ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(3): 621-625

www.fisheriesjournal.com
Received: 09-03-2016
Accepted: 10-04-2016

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A Study of Interrelationship between Physico-Chemical Characteristics of Water and Spermatological Qualities of *Cyprinus Carpio*

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Abstract
The main aim of this work was to study the effect of environmental factors on the spermatological characteristics of *C. carpio*. The temperature, pH, alkalinity, total hardness, ammonia and nitrate negatively correlated with all sperm quality parameters of *C. carpio*. But dissolved oxygen exhibit positive relationship with all sperm quality parameters (semen volume, semen pH, duration of sperm motility, motility %, motility score, sperm density, live and dead cell %) of *C. carpio* and showed significant positive relationship with spermatocrit (r= 0.994, $P<0.05$). The water pH showed negative correlation with all sperm quality parameters of *C. Carpio*. Alkalinity showed significant negative correlation with volume of semen (-0.994) at $P<0.05$ level. Total hardness exhibit significant negative relationship with duration of motility (r= -0.989), % motility (r= -0.999), motility score (r= -0.989) and live cell percentage (r= -0.995) at $P<0.05$ level. Ammonia showed significant negative relationship at $P<0.05$ level with spermatocrit. Nitrate exhibit perfect negative correlation with motility score at $P<0.01$ level and showed significant correlation with live cell percentage at $P<0.05$ level.

Keywords: *C. carpio*, sperm, temperature, Dissolved oxygen, semen pH, sperm motility, alkalinity, hardness.

1. Introduction
The global aquaculture production in the world was 90.43million tons in the year 2012 [31]. According to the report of FAO [6] the food fish production in 2010 was 59.9million tons and it as increased to 66.63million tons in 2012. The food demand will be increased in forthcoming years due to population explosion and capture fisheries alone can't meet this requirement. China holds first place in aquaculture production from 1970 to 2012 and India has the second level next to China [7]. It is important to analyse the factors affecting fish reproduction and enhance the aquaculture production. A perceptive knowledge about gamete biology is very essential for improving aquaculture industry. At the same time evaluating gamete quality is also an important one to increase the reproductive success in aquaculture
C. carpio is mostly preferred for pond culture for its fast growing and omnivorous feeding habit. *C. carpio* is included as exotic species in India. Seasonal changes greatly influence the spawning behaviour of *C. carpio* [29, 30]. The growth and survival of aquatic organisms are greatly influenced by the physico-chemical characteristics of water. Seasonal changes influence the fish reproduction [32, 33]. The main aim of the present study is to understand the relationship between physico-chemical characteristics of water and sperm qualities of *C. carpio*, this will helpful for successful reproduction and increase in the *C. carpio* population.

2. Materials and methods
2.1. Experimental fish
The experimental fish *C. carpio* size ranged from 300 ± 50g collected from Tamil Nadu Fisheries Development Corporation, Aliyar Nagar, Pollachi, Tamil Nadu, India. The fishes were stocked in cultured tanks for 10 days of acclimatization. Then they were divided into two groups of 100 fishes each. These were fed with traditional feed. This experiment was carried out for 90 days. The physico-chemical factors such as temperature, pH, Dissolved oxygen (DO), alkalinity, total hardness, ammonia and nitrate was noted every week of experimental period. Sperm quality parameters were analysed bimonthly.

2.2. Collection of fish milt
Ten mature Common carp males were randomly selected from the stock and were used as semen donors. The semen was collected first week of every month. The fish were not fed 48 hrs prior to the semen collection. Each male was stripped only once and the total amount of expressible milt was collected individually by gently pressing the abdomen. The semen was collected directly into clean and dry glass tubes. Care was taken to avoid the contamination of semen with water, urine, blood or faecal matter. The tubes were covered and immediately transported to the laboratory for analyses.

2.3. Evaluation of water quality parameters
Water temperature was noted by using mercury thermometer and dissolved oxygen was estimated by winkler's iodometric method. pH was measured by using digital pH meter. Alkalinity and total hardness, ammonia and nitrate were evaluated by APHA [1].

2.4. Evaluation of milt quality
2.4.1. Semen volume and pH
Sperm was sampled into 20 ml calibrated glass tubes and the volume was expressed as ml. Sperm pH was measured using digital pH meter within 30 minutes of sampling.

2.4.2. Spermatozoa motility
Semen sample was collected by abdominal stripping of male fish. Semen was diluted with medium water in ratio of 1: 100. Then 10µl of semen sample was placed on a glass microscopic slide and observed under a light microscope. Duration of spermatozoa motility was noted by using stopwatch. Duration was noted from the time of water dilution and expressed as seconds. Each motility determination was performed in triplicate [2].

2.4.3. Determination of motility score and motility percentage
The motility score was determined arbitrarily on a 0 to 5 point scale [4]. The percentage of motility was determined based on the motility score, 0 denoting 0% motility and 5 denoting 100% motility. Motility percentage was calculated by the percentage of actively moving spermatozoa. Forward moving sperm were considered as motile others considered as non-motile. Observations were made within 2 hrs of semen collection.

2.4.4. Sperm density
Sperm density was determined according to the haemocytometric method [5]. Semen sample was diluted in medium water at a ratio of 1: 100 (semen: medium water). The diluted semen sample (10µl) was placed on the haemocytometer slide (depth 0.1 mm) with a coverslip, the sperm were allowed to settle for 3-5 minutes, then the number of spermatozoa was counted in 16cells and calculated according to Caille *et al* [3] using compound microscope (40X), spermatozoa density was expressed as $x10^9$cells/ml. This process was carried out in triplicate.

2.4.5. Spermatocrit
Spermatocrit is defined as the percentage volume of white packed cells to the total volume of semen. Measurements were done in triplicate for each sample and the average of three measurements was used in subsequent statistical analysis. Spermatocrit was measured within 1hr of the semen collection. For spermatocrit measurement, the volume (length) of semen in capillaries was measured by meter scale in mm and centrifuged for 3min at 1000g [24]. The volume of white packed cells was measured in mm.

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

2.4.6. Live and dead cells
Live and dead sperm cells were counted by using eosin-nigrosin staining method [27].The live-dead ratio was calculated by counting the number of live cells (without color) and dead cells (pink) using optical microscopy (400X), after combining 1µl of semen with 1µl of eosin-nigrosin.

3. Results
The physico-chemical characteristics of water in the experimental tank showed significant difference during the study period from January to March, 2013 (Table.1). The high temperature of 28^0C was observed in March, 2013 compared to January, 2013 (25.5^0C) and February, 2013 (26.6^0C). The DO content in fish culture tank was high in January, 2013 (6.37) and low level of DO was found in March, 2013. The other physico-chemical characteristics of water included pH, alkalinity, total hardness, ammonia and nitrate was found high level in March, 2013 compared to January, 2013 and February, 2013.

The spermatological properties of *C. carpio* showed significant differences. The sperm quality parameters were high in January 2013 and gradually decreased in February and March, 2013 shown in Table 2.

Correlation analysis indicated that physico-chemical characteristics of water in experimental tank (Temperature, pH, alkalinity, total hardness, ammonia and nitrate) showed negative relationship with all sperm quality parameters of experimental fishes (Table 3). Temperature showed significant negative relationship with % motility (r= -0.999), motility score (r= -0.988) and live cell percentage (r= -0.995) at $P<0.05$ level. The DO showed positive correlation with all sperm quality parameters of *C. carpio* and showed significant positive relationship with spermatocrit (r= 0.994, $P<0.05$). The water pH showed negative correlation with all sperm quality parameters of *C. carpio*. Alkalinity showed negative relationship with all sperm quality parameters and exhibit significant correlation with volume of semen (-0.994). Total hardness exhibit significant negative relationship with duration of motility (r= -0.989), % motility (r= -0.999), motility score (r= -0.989) and live cell percentage (r= -0.995) at $P<0.05$ level. Ammonia showed significant negative relationship at $P<0.05$ level with spermatocrit (r= -0.992). Nitrate exhibit perfect negative correlation with motility score (r= -1.000, $P<0.01$) and significant with live cell percentage (r= -0.997) at $P<0.05$ level.

Table 1: Physico-chemical characteristics of water observed during the experimental period.

Parameter	January, 2013	February, 2013	March, 2013	Mean±SD
Temperature	25.5	26.6	28	26.68±1.26
DO	6.37	6.35	5.55	6.09±0.47
pH	8.47	8.42	8.87	8.59±0.25
Alkalinity	44.17	54.33	57.5	52±6.96
Total hardness	36	39.67	44.67	40.11±4.35
Ammonia	0.75	0.75	0.92	0.81±0.1
Nitrate	0.043	0.047	0.05	0.047±0.004

From January to March, 2013
Values are mean ± SD

Table 2: Spermatological parameters of *C. carpio* during the experimental period from January to March, 2013.

Sperm quality parameter	January, 2013	February, 2013	March, 2013	Mean±SD
Semen volume (ml)	1.98	1.85	1.83	1.89±0.08
Semen pH	8.32	7.25	7.32	7.63±0.6
Motility duration (sec)	127	113.67	81.33	107.33±23.49
% motility	53	47.67	39.17	46.61±6.98
Motility score	2.67	2.3	2	2.32±0.34
Sperm density ($X10^9$/ml)	2.43	2.33	1.98	2.25±0.24
Spermatocrit (%)	70.83	68.33	53	64.05±9.65
Live cell %	76.33	69.83	63.5	69.89±6.42

Values are mean ± SD

Table 3: Correlation analysis between physico-chemical characteristics of water and spermquality parameters of *C. carpio*.

Parameter	Volume of semen	Semen pH	Motility duration	%motility	Motility score	Sperm density	Spermatocrit	Live cell %
Temperature (^{0}C)	-0.881	-0.781	-0.990	-0.999*	-0.988*	-0.976	-0.955	-0.995*
DO	0.619	0.468	0.965	0.932	0.846	0.982	0.994*	0.873
pH	-0.519	-0.356	-0.925	-0.881	-0.774	-0.951	-0.973	-0.806
Alkalinity	-0.994*	-0.959	-0.863	-0.911	-0.973	-0.823	-0.773	-0.959
Total hardness	-0.883	-0.784	-0.989*	-0.999*	-0.989*	-0.975	-0.954	-0.995*
Ammonia	-0.603	-0.449	-0.959	-0.924	-0.834	-0.977	-0.992*	-0.862
Nitrate	-0.950	-0.878	-0.950	-0.977	-1.000**	-0.924	-0.889	-0.997*

*P<0.05
**P<0.01

4. Discussion

In the present study the physico-chemical characteristics of water in the experimental tank showed significant differences during the period of the study. The *C.carpio* is able to tolerate temperature ranged between 25^{0}C to 35^{0}C. But the optimum temperature required for spawning in *C. carpio* is 18^{0}C to 23^{0}C [8]. The intensity and duration of motility changes during spermiation period and also depends on the species and temperature of the water medium [9]. The temperature observed during the present study ranged from 25.5^{0}C to 28^{0}C. The DO level below 4mg/l reduced the growth rate in lake trout, *Salvelinus namaycush*. The oxygen requirement in rainbow trout *O. mykiss* ranged from 1.0 to 5.0mg/l [10]. Similar reports were observed in the present study DO level ranged from 5.55 to 6.37mg/l. The pH ranged from 5 to 10 is essential for activation of sperm motility in all species of fish especially in *C. carpio* [11]. Similar findings were recorded in the present study pH ranged between 8.42 to 8.87. The recommended value of alkalinity in fish culture tank is 10-100ppm [12]. Similar findings recorded in the present study that the alkalinity ranged from 44.17ppm to 57.5ppm. The total hardness analysed in the present study was ranged from 36ppm to 44.67ppm. This is inagreement with the report of Santhosh and Singh [13]. They observed that 0.1mg/l to 4.0mg/l is the suitable range of nitrate in fish culture tank and the similar reports were recorded in the present study. Bieniarz *et al*. [14] have reported that nitrate level in water bodies increased by the use of fertilizers in agriculture activities and the combustion of fossil fuels. The *C. carpio* reproduction was greatly affected by such eutrophic pond conditions and lowers the sperm quality. Generally freshwater fishes are more tolerant to ammonia toxicity compared to marine fish. The NH_3-N concentrations below 0.05mg/l and Total Ammonia Nitrogen (TAN) below 1.0mg/l should be maintained for long-term exposure of fish [16]. This was supported by the present study the NH_3 level ranged between 0.75mg/l to 0.92mg/l.

The sperm quality parameters of *C. carpio* also showed significant differences during the period of this study from January to March, 2013. Bozkurt [15] have reported that the volume of milt in scaly carp was 2.75ml. The volume of milt observed in the present study ranged from 1.83 to 1.98ml. The milt pH is one of the essential factors to induce sperm motility. The milt pH of *Oreochromis* varied from 6.2 to 8.2 [17]. Similar findings were recorded in the present study the pH of milt varied from 7.32 to 8.32. Alkaline pH of 8.0 to 8.2 increased the fertilization success in *O. mykiss* [18]. Sperm motility behaviour used to analyse the semen quality of fishes [19]. The sperm motility duration is significant due to the time required by the sperm to reach the egg for fertilization. Generally externally fertilizing fish species exhibit very brief period of motility duration usually ranged from 30s to 60s. Verma *et al.* [20] have investigated the motility duration in different species of carp and he reported that mrigal species showed high duration of motility (110s) and short duration of motility noted in silver carp (80s) and catla (85s). This was supported by the present study that the duration of sperm motility was high (127s) in January, 2013 and slowly decreased (113.67s) in February, 2013 and short duration of motility (81.33s) noted in March, 2013. The percentage of motility in grass carp was 77.0±8.89% [26]. But in the present study the percentage of motility in January, 2013 was 53% and it was gradually decreased in February, 2013 (47.67%) and March, 2013 (39.17%). The motility score were recorded in the present study ranged from 2 to 2.67. The motility score observed in *Prochilodus lineatus* varied from 4 to 5 [21]. Sperm density is an important parameter to evaluate the milt quality [28]. Chutia *et al*. [23] have found out the sperm density of $6.6x10^9$sperm cells/ml in *C. carpio*. In the present study the average sperm density of $2.25x10^9$spermcells/ml was recorded in *C. carpio* during the period of study from January to March, 2013. Tekin *et al.* [25] have reported that spermatocrit value decreased with increasing age of fish. The spermatocrit value observed in the present study ranged from 53% to 70.83%. The spermatocrit value was higher in January, 2013 and low value noted in March, 2013. Live cell percentage determine the success of animal production [22]. The live cell percentage was high (76.33%) in January, 2013 and low (63.5%) in March, 2013.

5. Conclusion

In the present study it was clearly understood that the sperm quality parameters was high in January, 2013 compared to February and March, 2013. Statistical analysis determined the relationship between physico-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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