International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2020): 7.803

Effects of Ovaprime on Spawning Induction and Larval Rearing of Tire Track Eel (*Mastacembelusfavus*)

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Abstract: ***The economically important Tire Track Eel serves as an aesthetic and table food species but increasing demand especially in Asia led to consequent depletion and pressure on wild fish stock. The present study was conducted to assess the best dose of ovaprim and comparing effects with other hormones (PG and HCG), and understanding the larval rearing process obtained from these hormones. The sex ratio was 1: 3 (male to female). The fish were given best dose of ovaprim (0.5 mg/kg), PG (5 ml/kg), and HCG (3000 IU/kg) with 3 treatment and 3 replicates. Later, larvae obtained from these hormone were reared up to 20 days using live foods (Moina, and Tubifex). The best dose obtained for ovulation of Tire Track Eel was 0.5 ml/kg ovaprim with an ovulation rate 100%, absolute fecundity 21588 ±3661, fertilization rate 78.8 ±9.52 %, and hatching rate 69.7 ±5.08 %. Larvae obtained from ovaprim hormone performed good growth rate and survival as compared to other hormones with weight gain 0.006 g, length gain 0.08 cm, and survival rate 61.17 ± 1.19 %. Therefore, it can be concluded that the optimal dose for inducing breeding of Tire Track Eel was 0.5 ml/kg.***

Keywords: Tire Track Eel, Ovulation, Live foods, Growth rate, Survival Rate

1. Introduction

Mastacembelus favus (Hora, 1923), is a member of a family Mastacembelidae under Synbranchiformes order, commonly known as tire Track eel and Flower spiny eel. This spiny eel species play a vital role as economic importance in mainland Southeast Asia as a fresh or dried product and sometimes as an aquarium trade [1]. Generally, this species is abundantly found along the bottom of tropical flowing water across Thailand, Lao PDR, Cambodia, Vietnam, and Peninsular Malaysia [2]. *Mastacembelus favus* has a lucrative size with high protein content. The eel flesh contains more than 303 cal/100g caloric value compared to 110 cal/100g in other average fish [3]. Generally, the morphology characteristic of *Mastacembelus favus* and *Mastacembelus armatus* are the same which creates misidentification as recorded in the international database of species taxonomy and DNA barcode. GenBank sequences of *Mastacembelus favus* formed a sister relationship to M. armatus although both have the same physical appearance, DNA barcoding helps to differentiate those two species [4]. In contrast to its significant value, the basic information related to its ecology and biology i. e. migratory pattern, breeding, and other spatial and temporal patterns of population dynamic and evolutionary trends remain in flux. The length of this species reaches up to 70 cm. This nocturnal fish feeds on benthic insect larvae, worms, small fishes, crustaceans, and some submerged plant matter [5].

Generally, the spawning period of Tire Track Eels starts from March to August, concentrating on June to July [6]. Most Mastacembelid family species spawn in freshwater i.e. Potamodromous. A species of Mastacembelid family usually found in the highland stream to lowland wetland, mostly in stream and river with sand, pebble or boulder substrate, flooded forest, coastal marshes and enter into the canal, lakes, and other floodplain areas during the flood season [7]. When the species get sexually mature, they lay the egg on the floating plants i. e. filamentous algae in the whirlpool. These species are relatively stationary species, which perform only short local migration. Habitat and spawning occur in the dry season. During the flood season, these species move to down - streams in the vicinity of the dry season for feed and spawn. When larvae attain its size, it again moves upstream [8]. Some spawning inducer i. e. photo - period, temperature, and monsoon rainfall plays a vital role in reproduction [9]. So far, the artificial ways of breeding eels for commercial farming have not been successful. Until the discovery of breed hatching from eel spawn, the industry will not try sustainability and will always depend on the supply of wild glass eels for its success [10].

Generally, fish were injected via intraperitoneal or intramuscular hormones for spawning. Only some experiments were conducted for the artificial breeding of *Mastacembelus favus* where most researchers used intramuscular hormonal treatment for the spawning. Intramuscular injections were used to broodstock using three different hormones i.e. HCG, PG, and LHRH + DOM up to now. An experiment used PG 5 mg/kg, HCG 300 IU/Kg, and LHRHa+DOM 150 µg/kg + 20 mg were best hormones whereas, overall LHRHa+DOM was considered to be the best hormone [11]. Similarly, Phan (2015) used a different dose of HCG and LHRHa+DOM and concluded with 2000 IU/kg HCG and 150 µg/kg +20 mg LHRHa+Dom were the best hormone for the spawning of Tire Track Eel [12]. Also, Nguyen et. al (2019) supported that the 3000 IU/Kg HCG was the best hormone for the artificial propagation of Tire Track Eel. However, Ovaprim was not used until this period for the artificial propagation of *Mastacembelus favus*. Ovaprim has unique advantages over PGE i.e. ready to use the liquid form in 10ml vial, consistent potency and reliable results, long shelf life, and can be stored at room temperature, formulated to prevent overdosing, male and female can be injected once simultaneously, reduce handling and post - breeding mortality, repeated spawning possible later in the season and high percentage of eggs, fertilization, and hatching. These overall reduces the handling of brood fish but also helps in saving a considerable amount of time and labor which will add to the cost of seed production. The potency of ovaprim is uniform and contains sGnRH which is known to be 17 times more potent than LHRH [13].

Fecundity and egg size of spiny eels can be influenced by food quantity [14], fishing pressure, and environmental parameter [15]. The fecundity increased with the size and weight of the fish. Generally, total length, weight can be used to predict fecundity of spiny eel but weight proved to be the best indicator of fecundity [9]. There are lots of theories related to the importance of knowledge regarding the fecundity of any fish. The knowledge of fecundity, its helps to determine spawning potential and its success [16]. fluctuation in the egg production potential of individual stock related to life processes such as age and growth [17], the effect of an environmental factor [18], and formulating the commercial management of fishery [19]. Since this spiny eel i.e. *M. favus* is new species that needs lots of research to conduct for accurate fecundity rate. Similar species under mastacembelidae is *M. armatus* with fecundity ranges from 3155 - 24684 eggs and the size was 260 - 535 mm (TL) [20]. Another species i.e. *Mastacembelus pancalus* has fecundity ranged from 765.48±231.68 (March) to 1691.96 ± 932.1 (August). A fish measuring 14.54 2.58 cm in length and 14.18 5.75 g in weight had the highest fecundity (1691.96) [21]. Ovulation in fish not only depends upon a high dose of hormone, rather depends upon the ecological condition, health status, and maturity level of fish gonads [22].

There are many species with economic significance, which can't reproduce spontaneously in captivity. So, the artificial propagation method is a reliable method to induce reproduction in these species using hormonal activity. The nature of the propagation in any fish is evaluated via the sexual maturity time/age, propagation season, the habitats, and the extent of the parental case [23]. Similarly, there are many external and internal mechanisms of fish spawning. The reproduction of fish species is mainly regulated by an external parameter that triggers the internal mechanism. By placing the fish in a favorable environment or by changing internal regulating factors with injected hormone or other substance effects on the final event of the reproduction cycle, egg and sperm release resulting in spawning. The internal mechanism in fish reproduction at a sequential level includes the brain, hypothalamus, pituitary, and finally to the gonad. The major thing to remember about appropriate hormones to be selected is based on the species to be spawned and the availability of the hormones [24]. The increase in the demand for quality seed in modern intensive and super - intensive culture systems is increasing day by day. To deal with this increased demand, artificial propagation play vital role in a better rate of fertilization and hatching, protection against enemies and unfavorable environmental condition, and better condition for growth and survival of species [23].

Larval rearing is the most sensitive part of any hatchery where, proper supervision and management in terms of feeding, environmental parameter, and nutrient are needed. The current trend of Intensification on fish culture is fully dependent on the availability of a well - balanced nutritionally complete diet and cost - effective compounded feeds. Usually, the larvae stage of any fish species and crustacean relies on the micro - algae and zooplankton. Commercial feed containing a high amount of protein, oil, and other nutrients is also useful during eel culture in which young prefer protein paste and pellets for the adult one. The young stage of these eels prefers zooplankton as feed, whereas grading should be emphasized to reduce cannibalism and competition for food [25]. *Mastacembelus favus* required a high proteinous diet for growth and development. Larvae feeding in Eels are very crucial, once the yolk is absorbed, and then they are fed zooplankton and boiled egg yolk [26]. The first exogenous feeding period of any larvae is the critical characteristic to manage them and this mostly depends on the absorption of the yolk sac. The initial larvae stage of any species is critical because the reserve of the yolk sac becomes exhausted and the fish is entirely dependent upon ingested food for nutrition [27]. An experiment was done on larval rearing of Mastacembelus erythrotaenia for 35 days. This experiment includes live feed i. e. Moina, tubifex, and homemade feed. It was found to be tubifex is the best feed for larval rearing whereas homemade feed should be provided at higher days old of larvae [28]. Generally, the fry of *Mastacembelus favus* can be reared at stocking density from 1000 to 1500 fry/tank/2 m3 and fed with live food like Artemia, Moina, Tubifex, Worms and some industrial feed for the proper growth and development of the species [6]. The riskiest and difficult phase in aquaculture is larval rearing which could be the most profitable venture if the larvae are properly fed with live feed and overcome the risk of mortality. After yolk absorption, the newly hatched larvae start exogenous feeding. During this period, they have a very small mouth size (<0.1mm), a primitive digestive system, and low digestibility. Hence, it's difficult to accept artificial supplementary feed. Here, live food playsa vital role as acceptable size and is easily digestible with the nutrient - rich diet for larvae. The combination of live food i. e. Moina and worm for 45 days of culture time showed good results in the survival and growth of *Mastacembelus favus* larvae [11]. Similarly, live feeds including artemia, Moina, and worms were used during the nursery stage of *Mastacembelus favus*, provided good results in growth and survival rate. The minimum survival rate of *M. favus* was 44.6% [6]. The main purpose of this experiment was to determine the effect of different doses of ovaprim to induce spawning of Tire track Eel and followed by a comparison with PG and HCG. Also, Analyze growth performance and survival rate of larvae with different hormonal treatments i. e. Ovaprimm, PG, and HCG.

2. Materials and Methodology

2.1 Research location

This study was conducted from June to July 2021. The experiments were carried out at the wet laboratory of the College of Aquaculture and Fisheries, Can Tho University, Vietnam. The experiments were conducted at the peak of the breeding season of Tire Track Eel (*Mastacembelus favus*).

2.2 Experimental Materials

Experimental fish

During the initiation of the experiment, active, healthy, sexually mature, body without deformation and wound, proper shape and proportion, and less aggressive fish was selected for breeding activities. The mature brood fish was 500 - 800g/tail. The broodstock fish were conditioning in the hatchery. They were fed with grinded trash fish combined with commercial pellets (40% CP). Selected broodfish were weighed and kept in the cistern to inject with different hormones. Two cisterns were used with size 1m3. The cisterns were provided with continuous water supply and aeration facilities for conditioning of broodfish. Nylon rope was used for the Substrate in the cistern tank. Male and female broods were identified considering their external appearances. The mature male has their flat abdomens and long protruded genital papillae whereas, females have swollen and round abdomen, urogenital papillae [29].

Hormones and other chemicals

In this experiment, experimental fish were weighed and injected with recommended doses. All male and female fish were injected intra - muscularly exactly above the lateral line with a half dose of female final injection to male. Both PG and Ovaprim were injected one time where HCG doses were given three times i. e. first 400 IU/kg, the second time was 1000 IU/kg and final doses was 1600 IU/kg. The time for the 2nd injection was 24 hours after the ist injection whereas, the 3rd time (decision) was from 6 to 8 hours after the 2nd time. Male fish was injected only one time along with 3rd injection for females. The eggs and milts were collected by the stripping method. In this experiment, 3 hormones were used for comparing the suitable doses of ovaprim with the best dose of Pituitary Gland (PG) and Human Chorionic Gonadotropin (HCG). Saline solution (9 ppt) was used to dilute hormones before injecting into the brooders. Milts were stored in NaCl solution before fertilizing the eggs. Later on, potassium permanganate (3ppm for 30 min) was used during the incubation period to remove fungus growth. During larval rearing, iodine (1ml), and potassium permanganate (3 ppm for 30 min) were used to clean the tank and treat for fungus infestation.

Feed

The larvae were fed with live foods i. e. Moina (Moina sp.), and Tubifex (Tubifex tubifex) for 20 days whereas the broodstock fish were fed with trash fish combined with pellet feeds (40% CP) twice a day at 8 am and 7 pm at the evening. The live food for larvae was fed 6 times a day (6 am, 9 am, 12 pm, 3 pm, 6 pm, and 9 pm) respectively.

2.3 Handling of brooders and conditioning

Proper handling and conditioning of broodstock fish during artificial propagation leads to successful breeding activities. Deformation, wounded and stressful conditions during transportation, and injection time can affect fish spawning results negatively than all other factors. Also, physiological changes can lead to a breakdown of eggs or re - absorption in females as a result of stress from injuries and a cultured environment. In this experiment, The broodstocks were conditioning at the hatchery in a 100 L tank with aeration at Can Tho University. Broodstock was fed 2% of body weight with trash fish combined with pellet feed (40% CP). Here, Brood fish were maintained in optimal conditions and good water quality parameters to avoid stress and enhance the success of hormone - inducing spawning. The conditioning period was one month. Furthermore, potassium permanganate was used to treat fungus infestation before stocking in the conditioning tank.

2.4 Determination of fecundity and egg diameter

Total or absolute fecundity measures the total number of eggs that females produce during the spawning period. Absolute fecundity was measured by counting the total eggs in a ripe gonad through a gravimetric sub - sampling method [30]. The procedure involved weighing the whole ripe gonads stored in 4% formaldehyde. A small portion from each posterior, middle and anterior region was weighted and the number and weight of ripe eggs were determined. The egg's size was determined by using a calibrated eyepiece in the ocular microscope. The egg size was found to be 3 - 3.7 mm from randomly selected eggs per replication. The total number of ripe eggs in the gonad of an individual fish was calculated by using the formula:

$$E = \frac{(Wg \; X \; Es)}{We}$$

Where,
E: the total number of eggs in a gonad
Wg: the weight of the gonad
Es: The number of eggs in the sample
We: the weight of eggs in the sample

2.5 Stripping and fertilization

Initially, eggs and milts were collected in a tray using the stripping method. Then, striped eggs and milts were mixed using a sterile feather. For the better results of fertilization, saline water was used and mixed well with clean water. Later on, a solution of NaCl 4 g was used for washing the eggs. And finally, the inseminated eggs were incubated on the grid net, provided with continuous droplets of water and aeration.

2.6 Experimental design

Experiment 1: Effect of different Ovaprim doses on spawning induction of Tire Track Eel (*Mastacembelus favus*)

This experiment was designed with 4 treatments and 3 replicates for each treatment. The experiment was based on

Paper ID: SR21920090527 DOI: 10.21275/SR21920090527

the finding of a suitable dose and its effects on fish breeding behavior. Each treatment included a 1: 3 sex ratio of males and females. In this experiment, both males and females were injected with hormone for only one time where the males were injected half of the final dose of female. Different doses of Ovaprim i.e. 0.25, 0.5, 0.75, and 1 ml/kg were used to assess the best hormone for artificial propagation.

Experiment 2: Comparative doses of Ovaprim with PG and HCG

This experiment was conducted after obtaining the best dose of ovaprim and later, compared with other hormones i.e. PG and HCG.

Table 1: *Comparative doses of Ovaprim with PG and HCG*

Treatment	T1	T2	T3
Hormone doses	Ovaprim	PG	HCG
	From 1st experiment	5mg/kg	3000 IU/kg

Experiment 3: Larval rearing technique obtained from different doses of Hormone

The last experiment followed by the second experiment was based on larval rearing obtained from different hormonal activities i.e. Ovaprim, PG, and HCG. The larvae were fed with the frequency of 6 times a day (6 am, 9 am, 12 pm, 3 pm, 6 pm, and 9 pm), every 3 hours using live food i.e. Moina and Tubifex. This experiment included 3 treatments and 4 replication for each treatment. Total larvae in each replication tank were 150, reared in 0.1 m3 of the tank. During these periods, water quality parameters i.e. Temperature, DO, pH, Ammonia, and Nitrite were monitored and recorded daily in the morning and afternoon.

Table 2: Larval rearing obtained from different hormonal activity

Treatment	Hormone	Moina	Tubifex
T1	Ovaprim	1 - 5 days	6 - 20 days
T2	PG	1 - 5 days	6 - 20 days
T3	HCG	1 - 5 days	6 - 20 days

2.7 Monitoring of Water quality parameters

Water quality parameters were monitored regularly daily using a thermometer, hand - held Oxy - Guard digital meter, and test kit for NH3 and NO2. Water quality parameters like temperature, DO, pH, ammonia, and nitrite were measured during the larval rearing period.

2.8 Analytical methods

Eggs quality analysis: After fertilizing eggs, the eggs were checked by using the following spawning parameters [31].
Spawned female broodfish:
Absolute fecundity (No/kg) = Total number of egg / 1kg of female
Fertilization Rate (%) = (No. of fertilized. Eggs / total No. of incubated eggs (fertilized+ unfertilized)) *100
Hatching rate (%) = (Total No hatched eggs / Total No of incubated eggs) *100
Egg size (mm): will be determined using Microscopy

Larval quality analysis: The parameter involved in larval rearing was evaluated by using the following data (El - Hawarry et al., 2016) :
Weight gain (WG): Final weight – Initial weight
Length gain (LG): Final length – Initial length
Average weight gain (AWG) = (Final weight – Initial weight) / Days
Average length gain (ALG) = (Final length – Initial length) / Days
Specific growth rate (SGR) = (lnFinal weight - ln Initial weight) / Days*100
Survival Rate (SR) % = (No of fish harvested / No of fish stocked) *100

2.9 Statistical Analysis

Statistical analysis, one - way analysis of variance, and Duncan's multiple range tests were used to evaluate the best dose of ovaprim and the difference of hormones efficacy in the experiments. Data were analyzed for mean, standard deviation, standard error mean, and One way ANOVA to analyze larval rearing data using statistical software SPSS 22.0 and Excel 2016 software. Differences were considered a p - value of 0.05 using computer software SPSS.

3. Results and Discussion

3.1 The effects of different hormones on spawning

The ripe and healthy male and female broodfish should be selected based on physical and visual examination of secondary sexual characteristics i.e. size, color, swollen abdomen, and genital openings to achieve successful artificial propagation. The Density of broodstock fish should be maintained at 1 - 2 kg/m3 for better survival of Tire Track Eel [12]. As the breeding experiment is conducted at the peak of spawning time, egg size will be bigger as compared to other months. During the acclimatization of broodstock, tolerance of stress and nutrition affect the egg size, timing of reproduction, or complete inhibition of reproduction [32]. Research conducted for artificial breeding of Tire Track Eel and fed with trash fish resulted in a maturity rate of 75.5 % in brood stocks [33]. The breeding season of *Mastacembelus favus* is March to August, concentrating on June - July [6].

3.1.1 Water quality parameters during spawning

The system was provided with a continuous aeration system. A centigrade thermometer within the range of 0°C to 120°C was used to record the water temperature, whereas, hand - held Oxy - Guard digital meter was used to record pH and Dissolved oxygen.

Table 3: Water quality parameter during spawning

S. N.	*Parameters*	*Range*	*unit*
1	*Temperature*	*27 - 29*	*°C*
2	*Dissolved Oxygen (DO)*	*4 - 6*	*mg/L*
3	*pH*	*7 - 8*	

3.1.2 Spawning parameters using different doses of Ovaprim

After conditioning of broodstock, the first experiment was conducted to determine the best dose of ovaprim. The sex ratio of male: female was 1: 3 in each treatment. Different doses of ovaprim i. e.0.25, 0.5, 0.75, and 1 mg/L were used. During this experiment, ovulation rate, absolute fecundity, fertilization rate, and hatching rate were determined. The parameters are mention below in Table 4.

Table 4: Spawning parameter using different doses of ovaprim

Parameters	Treatments			
	T1	T2	T3	T4
	0.25 ml/kg	0.5 ml/kg	0.75 ml/kg	1 ml/kg
Ovulation rate (%)	100 ± 0.00	100 ± 0.00	66.6 ± 57.7	33.3 ± 57.7
Absolute fecundity	2378 ± 3151^a	7649 ± 5754^b	1518 ± 856^a	519 ± 415^a
Fertilization rate (%)	55.8 ± 2.29^b	75.52 ± 5.14^b	26.67 ± 12.67^a	16.67 ± 28.87^a
Hatching rate (%)	59.7 ± 25.08^b	64.5 ± 4.2^b	25.65 ± 12.52^a	5.25 ± 4.23^a

Note: The values shown on the table are the mean ± SD. The different superscripts a, b represents significant differences ($p<0.05$).

The ovulation rate was similar when applied 0.25 ml/kg and 0.5 ml/kg ovaprim but absolute fecundity, fertilization rate, and hatching rate were higher when using 0.5 ml/kg ovaprim. T2 was significantly different ($p<0.05$) with other treatments in absolute fecundity whereas there was no significant difference with T1 in fertilization rate and hatching rate. On increasing the dose of ovaprim, there was a gradual decrease in spawning parameters. Upto now, there was not any fixed ovaprim dose for spawning. Now, Table 4 signify that the ovaprim 0.5 ml/kg was the best dose for artificial propagation of Tire Track Eel.

4.1.3 Spawning parameters using different hormonal doses i. e. Ovaprim, PG, and HCG

The second experiment was conducted with the best dose of ovaprim 0.5 mg/L and compared with the best doses of PG and HCG. Different parameters were determined i. e ovulation rate, absolute fecundity, fertilization rate, and hatching rate as listed below in Table 5.

Table 5: Spawning parameters using different hormonal doses

Parameters	Treatments		
	T1	T2	T3
	Ovaprim (0.5 mg/L)	PG (5mg/kg)	HCG (3000 IU/Kg)
Ovulation rate (%)	100 ± 0.00	33.3 ± 57.7	66.6 ± 57.7
Absolute fecundity	21588 ± 3661^b	8681 ± 5924^a	2558 ± 4516^a
Fertilization rate (%)	74.8 ± 9.52^b	35.82 ± 10.94^a	29.97 ± 19.17^a
Hatching rate (%)	69.7 ± 5.08^b	21.5 ± 18.2^b	25.65 ± 19.92^a
Egg size (mm)	3.35 ± 0.17^a	3.37 ± 0.17^b	3.35 ± 0.18^a

Note: The values shown on the table are the mean ± SD. The different superscripts a, b represents significant differences ($p<0.05$).

The delay time between hormone injection and collection of ova is a major factor in the success of reproduction techniques that involve hormone - induced ovulation and artificial fertilization of fish [34]. Delayed collection of gametes after ovulation leads to over - ripening of ova that impacts on low fertilization rate, a large number of deformed embryos, and increased mortality rate for embryos and larvae [35]. The ovulation rate of the present study was 100% (Ovaprim), 33.3 ±57.7 % (PG), and 66.6 ±57.7 % (HCG). Overall, the ovulation rate obtained from Ovaprim was significantly higher than PG and HCG. The ovulation rate when used LHRH+DOM (150 µg/kg+20 mg) was 95.0±7.07 % [11], which was comparatively higher than the present study when use HCG (3000 IU/kg) i.e. 87.7±8.80 %.

Different hormonal doses gave different effects on induced breeding parameters i.e. ovulation rate, fertilization rate, hatching rate, and eggs size. The reproductive potential of the fish can be measure in terms of fecundity which increased with the size and weight of the fish [9]. Estimation of fecundity is a prerequisite not only in assessing the stock and life history of the species but also in developing a successful breeding program [36]. The number of eggs stripped depends upon the maturity of fish and the GSI of female brood fish. The total number of egg obtained from a single female differ considerably and depends upon several factors like age, size, condition, and species [37]. As egg number in tilapia is inversely related to the average weight of a female brood fish i.e. fish produce either a large number of small eggs or a small number of large eggs [38]. In this experiment, absolute fecundity was measured by using three different hormones i.e. Ovaprim, PG, and HCG. The results of this experiment signify that the ovaprim dose of 0.5 ml/kg had absolute fecundity of 21588 ±3661, PG (5 ml/kg) had 8681 ±5924, whereas HCG (3000 IU/Kg) had 2558 ±4516. The absolute fecundity of the present study was comparatively high as compared to *Mastacembelus armatus*, with absolute fecundity 580 - 10, 980 [39], 2, 235 - 19, 493 [40], and *Macrognathus pancalus* with absolute fecundity 765.48 ± 231.68 (March) to 1691 ± 932.1 (August) [21].

The fertilization and hatching rate of species largely depends upon water quality parameters [41]. Also, the fertilization rate depends on the quality of brood fish, maturity and size of the egg, sperm quality, and mobility of the male [42]. In this study, three different hormones were used i. e. Ovaprim, PG, and HCG. Overall fertilization and hatching rate obtained from ovaprim hormone (74.8±9.52% and 69.7±5.08%) were relatively higher than PG (35.82±10.94% and 21.5±18.2%), and HCG (29.97±19.17% and 25.65 ± 19.92%) respectively. In a similar study, some researchers used PG, HCG, and LHRH for the artificial propagation of Tire Track Eel. An experiment reported that HCG (3000 IU/Kg) and LHRH+DOM (150µg/kg+20mg) were the best hormonal doses for artificial propagation of Tire Track Eel [11]. Researcher used HCG (200, 2500, and 3000IU/KG), PG (3, 4, and 5 mg/kg), and LHRH+DOM (100, 150, and 200 µg/kg+20 mg) for induce breeding. The fertilization rate and hatching rate while using PG 5 mg/kg were 59.0 ± 4.10 and 66.0 ± 4.10 respectively, which were higher as compared to the present study. This might be due to the size, weight and, age of broodstock which were higher as compared to the present study. Overall, the highest

Paper ID: SR21920090527 DOI: 10.21275/SR21920090527

fertilization rate obtained from HCG (3000IU/kg) was 71.3 ±10.2, which is lower as compared to the present study (74.8±9.52), whereas, highest hatching rate obtained from LHRH+DOM (150µg/kg+20mg) was 77.3±3.10 which is higher as compared to present study by using ovaprim (0.5mg/kg). Similarly, the fertilization rate was found to be 60% while using HCG hormone [6] which was comparatively higher than the present study when HCG was used for propagation. The hatching period was 46 - 55 hours of fertilization [6] 46 - 49 hours [11] whereas, the present study shows 43 - 44 hours of hatching time after fertilization of the egg.

The quality of eggs is directly related to the quality of feed provided. As trash fish was used during artificial breeding of Tire Track Eel, provide a good result in maturity rate [34]. Some theories believed that egg size varied only within a relatively narrow window. A regression analysis revealed that there is a strong and significant relationship between fish size to fecundity and egg size [44]. In tilapia, there is unclear data on whether maternal age or size is the major factor influencing egg size [43]but still, some researchers claimed that maternal age may be a very important factor [44]. Egg diameter differs with developmental stage, spawner age/size, water quality parameter, and best timing of spawning season [45]. At the peak of spawning time, the egg size was bigger as compared to other months. Similarly, acclimatization of broodstock, stress tolerance, and nutrition affect the egg size, timing of reproduction, or complete inhibition of reproduction [32]. This experiment shows that the egg size obtained from different hormonal activities were range from 3.5 - 3.7 mm. As compared to other species from the same genus, M. armatus has an egg size of 1.46 - 2.05 mm which was large in number [9].

4.2 The effect of different hormones on the growth and survival rate of Tire Track Eel larvae

The Tire Track Eel is a highly important species that serves both as a food fish as well as aquarium fish species and hence has an excellent culture potential. To expand the culture of this species, knowledge of early larvae development and feeding is imperative. Hormonal practices enhance the spawning as well as larvae rearing practice i.e. growth and survival rate. But the overall larvae culture of this fish is poorly understood and only a few studies have ever been made. Therefore, the present study was conducted to develop a larvae growth strategy from different hormonal practices i. e PG, HCG, and Ovaprim

4.2.1 Water quality parameters of larval rearing tanks

The ranges of water quality parameter i.e. temperature (morning 26 - 31°C and afternoon 30 - 33°C), DO (4 - 7 mg/L), pH (6 - 8), ammonia (0 - 1 mg/L), and nitrite (0 - 4 mg/L) were recorded during rearing period.

Table 6: Water quality parameters during larvae rearing

Parameters	Treatments					
	T1		T2		T3	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Temperature (°C)	28.62±1.3	31.49±0.6	28.76±1.3	31.56±0.6	28.94±1.3	31.56±0.6
DO (mg/L)	5.32±0.5	5.08±0.8	5.23±0.6	5.21±0.8	5.27±0.6	5.19±0.8
pH	7.47±0.2	7.57±0.2	7.5±0.2	7.6±0.2	7.52±0.2	7.56±0.2
Ammonia (mg/L)	0.23±0.1	0.25±0.12	0.23±0.1	0.25±0.12	0.23±0.1	0.25±0.11
Nitrite (mg/L)	1.65±1.3	1.45±1.2	1.5±1.3	1.65±1.2	1.6±1.3	1.7±1.3

Note: Values shown in the table are in mean±SEM.

4.2.2 Fish growth and specific growth rates

Variation in the mean values of growth parameters (initial weight, weight gain, final weight, initial length, final length, length gain, Average Daily Length Gain, Specific Growth Rate, Average Daily Weight Gain), and Survival rate under different treatments are presented in Table 7.

Table 7: Fish growth rate parameters of *M. favus* larvae

Parameters	Treatment		
	T1	T2	T3
Initial weight (g)	0.019 ± 0.0002^{a}	0.018 ± 0.0004^{a}	0.019 ± 0.0001^{a}
Initial length (cm)	0.87 ±0.00^{a}	0.87±0.002^{a}	0.86±0.006^{a}
Final weight (g)	0.14 ± 0.004^{c}	0.10 ± 0.004^{a}	0.12 ± 0.004^{b}
Final length (cm)	2.46±0.005^{c}	2.30±0.010^{a}	2.38±0.005^{b}
Weight gain (g)	0.12 ± 0.004^{c}	0.08 ± 0.004^{a}	0.10 ± 0.004^{b}
Length gain (cm)	1.59±0.005^{c}	1.43±0.010^{a}	1.51±0.009^{b}
Average daily weight gain (g)	0.006 ± 0.0002^{c}	0.004 ± 0.0002^{a}	0.005 ± 0.0002^{b}
Average daily length gain (cm)	0.079±0.0002^{c}	0.072±0.0005^{a}	0.076±0.0005^{b}
Specific growth rate (%)	10.00 ± 0.17^{c}	8.50 ± 0.32^{a}	9.27 ± 0.18^{b}
Survival rate (%)	*61.17 ± 1.19*^{c}	*40.67 ± 1.87*^{a}	*50.00 ± 2.36*^{b}

Note: The values shown on the table are the mean ± SEM. The different superscripts a, b, and c represents significant differences (p<0.05).

Mastacembelus favus is a nocturnal carnivorous inhabits and feeds on benthic insect larvae, worms, small fishes, crustaceans, and some submerged plant matter [5]. In this experiment, the culture period was 20 days where live foods (Moina and Tubifex) were used because it was very hard for the *M. favus* larvae to accept the commercial feed [46]. Moina and tubifex being comparatively smaller in size are the starter proteinous diet of most fish larvae which contains a high amount of nutrients that enhance growth and development [47]. The larvae were obtained from 3 different hormones i.e. PG, HCG, and Ovaprim. Later on, the larvae were reared in 50L of the tank with the same density i.e. 150 individual/tank. Majorly high proteinous live foods Moina was used for first 5 days and rest 15 days tubifex was used to feed larvae. During the stocking period, the weight and length of larvae were 0.018 - 0.019 g and 0.86 - 0.87 cm respectively. Proper management and care were taken during the culture period where temperature, pH, DO, Ammonia, and nitrite parameters were measured daily during culture period. The values of water quality parameters i.e. temperature range from 26 - 31℃ (morning), and 30 - 33℃ (afternoon), pH ranges from 6 - 8, DO ranges from 4 - 7mg/L, Ammonia ranges from 0 - 1 mg/L and Nitrite ranges between 0 - 4 mg/L during culture periods.

In the present study, the final length after 20 days of culture was approx 2.46±0.005 cm which is lower as compared to 45 days of reared larvae (6.50±0.18) [11]. The newly hatched larvae of *Macrognathus aculeatus* was 2.08±0.02 mm [48], *Mastacembelus pancalus* was 2 mm [49], and 2.9 mm for Japanese eel (*A. japonica*) [50]. The growth of larvae was higher in M. aculeatus as compared to *M. favus* and other mastacembelidae species. The variation may be due to different species and geographical habitats. Also, water quality parameters play a vital role in the growth and survival of species. It was recommended that 27 - 31°C for proper growth and survival of larvae [49], which is comparatively the same as the present study (26 - 32℃).

During the early days, the digestive system of larvae was not completed. cladocerans and tubifex worm have a significant effect on the growth and survival rate of *Mastacembelus favus* when used in the early days of larvae rearing [51]. Digestibility of live food is higher compared with commercial feed and hence, the highest growth can be achieved when live food were used during larvae rearing [52]. Specific growth rate, daily weight gain, and survival rate in T1 were 10%, 0.006 g, and 61.18 % which were significantly different (p>0.05) than other treatments. During the culture period, an infestation of fungus was treated using KMnO4 and Iodine. Proper management practices were applied during the larvae rearing period. However, fungus from tubifex affected the larvae which caused high mortality in some tanks, which resulted in the lower survival rate of larvae in all treatments. The young larvae have an insufficient amount of digestive enzymes to thrive on commercial feed powder, which live food provide exogenous enzyme to trigger early growth stage of larvae [53].

4.2.3 Survival rate of *M. favus* larvae in different hormonal activity

The survival rate of *M. favus* larvae obtained from different hormones (Ovaprim, PG, and HCG) was mentioned in Table 7. The highest survival rate was 61.17±1.19 % obtained from the first treatment (Ovaprim) whereas, the lowest survival rate was 40.67 ± 1.87 % in the second treatment where PG hormone was used. Statistical test of variance (one - way ANOVA) and Duncan multiple tests showed that larvae from ovaprim hormone had a significant difference (p>0.05) on the survival rate of larvae. Larvae obtained by use of HCG hormone show a 44.6% survival rate in *M. favus* when reared at 750 tail/m3 [6]. Here, the present study shows the highest survival obtained from 0.5 ml/kg (Ovaprim) and if compared with HCG, the survival rate is 50 ± 2.26 which is comparatively higher. A experiment conducted by using various hormone i. e PG (3, 4, and 5 mg/kg), HCG (2000, 2500, and 3000 IU/kg), and LHRH+DOM (100, 150, 200 μg/kg +20 mg) on Tire Track Eel. Later on, the total survival rate after 45 days of rearing was found to be 60%, [11] which is almost the same as the present study.

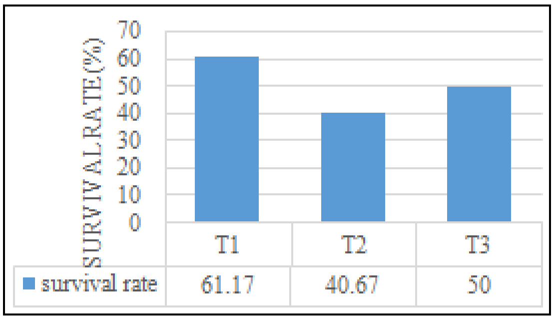

Figure 1: The survival rate of larvae in different treatment

4. Conclusion and Recommendation

From the results of this study, it was concluded that the best ovaprim dose for ovulation of *Mastacembelus favus* was 0.5 mg/kg. later on, when compared with other hormones PG (5ml/kg) and HCG (3000IU/kg), still ovaprim was found to be best with Ovulation rate 100±0.00%, fertilization rate 78.8 ±9.52%, and hatching rate 69.7 ±5.08%. Results of larvae rearing for 20 days signify that the growth rate and survival rate were higher from the ovaprim doses, which was 0.006 g/day and 61.17% respectively. Further research is still needed to be conducted. Determining the effect of different hormones on re - maturation and re - spawning of Tire Track Eel should be carried out.

5. Acknowledgement

We would like to thanks the College of Aquaculture and Fisheries, Can Tho University for providing research facilities to carry out this work.

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