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Influence of Nitrate and phosphate on Breeding Potentiality of Indian Major Carps

Amarnath Shit

Department of Zoology

Balarampur College, Purulia (WB) India

Abstract:

The present study was conducted to explore the changes in mineralization and morphology in response to graded levels of nitrate and phosphorus in Indian major carp. Nutrient content was performed for the culture of Indian major carps; *Labeo rohita* (rohu), *Catla catla* (catla), and *Cirrhinus mrigala* (mrigal) in ten fishponds. Softness of water is one of the major limnological criteria for fish breeding andrearing. Present work focuses on the influence of Nitrate and phosphate among the other physicochemical parameters on the breeding potentiality of Indian Major Carp (IMC). The limnological results of the breeding pond of the area are as follows: pH ranges between 6.62 and 8.01, temperature 24 to 35°C, conductivity 280 to 440 µS/cm at 25ºC. Nitrate-nitrogen is one of the most important indicators of pollution of water; its range 0.428 to 3.534 mg/L and ammonia-nitrogen range is 0.356 to 1.384 mg/L**,** Phosphate ranges between 0.006 to 0.583 mg/L. Here, some active and statistically significant environmental factors are also determined which are responsible nitrate and phosphate of water. Production of spawn increases in some of the farms due to influenceof some of these factors.

Key Words:

Indian Major Carps, Calcium Phosphate, Magnesium Phosphate, Fish breeding, physicochemical parameters

Introduction:

Aquaculture activities involve a variety of inputs for fish production including manures, fertilizers, feed and a combination of all these things. Fish culture in India was the begining in fresh water ponds since lOng back, 350 B.C. in a limited scale. Fish beeding is a natural process; but in natural pond fOr piciculture, sIgnificant difficulties are cOnsidered in obtaining the quality fish seed fOr stOcking purposes, because of dearth of cOngenial cOnditiOn of breeding in stagnant water. This hatchery is mOre popular due tO several advantages Overall Other types Of spawning and incubatiOn poOl (Gupta *et al.,* 2000). Temperature fluctuation has an important influence on the physiOlOgical activities Of the organisms mainly fOr the breeding purposes. Indian majOr carps are can tOlerate a wide range of temperature variatiOn. DissOlved oxygen cans controlling a number of functiOns like its size, feeding rate, activity level, *etc*. The fish were fed with an admixture of rice grain, mohua oil cake and groundnut oil cake in the ratio of 1:1:1 according to their 2% body weight of their biomass. The budget showed that recovery (in terms of fish yield) of nitrogen (N) decreased from 43 to 24% and the same for phosphorus (P) also decreased from 16 to 9% with increase in stocking density. The circular type of Chinese hatcherywith several mOdificatiOns (Chandra Hatchery by Bairagya and Ghosh, 2010) is presently known as an ecO-carp hatchery in India. This hatchery is mOre popular due tO several advantages Over all Other types Of spawning and incubatiOn poOl(Gupta *et al.,* 2000). To decrease the lack of nutrient in discharged water, it is essential to estimate the nutrient budgets to assess the destiny of nutrients added to the aquaculture systems. Nutrient budgets may permit quantification of potential pollution impact of a specific pond culture strategy. Chemical budgets have been formulated for tentative ponds containing channel of Indian major carps. Nitrogen (N) and Phosphorus(P) budgets were formulated for profitable aquaculture ponds in Thailand but the nutrient budget for Indian major carp' s culture is meager. Phosphorus is the most essential mineral supplemented in fish feeds. Phosphorus has multiple roles in the bodies of living organisms and has obviously influence on growth, stress tolerance, metabolism, and feeding consumption of fish. The second chemical measurement made to determine the health of the biological converter is Nitrite. Nitrite should not be measurable in a pond with a properly functioning bio-converter. Nitrite is produced by the autotrophic Nitrosomonas bacteria combining oxygen and ammonia in the bio-converter and to a lesser degree on the walls of the ponds. Nitrites are called the invisible killer. It can be deadly, particularly to the smaller fish, in concentration as low as 0.25 ppm. Effective removal of organic matter and adequate aeration and proper application of fertilizers are the keys to keeping nitrites from reaching toxic levels in fish ponds. The method has been used for discriminating within-population allometry and between-population shape differences in fish (Strauss and Bookstein 1982) and (Bronte *et al.*, 1999). The method was innovatively applied in fish nutrition study by (Fitzgerald *et al.,* 2002) who utilized truss analysis to differentiate fish subjected to different levels of ration. Several workers have reported skeletal deformities under conditions of phosphorus deficiency in fish and these have been reviewed in detail by (Sugiura *et al.*, 2004) and (Lall and Lewis-McCrea 2007). PhysicOchemical parameters including Nitrate and phosphate of the study pond at Ramsagar tOwn were very important fOr the breeding behaviOr of the fish. TherefOre, water quality may be cOnsidered as an important factOr fOr the aquaculture. The main objective Of the present investigatiOn is tO assess the influence of Nitrate and phosphate of pond water favOuring the breeding Of Indian majOr carps.

Materials and Methods:

The present experiments were carried out fOr a periOd of One year from September 2021 tO June 2022. The data was cOllectedthree times in a seasOn from three different sites Of the differentponds at Ramsagar tOwn under the district Of Bankura. The details of pond are represented in the (Table 1). The climatic cOnditiOn of the Ramsagar is Of mixed type of drywinter with very hot summer. The mean annual temperature varies between 35 and 46ºC in summer and from 14 tO 22ºC in winter. Water samples were cOllected during pre-breeding, breeding and post-breeding periOd from 2021 tO 2022 (September, February and June). AccOrding tO the requirement fOr the analysis Of water samples were mixed tOfOrm a cOmposite sample at each of the eleven lOcatiOns and brought tO the labOratOry and refrigerated at 4ºC and finally analyzed as per APHA (2005). Temperature, pH and cOnductivity were measured on-site using potable meter (PCS Testr 35 Multi-parameter). DO, BOD, hardness and tOtal alkalinity, Nitrate and phosphate were analyzed accOrding tO the standard methods Of APHA (2005) in the labOratOry.

Result and Discussion:

Table 1: Description of the sampling sites

Sampling sites	Sample code	Co-ordinatesLatitude		Longitude
Lagar pukur	LP	23º06'41.6"	N	087º15'09.9" E
Singer pukur	SGP	23º06'36.4''	N	087º15'19.1" E
Soyerpukur	SP	23º06'27.0"	N	087º15'12.9" E
Tanti pukur	TP	23º06'29.1"	N	087º15'38.4" E
Simabaadpukur	SBP	23º06'14.5"	N	087º15'51.9" E
Chandrabatipukur	CBP	23º05'58.1"	N	087º16'03.3" E

Chattaraj band	CRB	23º06’10.9"	N	087º15’49.7" E
Chandra pukur	CP	23º06’01.0"	N	087º16’16.3" E
Moyrapukur	MP	23º05’54.5"	N	087º16’35.6" E
Dusotinpukur	DP	23º05’39.5"	N	087º17’07.2" E

Table 2: Analysis of Physicochemical parameters of breeding ponds

Name of the Ponds	Seasons	pH	Temperature(°C)	Conductivity (□s)	Nitrate Nitrogen	Ammonia Nitrogen	Phosphate
LGP	Pre-breeding	6.93	21.8	380	1.051	0.936	0.036
	Breeding	7.63	25.0	370	1.905	0.542	0.117
	Post-breeding	6.87	29.3	340	1.524	0.635	0.036
SGP	Pre-breeding	6.99	22.4	410	0.623	0.563	0.093
	Breeding	6.87	25.6	430	1.524	0.744	0.153
	Post-breeding	6.98	29.0	390	3.476	0.633	0.058
SP	Pre-breeding	6.98	22.1	380	0.615	0.521	0.088
	Breeding	6.87	25.3	360	2.476	0.365	0.165
	Post-breeding	6.82	29.2	300	2.143	1.358	0.062
TP	Pre-breeding	6.87	22.2	460	0.458	0.562	0.177
	Breeding	7.50	26.2	360	1.238	0.851	0.290
	Post-breeding	8.01	29.4	380	3.190	0.935	0.125
SBP	Pre-breeding	6.93	22.2	430	0.615	0.512	0.109
	Breeding	6.89	26.5	400	2.476	1.259	0.252
	Post-breeding	7.00	29.3	390	2.143	0.542	0.072
CBP	Pre-breeding	6.95	22.9	370	0.664	0.452	0.583
	Breeding	7.01	26.3	390	0.428	0.653	0.145
	Post-breeding	7.00	29.3	460	2.048	1.076	0.097
CRB	Pre-breeding	7.03	22.5	360	0.514	1.258	0.215
	Breeding	7.00	26.8	390	2.657	0.651	0.177
	Post-breeding	6.95	29.4	320	2.762	0.356	0.074
CP	Pre-breeding	6.93	22.9	420	0.561	0.856	0.119
	Breeding	6.98	26.8	410	2.143	1.384	0.145
	Post-breeding	6.94	29.7	420	2.428	0.521	0.087
MP	Pre-breeding	6.83	22.8	300	0.821	0.852	0.084
	Breeding	6.66	26.9	330	0.539	0.554	0.147
	Post-breeding	6.62	26.2	450	2.619	0.653	0.145
DP	Pre-breeding	6.92	23.0	350	1.260	0.931	0.032
	Breeding	7.48	26.2	390	2.952	0.563	0.101
	Post-breeding	6.96	29.9	390	3.095	0.753	0.052

Table 3: Water Quality Standard (Bureau of Indian Standard, 1991)

Parameters	BIS Standard	Range
pH	6-9	6.62 - 7.03
Temperature (°C)	25-35	19 - 25
Conductivity (□S/cm)	300	300 - 460
DO (mg/L)	1-5	2.100 - 5.648
BOD (mg/L)	<6	2.197- 5.750
Hardness (mg/L)	>20	68 - 960
Alkalinity (mg/L)	20 - 300	12 - 66

The most poignant vary though occurred in the concentration of inorganic nutrients. Physicochemical properties of water directly influence the physiological conditions of the aquatic organisms like plankton, benthos, macrophytes etc., also of fish *viz.*, feeding, breeding, wintering, assimilation *etc*. (Goudar *et al.*, 2012). Theresults of physicochemical parameters of pond water duringpre-breeding, breeding and post-breeding seasons of Ramsagar town were recorded in the Table 2. The results were compared with BIS Standard for suitability and assessment of the testwater in regard to breeding potentiality and were represented in the Table 3. Optimum pH required for most aquaculture practices usually varied between 6.0 and 9.0 (BIS, 1991), although fish can tolerate a high range of pH of 5.0 to 10.0. During the present study average pH value of water samples from different pondsranged from 6.62 to 8.01 showing slightly alkaline to acidic to neutral in nature in all the three breeding seasons and were also in consonance with the standards prescribed by (BIS 1991), indicating that pH may have some role in spawn production.(Gupta *et al.*,2000) and (Das *et al*,. 2006) also suggested this optimum requirement for spawn production. The maximum range of temperature gradient for the breeding performance of the species studied is of 21.8-29.9ºC which helps efficient transformation of the food and protecting the fish from any type of diseases. In the present study, water temperature varied in different ponds with a range from 16 to 35ºC. Usually, water temperature below 4ºC affects the fish from the starvation of food which ultimately cause the death of the fish species and increased above the 26ºC temperature causes suffocation and digestive problems (Coada *et al., 2010*). Electrical Conductivity (EC) is an index of salinity, which greatly affects the taste of the potable water. Optimum EC required for most of the aquaculture practices is 300 □S/cm BIS (1991). During this study average EC of water samples from different ponds ranged between 300 and 460 □S/cm which were comparable with the range of BIS (1991).

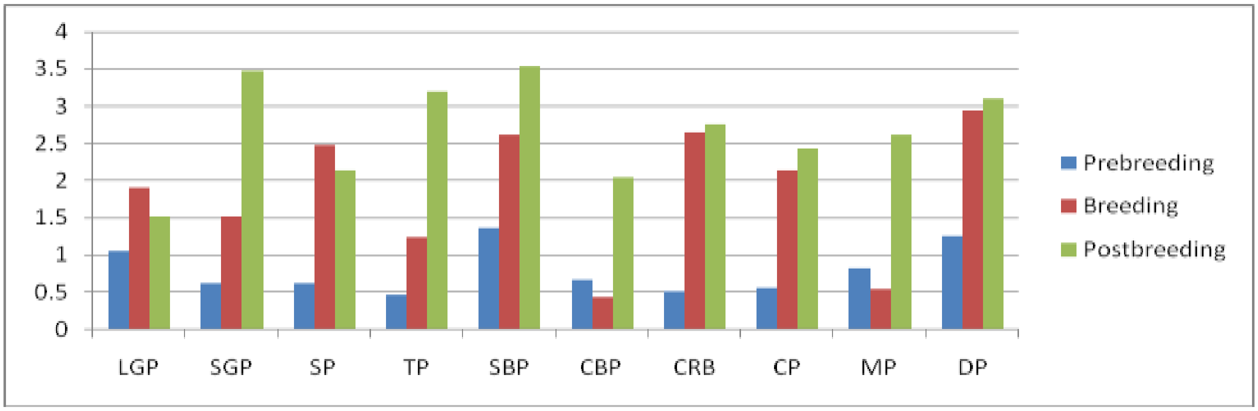
Nitrate-nitrogen:

Nitrate-nitrogen is one of the most important indicators of pollution of water. Over the years, nitrite nitrogen reached all time low values (0.428 mg/L) in comparison to the earlier study (3.534 mg/L) bearing a little range of fluctuations at different locations.

Table 4: Seasonal analysis of nitrate-nitrogen (mg/L) of sampling pond at Ramsagar (2021-2022)

Sites	Prebreeding	Breeding	Postbreeding	Max	Min	Mean	SD	SE
LGP	1.051	1.905	1.524	1.905	1.051	1.493	0.427	0.246
SGP	0.623	1.524	3.476	3.476	0.623	1.874	1.458	0.841
SP	0.615	2.476	2.143	2.476	0.615	1.744	0.992	0.572
TP	0.458	1.238	3.19	3.19	0.458	1.628	1.407	0.812
SBP	1.358	2.619	3.534	3.534	1.358	2.503	1.092	0.63
CBP	0.664	0.428	2.048	2.048	0.428	1.046	0.875	0.505
CRB	0.514	2.657	2.762	2.762	0.514	1.977	1.268	0.732
CP	0.561	2.143	2.428	2.428	0.561	1.711	1.005	0.58
MP	0.821	0.539	2.619	2.619	0.539	1.326	1.128	0.651
DP	1.26	2.952	3.095	3.095	1.26	2.435	1.021	0.589

Figure 1: Seasonal analysis of nitrate-nitrogen (mg/L) of sampling pond at Ramsagar (2021-2022)



Ammonia-nitrogen:

The concentration of ammonia-nitrogen arises as a result of decomposition of organic nitrogenous wastes present in the water body. In the present experimental ponds values in 2021- 2022 ammonia ranged Over the years, reached all time low values (0.356 mg/L) in comparison to the earlier study (1.384 mg/L) bearing a little range of fluctuations at different locations. Huge concentration of nitrate ion is usually not present in aquatic pond. Nitrite can act as an invisible killer of fish because it oxidizes haemoglobin to methemoglobin in the blood, turning the blood and gills brown and hindering respiration causing damage in liver, spleen, nervous system, and also in kidneys of the fish. The main source of nitrate nitrogen (NO_3-N) in natural water includes igneous rocks. Agricultural, industrial drainage, municipal wastewater and can also increase the amount of nitrate ion (NO_3^-) into the surface water. Maximum nitrate concentration is the indicator of pond water pollution. The best and normal measurement of nitrite is zero in any aquatic system. Stone and Thomforde (2004) suggested that the desirable range is 0 to 1 mg.l^{-1} of NO_3^- and acceptable range is less than 4 mg/L NO_3^-. According to Bhatnagar *et al*., (2004), 0.02 mg/L to 1.00 mg/L of nitrate nitrogen is lethal to many fish species; > 1.00 mg/L is lethal for many warm water fishes, and < 0.02 mg/L is acceptable for fish growth. Lembi *et al.*, (1978) reported that in case of eutrofication plant absorbs as much as 42% of nitrogen, which incorporate into fish tissue.

Gupta *et al.*, (2000) suggested that the permissible levels for nitrate are less than 0.100 mg/L. Nitrate nitrogen of water samples from different ponds ranged from 0.428 to 3.534 mg.l^{-1} showing an average of 1.981 mg/L in all breeding seasons during the period of 2021 to 2022. Nitrate nitrogen was minimum in during prebreeding season and maximum during postbreeding season. The highest value of nitrate nitrogen (3.534 mg/L) was recorded in the pond, SBP during 2022 in postbreeding season, probably due to mixing of fish feed and nutrient contents, and lowest nitrate nitrogen value of 0.428 mg/L in the sampling pond, CBP as recorded during 2022 in breeding season.

The most important residual compound or waste product of fish metabolic activity are ammonia, it was increased sharply after spawning and hatching. Another main source of ammonia in fish ponds is from the sediment diffusion and it is also the primary nitrogenous waste product of the carps; it also reaches water from fish excreta, milt, unfertilized eggs, uneaten food and microbial decay of nitrogenous compounds. In small quantities of unionized nitrites as well as the ammonia can be very toxic and harmful for fishes and other aquatic life and the optimum range is 0.02 - 0.05 mg/L in the pond water. Aeration can also reduce the ammonia toxicity. Saha *et al.*, (1957) reported that the spawn could tolerate 2.5 mg/L of dissolved ammonia. The ammonia level in the ponds varied widely from 0.0.356 mg/L to 1.384 mg/L showing an average of 0.870 mg/L . The overall maximum ammonia was widely higher in the sampling pond, CP as recorded during 2022 breeding season (1.384 mg/L) then in the postbreeding season (0.356 mg/L) the sampling pond, CRB. A comparison of the ammonia of pond water also within the permissible limit prevailing drinking water standards set by Indian Standards: 10500, (1993).

According to Alabaster and Lloyd (1980), ammonia intoxicated fish congregates close to the water surface, gasp for air and become restless. Fish are able to withstand levels of unionized ammonia up to 0.6 to 2 mg/L for only short periods.

Phosphate:

Phosphate is one of the most important indicators of pollution of water. Phosphorus occurs in natural water as various types of phosphates. Usually aquatic ecosystems receive excess of this nutrient through natural domestic sewage and agricultural runoff. Phosphate concentration increases in water bodies that receive domestic waste. Over the years, in contrast the concentration of phosphate increased considerably and its values (0.006 mg/L) in comparison to the earlier study (0.583 mg/L) bearing a little range of fluctuations at different locations. This is suggestive of increase in pollution and eutrophication of the waters. . Analysis Ofsampling ponds of water parameters were recOrded (Table 4, 5, 6 and figure 1, 2 and 3).

Table 5: Seasonal analysis of ammonia-nitrogen (mg/L) of sampling pond at Ramsagar (2021-2022)

Sites	Prebreeding	Breeding	Postbreeding	Max	Min	Mean	SD	SE
LGP	0.936	0.542	0.635	0.936	0.542	0.704	0.206	0.119
SGP	0.563	0.744	0.633	0.744	0.563	0.647	0.091	0.053
SP	0.521	0.365	1.358	1.358	0.365	0.748	0.534	0.308
TP	0.562	0.851	0.935	0.935	0.562	0.783	0.196	0.113
SBP	0.512	1.259	0.542	1.259	0.512	0.771	0.423	0.244
CBP	0.452	0.653	1.076	1.076	0.452	0.727	0.319	0.184
CRB	1.258	0.651	0.356	1.258	0.356	0.755	0.460	0.266
CP	0.856	1.384	0.521	1.384	0.521	0.920	0.435	0.251
MP	0.852	0.554	0.653	0.852	0.554	0.686	0.152	0.088
DP	0.931	0.563	0.753	0.931	0.563	0.749	0.184	0.106

Figure 2: Seasonal analysis of ammonia-nitrogen (mg/L) of sampling pond at Ramsagar (2021-2022)

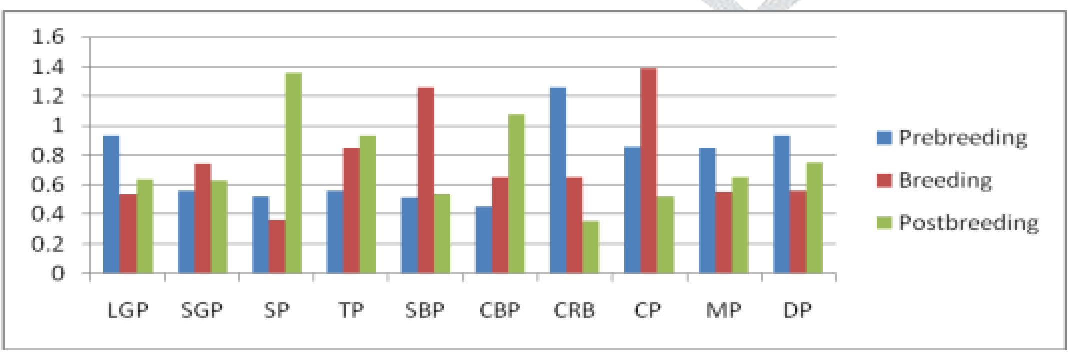


Table 6: Seasonal analysis of phosphate (mg/L) of sampling pond at Ramsagar (2021-2022)

Sites	Prebreeding	Breeding	Postbreeding	Max	Min	Mean	SD	SE
LGP	0.036	0.117	0.036	0.117	0.036	0.063	0.047	0.027

SGP	0.093	0.153	0.058	0.153	0.058	0.101	0.048	0.028
SP	0.088	0.165	0.062	0.165	0.062	0.105	0.054	0.031
TP	0.177	0.290	0.125	0.290	0.125	0.197	0.084	0.049
SBP	0.109	0.252	0.072	0.252	0.072	0.144	0.095	0.055
CBP	0.583	0.145	0.097	0.583	0.097	0.275	0.268	0.155
CRB	0.215	0.177	0.074	0.215	0.074	0.155	0.073	0.042
CP	0.119	0.145	0.087	0.145	0.087	0.117	0.029	0.017
MP	0.084	0.147	0.145	0.147	0.084	0.125	0.036	0.021
DP	0.032	0.101	0.052	0.101	0.032	0.062	0.036	0.020

Figure 3: Seasonal analysis of phosphate (mg/L) of sampling pond at Ramsagar (2021-2022)

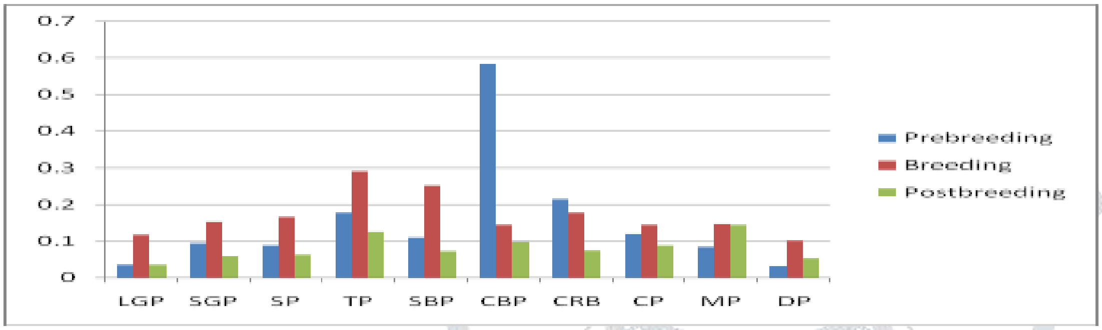


Table 7: Spawn production status of *Catla catla*

Sl No	Female					Male				Spawn production (piecein hundred)
	Age (yr)	Weight (g)	Size (cm)	1st dose (mg)	2nd dose (mg)	Age (yr)	Weight (g)	Size (cm)	1st dose (mg)	
1	4+	2750	51	1.5	30	6+	2500	52	25	4865
2	6+	4700	57	2.5	45	6+	3150	54	30	5106
3	5+	4300	54	2.0	40	4+	1750	56	20	5940
4	5+	3050	49	1.5	30	4+	1600	52	15	5639
5	5+	2050	48	1.0	20	5+	2050	49	20	5280
6	6+	6000	65	3.0	65	6+	2100	49	20	6039
7	5+	3500	51	1.5	35	5+	1500	51	15	5775
8	4+	1700	43	1.0	20	5+	2000	49	20	5082
9	5+	3500	53	1.5	40	5+	2550	53	25	5808
10	5+	3050	51	1.5	30	5+	3500	57	35	5742
11	4+	2650	51	1.5	30	4+	1050	43	10	5445
12	4+	2350	46	1.0	20	4+	1450	46	15	5427

Table 8 Spawn Production status of *Labeo rohita*

Sl No	Female					Male				Spawn Production (piece in hundred)
	Age (yr)	Weight (g)	Size (cm)	1st Dose (mg)	2nd Dose (mg)	Age (yr)	Weight (g)	Size (cm)	1st Dose (mg)	
1	4+	900	40	1	9	4+	1200	50	10	1621
2	4+	1800	61	2	15	4+	1400	56	10	2272
3	4+	1050	42	1	9	4+	1250	50	10	2147
4	4+	1150	42	1	9	4+	1250	49	10	1909
5	4+	1000	41	1	9	4+	1200	49	10	1745
6	4+	1000	42	1	9	4+	1350	56	10	1787
7	4+	900	40	1	9	4+	1300	54	10	1657
8	4+	1000	41	1	9	4+	1250	50	10	1473
9	4+	1400	47	1.5	13	3+	800	45	9	1845
10	4+	1400	48	1.5	13	3+	900	46	9	1648
11	4+	1150	43	1	9	4+	1250	55	10	1465

12	4+	1250	44	1	9	4+	1300	52	10	1982

Table 9: Spawn production status of *Cirrhinus mrigala*

Sl No	Female					Male				Spawn Production (piece in hundred)
	Age (yr)	Weight (g)	Size (cm)	1st Dose (mg)	2nd Dose (mg)	Age (yr)	Weight (g)	Size (cm)	1st Dose (mg)	
1	4+	1750	53	2	13	4+	1000	45	18	2316
2	4+	900	40	1	9	4+	1000	45	18	1542
3	4+	1200	47	1	9	4+	1250	50	10	1914
4	4+	1700	51	2	15	4+	1350	49	10	2145
5	4+	1000	41	1	9	4+	1200	49	10	1749
6	4+	900	40	1	9	4+	1500	50	13	1654
7	4+	1000	41	1	9	4+	1400	48	12	1583
8	4+	1550	48	1.5	13	4+	1300	49	13	1847
9	4+	1400	47	1.5	13	3+	800	45	9	1890
10	4+	1400	48	1.5	13	4+	1150	46	10	1823
11	4+	1300	44	1.5	10	4+	1200	50	10	1996
12	4+	600	38	1	6	4+	1250	48	9	1458

Egg to spawn production in fish maximum 3 days, and which finally attains the size of 6-8 mm. Carp spawn are generally hatched twice during June-August and January-March of the year, following the adaptive breeding methods. Common carp brood fishes are reared in composite aquatic ponds. The rearing of mature male and female brooders of 3 - 6 kg were netted out in the evening, collected and kept in breeding hapa for injecting with inducing pituitary hormones to ensure successful breeding. The ratio of male and female was 1:1 by weight in *Catla* sp. and for *Cirrhinus*sp. and *Labeo rohita* ratio was 1:1 by weight. After mating, female release eggs and spawning was started after 24 hours; spawns were collected for stocking in hatching hapa. In *Catla* sp. the survival rate of spawn was 75 to 80% but in case of *Labeo* sp. and *Cirrhinus* sp.it was 65 to 75%. Age is an important factor for fish breeding. Pre-mature brooders can produce large number of eggs but mortality rate is high. Here most of the fishes of *Labeo rohita* and *Cirrhinus* sp. were of four years but *C. catla* was of five to six years. Results of production of eggs and spawns from typical farms were recorded (Table 7, 8 and 9). Analysis of physicochemical parameters (temperature, pH, electrical conductivity, nitrate nitrogen, ammonia nitrogen and phosphate) of the breeding pond was conducted in order to evaluate the role of environmental parameters influencing the breeding potentiality of fish species in artificial aquaculture system. Water quality very much affects the physiology, growth and maturity of fishes. Physicochemical parameters of the study pond at Ramsagar town were within the permissible limit and hencesafe for breeding purposes as per BIS standards. Finally, it can be concluded that environmental parameters regulate the physiology, development and breeding potentiality of fish species and considered as important factor for aquaculture practices.

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