Bangladesh J. Fish. Res., 6(1), 2002: 11-18

Early developmental stages of *Nandus nandus* (Ham.)

M. Das*, M.A.K. Tarafder and S. Pal
Department of Aquaculture, Bangladesh Agricultural University
Mymensingh 2202, Bangladesh
* Corresponding author

Abstract
In order to study the early developmental stages of *Nandus nandus* an experiment was conducted, where eggs and milt were obtained from the laboratory reared *N. nandus* by stripping after 15 hours of 150 mg/kg body weight of carp PG extract injection. Then the eggs were fertilized in the laboratory and subsequent developmental stages were studied. First cleavage (two cell), four cell, eight cell, sixteen cell and multi cell stages were found 30, 50, 70, 105 and 160 minutes after fertilization respectively. Morula, early gastrula, middle gastrula, late gastrula and yolk plug stages were found 5, 8, 9, 11 and 13 hours after fertilization respectively. Hatching occurred within 20±2 hours after fertilization, and larvae were measured 1.60 mm in diameter. After one hour of hatching two melanophore bands were found at the caudal region of the body of the larvae. Eyes were first observed in 10 hours, pectoral and pelvic fin buds appeared in 22 hours and well developed in 38 hours old larvae. Mouth cleft and brain lobes were visible when the larvae were 34 and 38 hours old respectively. Myomeres partially appeared in 16 hours, which were clearly visible in 74 hours old larvae. Larvae started wandering and searching for food after 56 hours of hatching. The yolk sac was completely absorbed when the larvae became 62 hours old.
Key words: Early developmental stages, *N. nandus*

Introduction

Nandus nandus locally known as 'Nondoi', 'Meni' or 'Veda' is a common freshwater small carnivorous fish (Mustafa *et al.* 1980) of Bangladesh. It was commonly found in natural water bodies of the country. The fish is going to be rarely available and now a days is considered to be an endangered or threatened species. The species should be protected from being extinct. In view of this the species should be studied thoroughly to take measures for its available in quantities in the natural water bodies of Bangladesh. In view of this, proper domestication techniques should be developed and for this, biological data on the species should be as complete as possible.

Embryonic and larval stages of a fish are the most delicate part of their life. Fish early life stages are especially sensitive to stress and some times succumbed to death in mass due to the unavailability of appropriate quality and quantity of larval food especially at the first feeding. For successful rearing what should be done and when should be done is very important. Although the species have been studied for collecting

data on disease and parasites (Chandra and Golder 1987, Golder *et. al.* 1987, Golder and Chandra 1987), on the food and feeding habits and fecundity (Mustafa *et al.* 1980), on the predatory behaviour and fecundity (Akther 1999, Das *et. al.* 2001) and on laboratory rearing of *N. nandus* from young to sexual maturity (Das and Zamal 2000), no published information about early development of the species is available. So it was felt necessary to study and characterize its various stages of embryonic and larval development to understand the biological clock of the species, identify the early life history stages and to detect first feeding time. These biological information will allow us to take appropriate measures in evolving culture techniques of the species.

Materials and methods

The experiment was conducted from August'99 to August'00 in the laboratory of the Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. Eight glass aquaria each having size of 60 × 35 × 30 cm were used to stock the brood fish for obtaining experimental embryo and larvae. Six whole glass aquaria each having size of 30 × 30 × 25 cm were used as egg incubation chamber and subsequently as larval rearing tank. Early developmental stages of *N. nandus* were studied up to 96 hours starting from egg fertilization.

The aquaria were cleaned properly and filled with fresh tap water for the experiment. Sixteen experimental fish of *N. nandus* (8 male + 8 female) were used from a previous experiment conducted by Das and Zamal (2000). Two fish were reared in each aquarium feeding live prawns (*Macrobrachium lamarrei*) once a day (10 am) as has been reported by Das and Zamal (2000). Maturity of fish was first perceived by observing body colour and ejection of eggs and milt by means of gentle pressure on the abdomen of the female and male respectively. The dark brown colour and even size of the eggs also confirmed ripening of the eggs. To obtain fertilized eggs six pairs of mature fish were kept in different aquaria at 10 am July 14'00. 150 mg/kg of carp PG solution was administered intramuscularly on the dorsal region above the lateral line just beneath the base of the dorsal fin at 5 p.m. Injected breeders were kept in pairs in each aquarium. At 6 a.m. of the next morning fishes (2 pairs) started spawning. To observe the exact time of fertilization, male and female fishes of the remaining four pairs were stripped. Ovulated ova were collected on an enamel plate and milt was collected in a glass capillary tube. A drop of milt was poured on the ova. Ova and milt were mixed thoroughly and small amount of water was added to enhance fertilization and water hardening of the eggs. The fertilized eggs on plate were allowed to remain undisturbed for five minutes on the top of the table. The eggs thus obtained were delicately washed several times with tap water and finally transferred in aquaria for incubation. The fertilized eggs were incubated at ambient temperature 28-29°C in the aquaria. Aeration was given to the water of aquaria to keep the oxygen concentration at high level.

Sample was collected (five eggs) randomly from the aquaria every 5 to 10 minutes interval till the completion of morula and then after every one hour interval up to hatching. The larval sample (five larvae) was collected right from hatching up to 96

hours after hatching at hourly interval for first 22 hours and then every 6 hours, as the development stages did not vary very much. Samples were preserved in 70 percent ethanol for further study. Early developmental stages were studied under a stereomicroscope (Olympus SZH10) and an ocular micrometer was used to measure the eggs and larvae. Three individuals from each of the samples were examined for confirmation of developmental stages and the timing of development. Individuals were temporarily stained with methylene blue for clear observation and early stages of *N. nandus* were drawn by hand using a Camera Lucida (Olympus 306681) setting on stereo microscope.

Results

Present study was performed to find out the developmental clock of *N. nandus* for early developmental stages. The stages of embryonic and larval development of *N. nandus* with relation to the time period after fertilization and hatching respectively and characteristic features of each of stages are shown in Tables 1 and 2, and Figs. 1 (A-O) and 2 (A-Q). First cleavage (two cell), four cell, eight cell, sixteen cell and multi cell stages were found 30, 50, 70, 105 and 160 minutes after fertilization respectively. Morula, early gastrula, middle gastrula, late gastrula and yolk plug stages were found 5, 8, 9, 11 and 13 hours after fertilization respectively. Starting of heartbeat was first found at the age of 16 hours. Hatching occurred within 20±2 hours after fertilization, and larvae were measured 1.60 mm in diameter. After one hour of hatching two melanophore bands were found at the caudal region of the body of the larvae. Eyes were first observed in 10 hours, pectoral and pelvic fin buds appeared in 22 hours and well developed in 38 hours old larvae. Mouth cleft and brain lobes were visible when the larvae were 34 and 38 hours old respectively. Myomeres partially appeared in 16 hours, which were clearly visible in 74 hours old larvae. Larvae started wandering and searching for food after 56 hours of hatching. The yolk sac was completely absorbed when the larvae became 62 hours old.

Table 1. Embryonic development of *Nandus nandus* in the laboratory

Phase	Stage No.	Fig. No.	Time after fertilization (hr : min)	Temp. (°C)	Mean total diameter (mm)	Characteristic
Unfertilized eggs	I	1.A	0	28.5	0.6	Eggs spherical, brownish-yellow, demersal and slightly adhesive
Fertilized eggs	II	1.B	0	29	0.8	Eggs slightly adhesive, spherical, demersal and brownish-yellow.
		1.C	0:15	29	0.8	Blastodisc formed at animal pole.
Blastulation	III	1.D	0:30	29	0.9	Start of 1st cleavage which was restricted to small disc of cytoplasm at animal pole, dividing blastodisc into two blastomeres
	IV	1.E	0:50	29	0.9	The second division of the two blastomeres resulted four blastomeres
	V	1.F	1: 10	29	0.9	8 blastomeres formed
	VI	1.G	1: 45	29	0.9	16 cell seen
	VII	1.H	2:55	29	0.9	Multiple cell was visible

Morula	VIII	1.I	5:00	29	1.00	Cap-like structure of blastomeres was visible at the animal pole which gradually increased in size by time
Gastrulation	IX	1. J	8:00	29	1.00	Blastomeres started invading the yolk by spreading over the yolk in the form of a thin layer.
	X	1. K	9:00	29	1.00	Germinal ring was visible which occupy about half of the yolk by blastoderm
	XI	1.L	11:00	29	1.00	Blastoderm covered 3/4th of the yolk. Embryo shield was visible
Yolk plug stage	XII	1.M	13:00	29	1.10	The yolk invasion completed. The head and tail ends become differentiated
Starting o heart beat	XIII	1.N	16:00	29	1.10	Both tail and head-end were clearly visible. Heart was beating
Just befor hatching	XIV	1.O	19±2	29	1.60	Twisting movement become more vigorous and the embryo ruptured the egg capsule, started hatching.

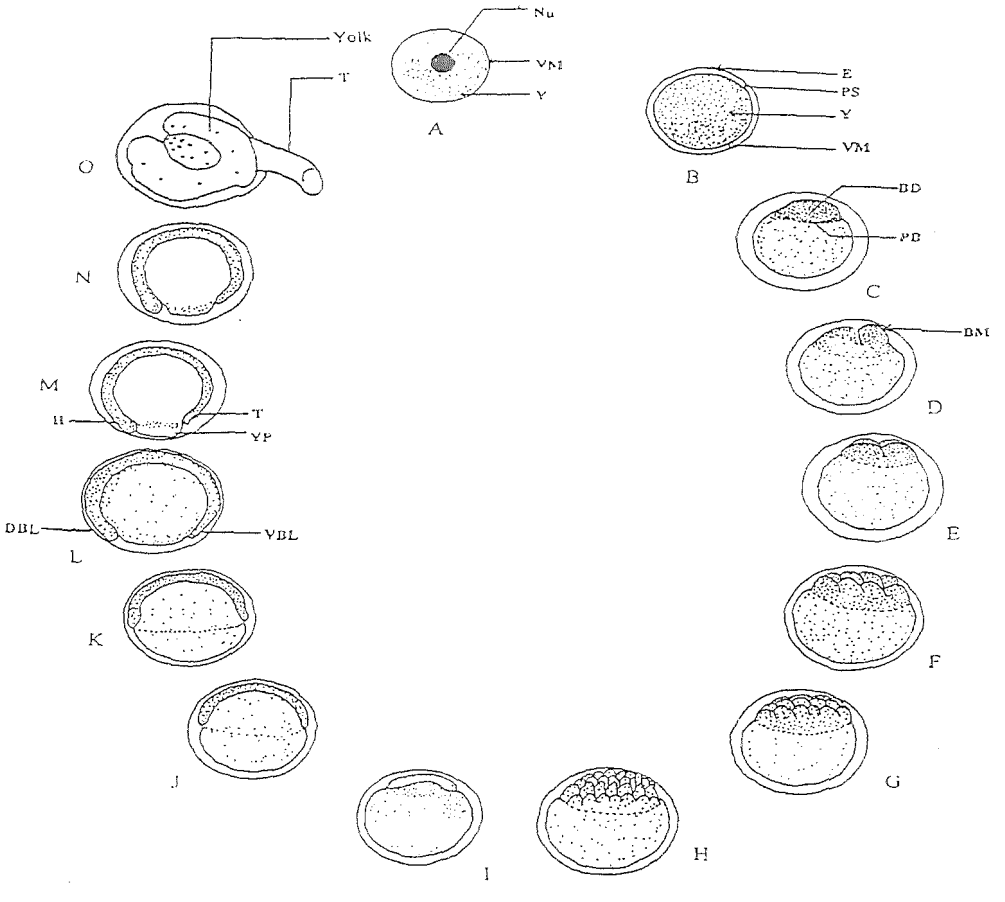

Fig. 1. Embryonic development of *N. nandus* in different time interval.
(A) Unfertilized egg, 0.6 mm in diameter, just after release (B) Fertilized egg, 0.8 mm in diameter, just after fertilization (C) Blastodisc formation, 0.8 mm in diameter, 15 minutes after fertilization (D) Two celled stage,

0.9 mm in diameter, 30 minutes after fertilization (E) Four celled stage, 0.9 mm in diameter, 50 minutes after fertilization (F) Eight celled stage, 0.9 mm in diameter, 70 minutes after fertilization (G) Sixteen celled stage, 0.9 mm in diameter, 105 minutes after fertilization (H) Multi celled stage, 0.9 mm in diameter, 175 minutes after fertilization (I) Morula stage, 1.00 mm in diameter, 5 hours after fertilization (J) Early gastrula, 1.00 mm in diameter, 8 hours after fertilization; (K) Middle gastrula, 1.00 mm in diameter, 9 hours after fertilization; (L) Late gastrula, 1.00 mm in diameter, 11 hours after fertilization (M) Yolk plug stage, 1.1 mm in diameter, 13 hours after fertilization (N) Organogenesis, 1.1 mm in diameter, 16 hours after fertilization (O) Hatching stage,1.6mm, 19±2hours after fertilization.

Table 2. Larval development of *Nandus nandus* in the laboratory

Stage No.	Fig. No.	Age (h)	Mean TL (mm)	Characteristic
I	2.A	0	1.8	Larvae were slender, transparent showing internal organs. The larvae with oval shaped, brownish colored yolk sac.
II	2.B	1	1.9	Body of the larvae yellowish in colour. Yolk sac still remained attached to the body. Two vertical melanophore bands were appeared at the caudal region.
III	2.C	5	2	Body of the larvae was more transparent. Head and body laterally compressed. Yolk sac partially decreased.
IV	2.D	6	2.1	Three melanophore bands were appeared. More melanophores appeared on head, around and/or on the yolk sac. One melanophore band appeared at brain region..
V	2.E	8	2.14	The yolk sac partially reduced. No change in melanophores distribution.
VI	2.F	10	2.19	Yolk sac reduced. Eyes and anus became slightly visible. Intestine was visible.
VII	2.G	14	2.25	Vertical melanophore bands were very much prominent. Melanophores were forming a slander band above the eye around the yolk sac and/or on the yolk sac.
VIII	2.H	16	2.38	Yolk sac slightly decreased. Myomeres were partially visible. Melanophore concentration increased.
IX	2.I	22	2.80	The eyes became pigmented and dark in colour. External melanophore appeared dorsally on head. Myomeres were partially visible. Yolk sac had become thin. Pelvic and pectoral fin-bud appeared.
X	2.J	28	2.92	Prominent pectoral and pelvic fin-bud appeared. Myomeres were slightly visible.
XI	2.K	34	3.10	The colours of larvae were changed to yellowish black. Mouth cleft formed.
XII	2.L	38	3.18	The eyes were increased in size and had become densely pigmented. Mouth cleft became more prominent. Brain lobe was visible. Pectoral and pelvic fin fold well developed.
XIII	2.M	44	3.25	Mouth cleft easily distinguished. Opercula fold appeared. Brain lobe clearly distinguished.
XIV	2.N	50	3.28	The yolk sac very much reduced. Myomeres became more developed. The body became more pigmented.
XV	2.O	56	3.32	Pectoral fin bud became more pronounced. Eyes were fully pigmented. The jaws became well distinguished. The larvae started wandering here and there in search of food.
XVI	2.P	62	3.34	Brian lobe clearly visible. The yolk sac was completely absorbed and the larvae had started feeding.
XVII	2.Q	74	3.38	Myomeres clearly visible and counted 18 post-anal and 5 pre-anal. Larvae were blackish transparent in colour.

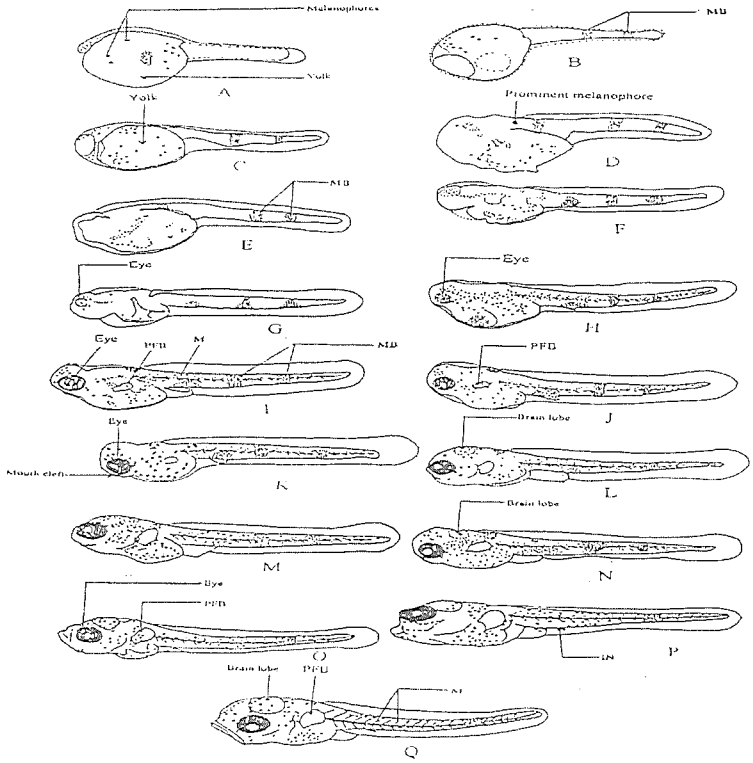

Fig. 2. Larval developmental of *N. nandus* in different time interval.
(A) Hatchling, 1.8 mm in TL (Just after hatching) (B) One hour old larvae,1.8 mm in TL (C) Five hours old larvae, 1.9 mm in TL (D) Six hours old larvae, 2.1 mm in TL (E) Eight hours old larvae,2.14 mm in TL (F) Ten hours old larvae, 2.19 mm in TL (G) Fourteen hours old larvae, 2.25 mm in TL (H) Sixteen hours old larvae, 2.38 mm in TL (I) Twenty two hours old larvae, 2.80 mm in TL (J) Twenty eight hours old larvae, 2.92 mm in TL (K) Thirty four hours old larvae, 3.10 mm in TL; (M) Forty four hours old larvae, 3.25 mm in TL (N) Fifty hours old larvae, 3.28 mm in TL (O) Fifty six hours old larvae, 3.32 mm in TL (P) Sixty two hours old larvae, 3.34 mm in TL and (Q) Seventy four hours old larvae, 3.38 mm in TL.

Discussion

Diagnostic bright reddish colour with black strip was observed in the dermis/skin of male *N. nandus* at the month of April. Female was comparatively dull in colour and their abdominal region was found swollen up. Genital aperture of female fish was found protruded. Eggs and milt were extruded by gentle pressure on the abdomen of the female and male respectively. The mature fish were injected with carp pituitary at the dose of 150 mg/kg body weight (Pal, 2000). The fecundity of *N. nandus* varied from 4858 to 15286. In the present study, the colour of unfertilized eggs of *N. nandus* was found brownish yellow. The findings agree with the findings of Akther (1999) for the same species. Das and Das (1999) found yellowish brown eggs in case of *Notopterus notopterus* and the intensity of that coloration was found varied with the variation of the sources of fish used. The colour of the egg is the complex result of the species specificity, type and amount of food and the inhabiting environment.

Average diameter of unfertilized eggs of *N. nandus* was 0.6 mm immediately after fertilization and 0.8 mm after water hardening. The two cell stage, four cell stage, eight cell stage and 16 cell stage was found 31, 50, 70 and 95 minutes after fertilization. The diameter of the eggs did not change (Table 1) after water hardening although so many physiological and developmental activities were going on. According to Rahman (1975) in case of *Anabus testudineus* same series of stages appeared after 15, 20, 45 and 97 minutes of fertilization respectively. In the present study morula, gastrula and yolk plug stages were found 5, 8-11 and 13 hours after fertilization. The study revealed that heartbeat was observed at 16 hours after fertilization. Whereas Rahman (1975) observed the same in 16.5 hours in case of *A. testudineus* which strongly supports the findings of this experiment. Generally closely related species are also close in their biological clock (Hoar and Randal, 1988). In *N. nandus* hatching was observed 19±2 hours after fertilization. Information is not available on this aspect of the species but several authors (Chakrabarty and Murty 1972, Thakur 1980) observed incubation period of fertilized eggs of some fishes lied between 18-32 hours.

The length of the newly hatched larvae of *N. nandus* was found to be 1.8 mm. Rahman (1975) found the length of the fresh hatchling in case of *A. testudineus* was 1.9 to 2 mm which was more or less similar to the present study. From this study it was found that the pectoral and pelvic fin bud appeared in 22 hours old larvae. Whereas, in case of *A. testudineus* fin buds were found in 14 hours after fertilization (Rahman 1975). This difference might be due to the species variation. Myomeres partially appeared in 16 hours old larvae but in 74 hours old larvae myomeres were clearly visible and counted 18 post-anal and 5 pre-anal. Whereas, Kohinoor *et al.* (1997) found the greater number of thirty six to forty myomeres at newly hatched larvae in case of *O. pabda* of which ten were pre-anal. In this context it can be said that the muscle arrangement is strictly own by the species. Mouth cleft of *N. nandus* had appeared in 34 hours old larvae. The brain lobe was appeared in 38 hours old larvae in the present experiment. Kohinoor *et al.* (1997) found mouth cleft in 12 hours old larvae in case of *O. pabda.* The larvae started feeding at 56 hours after hatching. Barua (1990) found *C. batrachus* larvae started feeding on the 4th day. The larvae started feeding before the completion of yolk absorption keeping required nutrition in hand in case of emergency. The same phenomena of starting external feeding keeping a part of internal food in the yolk sac was reported by Das (1995) for *Carassius auratus* larvae. Conservation is of evolutionary importance for the perpetuation of the species.

In the present experiment it was observed that the yolk sac fully absorbed in 62 hours old larvae. Rahman (1975) and Kohinoor (1997) reported complete yolk absorption in 144 hours old larvae of *A. testudineus* and 48 hours old *O. pabda* larvae at room temperature, which were not similar to the present study, perhaps due to the species variation. The timing of first feeding have evolutionary value and depends on the availability of natural food and synchronized activities of dependable communities in nature. Perhaps those determine the different species to act differently (Nikolsky 1963).

The present experiment provides information on early developmental stages of *N. nandus,* first feeding time for larval rearing enriching the knowledge of biology and

ecology of the fish. The knowledge will help sustainable development of culture as well as management technology of *N. nandus* to protect the species form being extinct. Developments of culture as well as management technology of *N. nandus* will play a substantial role in the overall nutrition of rural people of Bangladesh as it had been playing for long time.

References

Akther, N., 1999. Study of predatory behaviour and fecundity estimation of laboratory reared *Nandus nandus* (Hamilton). M.S. Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. 58 pp.

Barua, G., 1990. Gonadal development and fry rearing of *Clarias batrachus* (Linn.). Ph. D. Thesis, Department of Fisheries Biology and Limnology, Bangladesh Agricultural University, Mymensingh. pp. 205-241

Chakrabarty, R.D. and D.S. Murty, 1972. Life history of Indian major carps. *Cirrhina mrigala* (Ham.), *Catla catla* (Ham,) and *Labeo rohita* (Ham.). *J. Inland Fish. Soc. India*, 4: 132-161.

Chandra, K.J. and M.I. Golder, 1987. Effects of helminth parasites on a freshwater fish *Nandus nandus*. *Environ. Ecol.*, 5: 333-336.

Das, M., 1995. The energetics of stress tolerance in the early life stages of the goldfish *Carassius auratus* L. Ph. D. Thesis, Institute of Aquaculture, University of Stirling, Scotland. 210 pp.

Das, M. and D.R. Das, 1999. A study on the fecundity of feather back, *Notopterus notopterus* (Pallas) from two different sources (pond and beel). *Bangladesh J. Aquaculture*, 21: 53-60.

Das, M., N. Akther, M.A.K. Tarafder and S. Pal, 2001. Predatory behaviour of a perch, *Nandus nandus* (Ham.). *Bang. J. Fish. Res.*, 5(2): 197-200.

Das, M. and N. Zamal, 2000. Domestication of an endangered fish species *Nandus nandus* (Ham.). I. Laboratory rearing of young fish up to sexual maturity. *Bang. J. Fish. Res.*, 4: 135-140.

Golder, M.I. and K.J. Chandra, 1987. Infestation of *Isoparorchis hypselobagri* on the host fish *Nandus nandus*. *Environ. Ecol.*, 5: 337-341.

Golder, M.I., K.J. Chandra and A.K.A. Rahman, 1987. Helminth parasitism in *Nandus nandus* (Hamilton). *Bangladesh J. Fish.*, 10: 11-22.

Hoar, W.S. and D.J. Randall, 1988. *Fish Physiology*, Vol. XI. The physiology of developing fish. Part A. Eggs and Larvae. Accademic Press, London.

Kohinoor, A.H.M., M.S. Islam, D.A. Jahan and M.G. Hussain, 1997. Embryonic and larval development of *Ompok pabda* (Hamilton). *Progress. Agric.*, 8: 37-41.

Mustafa, G., A.T.A. Ahamed and K.R. Islam, 1980. Food, feeding habits and fecundity of a freshwater perch, meni fish. *Bang. J. Ag.*, 5: 205-210.

Nikolsky, G.V., 1963. *The Ecology of Fishes*, Accademic press, London. 352pp.

Pal, S., 2000. Artificial breeding of laboratory reared *Nandus nandus* (Hamilton) by using carp pituitary gland. M.S. Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. 58 pp.

Rahman, M.M., 1975. Developmental and life history of *Anabas testudineus* (Bloch) (Perciformes: Anabantidae). *Bangladesh J. Zool.*, 3: 11-16.

Thakur, N.K., 1980. Notes on the embryonic and larval development of an air-breathing catfish *Clarias batrachus* (Linn.). *J. Inland Fish. Soc. India*, 12: 30-43.

(Manuscript received 6 June 2001)