

***In situ* conservation and stock enhancement of endemic fish resources through captive breeding and artificial sanctuaries**

K G PADMAKUMAR¹, L BINDU and P S MANU

Kerala Agricultural University, Regional Agricultural Research Station, Kumarakom

ABSTRACT

Pearlspot, *Etroplus suratensis* and Golden catfish *Horabagrus brachysoma*, are endemic to Peninsular India, facing serious depletion in the Vembanad wetlands due to environmental alterations. In order to develop viable breeding techniques of these species under controlled conditions, habitat requirements and critical reproductive traits were closely monitored. Both the species are omnivorous, the former is an asynchronous spawner while the latter a group synchronous spawner. Breeding behaviour of the species were closely observed in natural and experimental conditions and based on this, captive breeding protocols were developed. *E. suratensis* was successfully bred under controlled conditions in artificial raceways of 70 m². The percentage success of breeding in the devised system (71%) was higher than that of pond breeding. Hatchling survival was also higher in the larval rearing system. Induced breeding of *H. brachysoma* was carried out by the administration of Ovaprim @1ml. kg⁻¹ body weight or fish pituitary extract @50-60 mg.kg⁻¹ body weight, the former being more effective. Fertilisation upto 100% and hatching rate of 73.1% were obtained. The present investigations on captive breeding is a major advance towards development of a standardized mechanism for conservation of indigenous species.

Key words: Captive breeding, *Etroplus*, *Horabagrus*, *In situ* conservation

The Western Ghat region in Kerala, on the south west coast of India, covers over 42.5% of the entire Ghat region. Richly endowed with plentiful rainfall, and with precipitation over 3000 mm per annum, the river network in these places, results in near water logged conditions in almost 20% of the total geographic area. The steep and undulating topography results in physiographic divisions, viz. the highlands, midlands and lowlands, identified based on heights above mean sea level. The Western Ghat river system thus becomes the biological hotspot, extremely rich in fish biodiversity due to its unique topographical characteristics, altitude and depth gradient. The steep and short rivers change gradually from fast flowing highland streams to slow meandering lowland rivers and generate habitats used by diverse fish species and the biological diversity gradually increases downstream. The river systems that confluence in a continuous chain of backwaters of over 350 km and lie parallel to the coastline, also sustain very high biodiversity and exert profound influence on the coastal fisheries.

The Vembanad estuarine system, the floodplain wetlands of five rivers, viz. Moovattupuzha, Meenachil, Manimala, Pamba and Achencoil in central Kerala, originating from the forested hills of Western Ghats, has been home to rich biodiversity. Although agriculture and fisheries have been

the two most important attributes of these wetlands; during the last century, the shallow flood plains and deltaic upper reaches of this wetland has been subjected to a series of human interventions, all to facilitate and intensify rice farming. The construction of a salt water regulator across the estuary at Thanneermukkom, to check tidal ingress of salinity in to the adjoining rice lands has been most catastrophic, as it affected the biological continuity of the lake. Its effect on the endemic fishery resources has also been widely documented (KWBS, 1989; Unnithan *et al.* 2001; Padmakumar *et al.* 2002).

Pearlspot, *Etroplus suratensis*, the commercially important omnivorous fish species in the lake, though tolerant to lower salinities is one such species that suffered rapid impoverishment. This is attributed *inter alia* to large scale reclamation of shallow wetlands and destruction of the fringing vegetation and mangroves, that served as favored nursery areas of the species. Apart from this, their unique breeding behavior also impose severe constraints on their natural recruitment. Another fish species considered endemic to the Western Ghat region is *Horabagrus brachysoma*, popularly known as 'Golden catfish', or yellow catfish. Once abundant in the lowland reaches of the riverine systems, this species has also become an extreme rarity owing to illegal fishing practices, indiscriminate exploitation and destruction of natural habitats.

Email: ¹kgpadman@gmail.com

Two main strategies for conservation of biodiversity in such situations are (i) stock enhancement by conservation stocking and (ii) promotion of natural recruitment by establishing protected breeding zones or sanctuaries. The present study documents the development of captive breeding protocols for mass production of seeds of *E. suratensis* and *H. brachysoma*, for species restoration and the establishment of safe reproduction protection zone and sanctuary in Vembanad for *in situ* conservation of *E. suratensis*.

MATERIALS AND METHODS

A detailed survey on the fisheries production and exploited resources of fish species of the wetland region was undertaken during 1999–2001. The survey covered the entire flood plain riverine areas on the upstream portions of the Vembanad lake south of Vaikom and the Thanneermukkom barrage (Fig. 1). The exploited catches of *E. suratensis* and *H. brachysoma* were assessed based on actual daily landings registered at thirty landing centers in the region. The predominant size groups of fishes in the collections and commercial catches were analyzed. Experimental fishing was conducted at different reaches using diverse gears, viz. cast nets, gill nets, drag nets and other local fishing devices to elicit information on the changes in the population status,

seasonal distribution of juveniles and spawning adults in the flood plains.

Detailed investigations on feeding and breeding biology of the two species, viz. *E. suratensis* and *H. brachysoma* were undertaken in order to identify the cues and triggers that are necessary for successful reproduction and recruitment. In addition to habitat requirement, critical reproductive traits, viz. fecundity, Gonadosomatic index and gonadal changes etc., were also monitored. Breeding behaviour of both species were closely observed in natural and experimental conditions and based on their unique breeding attributes, hormonal and environmental manipulation protocols for captive breeding of each of the species were developed. *H. brachysoma* were subjected to induced ovulation by using varying doses of inducing agents such as Carp Pituitary Extract (CPE) @ 50–60 mg.kg⁻¹ body weight or synthetic hormonal analogues such as Ovaprim® @ 1ml.kg⁻¹ body weight, in single dose. For breeding of *E. suratensis*, artificial nesting substrates comprising wooden logs fixed on to movable cement concrete base were deposited as nest substrates. Breeding was successfully accomplished under controlled conditions using the attached pairs. The nest containing the attached eggs were transferred to incubation tanks, provided with continuous aeration. Artificial breeding pits, fabricated on cement concrete, of 6 cm diameter and 4 cm deep, were provided as larval habitats during hatchling nursing. In order to promote natural recruitment for conservation, an engineered breeding habitat/sanctuary of 25 acres was established in the open lake, at Kumarakom on the eastern bank of the Vembanad lake. While developing this, the habitat requirements of the species were assessed very carefully and the situations were simulated. For this, a circular area of 10 ha in the open Vembanad lake, near Kumarakom was cordoned off, by planting coconut piles and bamboo poles at close intervals to hinder fishing and obstruct operation of crafts and fishing gears. Artificial hills and valleys were formed on the lake bed in the designated sanctuary to facilitate substratum characteristics and depths appropriate for natural breeding of the fish. Apart from this, a variety of artificial 'nest' and 'reef' substrates were installed on the lake floor to provide artificial nesting surfaces for the fish, as the fish is known to attach their eggs on underwater substrates at appropriate depths. Half split coconut shells, large boulders of laterite blocks, and specially designed cement concrete tetrapods were used as 'paaru' and 'reefs' for simulated breeding. Additionally, fourteen mangrove islands were formed by fixing cement concrete rings and filling them with excavated silt from the lake bed. These artificial mounts were planted with mangrove seedlings, and developed as mangrove islands around the margin of the designated sanctuary. Extent of utilization of the protected habitats and deposited substrates in the sanctuary was carefully evaluated through fish population census and visual counts.

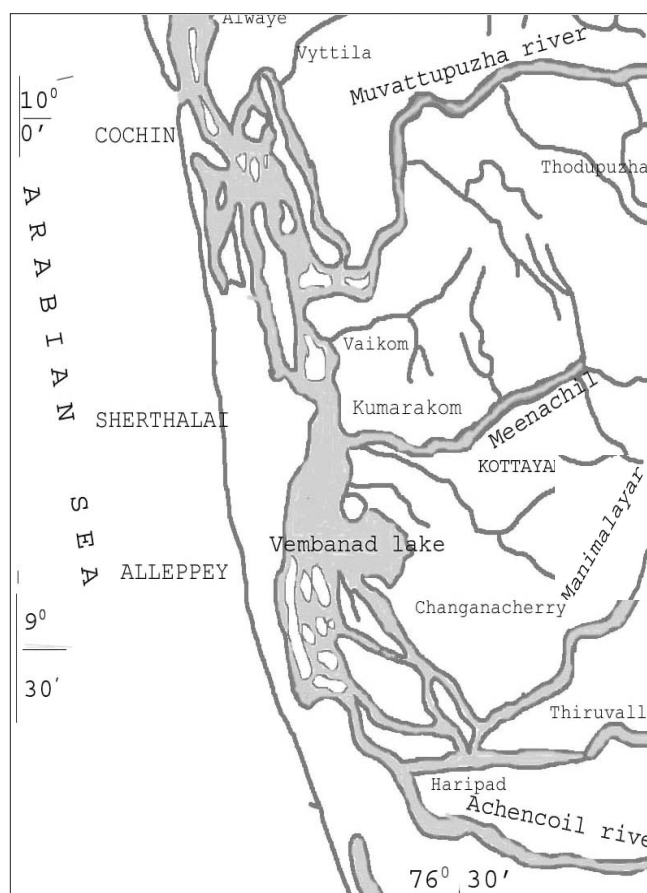


Fig 1. Riverine stretches of Vembanad lake

RESULTS AND DISCUSSION

The total annual fish production in the upstream locations of the open lake was observed to be 440 and 434 tons, respectively, during 1999–2000 and 2000–2001. Although 56 fish species were observed to inhabit the freshwater zones, thirty-six fish species were encountered in the commercial catches. A comparison of the fish catches on either side of the salinity barrage revealed that the brackish water zone, immediately north of the barrage which accounts for only 15.3%, contributed over 20% of the *E. suratensis* landings.

Captive breeding of Pearlsplit, *Etroplus suratensis*

The indigenous cichlid, *E. suratensis* popularly known as pearlsplit, alone contributed to 200.6 tons per annum (46%). The catches were highest in the Kumarakom zone, constituting almost 48% of the total landings of this species in the lake (Fig.2, 3). A detailed evaluation of the production trend of pearlsplit in the Kumarakom region for four years (2002–2005), revealed a perceptible reduction in production and productivity, in terms of catch per unit effort (Fig.4).

Information on biological features is indispensable for

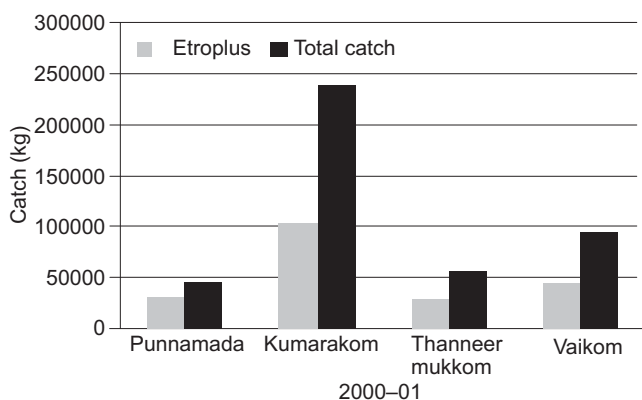


Fig 2. Catch variations of *Etroplus suratensis* in different zones

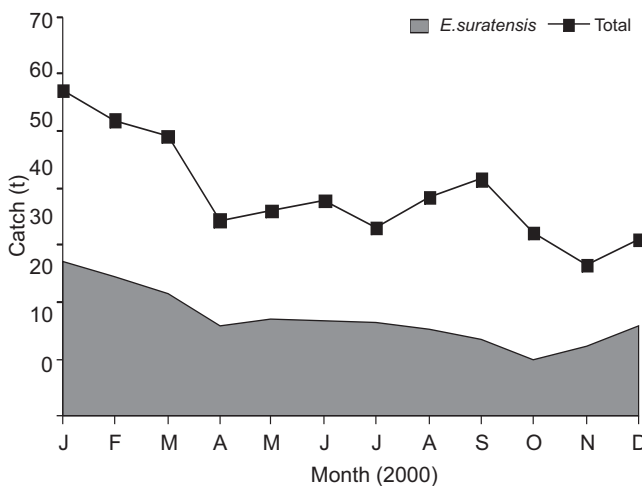


Fig 3. Exploited landings of *Etroplus suratensis* in Vembanad lake

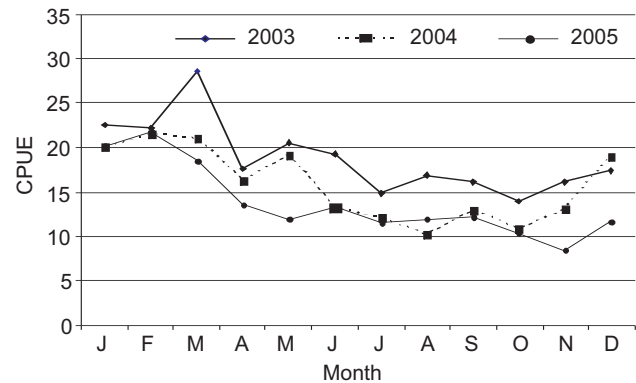
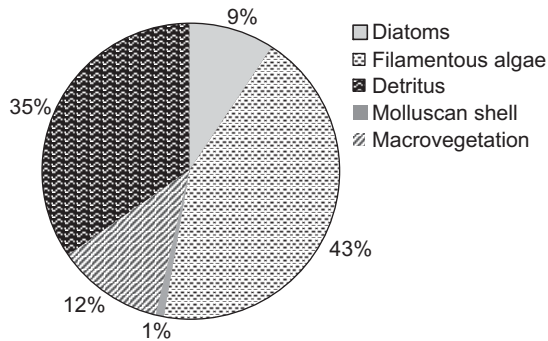


Fig 4. Catch Per Unit Effort of *Etroplus suratensis* in scare line fishing.

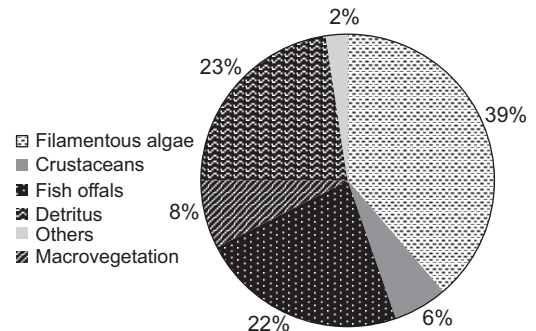
devising valid programs for conservation. Studies on biological attributes of *E. suratensis* indicated that the fish prefers a herbivorous diet, comprising filamentous algae (43%), detrital matter (42%), macro-vegetation (12%) and miscellaneous items (3%) comprising aquatic insects, molluscs (Fig.5.1). Fecundity of pearlsplit was found to range from 874 to 7554. Gonadosomatic index (G.S.I) for different size groups of females varied from 1.84 to 4.4. Highest G.S.I was observed during April and June coinciding with the pre-monsoon and monsoon season (Fig.5.2). Ova diameter measurements indicated two distinct batches of eggs in the same ovary (Fig.5.3) implying that the fish spawns twice a year. The two G.S.I peak during March-April and June-July conform to this findings. The fish exhibited typical biparental monogamy and was found to form mating pairs close to breeding and courtship commences only between such 'attached' pairs. In nature, the paired fishes were observed to utilize stationary solid objects such as coconut leaves, coconut husk, wooden logs, stones and any other solid objects, 11 to 45 cm above the ground. Both male and female partners were found to engage actively in nest preparation. After the substrates are cleared off, the female lay flat on the spawning site and gently move from end to end and attach their eggs carefully on to the nest substratum with the help of its tubular and fleshy ovipositor. The male fish follow close behind and fertilize the deposited eggs instantly by releasing a spray of milt. This process of egg laying and fertilization is continued several times and the eggs are seen placed closely in a patch without touching each other. The number of eggs per nest varied from 250 to 1573 with a mean of 854 and nest area per brood varied from 20 to 49.5 cm² (Fig.6). After the eggs are laid, the female with their rhythmic fanning and mouthing activity, continually aerate the eggs; males guard the territory and chase away all intruders. Results of breeding trials is summarized in Table.1.

The very next day after egg fixing, the parent fish started excavating small cup like pits, on the ground, just below the nest, by scooping out mud and clean the vegetation around by vigorous mouth picking movements. The bottom pits

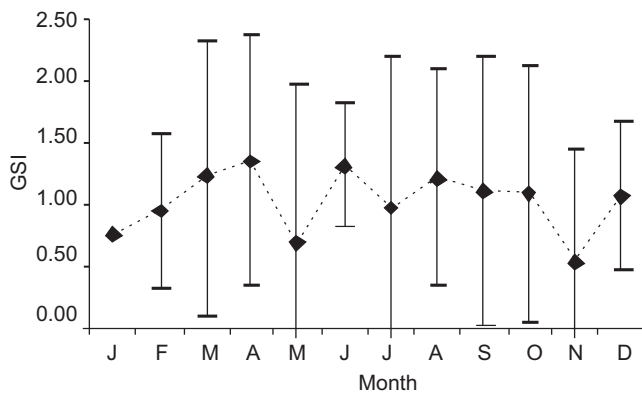
5.1 (a)



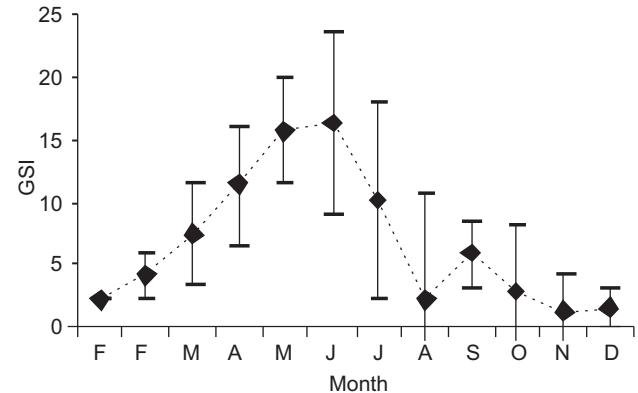
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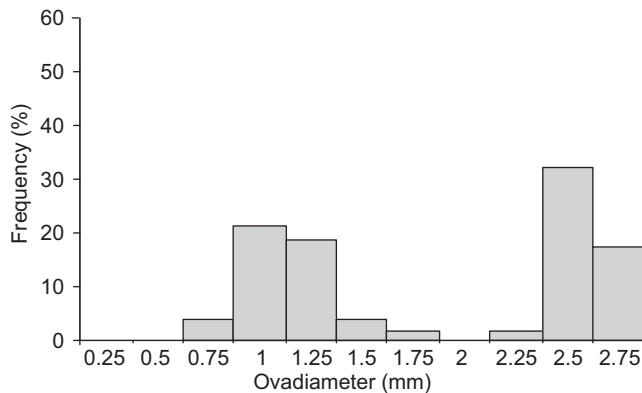
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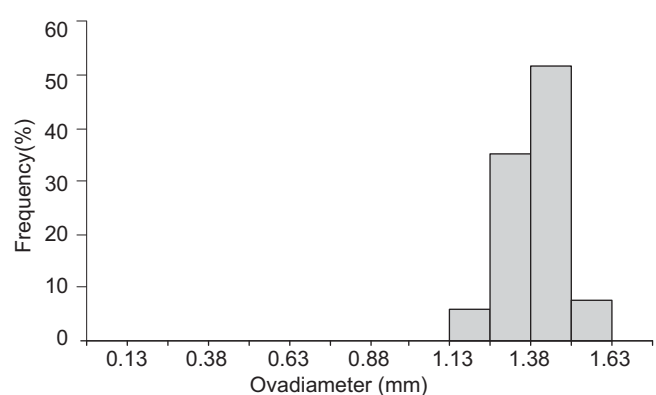
5.2 (b)



5.3 (a)



5.3 (b)

Fig 5. Critical life history parameters of *E.suratensis* and *H.brachysoma*.

5.1 Food composition—(a) *Etroplus suratensis* (b) *Horabagrus brachysoma*; 5.2 Gonadosomatic index—(a) *Etroplus suratensis* (b) *Horabagrus brachysoma*; 5.3 Distribution pattern of eggs in a mature ovary—(a) *Etroplus suratensis* (b) *Horabagrus brachysoma*

varied in number from 7 to 15 (Table 2). Both the couple participate in the process and males are more actively involved in this laborious job. The eggs hatch out in 70–72 hours and the hatchlings or 'wrigglers' are picked up by the brooding female in its mouth and transferred to the breeding pits. The parent fish actively engages in 'pit guarding', a behaviour characteristic to this species. During this period also, fanning and mouthing of the brood is continued. When the yolk attached to the hatchlings is fully utilized, in a week, the 'wrigglers' become free swimming and gradually move

out of the pits and swim freely in to the open waters escorted by the parents.

In the context that *E. suratensis* exhibited such a characteristic parental behaviour, to facilitate captive breeding, breeding situations were simulated in an artificial raceway system (70 m²). The raceway system consisted of a shallow cement tank with sloping floor that facilitated a mild flow of water and high transparency. The heavily yolked hatchlings were found to congregate instinctively in the artificial pits placed in the larval rearing tank. Around 900–

1200 eggs were produced per brood and over 95% survival was obtained in the larval rearing system.

Captive breeding of Golden catfish, Horabagrus brachysoma

The survey revealed that *H. brachysoma*, once abundant in these wetlands, constituted only 1 to 1.8% in the local fishery in the lake. Their incidence was more pronounced in river systems confluent to the Vembanad lake. The fish was observed to inhabit dark locations adjacent to granite side-pitching areas of the irrigation canals and weed infested shallow riverine locations, especially where submerged herbs of *Aponogeton* provide natural shelter habitats.

Studies on food and feeding revealed that the fish apparently feed on filamentous algae (39%) followed by detritus (23%), fish offal (22%), macrovegetation (8%), crustaceans (6%) and other items (2%) (Fig.5.1b). The fish attains maturity during the first year of age and males apparently mature earlier than females. Females exhibited a group synchronous ovarian development (Fig 5.3b). Absolute fecundity was found to vary between 1,140 and 1,23,968 (mean 20,472). The fecundity of *H. brachysoma* observed in the study was higher than that reported for most other catfishes, viz. *Wallago attu* (Das, 1994). *Clarias batrachus* (Rao *et al.* 1994), *C. macrocephalus*, (Saidin, 1986). Relatively high fecundity and omnivorous feeding behavior make this indigenous catfish, a potential candidate for culture.

Investigations on artificial breeding of *H. brachysoma* was undertaken for the first time, by the Regional Agricultural Research Station, Kumarakom as part of the National Agricultural Technology Project in collaboration with the National Bureau of Fish Genetic Resources, Lucknow. It was demonstrated that *H. brachysoma* can be brought to final maturation and ovulation by the administration of a single dose of Ovaprim @1ml.kg⁻¹ body weight or fish pituitary

extract @50–60 mg.kg⁻¹ body weight, the former being more effective. Captive breeding was carried out successfully using farm reared stocks (Padmakumar *et al.* 2004). The performance of captive breeding trials and breeding protocols are summarised in Table 3.

Historically, *H. brachysoma* was locally abundant in the rivers of central Kerala, but populations have declined drastically and the species is now restricted to sparse populations. The species is listed as endangered and is categorized as a species of special concern by the IUCN (CAMP, 1998; Shaji *et al.* 2000; Gopalakrishnan and Ponniah, 2000). Stocking of natural waters using hatchery raised seeds is an important and, in some cases, very successful tool for species conservation. There has been heavy demand for the seed of this species for aquaculture and the Regional Agricultural Research Station, Kumarakom is engaged in a program for mass production of seeds of *H. brachysoma* for aquaculture and open stocking. More recently, during 2007–08, the seeds produced under this project was used for stocking the Sasthamcottai lake, a Ramsar site in the Western Ghat region, by the State Department of Fisheries. With these efforts, the Golden catfish is on a comeback trail in the Vembanad system.

Among catfishes, males are generally not free milting. Males in most silurids are considered not amenable to stripping *eg. C. batrachus*, *Heteropneustes fossilis* (Pandian and Koteeswaran, 1998). Therefore, in *Clarias*, the males are sacrificed for collecting the milt since it is not possible to extract milt through stripping (Rao *et al.* 1994). *H. brachysoma* appears to be an exception, as mature males could be freely stripped, like *Pangasius hypophthalmus* (Legendre *et al.* 2000) and *Pangasius sutchi* (Chattopadhyay *et al.* 2002).

Asynchrony in maturity, with males becoming mature

Table 1. Captive breeding and nesting in *E. suratensis*

Nesting substrate	Nest area (cm ²)	Eggs (No.)	Egg density (no./cm ²)	Fertilisation (%)	Hatching (%)
Earthen pots	56	951	17	76	90
CC. substrate	32	420	13	100	63
Wooden poles	18	792	44	82	20
Wooden poles	37.8	1966	52	98	29
Hose tube	19	382	20	100	99
CC. substrate	18	633	35	95	63
CC. substrate	54.4	390	7	98	90
CC. substrate	27	1350	50	99	34
CC. substrate	28	477	17	96	65
Coconut-petiole	49.5	990	20	71	70
Wooden poles	20.3	1215	60	92	13
Granite block	22.5	260	12	100	48
Coconut leaf	43.7	1573	36	100	10
Fire brick	22.9	642	28	99	32
Coconut husk	28.26	1243	44	98	38
Hose tube	48.0	250	5	100	33

Table 2. Characteristics of pit nursing in *Etroplus suratensis*

Water column (cm)	No. of pits by a pair	Distance between pits (cm)	Diameter of pits (cm)	Depth of pits (cm)
18.9	10	10.43	7.24	5.0
22.0	13	7.5	6.21	4.0
23.0	11	5.82	8.5	4.3
24.4	15	3.91	6.0	6.0
25.0	9	5.42	5.79	5.0
25.8	9	10.33	4.33	3.67
26.2	14	7.10	5.0	4.0
27.6	15	5.13	5.63	4.8
27.8	7	6.08	4.5	4.04
33.2	13	5.44	5.7	3.7
33.8	13	6.63	4.5	3.76
37.7	15	5.56	3.8	3.32

earlier than female was observed in *H. brachysoma*. In the context of the observed asynchrony in final maturation in this species, the standardization of protocols for cryopreservation of milt of *H. brachysoma* (Gopalakrishnan *et al.* 2000) assumes significance. This also opens up possibilities for utilization of cryopreserved milt not only for germplasm conservation but also for artificial insemination. *H. brachysoma* was found to abound in weed infested slow flowing habitats, where high turbidity and low oxygen content prevailed. It appears that catfishes have a competitive advantage over many oxyphilic species, to survive in such environments. The low diversity of catfishes as pelagic communities, compared to littoral communities, is attributed to their ancient evolution of coping with hypoxic and turbid waters (Arratia *et al.* 2003), as rivers are old while lakes are much more recent. This also indicates their extreme resilience to a broad range of environmental condition.

In contrast, murky waters appeared to be inimical to cichlid, *E. suratensis* (Cole and Ward, 1969; Samarakoon, 1981). Apparently, decreased water turbidity favored visual displays for both feeding and breeding in *E. suratensis*. Visual stimuli favor and hasten ovulation in cichlids (Jalabert and Zohar, 1982) and being a visual feeder pearlspot prefer clear

water for breeding. This could be an adaptation that ensured better feed availability for the young ones (De Silva *et al.* 1984). As visual contact between the parents and the offspring is a critical requirement for spawning, the increased turbidity of water in the lake by siltation and high sedimentation during monsoon is one of the factors that limit their natural recruitment, a situation that gradually changes with the infiltration of saline water during summer.

With increasing pressure on inland fish biodiversity, a range of strategies are suggested for enhancement of natural populations of endangered fish stocks. One of the strategies for conservation of such species is stock enhancement by artificial stocking by developing breeding techniques in controlled conditions. Despite resolute utilization of captive breeding in species recovery for a variety of species in recent years, the limitations of this approach also cannot be overlooked. It is not an alternative to habitat and ecosystem protection and hence may be invoked most judiciously in the absence of other comprehensive efforts to maintain or restore populations in wild habitats. Merely demonstrating that a population of a particular species is declining or has fallen below what may be a minimum viable size does not constitute enough reasons to justify captive breeding as a

Table 3. Captive Breeding protocols of *Horabagrus brachysoma*

	Ovaprim*(N=78)		CPE**(N=19)	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
Fish body weight (male)(g)	200-534	372.57 $\pm$ 76.64	230-255	242.50 $\pm$ 17.68
Fish body weight (female) (g)	140-390	223.29 $\pm$ 64.05	280-320	300.00 $\pm$ 28.28
Hormone dose (ml)	0.14-0.39	0.22 $\pm$ 0.06	0.27-0.33	0.30 $\pm$ 0.04
Latency period (h)	8.0-16.0	13.19 $\pm$ 1.83	8.0-14.0	11.00 $\pm$ 4.24
Incubation time (h)	23.0-29.0	25.36 $\pm$ 1.74	22.0-25.0	23.50 $\pm$ 2.12
Fertilisation success (%)	80.0-100.0	97.05 $\pm$ 5.17	50.0-98.0	74.00 $\pm$ 33.94
Hatching success (%)	23.0-100	73.09 $\pm$ 30.56	6.0-10.0	8.00 $\pm$ 2.83
Water temperature ($^{\circ}$ C)	20.0-27.0	24.84 $\pm$ 1.99	25.0	25.0 $\pm$ 0.00
Water pH	6.5-7.8	6.87 $\pm$ 5.17	7.0-8.5	7.75 $\pm$ 1.06

* Ovaprim @ 1 ml.kg⁻¹ ; N - No. of trials; **Carp Pituitary Extract @ 50–60 mg.kg⁻¹



Figs 6–8. 6. Eggs on nesting substrate – *E.suratensis*. 7. Depositing breeding habitats/substrates in the fish sanctuary; 8. Scare line fishing.

recovery measure (Collares-Pereira and Cowx, 2004). This is principally due to unavoidable genetic and phenotypic changes that can occur in captive breeding. Apparently this means that ranching of seeds is not a panacea for all endangerment and should be invoked most judiciously in the absence of any other ways to maintain or restore natural populations. Nevertheless, captive breeding operated under carefully defined conditions of disease prevention and genetic management is the crucial method for recovery of species when effective alternatives are not available. All these highlight the dire need to develop simultaneously valid programs in biodiversity conservation through concerted public education, and *in situ* conservation efforts.

In the context that habitat management is the corner stone of species conservation, the unique breeding behavior of the species was utilized for the development of a 'fish sanctuary' for pearlspot in open waters in the Vembanad system (Fig.7). Investigations indicated that fish congregate in large numbers in this protected breeding ground and spontaneously utilize the deposited substrates for breeding. A perceptible increase in fish abundance and catches almost six fold (120 kg c.p.u.e) was evident for scare line fishing, *Vellavali* (Fig.8), near the sanctuary zone during the succeeding season (Padmakumar *et al.* 2002). Although, the major objective of the 'fish reserve' has been protection of a minimum spawning stock to ensure recruitment to fished areas, the spill over effects of the sanctuary by adult fish movement from fish reserve to the adjacent waters was reflected in increased fish yield in fishing zones close to the sanctuary. With the increased availability of fish, the fish sanctuary zone in Kumarakom has also become favored resting places for water birds. A perceptible increase of water bird counts, as high as 100 percent was reported from the sanctuary zone in Vembanad lake (VWBC, 2004). Apparently, these results indicate that habitat management resulted in positive benefits for other biota, as well. Indications are that such fish conservation efforts through habitat restoration can accrue wider environmental benefits than mere enhancement. Noble *et al.* (2004) has also demonstrated that rehabilitation of reed beds in United Kingdom was a major

factor that enhanced the conservation of the bitterns, *Botaurus stellaris*, an extremely rare fish eating bird.

Rehabilitation of endangered species by habitat preservation by establishing sanctuaries is a recognized method for conservation management. Partial rehabilitation of fish species in fish sanctuaries by dropping trunks of trees *etc.*, as convenient artificial habitats and spawning areas has been reported (Thuok, 1998). Use of spawning substrates installations and deployment of shelter devices for fish enhancement are rarely tried in river systems. Therefore, the present investigations on conservation management of indigenous fish species is a major advance towards development of a standardized mechanism for conservation of indigenous species. The foremost challenge haunting mankind in the new millennium is the unabated loss of biodiversity, caused by degradation and destruction of unique habitats. Restoration of aquatic habitats towards pristine condition is an utopian view (Cowx and Gerdeaux, 2004), as our river systems have already experienced extensive land use changes. Hence, a practical approach for rehabilitation of fisheries shall be to recreate functional habitats, with focus on reinstating lateral connectivity, besides improving flow regimes and water quality.

ACKNOWLEDGEMENT

We place on record our deep sense of gratitude to Shri. K.R.Viswambharan IAS., Hon. Vice Chancellor, Kerala Agricultural University for constant support and encouragement. We are grateful to Dr S. P. Singh, and Dr. A.G Ponnaiah, National Bureau of Fish Genetic Resources, Lucknow for support and guidance. We are also deeply indebted to Dr. D.Alexander, Director of Research, Kerala Agricultural University and Dr. Joseph Philip, Associate Director of Research, Regional Agricultural Research Station, Kumarakom for encouragement.

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