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Early Development of a Threatened Freshwater Catfish *Mystus montanus* (Jerdon)

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ABSTRACT

Mystus montanus eggs contain a relatively small yolk (1.0–1.2 mm) and are strongly adhesive. The egg envelope is thick and transparent. Embryos hatched 23.0–24.0 h after activation, and the incubation period was 23.0 h long. The circulatory system appeared early (at 17.75 h) and was characterized by a complex anastomosing vitelline system and by well-developed segmental vessels suggesting that *M. montanus* embryos can exploit available oxygen sources very efficiently. The post larvae were observed to have begun aerial respiration on the seventh-ninth day after hatching. After 20 days, the length of fry ranged 15.0–16.0 mm and resembled the adult in its external features. The length of fingerlings ranged from 25.0–30.0 mm after 30 days, and their external features were just like those of an adult except that they were not sexually mature.

Key words: Early development, ontogenic events, threatened catfish, *Mystus montanus*.

INTRODUCTION

The freshwater catfish *Mystus montanus* (Jerdon, 1849) which accounts for about 10% of commercial landings of air-breathing fish in Tamil Nadu, India, is one of the most highly priced fish due to its tender flesh, few bones, and good taste. It is endowed with remarkable powers of respiration, which enables it to lead an amphibious life and to survive in adverse conditions in swampy areas. It is endemic to catchments of the River Tambaraparani, Tirunelveli Dt (Tamil Nadu, India). Its status varies from threatened (Haniffa *et al.*, 2001) to susceptible (CAMP, 1998);it lives in lentic parts of the submontane zone or in main streams of lowland areas (Arunachalam, 1978) and requires special habitats which are at risk due to habitat destruction, pollution, construction of dams, introduction of alien species etc. In the Tambaraparani River, *M. montanus* occurs occasionally or in isolation (Pandian, 1970) and its abundance has declined dramatically in the recent past (ICAR-NATP, 2002). Hence extensive studies on mass seed production of threatened fish species by induced breeding have been undertaken by the Centre for Aquaculture Research and Extension (CARE) (ICAR-NATP, 2002). The present investigation deals with the early development of *M. montanus.* and the ultimate objective is commercial culture in derelict water systems.

MATERIALS AND METHODS

Mystus montanus brooders were collected from the Tambaraparani River ($8^0$42'N $7^0$24'E) fed systems using drag nets, cast nets, and gill nets in the early morning (05.00–06.00) and late evening (17.00–18.00), after which they were transported to CARE. After a brief dip in potassium permanganate, the brooders were acclimatized in stocking ponds (8 x 4 x 1 m) and fed with finely chopped chicken intestine (Haniffa *et al.*, 2002). Spawning was induced by an intramuscular injection of 0.3 ml/kg body weight of ovaprim (Haniffa *et al.*, 2000). Each breeding set

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consisting of two males and one female was released into a breeding tank (3 x 1 x 1 m) after injection. Aquatic macrophytes like *Hydrilla verticiellata* and *Eichhornia crassipes* were introduced into the breeding tanks to provide places to hide (Haniffa and Sridhar, 2002). The behavior of brooders was observed every hour after the injection. Spawning was noticed within 13.0–14.0 h after the injection. Fertilized eggs were collected with the help of a dropper and were reared at a density of 30/cement tank (3 x 1 x 1 m) provided with well-aerated water (DO 5.4–5.8 mg/l, pH 7.2–7.4 and temperature 26–28 ^{0}C). Larvae were fed with zooplankton (rotifers and moina) two times (at 10.00 and 14.00) daily (*ad libitum*) after yolk absorption (third day onwards). Samples of eggs before fertilization and thereafter at every 30-min interval were taken for further studies. Descriptions of the developing stages were made on the basis of examining live specimens under a microscope (Nikon Eclipse E 400–UIII), and microphotographs of the developmental stages of eggs and larvae were made.

RESULTS

In the present study, spawning was observed within 13.0–14.0 h after injection of the hormone. Fertilized eggs (Fig. 1) of *M. montanus* were adhesive, demersal, and spherical in form. The yolk sphere contained no oil globule. Due to the adhesive nature of the egg, considerable debris adhered to the capsule of the egg. The grayish-white egg capsule was transparent, while the yolk was green or brown. The previtelline space was large measuring 0.1-0.2 mm in width. The eggs became translucent as development progressed. The diameter of the egg capsule ranged 1.2-1.3 mm, while the yolk sphere ranged 1.0-1.2 mm. The developmental stages of *M. montanus* were divided into six stages: embryo, hatchling, larva, post-larva, fry, and fingerling (Jhingran and Pullin, 1985), with each stage having typical anatomical and physiological features. A summary of the timing of the important ontogenic events and structures is presented in Table 1.

Embryo

The time required to develop from the first cleavage to formation of an embryo was about 1 h. The embryonic development of *M. montanus* was usually completed within 22.0–23.0 h after fertilization. The first cleavage commenced 20 min after fertilization when the blastodisc divided into two blastomeres (Fig. 2). Within another 5 min, the four-cell stage was obvious. The eight-cell stage was reached after 10-15 min. Sixteen blastomeres (Fig. 3) were noticeable within the next 15-20 min. The 32-cell stage was attained in the next 20 min, and the number of cells doubled (64-cell stage) in the following 10 min. The morula stage (Fig. 4) was visualized within 1.5 h after fertilization. By about the seventh hour, the head and tail ends of the embryo were distinguishable (Fig. 5). Myomeres differentiated between10 and 12 h of development (Figs. 6–8). In the 15-somite stage, the optic vesicles appeared, and in the 14^{th}-16^{th} hour (Figs. 9, 10), the number of myomeres increased to 20 and the position of kupffer's vesicle and a pulsating mechanism, the forerunner of the heart were discernible. In the final stage of embryonic development, the growing embryo occupied the entire previtelline space, and by about 1.5–2.0 h before hatching, it exhibited frequent twitching movements (Fig. 11). After a pause of about 30 sec, this frequent movement suddenly culminated in a violent jerk breaking the previtelline membrane, and the hatchling emerged tail first (Fig. 12).

Hatchling

Length measurements of newly hatched larvae are given in Table 2. Hatchlings (Fig. 13) of *M. montanus* showed a laterally compressed transparent body, characterized by the presence of an almost round yolk sac, occupying about 45% of the total length. Chromatophores were completely absent. The mouth, alimentary canal, and gills were not yet differentiated. The primitive streak of the notochord was quite prominent; about 25-27 myomeres were distinct and another 7-8 were apparent in the tail region. The tip of the tail was rounded and the fin fold was differentiated, but not very distinctly. Newly hatched larvae were not active and generally remained resting on their sides at the bottom of the container.

Table 1. Ontogenic events in the early development of *Mystus montanus*. Each value is the average of five observations.

Age (h)	Ontogenic events
	Cleavage
0.20-1.0	2–16 cell stage
1.0–1.5	Morula stage
	Formation of embryo
2.0– 2.5	Blastula stage
3.0–5.0	Germinal ring formed; embryonic shield formed; more than half of yolk invaded
7.0	Yolk invasion two-thirds complete
8.5	Yolk invasion complete; yolk-plug stage almost Complete
	Differentiation of embryo
10.0	Embryonic rudiment distinct 2–3 myomeres; eye vesicles demarcated
12.0	7-8 myomeres; heart rudiment visible; demarcation of brain
14.5	12-17 myomeres; head and tail differentiated
17.75	Entire space inside egg occupied by embryo; heart beat visible; tail beginning to separate from yolk; blood circulation commenced; embryo making frequent movements
22.0	Hatching begins
23.0-24.0	Larva just hatched
	Larva (h post-hatching)
Larva just hatched	3 mm long; unpigmented eyes; no fin buds; mouth not yet formed; stellate pigment scattered in the yolk mass; head and body faintly yellow
3.0–8.0	4 mm long; displaying unpaired dorsoventral fin; heart, brain, and ventricles distinct; brownish; yolk sac elongated
15.0–24.0	Caudal fin beginning to separate; faint pigmentation of eyes; alimentary tract distinct; pectoral fin bud appeared; pigment accumulation in yolk sac; formation of barbels
30.0–35.0	Mouth beginning to differentiate; tail fin demarcated
40.00 – 47.00	Mouth opens; jaw movements begin; caudal fin rays distinguished; barbels formed
	Post larva
Third day	5 mm long; head prominent; pectoral fin flap-like; yolk sac absorbed
Fifth day	6 mm long; body golden-yellow; pectoral fin clearly recognizable

Larva

A relatively broad space appeared between the head and anterior margin of the yolk in 2.0–3.0-h-old larvae. This space facilitated the accommodation of the developing heart. Buccal invagination was apparent in 6.0–8.0-h-old larvae, and the alimentary canal formed as a straight tube emerging from the posterodorsal aspect of the yolk sac. The anal opening was closed and was situated between the 10^{th} and 12^{th} myomeres, which was slightly less than half the length of the larva at this stage. The heart commenced to beat at a rate of 117-122 beats/min. Barbels appeared in the form of tiny protuberances in 1-day-old larvae. The Upper and lower jaws were formed, and the lower jaw showed occasional movements. The urinogenital opening was distinct and situated just posterior to the anal opening in 2-day-old larvae (Fig. 14). The heart beat at a rate of 140 beats/min. The pectoral fin bud appeared as a moderate elevation. Intestinal coiling of the alimentary canal was

Table 2. Average measurements (in mm) of hatchlings and post-larvae of *Mystus montanus* at various stages of development. Each value represents an average of 25 samples ± S.D.

Parameter (mm)	At Hatchling	At 6-8 h old	Time after hatching (day)						
			1	2	3	4	5	10	15
Total length	3.0 ± 0.1	3.20 ± 0.13	4.40 ± 0.21	5.50 ± 0.18	6.30 ± 0.26	7.10 ± 0.31	8.50 ± 0.3	10.30 ± 0.28	12.50 ± 0.44
Length up to tip of the notochord	2.91 ± 0.11	3.10 ± 0.15	4.10 ± 0.11	5.20 ± 0.25	5.90 ± 0.36	6.10 ± 0.28	7.20 ± 0.22	8.70 ± 0.27	10.20 ± 0.25
Length up to vent	-	2.05 ± 0.25	2.10 ± 0.15	2.75 ± 0.19	2.95 ± 0.20	3.10 ± 0.33	3.75 ± 0.21	4.20 ± 0.28	5.10 ± 0.25
Length of yolk sac	1.56 ± 0.11	1.10 ± 0.12	0.95 ± 0.02	0.90 ± 0.02	0.80 ± 0.07	0.70 ± 0.05	-	-	-
Maximum height of yolk sac	1.10 ± 0.12	0.90 ± 0.04	0.80 ± 0.04	0.70 ± 0.01	0.60 ± 0.03	0.50 ± 0.03	-	-	-

noticeable. The yolk was exhausted by the end of the third day of development, and larvae commenced exogenous feeding even before completion of yolk absorption.

Post- larva

In 5-day-old post larvae (Fig. 15), streaks denoted rudimentary rays, which appeared in the caudal fin. The pectoral fin was differentiating and was in the form of a flap just behind the operculum; at this time, sidewise movement of the larvae commenced. The yolk was completely absorbed, and larvae began wandering in search of food.

Ten-day-old post-larvae were endowed with eight branched rays in the dorsal fin and seven-eight in the caudal fin, and at this stage, the outline of the brain in the cranial cavity could clearly be seen under a microscope. The phenomenon of aerial respiration began on the seventh day of development. Fifteen-day-old post-larvae showed seven-eight anal fin rays, and the pectoral spine had become stout. The embryonic fin folds had yet to disappear. Vertebral segmentation of the notochord took place with distinct neural and hemal spines especially in the caudal region. Pigmentation was more pronounced throughout the head and body.

Fry

Twenty-day-old fry (Fig. 16) ranged 15.0-16.0 mm in length. Fry swam actively and were observed to voraciously feed on plankton. Fry displayed a dorsal fin with branched rays. The body became opaque due to the accumulation of pigments.

Fingerling

This stage (Fig. 17) began on the 30th day and lasted for the next 15 days. Thirty days after hatching, the pectoral, pelvic, dorsal, caudal and anal fins showed seven-eight, six-seven, eight, ninteen and seven-eight rays, respectively, representing the full complement of rays. On day 30, fingerlings were 25.0–30.0 mm in total length and externally resembled the adult (Fig. 18) suggesting the end of the fingerling stage.

DISCUSSION

Changes in the pattern of the entire structure of an organ or of a specific organ in relation to the environment are decisive for evaluating the developmental patterns of a species (Balon, 1999). Since the egg envelope is thick, transparent, and sticky, observations on the development of *M. montanus* are difficult (Kovac, 2000). Ontogenic events during the ovular phase (cleavage stage) did not markedly differ from those in *Heteropneustes fossilis* or *Channa marulius* (Khan, 1926; Mookerjee, 1945). Changes in structure emphasize the thresholds between embryonic, larval, and post-larval development from the onset of

cleavage or epiboly, or at the time of organogenesis, respectively (Kovac, 2000; Carlos *et al.*, 2002).

The yolksac of *M. montanus* was fully resorbed by the third day, when the larvae measured 5.0-5.5 mm in length. Disappearance of the yolk sac was observed on the third day in *Clarias lazera* (Panjionghua and Zhengwenbiac, 1987) *C. fuscus* (Panjionghua and Zhengwenbiac, 1982), *C. batrachus* (Landge, 1995), *Channa striatus* (Alikunhi, 1953), and *Mystus macropterus* (Wang *et al.*, 1992). *Mystus montanus* larvae gradually changed to an orange color in the early post-larval stage. Similar color changes (purple red) were noticed in *C. striatus* larvae in the late post-larval stage (Yackob and Ali, 1992).

The mouth and the tip of the notochord in *M. montanus* were directed upwards on the fourth-sixth day. In *C. striatus*, *C. marulius*, and *M. macropterus* the tip of the notochord turned upwards on the fifth-seventh, ninth-thirteenth and second day respectively (Khan, 1926; Parameswaran and Murugesan, 1976; Wang *et al.*, 1992). In the present study, the air breathing habit developed 7–9 days after hatching. Similar reports are available in the literature for *Anabas testudineus* (12–13 days; Munshi and Hughes, 1992), *C. marulius* (13 days; Parameswaran and Murugesan, 1976), and *H. fossilis*, and *Clarias* sp (5 – 8 days; Dehadrai, 1972).

Fish farmers are much less familier with the culture of catfish species because of the lack of breeding and feeding techniques and non-availability of seeds from the wild (Meehan, 2002). Despite this small-scale operations have been attempted for *M. gulio* and *M. oculatus* (Hunter and Kimbrell, 1980) and the successful culture of larger catfishes e.g., *Heteropneustes fossilis* and *C. batrachus*, have also been achieved in the past (Marguiles, 1997). The high fecundity, short embryonic period, fast development of sense organs and air-breathing habit of *M. montanus* suggest that it is a suitable species for commercial culture.

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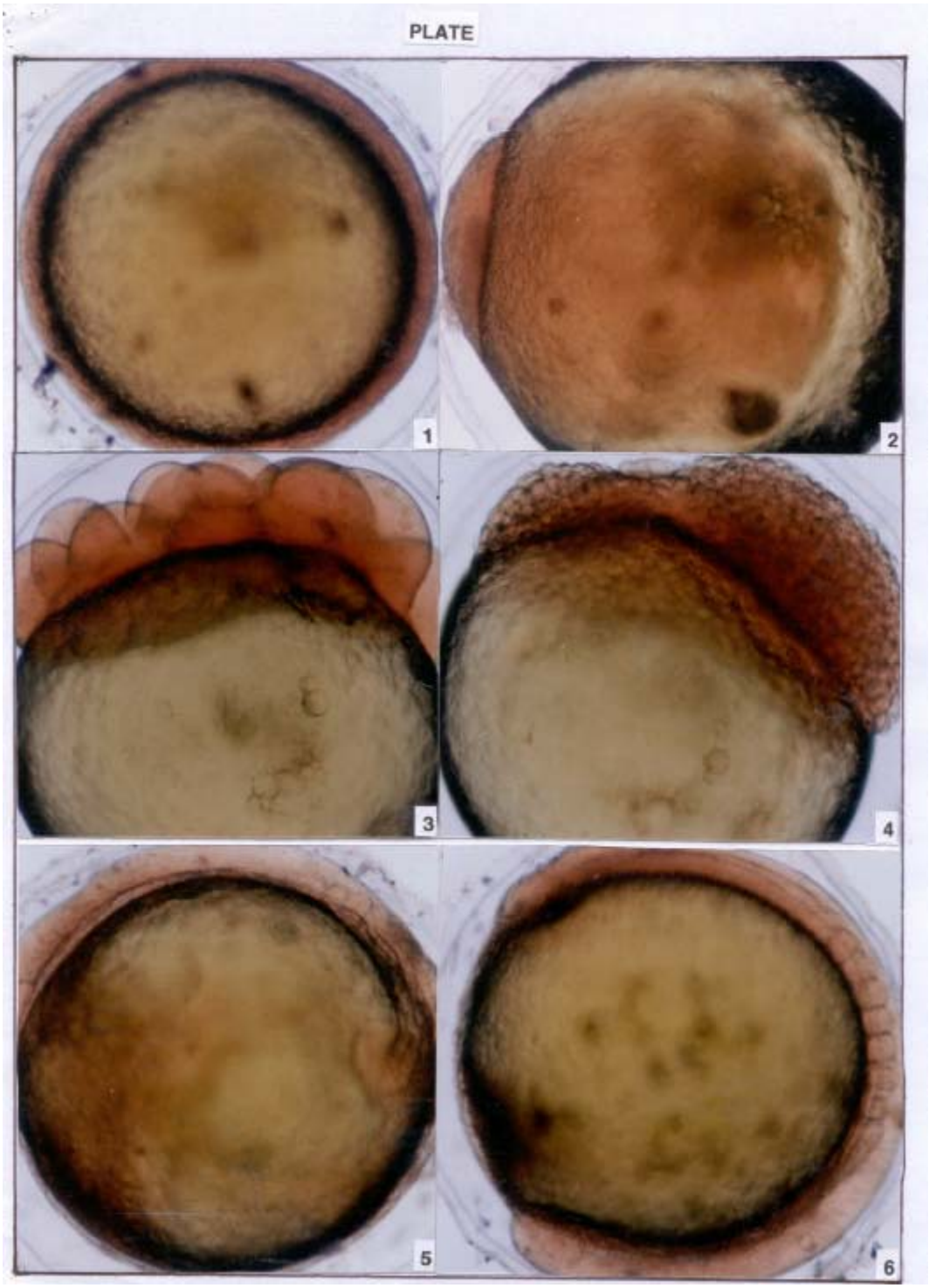

Plate. 1 Developmental Stages of *Mystus montanus.* Fig.1: Fertilized egg; Fig.2: Formation of two blastomeres; Fig.3: Sixteen-cell stage; Fig.4: Morula stage; Fig.5: Seven-hours old embryo; Fig.6: Ten-hours old embryo.

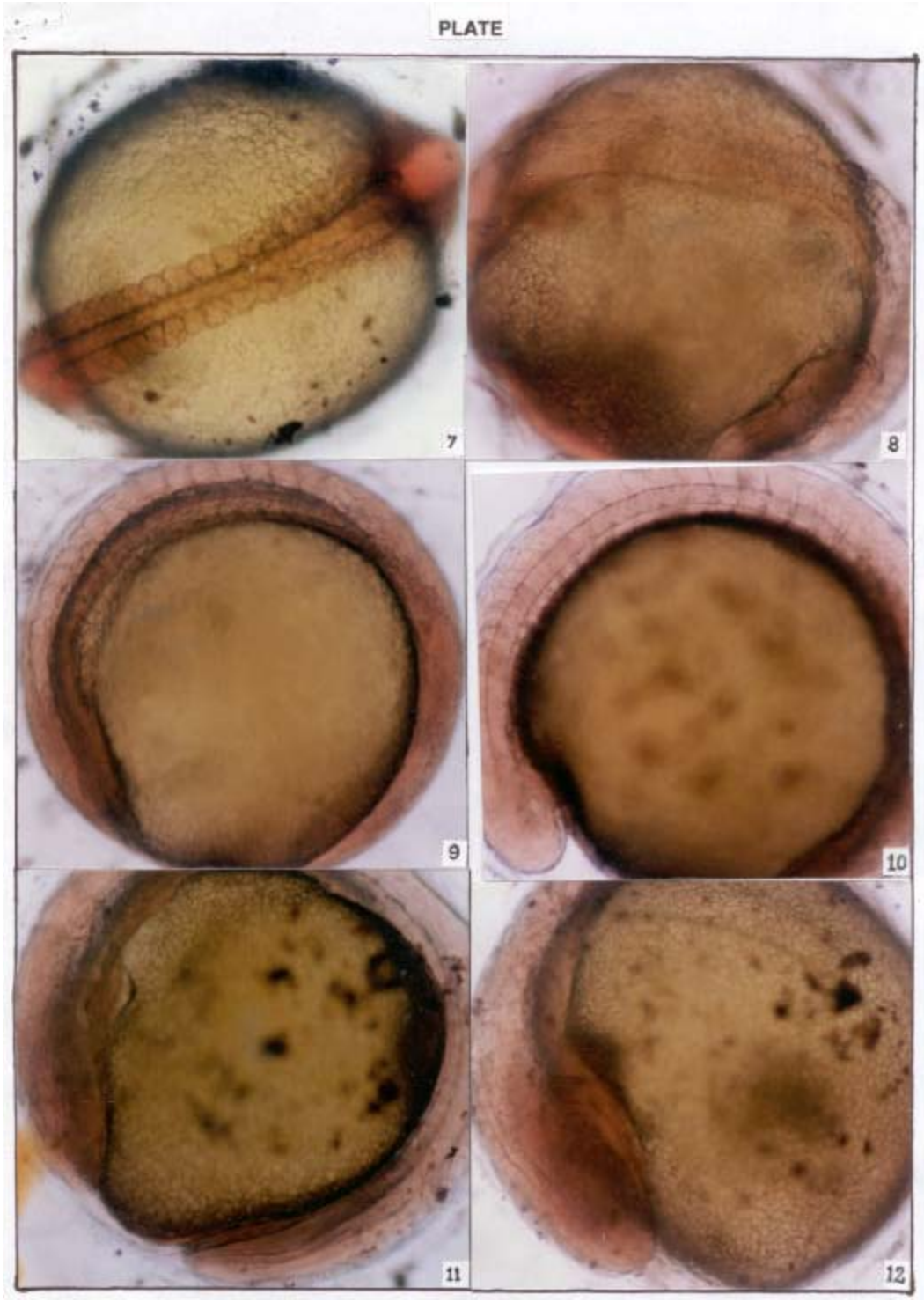

Plate. 2 Developmental Stages of *Mystus montanus.* Fig.7: Eleven-hours old embryo; Fig.8: Twelve-hours old embryo; Fig.9: Fourteen-hours old embryo; Fig.10: Sixteen-hours old embryo; Fig.11: Eighteen-hours old embryo; Fig.12: Twenty-two-hours old embryo.

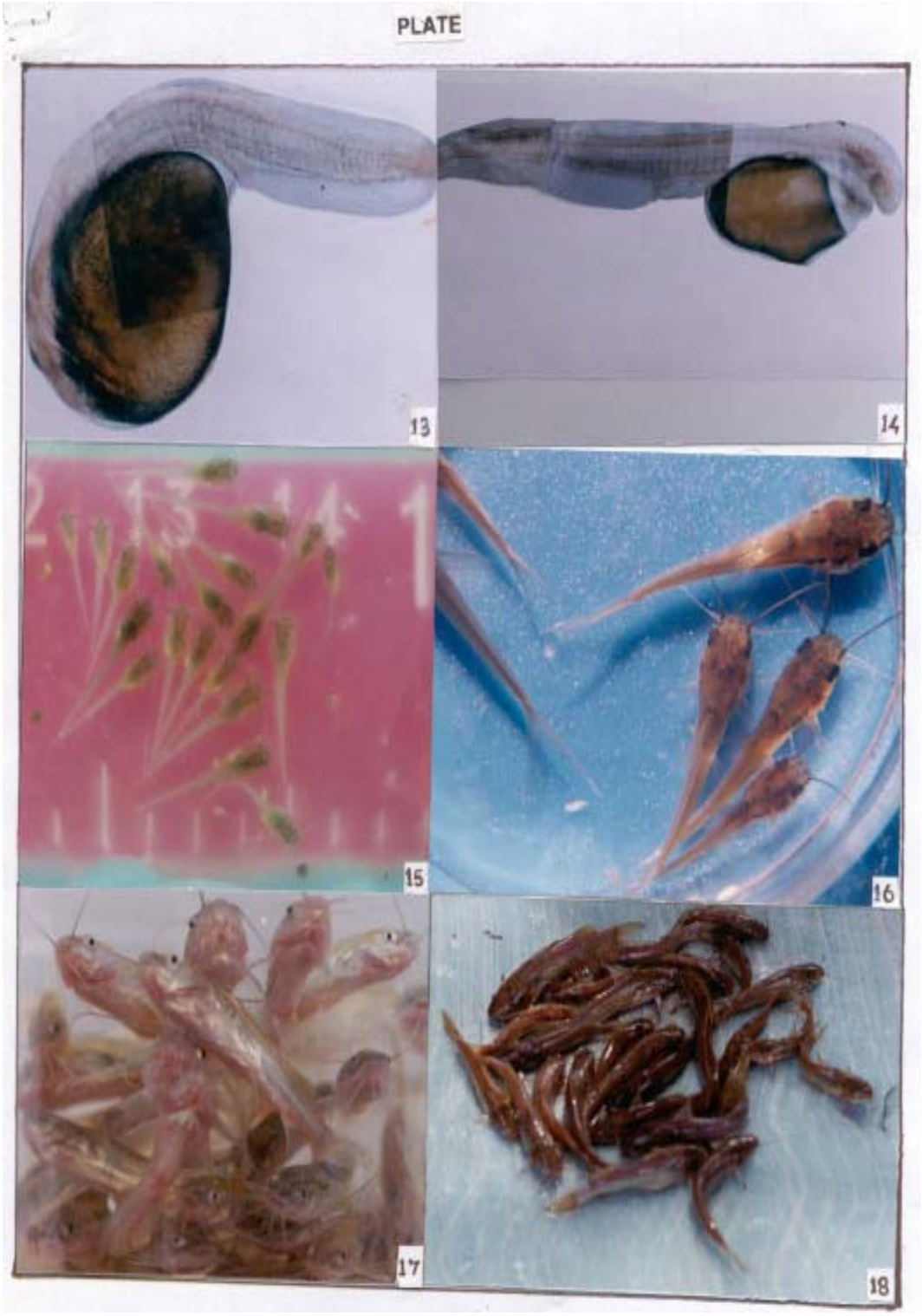

Plate. 3 Larva, Fry, and Fingerlings of *Mystus montanus*.Fig.13: Just hatched larva; Fig.14: Forty-eight-hours old larva; Fig.15: Five-days old post larvae; Fig.16: Fry; Fig.17: Fingerlings; Fig.18: Adult.

受威脅種淡水鯰魚 *Mystus montanus* (Jerdon) 之早期發育

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摘 要

Mystus montanus 的卵具相對較小（1.0 -1.2 mm）的卵黃，以及有著高黏度、厚且透明的卵膜。胚胎在活化之後歷經 23.0 小時的孵化期，於 23.0-24.0 小時孵化。胚胎的循環系統較早出現（約在 17.75 小時），我們可由複雜的網狀卵黃系統及發育良好的體節血管辨識出：*M. montanus* 胚胎可以非常有效地利用各種可能的氧氣來源。我們觀察到孵化後第 7 至第 9 天的後期幼體已經可以開始直接呼吸空氣；20 天以後的魚苗體長約 15.0 -16.0 mm，其外型與成魚大致相似。孵化 30 天後的小魚體長約在 25.0 -30.0 mm 的範圍內，此時除了未臻性成熟外，其外部形態已和成魚幾乎相同。

關鍵詞：早期發育, 個體發生事件, 受威脅鯰魚, *Mystus montanus*