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The Effect of Body Weight on Fresh Sperm Movement Characteristics in African Catfish (*Clarias gariepinus*)

[1]K. Noor Azlina, [2]W.E. Wan Khadijah and [2]R.B. Abdullah
[1]Department of Biological Sciences, Faculty of Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia
[2]Animal Biotechnology-Embryo Laboratory (ABEL), Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

Abstract: Fresh sperm of African catfish were analysed with the aim to determine sperm movement characteristics based on individual fish body weight. Three Body Weight (BW) of fish were grouped as small (<1 kg), medium (1-1.5 kg) and large (>1.5 kg). The fish was sacrificed (euthanasia) and semen was collected by perforation of the testis. Large Body Weight (BW) of African catfish gave the highest fresh sperm total motility (82.40±4.59%) followed by medium body weight (51.64±9.82%) and small body weight (40.40±12.16%). In sperm velocity distributions, the values for rapid, medium, slow and static velocity for fresh sperm were ranged from 14.00±6.63 to 25.80±4.97, 5.60±2.32 to 15.40±2.82, 20.80±6.49 to 41.20±5.18 and 17.60±4.59 to 59.60±12.16%, respectively. The values of VAP (83.34±9.31 μm sec^{-1}), VSL (73.44±11.60 μm sec^{-1}) and VCL (108.12±5.51 μm sec^{-1}) for small body weight group were significantly higher ($p<0.05$) than those of the large body weight group (49.70±6.42, 41.90±4.94 and 74.60±9.47 μm sec^{-1}, respectively). It is concluded that larger body weight of the fish gives better sperm quality compared to medium and small BW.

Key words: Fresh sperm, Sperm Movement Characteristics (SMCs), African catfish, body weight, fish, motility, Malaysia

INTRODUCTION

Fish sperm show species differences in the initiation (Morisawa, 1985; Cosson *et al*., 1995), duration (Billard, 1978; Billard and Cosson, 1992) and pattern of motility (Boitano and Omoto, 1992; Ravinder *et al*., 1997). The difference in K^+ ion concentration (in salmonids) or osmotic pressure (in cyprinids, clariids and other families) between the seminal plasma and water are among the factors to trigger the initiation of movement (Morisawa *et al*., 1983; Billard, 1986). With the advances in reproductive technology, analyzing of sperm movement can be done easily using computer-assisted sperm trackers, it comprises essentially a microscope coupled to a CCD camera which conveys a signal to a monitor, VCR recorder and computer (Boyer *et al*., 1989). Sperm movement is usually recorded onto videotape which is later analysed by the computer software. Various parameters can be measured using CASA such as percent motility, Velocity Curvilinear (VCL), Velocity Average Path (VAP), Velocity Straight Line (VSL), Linearity (LIN) and Straightness (STR). From studies using such sperm tracking systems conducted on African catfish, carp, goldfish, roach, Eurasian perch, trout, lake sturgeon, the most useful parameters of velocity are the curvilinear velocity (VCL, the actual velocity along the trajectory) and the straight line Velocity (VSL, the straight line distance between the start and end points of the track divided by the time of the track) (Ciereszko and Dabrowski, 1996; Kime *et al*., 2001; Rurangwa *et al*., 2001, 2002; Jobling *et al*., 2002). If the trajectory is a straight line then VCL and VSL are identical. The angular path velocity (VAP, the velocity along a derived smoothed path) is generally of little use in most fish since unlike mammalian sperm, the tracks are general smooth curves so that VAP and VCL are identical. A considerable amount of literature has been published on factors affecting sperm quality in fish. The most critical factors are rearing photoperiod and temperature, nutrition, water and food contamination, stress, age of broodstocks, breeding season, diseases of broodstocks, hormonal induction and spermiation which are reviewed. The summary of main factors that can influence gamete quality in fish and main parameters that can be recorded fully characterised gamete quality was reviewed by Bobe and Labbe (2010). Up to now, there is no study related to the effect of body weight on sperm motility characteristics in African catfish. Thus, the present study was the 1st reported study which

Corresponding Author: W.E. Wan Khadijah, Animal Biotechnology-Embryo Laboratory (ABEL), Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

gave the baseline information on sperm quality in different body weight groups. The objective of this study was to determine the fresh sperm movement characteristics of African catfish (*Clarias gariepinus*) with special attention to different body weight groups.

MATERIALS AND METHODS

Experimental fish and maintenance: Total 80 adult males of African catfish, *Clarias gariepinus* that were sexually mature (aged between 1-2 years; body weight of 1-2 kg), established for this study was bought from a local fish farm in Rembau, Negeri Sembilan. The broodstocks were acclimatised in a fibreglass tank in the fish house at Institute of Biological Sciences Mini (Livestock) Farm, the University of Malaya. Routine management of fish was scheduled accordingly to avoid stress-aggravated during experimental period. The broodstocks were hand-fed with commercial finisher layer mash twice a day, *ad libitum* and daily monitored.

Hormonal induction: Body weight of selected catfish was measured to quantify the dosage of hormone per body weight for each individual fish to be administered. Then, Ovaprim (Syndel, Vancouver, Canada) 0.5 mL kg^{-1} Body Weight (BW) was injected intramuscularly into the dorsal muscle of catfish. Prior to this, the head of the catfish was covered by a wet towel in order to keep it quiet during injection. After receiving the hormone treatment, males were individually housed in separate tank to avoid aggressive interaction and to maximise care during the experimental period.

Collection of milt: African catfish were sacrificed to collect the milt. First, the individual fish was weighed to get an actual body weight. The actual body weight was used to categorize three respective sizes, namely small (<1 kg), medium (1-1.5 kg) and large (>1.5 kg). Two individual males were sacrificed for each time and the body surface thoroughly dried after which the testis was dissected out from the body cavity. Subsequently, the testis was cleaned with tap water to rinse the blood. Then, the testis was gently perforated with needle to collect the milt. Precaution has to take into account to avoid the needle pierce into the capillary. The whitish-like semen was flowing out and the semen was collected using eppendorf tube.

Analysis of Sperm Motility Characteristics (SMC): The milt collected was diluted with diluents in a ratio of 1:10 facilitate analysis of sperm motility characteristics. Without dilution, the analysis of sperm using IVOS was difficult because the sperm was too concentrated. Fresh sperm was analysed using the Automated Semen Analyzer (IVOS; Hamilton Thorne, USA) to evaluate the sperm movement characteristics. The parameters which were assessed include total motility, progressive motility, velocity distribution (rapid, medium, slow and static) and sperm motion characteristics (VAP: Velocity Average Path; VCL: Velocity Curvilinear; VSL: Velocity Straight-Line ; LIN: Linearity; ALH: Amplitude of Lateral Head displacement; BCF: Beat-Cross Frequency and STR: Straightness.

Data analysis: All data were subjected to Analysis of Variance (ANOVA) followed by comparison of means using Duncan's Multiple Range Test (DMRT). All statistical analysis was performed using SPSS (Statistical Package for Social Sciences) for windows Version 12.0. The data was presented as (mean±SEM). To determine correlations among the parameters measured, Pearson correlation was used which was significant at $p<0.01$.

RESULTS AND DISCUSSION

Table 1 shows the total motility and progressive motility of fresh sperm obtained from 24 randomly selected fish which were grouped into small (5), medium (14) and large (5) Body Weight groups (BW). Large body weight of African catfish gave the highest total motility (82.40±4.59%) followed by medium body weight (51.64±9.82%) and small body weight (40.40±12.16%) whereby small body weight fish were significantly different in total motility compared with the other two groups. The values for progressive motility for small, medium and large bodyweight of fish were 8.20±3.65, 14.00±4.29 and 17.40±3.36%, respectively ($p>0.05$). Velocity distributions for fresh sperm of African catfish according to body weight group of fish are shown in Table 2.

Table 1: Total motility and progressive motility (mean±SEM) for fresh sperm of African catfish according to body weight group of fish

Body size (BW, kg)	N*	Total motility (%)	Progressive motility (%)
Small (<1)	5	40.40±12.16[a]	8.20±3.65[a]
Medium (1-1.5)	14	51.64±9.820[ab]	14.00±4.29[a]
Large (>1.5)	5	82.40±4.590[b]	17.40±3.36[a]

Table 2: Velocity distributions (mean±SEM) for fresh sperm of African catfish according to body weight group of fish

Body size (BW, kg)	N*	Rapid (%)	Medium (%)	Slow (%)	Static (%)
Small (<1)	5	14.00±6.63[a]	5.60±2.32[a]	20.80±6.49[a]	59.60±12.16[b]
Medium (1-1.5)	14	18.79±6.13[a]	6.21±1.86[a]	26.50±5.25[a]	48.36±9.820[ab]
Large (>1.5)	5	25.80±4.97[a]	15.40±2.82[b]	41.20±5.18[a]	17.60±4.590[a]

N* = No. of individual fish; [ab]Means with different superscripts within a column were significantly different ($p<0.05$)

The values of sperm with rapid and slow velocities for the three body weight groups did not show any significant differences ($p>0.05$). However, sperm for large body weight group with medium velocity showed the highest significant value (15.40±2.82%) as compared to medium body weight group (6.21±1.86%) and small body weight group (5.60±2.32%).

In static velocity, there were no significant differences among the different body weight groups studied ($p>0.05$). The values for rapid, medium, slow and static velocity distributions for fresh sperm were ranged from 14.00±6.63 to 25.80±4.97, 5.60±2.32 to 15.40±2.82, 20.80±6.49 to 41.20±5.18 and 17.60±4.59 to 59.60±12.16%, respectively. Interestingly, large body weight group of fish gave significantly higher medium velocity compared to the other groups. Conversely, small and medium body weight groups were significantly higher in static velocity compared to large body weight group.

Analysis of sperm motion characteristics for fresh sperm of African catfish are shown in Table 3. There were no significant differences for values of ALH (range: 5.36±0.60-6.20±0.49 μm), BCF (range: 10.78±2.82 to 16.10±2.66 Hz), STR (range: 83.33±1.45 to 88.36±1.53%) and LIN (range: 62.00±1.97 to 66.80±7.88%) among the three body weight groups ($p>0.05$). However, the respective VAP (83.34±9.31 μm sec^{-1}), VSL (73.44±11.60 μm sec^{-1}) and VCL (108.12±5.51 μm sec^{-1}) values for small body weight groups were significantly higher ($p<0.05$) than those of the large body weight group (49.70±6.42, 41.90±4.94 and 74.60±9.47 μm sec^{-1}). The presumptive reason behind effects of body weight might be associated with the age of male African catfish broodstocks which can produce a high sperm total motility but this is not an absolute reason, since a limited sample size of fish were used for each groups. These findings further support the idea of Vuthiphandchai and Zohar (1999) who suggested age of broodstocks have a significant influence on the sperm quality and may affect the success of storing sperm. In captive-reared striped bass (*Morone saxatilis*), 3 years old fish had higher sperm quality than the 1 year or 12 months old fish based on higher sperm production and increased sperm longevity during short-term storage. However, the fertilising capacity of virgin and repeat spawners was comparable in Atlantic cod, *G. morhua* (Trippel and Neilson, 1992).

Another possible explanation for this is that the nutrition shows an important factor that affects the body weight of African catfish. Nutrition is susceptible to affect not only fecundity and gametogenesis but also gamete quality and existing work has been extensively reviewed (Kjorsvik *et al*., 1990; Brooks *et al*., 1997; Izquierdo *et al*., 2001). Improvement in broodstock nutrition and feeding greatly improves gamete quality and seed production (Izquierdo *et al*., 2001).

According to Alavi *et al*. (2009), they found that significant relationships were observed between weight and length of broodfish (*Barbus barbus* L.) with total number of sperm and sperm concentration but not with sperm volume. The evidence of this was associated with endocrinological mechanism regulating hydration of sperm during spermiation (Nagahama, 1994; Billard *et al*., 1995; Alavi and Cosson, 2006). Table 4-6

Table 3: Sperm motion characteristics (mean±SEM) for fresh sperm of African catfish according to body weight group of fish

Body size (BW, kg)	N*	VAP	VSL	VCL	ALH (μm)	BCF (Hz)	STR (%)	LIN (%)
		(μm sec^{-1})						
Small (<1)	5	83.34±9.31^{b}	73.44±11.60^{b}	108.12±5.51^{b}	6.20±0.49^{a}	10.78±2.82^{a}	86.00±3.81^{a}	66.80±7.88^{a}
Medium (1-1.5)	14	58.82±6.22^{ab}	52.55±5.610^{ab}	80.45±6.87^{ab}	5.36±0.60^{a}	11.21±1.64^{a}	88.36±1.53^{a}	62.00±1.97^{a}
Large (>1.5)	5	49.70±6.42^{a}	41.90±4.940^{a}	74.60±9.47^{a}	5.53±1.47^{a}	16.10±2.66^{a}	83.33±1.45^{a}	62.67±2.32^{a}

N* = No. of individual fish; ^{ab}Means with different superscripts within a column were significantly different ($p<0.05$)

Table 4: Correlations among fresh sperm motility characteristics for small body weight group of African catfish

	Total motility	Progressive motility	Rapid	Medium	Slow	Static	VAP	VSL	VCL	ALH	BCF	STR	LIN
Total motility	1	0.768	0.632	0.857	0.845	-0.959**	-0.324	-0.364	-0.014	-0.375	0.362	-0.439	-0.406
Progressive motility	-	1	0.795	0.369	0.310	-0.669	-0.213	-0.356	0.378	-0.471	0.240	-0.636	-0.465
Rapid	-	-	1	0.202	0.245	-0.714	-0.193	-0.325	0.313	-0.909*	0.402	-0.569	-0.420
Medium	-	-	-	1	0.970**	-0.818	-0.430	-0.374	-0.406	-0.018	0.391	-0.250	-0.346
Slow	-	-	-	-	1	-0.852	-0.263	-0.203	-0.303	-0.117	0.284	-0.087	-0.176
Static	-	-	-	-	-	1	0.328	0.357	0.069	0.561	-0.445	0.404	0.389
VAP	-	-	-	-	-	-	1	0.983**	0.786	0.140	-0.948*	0.837	0.950*
VSL	-	-	-	-	-	-	-	1	0.661	0.237	-0.945*	0.923*	0.991**
VCL	-	-	-	-	-	-	-	-	1	-0.198	-0.715	0.321	0.554
ALH	-	-	-	-	-	-	-	-	-	1	-0.425	0.403	0.302
BCF	-	-	-	-	-	-	-	-	-	-	1	-0.827	-0.922*
STR	-	-	-	-	-	-	-	-	-	-	-	1	0.966**
LIN	-	-	-	-	-	-	-	-	-	-	-	-	1

No. of fish = 5; **Pearson correlations were significant ($p<0.01$); *Pearson correlations were significant ($p<0.05$)

Table 5: Correlations among fresh sperm motility characteristics for medium body weight group of African catfish

	Total motility	Progressive motility	Rapid	Medium	Slow	Static	VAP	VSL	VCL	ALH	BCF	STR	LIN
Total motility	1	0.813**	0.776**	0.783**	0.692**	-1.000**	-0.335	-0.353	-0.360	0.090	0.325	-0.167	-0.181
Progressive motility	- -	1 -	0.993**	0.611*	0.149	-0.813**	-0.289	-0.299	-0.296	-0.001	0.098	-0.162	-0.255
Rapid	-	-	1	0.519	0.103	-0.776**	-0.269	-0.279	-0.274	-0.006	0.060	-0.153	-0.250
Medium	-	-	-	1	0.509	-0.783**	-0.207	-0.227	-0.234	0.211	0.354	-0.172	-0.078
Slow	-	-	-	-	1	-0.692**	-0.262	-0.282	-0.298	0.136	0.513	-0.090	-0.003
Static	-	-	-	-	-	1	0.335	0.353	0.360	-0.090	-0.325	0.167	0.181
VAP	-	-	-	-	-	-	1	0.979**	0.963**	0.561*	0.007	-0.370	0.577*
VSL	-	-	-	-	-	-	-	1	0.896**	0.597*	-0.139	-0.200	0.705**
VCL	-	-	-	-	-	-	-	-	1	0.435	0.116	-0.508	0.366
ALH	-	-	-	-	-	-	-	-	-	1	0.033	-0.099	0.659*
BCF	-	-	-	-	-	-	-	-	-	-	1	-0.742**	-0.392
STR	-	-	-	-	-	-	-	-	-	-	-	1	0.384
LIN	-	-	-	-	-	-	-	-	-	-	-	-	1

No. of fish = 14; **Pearson correlations were significant ($p<0.01$); *Pearson correlations were significant ($p<0.05$)

Table 6: Correlations among fresh sperm motility characteristics for large body weight group of African catfish

	Total motility	Progressive motility	Rapid	Medium	Slow	Static	VAP	VSL	VCL	ALH	BCF	STR	LIN
Total motility	1	0.462	0.400	0.225	0.407	-1.000**	0.062	-0.056	0.025	-0.032	0.116	0.296	0.092
Progressive motility	-	1	0.993**	-0.004	-0.507	-0.462	-0.984*	-0.995**	-0.979*	-0.998**	0.019	0.941	0.998**
Rapid	-	-	1	-0.084	-0.531	-0.400	-0.986*	-0.999**	-0.989*	-0.994**	0.109	0.963*	0.989*
Medium				1	-0.251	-0.225	0.170	0.138	0.233	0.067	-0.892	-0.212	0.018
Slow	-	-	-	-	1	-0.407	0.847	0.822	0.797	0.869	0.452	-0.635	-0.883
Static	-	-	-	-	-	1	-0.062	0.056	-0.025	0.032	-0.116	-0.296	-0.092
VAP	-	-	-	-	-	-	1	0.993**	0.996**	0.993**	-0.086	-0.916	-0.978*
VSL	-	-	-	-	-	-	-	1	0.994**	0.996**	-0.109	-0.953*	-0.988*
VCL	-	-	-	-	-	-	-	-	1	0.986*	-0.171	-0.941	-0.968*
ALH	-	-	-	-	-	-	-	-	-	1	-0.022	-0.928	-0.996**
BCF	-	-	-	-	-	-	-	-	-	-	1	0.307	-0.039
STR	-	-	-	-	-	-	-	-	-	-	-	1	0.923
LIN	-	-	-	-	-	-	-	-	-	-	-	-	1

No. of fish = 5; **Pearson correlations were significant ($p<0.01$); *Pearson correlations were significant ($p<0.05$)

show correlations of fresh sperm motility characteristics in African catfish for small, medium and large body weight groups. For small body weight group, positive correlations ($p<0.05$) were shown between medium and slow; VAP and VSL; VAP and LIN; VSL and STR; VSL and LIN and STR and LIN. In contrast, total motility and static; rapid and ALH; VAP and BCF; VSL and BCF and BCF and LIN showed high negative correlations ($p<0.05$). In medium body weight group, total motility and progressive motility; total motility and rapid; total motility and medium; progressive motility and rapid; progressive motility and medium; VAP and VSL; VAP and VCL; VAP and ALH; VAP and LIN; VSL and VCL; VSL and ALH; VSL and LIN as well as ALH and LIN were high positively correlated ($p<0.05$).

On the other hand, high negative correlations were shown between total motility and static; progressive motility and static; progressive motility and VAP; progressive motility and VCL; rapid and static; rapid and VAP; rapid and VCL; medium and static; slow and static; VAP and LIN; VSL and LIN; VCL and STR; VCL and LIN and BCF and STR ($p<0.05$). In large body weight group, positive correlations ($p<0.05$) were shown between progressive motility and rapid; progressive motility and LIN; rapid and STR; rapid and LIN; VAP and VSL; VAP and VCL; VAP and ALH; VSL and VCL and VSL and ALH. In contrast, negative correlations ($p<0.05$) were shown between total motility and static; progressive motility and VAP; progressive motility and VSL; progressive motility and VCL; progressive motility and ALH; rapid and VAP; rapid and VSL; rapid and VCL; rapid and ALH; VAP and LIN; VSL and STR; VSL and LIN; VCL and LIN and ALH and LIN.

Table 7 shows correlations among fresh sperm motility characteristics of African catfish for overall body weight groups. There were positive correlations ($p<0.05$) between total motility and progressive motility; total motility and rapid; total motility and medium; total motility and slow; progressive motility and rapid; progressive motility and medium; rapid and medium; medium and slow; slow and BCF; static and VSL; VAP and VSL; VAP and VCL; VAP and ALH; VAP and LIN; VSL and VCL; VSL and ALH; VSL and LIN; VCL and ALH and STR and LIN. In contrast, negative correlations ($p<0.05$) were

Table 7: Correlations among fresh sperm motility characteristics of African catfish (*Clarias gariepinus*) for overall pooled body weight groups

	Total motility	Progressive motility	Rapid	Medium	Slow	Static	VAP	VSL	VCL	ALH	BCF	STR	LIN
Total motility	1	0.776**	0.739**	0.778**	0.745**	-0.995**	-0.407	-0.428*	-0.375	0.027	0.419	-0.295	-0.234
Progressive motility	-	1	0.971**	0.509*	0.184	-0.767**	-0.357	-0.364	-0.346	-0.144	0.132	-0.170	-0.155
Rapid	-	-	1	0.439*	0.145	-0.748**	-0.321	-0.336	-0.300	-0.179	0.152	-0.195	-0.146
Medium	-	-	-	1	0.566**	-0.774**	-0.297	-0.318	-0.262	0.155	0.362	-0.298	-0.152
Slow	-	-	-	-	1	-0.746**	-0.294	-0.307	-0.277	0.184	0.543**	-0.217	-0.218
Static	-	-	-	-	-	1	0.407	0.427*	0.380	-0.011	-0.433*	0.287	0.230
VAP	-	-	-	-	-	-	1	0.976**	0.940**	0.511*	-0.266	-0.015	0.518*
VSL	-	-	-	-	-	-	-	1	0.849**	0.507*	-0.403	0.182	0.650**
VCL	-	-	-	-	-	-	-	-	1	0.454*	-0.087	-0.288	0.247
ALH	-	-	-	-	-	-	-	-	-	1	-0.011	-0.099	0.200
BCF	-	-	-	-	-	-	-	-	-	-	1	-0.736**	-0.494*
STR	-	-	-	-	-	-	-	-	-	-	-	1	0.602**
LIN	-	-	-	-	-	-	-	-	-	-	-	-	1

No. of fish = 24. ** Pearson correlations were significant ($p<0.01$); *Pearson correlations were significant ($p<0.05$)

shown between medium and static; slow and static and BCF and STR. The pattern of positive correlations among sperm motility for respective small, medium and large body weight group was different. In small body weight group, the pattern of sperm motility characteristics correlations were medium and slow; VAP and VSL; VAP and LIN; VSL and STR; VSL and LIN and STR and LIN.

This pattern of correlations differ in medium and large body weight group which showed correlations of total motility and progressive motility; total motility and rapid; total motility and medium; progressive motility and rapid; progressive motility and medium; VAP and VSL; VAP and VCL; VAP and ALH; VAP and LIN; VSL and VCL; VSL and ALH; VSL and LIN as well as ALH and LIN. In large body weight group, the patterns of correlations was progressive motility and rapid; progressive motility and LIN; rapid and STR; rapid and LIN; VAP and VSL; VAP and VCL; VAP and ALH; VSL and VCL and VSL and ALH. In general, therefore, percent total motility and sperm velocity have been correlated to reproductive success in fish; reductions in these parameters may decrease fecundity (Blaxhall and Daisley, 1973; Bustos-Obregon and Flechon, 1975; Brunette *et al*., 2001; Cabrita *et al*., 2003). The variation of pattern in correlation among sperm motility characteristics across the body weight group was assumed to be related with the average gamete quality of each group.

CONCLUSION

Results from the present study suggested that larger body weight of African catfish gave better sperm quality in comparison to medium body weight and small body weight groups. This indicated that age of African catfish plays a major role in determining the maturity of sperm production thus, affects the quality of sperm motility characteristics.

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