Eco. Env. & Cons. 20 (3) : 2014; pp. (929-941)
Copyright@ EM International
ISSN 0971–765X

Studies on embryonic and larval development of induced bred *Channa striatus*

Bilal Ahmad Paray[1], M.A. Haniffa[1]* and Y. Ananth Kumar[2]

[1]Centre for Aquaculture Research and Extension, St.Xavier's College (Autonomous), Palayamkottai
[2]Department of Zoology, St.Xavier's College (Autonomous), Palayamkottai

(Received : 15 April, 2014; accepted 20 May, 2014)

ABSTRACT

Synthetic hormone Luteinizing Hormone Releasing Hormone analogue (LHRHa) with different doses (Low - 50 µg/kg BW, Medium – 60- µg/kg BW and High —-- 70 µg/kg BW) was used for induced spawning. Latency period, incubation period, fertilization rate and hatching rate were studied as a function of different dosages of hormone. By overall performance with regard to number of eggs spawned, latency period, fertilization rate and hatching rate, the present investigation confirms that High dose of LHRHa (70µg) was found to be the most potent ovulating agent in *Channa striatus.* The embryonic stages occurred inside the chorion was completed before hatching. The diameter of fully swollen fertilized eggs varied from 1.22-1.45 mm. The first cleavage commenced 20 min after fertilization and after 5 - 6 hr embryo attained blastula stage. The embryonic development of *Channa striatus* was completed within 22- 26 hr. Hatchlings of *Channa striatus* ranged from 2.8 - 3.6mm (mean ± SD: 3.2 ± 0.2) mm in total length characterised by presence of an almost round yolk sac, occupying about 45% of total length. Mouth was formed as a terminal opening when the larve was 36h old(5.1±0.2 mm) and the reserved yolk material was completely absorbed in 3 days old larva (5.8 ±0.5 mm) and ontogeny was completed inn 20 days old larva (22.9±0.5 mm) and the fry resembled the adults in all characteristics except maturity.

Key words : *Induced breeding, LHRH, Channa striatus, Embryonic development*

Introduction

Interest in commercial production of murrels commonly called snakeheads has increased in recent years. But reliable spawning methods remain a problem till date. It is well known that reproductive processes in fishes are controlled by endogenous biological rhythms as well as by environmental cues (Munro, 1990). The final event of the reproductive cycle, the release of eggs (ovulation) and sperm (spermiation) known as spawning, can be induced by either exposing the fish in an appropriate environment or by changing the fish's internal regulating factors by hormones and/or other substances. Many factors which have impact on ability of induced spawning, include: 1) Condition of the fish, 2) Stage of sexual maturity, 3) Size of the fish, 4) Previous spawning history, 5) Water temperature, 6) Season of the year and 7) Dosage of hormone.

Murrels breed naturally during southwest monsoon and northeast monsoon in flooded rivers and ponds in India. Since monsoon failure often limits their seed production, Parameswaran and Murugesan (1976) attempted induced breeding by injecting carp pituitary glands. Hypophysation is a simple practical technique but suffers from the disadvantage that often gonadotropic potency of pituitary glands used is unknown and difficult to standardise. Hence alternative sources *viz.* Human Chorionic Gonadotropin (HCG) (Mollah and Tan, 1983; Zairin *et al.,* 1992; Inyang and Hettiarachchi, 1994) Luteinizing Hormone Releasing hormone ana-

***Corresponding author's email:** haniffacare@gmail.com

logue (LHRHa) (Fermin, 1992) and Ovaprim (Alok *et al.* 1993; Francis, 1996; Haniffa *et al.* 1996) have been attempted in air-breathing fishes.

The appropriate hormone preparation should be selected on the basis of the species to be spawned and the availability of the hormones. One such hormone is Luteinizing Hormone Releasing Hormone analogue (LHRHa) - Sigma Aldrich, Bangalore. Information on early embryonic and larval development is of critical importance in understanding the basic biology of a particular species and its dietary needs and environmental preferences (Koumoundouros *et al.* 2001; Borcato *et al.* 2004). Further, studies on embryonic and early larval development are imperative and consequential to the successful rearing of larvae for large scale seed production in aquaculture (Khan and Mollah, 1998; Rahman *et al.* 2004). In sexual reproduction, ripening of the egg and formation of spermatozoon constitute the first phase. The second phase of development is fertilization, third phase is the period of cleavage, fourth phase is gastrulation and the next phase is organogenesis, which is followed by growth and histological differentiation. The development of fish larvae to juvenile stage constitutes the most critical period in the life cycle of a fish.

The larval development of air breathing fish species *viz., Channa marulius* (Mookerjee, 1945) *C.punctatus* (Banerji, 1974; Haniffa *et al.* 2003);); *C. macrocephalus* (Mollah and Tan, 1983) ; *Anabas testudineus* (Munshi and Hughes., 1991); *Clarias batrachus* (Thakur, 1976 and 1980); *Heteropneustes fossilis* (Thakur *et al.*, 1974); *Clarias gariepinus* (Bruton, 1979; Verreth *et al.* 1992; Segner *et al.* 1993and *Mystus montanus* (Arockiaraj *et al.* 2003) have been reported. The embryonic and larval stages are very sensitive to of environmental disturbances. Moreover studies on larval development of any cultivable species are useful in directing the hatchery efforts of fish farmers to succeed in their efforts on seed production by promoting larval growth and survival. Hence the present study was attempted to provide detailed information on the embryonic and larval development of the induced bred striped snakehead, *C. striatus* under captive conditions.

Materials and Methods

Brood fishes of *C. striatus* were collected from river Tamirabarani and transported to CARE Aquafarm with least disturbance and reared in CARE stocking pond (15 m x 5 m x 1 m). The brooders were acclimatized to laboratory conditions for a month by feeding semi moist feed consisting of anchovy (35%), jawala (25%), tapioca (10%), wheat flour (14%), and rice flour (14%) with vitamin and mineral mix (2%) and chopped chicken intestine *ad libitum*. The length of brooerds ranged from 27 - 36 cm and 680 - 790 gm weight. After 2 months of rearing the fishes were found mature enough for captive breeding experiments.

Induced Breeding

The induced breeding experiments were conducted during the north-east monsoon (October; 2009), the natural breeding season of *C. striatus* in fibre tanks of (1000 L) capacity . Mature healthy males and females were selected by sexual dimorphism. A day before experiment, the required fishes were selected and transferred to fibre tanks (1000 L capacity) filled with dechlorinated water. Each breeding set consisted of two males and one female (Jhingran and Pullin, 1985; Haniffa *et al.* 1996). Synthetic hormone (LHRHa) was used for induced spawning. Three doses of LHRHa were used and for each dose, three breeding trials were made to find out the response of the fish and to observe the variation in latency period, fertilization and hatching (Table 1).

Injections were administered intramuscularly in the dorso lateral region of the body. The injections were given during late afternoon and early evening (17-19 h). Immediately after administration the hormone, the breeding sets were released into the spawning tanks (3m X 1.5m X 1.5m). Each breeding tank was covered by a mosquito net and provided with aquatic weed *Hydrilla verticillata* for hiding purposes and for holding the floating eggs together without dispersion. The water quality parameters recorded during the study were as follows: water temperature (30 ± 1.5^0C), pH (7.5 ± 0.23), and dissolved oxygen (5.2 ± 2.3 mg/L).

In the early hours of the day, the brooders were closely observed for their responses to hormone administration and breeding behaviour. After spawning, eggs were collected from each breeding tank using a beaker and immediately total eggs were enumerated and percentage of fertilization was estimated. The hatchability was determined as the percentage of normal larvae from the total number of fertilized eggs in each sample (Haniffa *et al.*, 2000) and the remaining eggs were allowed to hatch out and grow along with the parents in the breeding

tanks to observe parental care.

Embryonic and Larval Development

To study embryonic and larval development and their morphometric and meristic characteristics, samples were collected from the brooders injected with LHRHa hormone at the dosage of 70µg/Kg BW. After spawning, the fertilized eggs were carefully collected from the breeding tanks using a 500 ml beaker and transferred to a glass aquarium containing 25 *l* of water under gentle aeration. Developing eggs were sampled every hour during the first 48 hours and every 4 hours during the next three days and then only once a day. The observations of the larvae were carried out under a microscope (Nikon microscope - U III E-400 Eclipse) till the end of the larval period. The embryo and larvae were subjected to Magnus Pro microscope software and data were analyzed by Biostat (2007).

Results

Spawning behaviour and parental care

Males approached the female side by side in position and started to quiver. One of the males was active and showed more aggressive and active participation in mating which was preceded by an elaborate courtship. In the present study the breeding behavior of *C. striatus* was observed 6 hours after administration of the hormone irrespective of the dosage of the hormone used and continued till spawning upto 22-26 hrs. The active male chased the female and frequently excited its movement which commenced from 10-12 hrs after the hormone injection. In all the spawning attempts, the important observation was that the active male involved in the courtship and spawning by hitting the snout and vent region of the female more frequently. The other male was passive and idle at one corner of the breeding tank. The mating pair inclined slightly to one side, keeping their anal regions close to each other forming an X- shaped appearance. The spawning activity was keenly observed till the gametes were released. At the time of courtship, the male starts to tilt its body close to genital papilla of the female and the breeders joined together which ultimately resulted in spawning after about 24±2 h duration followed by external fertilization. The fertilized eggs were yellow in colour which usually floated and adhered to each other forming an egg mass of 10-15 cm in diameter; while the unfertilized eggs were white in colour and transparent without any adherence.

Aggressive behaviour was shown by male parent who remained curving around the fertilized eggs by circular movement and fanning the eggs with its pectoral fins. After 24 h, fibre tanks were found full of hatchlings black in colour. Both the parents showed care of hatchlings by guarding them right from the stage of fertilized egg till the fry stage but paternal care was more dominant. In the present study it was observed that the eggs guarded by the parents remained clean, showed good development and reached post-larval stage with high survival rate. It was also observed that the parental care is much necessary to increase the hatching rate and to protect the eggs from fungal infection as well as predation.

Induced spawning using synthetic hormone LHRHa

In the low dose of this hormone (50µg/kg BW), the average latency period was found to be 23.3 ± 0.30 h and the average fertilization rate was 45.6 ± 1.34%. In all the three trials conducted with low dose, partial spawning was observed. Each female released an average of 2764 ± 165.65 eggs with fertilization and hatching rate of 45.6 ± 1.34% and 60.4 ± 1.85% respectively. In the medium dose of LHRHa, (60µg / Kg BW) the average latency period was 24.5 ± 0.40 hrs. Each female spawned an average of 5655 ± 151.18 eggs. A fertilization rate of 65.5 ± 2.18 % and hatching rate of 69.3 ± 1.45% were observed in the medium dose of LHRHa. In the high dose (70µg / Kg BW), an average latency period of 22.4 ± 0.45 hr was observed, which was the lowest among all the trials of induced breeding. Each female spawned 7880 ± 28.21eggs and fertilization rate was 80.2 ± 1.92 % and hatching rate was 78.7 ± 1.22 % as a function of high dose of LHRHa (Table 1).

Statistical analysis indicated that, there was no significant difference ($P>0.05$) between the doses with regard to latency period. Whereas, a significant difference ($P<0.05$) was observed between the three doses with regard to number of eggs spawned. Statistical inference indicated that the high and medium doses significantly differed from the low dose. The fertilization rate as a function of the high dose of LHRHa was significantly different ($P<0.05$) from those of low and medium doses. A significant difference in the hatching rate was found between the medium and high doses and the medium and low doses.

Embryonic and larval development

A summary of the timing of the important ontogenic events and structure are presented in Tables 2 &3. In the present study spawning was noticed within 24±2 hrs after administration of the hormone. Fertilized eggs of *C. striatus* were free floating, spherical, non-adhesive and straw yellow in colour. The eggs became translucent as the development progressed. The diameter of the fully swollen fertilized eggs varied from 1.22-1.45 mm. Eggs with narrow perivitelline space had a single large oil globule which occupied the greater part of the ovum and was immersed in the oval shaped golden yellow yolk. It was adjacent to the upper pole of the egg and in surface view under the microscope, was seen to be surrounded by a narrow zone of yolk, the whole being contained within a space bounded by the vitellien membrane.

The embryonic development of *C. striatus* was completed within 22- 26 hrs. The first cleavage commenced 20 min after fertilization that divided the blastodisc into two blastomeres. The segmentation was typically meroblastic. Within another 15 min the second cleavage occurred followed by 16 cell stage within next 30 min. As successive cleavage proceeded, the blastomeres decreased in size and the morula stage was visualised within 1.30 – 2.0 hr after fertilization. After 5 - 6 hrs of fertilization the embryo attained the blastula stage. After half an hour, the spread of blastoderm was evident and 6 hrs after fertilization it was flattened at the top resulting in the formation of the germinal ring. Embryonic shield appeared within next 2 hrs and by that time more than half of the yolk was invaded and at this stage the head and tail ends of the embryo were distinguishable. Gastrulation was in progress approximately 9.30 hrs after fertilization and the blastopore was evident. In another 30 min yolk invasion was completed and the blastopore was almost closed.

Observation made at 10.30-11.00 hrs revealed that antero-posterior axis was distinguishable;

Table 2. Summary of Embryonic development of *Channa striatus*

Time after spawning	Stage	Characteristics
0.00 min	Fertilized egg	Eggs were free floating, spherical, non-adhesive, transparent and straw yellow colour. Diameter varied from 1.22-1.45 mm
15-20 min	2 cell stage	First Cleavage
40 -55 min	16 celled stage	Fourth cleavage
1.30-2.00 hr	Morula	Blastulation progresses to form a multicellular blastodisc
5.00-6.00 hr	Blastula	Embryonic shield formed more than half the yolk invaded, anterior and posterior differentiation evident.
8.00-9.00 hr	Gastrula	Gastrulation converts the embryo into a two- layered structure, with an outer epiblast and inner hypoblast.
9.00-9.30 hr	Post gastrula	The germinal ring and embryonic shield get established, yolk invasion completed
9.30-10.00 hr	Early neurula	Cephalic region broader with distinct fore brain.
10.30-11.00 hr	Neurula somite	Embryonic rudiment becomes distinct, two myotomes and optic vesicle are demarcated.
13.00-14.00 hr	Late Neurula	Melanophores appear, six myotomes formed, notochord laid, heart rudiment visible.
15.00-16.00 hr	10 Myotome	8-10 myotomes, demarcation of brain, cephalic region broadened.
17.00-18.00 hr	15 Myotome	12-15 myotomes laid, eyelens formed in the rudimentary eyes, kupfer's vesicle visible.
20.00-21.00 hr	22 Myotome	18-20 mytomes formed, eye lens fully formed, heart formed and blood circulation commenced, heartbeats at the rate of 140-145/min, a few melanophores appear over the yolk sac.
22.00 hr	Pre hatchedembryo	The embryo encircled the entire yolk. Olfactory pits and the concre tions on the auditory vesiclesformed, blood colorless, kupfer's vesicles has disappeared, heartbeats at the rate of 180/min, a fewmelanophores appeared all over the body embryo makes frequent twitching movements.
23.00-24.00 hr	Hatching	Hatching of embryo.

cephalic portion being broader and embryonic rudiment became distinct with two somites. The anterior protuberance formed a head fold and the posterior part elongated further to form tail fold. By the time maximum diameter of the coiled embryo was 0.958 mm. The eye vesicles were demarcated. About 6-8 sometimes were formed after 14 hrs and optic cups were clearly distinguished. In the 15-16 hr old embryo, more than three fourth of the egg peripheral space was occupied by the embryo. Number of mesodermal somites gradually increased from 8 to 10 and pigmentation was noticed in somites. Notochord was more clearly seen. Cephalic portion was broadened and embryo was embedded in the yolk mass all over its length. At 18 hr old embryo, the whole space inside the egg was fully occupied by the embryo. The mesodermal somites ranged from 12-15 in numbers. Blood circulation was observed. Ectodermal thickening to form lens of the eye was noticed. The caudal tail region started to detach from the yolk mass. Embryonic fin fold appeared, and Kupffers vesicle was noticed. Motility in the embryo was observed with 18-20 contractions per minute. In the 20 hrs old embryo 18-20 somites were observed. Embryonic fin fold on the ventral side extended upto the 11th somite. Eye lens was fully formed in the eye and olfactory placode was also observed. Blood circulation commenced over the yolk into the rudimentary heart lying anterior to the yolk sac. The heartbeat ranged from 140-145 beats per minute. Few dendritic melanophores appeared over the yolk.

In 22 hr old embryo, head was prominent and assumed piscine condition; pigment deposition occurred at the anterior end of embryo. Prominent pectoral fin rudiment and tremors in the trunk region were observed. Somites number increased to 24-25 and the yolk was completely encircled by the embryo. The tail end was free from the first two somites. Melanophores were scattered above neural chord over the trunk and caudal regions. Heartbeat ranged from 175-180 per minute. In the final stage of embryonic development, the growing embryo occupied the entire previtelline space, and about 1.5 – 2 hrs before hatching, it exhibited frequent twitching movements. After a pause of about 30 sec, this frequent movement suddenly culminated in a violent jerk breaking the previtelline membrane and the hatchling emerged with its tail first. Hatching took place about 23-24 h after fertilization and the embryo lied horizontally at the bottom. The embryo showed vigorous movements and lashed its tail vigorously against the capsule, there by rupturing the capsule towards the head region and finally emerged out from the capsule. All the eggs of the same brood did not hatch out simultaneously and hatching continued for nearly four hours.

Newly hatched larva

Hatchlings of *C. striatus* were transparent and faintly brown in colour, characterised by the presence of an almost round yolk sac, occupying about 45% of the total length.Body length (BL) of day 0 larvae ranged from 2.8 to 3.6 mm (mean ± SD: 3.2 ± 0.2) (n = 8) . Newly hatched larvae (n = 8) had a large oval yolksac [vertical axis 1.10 ± 0.05 mm (mean ± SD), horizontal axis 0.89 ± 0.04 mm], extending 36% BL, capped by a distinct blastodisc and contained an oil globule liable to disintegration to 4-6 globules, unequal in size. Yolk was rapidly utilized during the first 0–2 days after hatching and was completely absorbed in flexion larvae by day-3 (5.8 ± 0.5 mm BL, n = 10). Head was initially bent and subsequently separated from anterior margin of yolksac in YS flexion larvae on day 2 (5.4 ± 0.3 mm, n = 10).

Faint pigmentation was noticed under the microscope. The mouth, alimentary canal and gills were not yet differentiated. The hatchlings had unpigmented eyes and devoid of distinct mouth and fins. Since the head was very small, it was not distinctly separated from the yolk sac. Functional heart and the blood circulation were noticed but the blood was unpigmented. The head and the yolk sac together appeared as a bulb like structure when viewed from the above. Newly hatched larvae were not active and floated passively on the water surface and occasionally swam upside down in an inclined manner. Larvae started a nonstop tail wagging movement.

Four hour old larva

Average length of the 4 hr old larvae was about 3.5 ± 0.2 mm and brownish in colour. The mouth was not yet developed and the anal invagination appeared on the ventral side. A conspicuous depression identified the position of the mouth. Eyes were unpigmented. Circulation of body fluid was seen around the notochord in addition to the brain and yolk sac. Blood corpuscles were reddish yellow showing formation of hemoglobin. The pigmentation was dark in anterior region and melanophores were scattered on the yolk sac and were present on the unpaired fin. Larvae were seen in clusters and

few started swimming to long distance. They covered a distances of 30 cm^2 and swam in a spiral fashion.

Eight hour old larva

Average length of 8 hr old larva was about 3.9±0.3 mm. Bulged yolk became gradually elongated at this stage. The larva displayed dorso-ventral unpaired fin. Some melanophores appeared on the head region, ventral side of the notochord and dorsal side of the body. Organs like heart and brain were clearly distinct. Ray like markings were faintly noticeable at the end of the caudal region. Circulation was conspicuous at the optic region. Some pigments were visible on the iris. The heartbeat was observed as 145-150/minute. At this stage many larvae became active and swim passively on the water surface and were negatively phototrophic and sensitive to light.

Sixteen hour old larva

The average length of the 16 hr old larvae ranged 4.2±0.4 mm. The auditory capsule near the eye became prominent. Dark prominent eyespot on the anterior part of the head was noticed and caudal fin began to separate. Buccal invagination appeared. Pectoral fin buds and swim bladder formed, heart was noticed in front of the yolk and the pulsation of the heart was clear.

Twenty four hour old larva

The average length of the 24 hr old larvae measured about 4.8±0.4 mm. The eyes became darker gradually with black pigmentation. Another striking new character of this stage was the first appearance of the pectoral fins as rudimentary buds above the yolk sac. At this stage 32 myotomes were seen. Buccal invagination appeared but was not connected with pharyngeal tube. Pectoral fin buds were seen as a small protuberance and the alimentary tract was distinct. The air sac was differentiated as small tube below the pectoral fin bud. Eyes were fully pigmented. The heart was seen in front of the yolk. Pigmentation extended to the yolk sac both dorsally and ventrally, whereas melanophores were scattered on the dorsal fin fold and trunk region.

Thirty six hour old

The 36 hr old larva measured an average length of 5.1±0.2 mm and post anal length of 2.6 mm. The eyes were dark pigmented. Pectoral fin was round in shape and was actively used for free movement. Heart was distinctly visible and located behind the head and showed regular beats. Mouth was formed as a terminal opening. The lower jaw was well developed and the vent was just formed. Rudimentary gill opening and nartial pits were differentiated. A thick band of melanophores were noticed from the post orbital region to base of the pectoral region. The yolk reserve was further diminished.

Sixty hour old larva

The average length of larva at this stage was measured about 5.4±0.3 mm in length and post anal length was 2.7 mm. For two days after hatching the larvae remained at the surface of the water, resting on one side with the yolk sac. The eyeball was dark and prominent; the mouth cleft was well formed with well developed lower jaw. The yolk reserve was further diminished. The pectoral fin became paddle shaped with undulating dorsal margin. The anal aperture and opercula were well developed and distinct.

Three days old larva

After 3 days, the larva measured an average length of 5.8 ±0.5 mm and post anal length of 2.9 mm. The pectoral fin showed vigorous movements and were, vascularised with a distinct circular vessel running across them. The head was prominent and free movements of the eyeball were observed. Melanophores were scattered on both sides of the vessel. The reserved yolk material was completely absorbed. The abdomen appeared as heart shaped when viewed from the ventral side. The body was brownish in colour. Five rudimentary rays were noticed in the caudal fin and the pectoral fins were flap like and vascularised. Pigments were more concentrated in the anterior region, however the density decreased gradually. Larvae exhibited vigorous movements and swam close to the water surface and occasionally sank to the bottom. At this stage schooling behaviour of larvae was observed the air-sac appeared, the pectoral fins began to flap, and respiratory movements commenced.

Post larval development

Six days old larva

The average length of 6 days old larva was measured as 7.4±0.3 mm and post anal length as 3.5 mm. The larvae were now leaving the surface and were

seen moving at all sides freely. The body was brownish in colour and bright yellow spots appeared over the eyes. The sides of the body were free from pigment, and were consequently traversed by a pale longitudinal band parallel with the notochord. More or less interrupted pale band was noticed in the middle dorsal line of the fore-body, in front of the embryonic fin. Eyeballs were large and distinct. Pectoral and caudal fin rays were clearly noticeable. The yolk was completely absorbed, and larvae began wandering in search of food.

Ten days old larva

Average length of 10 days old larvae measured 10.8 ±0.5 mm and post anal length was 5.2 mm. Dorsal and anal fins were clearly demarcated and were almost separated from the caudal fin. The colour bands were distinct. The caudal fin rays were clearly seen as five in number and hypurals were indicated as basal thickening. Ventral fin buds were formed. Up to this time the body of the larva was colourless, except for the black pigment. The phenomenon of aerial respiration was observed after 13 days of development.

Fifteen days old post larva

At this stage average length of the larvae was measured as 14.8±0.2 mm and post anal length was 6.4

Table 3. Summary of Larval development of *Channa striatus*

Time after hatching	Characteristics
0 hr(at hatching)	Average length 3.2 ±0.2 mm, faintly brown in colour, well defined yolk sac, with a transparent fin fold encircling the body. Heart was functional, mouth and anus were absent.
4.00 hr	3.5 ± 0.2 mm long, un pigmented eyes a conspicuous depression identified the position of the mouth. Larvae converged in cluster, few started swimming to a long distance. They cover a distances of 30 cm^2 swimming in a spiral fashion.
8.00 hr	Length 3.9±0.3 mm, displays dorso ventral unpaired fin, heart, brain and ventricles distinct, few small sized melanophores appeared on the head region, ventral side of the notochord and the dorsal side of the body. Many larvae became active and would passively to the water surface and negatively phototrophic. They were very sensitive to bright light.
16.00 hr	Average length 4.2±0.4 mm, dark prominent eyespot on the anterior part of the head, caudal fin begins to separate. Buccal invagination appeared. Pectoral fin buds and swim bladder formed, heart positioned in front of the yolk.
36.00 hr	Average length 5.1 ±0.2mm and post anal length 2.6 mm, pectoral fin round shaped, mouth formed as a terminal opening, the lower jaw is less developed, vent formed. Rudimentary gill opening and nartial pits differentiated.
48.00 hr	Average length 5.4 ±0.3mm and anal length 2.7 mm mouth formed with well-developed lower jaw. Vent and gill rudiments clearly visible, pectoral fins paddle shaped, Larvae move horizontally in shoals and swam haphazardly to the water surface. Larvae feeding exogenously.
3 day	Average length 5.8 ±0.5mm and post anal length 2.9 mm, head prominent, pectoral fin flap like, yolk sac absorbed, body bilobed, respiratory movements arise and larvae swam vigorously.
6 day	Average length 7.4±0.3 mm and post anal length 3.5 mm, yellow pigments on the dorsal and lateral sides appear as bands, pectoral fins clearly recognizable. caudal cartilage appear, eyeballs are large and distinct.
10 day	Average length 10.8 ±0.5 mm and post anal length 5.2mm, colour bands are more distinct, five caudal fin rays. Dorsal fin separate from caudal fin, ventral fin buds formed, fry come to water surface to gulp air
15 day	Average length 14.8±0.2 mm and post anal length 6.4mm. Basal thickening appears in the dorsal and ventral fin folds. Eight caudal fin rays distinguished. Silver and greenish pigments appeared on the orbital rim and on the postorbital regions.
20 day	Average length 22.9±0.5 mm and post anal length 14.2 mm, fry assumes adult character, dorsal, anal and caudal fins clearly differentiated. Larvae continuously hunt for feed with only few hours of rest. These behaviours continued till the end of larval period.
35 day	Average length 42.4 ± 0.2 mm and post anal length 22.6 mm, fry assumes almost all the adult characters, dorsal, anal and caudal fins become clearly differentiated

mm. The post larvae metamorphosed to orange colour. Basal muscle thickening appeared in the dorsal and ventral fin folds.

Twenty days old fry

Average length of 22.9±0.5 mm and post anal length of 10.2 mm were measured at this stage. The characteristic yellow bands and dark lateral bands were prominent. At this stage ontogeny was completed and the fry assumed nearly the adult stage, except the colour pattern. Fry swam actively in shoals and were observed to voraciously feed on plankton. Caudal, anal, dorsal and pectoral fin rays were fully developed. The anal and the dorsal fins were confluent with caudal by narrow flanges.

Thirty days old fingerling

At this stage average length was 42.4±0.2 mm and post anal length was 22.6 mm;fry assumed almost all the adult characteristics, dorsal, anal and caudal fins became clearly differentiated. Cannibalism was observed until size difference was minimized. The anal rays appeared in the middle of the fin, separated by an interval from the basal line and surrounded by dense pigment. The dorsal rays raised from the basal line and the pigmentation was sparse. The larvae were now swimming near the bottom of the shallow aquarium in which they were reared and came to the surface to take air. On the thirtieth day the larva gulped the air once a minute, eight times in eight minutes, each time leaving a small air-bubble, at the surface. As soon as the fin-rays were properly laid down and their outlines darkened by pigment, the amount of pigment in the dorsal and anal fins became equalized. The other characters, at this stage were; the broad yellow lateral stripe, a short yellow band in front of the dorsal fin culminating in a shining golden occipital spot, and a golden yellow mark over each eye.

Fin development

Dorsal and anal fin rays started appearing in flexion larvae larger than 7.4 mm BL after day 6. These fins showed segmentation on day 15th onwards and the development was completed on day 28 (26.6 mm BL). The number of fin rays was 30 – 33 in dorsal and 20 – 21 in anal fin. Pectoral, caudal and pelvic fins were seen on day 1 (4.8 mm BL), day 4 (6.5 mm BL) and day 8 (10.5 mm BL) respectively and segmentation was observed on day 13 (14.2 mm), day 10 (10.8 mm) and day 19 (22. 7 mm) respectively. The development of pectoral and pelvic fins was completed on day 26 (26.6 mm), whereas caudal fin completed its development on day 16 (14.9 mm). Dorsal, anal and caudal fins were differentiated in juvenile stage where 43 – 47 dorsal fin rays, 16 – 20 caudal fin rays, 25 – 28 anal fin and 20 – 24 pectoral fin rays were clearly seen.

Discussion

Synthetic analogues of LHRH have been effectively used to induce ovulation and spawning in a number of commercially important finfishes (Tucker, 1994; Mylonas and Zohar, 2001). Analogues of LHRH have often been favoured over gonadotropic hormones (pituitary extracts and HCG) because they are non-immunogenic and provide more integrated control of final oocyte maturation (FOM) and ovulation (Zohar and Mylonas, 2001). In the present study, ovulation was induced in striped murrel *C. striatus* during the natural spawning season (October, 2009). Under these conditions, LHRHa administered as a single dose was effective for inducing ovulation. Considering striped murrel in spawning condition, it appears that the degree of ovarian development may be more important than the gonadotropic-inducing agent used to stimulate ovulation. Given the success obtained using HCG to induce ovulation in many serranid species (Tucker, 1994; Watanabe *et al.* 1995) and its recent approval by the U.S. Food and Drug Administration for use on broodstock, a direct comparison of LHRHa and HCG is justified. The efficacy of LHRH analogues has also been shown to be influenced by dose, primary peptide structure, and water temperature (Harmin and Crim, 1992; King and Pankhurst, 2004).

Under captive conditions striped murrel *C. striatus* was successfully bred by the administration of LHRHa. Previous studies of black sea bass dem-

Table 4. Body length (mm) and age in day at each developmental stage

Stage	BL (mm)	Age in day	*n*
Yolksac stage	3.2 – 6.8	0 – 5	36
Pre flexion larva	6.8 – 12.2	5 – 12	20
Flexion larva	12.2 – 22.7	12 – 19	25
Post-Flexion larva	22.7 – 26.5	19 - 25	20
Juvenile	> 42.4	> 30	10

BL- Body Length; *n* – -No of fishes

onstrated that a single cholesterol– cellulose pellet containing 50 µg LHRHa (approximately 50 µg/kg) successfully induced multiple spawning events over a prolonged period (Watanabe *et al.* 2003). In the present study, the same dose of 50µg/Kg was also administered to pre-spawning striped murrel. All fish in this condition ovulated low quantities of eggs with low fertilization rate. While the optimal dose of LHRHa has not been established for striped murrel, administration of this hormone at the dosage of 70 µg/kg was effective for inducing ovulation without decreasing fecundity or fertility. The responsiveness of *C. striatus* to LHRHa may change over time and for the first and subsequent spawns. Further research is necessary to establish the ovulatory cycle and period of over-ripening in order to minimize fish handling and preserve egg quality.

Breeding behaviour or courtship behaviour is a very important act in fish breeding. It varies from the simple swimming of the breeders along the side of each other to the elaborate act of nest building and intense male competition inherent in group spawning. Spawning depends not only on gametogenesis but also on behaviour such as pre spawning migration, habitat selection, mate selection and courtship. In *C. striatus* the breeding behaviour was noticed within 6 hrs after the hormonal injection and continued till spawning upto 22 – 26 hrs. These results are similar to those reported previously viz: *Channa punctatus* (Haniffa *et al.,* 2004), *Ananbas testudineus* (Johannessen *et al.,* 1993), *Clarias batrachus* (Moitra *et al.,* 1979), *Heteropneutes fossilis* (Thakur, 1976), *Hopilas malabaricus* (Prado et al. 2006) and *Channa gachua* (Milton, 2011). Similarly the observation of the unpaired male remained passive in a corner of the breeding tank was observed in *A. testudineus* (Moitra *et al.,* 1979) and *C. batracus* (Thakur, 1976).

Egg guarding is the most common form of parental care (Clutton – Brock, 1991) and in a majority of species only one parent involves in parental care. Among the teleost families male care is much more common than female care with 61% against 39% respectively; biparental care occurs in less than 25% of the families (Gross and Shine, 1981) as reported by Haniffa *et al* (2004) in induced bred spotted murrel *Channa punctatus*. In the present study parental care was observed in the brooders induced by LHRHa. According to Alikuni (1957), the murrel breeds in natural conditions and both the parents were involved in parental care of eggs upto fry stage. In our study the induced bred striped murrel *C. striatus* bred in fibre tank which is the first of its kind and showed biparental care but male showed much care towards their youngones. Moreover when the eggs were removed and incubated without parental care, they were affected by fungal infection resulting in poor hatching.

In the present study the dosage of the hormone LHRHa was selected based on previous reports for induced spawning in murrels and catfishes and found to be in the range of 50µg - 70 µg for LHRHa (Haniffa *et al.* 2000; Francis 1996; Mollah and Tan 1983; Zarrin *et al.* 1992 and Zonneveld, 1988). Spawning was observed in almost all attempts irrespective of doses of LHRHa but in case of low dose injected fish, no spawning was observed in one trial. Spawning was complete in the medium and high doses, whereas partial spawning was observed in the low dose. Similar results were reported in *C. striatus* showing complete spawning for medium and high doses of HCG and Ovaprim, whereas low dose of Ovaprim injected fish did not respond (Haniffa *et al.* 2000).

In the present study average latency period of *C. striatus* was noticed as 22.4 ± 0.8 hrs. Froud Bosak (2010) recorded a latency period of 24.42 ± 0.2h for *Barbus sharpeyi* when injected with LHRHa + CPE. The latency period was longer than those of LHRHa + MET treated *Cyprinus carpio* (14 – 16 hr) (Drori *et al.* 1994) and Ovatide treated *Clarias batrachus* (17 hr) (Sahoo *et al.* 2005). In contrast, a latency period of 28 – 30 hrs was observed for LHRHa administered Common carp (Muhammed *et al.* 2001). Seyed Abdolsaheb (2010) reported a latency period of 27 hr for *Barbus xanthopterus* injected with LHRHa. In the present study, among the three doses of LHRHa, better result was obtained in the high dose (70µg / Kg body weight) with regard to number of eggs spawned, fertilization rate and hatching rate. Kumarasini and Seneviratue (1988) conducted experiments on induced breeding of *Cyprinus carpio* using LHRHa and reported 100% fertilization rate.

Considering the overall performance of three doses of LHRHa with regard to, latency period, number of eggs spawned, fertilization rate and hatching rate, the present investigation confirms that high dose (70µg / Kg body weight) was found to be the most potent ovulating dose in *C. striatus.* The efficacy of the different dosages can then be summarized as high dose > medium dose > low dose. Thus it is obvious that LHRHa at a dosage of

70µg / Kg BW can be recommended for higher and better results in breeding attempts of *C.striatus*. Hence in the view of conservation of striped murrel *C.striatus,* LHRHa at the dosage of 70µg / Kg body BW can be suggested for successful breeding.

The present study was conducted to investigate and also to provide detailed information about the embryonic and larval development with morphological and meristic characteristics of this important food fish species. The egg membrane is fully separated from the egg and has a small privitelline space which is filled with fluids and this fluid cushion may protect the eggs and the embryo from any external injury (Khan, 1972). The fertilized egg diameter of *C. striatus* ranged from 1.22 to 1.45 mm. High variation in egg sizes is also recorded in African catfish, *C. gariepinus* by different authors (Bruton 1979; Zaki and Abdula 1983; Herath 1988; Verreth *et al.* 1993). This might be related to the existence of different strains, conditions, and size of the female in the wild conditions (Thakur, 1980). The fertilized eggs of *C. striatus* were adhesive, as in the case of *Clarias batrachus, Mystus montanus* (Jerdon) and *Pangasius sutchi* (Fowler) (Arockiaraj *et al.* 2003; Islam, 2005).

All the teleostean fish species showed a discoidal meroblastic cleavage, where the large yolk materials restrict cell division to a small area at the animal pole close to the micropyle (Hall *et al*., 2004). The mode of cleavage recorded in the present observation is similar to other catfish species *Pungasius Sutchi* (Islam, 2005) and *Mystus montanus* (Arockiaraj *et al.* 2003). However, inter- and intraspecific variation of the cleavage pattern was reported in the embryo of Atlantic cod, *Gadus morhua* (Hall *et al.* 2004).

In the present study the first cleavage occurred within 15-20 min and the 16-cell stage was reached in 40 -55 min of postfertilization. Thakur *et al.* (1974) observed that the first cleavage, 16 cell, and morula stages in *Heteropneustes fossilis* were attained within 30, 70-80 and 100 min, respectively after fertilization. In *C. punctatus*, eggs attained the 16-cell stage in 45 min after fertilization (Banerji 1974). In the present observation, the gastrula stage was reached in 9 hrs after fertilization. Banerji (1974) and Munshi and Hughes (1991) reported in the blastula stage appeared after 2-3 h *C. Punctatus* and the yolk invasion was completed 9 hrs after fertilization. In *A. testudineus*, the invasion of the yolk by the blastoderm was completed about 10 hr after spawning (Munshi and Hughes, 1991).

Just 1-2 hr before hatching, the embryo of *C. striatus* showed twisting movements inside the egg envelops. Similar hatching behaviour is reported in *H. fossilis* (Thakur *et al.* 1974), *C. batrachus* (Thakur, 1980) and *P. sutchi* (Islam, 2005). The observation of early development and pre hatching behaviour of embryo in *C. striatus* agrees well with the results obtained in *C. gariepinus* (Zaki and Abdula, 1983; Herath, 1988) and in *M. montanus* (Arockiaraj *et al.* 2003).

In the present study hatching took place about 22-26 hr after fertilization at a temperature of 30 ± 1.5^{0}C. Kohli and Vidyarthi, (1990) reported in *H. fossilis* at a temperature of 26°C the incubation period of the eggs varied from 16-18 hrs. Banerji (1974) reported hatching of *C. punctatus* took place 24 hrs at a temperature of 28°C. Munshi and Hughes (1991) reported the incubation period in *A. testudineus* was around 10.5 hr after fertilization. The development and incubation periods of embryo in most fishes are fully temperature-dependent and varied from species to species (De Graaf and Janssen, 1996).

The newly hatched larva of the candidate species was 2.8 - 3.6 mm in length. According to Parameshwaran and Kamal (1988) the length of newly hatched snakehead hatchlings were as follows: 3.88-4.47 mm in *C. marulius,* 2.81-3.22 mm in *C. striatus,* 2.49-2.70 mm in *C. punctatus* and that of the yolk absorbed larva were 6.8-7.1 mm in *C. marulius,* 5.3-6.1 mm in *C. striatus and* 4.6-4.9 mm in *C. punctatus.* Observations made on the newly hatched larvae in *H. longifilis* recorded the length of 4.09-4.9 mm by Ogunji and Rahe (1999) but females of different ages and sizes may produce larger larvae. Mookerjee and Mazumdar (1950) reported a mean length of 5.8 mm for *C. batrachus,* while Bruton (1979) reported 3.6 mm for *C. gariepinus.* These variations can be related to the size of eggs. According to Bagarinao and Chua (1986) egg diameters are positively correlated with larval length and weight at hatching. The larvae actively exhibited tail-wagging movements. This may probably be useful to cutaneous respiration and aid to free the larvae from the substrate (egg shell), which could be a potential site for bacterial growth and infection (Ogunji and Rahe, 1999).

In newly hatched larvae the mouth did not open but the heart was functional. The heart of *C. punctatus* became distinct and the anal invagination appeared on the ventral side 3 hrs after hatching

(Munshi and Hughes, 1991). In the present study mouth was opened 36 hr after hatching. But in *H. longifils* Ogunji and Rahe (1999) reported the mouth opening 3-4 hr after hatching, and its first feeding took place 12 hr after the mouth opening. Ogunji and Rahe (1999) also reported the first feeding of *H. longifilis* larvae 48 hr after hatching. In the present study alimentary canal was observed 48 hr after hatching. However it was not fully developed physiologically.

The reserved yolk material was completely absorbed in 3 days old larvae in *C. striatus.* The yolksac of *H. longifilis* was fully resorbed 55 hr after hatching, (Ogunji and Rahe, 1999). The yolk sac of *Mystus montanus* was fully reabsorbed only after 3rd day when the larvae reached a length of 5-5.5 mm (Raj *et al.* 2003). Verreth *et al*. (1992) observed that the morphological and functional development of the stomach was not completed at the onset of exogenous feeding in *C. gariepinus.* In the *C. striatus* yolk sac was absorbed within three days after hatching.

In the present study aerial breathing of larvae was observed on 10th day after hatching. Similarly in other air breathing fishes the habit was observed from 12-13 days after hatching in *A. testudineus* (Munshi and Hugesh, 1991) and 14th day in *C. marulius* (Parameshwaran and Murugesan (1976b). The active movement and capture of prey by larva were noticed when it attained an average length of 10.8 mm on the 10th day onwards. This is likely due to the development of the extended caudal fin. It was noticed that *C. striatus* larvae metamorphosed only when they grew to a total length of 22.9 mm on 20th day.

Feeding was started on day 2 and yolk absorption was completed by day 3 in the present study, indicating that *C. striatus* has a preparatory period of less than one day for the shift from endogenous to exogenous energy-dependent period. Such a shift has been examined in several marine fish species, covering aspects of development and survival during the larval stages (Kohno, 1998; Moteki *et al.* 2001). However, similar investigations on freshwater fishes, including *C. striatus*, are still limited despite being necessary for any improvement in seed production, although few findings have been presented in recent years; e.g., North African catfish *Clarias gariepinus* (Matsumoto *et al.* 2001), climbing perch *Anabas testudineus* (Morioka *et al.* 2009) and snakeskin gourami *Trichogaster pectoralis* (Morioka *et al.* 2009). The preparatory periods for the shift from endogenous to exogenous energy-dependent periods in the aforementioned three species were 5, 5, and 10 days, respectively—much longer than that observed for *C. Striatus* suggesting that earlier feeding success is more important in *C. striatus*.

Conclusion

Freshwater aquaculture entrepreneurs and fish farmers in India are fully engaged in carp and catfish culture. Fish farmers are lacking knowledge about breeding, feeding of early larval stages and non availability of seeds of snakeheads. The short embryonic period or incubation period and fast organ development and air breathing habits starting at 10th day after hatching of this species *C. striatus* suggest that it is a suitable and potential species for small scale fish farmers for commercial culture and income generation.

Acknowledgement

We acknowledge the financial assistance received from Indian Council of Agricultural Research – National Agricultural Innovation Project (ICAR-NAIP F.No. 1 (5) 2007-NAIP dt.22 August 2008) to carry out this study. We are grateful to Rev. Dr.Alphonse Manickam S.J., Principal and Consortium leader, for providing necessasary facilities.

References

Alok, D., T. Krishnan, Talwar, G.P. and Garg, L.C. 1993. Induced spawning of cat fish *Meter opneustes fossilis* (Bloch), using D-Lys super (6) salmon gonadotropin releasing hormone analog. *Aquaculture,* 115: 159-167.

Alikunhi, K. H. 1957. Fish culture in India. *Fm.Bull. Indian. Coun. Agric. Res.* 20 : 144 p.

Arockia Raj A.J., Haniffa M.A., Seetharaman S., and Singh S.P. 2003. Early development of threatened freshwater catfish *Mystus montanus* (Jerdon), Acta Zoologica Taiwanica. 14(1) : 1-10.

Banerjee, S.R. 1974. Hypophysation and life history of *Channa punctatus* (Bloch). *J. Inland Fish. Soc. Ind.,* 6: 62-73.

Bagarinao, T. and Chua, T.E. 1986. Egg size and larval size among teleosts: Implications to survival potential. *In*: Maclean, J. L., L. B Dizon and L. V Holsilos (eds.), The first Asian fisheries forum. *Asian Fisheries Society,* Manila, Philippines, Pp. 651-656.

Borcato, F.L., Bazzoli, N. and Sato, Y. 2004. Embriogenesis and larval ontogeny of the Pirugordura, *Leporinus piau* (Fowler) (Pisces, Anostomidae) after induced

spawning. *Revista Brasileira de Zoologia.* 21: 117-122.
Bruton, M. N. 1979. The breeding and early development of *Clarias gariepinus* (Pisces: Clariidae) in lake sibaya South Africa, with a review of breeding in species of the subgenus Clarias (Clarias). *Trans. Zoo. Soc. Lond.,* 35: 1-45.
Clutton-Brock, T.H. 1991. The evolution of parental care. – Princeton University Press, Princeton.
De Graaf, G.J. and Janssen, H. 1996 Artificial reproduction and pond rearing of the African catfish Clarias gariepinus in sub Saharan Africa. *FAO Fisheries Technical Papers* 362: 1-73.
Drori, S., Ofir, M., Sivan, B.L. and Yaron, Z. 1994. Spawning inductionin common carp (*Cyprinus carpio*) using pituitary extract or GnRH superactive analogue combined with methoclopramide: analysis of hormone profile, progress of oocyte maturation and dependence ontemperature. *Aquaculture.* 119: 393-407.
Parameswaran, S. and Murugesan, V.K. 1976. Breeding season and seed resources of murrels in swamps of Karnataka state. *Journal of the Inland Fisheries Society of India.* 8 : 60-67.
Mollah, M.F.A. and Tan, E.S.P. 1983. HCG – induced spawning of the catfish, *Clarius macrocepalus* (Gunther). *Aquaculture*. 35 : 239–247.
Inyang, N. M. and Hettiarachchi, M. 1994. Efficacy of human chorionic gonadotropin (HCG) and crude extract of fish and frog in oocyte maturation and ovulations in African catfish *Clarias gariepinus* Burchell. *Aquacult. Fish. Manag.* 25 : 245-258.
Fermin, J. D. T. 1992. Induction of oocyte maturation and ovulation in the freshwater Asian catfish. *Clarius macrocephalus* by LHRHa and pimozide. *J. Appl. Ichtyol*. 8: 90-98.
Francis, T. 1996. *Studies on the effect of pituitary hormones and feeds on the reproduction of Heteropneustes fossilis* (Bloch). *Ph. D thesis.* Tamil Nadu Veterinary and Animal Sciences University, Madras, India.
Hall, T.E., Smith, P. and Johnston, I.A. 2004. Stages of embryonic development in the Atlantic cod *Gadusmorhua*. *J. of Morphology.* 259 : 255-270.
Haniffa, M.A., J. Shaik Mohamed. and T. Merlinrose 1996. Induction of ovulation in *Channa striatus* (Bloch) by SGnRH. *Fishing Chimes*, Pp. 23-24.
Haniffa, M. A., T. Merlin and Mohamed, J. S. 2000. Induced spawning of the striped murrel *Channa striatus* using pituitary extracts, human choronic gonadotropin and luteinzing hormone releasing analogue and ovaprim. *After. Icht. Piscat.* 30 : 53-60.
Haniffa, M.A. Nagarajan, M. Marimuthu, K. and Jesu Arockia raj, A. 2003. Embryonic and larval development of spotted murrel, *Channa punctatus* (Bloch). *Indian J. Fish.* 50(3) : 355-362.
Haniffa,M.A., Marimuthu K., Nagarajan M., Jesu Arockia Raj A. and Kumar D. 2004. Breeding behaviour and parental care of the induced bred spotted murrel *Channa punctatus* under captivity. *Curr Sci.* 86: 10.
Harmin, S.A. and Crim, L.W. 1992. Gonadotropic hormone-releasing hormone analog (GnRH-a) induced ovulation and spawning in female winter flounder, Pseudopleuronectes americanus (Walbaum). *Aquaculture.* 104: 375–390.
Herath, H.K.S. 1988. Hybridization, early development of embryos and production characteristics of larvae of African Catfish *Clarias gariepinus* (Burchell) and Asian catfish *Clarias batrachus* (Linnaeus). *Master Thesis*, Wageningen Agricultural University. Wageningen, the Netherlands.
Islam, A. 2005. Embryonic and larval development of Thai pangas (*Pangasius sutchi* Fowler 1937). Development. *Growth and Differentiation.* 47 : 1-6.
Johannessen T., Gjosaeter J. and Moksness, E. 1993. *Aquaculture*. 115 : 41–51.
King, H.R. and Pankhurst, N.W. 2004. Effect of maintenance at elevated temperatures on ovulation and luteinizing hormone releasing hormone analogue responsiveness of female Atlantic salmon (Salmo salar) in Tasmania. *Aquaculture.* 223: 583–597
Khan, H.A. 1972. Induced breeding of air breathing fishes. Central International Fish Res. Institute. Barrackpore. Indian farming. Indian Council of Agricultural Res. Krishi Bhavan, New Delhi.
Khan, M.M.R. and Mollah, M.F.A. 1998. Embryonic and larval development of African catfish, *Clarias gariepinus* (Burchell). Bangladesh *Journal of Fisheries.* 21 : 91-97.
Kohno, H. 1998. Early life history features influencing larval survival of cultivated tropical finfish. In: De Silva SS (ed) *Tropical Mariculture*. Academic, London, pp 71–111.
Koumonundouros, G., Divanach, P. and Kentouri, M. 2001. Osteological development of *Dentex dentex* (Osteichthyes: Sparidae). Dorsal, anal, paired fins and squamation. *Marine Biology.* 138 : 399-406.
Kumarasiri, W.S.A.A.L. and Seneviratne, P. 1988. Induced multiple spawnings of Chinese carps in Sri Lanka. *Aquaculture.* (74) : 57-62.
Rahman, M.R., Rahman, M.A., Khan, M.N. and Hussain, M.G. 2004. Observation on the embryonic and larval development of silurid catfish, gulsha (*Mystus cavasius* Ham). *Pakistan Journal of Biological Sciences* 7(6): 1070-1075.
Matsumoto, S., Morioka, S. and Kumagai, S. 2001. Development of African catfish Clarias gariepinus larvae during the transitional phase between endogenous and exogenous energy intake. In: Weyl OLF, Weyl MV (eds) *Proceedings of the Lake Malawi Fisheries Management Symposium.* Department of Fishery, Lilongwe, pp 227–232.
Milton, 2011. *Aquaculture Potentials of Threatened Snakehead by Seed Production and Larvi Culture Technologies.*

Ph.D thesis submitted to Manonmanium Sundaranar University, Tirunelveli, Tamilnadu, Pp 1-100.

Moitra, A., A. Pamdey., T.K. Ghose and J.S.D. Munshi 1979. Spawning behavior, postembryonic development and culture of *Anabas testudinues* (Bloch). In symposim on Inland Aquaculture held at CIFRI Barrackpore. *West Bengal.* Abst. 3 : 2-3.

Moteki, S., Yoseda, K., Sahin T., U¨stu¨ndag¢ C. and Kohno, H. 2001. Transition from endogenous to exogenous nutritional sources in larval Black Sea turbot *Psetta maxima*. *Fish Sci*. 67 : 571–578.

Mookherjee, H.K. 1945. Life histories of some carnivorous fishes of Bengal. *Sci and Cult.* 11 : 102-103.

Morioka, S., Kitamura, S. and Vongvichith, B. 2009a. Growth and morphological development of laboratory-reared larval and juvenile climbing perch Anabas testudineus. *Ichthyol Res*. 56 : 162–171.

Munro, A.D. 1990. General introduction. *In*: A.D. Munro, A.R. Scott and T.J. Lam (eds.) *Reproductive seasonality in teleosts environmental influences*. CRC Press, Boca Raton, Florida, USA. pp. 1-11

Munshi, J.S.D. and Hughes, G.M. 1991. Air Breathing Fishes of India. Oxford & IBH Publishing Co pvt. Ltd. New Delhi. P. 181 – 208: 289–294.

Mylonas, C.C. and Zohar, Y. 2001. Use of GnRHa-delivery systems for the control of reproduction in fish. *Rev. Fish Biol. Fish*. 10:463– 491

Ogunji, J.O. and Rahe, R.E. 1999. Larval development of the African catfish *Heterobranchus longifilis* VAL, 1840 (Teleostei; Claridae) and its larval behavior. *Journal of Aquaculture in the Tropics.* 14: 11-25.

Prado., 2006. Spawning and parental care in *Hoplius malabaricus* in the southern pantanal, Brazil. Braz. *J. Biol.* 66(2B) : 697-702.

Parameshwaran, S. and Kamal, M.Y. 1988. Synoposis of biological data on the faint murrel, *channa marulius,* and the spotted murel *Channa punctatus,* Central Indland Capture fisheries Research Institute, Barrackpore, India. *Bulletin.* 53 : 77.

Parameswaran, S. and Murugesan, V. K. 1976b. Observations on the hyopophysation of murrels (Ophiocephalidae): *Hydrobiologia.* 50(1): 289-316.

Raj, A. J., Haniffa, M.A., Seetharaman, S. and Singh, S.P. 2003. Early development of a threatened freshwater catfish *Mystus montanus* (Jerdon). *Acta Zoologica Taiwanica,* 14: 23-32.

Sahoo, S. K., Giri, S.S. and Sahu, A.K. 2005. Effect on breeding per- formance and egg quality of *Clarias batrachus* (Linn.) at various doses of Ovatide during spawning induction. *Asian Fisheries Science.* 18:77-83.

Seyed Abdolsaheb Mortezavizadeh., Mohammad, Y.F. and Froud, B.K. 2010. Efferct of GnRHa, LHRH and carp pituitary in artificial propagation of gattan, *Barbus xanthopetrus* (heckle, 1843). *World J.of fish and Mar. Sci*. 2(4) : 280 – 284.

Segner, H., Rosch, R., Verreth, J. and Witt, U. 1993. Larval nutritional physiology: Studies with *Clarias gariepinus*, *Coregonus lavaraetus* and *Scophthalamus maximus*. *Journal of World Aquaculture Society*. 24: 121-134.

Tucker Jr., J.W. 1994. Spawning by captive serranid fishes: a review. *J. World Aquac. Soc.* 25 : 345–359.

Thakur, N.K., Pal R.N. and Khan H.A. 1974. Embryonic and larval development of *H.fossilis* (Bloch). *J. Inland. Fish. Soc. India*. 6: 33-44.

Verreth, J., E. Torreele, E. Sparzier and A. Slurszen 1992. The development of a functional digestive system in *Clarias gariepinus* (Burchell). *J. World Aqua. Soc.* 23: 286-998.

Watanabe, W.O., Ellis, S.C., Ellis, E.P., Head, W.D., Kelley, C.D., Moriwake, A., Lee, C. and Bienfang, P.K. 1995. Progress in controlled breeding of Nassau grouper (*Epinephelus striatus*) broodstock by hormone induction. *Aquaculture*. 138 : 205– 219.

Watanabe, W.O., Smith, T.I.J., Berlinsky, D.L., Woolridge, C.A., Stuart, K.R., Copeland, K.A. and Denson, M.R., 2003. Volitional spawning of black sea bass (*Centropristis striata*) induced with pelleted luteinizing hormone releasing hormone-analogue. *J. World Aquac. Soc.* 34: 319–331.

Verreth, J., E. Torreele., E. Sparzier and A. Slurszen 1992. The development of a functional digestive system in *Clarias gariepinus* (Burchell). *J. World Aqua. Soc.* 23 : 286-998.

Zaki, M.I. and Abdula, A. 1983. The reproduction and development of *Clarias gariepinus* (Claridae) from Lake Manzala (Egypt). *Journal of Ichthyology.* 23: 48-58.

Zairin, M. Jr., K. Furukawa and Aida, K. 1992. Induction of ovulation by HCG infection in the tropical walking catfish *Clarias batrachus* reared under 23-25 °C

Zohar, Y., Mylonas, C. 2001. Endocrine manipulations of spawning in cultured fish: from hormones to genes. *Aquaculture* 197:99–136

Zonneveld N., Rustidja, W.J.A.R. Viveen, Mudana and Wayan, 1988. Induced spawning of egg incubation of the Asian catfish, *Clarias batrachus. Aquaculture.* 74: 41-47.

Gross, M.R. and Shine, R. 1981. Parental care and mode of fertilization in ectothermic vertebrates. *Evolution. 35*(4): 775-793.