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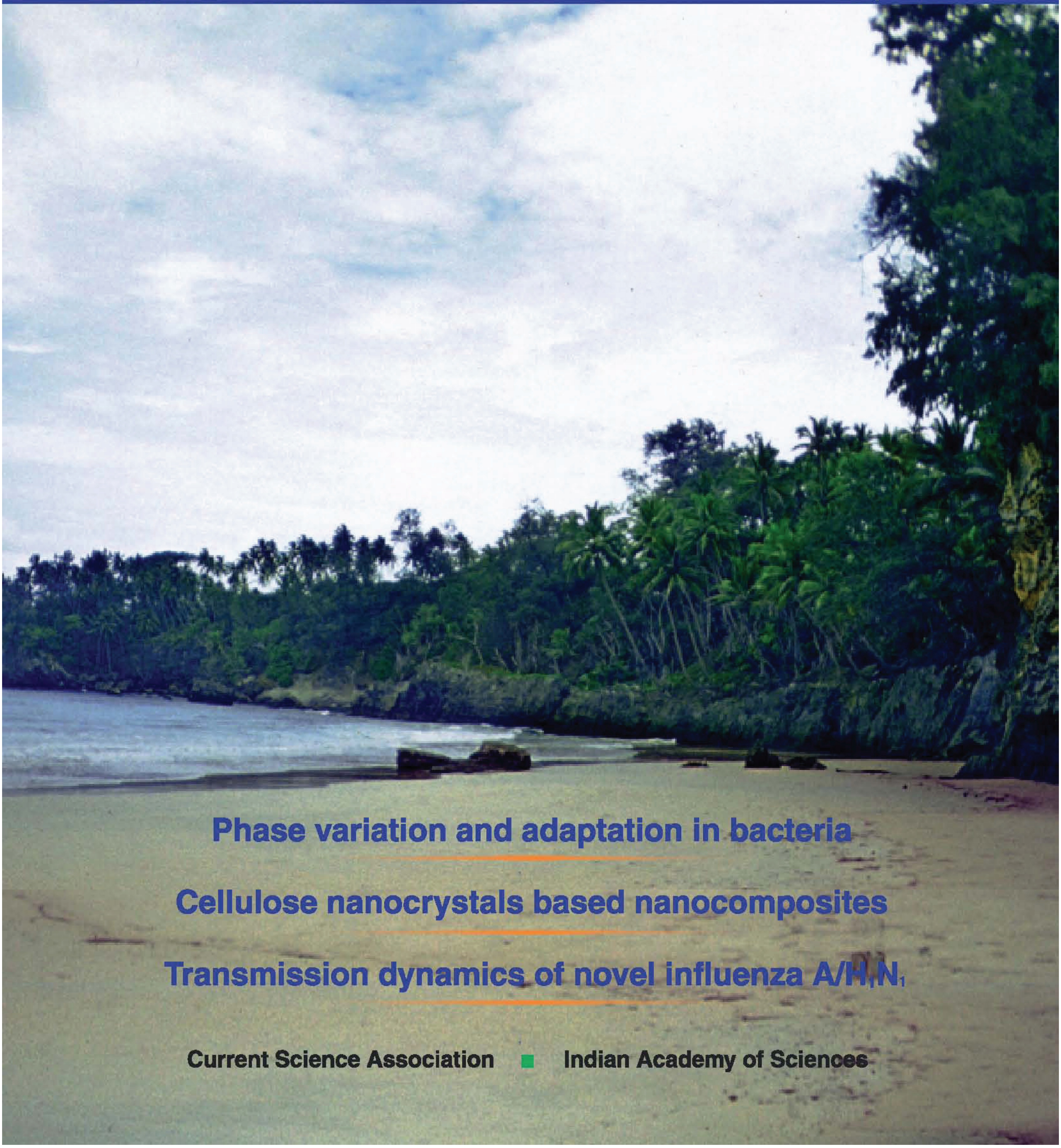
CURRENT SCIENCE

Volume 100 Number 8 25 April 2011



Phase variation and adaptation in bacteria

Cellulose nanocrystals based nanocomposites

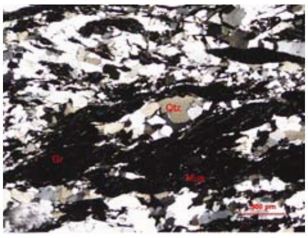
Transmission dynamics of novel influenza A/H_1N_1

Current Science Association ■ Indian Academy of Sciences

In this issue

Carbon isotopic evidence for the origin of Himalayan graphite

The carbon isotopic ratio ($^{13}C/^{12}C$) measured in natural graphite can discriminate in the origin of graphite occurring in diverse host rocks. This $\delta^{13}C$‰ of graphite can typically characterize the source of carbon involved in graphite formation as the graphite formed through the participation of biogenic carbon are enriched in ^{12}C, whereas the graphite formed from the abiogenic carbon comprises high ^{13}C. Sharma *et al.* (**page 1216**) present evidence for the origin of graphite found in Lesser Himalaya. The widespread graphite occur in Lesser and Higher Himalayan metasedimentary rocks particularly in the Kumaun Himalaya, wherein it is associated with the pelitic schists and gneisses. Small occurrences to sizable deposits of graphite are known to occur in



Precambrian crystalline rocks: Munsiari and its klippen such as the Almora Group of rocks. The carbon isotope data of the representative graphite from the Almora Group is presented in this study to delineate the origin of Lesser Himalayan graphite. The $\delta^{13}C$ values measured for studied graphite show enrichment of lighter carbon with $\delta^{13}C$ mean value as –29.08‰. These $\delta^{13}C$ values are, however, in narrow range and imply that the biogenic carbon participated in the formation of Lesser Himalayan graphite. The $\delta^{13}C$ values obtained for the graphite within the metasediments of Gumalikhet Formation are lower than those obtained for graphite from the Champawat Granodiorite Formation, close to the southern border of Almora nappe.

Golden catfish *Horabagrus brachysoma* on a come back trail

Captive breeding protocols of *Horabagrus brachysoma*, popularly known as golden catfish, endemic to the Western Ghat river systems, India were standardized and artificial breeding accomplished by hormonal manipulation, using ovaprim, an hormonal analogue of salmon gonadotropin releasing hormone (sGnRHa) and Domperidone. The fish, characterized by very high fecundity has been demonstrated to be amenable to artificial fertilization by stripping in 12–14 h after hormonal administration. This opened up opportunities for mass production of seeds of this species.

Being omnivorous, hardy and with high consumer preference and adapted to survive in oxygen-poor situations, *H. brachysoma* is an enormously potential food fish for farming and owing to its brilliant colouration, it is popular in the commercial ornamental fish trade as Asian sun catfish. The fish has been listed till recently as 'endangered' in river systems of the region. Artificial breeding technique has been of immense value in mass production of seeds for popularizing its farming as a preferred food fish and ornamental fish, and for open water stocking and species restoration.

Ranching of captive-bred young ones of the species, for conservation stocking has helped massive come back of the species in natural waters. This is a success story that demonstrates the value of captive breeding protocols not only for conservation and species restoration but also for commercial utilization. See **page 1232**.

Fighting adversity: bacterial style

Bacteria, especially pathogens, employ several strategies to counter life-threatening stresses. One of these is a process called phase variation which involves switching the expression of certain genes from the On state of expression to the Off state and vice versa. These genes are called 'contingency' genes and are characterized by some unique structural features and high frequencies of switching between the On and Off states. Generally, but not always, contingency genes are involved in the synthesis or modification of surface-associated cellular structures. The high frequencies of On/Off switching of their expression, several fold higher than spontaneous mutation frequencies, and presence of several contingency loci in a cell generate enormous population diversity in clonal populations of cells. Some contingency loci are not involved in the synthesis of surface-associated structures but are components of type I and type III restriction–modification (RM) systems. Phase variation in type III RM genes up or down regulate the expression of many unlinked genes, impacting on several properties of cells. This novel system of gene regulation has been called phase variable regulation or simply, phasevarion. In a review article appearing in this issue, Jayaraman discusses (**page 1163**) the organization of phase variable genes, the many mechanisms of phase variation and a related process called antigenic variation. How bacteria utilize phase variation to adapt to stressful life situations is discussed with examples.

Breeding of endemic catfish, *Horabagrus brachysoma* in captive conditions

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***Horabagrus brachysoma*, popularly known as golden catfish, is an endemic species in the Western Ghat rivers of peninsular India. Breeding of this catfish was successfully accomplished in captive condition. Induced fishes responded well and spawned naturally in 8–14 h and the fertilized eggs hatched in 22–29 h. Artificial fertilization by stripping was also successful when carried out within 1–2 h of the latency period after hormonal manipulation. Seed rearing was successfully accomplished in earthen ponds. This opened up opportunities for mass production of seeds of this species for restoration, stocking and conservation. Consequent to introduction of hatchery reared seeds produced by these techniques into natural waters, the species is now on a comeback trail.**

Keywords: *Horabagrus brachysoma*, incubation period, natural spawning, stripping.

WESTERN GHATS in peninsular India supports over 48% of the fish biodiversity in India[1]. This includes several rare and endangered fish species, many of which are potentially important either as cultivable food fish or ornamental fish species. Among the 617 freshwater fish species identified to exist in India, 210 are found in the Western Ghat river systems of Kerala, of which over 25% are exclusively endemic to the region[2]. Although, these river systems are projected as a 'goldmine' of fish biodiversity, only a small fraction of endemic fish diversity is utilized commercially.

In India, the three Indian major carps support about 80% of the total inland fish production[3]. Although many of the indigenous fishes have local taste preference and demand as food fishes, few of them have been incorporated into the culture systems. A wide array of anthropogenic disturbances have been implicated for the decline and extinction of many of these species[4]. With enormous publicity given to the ornamental value of several such fish species, many of them are removed deviously from these river systems for export. This poses a new threat to the native fauna, and even the last fish is being picked up for commercial exploitation from these river systems.

Horabagrus brachysoma, locally known as *manjakoori*, is an excellent table fish with high market demand and consumer preference. Their omnivorous feeding habit, high fecundity and nutraceutical properties make it a potential species for commercial aquaculture. Historically, *H. brachysoma* (Figure 1) was abundant in the rivers of central Kerala, but populations have declined drastically and the fish has became very rare and restricted to tributaries of Chalakkudy, Meenachil, Manimala and Pampa rivers opening to the Vembanad wetlands. A survey conducted during 2000–2001 along the riverine stretches of the Vembanad Lake[5], revealed that *H. brachysoma*, once abundant in these wetlands, constituted only 1.52% of the total riverine fishes in the lake.

The species has therefore been listed as 'endangered' and also considered as a species of special concern[2,6]. One of the proven techniques for saving endangered fish species from extinction is to increase its population size by developing scientific breeding techniques under controlled conditions and stocking of such artificially raised young fish into natural waters[7]. Recent attempts on development of captive breeding techniques for several endemic fish species of the Western Ghats river systems[4] has opened up opportunities for their restoration and conservation. The success achieved in the artificial breeding of golden catfish under controlled conditions is of enormous importance for restitution of the species in these river systems.

In this context, detailed studies were undertaken for fine tuning of captive breeding protocols and mass seed production of this species with reference to their biological attributes. The efficacy of different inducing agents for breeding them in captive conditions and the cues for spawning of the species with reference to critical life history parameters were also evaluated.

Artificial breeding and culture of the species is linked to important biological features, viz. food and feeding, sex ratio, gonadosomatic index (GSI), fecundity, oocyte size frequency profiles, etc. The sex ratio observed in the

Figure 1. The endemic golden catfish, *Horabagrus brachysoma.*

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commercial landings was found to be 1 male : 1.97 female[8]. These fishes were found to mature in the first year of age itself. The estimated mean size at first maturity (L_{50}) indicated that males mature at a smaller size (17.5 mm) than females (18.5 mm). Variations in GSI were also more pronounced during the breeding season, with a highest mean GSI of 6.6 ± 3.64 in male and 16.29 ± 7.89 in female. Females exhibited group synchronous ovarian development. The fish in nature spawn once a year and have a relatively very short breeding season. The absolute fecundity was found to vary between 1140 and 123,968 with a mean of 20,472 eggs. Egg diameter varied between 0.13 and 1.63 mm. Not only the GSI, the oocyte size frequency profile also indicated that *H. brachysoma* is a seasonal spawner, with peak breeding during June–July. The early maturity of *H. brachysoma* in the pond system at a size between 12.0 and 13.5 cm, also indicated the immense possibilities for breeding them using captive reared parental stocks.

As *H. brachysoma* do not breed spontaneously in confined situations, its spawning was facilitated under controlled conditions by hormonal treatment. Farm-reared broodstock was found to respond well to hormonal manipulation as compared to virgin wild spawners collected and utilized during the same season. A relatively protracted spawning season, from May to September, has been characteristic to *H. brachysoma*. Sexual dimorphism was most apparent among ripe fishes close to breeding season (Figure 2). Males were observed to attain maturity earlier than females. Ripe female possess a swollen abdomen and bright red vent from which eggs extrude under slight pressure. Males, have a streamlined body, look brighter and are generally smaller in size. In variance to most other catfishes, mature males exude milt freely at slight pressure.

Ripe fishes were collected from the farm pond and were subjected to induced ovulation by using varying doses of inducing agents, viz. carp pituitary extract (CPE) at 50–60 mg kg^{-1} body weight or Ovaprim® (a synthetic analogue of salmon gonadotropin releasing hormone (SGnRHa) 20 μg and 10 mg Domperidone in 1 ml) at 1 ml kg^{-1} body weight in single dose. Hormones were given intraperitoneally and males received half the dose of the females. Each breeding set comprised male and female fishes in the ratio of 1 : 1 by body weight or 2 : 1 by number. They were kept in 1.1 tonne fibre reinforced plastic (FRP) tanks for breeding. As the fish has the habit of hiding in dark corners, poly vinyl chloride (PVC) pipes of 30–40 cm were provided as hideouts inside the breeding tank and these shelter substrates were most effectively utilized by the spawners.

Shallow waters, with depth ranging from 20 to 30 cm, were found to be suitable for spawning *H. brachysoma*. Approximately in 3–4 h after injection, the males became more aggressive, chase the females and enter into sex play (Figure 3) resulting in spawning in 8–14.30 h. The spawning crescendo was found to be preceded by vibrant mating and courting behaviour. Female completed her egg expulsion in few minutes and spawning occur at water temperature between 23°C and 28.4°C. *H. brachysoma* is polygamous in nature and a single female is serviced by more than one male. Apparently, this behaviour helps the fish to maximize opportunities for mating with numerous individuals ensuring high genetic diversity.

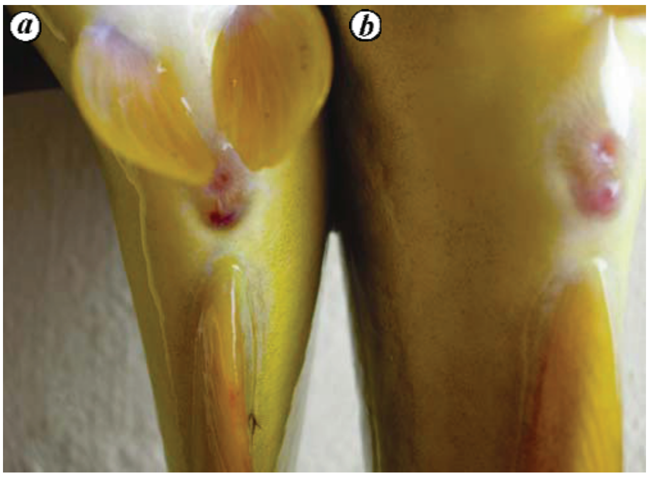

Figure 2. Sexual dimorphism in *H. brachysoma*. ***a***, Male; ***b***, Female.

Figure 3. Courting and mating in *H. brachysoma.*

Table 1. Response of *Horabagrus brachysoma* to hormonal manipulation (total number of trials, *N* = 178)

	Inducing agent	
	Ovaprim (*N* = 159)	CPE (*N* = 19)
Fertilization (%)	92.01	78.95
> 90%	74.10	60.00
70–90%	17.20	06.70
Hatching (%)	63.4	26.67
No. of females injected	190	26

Among the 178 breeding trials undertaken in the study, during 2000–2008, induced ovulation was successful in 92% of the trials using ovaprim and 79% with CPE (Table 1). Hatching percentage was also high in ovaprim-induced trials as compared to CPE. However, there was enormous variability in the latency response of females even at similar temperature regime and was observed to range from 8–14.30 h with ovaprim and 8–12 h with CPE.

Fertilized eggs of *H. brachysoma* are heavily yolked, translucent, spherical and golden yellow in colour. Unlike most other catfishes, the eggs are less adhesive but free and demersal. *H. brachysoma* is a broadcast spawner with high fecundity. The mean number of eggs expelled in natural spawning after hormonal manipulation was 89,756. Apparently, the realized fecundity is extremely high in *H. brachysoma*, comparable to that of major carps. The very high fecundity appears to compensate the lack of parental patronage among these fishes and helped to balance the loss of eggs and young ones in the hostile environmental conditions in rivers[9]. Egg incubation and hatching is better performed at shallow water conditions in FRP or glass aquarium tanks under continuous aeration.

Males of most silurids are not amenable to stripping[9,10] and in *Clarias* spp., the males are therefore sacrificed for collecting milt from the testis and the collected milt was utilized for artificial external fertilization[11]. However, in *H. brachysoma*, mature males were observed to be amenable to stripping, a characteristic observed very rarely among catfishes, viz. *Pangassius hypophthalmus*[12] and *P. sutchi*[13]. Stripping was observed to be most successful when done in less than 2–3 h on elapse of latency period. The correct timing of egg collection was found crucial for embryonic development and successful hatching. Induced females were found to ovulate in 12–14 h after injection and therefore the fish should be inspected at this time for readiness for ovulation and dry stripping shall be completed within 2–3 h, to achieve better success.

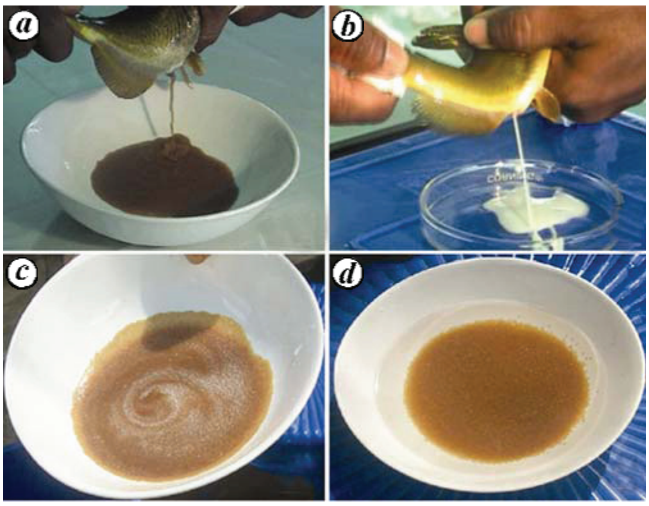

Figure 4. Dry stripping in *H. brachysoma*; ***a*** and ***b***, Collecting egg and milt after hormonal manipulation; ***c***, Artificial fertilization; ***d***, Fertilized eggs.

Hormonal manipulation followed by stripping (Figure 4) resulted in successful hatching only in 19% of the trials and hatching rate was also found to vary widely (10–95%). However, highest hatching rate was obtained in stripping preceded by administration of ovaprim. Unlike carps, *H. brachysoma* was found to be hardy and the stripped fishes were found to regain most rapidly. The stripped males also recoup its milting condition during the same season and could be used again for servicing fresh females. There were no perceptible variations in egg yield in natural spawning and stripping (Table 2). The relative weight of the stripped eggs ranged from 13.3% to 22.7% of the fish biomass. Highest GSI of female *H. brachysoma* at prime spawning season was 29.7 (ref. 8). It is therefore reasonable to infer that hormonal manipulation by administration of ovaprim followed by stripping will bring about a near complete ovulation and successful artificial fertilization in *H. brachysoma*.

The mean size of mature egg (1.4 ± 0.1 mm) is apparently one of the important determinants of egg quality and survival[14]. The diameter and quality of ova was found to be higher when pond-reared broodstocks were utilized for induced breeding. Embryonic development (Figure 5) in *H. brachysoma* is completed in 22–29 h at a temperature regime 24.99 ± 1.8°C and water pH 6.92 ± 0.57. Newly hatched hatchlings (4.13 ± 0.116 mm) subsist on nutrition from the stored yolk till 4th day of hatching and need exogenous feeding for further development. The young ones adapt fast to the new environment and the powdered yolk of boiled chick eggs is a highly relished artificial feed. The hatchlings can be gradually weaned to powdered commercial fish feeds. After a week, the fry attain a size of 6.75 ± 0.04 mm (Figure 6 *a*), and can be transferred to open nursery ‘hapas’ fixed in manured, plankton-rich earthen ponds for further rearing. The hapa-reared seeds (Figure 6 *b*) attain an average size of 6.5 ± 0.54 cm in three months and seeds raised in earthen ponds attain better growth than those in tank rearing.

Induced natural spawning through hormonal manipulation is the most useful approach for captive breeding of *H. brachysoma* and this is less stressful for the broodstock than stripping. The observed asynchrony in final maturation of male and female fishes in nature, even among same stock, and the short time tenure of retention of breeding condition, limits natural spawning and propagation in this species. This is serious impediment for natural recruitment, in open water systems where population size is too small. The milt cryopreservation technique developed for germplasm conservation of this

Table 2. Performance of *H. brachysoma* in natural and stripped spawning trials ($N = 24$)

	Natural breeding ($N = 12$)		Stripped breeding ($N = 12$)	
	Range	Mean ± SD	Range	Mean ± SD
Fish body weight (g)	110–500	248.33 ± 114.88	150–320	209.17 ± 52.99
Number of eggs/fish	4,248–72,500	22,345 ± 18,178	3,422–36,855	19,071 ± 10,893
Eggs/kg body weight	20,229–145,000	89,756 ± 49,766	21,388–140,952	88,697 ± 39,645
Latency period (h)	8.0–14.30	12.42 ± 1.79	13–18.15	16.31 ± 1.56
Fertilization rate (%)	3.0–98.4	74.97 ± 33.93	75–100	95.23 ± 7.33
Hatching rate (%)	0–98.0	35.15 ± 37.00	10–95.0	7.43 ± 22.78
Water temperature (°C)	23–27	24.48 ± 1.24	24–28.4	25.71 ± 1.47
Water pH	6.5–8.5	7.05 ± 0.65	6.0–8.5	7.26 ± 0.97

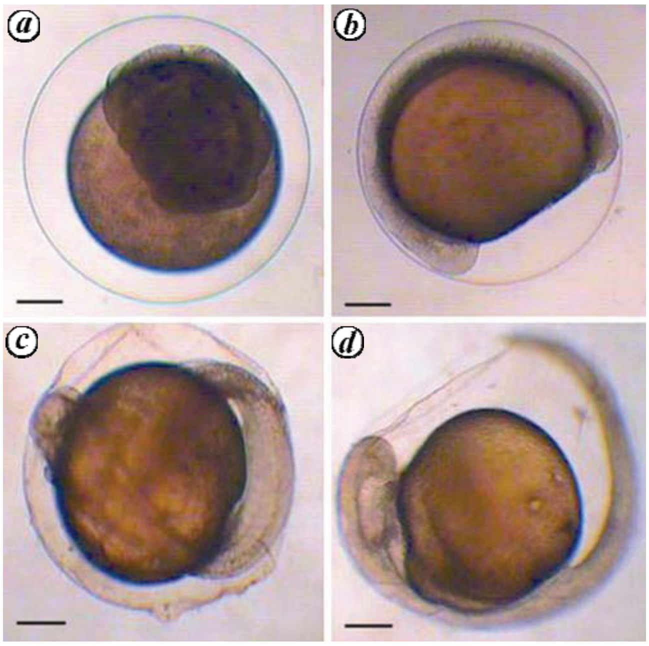

Figure 5. Early embryonic development in *H. brachysoma*. ***a***, 2 h; ***b***, 11 h; ***c***, 24 h – just before hatching; ***d***, Hatching (scale bar = 1 mm).

Figure 6. ***a***, Fry and ***b***, Fingerlings of *H. brachysoma*.

species[15] has opened tremendous possibilities for maintaining viability of sperm and for its use in artificial fertilization and propagation.

There is a growing demand for catfishes as food fishes and its commercial farming is expanding. Being omnivorous and hardy, adapted to survive even in oxygen poor situations and with high fecundity and high consumer preference, *H. brachysoma* is a highly potential candidate species for aquaculture in diverse environment. With the development of captive breeding, seeds of *H. brachysoma*, could be produced in large numbers and utilized for promoting aquaculture and open water stocking and the species is now on a comeback trail in natural waters. During 2007–2009, the seeds produced at the Regional Agricultural Research Station, Kumarakom were also utilized by the State Department of Fisheries for stocking the Sasthamcottai Lake, another Ramsar site on the Western Ghat region in India. With massive campaigns for ornamental fisheries development, there has been a heavy demand for seeds of *H. brachysoma*, popularly known as the Asian Sun Catfish in commercial trade. In the context that the species do not breed spontaneously in captivity, and natural seed availability is hardly reported, the breeding technique developed is of great value in popularizing its farming both as a high value food fish and ornamental fish.

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Received 22 September 2010; revised accepted 21 January 2011