stages of maturity in females, oocyte count as well as gonad somatic index (GSI) were recorded in all groups. Three stages of oocytes were found in the gonad (Previtellogenic, vitellogenic and matured egg) and the number of oocytes belong to each stage was counted for 100 mg of gonad sample in each group of fishes. The size of the egg (length and width) was also determined using ocular micrometry method.

Statistical analysis:

The data obtained in the present study were subjected for statistical 2-way ANOVA and regression analysis followed by Zar (1974).

RESULTS AND ANALYSIS

The biochemical indices like triglyceride, total protein and cholesterol were estimated in the blood, liver and gonad samples of *E. suratensis* brooders treated with synthetic hormones like ovaprim and HCG+LHRH. The level of triglyceride was more in all the tested samples (63.23±2.0 µg/ml in blood, 487.23±2.7 µg/g in liver and 51.2±1.5 µg/g in gonad) of fish administered with HCG+LHRH. But these exhibited lower values in fishes administered with ovaprim (30±2.0 to 41.0±8.00 mg/g) and in control (22.0±1.0 to 435±5.0 µg/g) groups (Table 1).

As like the triglyceride, a similar trend of result was observed in total protein level of blood (1.66±0.1 mg/ml), liver (9.12±0.19 mg/g) and gonad (4.02±0.09 mg/g) samples of *E. suratensis* administered with HCG+LHRH than the ovaprim (1.14±0.04 mg/g) as well as control

Table 1: Biochemical parameters of different tissues in *E. suratensis* in both experimental and control groups

Parameters		Blood (mg/ml)	Liver (mg/g)	Gonad (mg/g)
	Control	50.0 ± 4.0	435.0 ± 5.0	22.0 ± 1.0
Triglycerides	Ovaprim	37.0 ± 3.0	410.0 ± 8.0	30.0 ± 2.0
	HCG+LHRH	63.23 ± 2.0	487.23 ±2.7	51.20 ± 1.50
	Control	1.31 ± 0.05	7.02 ± 0.06	2.0 ± 0.08
Total Proteins	Ovaprim	1.14 ± 0.04	8.98 ± 0.28	3.75 ± 0.05
	HCG+LHRH	1.66 ± 0.11	9.12 ± 0.19	4.02 ± 0.09
	Control	12.2 ± 1.3	61.21± 3.0	3.0 ± 0.20
Cholesterol	Ovaprim	9.0 ± 1.0	144.0 ± 4.0	1.50 ± 0.05
	HCG+LHRH	14.4 ± 1.5	212.20 ±7.0	5.0 ± 0.50

(1.31±0.05 mg/ml to 7.02±0.06 mg/g) treatment (P <0.05). Apparently, the cholesterol level was significantly higher in the liver tissues of both HCG+LHRH (212.2±7.0 µg/g) and ovaprim (144±4.0 µg/g) treatment groups than the control (61.21±3.0 µg/mg) group (P< 0.05). Likewise the blood cholesterol level was in the order of 14.4±1.5 µg/ml, 9.0±1.0 µg/ml, and 12.2±1.3 µg/ml in HCG+LHRH, ovaprim and control groups, respectively. The gonad cholesterol level was 5.0±0.50 mg/g in HCG+LHRH, 1.5±0.05 mg/g in ovaprim and 3.0±0.20 mg/g in control groups. The statistical comparison of cholesterol level between the tested samples and tested groups were significant (P < 0.05) (Table 1).

Percentage distribution of different egg stages:

From matured gravid females of *E. suratensis*, based on the maturity, three different stages were identified (Fig. 1a, b and c). They are accordingly oocytes, which were spherical in shape with the length and width of 186.62 ± 12.25 µm and 176.62 ±14.2 µm, respectively. The second stage was pre-vitellogenic oocytes, which had the vitellogenic package of transparent yellow spheres with 599.85 ± 11.21 µm long and 333.25 ± 25.33 µm wide. Likewise, the third stage was vitellogenic (matured) eggs, prominent shape with 1932.85 ± 82.81 µm length and 1039.72 ± 92.22 µm wide (Table 2). Freshly ovulated mature eggs were slightly spherical in shape, visible to the naked eye and strong yellow in colour with opaque. The oocyte was obviously enhanced by hormone treatment as indicated by the increase of oocyte diameter. The percentage of matured egg was maximum (47.03%) in *E. suratensis* that received HCG+LHRH hormone. At the same time, the control group of fishes had 13.58% matured eggs, followed by fishes administered with ovaprim hormone 41.78%. Vitellogenic eggs (38.97%) and previtellogenic eggs (64.45%) were more in ovaprim administered fish and control fish, respectively (Table 2, Fig. 1a, b, c).

Table 2: Length and width of *E. suratensis* eggs

Egg stage	Length (µm)	Width (µm)
Oocyte	186.62 ± 12.25	176.62 ± 14.20
Pre-vitrllogenic egg	599.85 ± 11.21	333.25 ± 25.33
Mature eggs	1932.85 ± 82.81	1039.72 ± 92.22

The percentage of eggs in 100 mg of ovary showed that the hormonated experimental fish showed the highest percentage (41.78%), followed by pre-vitellogenic (38.96%) and the oocyte (19.24%) (Table 3).

For the assessment of internal milieu of fishes during the reproduction several biochemical indices should be

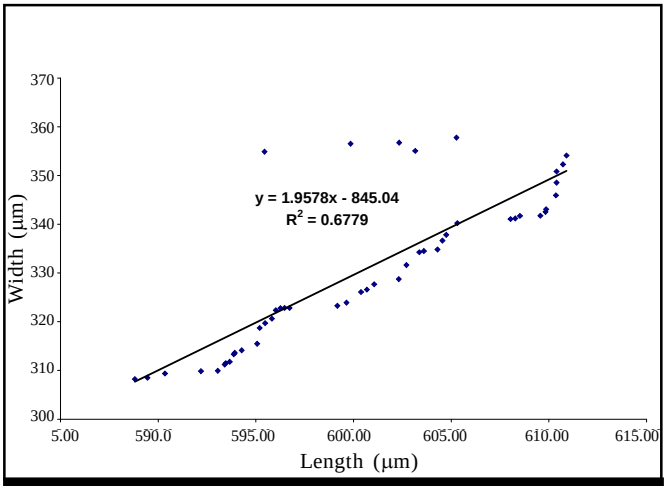

Fig. 1 a : Length-width relationship of *E. suratensis* pre-vitellogenic egg

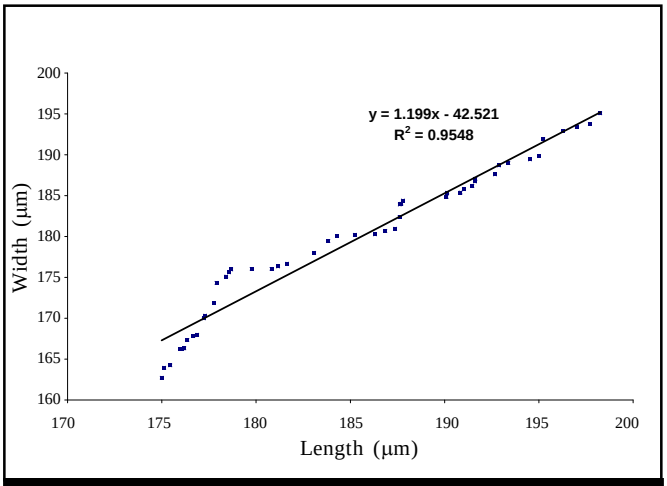

Fig. 1 b : Length-width relationship of *E. suratensis* oocytes

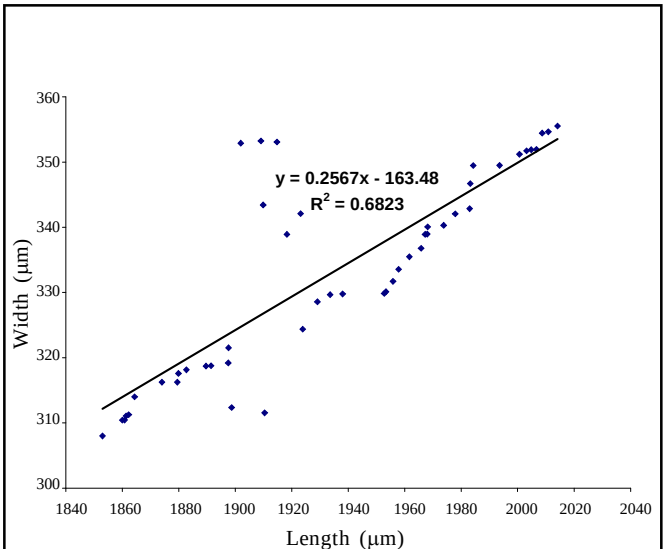

Fig. 1 c : Length-width relationship of *E. suratensis* mature eggs

Table 3 : Percentage of eggs in 100 mg of ovary

Stage of egg	Control egg % (in 100 mg ovary)	Experimental hormonated egg % (in 100 mg ovary)
Oocyte	64.45 ± 6.5	19.24 ± 1.8
Pre-vitellogenic egg	21.96 ± 2.3	38.96 ± 3.7
Vitellogenic egg (matured egg)	13.58 ± 1.2	41.78 ± 4.3

clearly resolved (Svoboda *et al*., 2001). The present study also had a part to analyze the possible biochemical variables such as triglycerides, total protein and cholesterol from blood, liver and ovary tissues, while administration with commercial synthetic hormones. Significant differences were observed between the control land hormone administered spawners. Results from the examination of triglyceride in the spawners tissues (liver and ovary) and blood indicate the significant positive regulation and high titer value in HCG+ LHRH a treated group. In the group administered with ovaprim failed to reserve the significant quality of triglycerides in blood and tissue, which is clearly reflected in the number of matured eggs in the ovary. Similar trend was observed in the blood plasma of fish tench (*Tinca tinca* L.) during pre and post-spawning period under the condition of hormonally induced artificial reproduction (Svoboda *et al*., 2001). Possible role of triglyceride in fish reproduction is to serve as the higher energy source as well as the precursor for the yolk protein synthesis (Luskova, 1997 ; Kovaqcheva and Tchekov, 1993).

Moreover, cholesterol which plays as the precursor in the synthesis of steroid hormones that involving in the fish maturation was significantly regulated the hormone treated animals than the control groups. The cholesterol that incorporates in the membranes and endogenous structures of the egg and its concentration in blood plasma of females found to be increased during the hormones administration (Diwan and Krishnan (1986). Diwan and Krishnan (1986) stated a fluctuation of serum cholesterol in male and females of *E. suratensis* as related to maturity. In the present study, cholesterol concentration in liver tissues of females that found to be lowest in the control females was reflected in the egg maturity.

The total protein level in the tissue samples from the hormone injected groups exhibited significant higher values than the control group in the present study. This result was in accordance to the data reported for some other fish species like trout – *Salmo trutta* (Mulcahy, 1971); carp – *Cyprinus carpio* (Svobodova and Parova, 1977); trout – *Salmo gairdeneri* (Hille, 1982); rainbow trout (Jirasek *et al*., 1993) and common carp (Rehulka, 1996). Total protein level in the blood determines the health status and reproductive ability in cichilids. In the present study also, higher levels of total protein was registered in HCG+LHRH group as it caused improved health status or reproductive ability of the fish *E. suratensis*.

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