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EtroBrood^{Plus} –A functional broodstock feed

for Pearlspot (*Etroplus suratensis*)

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Background

Etroplus suratensis is a cichlid fish endemic to estuaries, backwaters and fresh water streams of India and Sri Lanka (Chakrabarty, 2004; Sparks and Smith, 2004), and is widely known as green chromide. This is the largest among chromides and a geologically significant ancient cichlid. Locally known as pearlspot, *E. suratensis* is the popular food and aquarium fish (Padmakumar et al., 2012; Tavers, 2013) which is that has been transplanted to many foreign countries (Hornell, 1923). Pearlspot is a fish of delicacy in Kerala, a south Indian state, where this fish known as "Karimeen", and has been declared as the 'state fish' (Padmakumar et al., 2012). Diminishing wild supply (Abraham, 2011) and increasing demand as a table fish and aquarium hobby boosted the farming of green chromide (Padmakumar et al., 2012). Presently farmers produce green chromide seeds by stocking the adult fishes to spawn in groups in large ponds with vague feeding, and they harvest the seeds by unspecified means. Seed production with such a low fecund fish in a large system without proper feeding in the above manner is labour intensive, less productive and uneconomical.

Maximum reproductive capabilities of fish in terms of quantity and quality can be achieved only by offering a right feed and adopting an apt feed management strategy for the broodstock and their larvae. Studies on maturation in several farmed fish indicated that ideal amino acids, dietary pigments, lipids, and its component fatty acids are the primary nutrients directly affecting the reproductive performance (Watanabe and Vassalio – Aguis, 2003; Ng and Wang, 2011; Norambuena et al., 2013).

Environmental and communal needs of this fish are acceptably understood inclusively for chromides (Ward and Wyman, 1977; Ward and Samarakoon, 1981). However, the understanding on nutritional needs of green chromide is mostly restricted to juvenile stages (Pillai and Ali, 1997; Jeyaprakas and Sunil Kumar, 1999; Ali, 2004), and only a single study so far (Shiranee and Natarajan, 1996) is available on broodstock nutrition. Presently farmers feed the brood green chromides with mixture of rice bran and ground nut oil cake and commercial grow-out fish pellet feed, and produce the seeds by allowing the brood fishes in groups (communal breeding) in large ponds and collect the seeds latter, which is labour intensive, less productive and uneconomical.

There are studies reporting maturation and spawning of green chromides in wild and in captive conditions (Lazarus and Nandakumaran, 1986; Samarakoon, 1985; Padmakumar et al., 2009). However, all those studies reported a significantly less oviposited eggs and/or fry yield compared to the estimated potential fecundity in the wild matured females by counting the oocytes within the ovary. A breeding attempt using many pairs of green chromide (communal breeding) comparing the reproductive performance of green chromide in pond and raceways using conventional feed management practices (Bindu, 2006; Padmakumar et al., 2009) concluded that raceways were more efficient in seed production, and maximum number of eggs obtained per pair per annum grown in the race way was 1966 against 1577 under pond conditions.

Importance of this technology

Conventionally, young ones of the pearlspot fish is propagated by stocking the adult fishes in groups and allowing them to breed naturally in large ponds (Samarakoon, 1985) and race way tanks (Bindu, L., 2006; Padmakumar et al., 2009) using undefined feed and feeding methods. Breeding a substrate spawning, low fecund fish with complex parental care by the conventional methods in large tank and pond rearing systems is labor intensive, unpredictable and expensive. Therefore, adoption of the technique by the entrepreneurs still remains unrealistic, and lack of consistent seed supply continue to be a significant limiting factor in the commercial farming of the pearlspot fish.

It has already been proposed that, pearlspot can release eggs only two times is a year in a gap of around 6 months in between. Further, the estimated average of oocyte counts inside the ovary of female fish is to be >7500 (Ng and Tang, 2010; Bindu and Padmakumar, 2014), but the realised maximum egg yield reported was only 1966 (Padmakumar et al., 2009) from communal breeding method in large tanks using unspecific conventional feeds. Further, seed production by handling such a less fecund fish in a large ponds and/or tank system in above said manner would be labour intensive for harvest of seeds, unpredictable yields and expensive. Further keeping many brooding pairs together in one tank or pond is always dangerous because of filial cannibalism, a natural behaviour of this fish species, in which parents themselves consume their eggs and young ones. Parents displays this unique destructive behaviour if they gets external disturbance from other pairs of same fish or other species or from other means, in which they decides the chances of survival of their progeny is threatened.

Tracks of the technology development

Series of feed formulations were made with different levels of lipids and its compositions and evaluated for reproductive performance in several breeding trials over the last five years in captive conditions. Pearlspot is known for complex parental care, hence monitoring single pairs was needed and it provided more accurate knowledge on fecundity, spawning frequency and fry yield. We successfully standardised the process of breeding single pairs with multiple spawning in a season using cost effective green water systems. There were 132 spawning events accounted from several pairs of pearlspot to perfect the broodstock feed technology. The breeding experiments used both wild caught and captive domesticated broodstock for evaluation of the feed. The feed was able to support a higher reproductive performance not only in wild broodstock, but also was successful in sustaining a improved breeding performance for generations.

Different elements of the technology

Feed formulation

A broodstock diet was formulated to have 44% crude protein (CP) and 12 % crude lipid (CL) explained in Kumaraguru vasagam et al. (2009). Larval diet was also made in a similar way as pelleted broodstock feed,

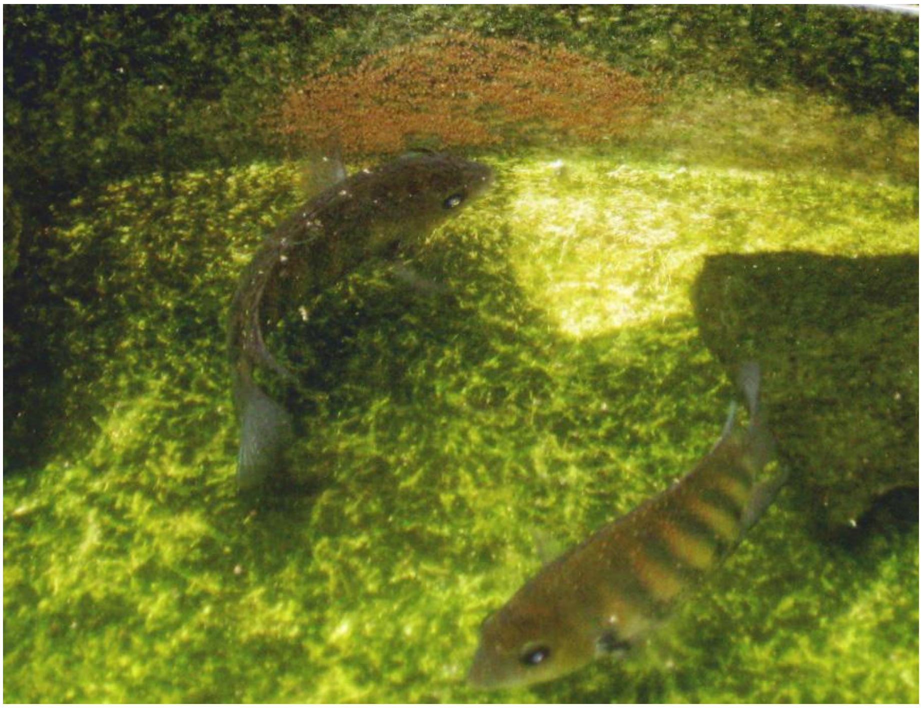
considering the minimum essential amino acid requirements optimized for Nile tilapia (Santiago and Lovell, 1988), a most studied cichlid fish for nutritional requirements. A larval diet was formulated to have 40% CP and 8% CL. Diets were formulated with help o f Feedsoft Professional 3.1 to contain practical feed ingredients. Feed was prepared in CIBA, pilot scale feed mill as and then it was crumbled to have 2 particle sizes such as 200 to 400µ and 400 to 600µ by manual crushing and sifting. Crumbled groundnut oil cake and commercial shrimp feed (CP, India) claimed to contain 34% crude protein and 6% lipid, which were offed to the brood fishes in alternate rations, similar in existing farmers practice served as control diet.

Brood fish and rearing system

An extended breeding experiment was conducted with wild caught adult fishes of two different ages which comprised of matured adults (average body weight: 187±48.5g) obtained directly from the wild. Both adult and pre-adult fishes for this study were obtained from Pullicat lake (13° 25' 40" N; 80° 17' 28" E), Tamilnadu, India. The fishes were caught by the local fisherman using either gillnet or cast net, and transported to the laboratory in plastic tanks (500 L) supplied with diffused oxygen. Upon arrival, the fishes were placed in 2000 L circular tanks and acclimated to the laboratory conditions. Acclimatized fishes were given 5 min freshwater dip to remove the external parasites if any. Pre-adults collected from the wild were grown up to adult size in cement tanks of 20 ton capacity, by feeding them grow-out feed for 4 months. For breeding experiment, about 24 adult fishes were stocked in four replicate tanks (1000 L), each to have a group of 6 unsexed fishes (4*6=24 fishes) and allowed for natural pairing. Breeding experiments was carried out in outdoor integrated green-water periphyton conditions. Twelve numbers of rectangular fiberglass (3 feed * 4 replications) reinforced plastic (FRP) tanks (2 x 1 x 0.5 m) of 1000 L volume kept under direct natural photoperiodicity served as the rearing system. The tank water was allowed to develop natural algae and microbial biota in the water (green-water) and on the tank walls (periphyton). Growth of suspended micro algae to yield green-water and succeeding formation of periphyton and biofloc are interwoven dynamic biological processes in outdoor rearing system. Having either one component minus the other is not practical on a longer run. Tank walls had thick periphyton throughout the experiment except for the initial days. Conical cement flower pots (volume: 30 L) and rectangular black 20 mm thick marble plates (30*20 cm) were placed in tanks as spawning shelter and/or spawning surfaces.

Pairing of broodstock and feed management

Green chromides are monomorphic and therefore it was difficult to identify the sex exactly. Sex ratio of six fishes was expected 1:1 based on an imprecise way of sex identification keys accumulated by our own experience. We used the genital papillae of the adult fishes, which appeared thin & pointed in males and large & broader in females . In females, the belly will be slightly bulged and presence of swollen ovary was felt by our finger on soft touch over the belly.



Feed ration was calculated as 3% of the biomass, and offered twice daily (equal portions; 10.00 and 17.00 h) using submerged feeding trays. Any uneaten feed was removed after; the feed level was adjusted further based on the presence and absence of feed in the feeding trays. Tanks were monitored closely for pairing of fishes from their behaviour. First spawning of all the pairs were allowed to happen in communal tanks. Noticing the pairing of fishes or spawning events, such paired fishes were retained in the same tanks or moved to new tanks. Likewise, we obtained 4, 8 and 7 single pairs in Exp.1and Exp.2, and were maintained for another 15 and 19 months respectively. Thereafter pairs were closely monitored daily for spawning activity, their behaviour, hatching and parental care. On end of conditioning period, any unpaired fishes were dissected out to confirm the sex and maturity status of the gonads. The maturity stages were identified as described by Jeyaprakas and Balakrishnan (1981). Gonads were removed and weighed in order to calculate the gonado somatic index (GSI = gonad weight/body weight/100).

To overcome the difficulties in assessing the fecundity by counting the deposited eggs and following loss of eggs due to filial cannibalism, fry yield was assessed instead. In every spawning and followed hatching, the fry were removed from the parents before they become free swimming (wrigglers) and counted manually. This was done routinely except for occasions like a larval rearing trial evaluating the influence of parental care duration on larval survival. To avoid loss of young ones due to filial cannibalism parents were not disturbed much during the spawning events.

Loss of eggs due to filial cannibalism by parents and hatchability rate of the eggs was assessed separately by comparing the counts of oviposited eggs and counts of fry (fry yield). In few spawning occasions, the oviposited eggs were digitally photographed after draining the water for a short duration, and number of eggs in the image was counted manually with help of image processing software (Adobe Photoshop CS 5) using varying colour eraser. Then the fry yield from respective clutch was matched to enumerate the hatchability in the given set of conditions. In many such attempts ended up in complete cannibalism of the eggs by parents. Nevertheless, we managed to get successful hatchings by minimal disturbance during this procedure.

On termination of conditioning period, any unpaired fishes were dissected out to confirm the sex and maturity status of the gonads. The maturity stages were identified as described by Jeyaprakas and Balakrishnan (1981). Gonads were removed and weighed in order to calculate the gonado somatic index (GSI = gonad weight/body weight/100).

Larval rearing in the absence of parental care

To assess development, growth and survival of the larvae, clutches of larvae from different spawning's were separated from the parents at four different ages (0, 4, 10 and 20 DPH) and reared in the outdoor green water system. Rearing container varied based on the needs of the life stages. Zero and four day old PHF were yolk bearing wirgglers, and they were grown in mesh strainers floating on the green water ( ). Fry of 10 and 20 DPH were free swimming larvae, and they were grown in 100 litters FRP tanks with green water + periphyton. Treatments started with wrigglers were provided freshly hatched artemia nauplii on 5DPH when they become free swimming, and continued until 10 DPH. From 8 DPH onwards they were slowly weaned to micro particulate diets by partially replacing artemia nauplii. From 10 DPH onwards all the survived larvae transferred to 100 litters FRP tanks with green water + periphyton. Irrespective of experimental variable the survived larvae were grown till 40 DPH. The larvae

separated from parents after 20 DPH got maximum survival and they were further reared up to juvenile size. About 300 number of 40 DPH fry from 100 l tank were transferred to 500 l rectangular FRP tank with green water + periphyton. The fry's were fed *ad libitum* with micro particulate diet for another 80 days. Survival and growth of the green chromide fry was measured fortnightly. Prevailed water quality parameters in both larval and fry rearing were in the range as in the case of broodstock development.

Water quality parameters

Continuous aeration was provided through air diffuser stones, and dissolved oxygen was maintained > 5 mg L^{-1}. Other water quality parameters like, water temperature, salinity, and pH were monitored regularly, and the values were in the range of 27 to 31ºC, 6 to 9 g L^{-1} and 7 to 7.8 respectively. The levels of total ammonia, nitrite and nitrate were maintained below 0.25mg/L. Tank bottom was siphoned for uneaten feed and faeces as and when required. About 50% of the water was replaced with new water from the source of same salinity when water gets so turbid to make fishes invisible under direct sunlight.

Reproductive performances of green chromide fed formulated broodstock diets

Growth, survival, reproductive performance of the *Etroplus suratensis* collected from wild as adults and fed test diets in one of the experiments

	DL60	DL90	DL120	DL150
Total pairs formed	0	2	8	6
Total spawning’s*		4	37	28
Average spawning/pair/annum		2	4	4.7
Average fry yield/ spawning		1805	2216	2148
Average relative fry yield/		7.8	9.8	10
Average inter-spawning days#			37.4	31
Highest inter spawning duration			55.0	40.0
Lowest inter spawning duration			18.0	21.0
Maximum no. of recurrent spawning by a single pair / annum		2.0	8.0	6.0

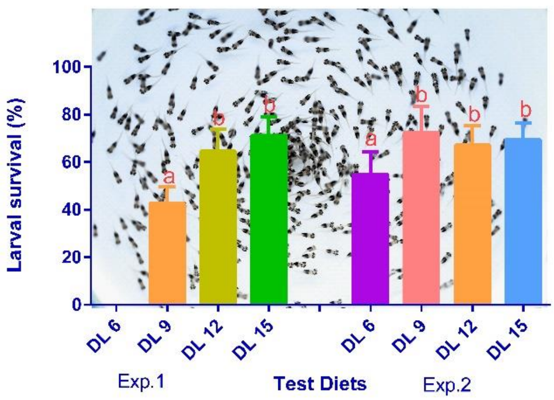
Data on growth, survival and recurrent spawning of the adult fishes kept in green-water tanks fed conventional feed and experimental broodstock feed for adults of different ages of conditioning is presented in table. Though the survival of the adult fishes (A2 and A2C) grown in captive conditions remains unaffected, growth and reproductive performance showed a significant difference ($P<0.05$) in most of the parameters tested (Table). The survival among the dietary treatments varied between 83 and 91% and it was not significantly different among treatments. Fishes (A1 and A2) which were on experimental broodstock feed started spontaneous spawning before the completion of 90 day conditioning phase, and we got 18 successful spawning pairs in total from all three treatments. Both of treatments, which fed broodstock feed irrespective of age, had significantly higher number of successful pairs and more number of repeated spawning up to maximum of 8 times in year compared to the fishes which fed conventional feed (A2C). Adult fishes fed broodstock feed showed signs of pairing within three months under broodstock care and a pair had their first spawning on 88th day of broodstock conditioning and care. The adult fishes fed conventional feed took more than 3 months for showing such signs, and we got only 3 pairs for the entire study duration. Over the entire one-year observation of the breeding performance, there were two distinct spawning phases in a year, with gap of around 2 months in between was apparent. Each spawning segment lasted for about four months, and was mostly between January to April and July to October of the year. On active recurrent spawning phase, the interval between subsequent spawning ranged between 25 and 43 days and there was no uniformity among the different pairs. The fry yields from a single spawning across the treatment ranged between

950 and 3670 with highest fry yields in fishes fed formulated broodstock feed. While average relative fry yield of fishes fed broodstock feed (A1 and A2) varied between 30.56 and 38.1 fry per g of female body weight, it was only 13.1 fry per g of female body weight in fishes fed control feed. While counting the eggs for hatchability assessment, in one instance we observed bigger egg clutch in which we counted 5244 eggs. In most spawning, eggs were attached on the vertical tank walls in an undefined manner with a partial cleaning of algal mat.

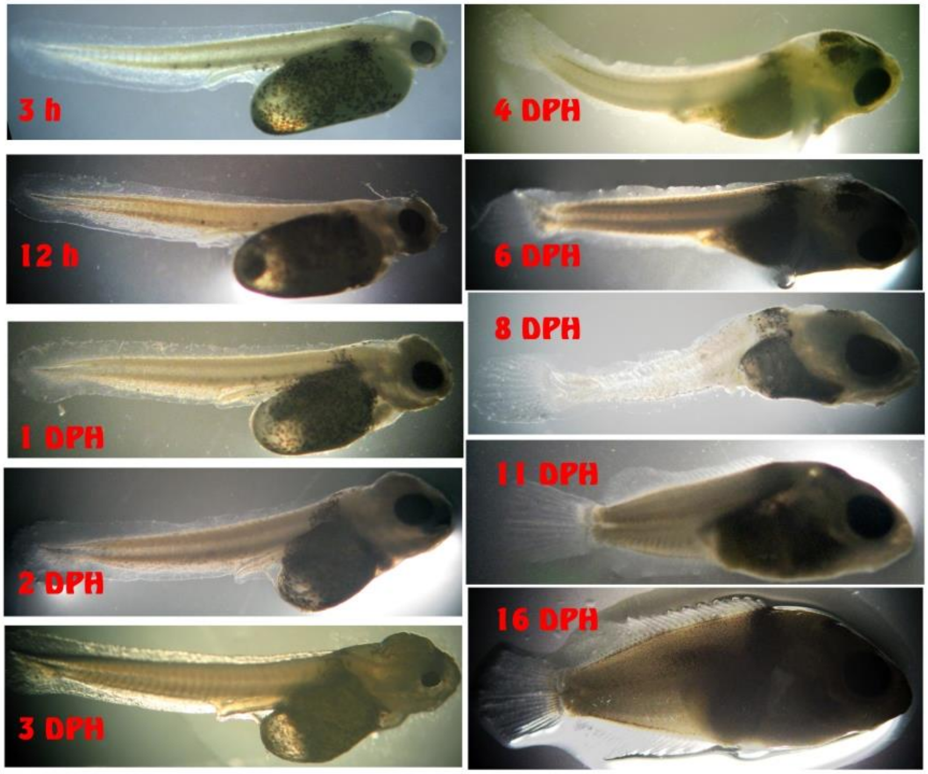
Larval survival and growth in the absence of parental care under different feeding regime

Survival of green chromide larvae separated from parents at their different ages is presented in figure. Larvae separated from parents on dph 1 (DPH 1A DPH 1F) and dph 4 (DPH 4A and DPH 4F) showed a survival of >90% on day 5 (dph 5) irrespective of the dietary treatment. There was a mass mortality of early larvae fed mircoparticulate feeds in treatments such as, DPH 1F and DPH 4F on day 6 and 7 respectively. On the other hand, similar aged larvae fed freshly hatched artemia nauplii were able to survive for another 40 days, but the number of survived larvae was very poor as 23% and 38.66% respectively. Larvae separated on dph 10 (10 DPH) and 20 (DPH 20) and fed formulated feed showed a higher survival of 81% and 87% after 40 days of post hatch.

Growth of newly hatched green chromide larvae in terms of length and weight for first 28 days is presented in figure. Average length and weight of green chromide larvae at the end of 28 days was 1.9 cm and 116 ± 20.4 mg respectively. Another larval feeding trial which tested natural feed and its combination with formulated feed in a 60 day



trial showed significant differences ($p<0.05$) in weight gain of larvae among the treatments. At the end of the trial, fry that which had access to the periphyton in addition to the formulated feed showed a maximum weight gain of 2168.9 ± 95.2 mg, while it was only 1002.3 ± 100.9 mg and 229.1±19.7 mg respectively in fry fed either periphyton or formulated feed alone. From day 36 onwards, there was a consistent divergence in fry growth between dietary treatments.



Technology Demonstration

Pearlspot being one of the most farmed fish species in Kerala, we identified two breeding facilities for demonstration of EtroBrood$^{Plus.}$. In real field conditions, a tank based hatchery produced 45000 seed using 24 kg of maturation feed and other farmer in pond based system produced 6000 seeds using 18 kg feed. The worked out feed cost per pearlspot seed produced was 5.8 paisa and 33 paisa in tank based and pond based systems respectively. Here we proved that single pair mating, integrated green-water periphyton rearing system, formulated broodstock feed and termination of parental care were instrumental in realizing a higher reproductive potential and simplifying the breeding protocol with green chromides. In this way of approach we were able to expose enormous reproductive potential of this larger cichlid in terms of recurrent spawning (> 4 times/year (average) and greater fry yield (average fry yield >2200/spawning) of single pair *E. suratensis* under tank conditions for first time. Separation of larvae in an optimum age and rearing them in the absence of parental care is critical to have repeated spawning and thereby a higher seed production.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