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Induced breeding of *Neolissochilus hexagonolepis* using pituitary and synthetic hormone under the agro-climatic condition of Meghalaya, India

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Abstract
The Chocolate Mahseer (*Neolissochilus hexagonolepis* McClelland, 1839) is an important food and sport fish of the region and its population is sharply declining due to various anthropogenic activities. However, for culture and conservation of the fish, there is no induced breeding protocol so far for the agro-climatic condition of Meghalaya. Thus it becomes necessary for standardized of doses of pituitary as well as synthetic hormone for artificial propagation of the fish and develop an induced breeding protocol. The fish was successfully bred at the Hatchery Complex, St. Anthony's College, Shillong, Meghalaya by using pituitary extract and synthetic hormone viz. ovaprim and gonopro-FH. The dose of pituitary extract is standardized as 4 mg/kg body weight to both male and female as 1st dose and at 7.9 mg/kg body weight only to female as 2nd dose. For ovaprim, a single dose as 0.8 ml/kg body weight to female and 0.3 ml/kg body weight to male for ovaprim was given and as 0.9 ml/kg body weight to female and 0.3 ml/kg body weight to male for gonopro-FH. The fecundity of the fish was 3500 eggs/kg body weight which was comparatively low. The final hatching percentage achieved was 60% and survival rate of hatchling was 50% up to fry stage.

Keywords: Induced breeding, chocolate mahseer, pituitary extract, synthetic hormone, protocol, fecundity

1. Introduction
The species *Neolissochilus hexagonolepis* (McClelland, 1839) commonly known as Chocolate Mahseer are an important sport and food fish of India, in general and Meghalaya, in particular [1, 2]. The fish is locally known as 'Khasaw', and has a high market value. This fish is considered as one of the endangered species, which is having tremendous scope for culture, sports and tourism. However, it has been observed that, the population of the species has declined in recent years from the waters of the state of Meghalaya due to various natural and anthropogenic factors [1]. Therefore, it needs immediate attention for replenishment of stock of this threatened species by induced breeding for its conservation in nature. The seed production will also help to promote its farming in artificial water impoundment.
The fish species, though live in lotic habitat, it has been observed that the species can survive and grow well in confined water as well. Therefore, they can be very good candidate species in culture system under the agro-climatic condition of the mid-altitudinal region of Meghalaya, which in turn improve socio-economic condition of the local tribal fishers. However, basic hindrance for culture of the fish is non-availability of seed. Thus, induced breeding of the fish is required to meet the demand of the seed for culture in the state. However, till now, artificial breeding was not possible due to limited knowledge on the feeding and breeding behaviour of the fish [3].
Induced breeding is a technique of fish breeding in confined water, stimulated either by gonadotropin from pituitary gland or by synthetic hormone administration such as ovaprim, ovatide and gonopro-FH etc. Pituitary extract was used for the successful spawning of *Labeo gonius* under climatic condition of mid-altitude of Meghalaya [4]. Successful induced breeding of *Tor putitora* was done by intramuscular administration of the synthetic hormone, ovaprim, with single dose (0.20 ml/kg body weight) [5]. The artificial propagation of the indigenous Tor species of Malaysia, *Tor tambroides* and *Tor duronesis* was successfully done in captivity using hormone treatments and with ovaprim (0.5 ml/kg body weight) being the most successful hormone treatment for both the species [6].

Similar work was also reported on induced spawning, larval development and rearing of the two indigenous Malaysian Mahseer, *Tor tambroides* and *Tor duronesis* [7]. The induced breeding, embryology and rearing of fry of Deccan Mahseer, *Tor khudree* was also observed from Mangalore [8]. The report on induced breeding of Chocolate Mahseer is scanty and the present study will help to understand and proceed for seed production of the species of fish.

However, accurate protocol for induced breeding of Chocolate Mahseer is not available for the artificial propagation under the climatic conditions of the state, Meghalaya. Therefore, this study attempts to standardize a protocol for induced breeding of *N. hexagonolepis* for production of quality seed with the use of pituitary extract and synthetic hormone viz. ovaprim and gonopro-FH.

The study also intends to understand the effect, if any, of synthetic hormone like ovaprim, etc. over natural gonadotropin from pituitary and advantage of the use of the synthetic hormone in the fish. It is expected that, the result would help in development of induced breeding protocol for the fish under the environmental condition of Meghalaya and production of seed which in turn will help in conservation of the fish in a natural habitat as well as increased fish protein production from culture system.

2. Materials and Methods

The species *N. hexagonolepis* used for the experiments were collected from different rivers of Meghalaya, viz. Amlayee River, Khri River, Umrynjah River and Janiaw River.

The experiments were conducted during May-August 2013 using pituitary hormone and repeated during the 2014, 2015 and 2016 using ovaprim and gonopro-FH. The collected specimens were brought to the hatchery complex of the Department of Fishery Science, St. Anthony's College, Shillong and reared in specially designed cemented ponds which have thick layers soil to help in circulation of soil nutrients. Replenishment of water was done every fifteen day to remove decompose and unwanted food materials. Continuous exchange of water was maintained through a flow-through system for better water quality, to increase the survival of the fish in captive rearing condition. The brooders were fed with supplementary feed during the development period. The amount of feed given was 30 gm/kg body weight per day having 25% protein. They were also given puffed rice periodically. The physico-chemical parameters of the stocking pond were analyzed with standard methods [9]. Fecundity was calculated prior to spawning [10].

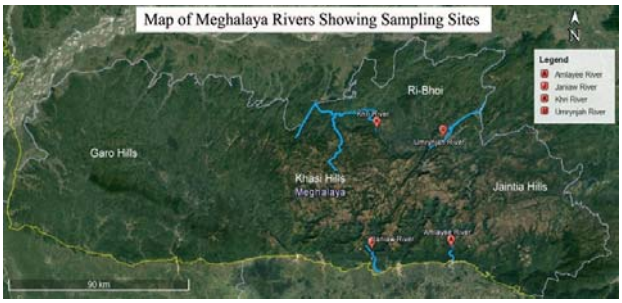

Fig 1: Map of Meghalaya showing the rivers from where fish specimens were collected.

The artificial breeding of Chocolate Mahseer was carried out on a sunny day (water temperature: 21-22°C and air temperature: 24-26 °C) [11, 12]. The males and females were segregated 5-6 h before injection. The operation was done using natural hormone viz. pituitary extract through hypophysation method [11, 12] and synthetic hormone viz. ovaprim and gonopro-FH. The dose of hormones was determined using trial and error methods under the environmental conditions of Shillong, Meghalaya.

3. Results

The length and weight of the selected brooders were between 18 cm and 25 cm in length and from 150 gms to 350 gms in weight. The physico-chemical parameters of the water were recorded (Figure 2 and 3). Injection was done during the evening time of a sunny day to avoid sudden drop of temperature which may affect spawning.

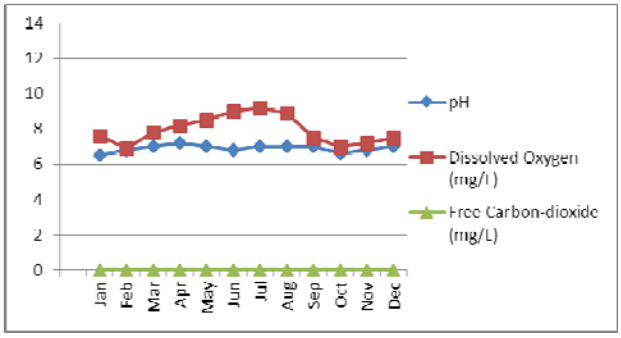

Fig 2: Average physico-chemical parameters (pH, dissolved oxygen and free carbon dioxide) of cultured pond water during 2013-2016

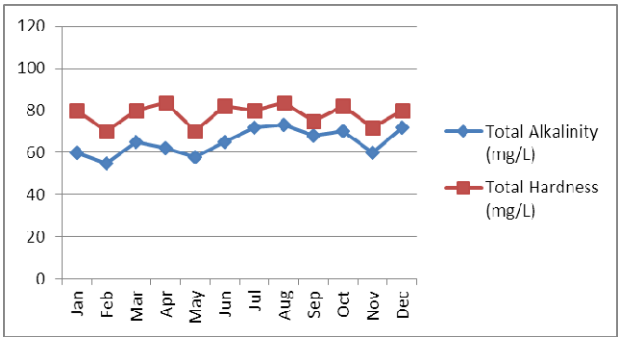

Fig 3: Average physico-chemical parameters (total alkalinity and total hardness) of cultured pond water during 2013-2016

The artificial propagation was successfully done with natural (pituitary) hormone (Table 1) and synthetic hormone, ovaprim (Table 2) and gonopro-FH (Table 3) after a series of trial and error experiments and the final dose for the protocol for artificial propagation were determined using pituitary hormone (Table 4) and synthetic hormones (Table 5).

The first operation was done using pituitary hormone through hypophysation technique. The first dose was given at 3.5 mg/kg body weight for both male and female brooder and the second dose was given for only the female at 7.5 mg/kg body weight. The second operation was done with the increase in the first dose at 4 mg/kg body weight for both male and female and the increase in second dose at 7.9 mg/kg body weight for female only. The experiment was repeated again using slightly different doses i.e. the first dose as 4 mg/kg body weight for both male and female and the second dose as 8.3 mg/kg body weight for female only. However fertilization rate was very low and the dose of 8.3 mg/kg body weight to the female, it blocked the vent thereby affecting the spawning of the fish.

Table 1: Doses of natural hormone injection during trial induced breeding of *Neolissochilus hexagonolepis*

Hormone used	No. of trials	First dose (mg/kg body weight)		Second dose (mg/kg body weight)		Results
		Female	Male	Female	Male	
Pituitary	I	3.5	3.5	7.5	0	Partial fertilization (No hatching take place)
	II	4.0	4.0	7.9	0	Fertilization percentage (60%)/ Hatching percentage (70%)
	III	4.0	4.0	8.3	0	Vent of female blocked /Partial Fertilization/ Low hatching rate (20-30%)

The induced breeding operation with synthetic hormones was done using ovaprim and gonopro-FH. The operation with ovaprim was done in three trials. The hormonal injection was given intramuscularly in the dorsal side near the caudal peduncle. The first trial was done with the dose of ovaprim given at 0.6 ml/kg body weight for female only. The male was not given any treatment as the males were mature and oozing freely under pond conditions. The second trial was done with an increased in the dose at 0.8 ml/kg body weight for female. The male was also given a dose of hormone at 0.3 ml/kg body weight. The experiment was repeated with the dose of ovaprim injection as 0.9 ml/kg body weight female and as 0.3 ml/kg body weight to male. However, the rate of fertilization and hatching remains the same indicating that the further increase in the doses of hormones does not have any effect on the fertilization process.

Table 2: Doses of hormonal injection during trial induced breeding of *Neolissochilus hexagonolepis* using synthetic hormone

Hormone used	No. of trials	Single dose (ml/kg body weight)		Results
		Female	Male	
Ovaprim	I	0.6	0	Partial fertilization/Hatching percentage low (20%)
	II	0.8	0.3	Fertilization rate (65%)/ Hatching rate (70%)
	III	0.9	0.3	Fertilization percentage not improved further

After ovaprim, another synthetic hormones used for trial induced breeding of the fish was gonopro-FH in four trials to see the effect of the hormone. The first trial was done with the first dose of hormone at 0.5 ml/kg body weight to the female and at 0.2 ml/kg body weight to the male. The second trial was done with the increased first dose of hormone injection at 0.7 ml/kg body weight to the female and at 0.2 ml/kg body weight to the male. The third trial was done with the increase in the first dose of hormone as 0.9 ml/kg body weight to the female and at 0.3 ml/kg to the male. The fourth trial was done with the dose of hormone as 1.0 ml/kg body weight to the female and at 0.3 ml/kg to the male. The result however affected the spawning of the fish as the vent in female was blocked and fertilization was not successful.

Table 3: Doses of hormonal injection during trial induced breeding of *Neolissochilus hexagonolepis* using synthetic hormone

Hormone used	No. of trials	Single dose (ml/kg body weight)		Results
		Female	Male	
Gonopro-FH	I	0.5	0.2	Partial fertilization (10-20%)
	II	0.7	0.2	Fertilization rate (30-40%)/Hatching rate (40%)
	III	0.9	0.3	Fertilization rate (80%)/ Hatching rate (85%)
	IV	1.0	0.3	Spawning affected/blocked vent in female

After 24 h of injection, the female and male brooders were stripped by dry-stripping methods in a white enamel tray. The eggs and milts were then mixed thoroughly with a sterilized bird feather. After that the eggs were washed with iron free clean water and any tissue or foreign particles were removed. The fertilized eggs were incubated in specially designed wooden tray fitted with nylon netting and placed in a long PVC tub where constant water flow was maintained and oxygen as supplied through an aerated pump. The water temperature, pH and dissolved oxygen were maintained at 22-26°C, 5-7 and 6-10 mg/L, respectively.

The fecundity of the fish was found to be very low (3500 eggs/ kg body weight). After stripping, fertilized eggs were incubated at 22-24 °C. Hatching took place after 72 h of incubation and the hatchling percentage was recorded to be 60%. The survival of the fry was 50%. The final doses of hormone for the induced breeding protocol developed for the fish *Neolissochilus hexagonolepis* was finalized based on the trial and error methods with pituitary hormone (Table 4) and synthetic hormones (Table 5).

Table 4: Final doses of natural hormone for induced breeding of *Neolissochilus hexagonolepis* in Meghalaya (Temperature range: 20°C-25°C)

Hormone	First dose (mg/kg body weight)		Second dose (mg/kg body weight)	
	Female	Male	Female	Male
Pituitary	4	4	7.9	0

Table 5: Final doses of synthetic hormone for induced breeding of *Neolissochilus hexagonolepis* in Meghalaya (Temperature range: 20°C-25°C)

Hormone	Single dose (ml/kg body weight)	
	Female	Male
Ovaprim	0.8	0.3
Gonopro-FH	0.9	0.3

4. Discussion

The natural habitat of the fish *Neolissochilus hexagonolepis* is predominantly lotic water bodies of hilly areas. The fish usually breeds in the catchment areas of the river with shallow

water with mild water current and reported to breed only once a year from May to September. However, the species has been reported to breed in natural habitats in the North-Eastern Regions of India during May-June and August-September [13]. During the present study, it has been established that the fish obtain sexual maturity in confined water as well and is possible to induce to breed with hormonal intervention. It has been observed that, *N. hexagonolepis* could breed only once a year under captivity from May to August. This was due to the delay in the maturation of gonad in female brooders under captivity in comparison to maturation of males. The availability of lower number of mature females also contributes to the problem.

The artificial propagation of Chocolate Mahseer, *N. Hexagonolepis*, was successful using natural hormone (pituitary) as well as synthetic hormone (ovaprim and gonopro-FH). The interval between the hormonal inoculation and the stripping of brooders was 24 h. This was in agreement with similar results (24-36 h) which was reported on *Tor khudree* [7]. The final dose for successful induced breeding of Chocolate Mahseer with pituitary extract was found to be 4 mg/kg body weight as first dose to both male and female and 7.9 mg/kg body weight as second dose only to female. Successful induced breeding of different fishes using different hormones have been reported by various authors [4, 6, 14-17].

The effective dose of synthetic hormone found as 0.8 ml/kg body weight and 0.3 ml/kg body weight (female and male respectively) for ovaprim whereas it was 0.9 ml/kg body weight and 0.3 ml/kg body weight (female and male respectively) for gonopro-FH. It has been observed that the rate of fertilization as well as hatching rate was lower with the use of pituitary extracts in comparison to the use of synthetic hormones. No adverse affects or unnatural behaviour of the fish has been noticed with the inoculation of synthetic hormone to the fish. The study proved that synthetic hormone is more effective in induced breeding of the fish as compared to natural hormone. However, the study should be continued further for the determination of micro changes in the hormonal dose for large scale production of seeds.

The positive response of the fish, *N. hexagonolepis,* to the synthetic hormone has an advantage over pituitary extract as the synthetic hormone needs only single dose whereas the pituitary needs double dose of injection with an interval of 5-6 h. The advantages of synthetic hormones also includes availability of the hormone commercially in the market, ready to use in liquid form, stable with long shelf life, consistent potency and assured breeding response. The synthetic hormones also reported a higher rate of spawning, fertilization, hatchling and survival compared to pituitary hormone [18].

5. Conclusion

The use of synthetic hormone with positive response of the fish to the hormone and finalization of the induced breeding protocol will go in a long way for production of quality seed of the fish, *N. hexagonolepis* in the country. The result observed that the fish responded better to the synthetic hormone than pituitary hormone. The protocol will help in the culture of the species as the fish can be a very good candidate species for the culture in India, particularly the state, Meghalaya. The protocol will also help in the ranching of the fish in the natural habitat by improving the population structure of the fish leading to conservation of the species.

6. Acknowledgement

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