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Role of Abiotic Factors in the Embryonic Development of Scale Carp

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These studies present the role of abiotic factors (Temp., DO, *p*H, light and pesticides) on the survival and hatching of eggs of scale carp (*Cyprinus carpio communis*). Eggs were exposed to various temperatures (10°–35°C); dissolved oxygen levels (0–12 mg/l); *p*H levels (2.35–12.0); photoperiodic regimes (10L:14; 14L:10D; continuous darkness and continuous illumination), and to different concentrations of pesticides viz., diazinon, fenitrothion, carbaryl, malathion and phosphamidon from fertilization to completion of hatching.

Survival and hatching rate of eggs increased directly with increasing temperature, dissolved oxygen concentration and *p*H values. Hatching time varied inversely with the incubation temperature ranging from 338 hr at 10°C to 42 hr at 30°C. The optimum range of temperature for successful hatching was observed to be 25–30°C. At the extreme temperatures (10°, 15° & 35°C), abnormal development of eggs showing irregular cleavage was observed. Low temperature also affected eye pigmentation and integumentary chromatophore appearance.

Dissolved oxygen also affected the hatching period which varied from 120 hr at 1.2 mg/1 to 68 hr at 12.0 mg/1—levels below 6.0 mg/1 appeared inadequate for proper survival. The retarding effect of reduced DO levels was less pronounced in early stages than at later stages of embryonic development. However, no abnormality in the scale carp larvae was observed at any DO concentration.

The lower and upper lethal limits of *p*H in case of eggs of scale carp were observed to be about 5.8 and 10.5 respectively. Light appeared to have no effect on the survival and hatching rate of eggs of scale carp. Survival and hatching rate decreased with increasing concentration of pesticides. Several deformities were observed in freshly hatched larvae of eggs exposed to different pesticides. On the basis of LC_{50} values, tested pesticides can be arranged in order of decreasing toxicity viz. diazinon $<$ fenitrothion $<$ carbaryl $<$ malathion and $<$ phosphamidon. The effect of pesticides was prominent on the later stages of embryonic development.

Key Words: Ecology, Developmental biology, Chordata (Pisces)

Introduction

Effect of environmental factors on the early life history stages of fish may limit fish production in areas suitable for older fish because early stages are more sensitive than

the adults (Smith & Oseid 1974). Therefore, the successful controlled rearing of fish, though achieved by the development and practice of empirical techniques, requires more specific knowledge of ranges of tolerance to limiting factors for successive developmental stages, to ensure that optimal rearing conditions are maintained.

The significance of abiotic factors (Temp., Dissolved oxygen (DO), *p*H, salinity etc.) in the survival of early life history stages of fish has been emphasised by many workers (Mayes et al. 1953, Alderdice et al. 1959, Kinne & Kinne 1962, Tatarko 1965, Forrester & Alderdice 1966, Gulidov 1969, 71, Mubbs et al. 1969, 71, Oseid & Smith 1971, Irvin 1974, Siefert & Spoor 1974, Dudley & Eipper 1975, May 1975, Tay & Garside 1975, and Mamor & Garside 1976). However to our knowledge no work has thus far been published on the effect of abiotic factors on the embryonic development and hatching of fish species of economic importance in India. No doubt, the literature pertaining to embryonic and larval development of freshwater fishes of India is voluminous.

The scale carp *(Cyprinus carpio communis)*, an important food fish in India, was therefore selected for present studies on the role of abiotic factors in embryological development and survival. As the hazards of pesticides to fishes are of great concern, therefore the study of their effect on survival and hatching of carp eggs was also considered essential. These experimentally based informations on early life history stages will help for starting fish culture in areas where conditions are optimum and for further progress in advancing fields of fish culture and fisheries research.

Materials and Methods

Freshly fertilized eggs of scale carp were collected from the Fish Seed Farm, Punjab Agricultural University, Ludhiana and immediately taken to the laboratory in plastic bags, where they were exposed to different abiotic factors (Temp., DO, *p*H, light and pesticides). Pond water from the cement spawning pond, used as test water during the experiments, was analysed according to the methods described by the American Public Health Association et al. (1971) and had the following characteristics (in mg/l): total alkalinity, 340–342; total hardness, 210–213; chloride content, 80.5–81.5; nitrates, 12.5–13; and phosphates 0.30–0.35. The *p*H ranged from 8.5 to 8.75 during the experiments. All experiments were conducted at room temperature (25±1°C).

Before use, the water was filtered to remove large particles. Effects of abiotic factors on the survival rate of development and hatching rate of scale carp eggs were studied separately. Test water was maintained at different temperatures, controlled by thermostat, ranging from 10° to 35°C (±0.5°C); at desired levels of oxygen (0–12.0 mg/l) by mixing supersaturated water (having 12.0 ppm) with oxygen free water produced by nitrogen stripping and at different *p*H levels, 2.35–12.0 (±0.05) by adding different quantities of hydrochloric acid and sodium hydroxide.

To study the effect of pesticides on the scale carp eggs, carbaryl, diazinon, phosphamidon, fenitrothion and malathion were selected. Various concentrations were made by using dilution technique (Shivaji Rao et al. 1969). Trial and error method was used to determine the sublethal concentrations of tested pesticides.

Carp eggs were also exposed to different photoperiodic regimes viz., 10L : 14D; 14L : 10D; continuous darkness and continuous illumination to evaluate the role of light in embryonic development of scale carp.

Eggs were incubated in glass chambers (20×15×15 cm). About 5 hours after fertilisation 200 eggs attached to small pieces of vegetation were placed in the experimental chambers. The oxygen concentration and *p*H was measured twice a day by the unmodified Winkler's method and by electric *p*H meter (ELICO) respectively.

At 6 hours intervals, samples of 10–20 eggs were gently removed from each of the test chambers to petri dishes containing water of same temperature, dissolved oxygen concentration and *p*H value, to observe the stage of development under a binocular microscope. Stages of embryonic development were identified from the schedule given by Varma (1970) and Kamaldeep (1976) for scale carp and mirror carp respectively and the average developmental times was recorded in each case. Dead eggs were counted and removed and live eggs were returned to the respective chambers. Hatched larvae were counted at the end of the experiments.

Results

Data pertaining to time taken for hatching, percentage hatching and rate of development of scale carp eggs exposed to different factors are presented in tables 1 and 2. Effects of different factors are as follows:—

Temperature—The hatching time varied inversely with incubation temperature ranging from 338 hours at 10°C to 42 hours at 30°C, survival of scale carp embryos, hatched at different temperatures ranged from 8–90%. Percentage hatch and rate of development increased with the increase of water temperature from 10° to 30°C and decreased above 30°C (table 1).

Average length of hatchlings varied inversely with the temperature. Rate of cleavage, twiching, heart beating, body movement before hatching and activity of hatchlings

Table 1 *Percentage hatching and incubation time of eggs of scale carp exposed to different temperatures, dissolved oxygen levels, pH levels,photoperiodic regimes*

Abiotic Factor	Average hatch	Average incubation time to hatching
I TEMPERATURE		
(°C)	(%)	(hours)
10	8	339
15	37.04	246
17.5	46.45	155
20.5	50.0	83
23	57.32	71
25	76.95	59
27.5	80.40	46
30	90.00	41
32.5	12.5	53
35	0	All embryos died during cleavage stages
II DISSOLVED OXYGEN (mg/1)		
0	0	All embryos died before optic vesicle was formed
1.2	4	120
3.0	40	76
6.0	65	73
9.0	92	70
12.0	98	68
III *pH*		
2.35	0	All embryos died before reaching morula stage
5.60	0	-do-
6.50	50	60
7.50	76.5	58
8.0	79.6	58½
9.0	95	58
9.5	60	58½
10.0	40	59
11.0	0	All embryos died during cleavage stages
12.0	0	-do-
IV LIGHT (*photoperiod*) *regimes*		
Continuous illumination	67	56
Continuous darkness	65	56½
10L:14D	66.6	54
14D:10L	65	55
Control (12L:12D)	68	55

Table 2 *Effect of pesticides on percentage hatching and development of scale carp eggs*

Conc. (ppm)	Average Hatch (%)	Average deformed larvae (%)	Deformities
DIAZINON			
1.5	8.3	8.3	
1.0	12	—	Undeveloped posterior region, deformed vertebral column
0.1	28.5	—	
0.05	30.7	25	
0.01	43.7	38.4	
Control	95	—	
FENITROTHION			
1.0	0	—	
0.85	8.3	43.75	Curved tail, undeveloped posterior region, deformed vertebral column
0.75	32.5	23.5	
0.65	45.4	—	
0.5	74	23.5	
0.25	84	23	
0.1	89	—	
0.02	93	—	
Control	95	—	
CARBARYL			
2.5	0	—	
1.0	76.7	3.3	
0.75	80	—	
0.5	85	—	Enlargement of periodical sac, circulatory soilure, coiling of the posterior region
0.25	90	—	
0.1	90	—	
0.01	100	—	
Control	95	—	
MALATHION			
5	0	—	
4.75	44.8	40	
4.0	66	—	Undeveloped head and posterior region
2.5	70	25	
1.0	76.6	—	
0.25	80	—	
0.01	100	—	
Control	98	—	
PHOSPHAMIDON			
135	0	—	
125	21.4	—	
110	62.5	10	Enlargement of pericardial sac, undeveloped pectoral fins, inwardly curved tail
100	70	107	
80	87.5	—	
50	96	—	
Control	100	—	

were observed to be temperature dependent as their rates increased with the increasing temperature. Irregular cleavage was observed in some eggs incubated at 10°, 15° and 35°C which caused the formation of different sized blastomeres. Such eggs could not develop beyond the gastrulation stage. Abnormality in the tail region was observed in case of scale carp larvae hatched at very low temperature (10° & 15°C). None of these deformed larvae appeared capable of normal locomotion and of survival. Low temperature also affected the intensity and time of appearance of both eye pigments and integumentary chromatophores. Larvae hatched at higher temperature (>20°C) were darkly pigmented than those hatched at temperatures below 20°C.

Dissolved oxygen—Survival of scale carp embryos hatched at different oxygen levels ranged from 0 to 98% (table 1). No eggs hatched at 0 mg/l and hatching was negligible (4%) at 1.2 mg/l. Percentage hatch increased with increasing DO concentration. Survival was uniformly high at DO concentration of 9.0 mg/l and above. Eggs maintained at low levels of DO developed more slowly and the time to hatching increased as the DO level decreased (table 1). The duration of incubation ranged from 120 hour at 1.2 mg/l to 68 hour at 12.0 mg/l.

In water lacking DO, all embryos died before optic vesicle formation, and most of the embryos died just before hatching in water with 1.2 mg/l DO. No abnormality in hatched scale carp larvae was observed at any DO concentration.

p*H*—The data presented in table 1 indicate that considerable hatching occurred at *p*H values ranging from 6.5–9.5 (±0.05). Survival of scale carp embryos at different *p*H levels ranged from 0–95%. No eggs hatched at 5.6 and 11 *p*H. Visual interpolation of percentage mortalities suggest the

lower and upper lethal limits to be *p*H 6.0 and 10.5 respectively. The time taken for hatching of eggs did not vary much. In acidic (<6.5) and alkaline (>10.0) water almost all the eggs showed exosmosis and endosmosis respectively and died before reaching the gastrula stage.

Light—Data given in table 1, clearly indicates that photoperiod has no effect on the percentage hatching and incubation time. The average percentage hatching and incubation time varied from 65–68 and 54–56½ hours respectively.

Pesticides—Survival and percentage hatching decreased with increasing concentration of pesticides. On the basis of LC_{50} values (conc. at which 50% hatching occurs), as presented in table 3, the tested pesticides can be arranged in order of decreasing toxicity, viz., diazinon<fenitrothion<carbaryl <malathion and<phosphamidon.

Table 3 *LC_{50} values for eggs and fry of scale carp*

Insecticide	LC_{50} values (ppm)	
	Eggs	Fry
Diaxinon	0.008	3.11
Fenitrothion	0.062	2.30
Carbaryl	1.4	10 36
Malathion	4.57	Not determined
Phosphamidon	112.2	163.4

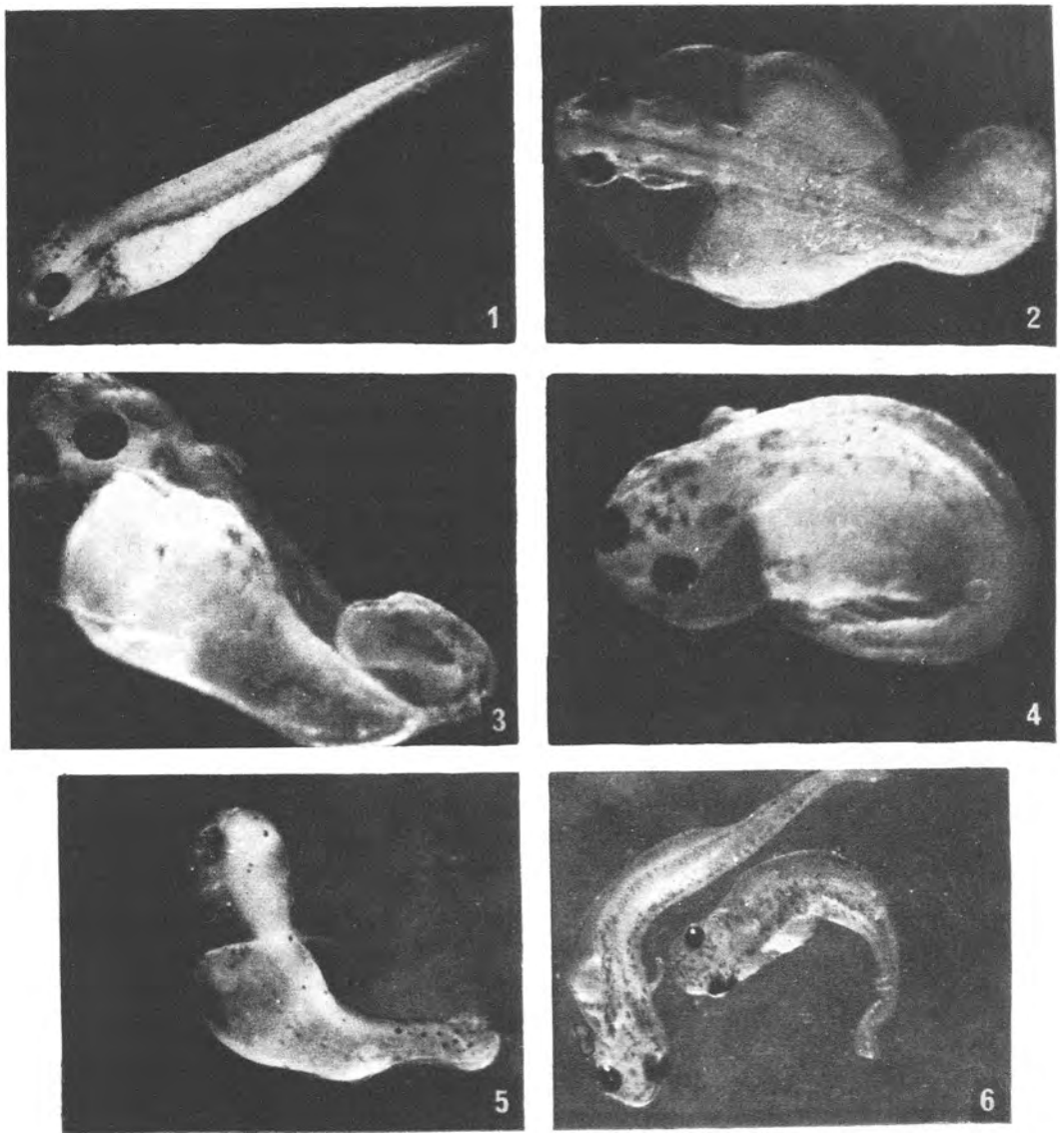
The time taken for 50% eggs to hatch, in all cases, varied from 55–74 hours. Temperature (26±1°C) affected hatching time more appreciably than the time of exposure to pesticides. On the basis of mortality at different stages of development it was found that early stages were less sensitive to tested pesticides than later stages of embryonic development. Effect of each pesticide on development of carp eggs is summarized in table 2.

Deformities caused by the pesticides in the larvae include stunted growth of body beyond the yolk sac (figures 5, 9, 11 & 13), upward and inward curving of the tail (figures 4, 6–8, 12), deformed head region (figure 13), enlargement of the pericardial sac (figures 2–4), deformed vertebral column (figures 6–8), circulatory failure and poorly developed eye pigments and chromatophores. In case of higher concentrations of pesticides, eggs developed through optic lobes formation but died before hatching. From failure of hatching it can be concluded that pesticides affect the activity of hatching enzymes.

Discussion

Temperature—Effect of temperature on the developmental rate of teleosts embryos have received the attention of several workers. Mayes 1949, Hayes et al. 1953, Kinne & Kinne 1962 and Garside 1966, 70 have reported that the early development of fish is strongly affected by the incubation temperature. In general, lower temperatures tend to retard and higher temperatures accelerate embryonic development. In scale carp, as observed during the present studies, the rate of development increased from 10 to 30°C, the optimum being at 30°C. However, Tatarko (1965) observed optimum hatching of pond carp at 16–20°C in Kiev (USSR).

The present observations are in conformity with the findings of Hayes et al. (1953) in *Salmo salar ;* Forrester and Alderdice (1966) in *Gadus macrocephalus;* Tatarko (1966) in scale carp; Edsall (1970) in *Alosa pseudoharengus;* Ehrlich and Farris (1971) in *Leuresthes tenuis* and Colby and Brouke (1973) in *Coregonus artedii* that there is direct and approximately linear relationship between rate of development and temperature. Although in case of *Limanda yokoshama* the relation is slightly curvilinear



Figures 1–6 (×10.08). *1*, Newly hatched larva of controlled eggs; *2*, Larva of eggs treated with carbaryl showing enlarged pericardial sac and coiled posterior region; *3,4*, Larva of phosphamidon treated eggs showing enlarged pericardial sac, poorly developed pectoral fins and inwardly curved tail; *5*, Note the retarded growth of posterior trunk region and knob-like tail without fin in larva of diazinon-treated eggs; *6*, Note deformed vertebral column of larvae of diazinon-treated eggs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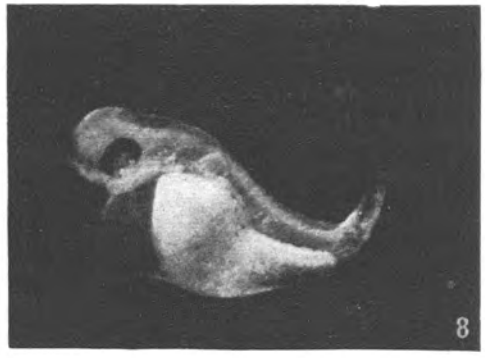
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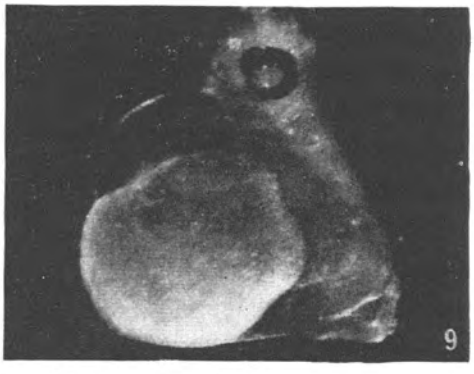
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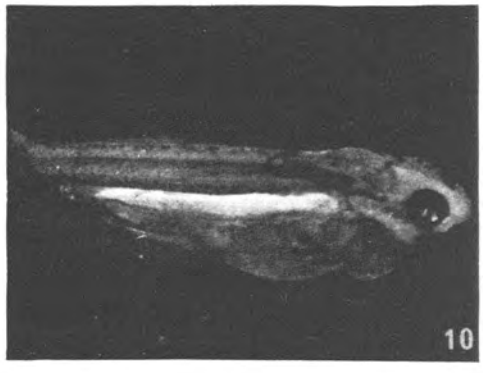
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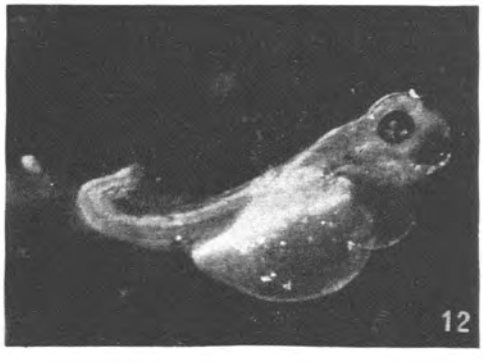
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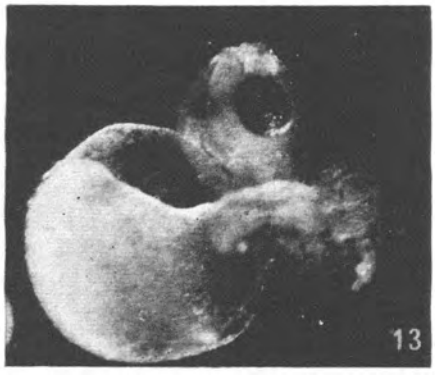
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(Yusa et al. 1971). Such inflections have also been reported for the embroys of *Salvelinus fontinalis* (Hayes et al. 1953), *Salmo gairdneri* and *Cyprinodon macularius* (Kinne & Kinne 1962). They indicated that developmental rate is not a linear function of temperature throughout the biokinetic range of developmental temperatures. Recently, Tay and Garside (1975) have also reported curvature in this relationship between developmental time and temperature for *Fundulus heteroclitus* embryos. According to them developmental time decreased disproportionately with the increase of temperature.

Our observation that the hatching period varied inversely with the temperature was similar to that of Lasker (1964), Swift (1965), Hubbs et al. (1969), Edsall (1970) and Florez (1972) for other fishes. It is observed during the present studies that both low (10°–15°C) and high (35°C) temperature retarded the mitotic division resulting in the failure of eggs to undergo normal cleavage and finally caused death before gastrulation. However, Shrivastava (1965) reported that cleavage abnormalities result from the unequal division of the blastodisc in *Cyprinus carpio*. In case of *Gasterosteus aculeatus* besides the unequal division of blastodisc, Swarup (1959 a,b,c,) observed other abnormalities like twins formation, spina bifida and microcephaly. Abnormal cleavage at very low and high temperature may be due to nuclear or cytoplasmic impairment. Lasker (1964) also reported that at temperature below 13°C all sardine *(Sardinops caerulea)* larvae failed to develop pigmented eyes and functional jaws. The undeveloped tail region in scale carp larvae may result from the effect of temperature on the closure of blastopore.

The present observation is in agreement with the observations of Kinne and Kinne (1962), Sweet and Kinne (1964) and Forrester and Alderdice (1966) that there was an inverse relationship between size of larvae and temperature. Recently Tay and Garside (1978) also obtained similar results in case of *Fundulus heteroclitus*. Hubbs (1926) reported that high temperature accelerate the differentiational aspect of development rate while at the same time it causes a relative diminution of growth aspect of the embryos. However, according to Tay and Garside (1975), the metabolic cost of maintenance in embryos at higher developmental temperature is disproportionately higher, so less of the yolk is available for structural synthesis.

Dissolved oxygen—As in our experiments, low DO concentration caused delayed hatching or retarded embronic development of Pacific salmon *Oncorhynchus* spp. (Alderdice et al. 1959); lake trout, *Salvelinus namaycush* (Gulidov 1969); and walleyes, *Stizostedion vitreum* (Oseid & Smith 1971). Developmental delay in coho salmon (*Oncorhynchus kisutch*) and northern pike (*Esox lucius*) also increased progressively with each reduction in DO concentration (Siefert & Spoor 1974). Our observation that the retarding effect of low DO on embryonic development is less pronounced at the earlier cleavage stages than at later stages conforms with the observations of Winnicki (1967); he reported that the decline in

Figures 7–13 *7*, Note deformed vertebral column of larvae of diazinon-treated eggs; *8*, Newly hatched larva of fenitrothion-treated eggs showing deformed vertebral column; *9*, Note the poor development of body beyond the yolk sac in larva of fenitrothion-treated eggs; *10*, Note the upward curvature of tail in larva of fenitrothion-treated eggs; *11*, Freshly hatched larva of malathion-treated eggs showing retarded growth of body beyond the yolk sac; *12*, Freshly hatched larva of malathion-treated eggs showing upward curving of tail; *13*, Note the deformed head region and retarded growth of body beyond the yolk sac in freshly hatched larva of malathion-treated eggs

developmental rate of embryos of brown trout (*Salmo trutta*) and rainbow trout (*S. gairdneri*) reared under unfavourable DO conditions was insignificant at early stages of morphogenesis but intensified at the blastopore stage. Hamor and Garside (1976) also reported that developmental rate was less affected by reduced DO levels at early stages but that after the first 10% of the development time had passed the retarding effect became progressively stronger. This increasing retardation has been demonstrated in three other salmonids by Garside (1959, 1966).

Our observation that the embryos became more sensitive to low concentrations (<6.0 mg/l) as development advanced was similar to that of Lindroth (1942) for salmonids. This increased sensitivity is presumed to be linked with the progressive increase of oxygen uptake as the embryos developed (Lindroth 1946). Banarjee and Sen (1969) also reported that oxygen consumption of eggs of the rohu (*Labeo rohita*) was very low during initial stages but increased as development proceeded; they assumed that oxygen uptake increased because the number of cells increased and not because the respiration of individual cells increased. Yurovitskii (1965) correlated the low sensitivity of embryos at early development stages to the absence of special respiratory organs, but as development proceeded or respiratory activity increased, the acceptable DO range was reduced. Interference with developmental processes of scale carp embryos by oxygen below 6.0 mg/l to the point of causing death, has also been reported for fish embryos by several authors (Alderdice et al. 1959, Eipper 1963, Doudorff & Shumway 1970, Saksena & Joseph 1972).

Our observations agreed with those of Gulidov (1969) and Turner and Timothy (1971), who reported that percentage hatching increased with increasing DO content of the water for northern pike and striped bass (*Morone saxatilis*),

The lack of abnormalities in hatched embryos and the tolerance for a wide range of DO seems attributable to natural selection in scale carp, whose eggs, laid on floating weeds, are exposed to fluctuating oxygen levels, as also reported by Gulidov (1969, 1971).

p*H*—Reports on experimental studies of lethal limits of *p*H for fish eggs are lacking. However, the lethal limits of *p*H have been determined for 12 fish species (see Daye & Garside 1975). Bandt (1936) concluded *p*H 10.4–10.8 lethal for carp (*Cyprinus carpio*) fingerlings which is coinciding the upper lethal limit of *p*H i.e. 10.5 observed for scale carp eggs during present investigation.

Recently Menedez (1976) and Trojnar (1977) reported that embryo hatchability was reduced significantly at all *p*H levels below 6.5 and 5.0 in case of *Salvelinus fontinalis* and white sucker (*Catostomus commersoni*) respectively. The lower lethal limit of *p*H for carp eggs is observed to be 6.0.

The present observation is in agreement with the findings of Krishna (1953) that eggs displayed exosmosis and endosmosis in highly acidic and highly alkaline medium respectively.

Light—Our observation that photoperiod has no effect on the hatchability and incubation time of scale carp eggs conforms with the observations of DeCiechomski (1967), Lillelund (1967) and Bieniarz (1973). However, MacCrimmon and Kawain (1969) reported that mortality, time taken to hatch, metabolic rate and number of vertebrae formed correlated positively with visible light intensity. Similar observations were also made by Lyubitskaya (1956), Canagaratnam (1959) and Eisler (1961). Bieniarz

(1973) reported negative effect of light of an intensity exceeding 330 lux, on the egg incubation of rainbow trout.

Pesticides—The low concentrations of pesticides which are toxic to fish eggs and fry frequently occur in polluted waters. Malone and Blaylock (1970) reported that carp embryos viability was not affected by any chemical (DDT, chlordane, dieldrin and endrin) at concentration less then 1.00 ppm and only diazinon and guthion induced mortality at 1.00 ppm. At concentrations 5 and 10 ppm all insecticides caused significant mortality of *C. carpio* embryos. During the present investigation, it is observed that 100% mortality of carp embryos is induced by 2.5 ppm carbaryl, 1.5 ppm diazinon, 135 ppm phosphamidon, 5 ppm malathion and 1 ppm fenitrothion.

Survival of developing embryos of *C. carpio* and their hatchability decreased in higher concentrations of tested pesticides and of DDT and PCB (Halter & Johnson 1974). Failure of hatching in higher concentrations may be due to the inhibitory effect of pesticides on hatching enzymes. However, more hatchability in very low concentration (001 ppm) of carbaryl, melathion and fenitrothion than controls may be due to slight stimulation of hatching enzymes by pesticides (Hart & Fouts 1965 and Bernstein et al. 1968).

On the basis of mortality at different stages of development it was found that early stages (upto optic vesicle formation) were less sensitive to tested pesticides than later stages of embryonic development in case of scale carp and rainbow trout (Olson & Marking 1973).

Comparison of LC_{50} values of pesticides (table 3) calculated for scale carp eggs and fries (Toor & Kaur 1974) clearly indicates that early life history stages are more susceptible than fry. Mironov (1969) also reported that earlier the stage of life greater is the effect of pollutant. But Johnson (1968) suggested that carp eggs and embryos are less susceptible to insecticides than adults.

Unlike the observations made by Halter and Johnson (1974) that mean time of hatching decreased with increase of concentration of PCB and DDT, the variation in hatching time of carp eggs is attributed to temperature and not to pesticides.

Weis and Weis (1974) reported that carbaryl and parathion caused developmental arrest prior to initiation of heart beat. Exposure for longer periods caused circulatory failure and death of embryos before hatching. Similar abnormalities have also been found to develop in pesticides treated embryos of scale carp during the present studies. These deformities may appear due to the interference of pesticides in cell differentiation and morphogenesis.

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