E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2018; 6(1): 111-115

www.fisheriesjournal.com
Received: 16-11-2017
Accepted: 17-12-2017

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Influence of biological factors in relationship with spermatological qualities of common carp (*Cyrpinus carpio*)

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Abstract
The main aim of this study was to identify the relationship between the environmental factors and the spermatological qualities of *Cyprinus carpio*. Different environmental factors like temperature, pH, alkalinity, total hardness, ammonia and nitrate are negatively correlated with all spermatological qualities like volume of milt, semen pH, duration of sperm motility, percentage of motility, motility score, sperm density, live and dead cell percentage of Common carp and show a significant positive relationship with percentage of motility (r = 0.996, p<0.01), motility score (r = 0.918, p<0.05), sperm density (0.979, p<0.05). The pH of the water showed negative correlation with all reproductive parameters in *C. carpio* and significance at p<0.05 (r = -923), motility percentage (r = -967) motility score, (r = -916) sperm density. Alkalinity shows the negative correlation with all sperm quality parameters and exhibits significant correlation with motility duration (r = -0.995) significant level of (p<0.01) level. Total hardness was negatively correlated with all spermatological parameter and show significant difference between pH of milt (r= -.940), motility percentage (r= -.944), sperm density (r = -.904), spermatocrit (r = -.941), live cell percentage (r = -.984), p<0.01 level. Ammonia showed significant negative relationship at p<0.05 level with pH of milt (r = -.924), motility percentage (r = -.910), sperm density (r = -.969). Nitrate showed negative relationship at p<0.05 level with pH of milt (r = -.915), spermatocrit (r = -.923) and live cell percentage (r = -.960).

Keywords: *Cyprinus carpio*, temperature, DO, volume of semen, semen pH, sperm motility, total hardness, live and dead cell

1. Introduction
Fish plays a vital source of protein, essential amino acids with micronutrients like zinc, magnesium phosphorus, vitamin A and vitamin C. “Rich food for all people”. Despite the significant contribution that fisheries and aquaculture make to employment, nutrition and trade [17, 18] The achievement of higher production of fish depends on its gamete quality. The successful fertilization propagation is enhanced by artificial propagation method [11]. The water quality is changed due to seasonal and climatic rhythms. Fishes are poikilotherms, so they can adjust or adapt the fluctuation of water quality parameters. The metabolic rate of fish is highly influenced by the water temperature. Temperature influences the reproductive accomplishment at various levels, gamete maturation, collection and perpetuation. Gametes maturation is principally guarded by endocrinological cues under the control of temperature [36]. Effect of pH and temperature on sperm motility in rainbow trout was studied [37] The experimental animal taken for the study was *Cyprinus carpio* (Common carp). It is suitable for pond culture due to its highly adoptive qualities. It is extensively distributed and it can resist in an undesirable conditions [28]. The fish has a wide range of tolerance with temperature. The reproduction in fish was greatly influenced by the environmental parameters. [32] [13].The metabolic activity in fish increased when the water temperature is high. Increased metabolic activities cause higher oxygen consumption and waste production (NH_3 and CO_2).The process of carp reproduction was also troubled by the extremely eutrophic pond condition: lower productiveness, lower quantity of sperm, decreased viability rate of embryos and adult fish. Understanding the factors that distress sperm quality could be useful for regulation and proficient management of them [22]. The factors have been split into effect of environmental factors (DO, pH, temperature, hardness, alkalinity, ammonia and nitrates) on the spermatological characteristics of *C. carpio* were analysed.

2. Materials and method

2.1 Experimental fish

Male *C. carpio* size ranged from 350.60 ± 0.10g collected from Tamil Nadu Fisheries Development Corporation, Aliyar Nagar, Pollachi, Tamil Nadu January 2015 to April 2015. The acclimatization of fish carryout for 15 days and the fish were stocked, the group of the experiment was carried out for 100 days. The biological factors like temperature, pH, Dissolved oxygen (DO), alkalinity, total hardness, ammonia and nitrate and it were analysed every week of experimental period. Quality of sperms were anlaysed.

2.2 Collection of fish milt

The *C. carpio* were randomly collected for the collection of milt. Collection of semen was carried out for 1 month. The fishes were starved for 48 hours before the milt collection. The milt was collected by gentle pressure in the ventral region of the abdomen. Care was taken to avoid contamination and the milt was collected in a clean airtight glass tube. The tubes were covered firmly and taken for laboratory analyses.

2.3 Estimation of water quality parameters

Temperature was measured using mercury thermometer, dissolved oxygen was analysed by Winkler's method. pH was analysed by digital pH meter, total hardness, alkalinity, ammonia and nitrate were estimated APHA [3]. Estimation of spermatological parameters (milt volume, pH of the milt, motility duration, motility percentage, motility score, sperm density, spermatocrit, live and dead cell in fishes *C. carpio* were analysed.

2.4 Assessment of sperm motility

Semen was collected by gentle abdominal press and the stripping of male semen was diluted with water in the ratio 1:100 and the 10 μl of semen sample was kept under the microscope and the motility of forward moving spermatozoa was observed [2]

2.5 Determination of motility score and motility percentage

The motility was determined arbitrarily on a 0 to 5 point scale. [22].

Motility percentage was calculated by the formula given below.

%MC = MC/WSC x 100

The percentage motility was considered only by the actively forward moving spermatozoa and observed within 1 hour of semen collection.

2.6 Evaluation of sperm density and spermatocrit

Sperm density was estimated by the harmocytometric method [34]. The semen sample of fish was diluted in the water 1:100 and 10 μl of sample was taken and placed on the haemocytometer slide (depth 0.1mm) with a cover slip allow to settle 3-5 minutes then the number of spermatozoa was counted in 16 cells. Using compound microscope (40X) and the density was expressed as $X10^9$ cells/ml [12] Spermatocrit is defined as a percentage of volume of white packed cells to the total volume of semen. Triplicates for each sample were carried semen was collected in a capillary tube and measured using meter scale in mm and centrifuged for 3 min at 1000gm [8]. The formula used to calculate the white packed cells was measured in mm.

$$\text{Spermatocrit} = \frac{\text{Volume of white packed cells}}{\text{Total volume of semen)}} \times 100$$

2.7 Evaluation of live and dead cells

Live and dead cells were evaluated by the modification of the eosin-nigrosin staining method. Live sperm exclude the eosin Stain and appear colorless and dead sperm appear in pink color. The percentage of live spermatozoa was determined by counting a minimum of 200 spermatozoa on the slide. [19]

3. Results

The physico-chemical parameters of water in the experimental tank show significant difference during the period of study from January, February, March and April, 2015. (Table 1). The temperature of April 2015 was observed high as compared to January, 2015 to March 2015. The dissolved oxygen (DO) was high in January 2015 (6.12 mg/l) and low level of dissolved oxygen (DO) was (5.36 mg/l) in April 2015. The other physico-chemical parameters like pH, alkalinity, total hardness, ammonia and nitrate was found high level in April 2015 compared to January 2015 and February 2015.

Table 1: Environmental Parameters of water analysed during the experimental period from January to April, 2015

Parameters	January	February	March	April	Mean ± SD
Temperature (C□□	25.8	26.6	27.3	30.7	27.60 ± 2.155
DO (Mg/l)	6.8	6.51	5.31	5.3	5.98 ± 0.788
pH	8.4	8.54	8.85	8.77	8.64 ± 0.207
Alkalinity (mg/l)	45.8	54.33	58.00	58.83	54.24 ± 5.956
Total hardness (mg/l)	36.0	40.33	43.33	47.23	41.72 ± 4.747
Ammonia (mg/l)	0.8	0.75	0.92	1.01	0.87 ± 0.117
Nitrate (mg/l)	0.03	0.038	0.04	0.05	0.039 ± 0.008

Values mean± SD

Table 2: Spermatological parameters of C. carpio during the experimental period from January to April, 2015

Sperm quality parameters	January	February	March	April	Mean ± SD
Volume of milt (ml)	1.5	1.85	1.55	1.900	1.70 ± 0.204
pH of milt	7.5	7.36	7.04	6.33	7.05 ± 0.521
Motility duration	113.3	83.31	70.18	72.15	84.73 ± 19.901
% Motility	56.0	52.83	43.00	41.51	48.33 ± 7.164
Motility score	2.5	2.25	2.05	2.11	2.22 ± 0.200
Sperm density$X10^9$/ml	2.1	2.1	1.35	1.18	1.68 ± 0.487
Spermatocrit (%)	67.61	55.66	52.73	50.16	56.54 ± 7.714
Live and dead cell (%)	66.75	62.45	58.46	50.50	59.54 ± 6.912

Values are Mean ± SD

Table 3: Correlation between water quality and spermatological parameters (January to April, 2015)

Parameters	Volume of milt (ml)	pH of milt	Motility duration	% motility	Motility score	Sperm density X10^{9}ml	Spermatocrit (%)	Live cell %
Temperature (C°)	0.663	0-.922**	0-.658	0-.817	0-.637	0-.834	0-.755	0-.975*
DO (mg/l)	0-.249	0.834	0.871	0.996**	0.918*	0.979*	0.850	0.876
pH	0.217	0-.724	0-.923*	0-.966*	0-.967*	0-.916*	0-.876	0-.797
Alkalinity (mg/l)	0.564	0-.763	0-.995**	0-.903*	0-.980**	0-.809	0-.995**	0-859
Total hardness (mg/l)	0.618	0-.940*	0-.897	0-.944*	-0.886	0-.904*	0-.941*	0-.984**
Ammonia (mg/l)	0.229	0-.924*	0-.616	0-.910*	0-.668	0-.969*	0-.645	0-.892
Nitrate (mg/l)	0.800	0-.915*	0-.844	0-.826	0-.796	0-.771	0-.923*	0-.960*

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

Correlation between the environmental parameters with the spermatological parameters of fresh water fish *C. carpio*. The reproductive parameters of *C. carpio* significantly differs with each other. The male *C. carpio* shows higher values in January 2015 and gradually decreased during February 2015 to April 2015 as shown in Table 2.

The correlation analysis indicates the physic-chemical parameters of experimental water in the feed tank (Temperature, pH, alkalinity, total hardness, ammonia and nitrate) shows negative of experimental fishes (Table 3). Temperature showed significant negative relationship with all reproductive parameters (volume of milt, pH, motility duration, % of motility, density, spermatocrit, live cell percentage). The DO showed positive relationship with all sperm quality parameters of *C. carpio* and showed significant positive relationship with percentage of motility (r = 0.996, p<0.01), motility score (r = 0.918, p<0.05), sperm density (0.979, p<0.05). The pH of the water showed negative correlation with all reproductive parameters in *C. carpio* and significance at p<0.05 (r = -923), motility percentage (r = -967) motility score, (r = -916) sperm density.

Alkalinity shows the negative correlation with all sperm quality parameters and exhibits a significant correlation with motility duration (r = -0.995) significant level of (p<0.01) level. Total hardness was negatively correlated with all spermatological parameter and show significant difference between pH of milt (r= -.940), motility percentage (r= -.944), sperm density (r = -.904), spermatocrit (r = -.941), live cell percentage (r = -.984), p<0.01 level. Ammonia showed a significant negative relationship at p<0.05 level with pH of milt (r = -.924), motility percentage (r = -.910), sperm density (r = -.969). Nitrate showed a negative relationship at p<0.05 level with pH of milt (r = -.915), spermatocrit (r = -.923) and live cell percentage (r = -.960).

4. Discussion

The present study shows the influence of biological factors in the spermatological parameters of *C.carpio* About 25°C to 35°C temperature was tolerated by the *C. carpio*. The spawning period required about 18°C to 25°C [4]. The intensity and duration of change in motility during spermiation period and it depends on species and environmental parameters [6, 7]. The temperature observed during the study ranged from 25.8°C to 30.7°C. The dissolved oxygen level below 4 mg/l reduced the growth in *Salvelinus namaycush* trout present in lake [9]. The oxygen requirement in trout *O. mykiss* ranged from 1.0 to 5.0 mg/l [25]. In the present study reveals the DO level from (5.3 to 6.82 mg/l). The pH ranges from 5 to 10 is essential for the activation of sperm and its motility in all kinds of species [27]. In the present study pH ranges from 8.42 to 8.77. The alkalinity suitable for the fish culture in the tank ranges from (10 – 100 ppm) [26]. The findings of alkalinity in the present study was (45.83 to 58.8 ppm). The total hardness of the present study ranges from 36 ppm to 44.67ppm [29]. The observation was 0.1 mg/l to 4.0 mg/l in the suitable range of nitrate in fish culture tank and the study also shows the range of nitrate as in the above statement [29].The nitrate level in the water bodies increased by the use of fertilizers and fuels [5]. The fish reproduction is greatly affected by the condition of pond and affects the fish reproduction fresh water fishes are more tolerable to the toxicity of ammonia. The ammonia toxicity below 0.05 mg/l and Total Ammonia Nitrogen (TAN) below 1.0 mg/l should be maintained for long term exposure to fish [33]. The NH_3 level ranged between (0.8 mg/l to 1.01 mg/l).

The spermatological parameters of *C. carpio* also have a significant difference during the period of study from January to April, 2015. The volume of milt in scaly carp was 2.75 ml [10]. The volume of milt observed in the present study ranged from (1.51 to 1.90 ml). The pH is essential factor to induce sperm motility. The milt pH of Oreochromis varied from 6.2 to 8.2 [16]. The present study shows the pH of milt varied from (6.33 to 7.51). Alkaline pH of 8.0 to 8.2 ranges induces the fertilization success in *O. mykiss* [23, 24]. The semen quality of fishes is analysed by sperm motility [36] fish species exhibits a brief period of motility ranges from 30s to 60s. The motility duration in different species of carp has been reported that Mrigal species shows high duration of motility (110s) and in silver carp short duration of motility noted as (80s) and in catla (85s) [14]. In the present study from January 2015 to April 2015 noted was 113s (January, 2015), 83.61s, (February, 2015), 70.18s (March, 2015) and 72.15s. (April, 2015). The present study the motility score recorded ranged from 1.18 to 2.13. In *Prochilodus lineatus* varied from 4 to 5 [21]. Sperm density is an important parameter to evaluate the milt quality [1]. Chutia, *et al.*, [15] have found out the sperm density of 6.6 x 10^9 sperm cells/ml in *C. carpio*. In the present study the average sperm density of 2.13 x 10^9 sperm cells/ml was recorded in *C. carpio* during the period of study from January to March, 2015. Tekin, *et al.*, [31] have reported that spermatocrit value decreased with increasing age of fish. The spermatocrit value observed in the present study ranged from 50.16% to 67.16%. The spermatocrit value was higher in January, 2015 and low value noted in March, 2015. Live cell percentage determine the success of animal production [19]. The live cell percentage was high (66.75%) in January, 2015 and low (50.50%) in April 2015.

5. Conclusion

In the present study it was clearly understood that the sperm quality parameters was high in January, 2015 compared to

February and March, April, 2015. Statistical analysis determined the relationship between physic-chemical characteristics of water and sperm qualities of *C. carpio*. The water temperature, pH, alkalinity, total hardness, ammonia and nitrate showed negative correlation with all sperm quality parameters but DO exhibit positive relationship with all sperm quality parameters of *C. carpio.*

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