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## First report on induced spawning of Indian pearl spot (*Eetroplus suratensis*)

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#### Abstract

Pearl spot (*Eetroplus suratensis*) belonging to the family cichlidae are commonly found in the brackishwater lakes and inland waters of India and Sri Lanka. In recent years, aquaculture of pearl spot undertaken in experimental cages in India. However, commercial aquaculture is hampered due to sustainable seed production and slow growth in the nursery rearing phase. The present study evaluated the suitability of synthetic human chorionic gonadotropin (hCG) hormone for induced spawning of pearl spot. The present study evaluated the application of hCG with doses ranging between 1000 and 2000 IU, through different route of administration at different spawning periods in captive maintained pearl spot. Doses of hCG ranging between 1500 and 2000 IU through subcutaneous injection resulted in superior performance. The results of the present study indicate the possibility of evaluating other synthetic hormones for induced spawning of pearl spot in captivity. Administration of hCG through subcutaneous injection in pearl spot produced better performance in comparison to the dietary administration in inducing spawning.

**Keywords:** Pearl spot, *Eetroplus suratensis*, induced spawning, captivity

#### Introduction

Pearl spot (*Eetroplus suratensis*), also known as green chromide is widely found in the freshwater and brackishwater ecosystems of India and Sri Lanka (Rishi and Singh, 1982) [26]. It is endemic to peninsular India extending from South Canara to Malabar on the west coast to Chilka lake on the east coast (Prasad, 1971) [24]. This species has been transplanted into inland water bodies of many states because of its ability to adapt to diverse habitats (Jhingaran and Natarajan, 1969) [13]. The distribution of this cichlid species has been reported in Malaysia, Singapore and other Asian countries (Lever, 1996; Ng and Tan, 2010) [18, 21]. In India, particularly in Goa, Kerala and Tamil Nadu, pearl spot is preferred as food and ornamental fish. Lately, this fish is proposed as game fish in Kerala (Sahadevan, 2017) [27]. This fish fetches a good price and considered as a delicacy, and commercial farming undertaken in Kerala (Joseph, 2016; Joseph and Ignatius, 2016) [14, 15]. The major constraint in the aquaculture of pearl spot is the lack of induced breeding techniques due to its inherent low fecundity, complex breeding behaviour and parental care (Padmakumar *et al.* 2009; Natarajan, 2013; Felix *et al.* 2017a) [23, 20, 9]. Recently, it was found that by curtailing parental care, large number of seeds can be obtained, when pearl spot broodstock maintained in plastic tanks with suitable substratum (Sukumaran *et al.* 2017a) [33]. Also, the authors recorded that the manipulation in the substratum and supplemental feeding influences growth of the pearl spot fry in low volume cages (Sukumaran *et al.* 2017b) [34].

In India, biology of pearl spot distributed in different aquatic habitats have been studied and in recent years, physiological studies indicate the use of this cichlid species for experimental biology (Prasad, 1971; Jayaprakash and Nair, 1981; Keshava *et al.* 1988; Jayaprakash *et al.* 1990; Rattan, 1994; Padmakumar *et al.* 2012; Sajan *et al.* 2013; Chandrasekar *et al.* 2014; Shivaprakash *et al.* 2012) [24, 12, 16, 12, 25, 22, 28, 4, 32]. Recently, the genetic characterization of pearl spot using mitochondrial DNA, ATPase indicated that the Pulicat lake, Tamil Nadu population completely divergent from those of Vellayani, Vembanad and Ashtamudi lakes of Kerala (CIBA, 2017) [6].

Our previous studies using the pearl spot distributed in the Pulicat lake demonstrated the size at first maturity, fecundity, and spontaneous spawning of pearl spot in captivity through environmental manipulation (Felix *et al.* 2016, 2017a; Selvaraj *et al.* 2016, 2017) [8, 9, 29, 30].

Induced fish breeding happens to be evolutionary history starting from bundh breeding to portable circular hatchery through a number of intermediate models (Chattopadhyay, 2017) [5]. In a previous report based on macroscopic observation, the authors recorded influence of inducing agent, human chorionic gonadotropin (hCG) on the increase in egg diameter but failed to demonstrate any spawning activity (Albin Dhas *et al.* 2010) [1]. In light of the above, the present study analyzed induced spawning of pearl spot using commercially available hCG (Fertigyn, 5000 IU).

### Materials and Methods

The trial was performed in FRP tanks (300 l) through hormonal manipulation in the feed and subcutaneous administration (Watanabe *et al.* 1993; Hodson and Sullivan, 1993) [36, 11]. The experimental FRP tanks and the breeding set up used in the study detailed in our previous publications (Fig. 1; Selvaraj *et al.* 2016a,b; Felix *et al.* 2017a) [29, 30, 9]. The doses 1000, 1500 and 2000 IU were administered to fish (Albin Dhas *et al.* 2010) [1]. BD Ultra-Fine 6 mm Needle Syringes (Becton Dickinson, USA) were used for subcutaneous injection. In addition, the response of hCG to induced spawning was recorded at the onset, during and completion of natural spawning periods. The experiment was performed in duplicates and performed between March and July 2017. During the experimental period, fish were fed with Growel floating fish feed at 3% of the body weight and 20% water exchange performed daily. Water temperature, pH and dissolved oxygen varied between 27-32°C, 6.5-8.5 and 4-8 ppm (Felix *et al.* 2017b) [7]. The length and weight of the experimental fish used for the study presented in Tables 1 and 2.

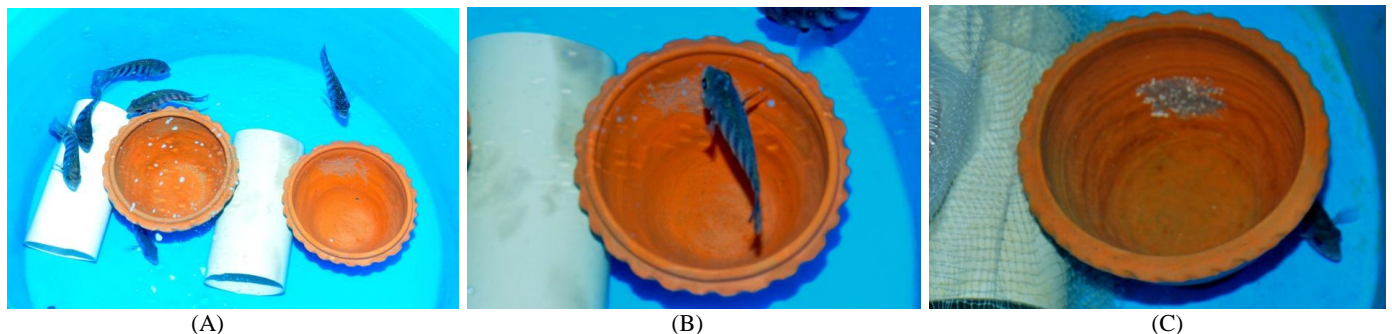
### Results and Discussion

Administration of hCG through feed resulted in lesser number of spawning, in comparison to the hCG injected fish. Hormone administration through feed resulted in poor performance at all hormonal doses. The response of hCG was delayed in induced spawning when administered through feed and even, lower dose failed to induce multiple spawning. Filial cannibalism is pronounced in the captive pearl spot and the same recorded during the experiment (Fitzgerald, 1992; Manica, 2002. Sukumaran *et al.* 2017a) [9, 19, 31]. In the previous study spontaneous multiple spawning recorded in the captivity and the same recorded with hCG injection at 1500 IU hCG/kg of body weight. Repetitive spawning was also

pronounced when hCG administered during the spawning season. During the spawning season period, hCG administration at 1500 and 2000 IU induced spawning in 36 hours post injection; however, lower dose resulted in first spawning after two weeks. Repetitive spawning in these hormonal treatments resulted after a week. The number of eggs varied between 300 and 1200 eggs, during each spawning. The fecundity of Pulicut lake pearl spot suggested to vary between 1258 and 2808. The number of eggs released during the single and spawning repetitive spawning is in agreement with the results reported previously (Felix *et al.* 2016a) [8]. Overall, the number of eggs released during each spawning was relatively higher compared to the spontaneous spawning in captivity (Selvaraj *et al.* 2017; Felix *et al.* 2017a) [29, 9].

In the natural spawning trial, it was found that when the paired individuals maintained separately for repetitive spawning, failed to undergo subsequent spawning after the first spawning (Selvaraj *et al.* 2016b; Felix *et al.* 2017a) [30, 9]. Similarly, when paired individuals that exhibited single spawning and maintained separately for a week, failed to result in spawning when administered with 1500 hCG/kg of body weight (Table 3). In contrast, when the paired individuals are maintained with the group of fish (n=6), multiple spawning observed. The results of induced spawning are in agreement with the natural spawning trials (Selvaraj *et al.* 2016a; Felix *et al.* 2017a) [28, 9].

In a previous study, the authors recorded an increase in egg diameter and biochemical parameters of pearl spot administered with combination of hCG and LHRH and ovaprim, in comparison to the control. Spawning activity not recorded; however, the authors recorded an increased level of total protein in the blood of hormone injected fish (Albin Dhas *et al.* 2010) [1]. The egg diameter analysis in adult pearl spot indicated different size of vitellogenic and mature eggs, coinciding with the results of spontaneous multiple spawning reported previously, and the multiple induced spawning recorded in the present study (Tables 4 and 5). Based on the results recorded in the present study, it is likely that hCG alone would induce spawning in captivity. In recent years several novel inducing agents such kisspeptin and neurokinin B, in addition to the existing GnRH based analogues shown to be potent for induced breeding in several freshwater, brackishwater and marine fish (Biran *et al.* 2012; Caraty *et al.* 2012; Selvaraj *et al.* 2016a) [2, 3, 29].



**Fig 1:** Induced breeding of pearl spot (*Etroplus suratensis*) in FRP tank (A), with substratum, flower pot and hide out, PVC pipes; parental care of eggs (B), fertilized (brown colour) and unfertilized (white colour) eggs attached to the substratum (C)

**Table 1:** Length-weight of pearl spot (*Etroplus suratensis*) used for testing influence of hCG administration through feed on induced spawning

Treatments					
1000 IU hCG/kg feed		1500 IU hCG/kg feed		Control	
Replicate 1					
Total length (cm)	Body weight (g)	Total length (cm)	Body weight (g)	Total length (cm)	Body weight (g)
14.4	88.7	16.0	118.2	14.3	88.0
16.5	99.3	16.6	117.8	14.6	82.0
16.0	97.0	15.0	98.6	16.3	125.5
15.3	91.2	13.0	77.4	17.5	120.0
15.4	82.1	14.2	78.2	14.8	106.8
17.2	134.3	14.9	89.3	17.1	111.7
Replicate 2					
15.3	93.5	16.7	122.4	15.2	107.2
12.0	58.0	14.6	71.1	17.1	111.7
14.3	90.0	14.3	107.2	16.2	125.2
12.8	58.4	13.6	66.2	12.6	64.1
15.3	88.9	14.3	79.7	14.0	82.6
15.2	107.5	14.6	81.3	14.6	81.3

**Table 2:** Length-weight of pearl spot (*Etroplus suratensis*) used for testing influence of hCG administration through subcutaneous injection on induced spawning in FRP tanks

Treatments							
1000 IU hCG/kg body weight		1500 IU hCG/kg body weight		2000 IU hCG/kg body weight		Control	
Total length (cm)	Body weight (g)	Total length (cm)	Body weight (g)	Total length (cm)	Body weight (g)	Total length (cm)	Body weight (g)
Replicate 1							
13.6	78.4	15.3	98.7	15.2	87.3	15.0	116.2
14.3	89.5	14.8	85.3	16.7	85.4	17.6	110.9
14.6	81.5	14.4	83.2	17.5	152.8	18.0	119.3
16.7	108.5	17.1	112.3	18.1	137.2	18.4	149.2
14.6	91.2	15.2	125.5	18.1	144.5	18.5	136.5
14.3	88.5	14.5	81.2	18.3	150.1	18.6	149.0
Replicate 2							
14.6	102.3	15.2		15.2	85.9	16.4	128.4
16.5	103.2	17.6		15.6	89.5	16.9	127.5
16.2	98.3	18.1		16.1	105.1	14.4	88.6
15.5	98.1	18.4	*	16.7	119.6	15.8	85.7
15.4	82.5	18.5		17.8	142.8	14.2	87.2
16.5	100.1	18.8		19.6	172.8	17.5	85.2

\* Due to technical difficulties in the field, individual weight could not be performed. Based on the previous results, mean body weight of 120 g was considered for individual dose calculation.

**Table 3:** Length-weight of pearl spot (*Etroplus suratensis*) used for testing influence of hCG administration through subcutaneous injection on induced spawning in paired pearl spot in FRP tanks

Parents	Male		Female	
	Total length (cm)	Body weight (g)	Total length (cm)	Body weight (g)
Pair 1	21.8	273.0	16.9	135.5
Pair 2	18.5	124.8	15.4	86.2

**Table 4:** Length-weight of pearl spot (*Etroplus suratensis*) used for egg diameter analysis

Vitellogenic female		Mature female	
Total length (cm)	Body weight (g)	Total length (cm)	Body weight (g)
14.3	71.2	13.8	78.3
14.3	68.9	15.2	84.8
14.0	60.9	15.2	85.6

**Table 5:** Egg diameter analysis in wild pearl spot (*Etroplus suratensis*)

Vitellogenic eggs (n=200)		Mature eggs (n=200)	
Range (mm)	Mean Percentage (%)	Range (mm)	Mean Percentage (%)
0.4-0.8	28.3	1.7-2.0	19.5
0.9-1.2	23.5	2.1-2.4	23.0
1.3-1.6	48.1	2.5-3.0	51.2
-	-	3.1-3.4	6.3

## Conclusion

In conclusion, the results of the present study indicate hCG as suitable inducing agent for pearl spot breeding and the hCG administration through injection at a dose varying between 1500 and 2000 IU/kg body weight found suitable for induced spawning in captivity.

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