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# Standardization of proper dose of synthetic hormone for induced breeding of three loaches of genus *Botia*

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**Abstract**
This study describes the dosage of WOVA-FH for induced breeding of *Botia sp.* (Indian loach). Synthetic hormone, WOVA-FH was used to induce the species at different doses (0.5 ml/kg as 1st dose, 0.25 ml/kg as 2nd dosage, 0.025ml/fish as 3rd dose and 0.0125ml/fish as 4th dose) in each breeding trial. The higher fertilization, hatching and survival rates were found with fish injected with 0.025 ml/fish. High dose (0.25ml/kg) of hormone affected the fishes and finally perished within 4 days. Fishes which were injected with given 0.5 ml/kg succumbed within 2 days. Fishes that were given 0.0125 ml/fish dose of WOVA-FH injection moved and fed but did not spawn. Loaches required very little amount of synthetic hormone for breeding in captivity. The present study showed that 0.025 ml/fish of WOVW-FH was optimum sufficient for induced breeding of the three loaches *Botia dario, Botia lohachata and Botia almorhae.*

**Keywords**: Genus *Botia*, Induced breeding and Dosage of hormone.

## 1. Introduction
*Botia* (Indian loaches) is a genus of freshwater fish of the loach family Botiidae. It is a large genus with about 20 species. Kottelat [1] identified the genus *Botia* into four related genera based on fish appearance and locality; *Botia* for "Indian loaches" (shorter body), *Chromobotia* for "Clown loach", Syncrossus for "Tiger loaches" (elongated body) and *Yasuhikotakia* for "Mekong loaches" (shorter body). Loaches are very beautifully coloured ornamental fish having value. These species have a pair of sharp spines under their eye sockets. These spines normally lie flat but may be extended when they feel threatened. Loach feed on snail, mosquito larva, shrimp, blood worms, *Tubifex*, *Daphnia* and so on. The most important thing for loach is that they always require clean and well oxygenated water. Frequent water changes every day are necessary for the loaches. Many of the loach of genus *Botia* are of vulnerable or threatened categories. The endangered status of the loaches are mainly because of the deterioration of the environment particularly, water quality which may be due to agricultural run offs or pesticidal effect of tea gardens. There is no literature on the artificial breeding of Genus *Botia*. The present study was, therefore, undertaken to standardize the proper dose of hormone for artificial breeding of genus *Botia*. The present study was done on three species of *Botia* which were *Botia dario, Botia lohachata* and *Botia almorhae.* Some investigation have shown results on spawning biology and fecundity of *Cobitis taenia* [2] , fecundity of *Botia dario* [3], spawning behaviour of *Sabanejewia vallachica* [4] and spawning biology of *Botia almorhae* [5] and diversity of loaches in Darjeeling, West Bengal [6]. No other literature is available on breeding of loaches.

## 2. Materials and Methods
### 2.1 Collection and Experimental site
The sampling sites were located at Bhelakopa, Dwitia Khanda of Cooch Behar lying at $26^0$18'North latitude and $89^0$34'East longitude. After collection the fishes were oxygen packed in sterile polythene bags and kept in cartons for transport to the wet lab of Aquaculture and Limnology Research Unit, Department of Zoology, University of North Bengal. In the laboratory the fishes were transferred to suitable aquariums for regular monitoring of their maturation.

### 2.2 Induced Breeding
2- phenoxy ethanol @ 2ml in 20 lit. Of water was used to anesthetize for easy handling prior to injection of the fish. This also prevented the fish from getting stressed. Twenty four (24) pairs of each group of matured fish were injected with different doses of synthetic hormone WOVA-FH (Biostadt India limited, Mumbai). Total 48 pairs of fish were taken (8 pairs of *Botia dario*,

8 pairs of *Botia lohachata* and 8 pairs of *Botia almorhae*) for the breeding experiment. Insulin syringe of 1 ml normally divided into 40 parts that is 1 part is 1/40 =0.025ml was used. The fish were injected at the base of the pelvic fin. Breeding tanks of 100 litres capacity hourly running water system were used. After injection the fish were transferred in the tanks. Different Set-up protocol details followed are given in Table1.

**Table 1:** Summary of the protocols of experimental Set-up and design for induced breeding of Genus *Botia* in100 litre tanks with running water system.

| No. of Set-up | Species name | Sex ratio | Number of Fish | Dose of hormone (WOVA-FH) |
|---|---|---|---|---|
| Set-up-1 | *B. dario* | 1:1 | 2 pairs | 0.5ml/Kg |
| Set-up-2 | *B. dario* | 1:1 | 2 pairs | 0.25ml /Kg |
| Set-up-3 | *B. dario* | 1:1 | 2 pairs | 0.025ml /fish |
| Set-up-4 | *B. dario* | 1:1 | 2 pairs | 0.0125 ml/fish |
| Set-up-5 | *B. lohachata* | 1:1 | 2 pairs | 0.5ml/Kg |
| Set-up-6 | *B. lohachata* | 1:1 | 2 pairs | 0.25ml /Kg |
| Set-up-7 | *B. lohachata* | 1:1 | 2 pairs | 0.025ml /fish |
| Set-up-8 | *B. lohachata* | 1:1 | 2 pairs | 0.0125 ml/fish |
| Set-up-9 | *B. almorhae* | 1:1 | 2 pairs | 0.5ml/Kg |
| Set-up-10 | *B. almorhae* | 1:1 | 2 pairs | 0.25ml /Kg |
| Set-up-11 | *B. almorhae* | 1:1 | 2 pairs | 0.025ml /fish |
| Set-up-12 | *B. almorhae* | 1:1 | 2 pairs | 0.0125 ml/fish |

## 3. Results and Discussion

4 different doses of WOVA-FH hormone (0.5 ml/kg as1st dose, 0.25 ml/kg as 2nd dose, 0.025ml/Fish as 3rd dose and 0.0125 ml/fish as 4$^{th}$ dose) were used, with the best response to reproduction obtained from the dosage of WOVA-FH of 0.025 ml/ fish. The higher fertilization, hatching and survival rates were found in fish injected with 0.025 ml/fish in Set-ups-3, 7 and 11. Breeding trails were done three times by applying different doses of hormone in each breeding trials. Same dose of WOVA-FH hormone was injected to both male and female. Injected fishes were released in tanks and observed that after 4-4.30 h they started spawning simultaneously. Spawning was observed in Set-up-3, 7 and 11 but there was no spawning in others Set-ups. All fishes died in Set-ups-1, 5 and 9 within 2days. In Set-up-2, 6 and 10 all fishes died within 4 days. In Set-up-4, 8 and 12 all fishes were most active and took feed properly but spawning was not observed.

**Table 2:** Summary of the different stages of breeding of Genus *Botia* in the different Set-ups.

| Time | Set-up -1(No 5 and 9) (0.5ml/Kg) | Set-up -2(No 6 and10) (0.25ml /Kg) | Set-up -3(No 7 and 11) (0.025ml /fish) | Set-up – 4 (No 8 and 12) (0.0125 ml/fish) |
|---|---|---|---|---|
| 06p.m. | Transferred to tank | Transferred to tank | Transferred to tank | Transferred to tank |
| 07p.m. | Black patch appeared at the injection site | All fish cuddled in a corner | All fish cuddled in a corner | All fish cuddled in a corner |
| 08p.m. | All fish cuddled in a corner | Some males active | All fishes were active | All fishes were active |
| 09p.m. | Some males died | Some males active | Fishes were swimming against flow of water | Males were active |
| 10p.m. | Females swelled up | Females swelled up | Females were being chased by the males at the same time the males were fighting with each other | Females swelled up and males started chasing. |
| 11p.m. | All fish cuddled in a corner | Some males were chasing the females; no spawning. | Spawning had started; the paired fishes were swimming with the current | Males started chasing the females; no spawning. |
| 12a.m. | All fish cuddled in a corner | No spawning | Spawning continued | No spawning |
| 01a.m. | All fish cuddled in a corner | No spawning | Spawning continued | No spawning |
| 02a.m. | All fish cuddled in a corner | No spawning | Spawning continued | No spawning |
| 03a.m. | All fish cuddled in a corner | No spawning | Spawning continued | No spawning |
| 04a.m. | All fish cuddled in a corner | No spawning | Spawning ceased | No spawning |
| 05a.m. | Not spawning | No spawning | Fishes moved actively | No spawning |
| After 1 day | Eggs came out on press the abdomen | Eggs came out on press the abdomen | Eggs no came out on press the abdomen | Eggs came out on press the abdomen |
| After 2 days | All fishes almost died | Feeding ceased | All fishes took feed and moved actively | All fishes took feed and moved actively |
| After 4 days | All fishes died | Almost all fishes died | All fishes took feed and moved actively | All fishes took feed and moved actively |

The present study demonstrated the successful breeding of three species of genus *Botia* in captive condition with little dose 0.025ml/fish WOVA-FH. In the same way, Ovatide induced breeding in *Ompok pabo* with a single dose of 0.5ml/kg and 0.6ml/kg respectively to the male and female fish [8]. Similar type of study was reported in *Puntius sarana* by Udit *et a*l., [7]. For induced breeding of *Puntius sarana* Ovatide at 0.2 ml per male fish and 0.3 ml per female fish was used.

## 4. Conclusion

*Botia sp.* can be easily matured and bred successfully under captive conditions similar to that of carps. This study documented the breeding of ornamental fish *Botia sp* in captivity with use of synthetic hormones at 0.025ml/ fish. For induced breeding of these loaches minute amount of WOVA-FH is needed. This paper is therefore useful for loach breeders and aquarium keepers.

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