Journal of Environmental Biology
©Triveni Enterprises, Lucknow (India)
Free paper downloaded from: www.jeb.co.in

31 301-306 (**May** 2010)
For personal use only
Commercial distribution of this copy is illegal

Variations in physico-chemical and microbiological characteristics of water during breeding of *Cyprinus carpio* in a closed hatchery system

Devendra Mohan* and Devendra Choudhary

Department of Zoology, Jai Naraian Vyas University, Jodhpur - 342 005, India

(Received: May 10, 2008; Revised received: May 27, 2009; Accepted: June 30, 2009)

Abstract: *Physico-chemical and microbial characteristics of culture water were examined during the induced breeding of Cyprinus carpio in a controlled environmental system. Water temperature, dissolved oxygen, nitrate, phosphate, pH, ammonia nitrogen, total bacterial count, hardness, salinity, carbonate and bicarbonate were estimated before and after spawning and hatching. Average alteration in water pH before and after spawning was 7.91-7.57 and 7.86-7.58 respectively. Total hardness, carbonate and bicarbonate showed insignificant variations. Nitrate, ammonia nitrogen and phosphate contents significantly increased after spawning and hatching $p<0.05$. The average increase in nitrate was from 2.94 to 8.62 $\mu g\ l^{-1}$ after spawning and 3.10 to 8.49 $\mu g\ l^{-1}$ after hatching. Ammonia nitrogen contents were sharply increased from an average of 0.011 to 1.87 $mg\ l^{-1}$ after spawning and 0.013 to 0.56 $mg\ l^{-1}$ after hatching. The average phosphates increased from 2.59 to 4.15 $\mu g\ l^{-1}$ after spawning and 2.61 to 4.03 $\mu g\ l^{-1}$ after hatching. Dissolved oxygen was sharply depleted even after a continuous aeration. Temperature played a vital role during breeding. No successful breeding was observed at a temperature of 17°C or below and 31°C or above. There is a significant association between temperature, spawning and hatching ($p<0.05$). By optimizing temperature, the breeding success of this carp was achieved with a statistical significance of $p<0.05$. Total bacterial count was significantly increased after spawning and hatching. It was related to the amount of discharge and may cause mass mortality of fish embryo and spawn in a closed hatchery system.*

Key words: *Cyprinus carpio, Induced breeding, Closed hatchery system, Physico-chemical factors, Microbial factors*
PDF of full length paper is available online

Introduction

Breeding in fishes is regulated by environmental factors that trigger internal physiological mechanisms. The final event of the breeding cycle, the release of eggs and milt resulting in spawning, can be controlled by placing the fish in an appropriate environment and by changing its internal regulatory mechanism by injecting hormones or other inducing substances. The internal mechanisms that regulate spawning are similar in most fish (Rottmann *et al.*, 1991). The external environmental factors that control reproduction vary considerably among species.

Abiotic and microbial factors play a significant role in fish breeding, early ontogeny and survival of spawn in high-density culture. A large-scale mortality of developing embryos and spawn of culturable carps in fish hatcheries due to unfavourable environmental factors is of serious concern to fishery scientists and fish farmers. The conditions considered optimal for survival and growth of older fish may be a limiting factor for embryo or spawn. A natural variation in some abiotic and microbial factors of water occurs during fish spawning and become even more serious in a closed hatchery system or in places where the quality water for fish spawning is limited.

Dwivedi *et al.* (1988) observed the optimum range of factors like temperature, pH and dissolved oxygen for carp breeding and spawn rearing. Singh and Das (2006) reported that the reproduction, mortality and growth of *Cyprinus carpio* is influenced by some abiotic factors. Alikunhi (1966) reported the variation in the size and weight of *Cyprinus carpio* at variable temperature ranges. Mohan (2000) studied the effects of some of the abiotic factors on the breeding of some Indian and exotic carps.

The proper environmental conditions stimulate the reproduction process, while unsuitable conditions can override any attempt of induced spawning. Variation in the water quality after egg laying and hatching significantly influence the survival of embryo and spawn and may directly influence the hatchery operation. In the present study, we examined the variations in abiotic and microbial factors during breeding of *Cyprinus carpio* in a closed system.

Materials and Methods

The brooders of *C. carpio* were collected from near by water bodies and were carried to the laboratory in open canvas happa. They were acclimatized in the brooders pond of the department until breeding in a closed system. Continuous flow of water was not maintained in the closed system breeding and hatching units. However, water circulation and aeration was maintained within the breeding unit. Water samples were taken before and after spawning and hatching. For induced breeding the male and female brooders were collected by cast net and transferred to the breeding unit where ovaprim was injected at their caudal peduncle at 0.2 to

* **Corresponding author:** mohandevendra@hotmail.com

0.4 ml kg^{-1} body weight. The male and females were weighed before and after spawning in an electric balance to measure the discharge. Eleven set of breeding experiments from November, 2006 to May, 2007 were conducted at a temperature range of 15ºC to 31ºC. For each set three experiments were run having similar conditions. Thus, a total of thirty three experiments were conducted and the average of the each set is compiled in the tables. For experiment number 1 to 9 no alteration in water temperature was required since the variations in water temperature were according to the seasonal thermal variations. In the month of November, 2006 in one set of the experiments the water temperature was raised by recirculating experimental water through water heater and after attaining the required temperature the thermostat was applied to maintain it. During the experiments the water was in continuous circulation through the bottom water circulation system. Continous aeration was also done to maintain the desired level of dissolved oxygen. The water temperature was dropped from 31 to 25°C by continuous showering and fanning in the month of May, 2007 and by adding ice prepared in an Icematic Machine from same water as and when required. Water circulation and continous aeration was maintained and the temperature was monitored after every two hours during experiments. Plastic strips of 10 cm width and 90 cm length were exposed in breeding units for sticking of the eggs. After spawning the strips containing sticky eggs were transferred from the breeding unit to the hatching unit. This unit contains fresh water with aeration and a sediment removal system. Water samples were collected from the breeding unit before and after spawning and then from hatching unit before and after hatching for Physico-chemical and Microbial examination. The associations were seen by Chi-square test.

Estimation of physico-chemical and microbiological parameters: Temperature was measured by digital thermometer and pH by digital pH meter. Dissolved oxygen was measured by modified Winkler's method, carbonate and bicarbonate alkalinity by using phenolphthalein+ methyl orange as indicators, Salinity by Argentometric method, Total hardness by using EDTA titration method and Ammonia nitrogen by Nesslerization method as given in APHA (1985). Free carbon dioxide was measured by titration method using phenolphthalein as an indicator (Welch, 1948), Phosphate by using stannous chloride (Murphy and Riley, 1962) and Nitrate by using copper sulphate and hydrazine sulphate as reductant (Mullin and Riley, 1955).

The total coliform count was estimated as suggested in APHA (1985). The culture media used was Mac-Conkey's Agar and the incubation period and incubation temperature were 24±2 hr and 37±0.5°C respectively. Water samples before and after spawning and hatching were poured on the Mac- Conkey's Agar plates and incubated. After incubation the appearance of red to pink colonies indicated the presence of members of the coliform group.

Results and Discussion

Eleven set of induced breeding experiments were conducted during 2006-2007 on *C. carpio*, which breeds twice in a year in this region (Table 1a,b). Variations in the abiotic and microbial parameters of water before and after spawning and hatching were recorded (Table 2). A mass mortality of embryo and spawn was observed whenever the water was not replaced after egg laying. Thus, in each experiment the water was changed after egg laying and hatching to refresh the system and to regulate the abiotic and microbial parameters. Among abiotic factors, temperature was recorded as one of the most vital factor for breeding of fish. No breeding was recorded whenever the temperature was 17°C or below and 31°C or above. Significant variations were observed in time of egg laying, fertilization percent and time of hatching with respect to changing temperature ($p<0.05$). At lower temperatures the time taken for egg laying and hatching was increased significantly ($p<0.05$). At 17°C the time taken for egg laying was 63 hrs with 10% fertilization and no hatching. However, when this temperature was raised to 25°C the time taken for egg laying was reduced to 30 hours and fertilization and hatching percentage was increased to 50% each and the hatching time reduced to 48 hr ($p>0.05$). This experiment was conducted in the month of November 2006. At 19°C the time taken for egg laying was 44 hr after the hormone injection with a fertilization percentage of 50% and hatching time of 62 hr. No breeding was observed in the month of May, when the temperature was 31°C or above but when this temperature was reduced to 25°C by continous showering, fanning and by adding ice, breeding occurred. The spawning time was recorded 20 hr after injecting ovaprim with 40% fertilization and 60% hatching. Thus by regulating this factor only, the breeding of this fish was possible during adverse thermal conditions as well. The optimum temperature range for the breeding of this species was 23 to 29°C. Balon (1975) reported that temperature seems to be the most important external factor for the incubation of the fish egg. Further, he reported (Balon, 1995) that 17°C as the lowest limit of spawning of common carp while Osipova (1979) reported 15°C as the lower limit of spawning for wild species. Embryonic development remains slow at lower temperature and accelerates with increasing temperatures with in the optimum limits.

Incubation temperature affects certain morphological features, the hatching rate along with the behaviour of the larvae (Bagenal and Braum, 1978). Penaz *et al*. (1983) studied the functional relationship between temperature and duration of embryonic development in *C. carpio* to determine the optimum range of temperature. Herzig and Winkler (1986) reported that the hatching period varies inversely with temperature and duration of development shows a curvilinear relationship with temperature. Barbara *et al*. (2000) reported that the incubation temperature below 17°C and above 26°C results in an increased production of defective larva. The dissolved oxygen contents were found decreased with increasing temperature. Das *et al.* (2006) studied the effect of four temperatures on the embryonic development of *Labeo rohita* and reported that at 31°C the hatching percentage was highest and the time taken for each ontogenic stage was lowest while at 36°C the hatching percentage was lowest and time taken for each ontogenic stage was highest. Different carp species have different ranges of optimum temperature and tolerance limits which

Table - 1(a): Induced breeding of *Cyprinus carpio*

Expt. No.	Water temp (°C)	Brooder weight (kg)					
		Male			Female		
		BS	AS	Weight loss	BS	AS	Weight loss
1	15	2.50±0.351	-	-	1.20±0.200	-	-
2	17	2.25±0.950	2.16±0.054	0.09±0.004	1.20±0.087	1.08±0.066	0.12±0.025
3	19	2.80±0.100	2.71±0.788	0.09±0.023	1.50±0.173	1.30±0.218	0.20±0.050
4	21	5.63±0.085	5.47±0.075	0.16±0.020	3.02±0.176	2.67±0.120	0.35±0.061
5	23	6.35±0.127	6.16±0.115	0.19±0.012	3.75±0.228	3.38±0.205	0.37±0.023
6	25	3.59±0.197	3.45±0.195	0.14±0.002	2.54±0.153	2.20±0.155	0.34±0.003
7	27	3.42±0.075	3.31±0.090	0.11±0.021	2.57±0.113	2.34±0.104	0.23±0.009
8	29	2.34±0.139	2.25±0.152	0.09±0.013	1.50±0.173	1.29±0.096	0.21±0.079
9	31	2.50±0.200	-	-	1.15±0.132	-	-
10	31 ↓25	2.95±0.606	2.85±0.604	0.10±0.010	1.73±0.243	1.45±0.298	0.28±0.064
11	17 ↑25	3.55±1.540	3.43±1.526	0.12±0.015	2.25±0.529	1.93±0.458	0.32±0.082

↑↓ rise or fall in temp., BS = Before spawning, AS = After spawning, - = No breeding, ± = S.E.

Table - 1(b): Induced breeding of *Cyprinus carpio*

Expt. No.	Water temp. (°C)	Time taken for egg laying (hr)	No. of eggs laid (in lakh)	Fertilization (%)	Hatching time (hrs)	Hatching (%)	Seed produced (in lakhs)
1	15	-	-	-	-	-	-
2	17	63±2.00	1.12±0.442	10±2	-	-	-
3	19	44±4.36	1.82±0.446	50±5	62±3.464	25±3.61	0.227±0.027
4	21	23±2.65	2.97±0.173	80±2	40±2.646	70±6.08	1.663±0.248
5	23	20±1.73	3.15±0.229	80±2	33±2.646	90±7.57	2.268±0.144
6	25	18±2.65	2.87±0.341	75±7	20±2.000	85±7.57	1.830±0.114
7	27	14±1.00	2.07±0.131	90±3	18±2.000	85±7.81	1.583±0.114
8	29	12±1.73	1.93±0.250	80±3	17±2.646	40±2.08	0.617±0.104
9	31	-	-	-	-	-	-
10	31 ↓25	20±5.29	2.42±0.252	40±4	28±1.732	60±3.46	0.580±0.131
11	17 ↑25	30±5.57	2.75±0.244	50±5	48±4.000	50±3.06	0.687±0.121

↑↓ rise or fall in temp., - = No breeding, ± = S.E.

should be managed in a successful breeding and high density spawn culture. During the present study also it was observed that spawning, hatching and seed production is influenced by changing temperature.

Dissolved oxygen (DO) is another important factor for a successful breeding operation of the fish. Mortality of developing embryos and hatchlings occur whenever DO drops below 0.8 mg l^{-1}. During the start of breeding experiments a higher level of 4.1 to 4.8 mg l^{-1} of DO was maintained which drops to 1.2-2.4 mg l^{-1} after spawning and 1.1-2.8 mg l^{-1} after hatching even after a continuous aeration of the system. The sudden decline in the DO may occur due to the increased oxygen demand of the embryo and spawn, and the decomposition of organic matter and increased bacterial load. The common carp is known to survive at a dissolved oxygen level of as low as 0.3 to 0.5 mg l^{-1} while this DO is fatal for the embryo and spawn. Gupta *et al.* (2000) suggested that 4.0 ml l^{-1} of DO is optimum for hatchery operation of Indian major carps. Fish need oxygen to breathe and the bacteria need oxygen to convert ammonia into nitrate. The bacteria need more oxygen than fish in a closed system. The dissolved oxygen decreases by both of these factors so a continuous aeration is required for breeding and high-density spawn culture in a closed hatchery system.

Nitrate significantly increased ranging from an average of 2.94 to 8.62 µg l^{-1} before and after spawning and from 3.10 to 8.49 µg l^{-1} before and after hatching showing an average increase of about 3 times during induced breeding experiment with *C.carpio.* Gupta *et al*. (2000) suggested that the permissible levels for nitrate and phosphate should be less than 0.1 and 0.01mg l^{-1} respectively. Nitrate commonly results from the decomposition of ammonia, which is a component of excretory product. Although nitrates are not as toxic as ammonia or nitrites, they must be monitored to avoid stressing of fish. In a closed hatchery system the nitrates increased significantly after spawning and hatching and can only be eliminated by replacement of experimental water, which is essential for a successful fish breeding operation. Bacterial oxidation of ammonia results in the formation of nitrite and nitrate which should also be regulated within the limits to prevent mortality of embryo and spawn.

Ammonia contents sharply increased after spawning and hatching. The average increase in ammonic nitrogen was from 0.011

Table - 2: Abiotic and microbial parameters during the induced breeding of *Cyprinus carpio*

Exp. No.	Water Temp. (°C)	DO (mgl^{-1})				Nitrate (μgl^{-1})				Phosphate (μgl^{-1})				pH				Ammonia-nitrogen (mgl^{-1})				Total bacterial count (colonies ml^{-1})			
		BS	AS	BH	AH	BS	AS	BH	AH	BS	AS	BH	AH	BS	AS	BH	AH	BS	AS	BH	AH	BS	AS	BH	AH
1	15	4.8	-	-	-	2.76	-	-	-	2.65	-	-	-	8.0	-	-	-	.010	-	-	-	40×10^2	-	-	-
2	17	4.4	1.2	4.2	-	3.25	9.55	3.04	-	2.88	4.16	2.37	-	7.8	7.4	7.8	-	.012	0.95	.017	-	50×10^2	750×10^2	35×10^2	-
3	19	4.6	1.9	4.4	2.2	2.67	9.46	2.74	9.47	2.42	3.95	2.23	3.25	7.9	7.5	7.8	7.7	.012	1.10	.016	.30	31×10^2	950×10^2	35×10^2	900×10^2
4	21	4.5	1.3	4.8	1.1	3.01	8.85	3.25	8.82	2.85	4.52	2.65	4.82	8.1	7.7	8.0	7.5	.012	2.25	.012	.62	30×10^2	980×10^2	35×10^2	720×10^2
5	23	4.6	1.2	4.3	1.4	3.54	7.90	3.06	8.95	2.01	4.01	2.45	4.52	7.9	7.7	7.8	7.4	.010	3.30	.016	.85	45×10^2	950×10^2	40×10^2	700×10^2
6	25	4.4	2.4	4.3	1.9	3.01	7.56	2.85	7.80	2.85	3.96	2.52	4.83	7.8	7.5	7.9	7.6	.010	2.50	.013	.66	50×10^2	1150×10^2	75×10^2	950×10^2
7	27	4.2	2.2	3.8	2.8	3.05	8.82	3.42	8.01	2.20	4.03	2.81	3.11	8.0	7.7	7.9	7.7	.009	2.10	.011	.52	25×10^2	900×10^2	40×10^2	700×10^2
8	29	4.1	1.9	4.2	2.8	2.85	8.21	3.01	7.92	2.43	3.54	2.65	4.05	8.1	7.8	8.0	7.7	.010	1.20	.012	.50	27×10^2	1500×10^2	31×10^2	1000×10^2
9	31	4.2	-	-	2.8	2.90	-	-	-	2.45	-	-	-	7.9	-	-	-	.012	-	-	-	35×10^2	-	-	-
10	31 ↓25	4.3	2.0	4.5	2.1	2.76	9.01	3.90	8.90	2.75	4.04	2.50	3.48	7.8	7.4	7.8	7.6	.011	1.15	.010	.42	33×10^2	900×10^2	45×10^2	1050×10^2
11	25 ↑17	4.8	1.3	4.5	2.8	2.54	8.25	2.65	8.06	3.01	5.15	3.33	4.23	7.8	7.5	7.8	7.5	.010	2.25	.012	.60	33×10^2	1000×10^2	30×10^2	750×10^2

BS = Before spawning, AS = After spawning, BH = Before hatching, AH = After hatching, DO = Disolved oxygen, ↑↓ rise or fall in temperature, - = absent

Table - 3: Variation in average value of the abiotic parameters during the breeding experiments

Abiotic parameter (*)	BS	AS	BH	AH
Hardness	33.45 ± 1.27	36.44 ± 1.69	33.55 ± 1.21	36.12 ± 1.10
Salinity	108 ± 40	136 ± 10	110 ± 40	120 ± 30
Free CO_2	22.18 ± 2.79	28.22 ± 1.80	22.22 ± 0.74	23.62 ± 0.27
Bicarbonate	1.63 ± 0.33	2.44 ± 0.46	1.77 ± 0.13	2.75 ± 0.27

BS = Before spawning, AS = After spawning, BH = Before hatching, AH = After hatching, * = All values are in mg l^{-1} ± = S.E.

to 1.87 mg l^{-1} before and after spawning and 0.013 to 0.56 mg l^{-1} before and after hatching. Shah *et al.* (1956) reported that the spawn could tolerate 2.5 mg l^{-1} of dissolved ammonia. Ammonia is the primary nitrogenous waste product of the carps; it also reaches water from fish excreta, milt, unfertilised eggs, uneaten food and microbial decay of nitrogenous compounds. High stocking density and uneaten food increases the ammonia level when the dissolved oxygen is low (Merkens and Downing, 1957). Abbas (2006) recorded 0.93 mg ammonia-nitrogen l^{-1} as 96 hr LC_{50} for *C. carpio* at a pH of 7.5.

The Phosphate contents significantly increased after spawning and hatching. The average increase was from 2.59 to 4.15 µg l^{-1} after spawning and 2.61 to 4.03 µg l^{-1} after hatching. Gupta *et al.* (2000) suggested the optimum range of 0.01 mg l^{-1} for hatchery unit and 0.01-0.5 mg l^{-1} for brooder pond. Although phosphate has no direct effect on fish, higher concentration causes algal and cyanobacterial blooms, which can adversely affect the embryo and spawn of the fish. Sharma and Chakrabarti (2004) reported that the values of Phosphate, Ammonia and Nitrate were significantly increased when the stocking density of the larvae of *Catla catla* and *Labeo rohita* was higher *i.e.* greater than 10,000 larvae m^{-3}.

Carbonate was found absent in most of the experiments while free carbon dioxide was mostly present ranging from an average of 22.18 to 28.22 mg l^{-1} before and after spawning and 22.22 to 23.62 mg l^{-1} before and after hatching. Thus a little increase in free CO_2 was reported after spawning and hatching. This may be due to increased oxygen demand because of bacterial decomposition and respiration by the embryo and hatchlings in high-density culture. Continuous supply of air may also affect this factor. Bicarbonate alkalinity fluctuates between 1 to 3 mg l^{-1} in all the experiments under both conditions. Salinity also showed a little variation during the experiments, increasing from an average of 108 to 136 mg l^{-1} after spawning and from 110 to 120 mg l^{-1} ppt after hatching.

Average hardness of water before and after spawning was 33.45 and 36.44 mgl^{-1} and before and after hatching it was 33.55 to 36.12 mg l^{-1}. The insignificant variation was recorded after spawning and hatching (Table 3). Mateen *et al*. (2004) reported higher hardness of water is more favourable and has no negative effects on the growth of *L. rohita* and its hybrid.

The average pH of the water before spawning was 7.91, which reduced to an average of 7.57 after spawning. Similarly, the pH reduced from 7.86 to 7.58 before and after hatching respectively. This reduction was also due to the discharge and decomposition of the organic contents. The pH remained alkaline throughout the experiments. This range comes with in the optimum limits as suggested by Gupta *et al.* (2000) and Das *et al*. (2006).

The total bacterial count was estimated during the experiments. The mean value of the bacteria in the water of breeding pool was 36.27×10^2 colonies ml^{-1}, which rose to 1008.88×10^2 colonies ml^{-1} after spawning of the fish. Thus there was an average increase of about 28 times. If the water was not replaced, a mass mortality was recorded which was time and number of bacterial colonies dependent. The egg strips from the breeding unit were carried to fresh water of the hatching unit where the mean value of bacterial count was about 40.66×10^2 colonies ml^{-1}. After hatching number of bacterial colonies further rose to a mean level of 846.25×10^2 colonies ml^{-1}. The highest increase in the bacterial count was 1500×10^2 colonies ml^{-1} after spawning and 1050×10^2 colonies ml^{-1} after hatching. Mass mortality of embryos and hatchlings was recorded whenever the bacterial count exceeds 1800×10^2 colonies ml^{-1}. Thus, microbial flora also plays vital role in the breeding operation in a closed system, although this factor is being ignored in most fish breeding operations.

By optimizing the physico-chemical and microbial factors, fish breeding can provide pure fish seed locally and help in the promotion of pisciculture in the areas like Western Rajasthan where natural fish breeding grounds are limited. Thus a closed fish hatchery system is suitable in areas particularly in the arid region where the quality water is limited.

Acknowledgments

The authors are thankful to the Department of Science and Technology, New Delhi for providing financial assistance and to the Head, Department of Zoology for providing laboratory facilities. We are also thankful to Dr. S. K. Bansal, Scientist 'F', DMRC, Jodhpur and Dr. J. LakshmiNarayan, Scientist 'E', who have helped in the statistical calculations.

References

Abbas, H.H.: Acute toxicity of ammonia to common carp fingerlings (*Cyprinus carpio*) at different pH levels. *Pakistan J. Biol. Sci.*, **9**, 2215-2221 (2006).

Alikunhi, K.H.: Synopsis of biological data on common carp, *Cyprinus carpio* Linnaeus, 1758 (Asia and the Far East). FAO Fisheries Synopsis, No. 31.1 (1966).

APHA: Standard methods for the examination of water and wastewater. 16th Ed. *Amer. Pub. Hlth. Assoc. Inc. Washington D.C.* p. 1193 (1985).

Bagenal, T.B. and E. Braum: Eggs and early life history. *In*: Methods for Assessment of Fish Production in Fresh Waters (*Ed.*: T. Bagenal). IBP Handbook No. 3, 3rd Edn., Oxford, London, Edinburgh and Melbourne. Blackwell Scientific Publication, 165-201 (1978).

Balon, E.K.: Terminology of intervals in fish development. *J. Fish. Res. Bd. Can.,* **32**, 1663-1670 (1975).

Balon, E.K.: Origin and domestication of the wild carp, *Cyprinus carpio*: from roman gourmets to the swimming flowers. *Aquacul.*, **129**, 3-48 (1995).

Barbara, J., K. Lugowska, M. Witeska and P. Sarnowski: Malformations of newly hatched common carp larvae. *Electronic J. Polish Agric. Univ.*, **3**, 1-13 (2000).

Das, P.C., S. Ayyappan and J.K Jena: Haematological changes in the three Indian major carps, *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton) exposed to acidic and alkaline water pH. *Aquacul.,* **256**, 80-87 (2006).

Dwivedi, S.N., A.K. Reddy and V.K. Tiwari: A carp hatchery for India with controlled environment. *J. Inland. Fish. Soc. India*, **20**, 6-12 (1988).

Gupta, S.D., S.C. Rath and S. Ayyappan: Designing and management of eco-hatchery complex for carps seed production. *Fishing Chimes*, **19**, 27-33 (2000).

Herzig, A. and H. Winkler: The influence of temperature on the embryonic development of three cyprinid fishes, *Abramis brama*, *Chalcalburns chalcoides mento* and *Vimba vimba. J. Fish. Biol.*, **28**, 171-181(1986).

Mateen, A., M. Afzal and I. Ahmad: Effects of hardness on the growth performance of Rohu (*Labeo rohita*) and its hybrid. *Intl. J. Agric. Biol.,* **6**, 71-73 (2004).

Merkens, J.C. and K.M. Downing: The effect of tension of dissolved oxygen on the toxicity of unionized ammonia to several species of fish. *Ann. Appl. Biol.*, **45**, 521-527 (1957).

Mohan, D.: Modified fish hatchery for the stressed ecosystem of Western Rajasthan. *Aquacult.*, **1**, 111-119 (2000).

Mullin, J.B. and J.P. Riley: The spectrophotometric determination of nitrate in natural waters with particular reference to seawater. *Anal. Chim. Acta.*, **12**, 464 (1955).

Murphy, J. and J.P. Riley: A modified single solvent method for determination of phosphate in natural waters. *Anal. Chim. Acta.*, **27**, 31-36 (1962).

Osipova, V.B.: A contribution to the ecology of the carp, *Cyprinus carpio*, in the cheremshan arm of Kuybyshev Reservoir. *J. Ichthyol.,* **19**, 151-154 (1979).

Penaz, M., M. Prokes, J. Koutil, and J. Hamackova: Early development of the carp, *Cyprinus carpio*. *Acta Sc. Nat. Brno,* **17**, 1-39 (1983).

Rottmann, R.W., J.V. Shireman and F.A. Chapman: Hormonal control of reproduction in fish for induced spawning. SRAC Publication No. 424 (1991).

Shah, K.C., D.P. Sen and P. Mazumdar: Studies on the mortality in spawn and fry of Indian major carps during transport. Pt.3. Effect of inimical substances from decomposition of metabolic products in the medium on spawn life and their control. *Ind. J. Fish.*, **3**, 135-140 (1956).

Sharma, J. and R. Chakrabarti: Role of stocking density on growth and survival of catla, *Catla catla*, and rohu, *Labeo rohita*, larvae and water quality in a recirculating system. *J. Appl. Aquacul.*, **14**, 171-178 (2004).

Singh, A.K. and P. Das: Status of common carp (*Cyprinus carpio*-L., 1758) and its environmental impact in India. *Aquacul.*, **7**, 219-231 (2006).

Welch, P.S.: Limnological methods. Mc Graw-Hill Book Co. Inc., New York. p. 381 (1948).