



## Physical Analysis of the Milt in Cultured and Wild Stocks of Scale carp, *Cyprinus carpio* var. *communis*

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### Abstract

The present study was conducted to compare the physical parameters of seminal plasma between the cultured and wild stocks of scale carp (*Cyprinus carpio* var. *communis*) during the year 2019-2020. The following physical spermatological parameters in wild conditions were found out: Mean sperm volume  $2.393 \pm 1.64$  ml, mean sperm motility  $75.038 \pm 10.162\%$ , mean movement duration  $50.367 \pm 13.92$  sec, mean sperm density  $3.534 \pm .272 \times 10^9$ /ml and mean pH  $8.29 \pm .494$  whereas in farmed condition, the mean sperm volume were  $1.486 \pm 0.88$  ml, mean sperm motility  $68.9 \pm 12.46\%$ , mean movement duration  $44.66 \pm 13.48$ s, mean sperm density  $3.84 \pm 0.18110^9$ /ml, and mean pH  $8.5 \pm 0.311$ . Significantly higher sperm volume and motility percentage were found in the wild ( $P < 0.05$ ) as compared to farmed fishes, whereas significantly higher sperm density was found in the farmed fishes ( $P < 0.01$ ) as compared to wild fishes. It can be concluded that the sperms of wild fishes were of better quality than the sperms of farmed fishes.

**Keywords:** *Cyprinus carpio* var. *communis*; Sperm Motility; Wild; Farmed

### Introduction

*Cyprinus carpio* var. *communis*, are widely cultivated around the world in tropical, subtropical, and temperate climates because of their outstanding ability to tolerate a wide range of climatic conditions. It is considered to be a completely domesticated fish and is one of the most frequently farmed fish in the world. *C. carpio* made up roughly 69.13% of the entire capture by weight in the Dal lake of Kashmir, making it the most dominating fish both in terms of population and weight [41]. According to [41] of the two fish species that live in the lake, *C.c. communis* has contributed 59.2% and *C.c. specularis* has made up 9.11% of the total capture by weight. According to [42] *C.c. communis* made up the majority of the catch from the Dal lake. Due to exploitation and contamination of water resources as well as declining catch fisheries, Kashmir's native species, particularly the Scale carp, are under extreme stress [37,38]. The lake has also seen a decline in the fecundity of *Cyprinus*

*carpio* var. *communis* [40,46,47]. More consideration should be given to the opportunities presented by efficient fish aquaculture methods due to the decline in brood stock captures and, as a result, a shortage of gametes (sperm and eggs). To encourage artificial fertilisation as a means of fish species reproduction, several trials have been carried out. The most crucial element in improving the effectiveness of artificial fertilisation is the selection of high-quality sperm.

Despite the fact that the sperm quality of male brood stock affects the production of healthy larvae, the quality of eggs is prioritised in the fish farming industry. An important method for assessing the reproductive capacity of a species is milt quality evaluation in fish reproduction [24,25]. Sperm quality is a critical factor in aquaculture management because it determines the percentage of eggs fertilised and hence the number of viable larvae produced from a particular brood stock.

In fish farms, gamete management is important to achieve high fertilization [7]. Basic knowledge for managing fertilization can be provided by studying sperm physiology [2]. It is essential to have sufficient understanding of the physical and chemical features of the milt to assess the reproductive capacity of farmed fish in order to achieve controlled and successful production in aquaculture systems [45]. Fertilisation is a crucial step in achieving better productivity in aquaculture. No single predictive parameter exists to evaluate the milt quality. To predict sperm quality and fertility, we could establish a consistent picture of the sperm quality by evaluating its physical characteristics collectively. The analysis of sperm samples is crucial for identifying males with improved reproductive performance and for tracking the ideal parameters for brood stock rearing in two separate settings [44]. Sperm count, sperm density, osmolarity and pH of seminal plasma, chemical composition of seminal plasma, enzymatic activity, adenosine triphosphate (ATP) concentration, motility, morphology and ultrastructure, fertilising capacity, and several other biomarkers of sperm quality have all been documented [5,14,15,18]. Sperm motility and sperm density determine the fertilization capability of spermatozoa and are often used to estimate the semen quality [6,17,20,32]. To ascertain the reproductive capacity of fish species, sufficient understanding about the physical features of milt is required. In order to effectively “pinpoint” and appropriately assess elements that have a negative impact on fish semen quality qualities, physical aspects of semen must be taken into account. As a result, our primary goals were to contrast the physical semen indices of wild and domesticated brood stock.

## Materials and Methods

Two groups of male brood stocks of Scale carp were taken for experiment: Group A (n = 30, TW = 293 ± 270.84), the cultured fishes: these brooders were reared under captivity conditions in the hatchery of Faculty of Fisheries and Pandach fish farm (J&K state Govt. owned farm). Group B (n = 30, 462.5 ± 393.06) the wild fish: these brooders were captured from the Dal Lake during the spawning season.

### Milt collection

For use as semen donors, 30 mature wild and 30 farmed male brooders were chosen at random. One time only, every male was stripped, and the whole amount of expressible milt was

collected by gently pressing the abdomen. Directly into clean 15 ml graded centrifuge tubes were used to collect the semen. Semen was carefully protected from contamination by water, urine, blood, or faeces. The tubes were covered and sent directly to the lab for analysis on ice (4°C).

### Estimation of physical parameters

- **Sperm motility:** Motility was calculated as a percentage of motile spermatozoa using a 40X light microscope (Olympus CX31). An activating solution containing 0.3 percent NaCl was used to measure the motility. For assessing semen motility, 10 µl of semen and 100 µl of activation solution were combined on a glass microscope slide [21].
- **Volume and pH:** The milt was collected in a 15 ml graduated centrifuge tube to compute the volume, and the pH was measured using a digital pH metre (pH ep® Hanna instruments, Italy).
- **Spermatozoa density:** At a magnification of 40 X, sperm was diluted in Hayem solution (5g Na<sub>2</sub>SO<sub>4</sub>, 1g NaCl, 0.5g HgCl<sub>2</sub>, and 200 ml double distilled water) at a ratio of 1:1000, and For each fish, the average spermatozoa count was calculated from three replicate samples. A haemocytometer counting chamber (Gem Industrial Corporation, Noida, India) was used to measure the spermatozoa density. On a haemocytometer slide (depth 0.1 mm) with a cover slip, a droplet of diluted milt was counted using light microscopy. After three to five minutes (to allow for sperm sedimentation), the quantity of spermatozoa was counted [27].

## Results

### Physical parameters

Biology of scale carp and summary of brood size sperm in wild and farmed conditions was determined as sperm volume (ml), motility (%), density (×10<sup>9</sup>/ml) and pH (Table 1 and Table 2). The mean values and standard deviation of the physical parameters of the milt of *Cyprinus carpio* var. *communis* in wild and farmed conditions is presented in Table 3 and Figures 1-5. In case of wild conditions, sperm volume, motility rate, duration, sperm density and pH were 2.393 ± 1.64 ml, 75.038 ± 10.162 percent, 50.367 ± 13.92 sec, 3.53 ± 0.272 ×10<sup>9</sup>/ml and 8.29 ± 0.494 respectively. Further, in case of farmed condition mean ± std of sperm volume,

motility rate, duration, sperm density and pH were  $1.486 \pm 0.88$ ,  $68.9 \pm 12.46$ ,  $44.66 \pm 13.48$  sec,  $3.84 \pm 0.181 \times 10^9$  and  $8.5 \pm 0.311$  respectively. Statistical analysis revealed non significant difference in motility duration and pH between the two environments. The level of sperm volume, and motility % between the two ecotypes

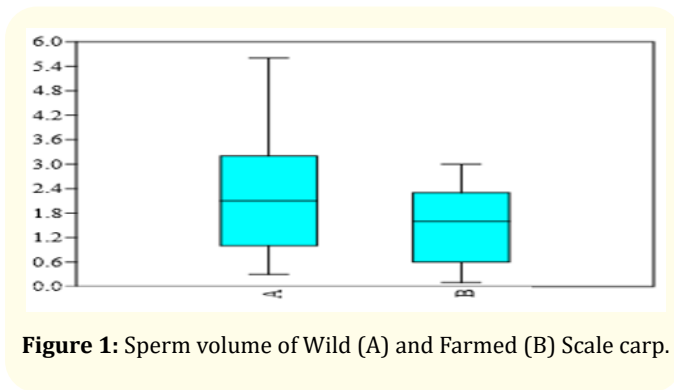
of the fish were significantly ( $P < 0.05$ ) different. The wild fish was having higher sperm volume and motility percentage than that of farmed fish. In addition, the Sperm density in the cultured population was significantly higher ( $P < 0.01$ ) than that in the wild type.

Parameter	Range	Mean	Standard error
Length (mm)	200-422	311.1	14.08
Weight (gm)	133-1350	462.5	71.76
Sperm volume (ml)	0.3-5.6	2.39	0.29
Motility (%)	61.2-94	75.03	1.85
Motility duration (seconds)	20-85	50.36	2.54
Sperm density ( $\times 10^9$ /ml)	3.16-3.98	3.53	0.04
pH	7.2-8.9	8.29	0.09

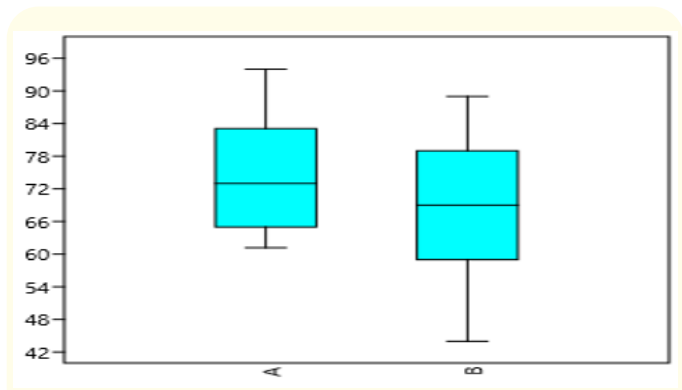
**Table 1:** Summary of brood size and sperm biology of Scale carp in wild conditions.

Parameter	Range	Mean	Standard error
Length (mm)	190-420	254.1	11.72
Weight (gm)	90-1180	293.1	49.44
Sperm volume (ml)	0.1-3	1.48	0.17
Motility %	44-89	68.9	2.27
Motility duration (sec)	20-69	44.66	2.46
Sperm density ( $\times 10^9$ ml)	3.27-4.21	3.84	0.03
pH	7.9-8.9	8.5	0.05

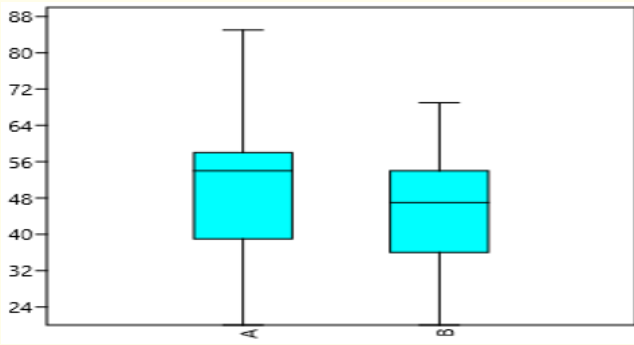
**Table 2:** Summary of brood size and sperm biology of Scale carp in farmed conditions.



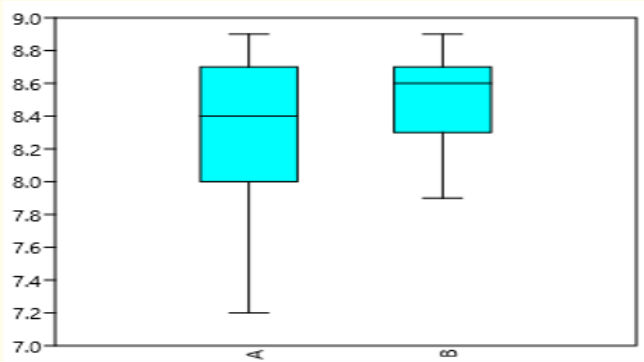
**Figure 1:** Sperm volume of Wild (A) and Farmed (B) Scale carp.



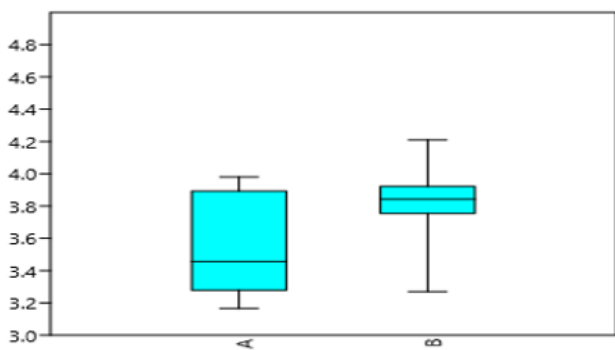
**Figure 2:** Motility % Wild (A) and Farmed (B) Scale carp.



**Figure 3:** Motility duration of Wild (A) and Farmed Scale carp (B).



**Figure 5:** pH of Wild (A) Farmed Scale carp (B).



**Figure 4:** Sperm density (A) Wild and Farmed (B) Scale carp.

Parameters	Mean ± std (Wild)	Mean ± std (Farmed)	t value	P value
Sperm volume (ml)	2.393 ± 1.64	1.486 ± 0.88	2.625	<0.05
Motility (%)	75.038 ± 10.162	68.9 ± 12.46	2.0899	<0.05
Duration (sec)	50.367 ± 13.92	44.66 ± 13.48	1.61	>0.05
Sperm density (×10 <sup>9</sup> /ml)	3.534 ± .272	3.84 ± 0.181	-5.148	<0.01
Ph	8.29 ± .494	8.5 ± 0.311	-1.905	>0.05

**Table 3:** Statistical analysis of physical parameters of the milt of *Cyprinus carpio* var. *communis* in wild and farmed conditions.

**Discussion**

In the present study, the differences in semen quality of wild and farmed Scale carp were examined. The present results showed

higher sperm volume, motility% and duration of wild compared to cultured fish. Mean sperm volume was higher in wild brooder (2.393 ± 1.64 ml) than in farmed individual (1.486 ± 0.88 ml).

Comparison of wild Scale carp with farmed fishes reveals the significant difference in sperm volume ( $P < 0.05$ ). Mean sperm volume in farmed and wild fish was found similar with the finding of [4] that recorded the sperm volume ranged from 1-9 ml. Mean seminal volume was also similar with the results reported by [23] they reported the sperm volume of Scale carp as 2 ml during the spawning season. [8] also reported the volume of milt in scaly carp as 2.75 ml. [36] also reported the volume of milt in the *Cyprinus carpio* as 1.83 to 1.98 ml. Higher sperm volume of wild fish than farmed has also been reported by [16]. [22] reported the cultured Caspian brown trout produced higher milt volume which is contrary to the present results. It is likely that cultured males of Scale carp with the application of an efficient osmoregulation, excrete the excess water of the body in response to hypotonicity of freshwater environment as reported by [22] in Caspian brown trout while studying the milt quality in the cultured and wild stocks on comparative basis. It is essential to say that the weight of wild males was higher than cultured individuals during the present study. [32,33] have reported that milt volume increases with increase of weight in Turbot (*Scophthalmus maximus*). Thus the higher weight of wild fish seems to be one of the reason for the higher milt volume of wild males than cultured fishes. The sperm volume has also been found higher in wild European flounder (0.7 ml; 29) than in cultured ones (0.2 ml; 3). [12] has found a low sperm quality and volume of stocked animals.

An important factor that determines the sperm quality is the sperm density [32] (Suquet, *et al.* 1992). It is important for the fertilization of spermatozoa [1,26]. In the present study, the average sperm density of  $3.534 \pm 0.27 \times 10^9 \text{ mL}^{-1}$  in wild and  $3.8415 \times 10^9 \pm 0.18 \text{ mL}^{-1}$  in farm was recorded for the fish which are in conformity with the results of [10] for grass carp ( $2.87\text{-}33.914 \times 10^9 \text{ mL}^{-1}$  in). [13] have reported the sperm density of  $6.6 \times 10^9$  sperm cells/ml in *C. carpio*. [36] recorded the average sperm density of  $2.25 \times 10^9$  sperm cells/ml in *C. carpio* from January to March, 2013. [19] found that the sperm density of *C. carpio* as  $0.5$  to  $1.0 \times 10^{11}$  cells per mL of milt. Comparison of farmed fish with wild revealed that sperm density of farmed fish was higher than wild individual. Similar results have been reported by [16]. Contrary to the present results [22] reported higher sperm density in wild than farmed fishes. Our results showed that the mean sperm density were higher in cultured males than in wild fish.

Motility is an important parameter to decide the quality of the spermatozoa [35]. Sperm motility is a key factor that determines fertilization success [7]. In the present study, the value of mean motility percentage and mean motility duration in case of wild fish was 75.03% and 50.36 sec respectively and in farmed fish the value was 68.9% and 44.6 sec respectively. Significant difference in motility percent was found between the groups but difference in motility duration was found non significant. [10] reported the mean sperm motility as  $63.18 \pm 7.1\%$  and mean motility duration was found  $56.81 \pm 20.3$  sec for Scale carp. [9] reported that motility percentage ranges from 70-95% and duration ranges from 35-117 sec in Grass carp. These results are in conformity with the present results for both farmed and wild fish. From the present study mean sperm/motility and mean motility duration were higher in wild brooder than in farmed individual. Similar results have been reported by [16]. [30] reported the wild males of Cod had higher sperm velocity, percentages of motile and progressive cells than their farmed counterparts. However many workers reported no difference in sperm motility between wild and cultured brooders [3,28,29]. Wild males with higher motility rate may be due to higher secretion of spermatid duct epithelium compared to the farmed ones. It is likely that with lower seminal fluid secretion by spermatid duct epithelium in farmed fishes, the quantity of materials involved in sperm motility decreases as well. This seems to be one of the reason for lower sperm motility rate in farmed Scale carp.

One of the sperm-activating factors in aquatic animals is a change in the external medium's pH [31]. In both of the fish conditions, the pH of the milt was found to be alkaline. Between wild and farmed fish, no discernible pH change was found. The pH of wild fish was 8.20.49 while that of farmed fish was 8.560.3 in the current study. The pH of the milt recorded in the present study is in the conformity with [10] who reported that pH of the milt of Scale carp from 6.9-9.2. [39] recorded pH of  $7.8 \pm 0.07$  in catla,  $7.3 \pm 0.06$  in rohu,  $7.9 \pm$  in mrigal,  $8.1 \pm 0.09$  in kalbasu,  $7.8 \pm 0.03$  in silver carp,  $7.9 \pm 0.06$  in grass carp.

The social dynamics between cultivated and natural circumstances are different, which may explain the variation in sperm features. High stocking densities are frequently used for fish rearing in ponds. They are dependent on artificial nutrition

and aren't given full exposure to the natural environment. Additionally, they experience physical stress during management rather regularly. While farmed fish are fed a diet of pellets, wild fish subsist on zooplankton and phytoplankton. Pellet diet reportedly has the potential to influence sperm characteristics (Bell, *et al.* 1996). Therefore, as proposed by Skjaeraasen (2009), a lack of social dynamics and differences in nutrition may account for the decreased sperm volume, motility, and longevity in Cod species.

## Conclusion

In a successful fish breeding, the quality of sperm is very essential. The present study revealed the higher sperm performance in wild fishes than farmed ones as wild fishes were found with higher sperm volume, motility % and duration but their sperm density was found lower than farmed fishes. The present results suggested that the type of environment has an effect on sperm quality.

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