ISSN: 2347-5129
IJFAS 2014; 1(6): 27-31

www.fisheriesjournal.com
Received: 06-05-2014
Accepted: 10-06-2014

M. Nahiduzzaman
(a) Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh (b) WorldFish, CSISA-BD, Rangpur, Bangladesh

S. Akter
Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh

M. M. Hassan
(a) Department of Fisheries Biology and Genetics, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh (b) School of Biological Sciences, Flinders University, Adelaide, Australia

A. K. M. Azad Shah
Department of Fisheries Technology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

M. A. R. Hossain
Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Correspondence:
Md. Nahiduzzaman
Fisheries Scientist/Hub Manager
WorldFish, Cereal Systems Initiative for South Asia (CSISA) in Bangladesh.
Rangpur Hub, House-46/2, Road-1, Parjaton purba Para Rangpur-5400, Bangladesh.

Sperm biology of artificially induced common carp, *Cyprinus carpio* (Linnaeus, 1758)

M. Nahiduzzaman, S. Akter, M. M. Hassan, A. K. M. Azad Shah, M. A. R. Hossain

ABSTRACT
Hatchery production of common carp seed has been practiced for several decades in Bangladesh but information on sperm biology of induced broods is limited. The study aimed to determine the sperm biology artificially induced broods to improve the current hatchery management. Sperm volume, motility, concentration and pH were 2.04±1.07 µl g^{-1} of fish, 93±3 %, 1.77±0.49×10^{10} cells ml^{-1} and 7.59±0.29, respectively. There has been a substantial variation ($p<0.05$) in volume (µl g^{-1} of fish), concentration (×10^{10} cells ml^{-1}) and motility (%) among the fortnightly collected sperm samples. The motility (%) of the fresh sperm was similar in all the activation media tested, however, sperm were motile for longer duration in 0.3% than in 0.2% NaCl, tap water and distilled water. Sperm biology of the induced broods would be useful in breeding programs advancing the development of aquaculture of the species.

Keywords: sperm biology, breeding, common carp, aquaculture.

1. Introduction
Common carp, *Cyprinus carpio* (Linnaeus, 1758) being one of the fastest growing fish species contributing 10% of the global freshwater aquaculture production [1]. The species is domesticated in lakes and ponds for aquaculture and the adaptability to a wider geographical location and temperature regime has contributed to the introduction of the species in many parts of Asia and Europe. Considering the potential of the common carp in small scale aquaculture in homestead and eutrophic ponds the species was introduced in Bangladesh in 1960 from China [2]. The contribution of exotic carps is 28% of the total aquaculture production in Bangladesh in which common carp is one of the leading species [3]. Although the species is considered invasive because of rubbing out pond dikes, however, the other attributes such as faster growth, disease resistance, adaptability to environmental fluctuations, short turnover time and suitability in polyculture has made the species a profitable choice in extensive and semi-intensive aquaculture.

In Bangladesh the seed production of common carp is entirely dependent induced gamete collection by hormone administration in hatcheries [4]. Better understanding of sperm biology of the species can improve the hatchery management efficiency of the species. In addition, sperm biology is intrinsically crucial for short and long-term sperm storage, which could also improve the current hatchery practice. Sperm biology provides a reasonable basis for developing a strategy for maximizing the fertility of a fish and also the development of preservation protocols [5].

The biophysical properties of sperm include its volume, concentration, pH and most importantly percentages of motile sperm. These attributes are also important indicators of sperm quality and artificial spawning. Usually sperm volume shows a seasonal variation in fish which is lowest at the onset that gradually reaches the highest during peak of spawning season [6]. Along with the seasonal variation, volume of sperm per unit fish body weight also varies with the age of species. A single sperm is capable of fertilizing an egg, however, millions of sperm are ejaculated by a male leading to sperm competition that ensure fertilization of eggs by the most competent sperm [7]. Therefore, concentration of sperm is crucial to maintaining sperm to egg ratios during artificial spawning.

Fish sperm are immotile inside the genital tract and after collection an osmotic gradient of extracellular solvent induce motility. Motility of sperm is activated by changing the external medium pH in many of the aquatic species [8], although it is not as much crucial as osmolality in carps. pH of the water which is added during artificial fertilization should conform with the pH of seminal plasma. In freshwater fish, a lower osmolality of extracellular medium (0-300 mOsmol l^{-1}) compared to the osmolality of seminal plasma induce motility whereas the opposite happens to marine species [9]. After activation, motility of cyprinid sperm is actively motility from few seconds to minutes in milli-Q water [10]. Therefore, sperm have to be in contact of the eggs soon after activation to ensure reaching micropile of eggs. Although common carp is one the major contributor in aquaculture in many parts of the world, including Bangladesh, information on sperm biology of artificially induced fish is limited. Therefore, the present study evaluated sperm biology of hormone administered common carp to improve current hatchery management practice in Bangladesh.

2. Materials and Methods

2.1. Rearing of broods

Broods were reared in brood-rearing earthen ponds (800 m^2) of Fisheries Field Laboratory Complex, Bangladesh Agricultural University (BAU) from July 2010-May 2011. Water quality parameters including temperature (25.07±5.12 ºC), pH (7.69±0.32), dissolved oxygen (5.63±0.42 mg l^{-1}) and total alkalinity (86.34±21.57 mg l^{-1}) of the ponds were recorded during the breeding season. The brood fish were fed with a commercial diet (35% protein; Paragon Feeds Limited, Bangladesh) twice daily at 4–5% of their body weight in the rearing period.

2.2. Sperm collection and spermatological parameters

Ready to spawn broods were collected from the ponds and conditioned for 12 hours in the hatchery tanks of the Faculty of Fisheries, BAU. The fish were injected with carp pituitary supernatant at 2 mg kg^{-1} of body weight and released in the conditioning tank. After 6 h of hormone injection, the males were captured from the tank using a scoop net and were laid on foam to wipe the urogenital pore. Gentle pressure was applied through the abdomen to remove urine, water, gut exudates and mucus to avoid contamination. Sperm were collected in glass vials by abdominal pressure and the vials were immediately placed at 4 °C. Ejaculated sperm volume was determined by the measuring pipette and expressed as µl. Sperm pH was determined with a pH indicator strips (pH: 0–14; Merck, Germany) immediately after collection. Concentration was determined in triplicates using a haemocytometer and expressed as the number of sperm cells × 10^{10} ml^{-1}. Sperm were diluted 4,000-folds in distilled water, and a droplet of the diluted sperm was placed in a haemacytometer (area of the smallest square = 1/400 mm^2, depth 0.1 mm) for counting sperm. The number of spermatozoa in five large squares (area mm^2) of the counting chamber was counted at 400 magnification.

2.3. Activation medium and sperm motility

The motility of sperm samples was evaluated using a light microscope (Novex K-range, Holland) at 400 magnifications. Sperm motility was estimated by adding 19 µl of distilled water (24 mOsmol kg^{-1}) as activating medium to 1 µl of fresh sperm on a glass slide. The motility was observed within 3 to 4 s after activation and was expressed as the percentage of actively forward moving sperm out of the total. Sperm spinning or vibrating in place were not considered to be motile. Four solutions (0.2% NaCl, 0.3% NaCl, distilled water and tap water) with different osmolalities were tested for motility activation of the fresh sperm. Motility of sperm in each of the solutions was observed in triplicates to assess the motility (%), duration of motility, and to compare motility among solutions from a pool of five males.

2.4. Statistical analysis

Motility percentage was subjected to arcsine transformation prior to statistical analysis. One-way analysis of variance (ANOVA) was carried out to determine the variation of sperm volume, motility percentage, concentration and pH. Pearson correlation was used to relate sperm biology and body traits of the broods. The Duncan's Multiple Range Test (DMRT) was used to compare means at 0.05 significant levels. All values were expressed as mean ± standard deviation (SD).

3. Results

The weight of male (n = 43) ranged between 370 and 2000 g (mean±SD; 681.88±375.06 g) and total length ranged between 28 and 56 cm (35.43 ± 6.93 cm). Biology of common carp sperm was determined as sperm volume (µl g^{-1} of fish), motility (%), concentration (cell ml^{-1}) and pH (Table 1). Sperm volume was correlated with total length and weight of the fish. Sperm volume was also positively correlated with sperm concentration and fresh sperm motility. Fish length and weight were negatively correlated with sperm concentration (Table 2).

Table 1: A generalized summary of brood size and sperm biology of common carp.

Items	Means ± SD	Minimum	Maximum
No. of fish	43	43	43
Total length (cm)	35.43 ± 6.93	28	56
Total weight (g)	681.88 ± 375.06	370	2000
Sperm volume (µl g^{-1})	2.04 ±1.07	0.68	4.67
Sperm pH	7.59 ± 0.29	7	8.4
Sperm concentration (×10^{10} ml^{-1})	1.77 ± 0.49	1	2.82
Sperm motility (%)	93 ± 3	85	95

Table 2: Correlations between sperm biology with length and weight of broods.

	Weight (g)	Sperm volume (µl g^{-1})	Sperm pH	Sperm concentration ($\times10^{10}$ ml^{-1})	Motility (%)
Length (cm)	0.972**	0.634**	-0.139	-0.396**	-0.147
Weight (g)		0.594**	-0.176	-0.359**	-0.127
Sperm volume (µl g^{-1})			-0.019	0.649**	0.504**
Sperm pH				-0.17	-0.162
Sperm concentration ($\times10^{10}$ ml^{-1})					0.515**

*p < 0.05, **p < 0.01

Spermatozoa showed a substantial variation in volume (µl g^{-1} of fish) (F=2.520; p=0.033), concentration ($\times10^{10}$ ml^{-1}) (F=9.578; p=0.00) and motility (%) (F=7.209; p=0.00) among the sampling fortnights (Table 3). Sperm pH did not show any significant variation (F=0.345; p=0.927) among the sampling fortnights.

Table 3: Temporal variations in sperm volume (µl g^{-1}), concentration ($\times10^{10}$ ml^{-1}), pH and motility (%) in a spawning season of the artificially induced common carp (Mean ± SD).

Fortnight*	Sperm volume (µl g^{-1})	Sperm concentration ($\times10^{10}$ ml^{-1})	Sperm pH	Sperm motility (%)
1	1.90 ± 0.09^{bcd}	1.44 ± 0.15^{b}	7.60 ± 0.22	94 ± 2^{ab}
2	1.86 ± 0.69^{bcde}	2.28 ± 0.11^{a}	7.63 ± 0.25	95 ± 0^{a}
3	2.92 ± 1.00^{ab}	2.15 ± 0.32^{a}	7.50 ± 0.00	94 ± 2^{a}
4	3.64 ± 0.96^{a}	2.02 ± 0.29^{a}	7.73 ± 0.40	95 ± 0^{a}
5	2.32 ± 1.01^{bc}	1.99 ± 0.49^{a}	7.55 ± 0.15	95 ± 0^{a}
6	1.70 ± 0.20^{cde}	1.42 ± 0.21^{b}	7.67 ± 0.29	93 ± 3^{ab}
7	0.96 ± 0.14^{de}	1.42 ± 0.18^{b}	7.70 ± 0.57	91 ± 4^{b}
8	0.77 ± 0.09^{e}	1.08 ± 0.11^{b}	7.56 ± 0.48	88 ± 3^{c}

*Fortnight 1-2: December; 3-4: January; 5-6: February; 7-8: March
Values with different letters within a column are significantly different (p < 0.05)

Percentage motility of the fresh sperm was almost same at the beginning of activation in the four activating media with different osmolalities. However, 0.3% NaCl (96 mOsmol kg^{-1}) yielded higher motility duration (141 s) than 0.2% NaCl (67 mOsmol kg^{-1}) that retained motility for 118 s. Both tap water (31 mOsmol kg^{-1}) and distilled water (24 mOsmol kg^{-1}) produced poor motility duration (58 and 56 s) (Table 4).

Table 4: Effect of activating media (0.3% NaCl, 0.2% NaCl, tap water and distilled water) at a range of osmolalities on sperm motility (%) and motility duration of common carp.

Activation solution	Osmolality (mOsmol kg^{-1})	Motility (%)	Motility duration (s)
0.3% NaCl	96	93 ± 3^{a}	141 ± 5^{a}
0.2% NaCl	67	93 ± 3^{a}	118 ± 6^{b}
Tap water	31	92 ± 2^{a}	58 ± 3^{c}
Distilled water	24	93 ± 2^{a}	56 ± 4^{c}

Means with different letters within a column are significantly different (p < 0.05)

4. Discussion

Several factors contribute to sperm biology, including age, length and weight [11, 12], rearing conditions of brood [13] and methods of spawning induction [14]. Sperm biology changes in a spawning season showing a gradual increase and decrease in motility percentage and concentrations at the beginning and end of spawning [15, 16, 17, 18]. Sperm volume of common carp was lower at the beginning of spawning season and it gradually increased with the time indicating the temporal variation of sperm volume in a spawning season. Sperm concentration of common carp varied between the sampling fortnights in which the highest concentration was found in the middle of the spawning season. Sperm concentration is an important parameter in hatchery management and it is highly variable depending on species, fish size and season [19]. Sperm volume and concentration of common carp were similar to other carp species including *Gibelion catla*, *Labeo rohita*, *Cirrhinus cirrhosus, L. calbasu* and *Hypophthalmichthys molitrix* [20, 21].

A change in pH of external medium is one of the sperm activating factors in aquatic species [22]. The pH of sperm was alkaline in common carp. There was no significant difference observed pH among different fortnight. The duration of sperm motility in *Petromyzon marinus* decreased with an increase in pH, but the percentage of motile cells did not change over the pH range 6.0–9.0 [23]. Ingermann *et al.* [24] reported pH

sensitivity of sperm in *Acipenser transmontanus*, and demonstrated that sperm at high pH (more than 8.2) had higher motility when active with water but lower motility at a comparatively low pH (less than 7.5). The motility activation in common carp sperm was tested conforming pH of salt solutions with seminal plasma only to test the effect of osmolality on the motility activation of sperm.

Motility of fish sperm may be affected by several factors such as pH, concentration, ions and osmolality [10, 25, 26]. Motility of the common carp sperm was triggered by tap water (31 mOsmol kg^{-1}), distilled water (24 mOsmol kg^{-1}), 0.2% NaCl (67 mOsmol kg^{-1}) and 0.3% NaCl (96 mOsmol kg^{-1}). Motility duration of common carp was very short like other freshwater fish at ambient temperature [27]. The duration of the forward movement of sperm at different osmolalities of these activating solutions showed great variations from 58 to 141 s which is similar to other carps [5]. Instant initiation of sperm motility could be achieved with hypotonic solutions in a wide range of osmolalities but sustaining motility over time requires an optimal osmolality. Sperm showed longer motility duration when suspended with 0.3% NaCl suggesting its applicability in artificial spawning as an activation media of common carp. In freshwater species, a hypo-osmotic shock can trigger sperm motility of mature spermatozoa [26]. In freshwater fishes, exposure of sperm to distilled water lead to flagellar damages after activation [28].

5. Conclusion

With the current state of demand for common carp seed for aquaculture in Bangladesh and hatcheries need information on the sperm biology of artificially induced broods to improve the current practice. This paper represents baseline information on the biology of common carp sperm which would contribute to future research and artificial breeding programs of the species.

6. Acknowledgements

This research was supported by the United States Department of Agriculture (USDA) through "*Ex situ* conservation of some indigenous fishes of Bangladesh by selecting the best stock through DNA markers" project (BGARS-120).

7. References

1. FAO (Food and Agriculture Organization of the United Nations). Global aquaculture production. FAO EIFAC, Rome, Italy, 2011.
2. Rahman AKA. Freshwater Fishes of Bangladesh, 2nd Edn. Zoological Society of Bangladesh, Dhaka, 2005, 394.
3. De-Silva SS, Nguyen TTT, Abery NW, Amarasinghe US. An evaluation of the role and impacts of alien fin fish in Asian inland aquaculture. Aquaculture Research 2006; 37(1):1–17.
4. Bhiyan AS, Musa ASM, Islam MK. Hatchery management in Bangladesh. Providing Claims Services to the Aquaculture Industry 2008; 10:20–24.
5. Hassan MM, Nahiduzzaman M, Al-Mamun SN, Taher MA, Hossain MAR. Fertilization by refrigerator stored sperm of the Indian major carp, Labeo calbasu (Hamilton, 1822). Aquaculture Research 2013; 45(1):150–158.
6. Alavi SMH, Rodina M, Policar T, Kozak P, Psenicka M, Linhart O. Semen of *Perca fluviatilis* L Sperm volume and density, seminal plasma indices and effects of dilution ratio ions and osmolality on sperm motility. Theriogenology 2007; 68(2):276–283.
7. Taborsky M. Sperm competition in fish bourgeois' males and parasitic spawning. Trends in Ecology & Evolution 1998: 13(6):222–227.
8. Alavi SMH, Cosson J. Sperm motility in fishes I Effects of temperature and pH a review. Cell biology international 2005; 29(2):101–110.
9. Morisawa M, Suzuki K, Morisawa S. Effects of potassium and osmolality on spermatozoan motility of salmonid fishes. Journal of Experimental Biology 1983; 107:105–113.
10. Alavi SMH, Cosson J. Sperm motility in fishes (II) Effects of ions and osmolality a review. Cell Biology International 2006; 30(1):1–14.
11. Trippel EA, Neilson JD. Fertility and sperm quality of virgin and repeat-spawning Atlantic cod (*Gadus morhua*) and associated hatching success. Canadian Journal of Fisheries and Aquatic Sciences 1992; 49:2118–2127.
12. Hoysak DJ, Liley NR. Fertilization dynamics in sockeye salmon and a comparison of sperm from alternative male phenotypes. Journal of Fish Biology 2001; 58:1286–1300.
13. Morisawa M, Hirano T, Suzuki K. Changes in blood seminal plasma composition of the mature salmpon (*Oncorhynchus keta*) during adaptation to freshwater. Comparative Biochemistry and Physiology 1979; 64:325–329.
14. Caille N, Rodima M, Kocour M, Gela D, Flajshans M, Linhart O. Quantity, motility and fertility of tench *Tinca tinca* (L.) sperm in relation to LHRH analogue and carp pituitary treatments. Aquaculture International 2006; 14:75–87.
15. Lahnsteiner F, Berger B, Weismann T, Patzner RA. Determination of semen quality of the rainbow trout, Oncorhynchus mykiss, by sperm motility seminal plasma parameters and spermatozoal metabolism. Aquaculture 1998; 163:163–181.
16. Lahnsteiner F, Berger B, Grubinger F, Weismann T. The effect of 4-nonylphenol on semen quality viability of gametes fertilization success and embryo and larvae survival in rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology 2005; 71:297–306.
17. Liley RN, Tamkee P, Tsai R, Hoysak JD. Fertilization dynamics in rainbow trout (Oncorhynchus mykiss): effect of male age social experience and sperm concentration and motility on in vitro fertilization. Canadian Journal of Fisheries and Aquatic Sciences 2002; 59:144–152.
18. Aral F, Sahinoz E, Dog˘u Z. Annual changes in sperm characteristics of young rainbow trout (*Oncorhynchus mykiss*, W. 1792) during spawning season in Atat rk Dam Lake Sanliurfa, Turkey. Journal of Animal and Veterinary Advances 2005; 4:309–313.
19. Glogowski J, Ciereszko A, Dabrowski K. Cryopreservation of muskellunge and yellow perch semen. North American Journal of Aquaculture 1999; 61:258–262.
20. Verma DK, Routray P, Dash C, Dasgupta S, Jena JK. Physical and biochemical characteristics of semen and ultrastructure of spermatozoa in six carp species.

Turkish Journal of Fisheries and Aquatic Sciences 2009; 9:67–76.
21. Nahiduzzaman M, Hassan MM, Roy PK, Hossain MA, Hossain MAR, Tiersch TR. Sperm cryopreservation of the Indian major carp *Labeo calbasu* Effects of cryoprotectants, cooling rates and thawing rates on egg fertilization. Animal Reproduction Science 2012; 136:133–138.
22. Stoss J. Fish gamete preservation and spermatozoan physiology. In: Hoar WS, Randall DJ, Donaldson (Eds.) Fish Physiology Reproduction Part B Behaviour and Fertility Control. Academic Press New York, 1983, 9:305–350.
23. Ciereszko A, Dabrowski K, Toth GP, Christ SA, Glogowski J. Factors affecting motility characteristics and fertilizing ability of Sea Lamprey spermatozoa. Transactions of the American Fisheries Society 2002; 131:193–202.
24. Ingermann R, Holcomb M, Robinson ML, Cloud JG. Carbon dioxide and pH affect sperm motility of white sturgeon (*Acipenser transmontanus*). Journal of Experimental Biology 2002; 205:2885–2890.
25. Cosson J, Billard R, Cibert C, Dre´anno C, Suquet M. Ionic factors regulating the motility of fish sperm. In: Gagnon C. (Ed.) The male gamete: from basic knowledge to clinical applications. Cache River Press, Vienna, IL, USA, 1999, 161–186.
26. Morisawa M, Oda S, Yoshida M, Takai H. Transmembrane signal transduction for the regulation of sperm motility in fishes and ascidians. In: Gagnon C. (Ed.) The male gamete: from basic knowledge to clinical applications. Cache River Press, Vienna, USA, 1999, 149–160.
27. Hulak M, Rodina M, Alavi SMH, Linhart O. Evaluation of semen and urine of pike (*Esox lucius* L.) ionic compositions and osmolality of the seminal plasma and sperm volume, density and motility. Cybium 2008; 32(2):189–190.
28. Cosson MP, Cosson J, Andre´ F, Billard R. cAMP/ATP relationship in the activation of trout sperm motility their interaction in membrane-deprived models and in live spermatozoa. Cell Mobility and the Cytoskeleton 1995; 31:159–176.