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Captive Breeding of Pufferfish (*Leiodon cutcutia*) from Assam, India

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Authors' contributions

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

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ABSTRACT

Background: *Leiodon cutcutia* has been exploited from its natural habitats which leads to a drastic decline of its natural socks ever since it has been reported its ornamental value. The development of effective breeding protocols, optimal management of environmental conditions, and the use of appropriate hormonal treatments can help stock improvement of the species.
Methods: Live specimens were collected from the Meleng River across the Jorhat districts. The maturity cycle was properly studied to determine the breeding season of the species. Acclimatization of the brooder and feeding were done following standard protocol. Breeding trials were conducted by habitat manipulation and also by administering hormonal injections.
Findings: Spawning was triggered by simulating habitat conditions of the breeding enclosure and extending daylight hours. The species exhibits sexual dimorphism; the females have white spots on the body and are always larger than the males. Males typically display courtship behaviour such as chasing and circling females. Territorial and aggressive behaviours complicate breeding efforts, requiring careful management of tank mates and breeding pairs. Adhesive eggs were attached to

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the substratum and males were found to guard the eggs until hatching. The breeding experiment had moderate success.
Conclusion: Captive breeding of *Leiodon cutcutia* presents unique challenges but offers significant benefits for conservation and the aquarium trade.

Keywords: Puffer fish; ornamental value; captive breeding; spawning behaviour.

1. INTRODUCTION

The pufferfish (family Tetraodontidae) comprises about 40 freshwater species under seven genera [1,2]. The only freshwater puffer fish, *Leiodon (Tetraodon) cutcutia* found in the Brahmaputra river system indeed has no food value but the species has a high ornamental value [3,4,5,6]. Since the species is not considered a food fish, due to its neurotoxic content; very little work was reported on the biology of *L. cutcutia* from this part of the globe [7,8,9]. However, a scan of the literature reveals that many researchers have studied the biology of puffer fish, especially reproductive biology. Chen [10] reported the induced ovulation and embryonic development of ocellated puffer (*Takifugu ocellatus*); Sirisha and Rao [11] worked on the reproductive biology of *Lagocephalus spadiceus*. Yang et al. [12] reported the differences between the reproductive strategies of obscure puffer (*Takifugu obscurus)* and ocellated puffer (*Takifugu ocellatus*). Again, Sirisha and Rao [13] studied on maturation and spawning of *Lagocephalus lunaris* while Math Piah et al.[14] worked on *Marilynapleuro sticta* and *Tetracten oshamiltoni*. Khalaf et al. [15] also studied the population structure and sexual maturity of the puffer fish, *Lagocephalus sceleratus.*

Due to its high potential as an ornamental fish, exploitation of *Leiodon cutcutia* from its natural habitats has also increased exponentially. Artificial propagation of the species is the need of the hour. In the present communication, therearing and breeding of *L. cutcutia*in captive conditions have been highlighted. Captive breeding and reintroduction of various species into the wild have demonstrated positive outcomes globally [16,17]. Although there are debates regarding its impact on fish biodiversity, captive breeding has significantly contributed to the management and conservation of fish species by alleviating pressure on depleted stocks and promoting the sustainable utilization of aquatic resources [18,19].

2. MATERIALS AND METHODS

Collection of brooders: A wild collection of *Leiodon cutcutia* was reared in the controlled conditions. Actual breeding experiment was conducted in April to July for consecutive two years during 2012 and 2013. For rearing feasibility studies, the differential size of *L. cutcutia* was collected from their natural habitat and then acclimatized in a separate enclosure (earthen tank).

Acclimatization: Live specimens, after collection, were kept in an aquarium (60 x 30 x 30 cm size) provided with a constant aerator. The fish specimens were acclimatized in a simulated environment (with artificial hideouts and aquatic plants) for about a week. The bottom of the aquarium was covered with fine sand mixed with mud and small stones (gravels) for a thickness of 3 cm and then filled with water up to a depth of 18 cm. Before releasing the fish, the aquarium was treated with 5% $KMnO_4$ solution. The initial length and weight of brooders were recorded at the time of release in the aquarium. Fishes were provided with live feed such as ant and mosquito larvae, earthworms as well as protein-rich commercial feed @ 5% body weight daily. Vital water quality parameters viz., water temperature, pH, DO, and free CO_2, were recorded at least once a week.

Natural breeding: Natural breeding of *L. cutcutia* was performed by habitat simulation i.e., keeping the breeding enclosure similar to its natural habitat.

Induced breeding: Substantial success has been achieved in the captive breeding of certain members of the genus *Channa* [16,17,18]. In the present study, the dosage of synthetic hormones, however, was slightly modified. The female brooders were injected with ovaprim intramuscularly @ 0.25- 0.29 ml/kg body weight. The second dose was given to both sexes after 6 hrs (if necessary). Three different aquaria with different sets of brooders were taken for each experiment. Like this, three sets of experiments were conducted in a year.

Breeding trial: The fully grown specimens were taken for the breeding experiment because only the matured specimen showed sexual dimorphism. After initial acclimatization in a specific enclosure for 5-7 days, the brooders were reared in the earthen tank for about 3 months (till the end of April). Feeding was done daily @ 10% of the body weight of the brooders. For captive breeding, a ratio of 2:1 (M: F) was maintained. The pH, DO, FCO_2, and water temperature of the tanks and the aquaria were maintained. The brooders were transferred to the aquaria for the administration of ovaprim. The bed of the aquaria is prepared with sand and gravel some aquatic plants like *Hydrilla* and water hyacinth. The female brooders were injected with ova-prim intramuscularly. The second dose was given to both sexes after 6 hrs (if necessary). Three different aquaria with different sets of brooders were taken for each experiment. Like this, three sets of experiments were conducted in a year.

Estimation of fertilization & hatching of spawn: The percentage of fertilization and hatching rates were recorded to determine the effectiveness of the hormone using the following formula:

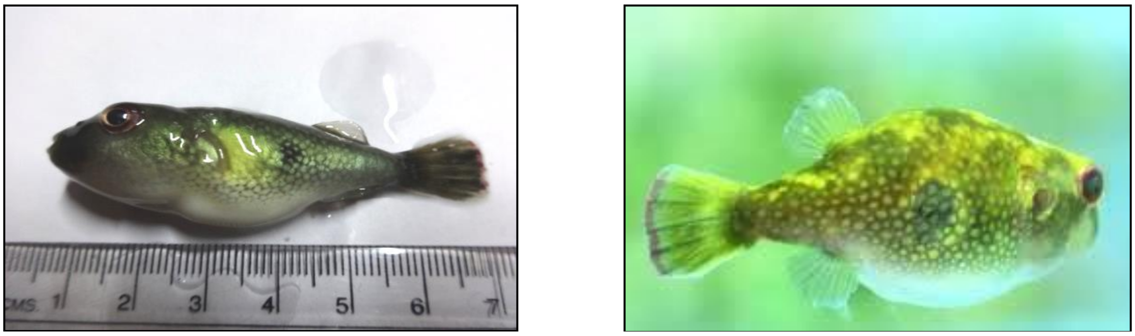
$$\%fertilization = \frac{No.of\ fertilized\ eggs\ \times\ 100}{Total\ no.of\ eggs\ (fertilized\ +\ unfertilized)}$$

$$\%\ hatching = \frac{No.of\ eggs\ hatched\ \times 100}{Total\ no.of\ eggs\ (fertilized\ +\ unfertilized)}$$

Statistical analysis: Simple correlation and regression equations were applied for analysis of certain biological parameters. The statistical packages viz., MS Excel, Minitab 11 were used in the present study.

3. RESULTS

Courtship and spawning behaviour: Male and female *L. cutcutia* display sexual dimorphism (Fig. 1). The gonado somatic index (GSI) of males and females in various months (Table 1) indicated that higher values of GSI from April to September. Between April and July, females were found to have fully mature gonads (Fig. 2). During this period, brooders were carefully selected for induced spawning. The doses of ovaprim were variable for different sets of brooders (between 0.2 and 0.3 ml/kg body wt.).The brooders showed aggressiveness after 6-10 hrs of administering (Fig. 3) 'ovaprim'. Females follow the dominant male and they move around with each other. During spawning, the male bent its body close to the female, hitting the vent region of the female more frequently which culminated in the release of gametes and the eggs were fertilized externally about 20 hrs after administration of ovaprim. The mating pair occasionally jumped above the water column and tried to escape from the breeding tank (aquarium). The unpaired male was passive and idled at a corner of the breeding tank most of the time; it was driven by the active male when it disturbed the mating pair. In *L. cutcutia,* the abdomen of the female enlarges and it indicates that the fish is gravid and ready to reproduce. The females have white colour spots on the body and the males have a net-like structure. During spawning time, the male chased the female around the aquarium (Fig. 4) and after that, the brooders moved around each other facing opposite directions. After that females sit on the egg-laying substratum (plastic pipes, earthen pots, aquatic plants, etc) and lay the eggs on it. Two males were there in the aquarium, among them the dominant male showed reproductive behaviour, and after spawning it fanned the eggs till hatched. The female and the other male were released from the aquarium. The female lays eggs in layered batches and about 300 eggs were released by the female. Spawning occurred on flat surfaces or among vegetation. As they grow, they can be transitioned to larger prey like *Daphnia* and eventually to formulated feeds.



Female   Male

Fig. 1. Sexual dimorphism of *Leiodoncutcutia*

Fig. 2. Gravid female

Fig. 3. Administeringovaprim to brooder

Fig. 4. Courtship display of male and female brooder

Fertilized eggs were found in the form of layered batches on the floor of the pipes and adhered to each other forming an egg mass, while unfertilized eggs were not adhesive and found scattered in the tank. The eggs were possessively guarded by the dominant male parent and fanned. The number of eggs varied from 186 to 394. The latency period too, ranged from 9 to 13 hrs. The fertilization rate was from 52.52% to 60.38% (Tables 2-4). The hatching percentage was 39.39 to 43.92 and hatchlings produced from 78 to 106 in case of the artificial breeding by ovaprim. The breeding trial with ovaprim was done in two trials. However, in the case of natural breeding, the rate of fertilization was 52.69%. The percentage of hatching was 36.56 and hatchlings produced 68. For the initial 6 days, the larvae depended mainly on the eggs' oil globules; after that, the spawn was fed live foods such as rotifers and brine shrimp nauplii. After attainment of the fingerling stage, they were shifted to another aquarium and fed with small earthworms, insect larvae and intestines of small fishes.

Table 1. Monthly maturity index (GSR) of *L. cutcutia*

Month	Male	Female
Jan	0.038±0.18	0.016±0.02
Feb	0.047±0.21	0.036±0.14
Mar	0.154±0.31	0.382±0.87
Apr	0.744±0.34	2.054±0.85
May	1.323±0.43	3.018±0.94
June	1.651±0.63	4.832±0.56
July	1.975±0.44	3.026±0.47
Aug	0.772±0.34	0.921±0.35
Sept	0.072±0.76	0.132±0.47
Oct	0.062±0.02	0.083±0.39
Nov	0.045±0.12	0.076±0.29
Dec	0.029±0.21	0.065±0.23

Table 2. 1st Breeding trial of *T. cutcutia* in June, 2013

Aq. No	Male		Female		Dose of ovaprimml/kg	Latency period (hr)	No of eggs laid	% of fertilization	Fertilized eggs	Hatching (%)	No of hatchling produced
	L (cm)	W (g)	L (cm)	W (g)							
1	4.5	3.63	4.9	5.22	0.2	10-12	No fertilization	--	--	--	--
	4.4	5.11	--	--	--	--	--	--	--	--	--
2	5.2	4.79	5.8	7.33	0.3	10-12	79	36.12	139	21.22	72
	4.9	5.92	6.4	7.99	0.2	--	No fertilization	--	--	--	--
3	6.3	5.65	6.9	8.11	0.3	9-12	81	33.17	131	18.11	71
	6.1	5.44	7.1	9.34	0.2	--	No fertilization	--	--	--	--
4	6.7	7.21	7.1	8.48	control	10-12	394	34.19	134	19.13	75
	6.3	6.98	--	--	control	--	--	--	--	--	--

Table 3. 2nd Breeding trial of *T. cutcutia* in June, 2013

Aq. No	Male		Female		Dose of ovaprimml/kg	Latency period (hr)	No of eggs laid	% of fertilization	Fertilized eggs	Hatching (%)	No of hatchling produced
	L (cm)	W (g)	L (cm)	W(g)							
1	5.2	5.121	5.9	7.17	0.3	9-12	212	60.38	128	39.62	84
	4.9	5.023	6.2	7.39	0.2	--	No fertilization	--	--	--	--
2	5.9	6.13	6.3	9.21	0.3	10-13	260	60.29	156	40.77	106
	5.6	6.112	7.39	9.35	0.2	--	No fertilization	--	--	--	--
3	5.9	7.231	7.6	10.113	0.4	11-13	274	61.55	168	44.38	117
	5.6	7.121	6.3	9.68	0.2	--	No fertilization	--	--	--	--
4	7.2	8.215	7.9	9.99	control	--	174	53.45	93	41.38	72
	6.9	8.112	7.6	8.84		9-12	167	49.68	88	38.46	69

Table 4. 3rd Breeding trial of *T. cutcutia* in June, 2014

Aq. No	Male		Female		Dose of ovaprimml/kg	Latency period (hr)	No of eggs laid	% of fertilization	Fertilized eggs	Hatching (%)	No of hatchling produced
	L (cm)	W(g)	L (cm)	W(g)							
1	5.2	5.12	5.9	7.17	0.3	9-11	198	52.52	104	39.39	78
	4.9	5.02	5.2	8.29	0.2	--	No fertilization	--	--	--	--
2	5.9	6.13	6.3	9.21	0.3	11-13	214	59.81	128	43.92	94
	5.6	6.11	7.2	8.92	0.2	--	No fertilization	--	--	--	--
3	6.9	7.23	7.6	10.11	0.4	10-13	222	59.98	136	49.28	98
	6.7	7.12	7.8	9.67	0.2	--	No fertilization	--	--	--	--
4	7.2	8.22	7.9	9.99	control	--	186	52.69	98	36.56	68
	6.9	8.112	7.6	8.84	control	--	--	--	--	--	--

4. DISCUSSION

Various factors regulate the spawning of fish. Therefore, it has always been a tedious task to breed fish in captivity as there is no natural environment to act as a stimulant. The morphological changes in the gonads, progression of the size of the ova and above all, the rise and fall in GSR values are indicators of spawning season. Establishing breeding pairs can be challenging due to the territorial nature of pufferfish. Providing ample space and monitoring interactions is also crucial. Occurrence of fish with fully matured (ripe) gonads from the last part of April to the first part of August [6]. The maturity index (GSR) was found maximum in June-July and after that, it sharply declined indicating the termination of gonadal products. The cycle of maturation and depletion of gonads synchronizes in both sexes indicating a regular seasonal rhythm throughout the population [9].

The changing pattern of structure and condition of gonads exhibit the successive changes of gonads in different months of the year. During summer large number of reproductively mature individuals of *L. sceleratus* found in the Mediterranean Sea is an indication of its breeding season [17]. While swimming together in a group, several males would actively pursue a female, nuzzling and biting. After sunset, the fishes suddenly swam rapidly out of the water and onto the beach, where they vigorously flopped and trembled their body. Since the present breeding trials were conducted with wild collection, the exact age of the brooders is not known. Like other fish, the feeding intensity and condition of the fish are important [19] in attaining sexual maturity. Kobayashi et al. [20] assumed that the male released sperm every spawning act but the female spawned only once in each spawning season. From observations on the annual cycle of maturation and depletion of gonads in *Uranostoma richei,* the breeding season for Lyttelton Harbour puffer fish was defined as October to March [21]. This was similar to the finding for Avon-Heathcote Estuary pufferfish [22].

In the case of puffer fishes, the male guards the eggs and the female lay eggs and the same pair spawned several times at an intervals of 14-30 days [23]. This may be true in the present case also, as the spawning season was found for 4 months. It was found that rainy weather favours breeding of *L. cutcutia*. The water temperature affects the reproductive processes of most fishes with species-specific optimal temperature regimes for development and hatching [24]. Karan et al. [25] reported that the spawning season of *Dayscieaena albida* from March to June. Agbugui [26] opined that *Pomadasys jubelini* spawns during the rainy season. Various workers [27,28] suggested that environmental factors like photoperiod, seasonal rainfall and temperature regulate the reproductive cycle in teleost fishes as also in *L. cutcutia*. Spawning of another pufferfish, *Fugu niphobles* is linked with the moon phase [29] whereas, *Canthigaster valentine* spawns a year round [30,31].

The dosage of hormones used for inducing spawning in fish can vary significantly among species due to differences in their physiological and biochemical responses. Marimuthu and Haniffa [32] and Yulintine et al. [33] noted success ful spawning in *Channa punctata* and *Channa striata* with hormonal dosages of 0.4 mL/kg and 0.3 mL/kg body weight, respectively. The success of induced breeding in fish hinges on several critical factors, including the proper administration of specific hormones, the correct dosage, the condition of the brood fish, and favorable environmental conditions. According to Miah et al. [34] and Afroz et al. [35], these elements collectively ensure the effective induction of spawning. In the case of *Macrognathus pancalus,* specific hormonal dosages have been identified as particularly effective [36]. These dosages help overcome the natural reproductive inhibitors and synchronize the spawning process [37].

With the rising temperature, fresh rainwater with high dissolved oxygen content, longer photoperiod and plenty of natural food during that period act as triggering factors for commencing breeding in most of the fish species in this region. Therefore, maintaining stable temperature and good aeration is vital during egg incubation, which typically lasts 5-7 days. Spawns were fed live foods such as rotifers and brine shrimp nauplii. Maintaining high water quality is crucial, as larvae are highly sensitive to pollutants and fluctuations in water parameters.

L. cutcutia needs a substratum for laying adhesive eggs. Richter [38] reported that the freshwater puffer fish, *Carinotetraodon lorteti* spawned in algal clumps, or primarily over rocks. In Japan, the marine puffer fish, *Fugu pardalis* is known to spawn in seagrass beds [39]. Arai and Fujita [40] reported that *Canthigaster rivulata* lay their eggs above the substratum. While swimming together in a group, several males would actively pursue a female, nuzzling and biting. In the present case, it was found that the males often devour the eggs if the brooders remain unfed for a prolonged period. It is, therefore, advisable to remove the brooders from the breeding enclosures as soon as eggs are laid to prevent predation.

5. CONCLUSION

Similar to other pufferfish, *Leiodon cutcutia* larvaehad a high mortality rate, necessitating meticulous care and monitoring. Territorial and aggressive behaviours complicate breeding efforts, requiring careful management of tank mates and breeding pairs. Moreover, maintaining pristine water in the breeding enclosure is important to prevent fungal infection. By addressing species-specific requirements and employing advanced aquaculture techniques, it is possible to achieve successful reproduction and rearing of this fascinating pufferfish species. Successful captive breeding programmes contribute to conservation efforts by providing insights into the species' biology and potential strategies for habitat restoration. R & D support and careful management are essential to improving breeding outcomes and ensuring the long-term viability of captive populations. Successful captive breeding programmes contribute to conservation efforts by providing insights into the species' biology and potential strategies for habitat restoration. Captive-bred *Leiodon cutcutia* can help meet the demand in the aquarium trade, reducing the pressure on wild populations.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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