THE EFFECTS OF DIFFERENT STRIPPING TERMS ON SPERM QUALITY PARAMETERS OF RAINBOW TROUT *(ONCORHYNCHUS MYKISS)*

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UTICAJ PERIODA ISTISKIVANJA MLEČA NA PARAMETRE KVALITETA SPERME KALIFORNIJSKE PASTRMKE (*ONCORHYNCHUS MYKISS*)

Apstrakt

U istraživanju su praćeni parametri kvaliteta sperme mužjaka kalifornijske pastrmke (Oncorhynchus mykiss) kojima je istiskivan mleč u januaru, februaru i martu. Rezultati nisu pokazali značajnu razliku u vrednostima koncentracije i gustine spermatozoida, pH, zapremine i spermatokrita mleča, kao i trajanje pokretljivosti spermatozoida ispitivanih muških matica ($P>0.05$). Statistički značajna razlika utvrđena je za vrednosti pokretljivosti spermatozoida u različitim periodima ($P<0.05$). Utvrđeno je opadanje svih parametara kvaliteta od januara do marta.

Ključne reči: parametri kvaliteta sperme, kalifornijska pastrmka, Turska
Keywords: Sperm quality parameters, rainbow trout, Turkey

INTRODUCTION

The use of high quality gametes is of great importance for ensuring the production of viable larvae, but gamete quality is difficult to assess in a quantitative and meaningful manner (Kjorsvik et al., 1990). It is very important to control and examine performances of broodstocks in hatchery stations of fish farms for their quality and productivity of gametes will enter into aquaculture systems. The determining quality of sperm is necessary to define its quality. Motility, duration of motility and density of active spermatozoa, pH, spermatocrit and seminal plasma contents of sperm has been used to determine quality of sperm (Billard and Cosson, 1986; Linhart et al., 1991). It determined that reproduction performance has been affected on level of stocking, quality, amount and rate

of feeding, quality of physicochemical parameters of water, method of spawning and stripping, age and size of salmonid broodstocks (Büyükhatipoğlu and Holtz, 1984).

The objectives of this research were (1) to assess of effects by different term stripping on sperm quality parameters, (2) to determine reproduction performance of male broodstocks in a trout farm.

MATERIALS AND METHODS

Sperm samples were obtained during 2010 spawning seasons from broodstock supplied at Fishery Department in Erzincan University, Kemaliye Hacı Ali Akın Vocational High School in Turkey. The mean weight and total length of the fish was 3156.64±434.32 kg and 61.00±2.45 cm, respectively. For this research, 15 mature males took out and stocked into 500-L fiberglass tank at reproduction term in this farm. Tank was supplied with a constant flow of well water. Fish for experiment adapted in this tank for 2 weeks. Feeding stopped 2 days before stripping. Water temperature (^{o}C), dissolved oxygen (DO) (mg^{-L}) and pH were measured in ponds daily (APHA, 1985). Stripping was performed by massage from the front to back of the fish abdomen. Freshly stripped milt was stored on ice in 30-ml plastic containers until used. A total of 10 usable milt samples were collected from 10 males in January (A) (14.01.2010), February (B) (17.02.2010) and March (C) (14.03.2010).

Sperm concentration was measured through a spectrophotometric method after dilution (2µl sperm:1998µl NaCl, 0.7%) by 605 nm (Ciereszko and Dabrowski, 1993). Sperm was sampled into 50-ml calibrated glass tubes and the volume was expressed as ml. Sperm pH was measured using standard pH-electrodes. Spermatocrit was determined using milt collected into microhaematocrit tubes without heparin (75 mm length, 1.1–1.2 mm inner diameter) and centrifuged at 10000 rpm for 5 minutes. Motility was evaluated at 400X magnification. The sperm motility duration was taken as the time at which 50% of the activated spermatozoa ceased movement. This was obtained using a stop-watch. This procedure was repeated tree times for each of males (Viveiros et al., 2003).

In statistics, ANOVA (with Duncan) was used to determine the significance of the observed all data by SPSS 15 software. Statistical difference was indicated when the p value was less than 0.05.

RESULTS

Sperm quality parameters in Rainbow trout in different stripping terms (January-A, February-B and March-C) have been showed in Table 2 in this study. It determined density, motility, duration of motile in spermatozoa and pH, spermatocrit and volume in sperm for each fish species. In the study, water temperature, dissolved oxygen (DO) and pH values were measured daily. Throughout the research period, water temperature was 10±0.1^{o}C, pH 7.3±0.2 and DO recorded as 9.5±0.31 mg/L. During the experimental period, water temperature, pH and DO values did not show much variations and this difference was identified statistically ($p>0.05$) as insignificant. All our results showed that were up and down value in volume of sperm, slightly decrease in pH, duration of motility, spermatocrit and density of spermatozoa but distinctly decrease in motility of spermatozoa at post stripping in this study.

Motility of spermatozoa was significantly ($p<0.05$) lower in C than A and B but was not significant ($p>0.05$) in all other parameters for stripping terms (Table 2-3, Fig.1).

Table 1. ANOVA test for sperm quality parameters between different stripping terms

		Sum of Squares	Mean Square	F	Sig.
Sperm Volume (ml)	Between Groups	1931.258	965.629	1.838	0.177
	Within Groups	15232.617	525.263		
pH	Between Groups	0.247	0.124	1.305	0.287
	Within Groups	2.745	0.095		
Motility (%)	Between Groups	21.169	10.584	14.735	0.000*
	Within Groups	20.831	0.718		
Duration (sec)	Between Groups	201.241	100.621	1.226	0.308
	Within Groups	2381.023	82.104		
Spermatocrit	Between Groups	148.728	74.364	1.445	0.252
	Within Groups	1492.072	51.451		
Density of Spermatozoa (x 10^9/ml)	Between Groups	7.510	3.755	0.276	0.761
	Within Groups	394.482	13.603		

***** Significant difference as statistically, $p<0.05$

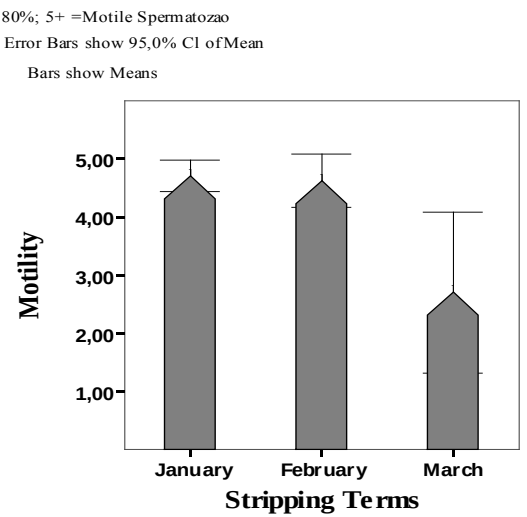

Figure 1. The values of motility of spermatozoa between different stripping terms

Table 2. Descriptive Statistics of sperm quality parameters between different stripping terms

		N	Mean±Std.Dev.	95% Confidence Interval for Mean		Min.	Max.
				Lower Bound	Upper Bound		
Sperm Volume (ml)	**A**	10	29.79±20.57[a]	17.91	41.66	9.00	79.00
	B	10	46.64±21.61[a]	32.12	61.16	11.00	70.00
	C	10	31.43±29.04[a]	4.57	58.29	5.00	87.00
pH	**A**	10	7.36±0.21[a]	7.23	7.48	7.00	7.75
	B	10	7.45±0.15[a]	7.35	7.56	7.00	7.50
	C	10	7.21±0.57[a]	6.69	7.74	6.50	8.00
Motility	**A**	10	4.71±0.47[b]	4.44	4.98	4.00	5.00
	B	10	4.64±0.67[b]	4.18	5.09	3.00	5.00
	C	10	2.71±1.50[a]	1.33	4.10	1.00	5.00
Spermatozoa Duration (second)	**A**	10	27.08±5.19 [a]	24.08	30.07	20.32	42.02
	B	10	25.27±4.11 [a]	22.51	28.03	20.24	33.05
	C	10	20.53±17.62 [a]	4.23	36.82	5.20	58.58
Spermatocrit (%)	**A**	10	25.02±9.09 [a]	19.77	30.27	8.70	37.50
	B	10	20.88±4.03 [a]	18.18	23.59	13.70	27.01
	C	10	26.02±6.53 [a]	19.98	32.05	18.52	35.29
Spermatozoa Density (x 10^9/ml)	**A**	10	9.86±3.18 [a]	8.03	11.70	3.78	13.16
	B	10	10.44±2.07 [a]	9.05	11.83	6.49	13.50
	C	10	9.12±6.06 [a]	3.51	14.72	1.08	18.00
Fish Weight (g)		15	3156.64±434.32			2591	3852
Fish Length (cm)		15	61.00±2.45			56.00	65.00

DISCUSSION

Density of spermatozoa, motility and duration of motility are the most commonly used parameters to evaluate sperm quality. From comparison between species, sperm production can be expressed in 10^9 sperm per g body weight: it was 7 for rainbow trout, 4 for carp, 2.7 for guppy, 0.6 for pike and 0.1 for *Leporinus*. Measurements of beat frequency on three species show that the duration of motility is very short in trout (20-25s) and lasts slightly longer than 1 min in carp and halibut. The beat frequency of the majority of sperm declines progressively within 20-25s (trout) and 80-90s (carp) (Billard et al., 1995).

In this study, the determined density of spermatozoa was 9.86(A), 10.44(B) and 9.12(C) x10^9 per/ml and duration of motility was 27.08(A), 25.27(B) and 20.53(C) second, respectively. Density of spermatozoa obtained in this study are close to the results reported by Billard et al. (1995), for rainbow trout. . Other authors reported for rainbow trout a density of spermatozoa 11.8 (Ciereszko and Dabrowski, 1993), 8.9 (Geffen and Evans, 2000); for Atlantic salmon, *Salmo salar*, 3.5 (Aas et al., 1991), 12 (Truscott and Idler, 1969). Several studies have mentioned that the differences in sperm production could be related to many factors including the age and weight of the male, ecology and spawning behavior of broodstock and sampling period and method (Piironen and Hyvarinen, 1983; Suquet et al., 1994, Suquet et al., 1998). Volume and pH of sperm were also

investigated as 29.79(A), 46.64(B) and 31.43(C) ml and 7.36(A), 7.45(B) and 7.21(C), respectively. The data of volume of sperm in this study was highest in term B and included to the normal standards for the sperm quality parameters which were reported by authors (Munkittrick and Moccia, 1987; Geffen and Evans, 2000).

Variation in sperm quality across the spawning season has been previously reported for a number of freshwater and marine fishes (Billard 1986; Munkittrick and Moccia 1987; Beirao et al., 2011). Results have shown that within and across species, seasonal changes in sperm quality can differ. Increases in sperm density throughout the spawning season have been found in Atlantic salmon (Piironen, 1985), and Atlantic cod (Butts et al., 2010). In contrast, studies on rainbow trout (Büyükhatipoğlu and Holt, 1984), snow trout, *Schizothorax richardsonii* (Agarwal and Raghuvanshi 2009), brown trout, *Salmo trutta* (Hajirezaee et al., 2010) and Atlantic salmon, *Salmo salar* (Aas et al., 1991) found that sperm density decreased throughout the season. As stated by Billard (1986), in salmonids, gametogensis is a discontinuous process where sperm is released from the sperm ducts over several months, aging throughout the spawning period.

According to our results, the values of semen volume and pH, motility, duration and density of spermatozoa declined by increasing of stripping frequency as previously reported for Atlantic salmon (Aas et al., 1991) and rainbow trout (Sanchez-Rodriguez et al., 1978).

As a conclusion, good suggestions on obtaining sperm quality parameters from broodstocks have been offered. It is hoped that this research would contribute to more production of this important species of fish in our country.

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