JEB
Website : www.jeb.co.in
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Journal of Environmental Biology

ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

Studies on the reproductive biology and captive breeding of *Channa aurantimaculata*, an endemic fish from Assam

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Publication Info

Paper received:
09 March 2014

Revised received:
21 February 2015

Re-revised received
27 April 2015

Accepted:
05 June 2015

Abstract

Channa aurantimaculata Musikasinthorn, an endemic murrel of Brahmaputra basin, is highly regarded as both food and ornamental fish. Five stages of gonadal development were observed in male and female fish. The sex-ratio was significantly male-biased (M: F= 1:0.5). 50% maturity (M_{50}) for male and female was observed at 26-30 and 21-25cm respectively. However, both sexes were found to attain 100% maturity at 36-40 cm size group. The highest value of GSR (8.23±0.078) in females was observed in April. *C. aurantimaculata* is total spawner, breeding season is fairly prolonged starting from March- April and continuing up to July- August depending on rainfall pattern in a particular year. The species is moderately fecund, the absolute fecundity ranging from 1189 to 7642. Correlation coefficient (r) indicated a close relation between fecundity and ovary weight than body weight or body length. 'Pairing' was noticed among brooders at the onset of pre-monsoonal rain. Captive breeding of the species was successful and about 200 spawn were raised to fingerling stage. The approach of entire breeding programme was based on habitat manipulation. Courtship behaviour and parental care of the species were also documented.

Key words

Channa aurantimaculata, Courtship behaviour, Parental care, Reproductive biology

Introduction

The water resource of Assam is very rich in fish fauna and over 250 species of fish have been reported from Assam (Biswas, 2003; Das and Biswas, 2008). But due to lack of proper knowledge of rearing, endemic species of the region are facing extinction. *Channa aurantimaculata* Musikasinthorn, an endemic murrel of upper Brahmaputra river basin, is one of them (Biswas *et al*., 2007). The species is a data deficient fish having ornamental value also. However, due to its high market value, the species is captured indiscriminately from *beels,* jeopardizing its very existence throughout its distributional range. Reproductive biology of a fish might be defined by its reproductive traits and it also expresses combination of the species specific reproductive mode (Murua and Saborido-Rey, 2003; Morgan, 2008; Prasad *et al.,* 2011). Reproductive biology of fish is an essential factor that enables the determination of appropriate management practice which should be done to conserve species of fish in their habitat. Captive breeding is one of the principal techniques employed to compensate for declining fish population and simultaneously to supplement and enhance yield for fisheries. Hazarika *et al*. (2014) performed a successful breeding experiment on *Channa aurantimaculata* in cemented cistern without any stimulation. However, virtually no other information is available on the biology and rearing of the species. Keeping the above points in view, an attempt was made to study certain aspects of reproductive biology and feasibility of *ex-situ* breeding of this endemic species.

Materials and Methods

Specimens of *Channa aurantimaculata* were collected from Maguri *beel* (27 º34' 22" N and 95º22' 29"E) in

Tinsukia District of Upper Assam from August 2011 to July 2012. A total of 206 specimens were collected during the study period and were brought to laboratory, and total length and weight of the fish were measured nearest to 1.0 mm and 0.1 g, respectively. Each specimen was dissected and gonads were removed. Sex of each specimen was identified by examining the gonads.

Proportion of these two sexes, relative to one another, was used to calculate the sex ratio. Gonadal stages were examined macroscopically and classified according to Nikolsky (1963) and is as follows: Stage I - Immature; Stage II- Maturing (developing); Stage III- Mature (developed); Stage IV- Ripe and Stage V- Spent. The number of males and females at different stages of gonadal development were counted and recorded. The length at first maturity was determined directly by plotting the percentage of mature fish against their length. The length at which 50% of the specimens attained Stage IV was considered as the length at first maturity (Hodgkiss and Mann, 1978).Ovaries from ripe specimens (Stage IV) were only considered for fecundity studies. Sub-sampling method (Bagenal and Braum, 1968 and Grimes and Huntsman, 1980) was employed for calculating fecundity. Similarly, relative fecundity was estimated by simply dividing absolute fecundity with total body weight (g). Relationship between fecundity and total length; between fecundity and body weight and between fecundity and ovary weight were calculated (Bagenal, 1978). Measurements of ova diameter were taken from formalin preserved ovary. Ova diameter using ocular micrometer was measured following the method of Biswas (1993).

Breeding trial was continued from September 2011 to June 2012 in controlled condition. For breeding trial, collected specimens were first acclimatized in a separate enclosure for 7-10 days. A cemented tank (2m x1.3m x1.2m) was selected for rearing and breeding of the species. About 15 cm of tank bottom was filled with mud and some floating (*Pistia stratiotes* and *Nymphoides indicum*) and submersed hydrophytes (*Hydrilla verticillata* and *Ceratophyllum demersum*) were introduced so as to give the environment a natural look. Further, aquatic macrophytes would act as substrates for adhering eggs. Some small herbivorous fish like *Danio rario*, *Puntius ticto* and *Amblypharyngodon mola* were also added to the artificial aquatic ecosystem to maintain the food web relationship in tank, as food habit of the studied species was hunting type predator. Brooders were then transferred to the cemented tank and proteinous food items like earth worm, chopped goat-liver; silk worm pupae, live cockroach and grasshopper were supplied @ 15g day^{-1} of body weight for gonadal maturation. Courtship behaviour and parental care, before and after spawning of the species were also documented. Physico-chemical properties of tank water like temperature, pH and dissolve oxygen of the tank were recorded fortnightly (APHA, 2012).

Results and Discussion

Among the 206 specimen studied 136 (66.01%) and 70 (33.98%) were observed to be male and female respectively. This gave a male-female sex ratio of 1:0.5 in favor of males ($\chi^2 = 21.15$), which might be attributable to the fact that females remain inside the holes guarding their young ones so males were susceptible to capture than females. A similar result was also reported by Hussain *et al.* (2007) in *Botia dario* and Singh *et al.* (2011) in *Macrognathus aral,* with male-female ratio of 1:0.61 and1:0.27 respectively. Adebiyi (2013)) reported that sex ratio of *Pomadasys jubelini* was significantly tilted towards male (1:0.85).

Five different maturity stages were identified based on their morphological structure, space occupied in the abdominal cavity by gonads and diameter of unspawned eggs: - .Stage I (Immature): Ovaries were translucent, ribbon like, pale yellowish in colour. Ova was not distinct to naked eyes. Stage II (Maturing): Ovaries were more yellowish and thicker; ova was visible to naked eyes; under microscope ova was spherical in shape, covered ½ of the abdominal cavity. Stage III (Mature): Ovaries were dark yellowish enlarged lobes with prominent ovules and blood vessel; occupied ¾ of the body cavity, much broader than stage I and II. Stage IV (Ripe): Ovaries were deep yellow in colour; with maximum size; occupied the entire body cavity. Under microscope, ova was spherical in shape and opaque due to huge amount of yolk present. At this stage, ova was of full size and started liberating through oviducts on putting light pressure on the abdomen. Stage V (Spent): Ovaries became flaccid, almost thread like in appearance resembling matured Stage I ovary; reduced in size and volume, became pale yellowish in colour.

Similarly five matured stages were recognized in males based on the progression of the development of testes. Stage I (Immature): Testes small; very fine thread- like structure and pale whitish in appearance. Stage II (Maturing): Testes were pale whitish; 'V' shaped in structure; slightly increased in volume and weight and occupied about ¼ of the abdominal cavity. Stage III (Mature): Testes were enlarged, became brush like structure; blood vessels were prominent; covered nearly ½ of the abdominal cavity. Stage IV (Ripe): Testes became more prominent and soft; blood vessels increased and milt ran with slight pressure on abdomen. Stage V (Spent): Testes shanked, reduced in size and weight and became pale white.

Females with immature gonads (Stage I) were observed from October to February, peak being in November. Stage II (Maturing gonad); females were

observed in November and available till May; highest percentage being observed in March. Stage III (Mature gonad); females were observed from February to June; highest percentage was observed in March. Ripe females (Stage IV) were observed from March to August with highest percentage being observed in April and lowest in August. Spent females (Stage V) were found from June to February, highest percentage was observed in September (Table 1).

Stages III to IV of gonadal maturity stage was considered as mature gonad. It was observed that 50% (M_{50}) of the population of *C. aurantimaculata* attained maturity at 26-30cm and 21-25cm respectively (Table 2), indicating that females matured at smaller size than their male counterparts. It was observed that in both the sexes, 50% of the population of *C. aurantimaculata* attained maturity at average length group of 21-30 cm. Hence, these length groups were considered as the length at which first maturity was attained. The species was found to attain 100% maturity at 36-40 cm size group. Choudhury and Biswas (2003) recorded 50% maturity of *C. barca* at 25.0-35.0 cm for male and 20.0-30.0 cm for female. Biswas (1993) viewed that size at first maturity in fish not only differed from species to species but also in same species in different habitats.

Maturity determination by gonadosomatic ratio (GSR) has proved significant role in the life of fish. It is helpful in fish breeding too. In the present study, GSR or maturity index of *C. aurantimaculata* was calculated for males and females separately. High GSR values recorded in April (8.23±0.078), May (7.2±0.49) and June (6.53±0.336) suggested these months as peak spawning period in female. Highest value of GSR in females was observed in April, while minimum value was observed in December (0.34±0.024). In male, high GSR values were recorded in April (1.47±0.23), May (2.53±0.227) and June (1.25±0.055) highest being in May while lowest in December (0.09±0.018). In the present study, GSR values showed increase in rainy season. Kapil *et al*. (2011) recorded highest maturity index of *Channa punctata* in rainy season and

Table 1 : Percentage occurrence of *Channa aurantimaculata* at different stages of maturation

Month	No. of males examined	Maturity stages (%)					No. of females examined	Maturity stages (%)				
		Immature	Maturing	Mature	Ripe	Spent		Immature	Maturing	Mature	Ripe	Spent
Jan	8	6.5	42.8	4.5	-	46.2	3	12.4	18.8	-	-	68.8
Feb	9	13.3	15.5	46.8	-	24.4	4	8.8	11.1	24.5	-	55.6
Mar	11	8.6	24.1	38.5	28.8	-	5	-	28.6	53.2	18.2	-
Apr	12	-	8.0	28.5	63.5	-	8	-	3.4	21.5	75.1	-
May	14	-	7.8	26.6	65.5	-	9	-	2.4	26.8	70.8	-
Jun	15	-	-	37.2	62.8	-	6	-	-	12.3	74.7	13
Jul	16	-	-	-	11.1	88.9	11	-	-	-	12.7	87.3
Aug	13	-	-	-	3.1	96.9	9	-	-	-	2.4	97.6
Sep	11	-	-	-	4.7	95.3	6	-	-	-	-	100
Oct	11	27.7	-	-	-	72.3	4	16.4	-	-	-	83.6
Nov	9	29.8	15	-	-	55.2	3	20.4	9.2	-	-	70.4
Dec	7	11.2	45.3	-	-	43.5	2	13.7	18.5	-	-	67.8

Table 2 : Percentage of maturity in different length groups of *Channa aurantimaculata*

Standard length group (cm)	Sex	%Immature (Stage I)	%Maturing (Stage II)	%Mature/ripe (Stage III & IV)
	Male	100	-	-
6.0-10	Female	100	-	-
	Male	59.9	40.1	-
11-15	Female	52.6	41.6	5.8
	Male	6.4	52.1	41.5
16-20	Female	5.3	54.9	39.8
	Male	-	68.8	31.2
21-25	Female	-	43.5	56.5
	Male	-	47.4	52.6
26-30	Female	-	36.4	63.6
	Male	-	32.3	67.7
31-35	Female	-	1.4	98.6
	Male	-	-	100
36-40	Female	-	-	100

Table 3 : Monthly variation in fecundity of *Channa aurantimaculata*

Months	Mean body length (cm)	Mean body weight (g)	Mean ovary weight (g)	Mean absolute fecundity	Mean relative fecundity
Jan	18.8±4.66	64.5±34.70	0.30±0.18	Spent	–
Feb	19.71±2.62	69.8±20.88	0.48±0.13	1188.71±218.24	17.03±5.23
Mar	23.6±4.42	130±33.78	2.73±0.74	5919.23±475.48	45.53±23.14
Apr	24.4±4.00	125 ±28.84	10.30±2.45	7642.34±982.12	61.14±27.34
May	25.3±4.11	133.1±28.74	9.62±2.42	6456.11±628.63	48.51±21.29
Jun	25.4±4.31	133.2±31.23	8.64±2.52	7591.93±943.31	56.99±26.41
Jul	25.7±3.17	142.4±29.33	3.98±0.78	5203.82±438.23	36.54±17.42
Aug	25.2±4.44	150.8±39.08	1.39±0.34	4562.75±673.41	30.26±14.18
Sep	24.3±3.59	114±10.23	1.04±0.09	3034.09±643.23	26.61±11.71
Oct	25.1±3.17	144.8±29.52	1.03±0.32	Spent	–
Nov	20.2±3.01	83.6±17.72	0.49±0.10	Spent	–
Dec	22±3.86	91.2±17.12	0.31±0.05	Spent	–

lowest value in winters. The lowest values of gonadosomatic index in winter season indicate depletion of gonadal products as a result of intense spawning activity, shedding of eggs and due to reabsorption of remnants of ova in the spent ovaries (Biswas, 1993).

In the present study, mean monthly ova diameter showed lowest value in February (0.38±0.05); then it started increasing gradually from March onwards; sharp by rose in April (0.62±0.03), reached peak in May (0.87±0.12), and showed a fall in August (0.77±0.15) and September (0.58±0.11), respectively. Choudhury and Biswas (2003) reported that the average size of ova was 0.67 (±0.25) mm at gravid stage of *C. barca*. Mean ova diameter value reached maximum during spawning month due to high percentage of ripe ova being available during this period in gonads and dropped had down next month with release of ripe ova during spawning.

Studies on fish fecundity is important to evaluate the reproductive potentials of the species, (Duarte and Araujo, 2002). Fecundity of *Channa aurantimaculata* was found to be highly variable. Absolute fecundity was relatively higher in April to June. Mean ovary weight was also recorded high during these months (Table 3). Absolute fecundity varied from individual to individual and ranged from 1188.71 to 7642.34 (Table 3). Relative fecundity was found to vary from 17.03 to 61.14. Monthly, as well as, age/length group variation in fish species was less reported in many fish species (Biswas, 2002). Choudhury and Biswas (2003) reported that absolute fecundity varied from 9853 to 13624 in *C. barca* with relative fecundity ranging between 46.5 and 57.2. Results of fecundity revealed that murrel is a moderately fecund fish.

Table 4 : Physico-chemical parameters of tank water

Month	Temp (°C)	pH	DO ($mg\,l^{-1}$)	Free CO_2 ($mg\,l^{-1}$)
March	18-18.6	8.2- 8.4	5.8-6	2.3-2.8
April	22-22.8	7.6-7.7	4.8-5.1	1.6-1.7
May	23-25.8	7.2-7.4	5.4-5.9	1.1-1.6
Jun	25-26.1	6.8-6.9	5.1-5.6	1.4-1.5

Fecundity with total length, total weight and ovary weight was calculated and is summarized as follows: Fecundity and TL: Log F= -0.53+3 log L (r = 0.85); Fecundity and BW: Log F=0.70+1.39log W (r = 0.85) and Fecundity and OW Log F=3.45+0.46log OW (r = 0.93). In the present study, fecundity was found to increase with increase in ovary weight. Correlation coefficient (r) indicated a close relation between fecundity and ovary weight than body weight, body length.

Sex differentiation in adult *C. aurantimaculata* is not difficult. Young fish are closely similar with *Channa punctata* and *C. striata,* but after fingerling stage (about 2 months old) appearance of black spots followed by yellowish vertical stripes on ventro-lateral side was noticed. A black and yellow colouration was also observed on caudal, dorsal, pectoral and anal fins. Appearance of round blush blemish at the base of both pectoral fins indicated adulthood (gonadal maturation). Adult male and female fish showed some extent of sexual dimorphism. During breeding season, the male was brightly coloured with yellow reticulation on head scale along with deep bluish colour on pectoral fins.

For captive breeding, about 20 adult specimens were reared separately. Initially, the fish did not show any interest to take the supplied (artificial) food. But exactly from third day they started taking food slowly and after eight days they were found to be normal in taking food. In the later part of the experiment, their behaviour was similar to domesticated animal because, when a person moved in and around the aquarium, movement of specimens was active because they expected that somebody was coming to give them food and if a person came closer to them, their excitement increased by quicker and erratic movement. Among all the food material

Table 5 : Growth and survivability of *Channa aurantimaculata*

Age of the fish (days)	Average length (cm)	Average weight (gm)	Typeof food	Mortality %
15	2.2	1.4	Mosquito larvae and plankton	2.1
30	3.4	2.2	Mosquito larvae	1.5
60	6.2	4.5	Chopped earth worm	1.2
90	11.7	14.8	Mosquito larvae, dry fish and chopped earth worm	nil

supplied, they preferred mostly earthworm (60%) followed by insects. Surprisingly, except for earthworm and silkworm pupae, they did not prefer any dead animal or even fish. Although, intake of food was mostly during day time, night time feeding was observed occasionally. Feeding keenness was temperature dependant, as soon as water temperature fell down below 20ºC, particularly during winter months; they did not like to feed upon any food and became lethargic. The initial length and weight of the brooders transferred to the cemented tank measured 28-35cm and 125-140gm respectively and food was supplied to them regularly @ 15 gm day^{-1}. During trial it was unable to observe the matting behaviour devotedly but at night they tried to jump frequently during pre-monsoonal rain (April). During first week of May, fry were noticed. As the created habitat (cement tank) was in open air, the rain drops encouraged the mating pair for natural breeding, though they were in captive condition.

More than two hundreds fry were recorded in the tank. The water quality of the rearing tank was monitored regularly and important parameters were kept within certain limit to avoid stress for the fish (Table 4). Although, fry were guarded mainly by male, the female too, spent time with the fry .Same behaviour was observed in *Channa punctata* by Haniffa *et al.* (2004) in captive environment. For initial 15 days, the young ones were only fed on planktons collected from a nearby pond. Thereafter, they were provided mosquito larvae thrice daily for the next three months. *C. aurantimaculata* is a voracious and aggressive feeder and they prefer live prey.

After 15 days, the hatchlings attained an average size of 2.2 cm. Live mosquito larvae were supplied to them and it was observed that a single fry could consume as many as 50 to 80 mosquito larvae within an hour. After attaining the fingerling stage, they were fed with chopped earthworms only. In aquarium, the fingerlings attained size of about 10-12 cm and weighed about 15-20 gm. In captive condition, the mortality rate was recorded less than 5% (Table 5). 100% survival during fry to fingerling stage can be achieved, provided adequate feeding and optimal water quality parameter are maintained.

Most of the wild species do not breed in confined water and require hormonal injection for induced breeding (Singh and Biswas, 2011). Also proper dose is important for successful breeding (Purkayastha *et al.,* 2012). Like other murrel, *C. aurantimaculata* too, hibernates during winter months (December to February). Low temperature as well as scarcity of water in *beels* (wetlands) may be the reason for such behavioural adaptation. As pre-monsoon starts in March, and water temperature rises, they come out from the burrowing hole and start feeding intensely. In the present study, *Channa aurantimaculata* was also found to breed naturally during April-June. Identical breeding period was also reported for *Channa punctatus* by Saikia *et al.* (2011) and also in *Channa bleheri* by Gogoi *et al*. (2013). 'Pairing' of males and females were noticed prior to pre-monsoon (March-April) and just after a downpour, chasing and jumping behaviour among the brooders was noticed. Choudhury and Biswas (2004) also observed similar spawning behaviour in *C. barca* and also opined that the species can be bred in captivity. Recently, Hazarika *et al*. (2014) was successful in breeding *C. aurantimaculata* simply by providing brooders with favorable environment. Therefore, it may be concluded that by manipulating the habitat, murrel can be bred in captive condition without much difficulty.

Acknowledgments

The authors gratefully acknowledge the Department of Life Sciences, Dibrugarh University and Head, Department of Zoology, Kakojan College for the laboratory facilities used during the study. Financial assistance from NFDB, Hyderabad is highly acknowledged.

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