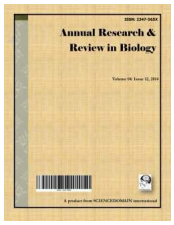

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Determination of Different Hormones Dose for Breeding of Green Back Mullet, *Chelon subviridis*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study was conducted in the hatchery complex of Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna, Bangladesh. The body weights of collected male fishes were ranged from 42-50 g and that of female fishes were from 50-58 g. The fish were divided into different groups, which were administered with different hormones: Pituitary Gland (3, 4 & 5 Mg of dried PG/Kg of both male and female fish), Human Chorionic Gonadotropin (2500, 3000 & 3500 IU of HCG/Kg of both male and female fish), Synthetic gonadotropin releasing hormone analogue (25, 30 & 35 Microgram of S-GnRHa/Kg), Only the group treated with S-GnRHa hormone exhibited spawning activity. Only 25, 30, 35 Microgram of S-GnRHaa (Ovupin)/Kg of dose injected fish showed spawning activity. In 25 Microgram/Kg S-GnRHa hormone dose, the spawning period, fertility rate, hatching period and hatching rate of green back mullet were 33-35 hrs, 72%, 21-24 hrs and 70%. In 30 Microgram/Kg S-GnRHa hormone dose, the spawning period, fertility rate, hatching period and hatching rate green back mullet were 33-35 hrs, 83%, 21-24 hrs, 81%. In 35 Microgram/Kg S-GnRHaa hormone dose, the spawning period, fertility rate, hatching period and hatching rate of green back mullet were 32-34 hrs, 80%, 20-22 hrs, 76%. All dose of S-GnRHa here shown more or less similar result. The present study might be useful for quality seed production on parse (*Chelon subviridis*) during induced breeding in hatcheries.

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Keywords: Green back mullet; fry; brackishwater; pituitary gland (PG); human chorionic gonadotropin (HCG); synthetic gonadotropin releasing hormone analogue (S-GnRHa); Bangladesh.

1. INTRODUCTION

Green back mullet, *Chelon subviridis* (Val. 1836) earlier known as *Liza subviridis* is a brackish water mugilid fish with a tropical Indo-pacific distribution. It is a euryhaline and eurythermal fish. This fish is locally known as parse/bata and commonly available in shallow coastal waters, estuaries and mangrove swamps of Bangladesh. The high quality of flesh, high economic value and wide temperature and salinity tolerance capacity make this species popular for aquaculture in the intertidal ponds [1].

There are about 1.5 million ha brackish water *ghers* (large hydrological units protected by embankment with provisions of controlled drainage and irrigation infrastructures connecting with coastal rivers) in the southwest region of Bangladesh [2]. At present about 2, 17,000 hectares areas are being used for brackish water aquaculture. Brackish water aquaculture in Bangladesh is mostly directed to traditional farming of brackish water shrimp, *Penaeus monodon* with or without fin fishes. The culture practice of this fish in the coastal impoundments (locally called *ghers*) of Bangladesh is getting much popularity. At present, the farmers depend upon wild seed for stocking to their *ghers*. Due to indiscriminate harvest from natural sources and some environmental reasons, the abundance of this fish is decreasing day by day. There is no alternate of supply of seed from artificial sources to conserve the natural biodiversity and increase production of this fish. This fish migrate to the sea during breeding period and again back towards coastal water where they pass their whole young life until the spawning period. Preliminary study on the breeding of *Liza subviridis* was conducted by Das in the south-west region of Bangladesh [3,4]. Hsu et al. reported that mullets are winter breeder and the suitable breeding temperature is 20-23ºC [5]. Age, growth, length-weight relationship, sex ratio, stages of maturity and fecundity of the greenback mullet were studied by Al-Daham and Wahab [6] and Rahman et al. [7]. Studies on the biology emphasizing length weight relationship, fecundity, and reproductive characteristics and spawning of some other mullet species were conducted by Ergen [8], Rheman et al. [9] and Cherif et al. [10]. Saha and Kabir reported [11] preliminary success of breeding of this fish in captivity.

Chelon subviridis has high demand in the national and international market. A lump sum amount of this fish is naturally produced as a wild catch in the *ghers* .It is now imperative to develop a suitable culture technology of this species to increase productivity of the *ghers.* But no potential attempt has yet been taken in this regard. Long back, a few attempts were undertaken by Bangladesh Fisheries Research Institute and studies were conducted on the production performance of this fish with shrimp using mullet seed from wild source [12,13,14]. Later on, no further attempt was undertaken in this regard for the development of either nursery management or culture technology due to unavailability of seed from artificial sources of this important fish. Realizing the importance of this fish, it has been evaluate to improve breeding and seed production by different hormonal doses of green back mullet, *C. subviridis*.

2. MATERIALS AND METHODS

2.1 Study Area and Time Duration

The experiments were conducted in Bangladesh Fisheries Research Institute, Brackishwater station, Paikgacha, Khulna. The study was performed laboratory of Brackishwater station, during the spawning season from December 2015 to March 2016 using the induced breeding facilitates in Bangladesh Fisheries Research Institute, Brackishwater station, Paikgacha, Khulna.

2.2 Experimental Fish

The experimental fish was green back mullet (*Chelon subviridis*). The body weights of female and male fish were 45-58 g and 35-45 g respectively.

2.3 Brood Fish Collection

About 70 to 80 brood fishes were collected from nature in Paikgacha area. After collection, the broods were reared in the rectangular ponds in Brackishwater station.

2.4 Brood Stock Management

The experimental fish samples were reared in 25 to 30 decimal rectangular ponds with 1 to 1.5 m

water depth. Salinity will be 10 to 12 ppt better for brood fish. To develop natural food used 2.5 ppm urea and TSP with 15 days interval. Commercial feed containing 32% protein was applied at the rate of 4 to 5% body weight of fish. Feed was supplied 2 times of day. Matured male and female were collected in spawning season from rearing pond were used for breeding. 40 male and 20 female reared fish from the collected fish were selected for experimental purpose on the basis of good size shape weight and colour. The selected fishes were kept in circular cistern, filled with filter pond water of 7 ppt salinity, which were gradually increased up to 20 ppt by adding 150 brine ppt in 72 hours, peleted feed was given in a tray and cleaned up time to time.

2.5 Brood Collection and Sex Determination

Initially male and female were distinguished by observing their morphological criteria. The gravid female has swollen belly with round and reddish genital papillae. The ripe male secretes milky white milt on gentle pressure in its anal region. Selected male and female brood were kept minimum 24 hours in conditioning tank before induced hormone.

2.6 Induced Breeding

The selected brooders were collected from the conditioning tank. The sex ratio of the spawners was kept at 2:1 for male and female. Induced breeding with Carp Pituitary Gland (PG) extract, the synthetic hormone Human Chorionic Gonadotropin (HCG) was produced by a commercial pharmaceutical company (Sumach:Infar, India), and S-GnRHa (Ningbo Sansheng Pharmaceutical Co. Ltd., Ningbo, China) each have three dose (Table 1).

Table 1. Determination of quality and doses of different hormones for breeding of *C. subviridis*

Types of hormones	Doses		
Pituitary extract (PG) (Mg of dried gland/kg)	3	4	5
HCG (IU/Kg)	2500	3000	3500
S-GnRHa (Microgram /Kg)	25	30	35

2.7 Preparation of Inducing Hormone

Locally available carp pituitary gland PG, HCG and S-GnRHa were collection from market in preserved condition. At first, the pituitary glands were gently removed from the vial with forceps and dried by using the filter paper for 2-3 minutes and then weighed by analytical electronic balance. The amount to be weight out was calculated on the bases of the body weight of all the fishes using the following formula:

$$\text{Weight of PG (mg)} = Wt \times Pt / 100 \quad (1)$$

Where, Wt represents total body weight (g) of all the fishes to be injected and Pt represent the ratio in mg PG to be injected/kg body weight under a particular treatment.

The weighed PG was transferred to a tissue homogenizer and thoroughly crushed. The crushed PG then diluted in distilled water to dissolve it and was centrifuged with a hand centrifuge for precipitation. The freshly prepared supernatant solution of hormone was then taken slowly in a 3 ml hypodermic syringe for injection. HCG was in liquid form preserved in vial and calculated in milligram unit. S-GnRHa, in inject able inducing hormone consisting of S-GnRHaa analogue in combination with dopamine antagonist, is also efficient in induced spawning [15,16].

2.8 Hormone Administration

For hormone administration the brood fish were collected from conditioning tank very carefully with net and place on sponge. Then they were wrapped with soft and moist cloth and injection needle was pushed near the base of dorsal fin. The amount of hormone solution for each fish was determined before according to the body weight of the brooders. In case of PG, HCG and S-GnRHa, female and male were administered single dose. The injected male and female fish were released at the ratio of 2:1 in 30 ppt saline water in fibre glass tanks (Cap. 500 liters) provided with gentle aeration. The temperatures of water of the tanks were maintained at a level of 24-25ºC. The fish spawned in the tank water.

2.9 Spawning Activity

After 30-36 hours of administering hormone, the fishes started to show spawning activity. At this stage, male and female fishes started pairing. The female release huge eggs with a jerk of its body and the male released spermatozoa and fertilized the eggs. The fertilized eggs were non-adhesive, spherical, transparent and shiny.

The diameter of the fertilized eggs varied from 750-850 μ with a single oil globule which made the eggs buoyant on the surface of water. The unfertilized eggs became opaque and whitish in colour. After spawning, the fertilized eggs were transferred to separate tanks with water having same salinity and temperature for incubation. After 19-21 hours of fertilization, the fertilized eggs were hatched out in incubation tank. The newly hatched larvae with yolk sac and oil globule were black in colour and planktonic in nature showing jerking movement. The lengths of the newly hatched larvae were 2.00-2.50 mm. After 3rd day of hatching, the yolk sac was absorbed and mouth opening became visible. At this stage feed was supplied to the fry. After hatching and yolk sac absorption, a portion of fries were fed with boiled and smashed egg yolk. For feeding larvae, *Brachionus rotundiformes* were cultured using *Nannochloropsis* and yeast as feed. *Nannochloropsis* was cultured in mass using Gillard's modified f/2 media [17] in the algal culture laboratory of Brackishwater Station. When the fries mouth opening became larger, nauplii of *Artemia* were supplied as feed.

2.10 Estimation of Fertilization Rate

The eggs were examined after 4 hours of mixing with sperm to determine the fertilization rate. For this purpose about 100 eggs was taken in a petridish from hatching jar. The eggs were observed under a magnifying glass and fertilized eggs were counted with the help of a soft thin brush. Fertilized eggs were easily separated from unfertilized eggs as the fertilized eggs of *Chelon subviridis* were transparent, non-adhesive have the presence of 'eye spot', round in shape while unfertilized eggs were white and opaque. The fertilization rate was calculated by using formula:

Fertilization rate (%) = No. of fertilized eggs×100/ Total no. of eggs (2)

Mean rate of fertilization (%) = Sum of fertilization rate (%) / Total no. of female (3)

2.11 Hatching of Fertilized Eggs

The fertilized eggs were placed in funnel type incubators which were previously washed and treated with malachite green. A continuous water flow was maintained in the incubators. Hatching started after 20±2 hours of fertilization. When hatching completed, the hatchlings were collected in a bowl and counted by visual observation using magnifying glass and recorded. Hatchling rate was calculated by following formula:

Hatchling rate (%) = No. of hatchlings × 100/Total no. of fertilized eggs (4)

Mean rate of hatching (%) = Sum of hatching rate (%) / Total no. of female (5)

2.12 Statistical Analysis

The results obtained in the experiment were subjected to analysis. Quantitative analysis of all data was carried out. MS Excel was also used for presentation of the tables and graphs obtained from different types of data set. Analysis of variance (ANOVA), Tukey Test for difference between means were used for analysis of the effect of different hormone on fecundity, fertilization rate and hatching rate of parse (*Chelon subviridis*) using IBM SPSS statistics v20 software.

3. RESULTS

Among the tested hormones, only S-GnRHa (Ovupin) inject fish showed positive response that means both male & female fishes were started pairing. But other hormone inject fishes were not started pairing and also were not release milt & egg.

Use of Ovupin showed the positive result and the results are given in Table 2.

In 25 Microgram/Kg, 30 Microgram/Kg and 35 Microgram/Kg Ovupin hormone dose, green back mullet responded for breeding. In 25 Microgram/Kg Ovupin hormone dose, the spawning period, fertility rate, hatching period and hatching rate of green back mullet were 33-35 hrs, 72%, 21-24 hrs, 70% (Table 2).

In 30 Microgram/Kg Ovupin hormone dose, the spawning period, fertility rate, hatching period and hatching rate green back mullet were 33-35 hrs, 83%, 21-24 hrs and 81% (Table 2). In 35 Microgram/Kg Ovupin hormone dose, the spawning period, fertility rate, hatching period and hatching rate of green back mullet were 32-34 hrs, 80%, 20-22 hrs, 76%.

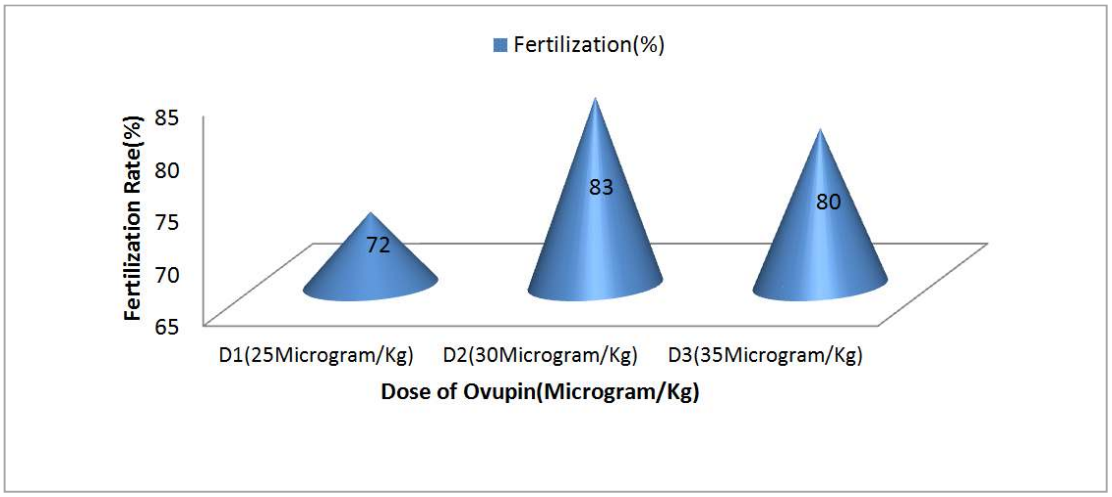

Figure 1. Fertilization rate (%) in different dose of hormone

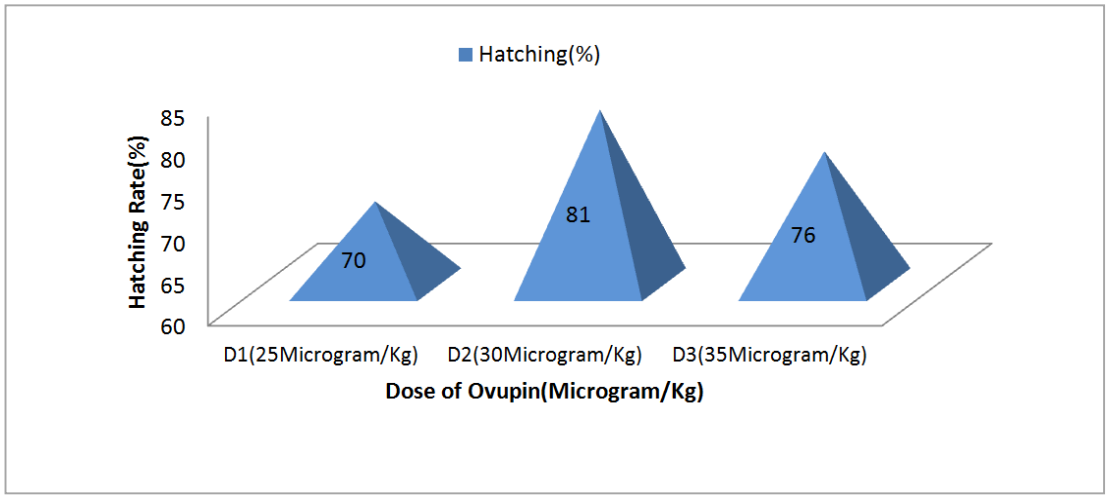

Figure 2. Hatching rate (%) in different dose of hormone

The larvae will be reared by using different live food like Rotifer, *Artemia*, shrimp larvae feed. In the present study, the fertilization and hatching rates were found more or less similar in fishes injected with S-GnRHa at different dose (Figures 1 & 2).

4. DISCUSSION

There are different types of fishes conducted successful work on induced breeding. In parse, to a successful work on induced breeding *Chelon subviridis* was initiated by using S-GnRHa hormone in hatchery of Brackish Water Station, Bangladesh Fisheries Research Institute, Paikgacha, Khulna [11]. In the present experiment, this experiment was further developed by comparing three spawning agents at different graded doses were tried. Pituitary extract and HCG hormone did not show positive response. Those hormones are usually useful in Freshwater fish but green back mullet is a Brackishwater fish so it might not showing positive response of breeding on those hormones. At dose of 3 mg/kg to 5 mg/kg PG *Chelon subviridis* did not respond by spawning this may be due to low quality of the product used or this species may require higher dose. HCG even at dose of 3500 IU/kg, *Chelon subviridis* was not effective. In the present study, S-GnRHa gave a positive spawning response. In 25 Microgram/Kg, 30 Microgram/Kg and 35

Table 2. Result of different hormones doses for breeding of *C. subviridis*

Ovupin (Microgram /Kg)	Response	Spawning period (hrs)	Fertility rate (%)	Hatching period (hrs)	Hatching rate (%)
25	Yes	33-35[a]	72±2[a]	21-24[a]	70±.05[a]
30	Yes	33-35[a]	83±1[a]	21-24[a]	81±2[a]
35	Yes	32-34[a]	80±3[a]	20-22[a]	76±1[a]

**Figures in the same column with the same superscripts are significantly indifferent (p>0.05)*

Microgram/Kg S-GnRHa hormone dose, green back mullet responded for breeding. But, in case of all those dose of S-GnRHa, the spawning period, fertility rate, hatching period and hatching are significantly indifferent. So it's showing that those entire doses are applicable for induced breeding of green back mullet. In the present investigation, positive results (spawning) were found from S-GnRHa treated fish. Saha and Kabir reported [11] successful breeding of green back mullet, *Chelon subviridis* using S-GnRHa. The result is in agreement with the work of Yeasmin et al. [18], where all male and female fishes injected with S-GnRHa and the fishes were successfully spawned.

The result of the current work was similar with the result found by Jamroz et al. [19] when S-GnRHa was used for *Labeo rohita.* Nayeem *et al*. conducted [20] experiment on induced breeding of Silver carp fish, where all the fishes were injected with S-GnRHa at the ratio of dose 0.6 mg/kg body weight and 100% spawn were found. Proper care of brood stocks is very important for assuring good production of eggs, fry and fingerlings. According to Bromage et al. [21], the food quality and feeding rates of brood stock diets have direct effects on fecundity and egg size. Nash et al. [22,23] reported the spawning procedures for rearing the grey mullet. Radhakrishnan et al. [24] fertilized the eggs of *Mugil parsia* with the milt of *M. macrolepis* through interspecies hybridization. Liao summarized [25,26] the work in Taiwan on the propagation of *M. cephalus* using artificial techniques of spawning first with brooders collected from the sea. In the present study, the brood stocks were managed carefully with well balanced feed comprising adequate amount of 32% protein, lipid and carbohydrate especially enriched with vitamin E.

In the present study, the fertilization and hatching rates were found more or less similar in fishes injected with S-GnRHa at 25, 30 & 35 Microgram/Kg body weight dose (Figures 1 & 2). The positive response of male & female to a single injection of S-GnRHa is very significant for the commercial seed production of *Chelon subviridis* fish as it saves a considerable amount of time and excessive handling of brood fish. This inducing agent can also be helpful for conservation of endangered species like parse (*Chelon subviridis*).

5. CONCLUSION

PG, Human chorionic gonadotropin and S-GnRHa (ovupin) hormone were tested in the present study. Among the tested hormone only S-GnRHa showed positive response. Among all the experiments s-GnRHa at the dose of 25, 30 & 35 Microgram/Kg body weight of female and male showed more or less similar fertilization and hatching rate. The present study suggests might be useful for quality seed production of parse (*Chelon subviridis*) during induced breeding in hatcheries.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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