

## Short Communication

# Enhancement of breeding frequency and reproductive performance of pearlspot *Etroplus suratensis* by curtailing parental care



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## ABSTRACT

Increasing the breeding frequency and reproductive performance of pearlspot, *Etroplus suratensis* in small tank system by manipulating parental care and salinity is reported. A total of 3 experiments were carried out to evaluate the reproductive performance of pearlspot. In the first experiment on breeding of pearlspot involving parental care (without larval separation), production of a single batch of offspring per pair of parent fish (294 larvae, 24–43 fry) was observed after 90 days. Whereas the second experiment on pearlspot breeding by curtailing parental care (with larval separation) demonstrated a higher breeding frequency; 6 in 120 days. The average inter-spawning interval and larval production were  $17.6 \pm 1.12$  days and  $1333.3 \pm 143.0$  respectively. The third experiment was to evaluate the breeding frequency of pearlspot at low ( $5.40 \pm 0.75\text{‰}$ ) and high salinity ( $27.20 \pm 0.80\text{‰}$ ) by curtailing parental care. The average inter-spawning interval was found to be significantly ( $p < 0.05$ ) lower ( $32.14 \pm 4.38$  days) at low salinity when compared to high salinity ( $64.37 \pm 12.95$  days). Similarly, the breeding frequency was also higher at lower salinity in 215 days ( $5.66 \pm 0.66$  at low compared to  $3.67 \pm 0.67$  at high salinity). The present study demonstrated that i) curtailing parental care through larval separation can be used to improve the breeding frequency and reproductive performance of pearlspot in captivity ii) relatively lower salinity reduced the inter-spawning interval of pearlspot iii) feasibility of breeding pearlspot in small tank system (1000 L).

## 1. Introduction

The fish species belonging to the family Cichlidae are the second major contributors (4.8 million tons) to the total aquaculture production (FAO, 2014). Pearlspot, *Etroplus suratensis* is a native cichlid species of India. It is economically valued as a high priced food fish and gaining importance as an ornamental fish. Pearlspot is farmed in polyculture ponds with other fish species. Low volume cage culture of pearlspot is found to be a viable option to improve the livelihood of small scale fish farmers (Pramod Kiran et al., 2014). However, aquaculture of the species is severely constrained by inadequate supply of quality seeds. Presently, farmers exclusively depend on wild seeds collected from natural water bodies or pond systems. Experimental seed production of pearlspot has been demonstrated in earthen ponds ( $100 \text{ m}^2$ ) (Abraham and Sultana, 1995) and large raceway systems ( $70 \text{ m}^2$ ) (Padmakumar et al., 2009). Larger systems involve more labor during seed collection and higher financial investment and this would emerge as a constraint in popularization of pearlspot seed production among small-scale farmers who are commonly involved in the farming of this species.

The most striking life history characteristic of the members of the family Cichlidae is the long duration of parental care of the young after spawning, a feature not found in most other fish species (Keenleyside, 1991). Although this behavior is directed at increasing the fitness of the offspring (Smith and Wooten, 1995), it is always compromised with fecundity of the fish (Noakes and Balon, 1982). This biological characteristic of pearlspot necessitates a hatchery operator to use a large number of broodstock and elaborate infrastructure which in turn reflects on the cost of production, space requirements and the efficiency of seed production of the species. The mass scale seed production of pearlspot therefore is limited by unique parental care, monogamous mating habits, small clutch size and their exclusive substrate breeding nature (Padmakumar et al., 2009).

In the present study, therefore, we explored the possibility to enhance reproductive performance of pearlspot by curtailing parental care through larval separation. The reproductive performance in the absence of parental care was also evaluated at two salinity regimes. Further, we have evaluated the potential of using small tanks (1000 L) for captive breeding of pearlspot.

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## 2. Materials and methods

The study was conducted at experimental Fish Hatchery, Muttukadu Experimental Station, ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India.

### 2.1. Broodstock management and selection of broodfish

Fifty pearlspot fish were collected from earthen ponds of ICAR-CIBA and maintained in cement tanks (35 t) for 15 days for acclimatization to experimental conditions. The pairs of broodfish (avg. wt.,  $152.25 \pm 19.01$  g; avg. TL,  $182.5 \pm 6.6$  mm) were then selected for stocking in breeding tanks based on the colour (pink to red) and appearance of genital papillae. It was presumed that fish with pointed genital papillae were males and enlarged ovipositor were females (Samarakoon, 1985; Bindu and Padmakumar, 2014). Four fish were stocked randomly in different breeding tanks at a sex ratio of 1:1. Feeding was done with commercial formulated feed (crude protein 35%, crude lipid 5%, pellet size - 2 mm) at 1.5–2% body weight twice a day.

### 2.2. Experimental set-up

Captive breeding was conducted in plastic rectangular breeding tanks (1.1 m × 0.9 m × 1 m, capacity - 1 ton), connected to a pressurized sand filter (approximate water flow rate; 1.5 L per minute) with provision for mild aeration. Till the completion of the first breeding, each tank was provided with a small rectangular plastic tray containing clayey soil (soil depth, 50–60 mm) to facilitate monitoring of pair formation and breeding.

### 2.3. Experiment 1 - captive breeding of pearlspot involving parental care

Breeding trials were conducted without larval separation (involving parental care) in triplicates. After the initial stocking of brooders, the tanks were left undisturbed and the seeds were counted after 90 days of stocking.

### 2.4. Experiment 2 - captive breeding of pearlspot by curtailing parental care

Following the same methodology (Section 2.3), one breeding trial (duration - 120 days) was conducted with larval separation (curtailing parental care). The breeding behavior was closely monitored and the tanks were observed for appearance of eggs and larvae. The newly hatched larvae were separated from the parent fish by siphoning and counted to record the larval number per spawning (Fig. 1).



Fig. 1. Pearlspot larvae separated from parent fish.

### 2.5. Experiment 3 - reproductive performance of pearlspot by curtailing parental care at two salinities

Following the same methodology (Section 2.4), a 215 day experiment was conducted in triplicates at two salinities; low salinity ( $5.40 \pm 0.75\text{‰}$ ) and high salinity ( $27.20 \pm 0.80\text{‰}$ ) to study the reproductive performance of pearlspot by curtailing parental care. The non-parent fish were separated following the first breeding and only the pair of parent fish guarding the larvae were retained in the breeding tanks.

In all the experiments the reproductive performance of pearlspot; breeding frequency, inter-spawning interval and larval production per spawning was recorded. Duration (days) between the appearances of two batches of newly hatched larvae was considered as the inter-spawning interval.

### 2.6. Water quality

The water temperature and salinity were monitored on a daily basis using a thermometer and refractometer (Atago, India) respectively. pH was measured using a pH meter (Eutech instruments, India). Dissolved oxygen (DO), ammonia-N, nitrite-N, and total hardness ( $\text{CaCO}_3$   $\text{mg L}^{-1}$ ) were measured fortnightly following standard protocols (APHA, 2012). For experiment 1 and 2, the salinity, temperature, pH, DO, ammonia-N, nitrite-N were  $12.85 \pm 1.12\text{‰}$ ,  $30.57 \pm 0.48$  °C,  $8.05 \pm 0.06$ ,  $6.70 \pm 0.18$   $\text{mg L}^{-1}$ ,  $0.09 \pm 0.01$   $\text{mg L}^{-1}$  and  $0.01 \pm 0.001$   $\text{mg L}^{-1}$  respectively. For experiment 3, salinity, temperature, pH, DO, ammonia-N, nitrite-N at low and high salinity were  $5.40 \pm 0.75$  and  $27.20 \pm 0.80\text{‰}$ ;  $27.25 \pm 0.35$  and  $27.18 \pm 0.33$  °C,  $8.23 \pm 0.09$  and  $8.16 \pm 0.34$ ;  $6.6 \pm 0.70$  and  $6.55 \pm 0.45$   $\text{mg L}^{-1}$ ;  $0.12 \pm 0.03$  and  $0.07 \pm 0.04$   $\text{mg L}^{-1}$ ;  $0.10 \pm 0.05$  and  $0.01 \pm 0.003$   $\text{mg L}^{-1}$  respectively.

### 2.7. Statistical analysis

Data analysis was performed using GraphPad Prism software version 5.00 (GraphPad, SanDiego, CA, USA) using two sample *t*-test at 5% level of significance.

## 3. Results and discussion

### 3.1. Captive breeding of pearlspot involving parental care

In the two breeding trials involving parental care, a fry production of 24 and 43 numbers (avg. wt.,  $413.33 \pm 107.55$  mg; TL-  $26.78 \pm 3.48$  mm) were recorded after 90 days. In the third trial two batches of seed guarded by separate pairs of fish was observed; a batch of 294 free swimming larvae (avg. wt  $3 \pm 0.1$   $\mu\text{g}$ , TL-  $2.87 \pm 0.85$  mm) and a batch of eggs in the hatching stage along with few newly hatched larvae. In this trial pearlspot exhibited filial cannibalism as the parent fish guarding the hatchlings cannibalised the entire batch of unhatched eggs and the hatchlings. This form of total filial cannibalism in fish, example pearlspot; is seen as a form of brood termination for recovering some energy under conditions when the survival of offspring is low (Manica, 2002).

### 3.2. Captive breeding of pearlspot curtailing parental care

In this pilot experiment where larval separation was adopted, the first breeding was observed after 24 days of the release of brooders in the tank. Subsequent breeding by the same pair was observed at intervals of 15–20 days (average:  $17.6 \pm 1.12$ ). The mean number of larvae obtained in each breeding ranged between 950 and 1350 with a mean of  $1333.3 \pm 143.0$ . Thus in 120 days, a total of 8000 larvae was produced from a single pair of pearlspot in six breedings. Larvae were reared on live feeds (*Artemia nauplii*), a survival percentage of 85–90%

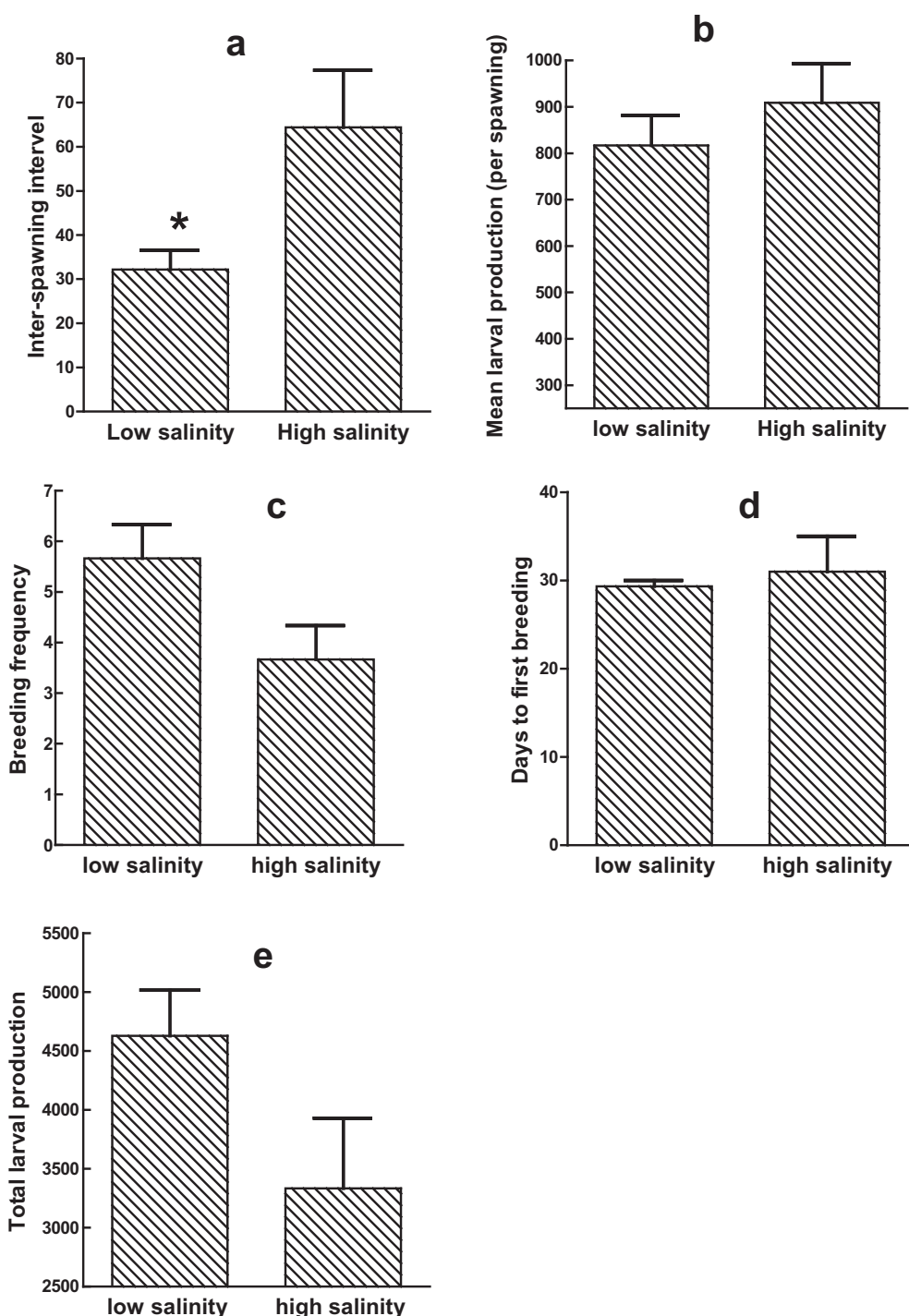


Fig. 2. Reproductive characteristics of pearlspot *Etroplus suratensis* by curtailing parental care at low and high salinity during 215 days a) inter-spawning interval b) mean larval production (per spawning) c) breeding frequency d) days to first breeding post-stocking e) total larval production per pair.

were observed. Previously, a pond-based trial using 50 brooders resulted in a production of 3500 pearlspot fry in a year from 5 sets of breeding (Abraham and Sultana, 1995). In a raceway tank system using 70 pairs, the total number of nestings, average egg released per spawning, fertilisation rate and hatching rate recorded were 50, 835, 75.7–100%, 63–99% respectively in one year (Padmakumar et al., 2009). The overall seed production from the system was not reported. The results demonstrated the successful captive breeding of pearlspot in small tank system of 1000 L capacity and a potential larval production per pair, 8000 numbers in 120 days by curtailing parental care.

### 3.3. Observations on breeding behavior of pearlspot in a small tank system

The breeding behavior of pearlspot in the captivity of 1 t tank system was mostly similar to the description of breeding behavior of pearlspot reported in previous studies (Ward and Samarakoon, 1981; Padmakumar et al., 2009) or that of other cichlid species. Observations such as an increasing aggression and territorial defense helped to confirm establishment of the breeding pair. Readiness to breed was indicated by low to negligible feed intake and appearance of cleaned a patch on the vertical sides of container or tank sides for egg attachment. Egg laying was followed by bi-parental care (fanning and guarding) with the dominant presence of one fish near the eggs. During the first breeding, small pit nests were constructed by the fish on the soil surface

of the container and the hatched out larvae deposited in one of the pits or aggregated as a single clutch at the tank bottom in the absence of soil container.

Ward and Wyman (1977) observed that in nature, adult pearlspot engage in altruistic parental care where several adults care for a single brood that presumably were spawned by two of the adults. On the contrary during captive breeding of two pairs of brooders stocked simultaneously in the same tank exhibited aggressive behavior towards each other, keeping the non-parent fish away from the vicinity of the egg or hatchlings. In experiment 2, a high degree of aggressiveness prior to spawning was observed in the parent fish in form of repeated chasing and nipping leading to injuries and mortality of the non-parent fish. The behavior may probably be due to the small tank size confining the non-parent fish to the territory of the guarding parents, thus getting subjected to continuous aggression. At the same time, simultaneous breeding by two pairs of pearlspot in one tank was also recorded indicating diverse and complex social interactions between the fish during breeding.

### 3.4. Reproductive performance of pearlspot by curtailing parental care at two salinities.

Pearlspot exhibits wide range of salinity tolerance (freshwater to seawater) (Chandrasekar et al., 2014). No study has so far been conducted on the reproductive performance of pearlspot with respect to salinity in captivity. In the present study, the reproductive performance of pearlspot was found to be superior at low salinity with significantly ( $p < 0.05$ ) lower inter-spawning interval,  $32.14 \pm 4.38$  days and higher ( $p > 0.05$ ) total larval production,  $4629.3 \pm 387.22$  (as compared to inter-spawning interval,  $64.37 \pm 12.95$  days; total larval production,  $3332.7 \pm 596.50$  at high salinity) (Figs. 2 and 3). The breeding frequency of pearlspot at low and high salinity was  $5.66 \pm 0.66$  and  $3.67 \pm 0.67$  respectively in 215 days. The highest breeding frequency (7 times) was recorded in low salinity. Salinity affects the reproduction of other cichlid species. In Florida tilapia a salinity of 1 ppt was found to be optimum for egg production while at salinity above 18 ppt, the fry production was found to be significantly reduced (Watanabe et al., 1989). In Nile tilapia, gonadal development was observed to be delayed at high salinities (Fineman-Kalio, 1988). Brackishwater (3–4 ppt) was found to be more suitable over seawater (38–41 ppt) for fecundity and hatching rates in *O. spilurus* (Al-Ahmad et al., 1988). However, the salinity did not significantly ( $p > 0.05$ ) affect the average larval production per spawning. The average larval

production per spawning at low and high salinity was  $816.94 \pm 64.58$  and  $908.91 \pm 84.86$  respectively.

Previous studies based on the reproductive biology of pearlspot from its natural habitats have suggested the breeding frequency of pearlspot to be one or two per year (Jayaprakas and Nair, 1981; Ward and Samarakoon, 1981; De-Silva et al., 1984). Based on the histology of mature ovary and appearance of mature specimens of pearlspot throughout or during most of the year, pearlspot has been reported to be multiple spawners (Costa, 1983; Pathiratne and Costa, 1984; Keshava and Joseph, 1988). The present study shows that pearlspot are multiple spawners, a breeding frequency of upto 7 was recorded at low salinity in 215 days. A breeding frequency of 6 in a 120 day period was also recorded in a pair. However in experiment 1, only a single batch of fry or larvae were observed with a pair of parent fish in 90 days indicating that breeding frequency in pearlspot is linked to parental care. According to Smith and Wooten (1995) the breeding frequency of parent fish is reduced when the fish cannot continue mating during egg or offspring care. Hence, one of the costs borne by parental care giving fish is the increased time interval until the next breeding attempt (Balshine and Sloman, 2011). One of the direct costs of parental care affecting the reproductive rates was demonstrated in *Sarotherodon galilaeus*, where females and males involved in parental care exhibited longer inter-spawning intervals than the fish which deserted the egg clutch (Balshine-Earn, 1995). Similarly, in the mouth-brooding cichlid *Haplochromis argens* the females that mouth-bred their eggs for 16 days took 33% longer time to re-spawn than non-parental caring females (Smith and Wooten, 1994). Further, a study in tilapia *Oreochromis mossambicus* showed that the postovulatory follicular tissue of females not exhibiting parental care behavior showed signs of degeneration after 10 days of spawning than mouth-brooding females in which the life span of post-ovulatory follicles was upto 25 days (Smith and Haley, 1987). Parental care is important for successful reproduction of Asian cichlids in their natural habitats (Ward and Wyman, 1977). However, the present study demonstrates that curtailing parental care can be used as an effective tool for enhancing the breeding frequency and overall larval production of the species under captivity.

### 4. Conclusion

For a hatchery, low fecundity of pearlspot would imply maintenance of a sizeable broodstock and elaborate infrastructure for mass scale seed production. Therefore, this work on increasing the breeding frequency by curtailing the parental care period can contribute to an increase in

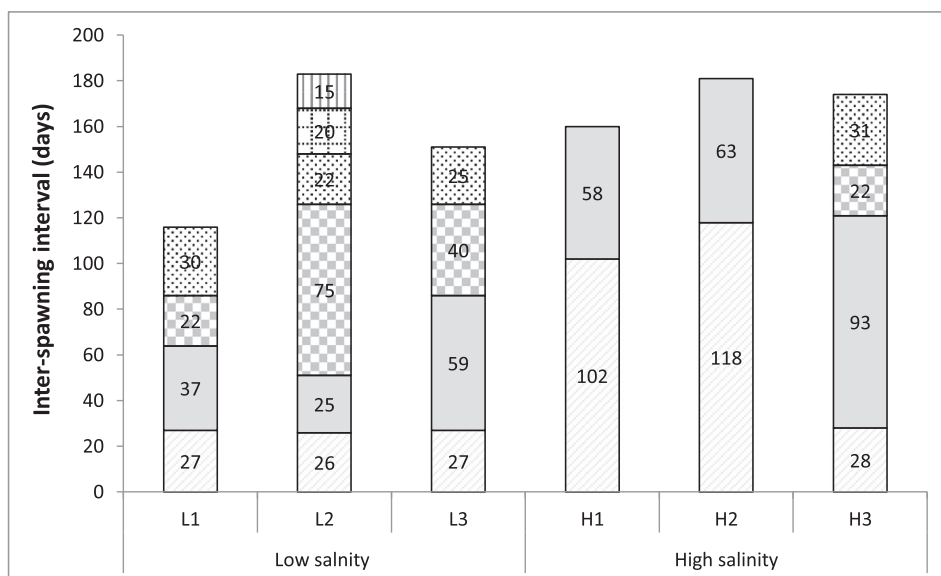


Fig. 3. Inter-spawning intervals of pearlspot *Eetroplus suratensis* by curtailing parental care at low and high salinity during 215 days.

the overall seed production per pair of pearlspot in captivity. Further, standardisation of pearlspot seed production in small tank system will facilitate popularization of pearlspot seed production technology among small scale farmers who are commonly involved in the aquaculture of this species.

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