Embryonic mortality of Indian major carps in some eco-hatchery systems

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ABSTRACT
Indian major carps viz. Catla catla (Ham.), Labeo rohita (Ham.), and Cirrhinus mrigala (Ham.) are induced bred in eco-hatchery system. Mortality of developing embryo in the incubation system are common which have been investigated at different eco-carp hatcheries in India during 1986-2000. Investigation and experiments were conducted by repeating breeding and incubating the eggs simulating the conditions under which mortality occurred earlier. The investigations have revealed that mortality incidences are due to various factors such as shedding of unprime gametes, teratogenic developments and premature hatchings which are usually followed by fungal infestations. Ovulated oocytes, if not activated, do not imbibe water and die instantly. Some eggs are activated but do not cleave and pass away. Some activated eggs undergo teratogenic development at different stages of embryogenesis such as unequal cleavage, erosion of active blastomeres and improper gastrulation. The teratogenic embryo either died before hatching or hatched out as deformed hatchling but later died due to innate deficiencies. Embryonic mortality instances are recorded more during early and delayed spawning. Partial or mass mortality is also caused by choriolysis and premature hatching. Choriolysis occur due to low oxygen tension (3 ppm) and higher total alkalinity (> 150 ppm) followed by fungal infestation. When embryonic mortality was considerably high (30%-40% of total eggs), usually it led to mass mortality. Some precautionary measures and simple hatchery management practices have resolved the problem.

Introduction
Induced breeding in eco-hatchery complex has revolutionized the seed production of Indian major carps in confined water. Administration of hypothalamic peptide combinations have further intensified the system (Nandeesha et al., 1991; Chondar, 1994; Lin and Peter, 1996). Instances of partial or mass mortality of developing embryos are obvious in the intensification process but their postmortem study are meagre (Saha, 1992; Rath et al., 1993, 1998; Gupta et al., 1998). Induced bred carp embryo in hatchery may die in the incubation system for the congenital problem of gametes (Chondar,
The basic studies of carp embryology have become important to understand and overcome problems faced by the hatchery managers. Chakraborty and Murty (1972); Aluko and Rath (1999) and Chondar (1999) have recorded the basic embryonic developmental stages of induced bred Indian major carps. The objective of the present study is to interpret the reason of the embryonic mortality incidences in carp hatchery through experimentation and embryological observations.

Materials and methods

Materials for this study have been obtained from fish seed hatchery project, Bhanjanagar, Orissa (1986); fish seed hatchery project, Saramanga, Orissa (1987); fish seed farm, Sitamarhi, Bihar (1989); carp hatchery, Central Institute of Freshwater Aquaculture (1993-1997); fish seed farm, Rawatvata, Rajasthan (1995); national fish seed farm, Jotisar, Haryana (1999); and fish seed farm, Gop, Orissa (2000). The mortality incidences were investigated and later experimentally induced for close observation. Brood of Catla catla, Labeo rohita and Cirrhinus mrigala were induced bred in breeding pool and eggs incubated in respective incubation system exactly as it was done during the last mortality incidences of the farm. The developing eggs were closely observed in situ incubation system and also studied under microscope for 72 h after hatching. Morphometry of the developing eggs were noted with camera lucida sketches and photomicrographs during different stages of uncommon embryogenesis. Inactivated eggs, uncleaved eggs and teratogenic embryos were observed till the death of embryos or hatchings under the microscope and compared with normal development. The physico-chemical parameters of water were recorded for each experiment and correlated with the embryonic mortality instances as situation warranted.

Results

All the species of carp embryo viz. Catla catla, Labeo rohita and Cirrhinus mrigala have shown common type of symptoms and responses during the study. The reason for mortality of ovulated eggs in incubation system after spawning are as follows.

1. Some ovulated eggs of size range 0.5-1.0 mm were not activated or imbibed water and died as such. It was often associated with the over dose of inducing agents.

2. Some eggs were activated, imbibed water (Fig. 1) but did not cleave. The blastodisc enlarged in size and regressed within 1 hr of spawning (Fig. 2). Frequency of such eggs were
more during early pre-monsoon breeding and in unripe brood of multiple breeding programme. Dead eggs provided good substrate for fungal development (Fig. 3).

3. Some eggs exhibited unequal type of
early cleavage (Fig. 5) over normal type of cleavage (Fig. 4), and produced two different groups of blastomeres during 1st few cleavages (Fig. 6). Embryo either died at this stage or continued to hatch out as deformed one (Figs. 21, 22). This is seen in all breeding operations (0.5 to 1%).

Fig. 6. Embryo at morula stage showing two different groups of blastomeres (arrow heads) and blastomere erosion. Note the further division of blastomere (arrow) in perivitelline space (4 h. of spawning).

Fig. 7. Normal cleaved embryo with 4 blastomeres of identical shape and size (50 min. of spawning).

Fig. 8. Activated egg showing abnormal cleavage. Note the unidentical blastomeres (arrow) of columnar shape. (50 min. of spawning).

4. At 4-8 cell stage (Fig. 7, 10), sometimes blastomeres were regressed either at animal pole end (Fig. 8, 11) or keeping the blastomeres intact vitelline membrane at vegetal pole was lysed followed by yolk disintegration (Fig. 12) leading to embryo mortality. This type of embryonic regression were seen en masse when water temperature in incubation chamber suddenly went up by 4-5°C of the ambient temperature during summer, where surface water was directly used in incubation system.
5. Some eggs showed very quick cleavage and produced a cluster of small blastomeres within 1 hr. of spawning act or when eggs after final maturation were retained in ovary for more time. This happens due to low dose of hormone administration or plugging.

6. Some eggs showed normal development up to blastula (Fig. 13) but since then blastomere production was completely ceased and embryos died at this stage (Fig. 14). This was observed once in the course of investigation due to the overcrowding of eggs in the incubation chamber.

7. Some eggs slowed down the production of blastomeres after blastula stages. Thus a small population of the blastomeres have produced a thin layer of blastoderm (Fig. 18). At this stage the embryos were either perished, or if continued, hatched out as "teratoma" without any differentiation of head, trunk and tail (Fig. 20). Such abnormalities were common (0.3 to 1.8%) during all breeding operations and unusually high (20-60%) during delayed breeding of early matured brood.

Fig.9. Activated egg showing regression in the morulation process. Note the cluster of micro blastomeres (arrow) at the animal pole (80 min. of spawning).

Fig.10. Cell stage of normal embryo. Note the uniform size of blastomeres (60 min. of spawning).

Fig.11. Developing egg of 8 celled stage showing blastomere regression at animal pole end (arrow) (75 min. of spawning).

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Fig. 12. Developing egg showing lysis of vitelline membrane at vegetal pole and oozing of yolk material (arrow) keeping animal pole intact (arrow head) with blastomeres. (75 min. of spawning).

Fig. 13. Developing embryo showing normal cell-mushroom and embryo shield. (180 min. of spawning).

Fig. 14. Disintegration of cell-mushroom embryo (230 min. of spawning).

Fig. 15. Cell-mushroom and embryonic shield stage showing erosion of blastomeres (200 min. of spawning).

8. Some of the developing embryos exhibited normal development till cell-mushroom stage, subsequently dropped down some of the potential blastomeres into the perivitelline space (Fig. 15). The isolated blastomeres divided independently (Fig. 6, 16) and got putrefied in the system. When only one or two blastomeres were dropped down, the
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Fig. 16. Inset of fig. 15, showing further division of isolated blastomeres.

Fig. 17. Normal cap stage with embryostick (arrow) and half covered yolk (arrow head) of developing eggs. (4 hr. of spawning).

Fig. 18. Thin population of blastomeres on blastoderm at the 'germ ring stage' (3 hr. and 30 min. of spawning).

Fig. 19. Embryo showing impaired gastrulation process and abnormal yolk plug (arrow), note the thin layer of blastoderm on body of the embryo. (5 hr. of spawning).

Embryo developed normally (Fig. 17). If the erosion of blastomeres was high (Fig. 6), it may pass through a defective gastrulation process (Fig. 19) leading to an abnormal embryo (Fig. 21, 22). This is encountered in the dry spell of spawning period, late monsoon breeding of multiple breeding programme and considerably high in overaged brood of more than 5 year.

9. Mass embryonic mortality resulted from (i) low oxygen tension (3 ppm) caused by slow inflow of ground water in incubation chamber. (ii) higher total alkalinity of above 150 ppm of hatchery pond water after heavy rain. (iii) mechanical pressure of water at duck mouth end.
Fig. 20. Undifferentiated 'teratoma' embryo (22 hr. of spawning).

Fig. 21. Axial impaired hatchlings (60 hr. of hatching).

Fig. 22. 'Tunicate' type of hatching (60 hr. of hatching).

Fig. 23. A pre-mature hatchling showing fungal hyphae (arrow) on its body (48 hr. of hatching).

10. Severe fungal infestation was evident on dead embryo or dead eggs (Fig. 3, 23) causing mass mortality.

Discussion

General account of teleostean embryogenesis is given by Lagler (1956) based on the work of Battle (1944) and Carr (1942) on Atlantic salmon. Only a few records are available on the embryogenesis of major carps (Okada, 1960; Chakraborty and Murty, 1972; Shireman and Smith, 1983; Murty et al., 1986). Recently Chondar (1999) has described the embryology of a few freshwater species including major carps viz. Catla catla, Labeo rohita and Cirrhinus mrigala. Some unprime ovulated eggs do not imbibe water (water hardening) and do not grow further. Chondar (1980) described such eggs as premature eggs spawned due to unstandardised overdose.
administration of inducing agents. Some ovulated eggs developed blastodisc but regressed soon. In the present study some water hardened eggs that developed ger-
minal disc, did not undergo cleavage and regressed. The developmental aberration mentioned above may be considered as a result of activation of eggs other than fer-
tilization. Development in some eggs have been regressed during first few cleavages, germ ring stage, blastulation and gastrulation process. In some in-
stances the embryo started disintegrat-
ing from blastomere at animal pole and in some, the process of disintegration is initiated by lysis of vitelline membrane with oozing of yolk into the peri-vitelline space, keeping blastomeres intact. Enmasse mortality is observed by this type of development if temperature of the incubation system is increased by 4-5°C above the ambient temperature. Similar observation was made by Nidhialkov (1981) in winter amur embryo. Some eggs show very quick cleavage leading to for-
mation of a cluster of small blastomeres within one hour of spawning and die. This type of embryonic regression is common in prolonged spawning, partial spawning and plugging which congre with the ob-
servation of Zonneveld and Van zon (1985) in grass carp breeding, that over retained oocytes in ovary (post-optimum phase) resulted in poor fertilisability and hatchability. Lagler (1956) described the term 'ringer' when the dense germring becomes lighter and embryo turns opaque in Atlantic salmon. This "ringer" condition of development is often ob-
served in the present study during de-
layed spawning instances of matured brood of multiple breeding programme. An embryonic shield appear next to morula at one side of the germ-ring show-
ing some syncitial layer spreading over the yolk mass. Some embryos of the present study die at this stage. Some of the carp embryos in the incubation sys-
tem during dry spell of breeding season show erosion of blastomeres from cell-
mushroom to the perivitelline space. This is more often seen in over aged brood. Erosion of blastomeres during embryogenesis has been described by Saha (1992) in silver carp. Makeeva and Saha (1985) have discussed the erosion of blastomeres as caused by chromosome displacement during second meiosis. Ero-
sion of blastomeres is considerably high in some cases under the present study giving rise to impaired gastrulation and teratoma embryo. Blastomere erosions cause lethal effects, which make post-
hatching existence impossible or pre-
hatching embryonic death (Rath et al.,
1995). Lysis of chorion observed in this study under low oxygen tension (3 ppm) and high total alkalinity (more than 150 ppm), lead to premature hatching. Blaxter (1969) explained the situation of low oxygen and pre-hatching choriolysis. In embryogenesis of Indian major carps the rate of oxygen consumption increased gradually from fertilization to hatching, (Mohan et al., 1986; Divakaruni and Sharma 1990). In addition to the increas-
ing demand of oxygen by the developing embryo various other factors such as fun-
gal growth, defective arrangement to duckmouth and use of untreated ground water in the incubation chamber caused the low oxygen tension and embryonic death. This support the observation of Gupta et al. (2000). Gupta et al. (1998) have demonstrated the effect of total al-
kalinity in incubation system of carp hatchery which is same as the present observation of choriolysis of developing eggs at higher total alkalinity (>150 ppm).

Instances where death of embryo is about or more than 40% of the total eggs, is very much susceptible to mass mortal-
ity of the stock.
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