Turkish Journal of Science & Technology
Volume 26(1), 81-87, 2014

Quantitative Characteristics of Rainbow Trout (*Oncorhynchus mykiss*) Semen Throughout the Reproductive Season

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(Received: 06.02.2013; Accepted: 10.10.2013)

Abstract

The aim of this study was to determine changes in quantitative characteristics of the rainbow trout semen throughout the reproductive season. Semen samples were collected from October to May from broodstock males (n = 12) kept under natural photoperiod regime and semen volume, density, motility, duration of motility, spermatocrit and pH parameters were investigated. In October, one of the 12 brooders (8.3%) were spermiating, increasing to 12 (100%) in December and dropping to 3 (25%) in May. Semen volume significantly increased in January and February. The total volume of expressible semen was maximal in January, increasing from a mean value of 1.7 ml to 20.9 ml. The spermatozoa density showed an increasing trend from October to January, with mean values ranging between 1.4 and 10.3 x 10^9 sperm/ml. The spermatocrit did not vary with the sampling date. The semen samples collected in November showed the lowest motility of spermatozoa (40%). But the percentage of motile spermatozoa collected from January to May showed more than 80% motility. The duration of motility for the monitoring period ranged between 58.0 and 375.6 sec. In conclusion, the highest quality semen was collected during the medial part of the reproductive season.

Key words: Rainbow trout, Sperm, Spermatological properties, Spawning season.

Üreme Mevsimi Boyunca Gökkuşağı Alabalığı (*Oncorhynchus mykiss*) Spermasının Nicel Özellikleri

Özet

Bu çalışmanın amacı üreme mevsimi boyunca gökkuşağı alabalığı spermasının nicel özelliklerindeki değişimleri belirlemektir. Sperma örnekleri doğal fotoperiyot altında tutulan damızlıklardan (n = 12) ekim kasım ayları arasında toplandı. Sperma miktarı ve pH, sperma yoğunluğu ve motilite, motil kalma süresi, spermatokrit belirlendi. Ekimde 12 damızlıktan bir tanesi (%8.3) sperm verirken, aralık ayında sperm veren birey sayısı 12'ye (%100) yükseldi ve mayıs ayında 3 (%3) bireye düştü. Sperma miktarı ocak ve şubat aylarında önemli ölçüde arttı. Sağılabilen sperma miktarı, 1.7 ml'den 20.9 ml'ye yükselerek, ocak ayında en yüksek düzeye çıktı. Ekimde 1.4 x 10^9 sperm/ml olan spermatozoa yoğunluğu ocak ayında 10.3 x 10^9 sperm/ml olarak saptandı. Spermatokrit değerleri örnekleme zamanına bağlı olarak değişim göstermedi. En düşük motilite kasımdaki örneklerde, ocak ve mayıs arasında toplanan örneklerde ise %80'den yüksek motilite saptandı. Motil kalma süresi, üreme mevsimi boyunca, 58.0 ve 375.6 s arasında değişti. Sonuç olarak, yüksek kalitede sperma üreme mevsimi ortasında toplandı.

Anahtar Kelimeler: Gökkuşağı alabalığı, Sperma, Spermatolojik özellik, Üreme mevsimi.

1. Introduction

The rainbow trout (*Onchorhychus mykiss*) has been cultured since the early 1970s and Turkey has become one of the top trout producing countries in Europe with an annual production of 107 936 tonnes, or 57 percent of the country's total aquaculture production [1]. However, they are not applied in a selection program to increase efficiency in commercial enterprises and the product remains within the boundaries of the natural yield. In aquaculture, supply of high-quality gametes from broodstock plays a very important role to obtain healthy offspring, thus increasing efficiency. In Turkey, the reproductive season of the rainbow trout starts in late November and lasts up to April, but the peak occurs during January-February [2]. On

the other hand, there seems to be large variations between stocks and individuals within the same stock. Synchronization disorders occur between male and female broodstock in breeding period in many farms, and sometimes eggs are taken but sperm can not be taken, or vice versa. In this case egg or sperm is wasted and this phenomenon leads to economic losses in terms of producers.

Sperm analysis throughout the year is important to determine the status of spermatozoa variation in broodstocks. Changes in the sperm characteristics of fish ocur naturally during the breeding season [3]. Parameters such as semen volume, spermatozoa concentration, percentage of motile spermatozoa, motility duration, pH and fertilizing capacity vary throughout the reproductive season [4]. In order to have controlled and successful production in aquaculture systems, it is necessary to have a sufficient information on sperm characteristics to identify the reproductive ability of fish [5]. Adequate knowledge of sperm characteristics provides information allowing fish farmers to determine the most appropriate time for sperm collection and to arrange optimal handling and storage protocols for sperm used in artificial fertilization [6]. Thus more economical and successful results can be taken from fish farming.

The objective of this study was to determine the monthly variations of sperm parameters from October (the beginning of spermiation period) to May (the end of spermiation period) in our hatchery conditions.

2. Materials and methods

2.1. Broodstock care and collection of sperm

Variations in sperm quality traits were assessed for 4-year old hatchery-reared Rainbow Trout at the Recep Tayyip Erdoğan University, Iyidere Fisheries Research Centre (IFRC), Rize, Turkey. All fish were housed in a fiberglas circular tank (6 m in diameter and depth of 0.8 m) fed by untreated water from a local stream source and were kept under a natural photoperiod and temperature regime. The mean water temperature was 12.9±0.58 °C (range 8.0-18.5 °C), water renewal was 100% day^{-1}. Fish were fed with a commercial trout diet at 2% of body weight per day. With an interval of one month (from October in 2011 to May in 2012) semen was collected from the same 12 individuals in the context of a repeated measures experimental design. For semen collection, fish were starved for 2 days before handling, in order to prevent faecal contamination during semen collection and anaesthetized using 30 ppm of benzocaine to minimize stress during handling and stripping. Semen samples were collected, using slight pressure to the abdomen and massaging towards the urogenital pore, in 10 ml graduated glass tubes and stored in crushed ice (4 ºC) before analysis. Special care was taken to ensure that urine, mucus, feces, blood cells or water did not contaminate the milt sample.

2.2. Evaluation of Semen

Semen was sampled into the calibrated glass tubes and the volume was expressed as ml. Sperm motility was estimated from freshly collected samples of milt according to the percentage of motile spermatozoa. The percent of spermatozoa exhibiting rapid, vigorous, forward movement was determined subjectively under a microscope (x 400 magnification) by diluting the semen in activation solution (0.3% NaCl) at a ratio of 1:100 (1 µl sperm to 99 µl activation solution). Microscopic observation at x400 magnification was carried out at room temperature (20°C). Three fields of view were examined for each slide and three aliquots of each milt sample were inspected for calculation of an average. Duration of spermatozoa movement was assessed using a sensitive chronometer (1/100) that was started simultaneously with the addition of activation solution into the samples. Spermatozoa concentration was determined using the haemacytometric method. Semen was diluted at ratio of 1:1000 with Hayem solution (5 g Na_2SO_4, 1 g NaCl, 0.5g $HgCl_2$, 200 mL bidistilled water) and 10 µl of the dilution were taken for counting on a Thoma hemocytometer slide (depth 0.1 mm). Mean spermatozoa count was calculated from three replicate samples for each fish at magnification of x400 and expressed as $x10^9$ sperm/ml. Spermatocrit was defined as

the ratio of white packed material volume to the total volume of semen multiplied by 100 [7]. Heparinized microhematocrit capillary tubes (75 x 1.1-1.2 mm) were filled with semen and one end was sealed with clay. The capillary tubes were centrifuged at 10,000 rpm for 10 min. Sperm pH was measured by using indicator papers (Merck 6.4-8).

2.3. Statistical analysis

All mean values represent mean ± SEM from triplicate. After controlling the normality of data by Shapiro-Wilks test, Kruskal-Wallis one-way ANOVA was used for statistical comparisons with Dunn's test ($P < 0.05$). The data were evaluated using SigmaPlot 12.0 statistical software.

3. Results

The expressible semen was available from only one of the twelve brooders at the beginning of the reproductive period which lasted eight months from October to May. All males produced semen between December and March (Fig. 1).

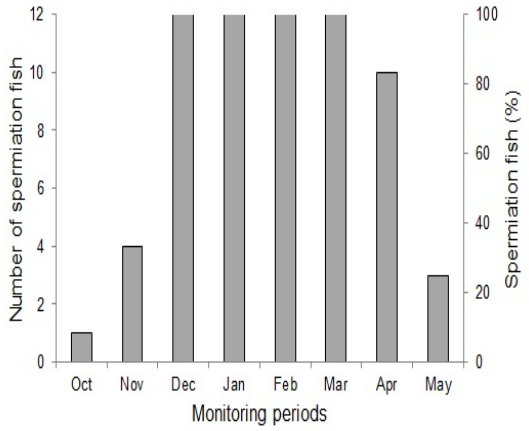

Figure 1. Monthly changes in number of the spermiation fish.

The smallest semen volume was obtained at the beginning of the breeding season (1.75 ml) ($P<0.05$). However, semen production increased during the monitoring period, reaching the maximum value at the middle of the season and the highest volume of semen was observed in January and February ($P<0.05$) (Fig. 2).

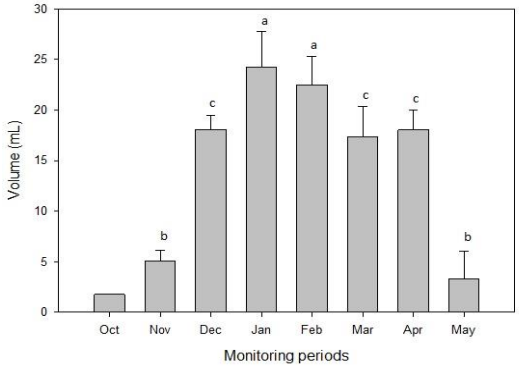

Figure 2. Monthly changes in semen volume. Different letters above each bar indicate a significant difference during the monitoring periods ($P < 0.05$). Data are represented as mean ± SEM. The value from an individual in October were not included in the statistical analysis.

The highest spermatozoa density were verified in samples collected at the middle (10.3 ± 0.95 x 10^9 sperm/ml in January, 8.9 ± 0.96 x 10^9 sperm/ml in February and 10.3

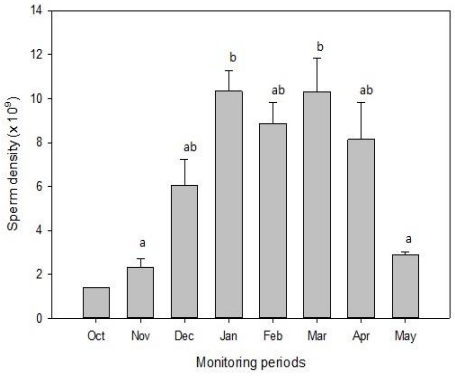

Figure 3. Monthly changes in the sperm density. Different letters above each bar indicate a significant difference during the monitoring periods ($P < 0.05$). Data are represented as mean ± SEM. The value from an individual in October were not included in the statistical analysis.

± 1.53 x 10^9 sperm/ml in March) of the breeding season ($P<0.05$) (Fig. 3).

The spermatocrit were highly fluctuating during monitoring periods but the differences were not statistically significant ($P > 0.05$) (Fig. 4). The lowest value was obtained in a sample collected in October, reaching only 18.8 ±

2.18%. The highest spermatocrit average was obtained in January (35.3 ± 0.86%).

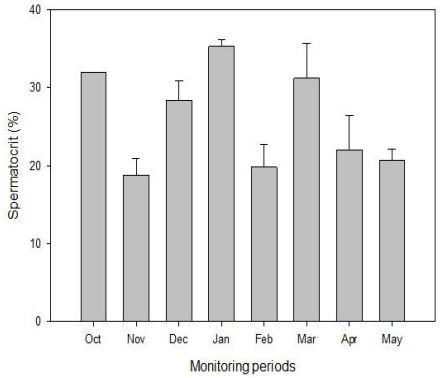

Figure 4. Variation of spermatocrit throughout the reproductive season of rainbow trout. There were no significant differences among the monitoring periods (P > 0.05). Data are represented as mean ± SEM. The value from an individual in October were not included in the statistical analysis.

Except for the first three months, no difference was found throughout the reproductive season regarding the percentage of motile spermatozoa (P>0.05). The sperm motility was superior to 40% and changed from 40% in November to 100% in May according to the motility scale used in this study (Fig. 5).

The motility duration increased throughout the breeding season (P<0.05). The highest motility duration was found in the middle (375.6 s in January) and in the end (303.7 s in May) of the monitoring periods (Fig. 6). At the beginning of reproductive season, the lowest values of total duration of sperm motility were found as 58 s (P<0.05).

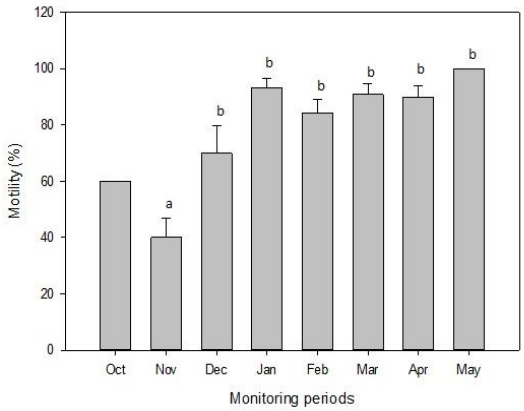

Figure 5. Monthly changes in the percentage of motile spermatozoa. Different letters above each bar indicate a significant difference during the monitoring periods (P < 0.05). Data are represented as mean ± SEM. The value from an individual in October were not included in the statistical analysis.

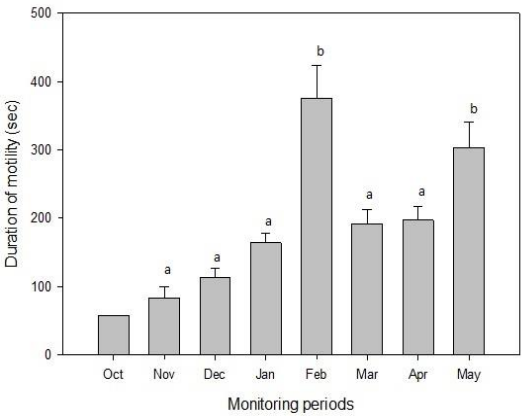

Figure 6. Monthly changes in the duration of motility. Different letters above each bar indicate a significant difference during the monitoring periods (P < 0.05). Data are represented as mean ± SEM. The value from an individual in October were not included in the statistical analysis.

4. Discussion

Determination of the seasonal changes in semen production and semen quality in fish is very important for estimating the number of needed males in the hatchery besides that the optimization of sperm to egg ratio at the time of fertilization. Semen produciton and variation in semen quality throughout the spawning season have been previously reported for several salmonid species [8-11]. The results of the research showed that seasonal changes in semen quality can differ. The results of the study on the semen quality of rainbow trout demonstrated that semen volume, motility, duration of motility and sperm concentrations varied significantly (P< 0.05) during the reproductive seasons and a decline in semen quality was observed. This declining trend throughout the reproductive season have been repoted by Legendre and Billard [12], Munkittrick and Moccia [8] for rainbow trout, by Piironen [13], Aas er al. [14] for Atlantic salmon and by Hajirezaee et al. [10] for Caspian brown trout due to spermatozoa aging [15].

The amount of semen produced from a fish is of vital importance in fertilization process. In the present study, eleven of twelve brooders failed to release semen after being hand stripped at the beginning of the breeding period and the amount of semen taken from an individual was smaller. As breeding season progressed, the semen volume was monthly changed. The mean volume of expressible semen increased in the middle of the spawning period, from about 1.7 ml on 5 October to about 20.9 ml on 3 January, and decreased again. All males produced semen between December and March. A similar bell-shaped curve of semen production, with a peak in the middle of the spawning season, was also observed by Büyükhatipoglu and Holtz [3]. These results suggest that the reproductive peak of this species occurs between December and March.

In hatcheries, sperm motility is considered to be the main parameter used to evaluate semen quality in fish. The sperm motility and its duration have great influence on successful fertilization. The percentage of motile spermatozoa exhibited a significant improvement during the spawning season. Increases in sperm motility towards the peak of the spawning season have been reported by Fostier et al. [16] and by Büyükhatipoglu and Holtz [3] for same species. During spermiogenesis, fully differentiated spermatozoa are released from the spermatocysts, but these intra-testicular spermatozoa do not yet have the capacity for forward motility [17]. According to Mylonas et al [18], at the beginning of the spawning season, when most of the spermatozoa had been just recently released from the spermatocysts, a significant percentage had not undergone the acquisition of motility capacity. As the season progressed, the residence time of the spermatozoa in the testicular duct increased and the acquisition of motility capacity was more complete. However, in rainbow trout sperm motility remained very high until the end of the spermiation period, for as long as expressible semen could be collected. The motility duration of sperm was long, except for one sampling time at the beginning of the study and motility duration remained high as long as expressible semen could be obtained, varying between 58 and 375.6 sec. This value was higher than reported by Büyükhatipoğlu and Holtz [3], Babiak et al. [19], Tekin et al. [20], Bozkurt et al. [21] and Tuset et al. [22] for same species. The duration and motility of sperm may vary depending on season [23], biochemical composition and osmolality of the seminal plasma [24].

In hatchery management, the evaluation of semen quality is important for the efficiency of artificial reproduction and storage of semen. For these operations, the sperm density is of vital importance. Sperm density may also effect the fertilization rate [14]. In the study, the sperm density was highly variable and increased in the middle of the spawning period, from about 1.4 x 10^9 sperm/ml in October (at the beginning of reproductive season) to about 10.3 x 10^9 sperm/ml in January (in the middle of reproductive season), and decreased again. Increases in sperm density throughout the spawning season have been found in Atlantic salmon *Salmo salar* [13]. In contrast, studies on rainbow trout [3], snow trout *Schizothorax richardsonii* [25], brown trout *Salmo trutta* [10] and Atlantic salmon [14] found that sperm density decreased throughout the season. In the present study, it was found that the sperm density was the highest in the middle of reproductive season, and was the lowest either at the beginning or end of reproductive season. Higher sperm density in semen collected in the middle of season could fertilize more eggs than when sperm density is lower toward the end of the season.

In conclusion, changes in the semen quality of rainbow trout occur during the reproductive season. The analyses of volume, sperm density, semen production and motility duration indicate that the reproductive peak of this species takes place between December and March which suggests that these periods might be better for artificial reproduction.

Acknowledgements

The authors thank Recep Tayyip Erdogan University, Center of Scientific Research Project, Rize, Turkey for the equipment and material support.

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