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Hormone induced spawning and embryogenesis of Cauvery carp, *Barbodes carnaticus* (Jerdon 1849): Implications on commercial culture and conservation

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The Cauvery carp *Barbodes carnaticus* (syn. *Puntius carnaticus*), an endemic and threatened species of the Western Ghats, was once commercially important species in the Krishnaraja Sagar reservoir, Mysuru but later became scarce due to exploitation. In this study, we attempted conservation and rehabilitation of this species. We raised wild-caught juveniles of *Barbodes carnaticus* to gonadal maturity in ponds, where male and female took 2 and 3 years to reach first maturity. The females exhibited a higher gonado-somatic index (GSI) than males. Sexing was accomplished based on morphological characteristics and subsequently the fish was induced to ovulate/spawn with ready-to-inject spawning agents viz. ovatide, ovaprim and human chorionic gonadotropin (HCG). The order of performance was ovatide >ovaprim >HCG. The ovatide-injected female produced higher relative fecundity (1462 eggs/kg body wt.) than the females administered ovaprim (814 eggs/kg body wt.). The ripe oocytes had a diameter of 0.98 mm, and the water-hardened eggs were of 1.90 mm. The injected fish responded to hormones between15 and 30 h at 25.6-28.5ºC. The fertilization rates were generally high (50-100%). However, the hatch rates were low (0-27%). The quantity of yolk-sac fry obtained was higher in ovatide trial (336 fry/kg body wt.) than the ovaprim trial (293 fry/kg body wt.). The performance of HCG was much lower than that of ovatide and ovaprim. The early development of *B. carnaticus* from activation of egg to yolk-sac absorption stage was recorded using live eggs, embryos and early fry. Five embryonic steps (blastodisc formation, cleavage, epiboly, organogenesis and blood circulation) and free embryo consisting of five larval steps were identified. Hatching occurred between 37 and 48 h (post-activation), the free embryo period lasting up to 5 days post-hatching. The transition from larva to juvenile occurred in 25 days when the fish were fully scaled and the lateral line organ clearly visible, with a distinct black blotch on caudal peduncle. A few deformed eggs with asymmetrical cleavage and embryos with yolk-sac malformations, spinal cord and tail curvatures were observed. When the fry were reared in manured and fed pond, they showed faster growth, reaching fingerling stage (3.5 g; 7.5 cm) in 50 days, with more than 95% survival. Spermatozoa motility duration (13-60 s) and percentage (16-63%) were moderate, while the spermatozoa density and spermatocrit were high.

Keywords: Brood-stock, Induced breeding, *Puntius carnaticus*, Spermatozoa

Barbodes (*Barbus* or *Puntius*) *carnaticus* (Teleostei: Cyprinidae), commonly known as Carnatic or Cauvery carp, locally *koracha*/*gende*, is endemic to several rivers draining from the Western Ghats. This species has been recorded from the Cauvery basins (Karnataka and Tamil Nadu)[1,2]; Chalakudy and Puyankutty rivers (Kerala)[3,4] and most recently from the Wainganga river, a tributary of the Godavari in Maharashtra[5]. With a maximum recorded size of 60 cm (12 kg)[6], *B. carnaticus* is considered a valued sport fish, superficially resembling and frequently confused by recreational anglers as the highly prized mahseers (*Tor* spp.), and represents a valuable table fish, similar to major carps, such as rohu, *Labeo rohita* and catla, *Catla catla*. Despite some authors communicating a decline in wild stocks[6] and describing the species as rare[7], the IUCN Red List currently classifies *B. carnaticus* as Least Concern[8], yet acknowledges that population data on which to inform this assessment are currently limited. The IUCN assessment also acknowledges that *B. carnaticus* is threatened by a wide range of factors including destructive fishing practices (e.g. poisoning and dynamiting, pollution, competition with non-native species and the degradation of functional habitats due to flow manipulation and river fragmentation via dam construction)[9]. Based on the combined commercial value, endemism and perceived anthropogenic stressors which threaten *B. carnaticus*, it has been declared as the `State fish of Karnataka' by the

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Department of Fisheries of Karnataka Government. In spite of the significance of this fish, to date, little attention has been afforded to the collection of baseline data on basic biology, distribution, reproduction and farming potential. Of the limited information available on growth of *B. carnaticus*, Manojkumar[10], based on commercial landings, estimated the length at first maturity to be 232 and 270 mm in males and females, respectively. Compared to other cultured major carps, this initial fast growth rate and early maturity has led to the recognition that *B. carnaticus* may represent an excellent candidate species for aquaculture[4,11]. The results of an earlier study on the possibility of inducing this species to spawn in captivity were reported[13]. Subsequently, DNA bar coding of *B. carnaticus* was carried out and cytochrome oxidase sub unit I gene sequence was submitted to NCBI (Accession number: KM 926561.1).

In this study, wes assessed the feasibility of artificial propagation for *B. carnaticus* commercial production. Studies on early ontogenic development and spermatozoa characteristics, such as motility, density and spermatocrit form a significant contribution to the biological knowledge base of *B. carnaticus*. These initial findings are discussed within the context of species ecology, hatchery management and conservation.

Materials and Methods

Collection, transportation and acclimatization of fish

Following initial field surveys to scope the abundance and availability of *B. carnaticus* stocks in different water bodies of Karnataka, the juveniles for raising brood-stock were collected from the river Cauvery at Ramanathapura in Hassan District and Dubare in Kodagu District. About 150 specimens were collected using a shore seine (mesh size 4 mm) or drag net (100 ft; mesh size 4 mm) and immediately anaesthetized with quinaldine (20 ppm) for 2 min before being transferred to high density polyethylene bags (capacity 20 kg) containing clear water (⅓) and packed with pure oxygen (⅔). The bags were then supported in a rectangular plastic crate and transported to Mangalore over a distance of 200 km, by road. During the course of the 6 h journey, partial water was exchanged and the bags were refilled with oxygen. Upon arrival at the College of Fisheries, Mangalore, the wild-caught specimens were acclimatized in Fibreglass Reinforced Plastic (FRP) tanks (400 L or 1000 L capacity) filled with stored and disinfected water. Every alternate day, the fish were subject to a bath treatment of KMnO4 solution (5 ppm for 2 min) to minimize infection risk.

Captive brood-stock development

Rectangular concrete ponds (50 m^2) with a soil base (6-8″) or earthen pond (300 m^2) were used for raising the brood-stock. The ponds were partially filled with water and then limed with $CaCO_3$ (1.0 kg/50 m^2). After four days, the ponds were fertilized with chicken manure (2.0 kg/50 m^2). Approximately 30% of the total water volume was exchanged every fortnight and natural productivity was maintained with additional applications of manure (0.5-1.0 kg/50 m^2) every month.

Ten days after manuring, the fish (50-200 g; av. total body weight: 122.0 g and av. total length 20.40 cm; range: 12-25 cm) were transferred to the ponds and initially fed with a specially prepared pelleted diet comprising of locally available ingredients, such as fish meal (30%), ground nut oil cake (24%), rice bran (25%), wheat flour (10%), tapioca flour (10%) and vitamin and mineral mixture (1%). The pellets were crumbled and fed once a day at a proportionate rate of 3% of stock body weight, for 6 months. Later, a commercially available floating diet (protein: 26% and fat: 3%, pellet size 3-4 mm) was used to raise brood fish. Upon maturity, the sex of the brooders was determined based on morphological features.

Induced spawning

To encourage successful ovulation and spawning, two common synthetic spawning agents, ovatide (Hemmopharma, Mumbai) containing synthetic gonadotropin releasing hormone-analogue (GnRH-a) + domperidone, a dopamine antagonist and ovaprim (Syndel Laboratories, Hyderabad) containing salmon gonadotropin releasing hormone-analogue (sGnRH-a) + domperidone and HCG (procured locally) were compared. Female brooders were injected with either ovatide or ovaprim at 0.8 or 1.0 mL/kg, while males received either 0.25 or 0.3 mL/kg, as a single dose. The HCG was tested at 1000 or 2000IU/kg female and 400 or 800 IU/kg male. The control female brooders were injected with 0.8 mL of 0.85% saline, whereas males received 0.3 mL saline/kg body wt. The intramuscularly-injected female and male (at a sex ratio of 1:2 or 2:3) fish were released in circular tanks (diameter: 3 m, depth: 1 m) for ovulation. After 12 h, the female brooders were checked to ascertain their readiness for stripping and the ovulated eggs

were obtained by stripping at 6 h interval. Ova were fertilized with similarly-stripped sperms using the dry method. Details on breeding response, egg quantity, fertilization rate, incubation period, hatching rate and the quantity of larvae obtained were recorded. The GSI, absolute and relative fecundities and the diameter of ova were calculated. The absolute fecundity was estimated by multiplying the number of ova present in a sample of ovary with total weight of ovary and dividing with weight of sample. The relative fecundity was calculated by dividing absolute fecundity with total weight of fish.

Collection of spermatozoa

Spermatozoa were collected from mature healthy males (2 or 3 males) and pooled to estimate their motility, density and spermatocrit. Prior to collection of spermatozoa, males were sedated using quinaldine (20 ppm for 2 min) and the region around the genital opening was cleaned with filter paper/small cotton gauze to remove water, mucus, urine and faecal material. Spermatozoa were drawn into chilled 2.0 mL Eppendorff tubes for immediate examination.

Estimation of motility of spermatozoa

The quality of spermatozoa, pooled from 2-3 males, was assessed by placing a small drop of milt (5-10 µL), with a micropipette, on a clean glass slide, mixing with clean tap water and observing under pre-focused inverted microscope (X400, Olympus). Percentage motility of spermatozoa was determined arbitrarily on a 0 to 10 point scale; 0 denoting 0% motility and 10 denoting 100% motility. The duration of motility was estimated by recording the time taken from activation to the complete cessation of activity of the last spermatozoa in that field. Spermatozoa were counted using a Nebular haemocytometer and expressed as total number of spermatozoa per ml milt. To measure spermatocrit, melting point capillary tubes (4″ long and 2 mm bore) were filled with spermatozoa, sealed with parafilm and were centrifuged at 10000 rpm for 10 min. Spermatocrit was reported as the proportion of the solid packed spermatozoa and expressed as a percentage of total volume.

Early development

Eggs (pooled from two females) were incubated either in rectangular wooden trays, with nylon mesh at the bottom, immersed in a concrete tank, with running water or in rectangular glass aquaria, with aeration (vortex blower) at ambient water temperature of 26-27ºC.

Post-hatching

Early endogenous nutrition (for the first four days following yolk absorption), was provided as freshly hatched *Artemia* nauplii supplemented with sieved zooplankton. On day five (post yolk absorption), larvae were transferred to an outdoor nursery pond (10×5×1 m; earthen and stagnant, well water; temperature 27-30°C) which had previously been limed and fertilized with poultry manure (5 tons/ha). Larvae were subsequently fed with a commercial fry feed (protein: 40%) at 100-200% of fish body weight once daily for 20 days, after which 50% of fish was removed (for separate rearing) and the feeding rate was reduced to 50% of fish body weight for both the groups.

Embryonic and early larval development was recorded under a stereo microscope and photographed (Motic Image Plus version 2.0). To facilitate future field studies to characterize the early ecology of *B. carnaticus*, ontogenetic staging follows the theory of saltatory ontogeny[14,15], previously applied to describe the early development of other Cyprinids[17,18].

Results

Weight and length of parental fish

Data on total body weight and total length of *B. carnaticus* recorded during captive rearing is presented in Fig. 1. During acclimatization, the survival was 100%, notwithstanding the injuries caused during fishing and subsequent handling. During the 31-month-rearing period, mean individual weight increased from 122.0 g to 1170.0 g and mean total length from 22.24 cm to 42.11 cm, when fed on prepared diets. The maximum individual weight of male and female attained during the period was 1160 and 2340 g. About 30% of females attained first sexual maturity in the beginning of third year in captivity, with males maturing at the end of second year. The size at first maturity is 232 and 270 mm in male and female, respectively.

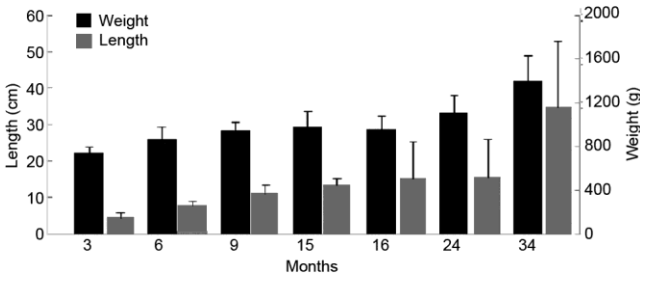

Fig. 1—Length (cm) and weight (g) of *B. carnaticus* recorded during grow-out period (mean ± SD, n = 10)

Identification of sex of brooders

Sex differentiation of brood stock was based on well-defined secondary sexual characteristics such as the swollen abdomen of females and papillomata's growth (white tubercles) on upper snout of males, which also freely discharged milt when pressure was applied to the abdomen.

Induced spawning

The results of the trials on the induced spawning are presented in Table 1. In the ovatide trial, four out of six injected females responded to the hormone injection, yielding relative fecundity varying between 1023 and 1,901 eggs/kg body wt. (Table 1). Of the two dosages tested, 0.8 mL yielded better results than 1.0 mL in terms of fecundity, breeding response, fertilization rate, hatch rate and fry survival (Table 1). However, the latter dosage resulted in a shorter ovulation period which facilitated three successful strippings from a single female. Five out of eight females injected with ovaprim (0.8 or 1.0 mL) also ovulated in one batch and produced a sum of 4300 eggs, with relative fecundity ranging between 694 and 935 eggs/kg body wt. (Table 1). The total quantity of yolk-sac fry (24 hah) produced was better with ovaprim (1,621 fry) than with ovatide (1549 fry). However, the number of fry obtained with ovatide was higher (336 fry/kg body wt.) than the quantity got from ovaprim (293 fry/kg body wt.) (Table 1). In the HCG trial, both dosages (1000 and 2000 IU) induced ovulation (partial), leading to the production of 450 and 319 eggs/kg body wt., respectively; the fertilization rate (65-88%), hatch rate (8.3-11.7%) and fry realisation (25-36) were also lower (Table 1). The maximum fertilization rate (100%) was observed in fish injected with ovatide or ovaprim, while the minimum fertilization rate (88%) was met with in HCG-injected fish. Ovatide and ovaprim treatment also lead to a higher hatch rate than that of HCG. All of the saline injected females in the control groups failed to ovulate 24 h after injection. A small proportion of deformed eggs and embryos were observed in all the trials.

The GSI of male and female was estimated to be 6.47±1.68 and 7.67±2.04, respectively. Absolute fecundity ranged between 30000 and 49200 ova/female, while relative fecundity ranged between 18750 and 37846 ova/kg body wt. The average diameter of oocyte was 0.98±0.31 mm, while that of the water-hardened developing egg was 1.9±0.25 mm.

Table 1—Breeding response, fertilization rate, hatching rate and number of fry obtained when injected with ovatide, ovaprim and HCG

Spawning agent	Ovatide		Ovaprim		HCG	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
No. and wt. of female brooder (g)	2/1260,2340	2/ 950, 1030	2/1260,2340	3/480, 570, 875	2/480, 520	2/420, 600
No. and wt. of male brooder (g)	3/520,1160,380	3/240,350, 420	3/520,1160,680	3/240, 460, 520	3/240, 450, 390	3/300, 410, 440
Age of brooder	2-3^{+}yr	2-3^{+}yr	2-3^{+}yr	2-3^{+}yr	2-3^{+}yr	2-3^{+}yr
Dosage : Female and Male	F: 0.8 mL/kg M: 0.25 mL/kg	F: 1.0 mL/kg M:0.3 mL/kg	F: 0.8 mL/kg M: 0.25 mL/kg	F: 1.0 mL/kg M:0.3 mL/kg	F:1000 IU /kg M:400 IU/kg	F:2000 IU/kg M: 800 IU/kg
Interval between injection and 1st stripping (h)	24	15 6* 18**	24	30	15	15
No. of eggs obtained	3681	1500, 400, 20	2500	1800	450	367
Breeding response (%)	Complete	Pr	Pr	Pr	Pr	Pr
Fertilization rate (%)	100	88, 50, 25	100	88	88	65
Hatching rate (%)	27	20.7, 12.5, 0	25.6	19.8	11.7	8.3
Hatching period (h)	38-48	41, 47, 0	42-46	41-47	41	43
Quantity of larvae obtained on 3rd day	1289	210, 50, 0	924	697	36	25
Temperature	Air:-33.5ºC Water: 28.5ºC	Air: 27.2ºC Water:26.5ºC	Air:-33.5ºC Water:28.5ºC	Air: 27.2ºC Water:26.5ºC	Air: 27.2ºC Water:26.5ºC	Air:27.2ºC Water:26.5ºC
Remarks	Free flow of eggs, free flow of milt; deformed fry: 250	No free flow of eggs; free flow of milt; deformed fry: 25	Slight flow of eggs, free flow of milt; deformed fry: 125	Slight flow of eggs; free flow of milt; deformed fry: 91	No free flow of eggs; free flow of milt; little flow of blood; deformed fry: 7	No free flow of eggs; free flow of milt; little flow of blood; deformed fry: 9

[Pr : Partial response, F : Female, M : Male, *Interval between 1st and 2nd stripping : 6 h; **Interval between 2nd and 3rd stripping : 18 h]

Early development

Hatching period

From activation to hatching, water temperature ranged between 25.6 and 28.5ºC. The time of activation to hatching lasted between 37 and 48 h and consisted of five steps: E1, activation; E2, cleavage; E3, epiboly; E4, organogenesis; and E5, onset of blood circulation (Fig. 2).

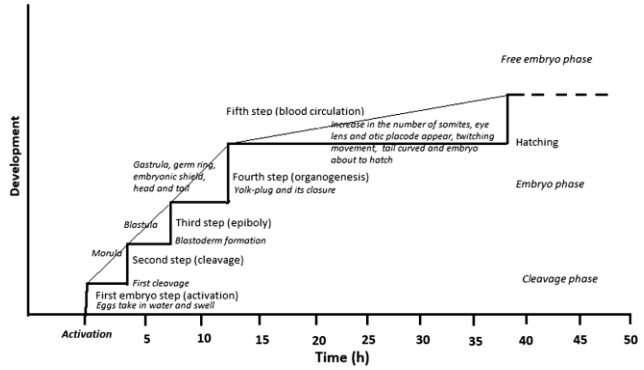

Fig. 2—Embryonic development model for *B. carnaticus* (average incubation temperature was 27.50ºC)

Cleavage phase

Step E1, activation began at 0 h after fertilisation (haf) (Fig. 3A and Table 2) and lasted for a period of 60 min. During this step the perivitelline space was formed and the orange coloured, spherical eggs swelled to a diameter of 1.89-2.0 mm, with the diameter of the yolk measuring 0.8-1.0 mm. The end of E1 was marked by the development of a blastodisc at the animal pole (Fig. 3B).

Step E2, cleavage started at 1.5 haf with the first division of the blastodisc and lasted for a duration of seven hours (Figs. 3 C-I). At 3½ haf a small-celled morula was visible (Fig. 3G). The increase in

Table 2—Stages of egg and embryonic development and their duration

Developmental stage	Development time (h:min)	Description
Fertilized egg	< 0:30	Zygote with a germinal disc (blastodisc) at animal pole (dorsal view); moderate perivitelline space (ps); egg diameter is 1.89 – 2.0 mm
Blastodisc formation	1:00	A prominent blastodisc (lateral view) formed after the migration of yolk-free cytoplasm to the animal pole ; divides to form two cells
8-cell	1:30	Third cleavage occurs after successive first and second cleavages; eight cells (blastomeres) formed in a 4 x 2 array of cells
16-cell	2:00	Fourth cleavage; 16 cells formed in an array of 4 x 4 blastomeres at right angles
32-cell	2:30	Fifth cleavage; 32 cells formed in an array of 8 x 4 in a perpendicular fashion to the previous stage
64/126-cell	3:00	Sixth/seventh cleavage; 64/128 cells layer formed; cells in this stage are smaller; the cell mound looks distinctly and for the first time some of the blastomeres completely cover other ones forming a second layer over the first one
Early morula	3:30	Several tiers of blastomeres formed; blastomeres rise high above the yolk
Mid morula	4:00	Further increase in the number of blastomeres; blastodermal region protrudes towards chorion and begins to spread over the yolk; successive cleavages lead to dome formation; less than a quarter of the yolk being invaded by the germ layer
Late morula	5:00	Further increase in the number of blastomeres; blastodermal layer spreads further on the yolk and becomes thickened and looks like a sphere; about ⅓ of the yolk being invaded by the germ layer
Early blastula	8:00	Epiboly continues and early germ ring appears; the blastoderm becomes flat
Late blastula	10:00	Epiboly continues and the germ ring increases in size; ½ of the yolk is invaded by germ layer
Gastrula	11:00	Epiboly continues and the germ ring increases in size; ¾ of the yolk is invaded by germ layer; the convergence movements continue rapidly, accumulation of cells takes place near the germ ring and over the yolk giving rise to the embryonic shield
Yolk-plug	12:00	At about 90% epiboly, yolk plug is generated by the uncovered yolk protruding near the vicinity of the vegetal pole and is characterised by the complete invasion of yolk by germ layer, excepting a pore known as blastopore; embryonic axis visible
Blastopore closure	12:30	The blastoderm covers most portion of the yolk, including the blastopore; the near end of the blastopore becomes the tail bud, while the end away from the blastopore becomes the head of the embryo; after the yolk plug closure, a tail bud appears
Embryo indication	14:00	The head and tail get differentiated; the notochord rudiment appears on the central axis on the dorsal side of the yolk; body segmentation starts; somites appear; the eye primordium and the Kupffer's vesicle appear
Pea-shaped embryo	18:00	Increase in the number of somites, eye lens and otic placode appear and the embryo assumes a 'pea shape'
Bean-shaped embryo	19:00	Further increase in the number of somites; the optic vesicles larger and the embryo assumes a 'bean shape'; perivitelline space (ps) is reduced very much
Comma-shaped embryo	20:00	The anterior part of the yolk is broad, while the posterior part is narrow; the tail elongates and the embryo assumes a 'comma shape'; there is a further increase in the number of somites and myotomes; mild twitching movement starts
Advanced embryo	28:00	There is a further increase in the number of somites; moderate twitching movement; optic vesicles more clear; tail elongates further and the embryo shows a prominent yolk sacs; no ps
Embryo just before hatching	37:00	Vigorous twitching movement; tail elongates further; the embryo occupies the entire space of the capsule; the tail curved, reaching up to the head; embryo about to hatch

blastomere number and progressive reduction in size of individual blastomeres continued until 8 haf.

Step E3, epiboly (Fig. 3J) began at 8 haf with the onset of the cell layer migrating across the yolk surface, and the formation of the germ ring (Fig. 3J). After another 2 h, the germ ring had increased in size to cover more than half of the yolk (Fig. 3K). Epiboly progressed until eventual closer of the blastopore at 12 haf (Fig. 3N).

Embryo phase

Step E4, organogenesis (Figs. 3O-T; Table 2) began at 12 haf with the development of the neural plate and cephalization of the embryo (Fig. 3O). This step continued further until hatching commenced at 37 haf. At 14 haf, the head and body became clearly differentiated, which was accompanied by the onset of myomere formation and the appearance of the eye primordium and the Kupffer's vesicle (Fig. 3O). At 18 haf, embryos became pear-shaped which was further characterized by an increase in the number of myomeres and the appearance of eye lens and otic placode (Fig. 3P). At 19 haf the number of myomeres and the size of the optic vesicles further increased (Fig. 3Q).

E5, onset of blood circulation, this step was signified by the onset of primary blood circulation accompanied by first muscular contractions at 20 haf (Fig. 3R). By 28 haf, moderate twitching movement was observed, the optic vesicles became more clearly visible, the caudal section became further elongated and embryos exhibited a prominent yolk sac with little space remaining (Fig. 3S). Over the course of the remaining nine hours until hatching which commenced at 37 haf, signifying the onset of the free embryo phase (Fig. 4A).

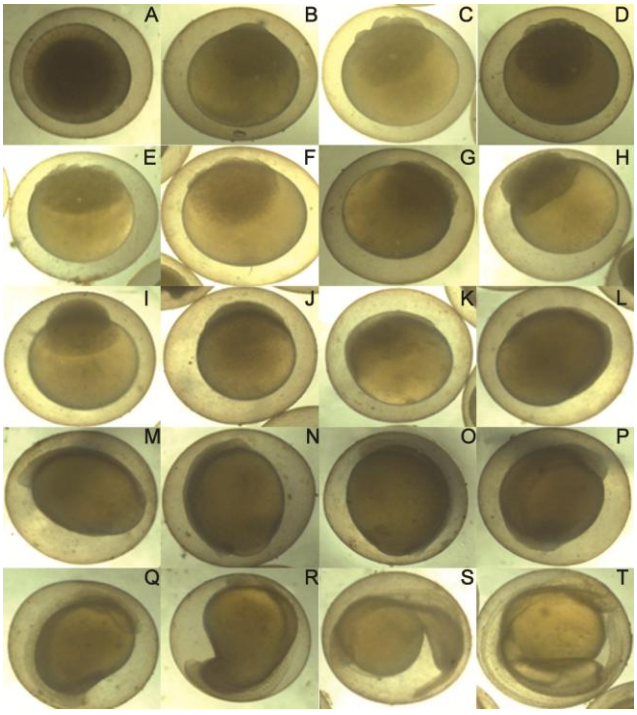

Fig. 3—Egg and embryonic stages of *B. carnaticus*. (A) fertilized egg with a blastodisc, dorsal view (<½ haf); (B) blastodisc formation, lateral view (1 haf); (C) 8-cell (blastomere) stage (1½ haf); (D) 16-cell stage (2 haf); (E) 32-cell stage (2 ½ haf); (F) 64/128-cell stage (3 haf); (G) early morula (3½ haf); (H) mid-morula (4 haf); (I) late morula (5 haf); (J) early blastula (8 haf); (K) late blastula (10 haf); (L) gastrula (11 haf); (M) yolk plug (12 haf); (N) blastopore closure (12½ haf); (O) embryo indication (14 haf); (P) pea-shaped embryo (18 haf); (Q) bean-shaped embryo (19 haf); (R) comma-shaped embryo (20 haf); (S) advanced (28 haf); and (T) embryo about to hatch (37 haf).

Post-hatching development

Free embryo phase

Step E6, free embryo (Fig. 4A; Table 3) began with hatching and ended with the onset of exogenous feeding. With hatching occurring between 37 and 48 haf, free embryos lacked pigmentation, had a total myomere count of 40-42, a slightly curved body typified by cyprinids and measured 5-6 mm total length (TL). Initially, free embryos exhibited little movement, were negatively phototactic and congregated in the corners of tanks with a slight movement (Fig. 4B). Four hours after hatching (hah) the morphology of the yolk sac began to elongate (Fig. 4C) with eyes becoming pigmented and pectoral fin buds, heart and otic placode becoming clearly visible, with the head being straight at 18 hah (Fig. 4E). Both external and internal melanophores were evident from the head to the tail, including the yolk-sac, after 24 h when the yolk had become evenly distributed. By the end of the free embryo phase, gill rudiments were also evident, with body pigment becoming more evident (Fig. 4F).

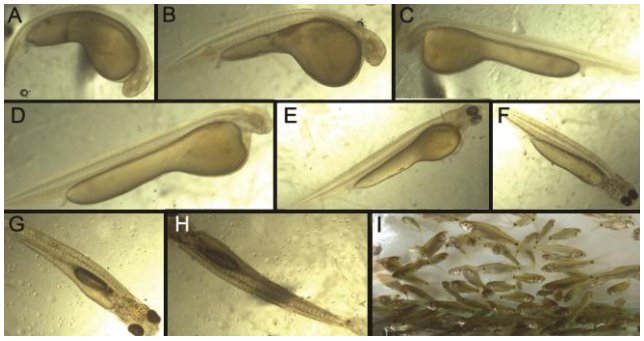

Fig. 4—Early larval stages. (A) newly hatched larva; (B) yolk-sac fry - 2 hah; (C) yolk-sac fry - 4 hah; (D) yolk-sac fry - 6 hah; (E) yolk-sac fry, 18 hah; (F) yolk-sac fry - 24 hah; (G) swim-up fry (head region) - 5 dah; (H) swim-up fry (tail region) - 5 dah; and (I) fingerlings - 25 dah.

Table 3—Morphological characteristics of early fry

Developmental stage	Development time (hours after hatching, hah)	Description
Newly hatched larva	0:00	The newly hatched larva has a slightly curved body with a tubular yolk-sac (ys); eye lens, notochord and somites (40-42) are prominent; the larva seldom moves quietly. The freshly hatched larva is 5-6 mm in length
Yolk-sac fry	2:00	The has a slightly curved body with a bulbous yolk-sac; optic vesicles, notochord and somites are more prominent; the larva shows forward movement; otic placard visible
Yolk-sac fry	4:00	Yolk sac is further reduced but elongated posteriorly; notochord is straight; no melanophores seen
Yolk-sac fry	6:00	Further reduction in yolk sac; melanophores absent
Yolk-sac fry	18:00	Yolk is broader in the anterior region while it is narrower in the posterior region; eyes are black; heart and pectoral fin bud are visible
Yolk-sac fry	24:00	Yolk is tapering towards the tail, eyes prominent, melanophores appear on the body and on yolk; heart is visible
Swim-up fry (head region)	5 dah	Yolk is almost absorbed; eyes prominent, melanophores appear on the body and the head; air bladder appears; gill rudiments appear
Swim-up fry (tail region)	5 dah	The caudal region extends with the presence of fin rays on the caudal fin; the density of melanophores is more on the head; air bladder present
Fingerling	25 dah	25-day-old fingerlings with a prominent black spot on the caudal peduncle, resembling a subadult

[Dah, days after hatching]

Table 4—Variation in spermatozoa motility, spermatozoa density and spermatocrit during Dec. '10 – Oct. '12 (Mean ± SE, n =3)

Month	Spermatozoa motility		Spermatozoa density (no./mL)	Spermatocrit (%)
	Motility duration (s)	Motility (%)		
Dec 2010	28.33±2.0	46.66±2.80	$7.18\times10^{7}\pm8.85\times10^{6}$	62.59±1.27
Feb 2011	13.3±5.80	36.66±3.90	$7.23\times10^{7}\pm9.28\times10^{6}$	72.09±1.50
April 2011	15.2±2.80	16.28±2.10	$8.18\times10^{7}\pm9.95\times10^{6}$	65.83±2.10
June 2011	20.3±2.70	17.31±3.50	$6.15\times10^{7}\pm3.32\times10^{6}$	60.37±1.89
Aug 2011	18.7±3.20	20.52±2.98	$7.18\times10^{7}\pm5.28\times10^{6}$	68.085±2.01
Oct 2011	20.5±2.31	25.28±5.28	$7.15\times10^{7}\pm2.22\times10^{6}$	70.0±1.20
Dec 2011	36.66±2.91	50.18±2.36	$8.13\times10^{7}\pm3.35\times10^{6}$	71.42±1.81
Feb 2012	25.31±2.18	63.33±1.89	$7.59\times10^{7}\pm4.28\times10^{6}$	66.66±1.23
April 2012	40.28±3.21	20.1±3.28	$4.83\times10^{7}\pm1.73\times10^{6}$	72.72±1.20
June 2012	60.2±3.21	35.0±2.90	$5.83\times10^{7}\pm2.18\times10^{6}$	76.74±1.38
Aug 2012	40±2.18	30.12±1.80	$5.92\times10^{7}\pm1.73\times10^{6}$	70.37±2.10
Oct 2012	60±1.80	45.0±2.90	$6.64\times10^{7}\pm1.82\times10^{6}$	67.53±2.12

Larva Period

The onset of L1 step (Figs. 4 G and H) was characterised by the onset of exogenous feeding and ended with the onset of flexion of the urostyle. The first free embryos reached this step on the fifth day after hatching (dah), when larvae measured 10 mm TL, the yolk sac was almost absorbed, the swim bladder (single chamber) inflated and the mouth and gut structure began to function. Fry from all the trials were reared in manured and fed ponds and reached 0.3 g (3.2 cm) in 25 d and 3.5 g (7.5 cm) in 50 d, with a survival rate of 95 and 100%, respectively. The fingerlings displayed a prominent black spot on the caudal peduncle (Fig. 4I).

Variation in spermatozoa characteristics

Spermatozoa characteristics were recorded at two-month intervals, from December 2010 to October 2012 (Table 4). Higher spermatozoa motility duration (40-60 s) was observed in the months of April-October 2012, while low vales were found during February-April 2011. While the highest motility (63%) was recorded in February 2012, the lowest (17%) was reported in June 2011 (Table 3). Spermatozoa density exhibited little variation between the months. Maximum and minimum spermatozoa densities of 8.18×10^{7} and 4.83×10^{7}no./mL were found in the month of April 2012 and April 2011, respectively (Table 3). Similarly, the spermatocrit values demonstrated limited temporal variation, with maximum and minimum values being recorded in the month of June 2012 and June 2011 (Table 4).

Discussion

Prior to the introduction of various major carps from North India, Peninsular India once harboured valuable fishery resources in the form of the medium

and minor carps; many of which are indigenous to the Cauvery and the Krishna rivers and their tributaries[20]. These fishes formed a major proportion of the harvest fishery of Karnataka, supporting the livelihood of millions of people due to their eating qualities and nutritional value. Within the Krishnarajasagar Reservoir fishery, where the Cauvery has been dammed near Mysore, *B. carnaticus* once formed a significant fishery (10% of the total catch), during the 1970s, yet now accounts for less than 1%, with the mean weight of individual fish also dramatically reduced from 6-8 kg to less than 0.5 kg[20]. Due to a range and in-combination effects of anthropogenic activity, many other indigenous and endemic species of the Cauvery basin have been dramatically depleted, including the iconic hump-backed mahseer which has historically attracted catch and release anglers from around the globe[21,22]. Despite their precarious conservation status and commercial culture potential, the indigenous carps which come under the genera *Puntius/Hypselobarbus*, *Labeo* and *Cirrhinus*, have remained unutilized and largely overlooked due to insufficient information regarding their biology and ecology. Several life history traits of *B. carnaticus* such as growth rate, maturity, length-weight relationship, condition factor, sex ratio and GSI were described and subsequently was suggested to be promoted as an aquaculture species[4,23].

Despite limited replication of treatments during the present study, the results qualify the culture potential of *B. carnaticus* and indicate the elevated efficacy of ovatide and ovaprim over HCG in stimulating ovulation. Small number of brooders employed in our study is attributed to difficulty in getting more female fish to full maturity due to prevailing higher temperature at this coastal place as against lower temperatures of its natural habitat (hilly area), where it is known to mature fairly easily. In spite of this constraint, the egg volume and fry quantity recorded in our study are comparable with those obtained for mahseers which share similar habitat[24,25].

Manojkumar[10] observed first maturity at the end of first year at a length of 232 mm in male and 270 mm in female *B. carnaticus* obtained from commercial catches. In the present study, commercial extruded feed successfully induced gonadal maturity in males during their second year and females in their third year, the same may be attributed to captive environment, more of prepared diet and handling stress that induce the fish (female in particular) to attain maturity more slowly in captivity. Also, the females attained higher weight than males, which is in agreement with that reported earlier[23]. The average GSI of both sexes constituted 5-9% of body wt., which is slightly less than that of golden mahseer (range: 5.6-10.8)[27], but lower than that of a major/medium Indian carp, *Labeo calbasu* (range: 18.8-20.7)[28].

Male *B. carnaticus* were observed to remain mature over a period of February to August with highest spermatozoa motility, density and spermatocrit recorded during June-August. This aligns with the observations from commercial catches[10] and suggests that the peak of natural spawning activity may be synchronised with the onset of the monsoon, thus guiding future ecological investigations to assess the importance of catchment connectivity to facilitate upstream and lateral migrations into side streams and tributaries[5].

Knowledge of the early development of vulnerable species is considered fundamental for the development of effective and robust conservation policies[19,29]. The embryological development of *B. carnaticus* revealed sequencing typical of other cyprinids[17,18]. The size of water-hardened eggs is known to influence the size of new-born larva[31] with potential to influence recruitment success[32]. The observed diameter of *B. carnaticus* eggs was consistent with those of mahseer *Tor khudree*. At~2 mm, *B. carnaticus* eggs are more than double the size of other related species such as *Puntius sarana*, which were observed to increase from 0.65 to 0.70 mm post-fertilisation[33], while the eggs of *Labeo bata* ranged between 0.7 and 0.8 mm[34]. At the other end of the spectrum, Chakraborty and Murty[35] observed the eggs of rohu, *L. rohita* (a major Indian carp) to range between 4.1 and 4.8 mm in diameter.

To date the spawning ecology of *B. carnaticus* has not been described. However, the post hatching ontogenetic sequencing described during this study provides some insight into the likely selection of spawning substrate and habitat utilisation of newly hatched free embryos. Specifically, the lack of eye pigmentation until 6 h post-hatching is consistent with some other cyprinids of the lithophilic spawning guild[36], such as European barbell *Barbus barbus*[17,19]. The lack of otic pigment and immediate function upon hatching typically corresponds with fishes hatching in areas of elevated flows. Exhibiting early photophobia, free embryos remain within interstitial gravels where yolk reserves allow development to proceed until late

free embryos/early larva are morphologically better equipped to survive within their immediate surrounding environment. These observations are in conformity with those recorded in the Deccan mahseer fry that shows similar morphogenesis[26]. Although this assumption requires validation, such observations usefully contribute to guiding future field studies to determine the selection of both spawning and early nursery habitats.

The growth and survival of the Carnatic carp fry during nursery rearing were moderate. The growth rate obtained was comparable with that reported for *Tor khudree*[37] and olive barb *Puntius sarana sarana*[38]. The fry and fingerlings were found to be very active and readily accepted artificial feed throughout the rearing period.

In spite of several *in situ* conservation strategies like declaration of river sanctuaries, protection of fish in temple pools, imposing fine for illegal fishing activities and confiscating fishing nets[39], natural stocks of *B. carnaticus* have continued to decline in response to growing anthropogenic pressures. The results of the present study indicate that *ex situ* conservation involving production of fry in hatcheries, ranching in streams and rivers and gamete preservation, represent feasible and valuable strategies to assist species conservation efforts and halt the further population declines of *B. carnaticus* in the wild. The efficiency of production will be further enhanced through controlled experimental design to determine optimal hormone dosing and the nutritional composition of artificial feeds.

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References

1 Dahanukar NR, Raghavan R, Ali A, Abraham & Shaji CP, The status and distribution of freshwater fishes of the Western Ghats. In: *The Status and Distribution of Freshwater Biodiversity in The Western Ghats, India*, (Ed. S Molur, KG Smith, BA Daniel & WRT Darwall; Chapter 3, 21, Cambridge, UK and IUCN, Gland, Switzerland and Zoo Outreach Organisation, Coimbatore, India), 2011, 116.

2 Jayaram KC, *The freshwater fishes of the Indian region.* (Narendra Publishing House, New Delhi), 1999.

3 Raghavan R, Prasad G, Ali A & Pereira B, Fish fauna of Chalakudy River, part of Western Ghats biodiversity hotspot, Kerala, India: patterns of distribution, threats and conservation needs. *Biodiv Conserv*, 17 (2008) 3119.

4 Manojkumar TG & Kurup BM, Age and growth of the Carnatic carp, *Puntius carnaticus* (Jerdon, 1849) from Chalakudy River, Kerala. *Indian J Fish*, 57 (2010a) 81.

5 Khedkar GD, Lutzky S, Rathod S, Kalyankar A & David L, A dual role of dams in fragmentation and support of fish diversity across the Godavari River basin in India. *Ecohydrology*, 7 (2014) 1560.

6 Talwar P & Jhingran AG, *Inland Fishes of India and Adjacent Countries (*Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi), 1991.

7 Thomas KR, George MJ & Biju CR, Freshwater fishes of southern Kerala with notes on the distribution of endemic and endangered species. *J Bombay Nat Hist Soc*, 99 (2002) 47.

8 Ali A & Raghavan R, *Barbodes carnaticus*, in *IUCN 2013*. IUCN Red List of Threatened Species. Version 2013.2.<www.iucnredlist.org> (2011).

9 Dahanukar N & Raghavan R, Freshwater fishes of the Western Ghats: Checklist v1.0 August 2013, *MIN* 1 (2013) 6.

10 Manojkumar TG, *Fish habitats and fish assemblage in the selected rivers of Kerala and investigation on life history traits of Puntius carnaticus* (Jerdon 1849). (Ph.D Dissertation, Cochin University of Science and Technology, Cochin, India), 2007.

11 Khan MA, Khan S & Miyan K, Aquaculture as a food production system: A review. *Biol Med*, 3 (2010) 291.

12 Sarkar UK & Lakra WS, Small indigenous freshwater fish species of India: Significance, conservation and utilisation. *Aquacult Asia*, 15 (3) 2010) 34.

13 Basavaraja N, Lun PB, Rather MA & Katare MB, Successful induced breeding of the Cauvery carp, *Puntius carnaticus* (Jerdon). *Fish Chim*, 32 (4) (2012) 44.

14 Balon EK, The theory of saltation and its application in the ontogeny of fishes: steps and thresholds. *Environ Biol Fishes*, 4 **(**1979) 97.

15 Balon EK, Reflections on some decisive events in the early life of fishes. *Trans Am Fish Soc*, 113 (1984) 178.

16 Balon EK, Epigenesis of an epigeneticist: the development of some alternative concepts on the early ontogeny and evolution of fishes. *Guelph Ichthyol Rev*, 1 (1990) 1.

17 Krupka I, Early development of the barbel [*Barbus barbus* (Linnaeus, 1758)], *Prace Ustavu Rybarstva A Hydrobiol*, 6 (1988) 115.

18 Gozlan RE, Copp GH & Tourenq J, Early development of the sofie, *Chondrostoma toxostoma. Environ Biol Fishes*, 56 (1999) 67.

19 Pinder AC & Gozlan RE, Early ontogeny of sunbleak. *J Fish Biol*, 64 **(**2004), 762. doi:10.1046/j.1095-8649.2004.00346.

20 Chandrashekharaiah HN, Rahman MF & Lakshmi Raghavan S, Status of fish fauna in Karnataka. In: *Endemic Fish Diversity of Western Ghats*, (Ed. AG Ponniah, A Gopalakrishnan; NBFGR – NATP Publication, 1, National Bureau of Fish Genetic Resources, Lucknow, Uttar Pradesh, India), 2000, 347.

21 Pinder AC, Raghavan R & Britton JR, Efficacy of angler catch data as a population and conservation monitoring tool for the flagship Mahseer fishes (*Tor* spp.) of Southern India. *Aquat Conserv*: *Mar Freshwater Ecosyst*, 2014a. DOI: 10.1002/aqc.2543.

22 Pinder AC, Raghavan R & Britton JR, The legendary hump-back mahseer (*Tor* spp.) of India's River Cauvery: an endemic fish swimming towards extinction? *Endang Species Res*, 28 (2015) 11.
23 Manojkumar TG & Kurup BM, Length-weight relationship and relative condition factor of *Puntius carnaticus* (Jerdon, 1849). *Fish Technol*, 47 (1) (2010b) 19.
24 Nandeesha MC, Bhadraswamy G, Patil JG, Varghese TJ, Sarma K & Keshavanath P, Preliminary results on induced spawning of pond-raised mahseer, *Tor khudree. J Aquacult Trop*, 8 (1993) 55.
25 Keshavanath P, Gangadhar B, Basavaraja N & Nandeesha MC, Artificial induction of ovulation in pond-raised mahseer, *Tor khudree* using carp pituitary and ovaprim. *Asian Fish Sci*, 19 (2006) 411.
26 Kangku ONS & Basavaraja N, Induced breeding, embryology and rearing of fry of Deccan mahseer, *Tor khudree* (Sykes). *J Aquacult Trop*, 25(1) (2010) 13.
27 Jan M, Jan N & Khan IA, Studies on the reproductive biology of an endangered coldwater fish golden mahseer, *Tor putitora* (Ham.) from Anji Mahseer Hatchery Reasi of Jammu region. *Int J Curr Res*, 6 (2014) 6710.
28 Shailja M & Saksena DN, Gonadosomatic index and fecundity of an Indian major carp, *Labeo calbasu* in Gohad reservoir. *The Bioscon*, 7(1) 2012) 43.
29 McDonald-Madden E, Probert WJ, Hauser CE, Runge MC, Possingham HP, Jones ME & Wintle BA, Active adaptive conservation of threatened species in the face of uncertainty. *Ecol Appl*, 20 (2010) 1476.
30 Hermoso V, Clavero M, Blanco-Garrido F & Prenda J, Invasive species and habitat degradation in Iberian streams: an analysis of their role in freshwater fish diversity loss. *Ecol Appl*, 21 (2011) 175.
31 Miller TJ, Crowder LB, Rice JA & Marschall EA, Larval size and recruitment mechanisms in fishes: toward a conceptual framework. *Can J Fish Aquat Sci*, 45(9) (1988) 1657.
32 Duarte CM & Alcaraz M, To produce many small or few large eggs: a size-independent reproductive tactic of fish. *Oecologia*, 80 (1989) 401.
33 Rather MA & Basavaraja N, Embryonic and larval development of olive barb, *Puntius sarana sarana* (Hamilton 1822), *Israeli J Aquacult-Bamidgeh*, *IJA*_64.2013.916. (2013) 10.
34 Miah MI, Harun M, Rahman MM, Haque MR & Hossain MA, Study on the embryonic and larval development of an endangered species of bata (*Labeo bata*). *Int J Sust Crop Prod*, 4(1) (2009) 72.
35 Chakraborty RD & Murty DS, Life history of Indian major carps *Cirrhinus mrigala* (Ham.), *Catla catla* (Ham.) and *Labeo rohita* (Ham.). *J Inland Fish Soc India*, 4 (1972) 132.
36 Mann RHK, Environmental requirements of European non-salmonid fish in rivers. *Hydrobiol*, 323(3) (1996) 223.
37 Basavaraja N, Hegde SN & Akash N, The fertility of cryopreserved Deccan mahseer, *Tor khudree* spermatozoa. *Asian Fish Sci*, 15 (2002) 193.
38 Chakraborty BK, Miah MI, Mirza MJA & Habib MAB, Rearing and nursing of local sarpunti, *Puntius sarana* (Hamilton) at different stocking densities. *Pak J Biol Sci*, 6(9) (2003) 797.
39 Basavaraja N & Keshavanath P, Conservation and management of fish genetic resources in Karnataka. In: *Endemic Fish Diversity of Western Ghats*, (Ed. AG Ponniah & A Gopalakrishnan; NBFGR – NATP Publication, 1, National Bureau of Fish Genetic Resources, Lucknow, Uttar Pradesh, India), 2000, 152.