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Effects of stocking density, feed and hormones on Artificial reproduction of Tire track eel *(Mastacembelus armatus)*

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The tire track eel (*Mastacembelus armatus*) is a species of ray-finned, spiny eels belonging to the genus Mastacembelus (Scopoli, 1777) of the family Mastacembelidae, and is native to the riverine fauna of India, Pakistan, Sumatra, Sri Lanka, Thailand, Viet Nam, Indonesia and other parts of South East Asia.
In Vietnam, this species is found commonly in Red river system and several other rivers in Northern and South Central Vietnam. It is reported to be a very good food-fish, and high commercial value. Because of its high value, the tire track eel is being overfished, lead this species resource to a serious decline. The study on effects of stocking density, feed and hormones on Artificial reproduction of Tire track ell (*Mastacembelus armatus*) is necessary to actively breeding, restoration of natural resources, conservation of biodiversity.
Our study consisted of 4 experiments. In experiment 1, the fish were randomly stocked at densities 1 kg/m^3, 2 kg/m^3 and 3kg/m^3, which were coded as MĐ1, MĐ2, MĐ3, respectively. The results showed that survival rate was lowest with MĐ3, but maturity rate and maturity index were equivalent to MĐ 1, MĐ2 ($\alpha = 0.05$). In experiment 2, the fish were fed with three diets of worm, commercial pellet, trash fish, which were coded as CT1, CT2, CT3, respectively. In the second experiment, the survival rate, maturity rate and maturity index of the CT1 were the highest, followed by CT3, and CT2, respectively.
Experiment 3, fish were divided into 6 treatments of different hormonic injections. The results showed that the treatment of CT6 with 2000UI of HCG) was the best treatment. 64,29% female broodfish were ovulated after 38.58 h from the last injection at the temperature of 23-28^{0}C. Last experiment, we used 3 different methods of fertilization. The results indicated that fertilization rate and hatching rates were the best at 68,0% and 55,17%, respectively when the dry fertilization method was applied. In conditions, the temperature was 27^{0}C.

Key words: Tire track eel, Artificial reproduction, hormone, Maturity rate, Maturity index, ovulated, fertilization rate, hatching rates

Introduction

The tire track eel (*Mastacembelus armatus*) is a species of ray-finned, spiny eels belonging to the genus Mastacembelus (Scopoli, 1777) of the

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family Mastacembelidae, and is native to the riverine fauna of India, Pakistan, Sumatra, Sri Lanka, Thailand, Viet Nam, Indonesia and other parts of South East Asia.

In Vietnam, this species is found commonly in Red river system and several other rivers in Northern and South Central Vietnam. This is a very valuable commercial fish and is greatly demanded due to its good taste. Because of its high value, the tire track eel is being overfished, lead this species resources to a serious decline. The study of Effects of stocking density, feed and hormones on Artificial reproduction of Tire track eel (*Mastacembelus armatus*) is necessary to actively breeding, restoration of natural resources, conservation of biodiversity.

In this study, we focused on two main issues. The fist issues was studies on the effects of diets and stocking density on the reproductive capacity of tire track eel. The second was to evaluate effects of some hormones on the breeding quality in Tire track eel

Materials and methods

Materials

Fish stocks: Tire track eel were captured from Red river and maintained in tanks. More one year old males and females fish (average weight 150g) were selected.

Hormones: Luteinizin Hormone-Releasing hormone analogue (Des-Gly10, [D-Ala 6] LH-RH Ethylamide) or LHRHa is a peptide that is similar in structure to native luteinizing hormone hormones (LHRH). The LRHa available on the market is a white powder and is combined with mannite as a filler (made in China).

Human chronic gonadotropin (HCG) is a polypeptide hormone with molecular weight 36000. At present, the ready-made material available on the market in china is "veterinary gonadotropin".

Methods

In the first experiment, 12 kg broodstocks (50% male and 50% female) were divided into 3 raceway tanks (size 1x2x1m). The fish were randomly stocked at densities 1 kg/m3, 2 kg/m3 and 3kg/m3, which were coded as MĐ1, MĐ2, MĐ3, respectively. Fish were fed the same foods (3% BWD, two times a day). The fish were raised in similar conditions, excepted experimental factor, repeated this experiment 3 times.

In experiment 2, 12 kg broodstock (50% male and 50% female) were divided into 3 raceway tanks(size 1x2x1m). The broodstock tire track eel were fed with three diffirent diets (blue worm, commercial peelet, trash fish, which were coded as CT 1, CT 2, CT 3, respectively). Fish were fed 3% body weight day two times a day (morning, sunset) throughout the experiment. In this experiment, the fish were raised in similar conditions, excepted experimental factor. repeated this experiment 3 times

Every week, Fish were checked for maturation. To determine survival at grading time (two times a month), number of fish was established and computed against the number at stocking. The experiments were taken place 2 months.

$$\text{Survival rate\%} = \frac{\text{Harvested amount}}{\text{Stocked amount}} \times 100$$

$$\text{Maturity rate\%} = \frac{\text{The number of maturation fish}}{\text{Stocked amount}} \times 100$$

$$\text{Maturity index\%} = \frac{\text{Ovary weight of female fish}}{\text{Body weigh of female fish}} \times 100$$

The third experiment, 24 female fish and 24 matured male fish were used. Females were selected for injections based on external characteristics reddish swollen vent and a soft rounded abdomen. Prior to injection, fish were individually weighted and we randomly divided into 6 treatment groups.

Groups of 8 fish were inject I.M. with different preparation (table 1.1). The Females Females were Intraperitoneal injected with different preparation: LHRHa combined with Dom (50 µg LRHa + 10mg DOM). For female fish, double injection were done in 1/3-2/3 ratio, 12 hours apart. The male fish were given one injection (same dose of female's first dose and first injection time)

Table 1. Dose of hormone treatment for tire track eel broodstock *1 kg^{-1}b.w female*

CT1	**CT2**	**CT3**	**CT4**	**CT5**	**CT6**
50 µg LRHa + 10mg DOM	100 µg LRHa + 10mg DOM	150 µg LRHa + 10mg DOM	1000UI HCG	1500UI HCG	2000UI HCG

After the second injection, the fish were placed in an indoor fiberglass tank with running water, temperature 23-28^0 C. The fish were checked for ovulation after

second injection every 6 hours interval up to ovulation. Each ovulated fish was stripped and the eggs were fertilized using sperm from male (Males were sacrifice to obtain their sperms). Used dry fertilization. Fertilized eggs were washed several times with clean water to remove the excess milt, blood etc. The fertilized eggs were transferred to for incubationTo observe the fertilization rate, 100 eggs were placed into mini plastic circular hatchery (20 L capacity) and after about 8 hours of gentle stirring, fertilization could be checked under a dissection microscope (10~100× magnification). Fertilization membranes formed when an egg was fertilized so we could use them as a standard to determine whether such an eventoccurred.

$$\text{Spawning rate\%} = \frac{\text{The number of ovulated fish}}{\text{Total number of injection fish}} \times 100$$

$$\text{Fertilization rates \%} = \frac{\text{The number of fertilized eggs}}{\text{The number of total eggs}} \times 100$$

$$\text{Relative- fecundity} = \frac{\text{The number of fish eggs}}{\text{Body weight of female (kg)}}$$

The data obtained from the trail were subjected to one way analysis of variance (anova) (using SPSS 16. Programme) to test for effects of induced treatments. When ANOVA identified significant difference among groups, multiple comparison tests among means were performed using LSD test. For each comparison, statistically significant differences were determined by setting the aggregate type I error at 5% ($P<0.05$)

Results

Survival and Maturity rates of broodstock Tire track eel breeding in different densities

Survival rate of broodstock Tire track eel breeding in different densities

The results of the survival rates of broodstock Tire track eel breeding in different densities are summarized in Table 2.

Table 2. Effects of breeding density on survival rate of broodstock Tire track eel

Breeding density Treatments/ Formulas	***Survival rate*** (mean, %)	
	15 days	30 days
MĐ1	89,52[a]	95,71[a]
MĐ2	89,36[a]	95,56[a]
MĐ3	81,45[b]	92,01[a]

Within columns, means marked/ followed by the same letter are not significant different according to P>0.05

The results shown in Table 2 indicate that, average survival rate of broodstock Tire track eel was 86.78% after breeding for 15 days as fish began to get familiar with artificial stocking environment. The survival rates of broodstock Tire track eel in the MĐ1 and MĐ2 treatments were 89.52% and 89.36%, respectively, which were higher than that in the MĐ3 treatment (81.45%). The survival rates were significant difference between the MĐ1, MĐ2 and MĐ3 treatments according to P <0.05.

In contrast, for longer breeding period: 15-30 days, broodstock Tire track eel got familiar with artificial conditions resulted in higher survival rate than this of the previous period of breeding, being 94.43%. The survival rates of broodstock Tire track eel in the MĐ1 and MĐ2 treatments were 95, 71% and 95.56%, respectively, which were higher than that in the MĐ3 treatment (92.01%). The survival rates were not significant difference between the MĐ1, MĐ2 and MĐ3 treatments according to P >0.05.

Different breeding densities of broodstock Tire track eel results in different survival rates. Thus, in order to get the highest survival rate, it is recommended that the breeding density of broodstock Tire track eel should be 1 - 2 kg / $1m^3$.

Maturity index, Maturity rates of Tire track eel breeding in different breeding densities

When Tire track eel bred in different density in broodstock *cement tanks,* the Maturity index of the MĐ1 and MĐ2 treatments were 18.01% and 18.25%, respectively, which were higher than that of the MĐ3 treatment, being 17.92%. However, the Maturity index of these three treatments was not significant difference among the MĐ1, MĐ2 and MĐ3 treatments according to P >0.05.

In addition, the maturity index was different among the iterations. The highest maturity index of all experimental formulas was observed at the second iteration. This result may be explained by the experiments of second iteration were coincided with the natural breeding season of Tire track eel in May leading to the highest maturity.

Table 3. Effects of breeding density on Maturity index and Maturity rate of broodstock Tire track eel

Breeding density Treatments/ Formulas	Maturity index %	Maturity rate %
MĐ1	18.01[a]	66.16[a]
MĐ2	18.25[a]	64.72[a]
MĐ3	17.92[a]	64.81[a]

Within columns, means marked/ followed by the same letter are not significant different according to P>0.05

The results shown in Table 3 indicate that, the highest Maturity rate of broodstock Tire track eel was obtained for the Formula 1, being 66.16%, followed by the formulas 3 and 2, being 64.81% and 64.72%, respectively. These differences among the three formulas were not significant. Thus, the results suggest that breeding density may not effect on the Maturity rate of broodstock Tire track eel.

From the above results, it is recommended that the breeding density of broodstock Tire track eel should be 1 - 2 kg / $1m^3$ in order to get the highest survival rate, Maturity index and Maturity rate.

Survival and Maturity rates of broodstock Tire track eel feeding with different kinds of feed

Survival rate of Tire track eel feeding with different kinds of feed

The results shown in Table 4 indicate that, survival rates of broodstock Tire track eel were significant different among the experimental formulas 86.78% after breeding for 15 days. The survival rate of broodstock Tire track eel was highest for the CT1 treatment, being 91.05%, followed by the CT3 and CT2, being 87.83% and 79.17%, respectively. The survival rates were significant different between the CT1, CT3 and the CT2 treatments (P <0.05).

Table 4. Effects of feed on survival rate of broodstock Tire track eel

Food formulas	***Survival rate*** (mean, %)	
	15 days	30 days
CT1	91,05[a]	100[a]
CT2	79,17[b]	84,17[c]
CT3	87,83[a]	95,56[b]

Within columns, means marked/ followed by the same letter are not significant different according to P>0.05

Survival rate of the formula 1 was higher than this of the formula 3, however this higher value was not significant different (P> 0.05). As the food used for the formulars 1 and 3 were obtained from natural sources, so the

broodstock Tire track eel adapt with these food easier after breeding for 15 days, resulted to high survival rate.

Similar to the results of the first 15 days of the breeding experiment, survival rates of broodstock Tire track eel were significant different among the experimental formulas after 30 days of the breeding process. Formula CT1 had the highest survival rate of 100%, followed by the formulas CT3, CT2, being 95.56% and 84.17%, respectively. These results were significant different ($p < 0.05$). Due to the quickly adaptation of the broodstock Tire track eel with food during the breeding process, survival rate for the later period of all formulas were higher than those for the first 15 days of the breeding experiment.

Maturity index, Maturity rates of Tire track eel feeding with different kinds of feed

Results on breeding show that broodstock Tire track eel got maturity weel in artificial conditions as the annual breeding season of Tire track eel runs from late April until the end of June.

Table 5. Effects of breeding feed on Maturity index and Maturity rate of broodstock Tire track eel

Food formulas	Maturity index %	Maturity rate %
CT1	20,01[a]	75,77[a]
CT2	16,42[c]	60,57[b]
CT3	17,54[b]	68,18[ab]

Within columns, means marked/ followed by the same letter are not significant different according to P>0.05.

Table 5 shows that Maturity rate of Tire track eel was highes for the formula CT1, being 75.77% when using blue-worm food for feeding, followed by the formula CT3, being 68.18% when using trash-fish for feeding. And the lowest Maturity rate was obtained from the formula CT2, being 60.57%. This difference was statistically significant according to $p < 0.05$.

The results show in Table 5 indicated that the Maturity index was similar trend to the Maturity rate. The formula CT1 obtained the highes Maturity index, being 20.01%, followed by the formulas CT3, and CT 2, being 17.54% and 16.42%, respectively. These results were significant different according to $P < 0.05$.

Induced reproduction

The maturity of all broodstocks was assessed before ịnecting of the hormones. Two dosage levels were used: a preparatory dose and a decisive, or

final, dose with a time gap of 24 hours between the two injections. Latency period between the last dose of hormonal stimulation and the ovulation of Tire track eel ranged from 30-48 h after at temperatures ranged from 23-28^{o}C (Table 6).

Table 6. Reproduction parameters of Tire track eel

Parameters	**Formulas**					
	CT1 50 µg LRHa + 10mg DOM	**CT2** 100 µg LRHa + 10mg DOM	**CT3** 150 µg LRHa + 10mg DOM	**CT4** 1000UI HCG	**CT5** 1500UI HCG	**CT6** 2000UI HCG
Water temperature (^{0}C)	23-28	23-28	23-28	23-28	23-28	23-28
Average weight (g)	126.62	127.79	122.73	130.14	129.20	125.8
Latency period	43 h 40’	41 h 46’	40 h 50’	40 h 38’	39 h 20’	38 h 35’
Spawing rate %	28.57^{c}	50.00^{ab}	57.14^{ab}	42.86^{bc}	50.00^{ab}	64.29^{a}
Relative- fecundity	2413^{e}	3096^{d}	5454^{b}	3591^{d}	4187^{c}	7176^{a}
Fertilization rates %	76,33	76	75,33	77	74,33	76

Within columns, means marked/ followed by the same letter are not significant different according to P>0.05.

Variations of the latency period depend on type of hormones, frequency of injections, the doses, the water temperature and the ecological conditions. The latency period varies among the the different species. In our experiments, the latency period ranged from 38 h 35’ to 43 h 40’ among the different formulas of hormone injection.

Results in Table 6 shows that the formula CT6 obtained the highest efficiency when spawing rate was 64.29%. By contrast, the spawing rate was lowest for the formula CT1, being 28.57%. The difference between formula 1 and 6 was significant (P <0.05). Thus the dosage and type of hormones in this study were also influenced to maturation and ovulation of Tire track eel.

For the case of using HCG, the results presented in Table 1 indicate that spawing of Tire track eel was highes for the formula CT6 (64.29%), followed by the CT4 and CT5, being 42.86% and 50.00%, respectively% (P<0.05). From such results, it is recommended that most effective concentration of HCG is 2000UI HCG for Tire track eel.

Fertilization rate in treated fish was in the range of 74,33-77% (Table 1) and CT5 was the lowest rate (74,33%), CT4 was the highest rate

(77%). But there was no significant difference in fertilization success among groups (P>0.05).

There was a significant difference among experimental groups in relative fecundity. The results showed that relative fecundity was highest in CT6 (7176 eggs/kg b.w. female), followed CT 3 and was lowest in CT(12413 eggs/kg b.w. female).

Discussions

Different biological aspects (mainly morphometric and ecological) of tire track eel have been studied sporadically (Day, F, 1878; Kachha, D., el at, 1993) Very little information is available on the culture and reproductive potentials of freshwater eel. Narejo et al. (Narejo, N. T., et al, 2002) and Rahman et al. (Rahman, A. K. A. 1989) did some works on its biology and rearing technique under laboratory condition. However, no initiative seems to have been taken to breed this species artificially (Mollah M. F. A., et al, 2013).

Mollah M.F.A, (Mollah M. F. A., et al, 2013) with studying Domestication and Observation on Induced Breeding of Spiny Eel found that survival rate of fish was higher in treatment II and III (96%) (trash fish and chicken viscera) than treatment I (Mega feed) (94%). Nutrition is very important for sound growth and maturation of gonads of the fish. The quality of eggs depends on the quality of feed provided. Tran Thuy Ha (Tran Thuy Ha *et al., 2013)* studied Artificial propagation on track eel. To data showed that Track eel brood stock were cultured in tank and fed by trash fish having Maturity rate of 75,5%.

In Mollah's (Mollah M. F. A., et al, 2013) experiment, different doses of PG extract were used as inducing agent in the breeding trials I, II, III and IV for females. PG doses of 100, 90, 80, 60, 55, 50, 45 mg.kg-1 body weight of fish did not show any response in female fish. But the dose of 40 mg PG.kg-1 body weight precipitated ovulation and successful striping of ovulated eggs was observed. The time interval between the injection of carp PG extract and ovulation (latency period) varied between 21 and 28 hr of injection in all cases. Fertilization rate was 82.00±2.21 at the same dose and embryonic development was observed up to gastrula stage. In the other hand 35 mg PG.kg-1 body weight of female showed partial ovulation of female but no fertilization occurred. On the other hand, the fish treated with 50-100 mg PG.kg-1 body weight showed high doses to induce ovulation.

Results of Tran Thuy Ha's study indicated that using a combination of 30 mg carp PG and 600 IU HCG per kg of female broodfish, there were 75-100%

female broodfish released the eggs after 28-30 hours at the temperature of 24-28^{0}C (Tran Thuy Ha *et al., 2013).*

The experimental results revealed that the suvival rate, maturity index, maturity rate of *M. armatus* was better in confined environment when fed with worm, trash fish than commercial peelet. The suvival rate, maturity index, maturity rate of *M. armatus* was the same at stocking densities 1 kg/m^3, 2 kg/m^3 and 3kg/m^3. Breeding trials with CT 3 (150 µg LRHa + 10mg.kg-1 body weight of fish) and CT6 (2000UI HCG.kg-1 body weight of fish) showed better performance.

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