

Role of pH on the initiation of sperm motility in rainbow trout (*Oncorhynchus mykiss*) and Çoruh trout (*Salmo coruhensis*)

Filiz KUTLUYER*

Munzur University, Fisheries Faculty, 62000, Tunceli, Turkey.

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Abstract

Experiments were designed to examine effects of pH (range 6.0–9.0) in different activation solutions on sperm motility of rainbow trout (*Oncorhynchus mykiss*) and Çoruh trout (*Salmo coruhensis*). Four activation solutions were prepared: a) NaCl (52 mM)-AS₁, b) NaCl (103 mM), CaCl₂ (63 mM), Glucose (4 mM), Tris (10 mM)-AS₂, c) NaHCO₃ (119 mM)-AS₃, d) NaCl (45 mM), KCl (5 mM), Tris (30 mM)-AS₄. Sperm was activated at the dilution ratio of 1:100 with different activation solutions. Highest motility rate and duration for *O. mykiss* were obtained from AS₂ at a pH of 9 while highest motility rate and duration for *S. coruhensis* were at a pH of 8. Data here reported also showed that non-progressive motility in a range of 6–8 was observed in AS₂ for two species. In conclusion, rainbow trout (*O. mykiss*) and Çoruh trout (*S. coruhensis*) sperm cells can be motile in a wide range of pH although sperm motility and duration was negatively influenced by low pH values.

Keywords: PH, *Salmo coruhensis*, *Oncorhynchus mykiss*, sperm quality.

Gökkuşığı alabalığı (*Oncorhynchus mykiss*) ve Çoruh alabalığı (*Salmo coruhensis*) sperm motilitesinin başlamasında pH'ın rolü

Özet

Çoruh alabalığı (*Salmo coruhensis*) ve gökkuşığı alabalığının (*Oncorhynchus mykiss*) sperm motilitesi üzerinde farklı aktivasyon solusyonlarının pH (6.0–9.0)'unun etkilerini belirlemek için denemeler dizayn edilmiştir. Dört aktivasyon solusyonu hazırlanmıştır: a) NaCl (52 mM)-AS₁, b) NaCl (103 mM), CaCl₂ (63 mM), Glikoz (4 mM), Tris (10 mM)-AS₂, c) NaHCO₃ (119 mM)-AS₃, d) NaCl (45 mM), KCl (5 mM), Tris (30 mM)-AS₄.

* FİLİZ KUTLUYER, filizkutluyer@hotmail.com, <https://orcid.org/0000-0001-8334-5802>

Sperm farklı aktivasyon solusyonlarıyla 1:100 sulandırma oranıyla aktive edilmiştir. S. coruhensis türünde en yüksek motilite oranı ve süresi pH 8'den elde edilirken O. mykiss türünde en yüksek motilite oranı ve süresi AS₂ solusyonu pH 9'da elde edilmiştir. Veriler, iki tür için AS₂'de pH 6-8 aralığında non-progresif motilite olduğunu göstermiştir. Sonuç olarak, sperm motilitesi ve süresi düşük pH değerlerinden olumsuz etkilemesine rağmen, Çoruh alabalığı S. coruhensis ve gökkuşağı alabalığının O. mykiss spermi geniş pH aralıklarında motil olabilir.

Anahtar kelimeler: PH, *Salmo coruhensis*, *Oncorhynchus mykiss*, sperm kalitesi.

1. Introduction

Salmo trutta and *Oncorhynchus mykiss* are Salmonid fish species and have great economical value and recreationally importance [1]. *Salmo coruhensis* lives in Eastern Black Sea and migrates to freshwater for reproduction purposes [2-5]. Because of its great economical value, *S. coruhensis* has been massively captured, leading to a decline of this trout population in Black Sea. Furthermore, the construction of hydroelectric power plants over the River Basin impaired the upstream migration and spawning of *S. coruhensis* and therefore, its reproduction sites were diminished. Also, the pollution of natural habitats and the river bottom modifications have negatively influenced their reproduction. [6-9]. Sperm motility is important for continuation of generation due to affect fertilization and hatching success [10]. Releasing into the water of sperm cells is needed for initiation of sperm movement in Salmonids [10-12]. For these reasons, activation media and its concent is important for progression and initiation of sperm movement [13-14].

Most studies to date have conducted on the impact of pH on sperm motility of different fish species: mullet *Mugil capito* [15], rainbow trout *Parasalmo mykiss* [16-18], halibut *Hippoglossus hippoglossus* [19], sea bass (*Dicentrarchus labrax*) [10, 20-22], paddlefish *P. spathula* [23, 24], turbot [25], white sturgeon, *A. transmontanus* [11], Siberian sturgeon, *A. baeri* [26], *Larimichthys polyactis* [27], shabut *Barbus grypus* [28], *Merluccius australis* [29], European eel *Anguilla Anguilla* [30] and waigieu seaperch *Psammoperca waigiensis* [14]. To the authors' knowledge, any study has not been performed about effect of pH of different activation medium sperm motility of Çoruh trout *S. coruhensis* and *O. mykiss*, although studies about effect of pH have been performed in rainbow trout *O. mykiss* [31] and salmonid species (*Salvelinus fontinalis*, *Salmo trutta*, *Salmo salar*, *Thymallus thymallus*) [32]. In this context, the aim of the report was to assess the effect of pH (range 6.0–9.0) of different activation solutions on the sperm movement and longevity of *O. mykiss* and *S. coruhensis*.

2. Material and methods

Mature Çoruh trout males and rainbow trout were provided from commercially farm, Trabzon, Turkey for sperm collection. Routine semen collection was performed through abdominal massage after anesthetized with 2-phenoxyethanol (0.6 ml L-1). The special care was provided to avoid blood, urine, or faecal contamination in sperm collection and sperm samples were stored in 50 ml vials on ice until analysis.

Progressive motility was assessed using a phase-contrast microscope (Nikon CI, Tokyo, Japan) with a magnification of 200X, captured by a CCD camera (Nikon DS-Fi, Nikon, Japan) and using NisArElements software (Nikon, Tokyo, Japan). Motility analysis from the video sequences were performed using the computer-assisted sperm analysis (SCA) using the software (Zeiss Axio Scope with AxioVision). Motile sperm percent was assessed as actively moved sperm. Duration of forward motility was time from activation initiation to sperm stop move. Pooled fresh sperm was used and sperm with normal pH, volume and motility >70% was selected for the experiment. Spermatozoa density was evaluated using a Burker cell hemocytometer. Spermatocrit was assessed according to Rurangwa et al. (2004) and expressed as percent (the ratio of white packed material volume/the total volume of semen $\times 100$).

Four activation solutions were prepared: a) NaCl (52 mM)-AS₁ (Bozkurt et al., 2011), b) NaCl (103 mM), CaCl₂ (63 mM), Glucose (4 mM), Tris (10 mM)-AS₂ (Valdebenito et al., 2010), c) NaHCO₃ (119 mM)-AS₃, d) NaCl (45 mM), KCl (5 mM), Tris (30 mM)-AS₄. The impacts of pH were determined at a range of 6.0–9.0.

Results are presented as means \pm standard deviation (S.D.). Comparisons among groups were made by one-way ANOVA followed by Duncan test. The significant level considered was $P < 0.05$. Statistical analyses were performed with the software SPSS version 14.0. Pearson's correlation among qualitative sperm quality parameters was performed with SPSS.

3. Results

Sperm quality parameters (mean \pm SD) are given in Table 1. The percentage and duration of motile spermatozoa in *S. coruhensis* and *O. mykiss* are presented in Fig. 1, 2, 3 and 4 for different activation solutions.

Table 1. Sperm parameters (Mean \pm SD) of *Salmo coruhensis* and *Oncorhynchus mykiss*.

Parameters	<i>Salmo coruhensis</i>	<i>Oncorhynchus mykiss</i>
Colour	White	White
Volume (ml)	6.78 \pm 0.12	7.49 \pm 0.36
pH	7.89 \pm 0.14	7.36 \pm 0.24
Spermatocrit (%)	49.87 \pm 0.24	45.25 \pm 0.39
Sperm density ($\times 10^9$)	6.25 \pm 0.29	4.58 \pm 0.22

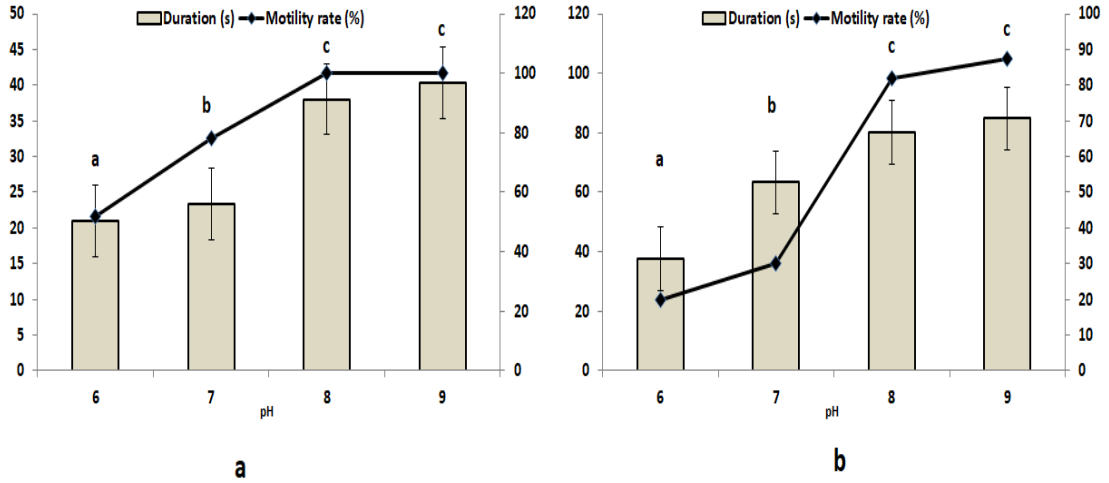


Figure 1. Effects of pH (6, 7, 8 and 9) of activation medium (NaCl, 52 mM) on percentage and of sperm motility and duration of a) Çoruh trout *S. coruhensis* and b) rainbow trout *O. mykiss*.

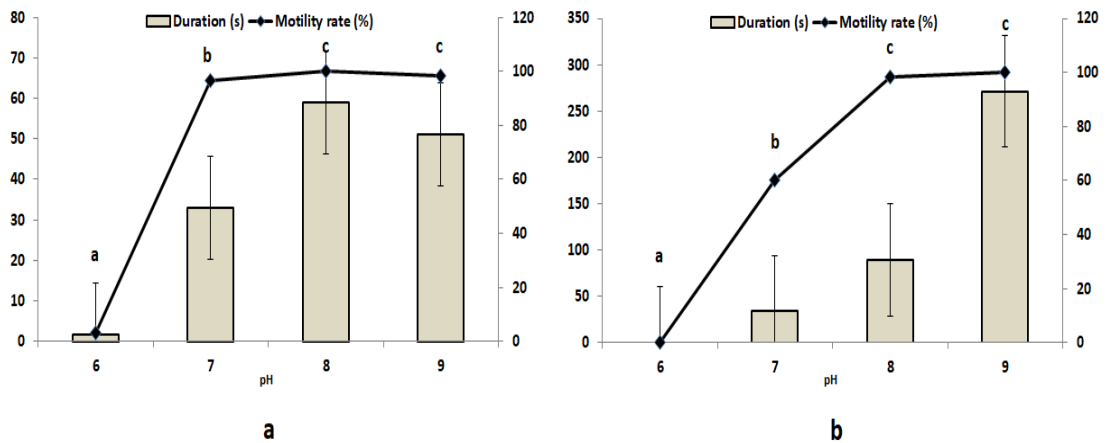


Figure 2. Effects of pH (6, 7, 8 and 9) of activation medium [NaCl (103 mM), CaCl₂ (63 mM), Glucose (4 mM), Tris (10 mM)] on percentage and of sperm motility and duration of a) Çoruh trout *S. coruhensis* and b) rainbow trout *O. mykiss*.

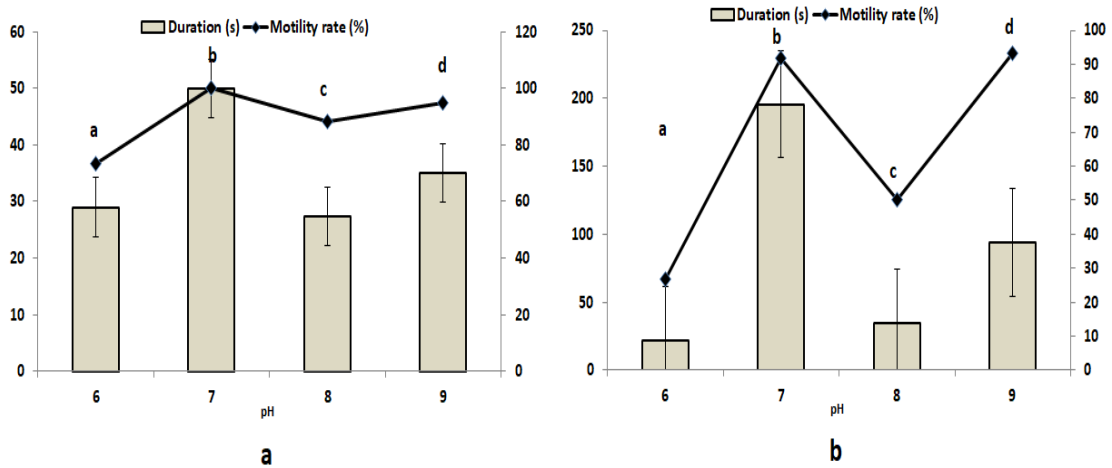


Figure 3. Effects of pH (6, 7, 8 and 9) of activation medium (NaHCO₃, 119 mM) on percentage and of sperm motility and duration of a) Çoruh trout *S. coruhensis* and b) rainbow trout *O. mykiss*.

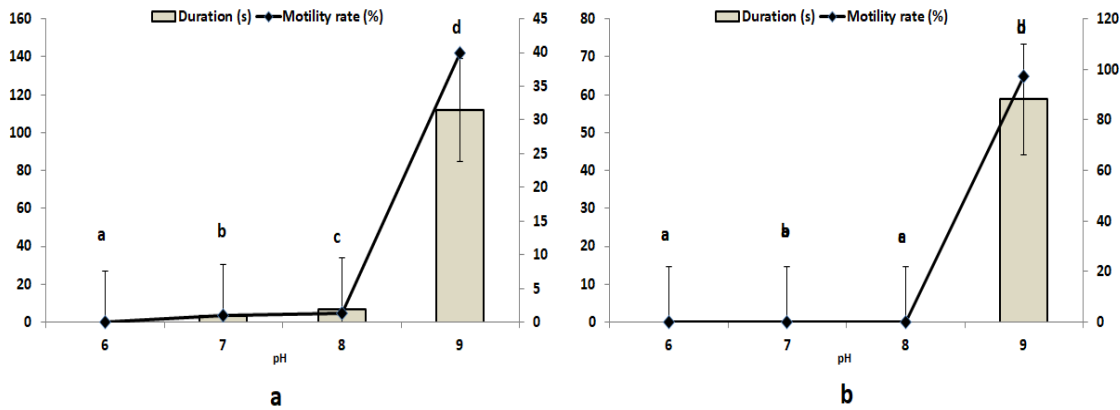


Figure 4. Effects of pH (6, 7, 8 and 9) of activation medium [NaCl (45 mM), KCl (5 mM), Tris (30 mM)] on percentage and of sperm motility and duration of a) Çoruh trout *S. coruhensis* and b) rainbow trout *O. mykiss*.

Highest motility rate and duration in *S. coruhensis* and *O. mykiss* were at a pH of 9 for AS₁. Highest motility rate and duration in *S. coruhensis* were at a pH of 8 for AS₂ while best results in *O. mykiss* were obtained at a pH of 9. Non-progressive motility was observed in at a pH of 6. Highest motility rate and duration in *S. coruhensis* and *O. mykiss* were at a pH of 7 for AS₃. Highest motility rate and duration in *S. coruhensis* and *O. mykiss* were at a pH of 9 for AS₄. Non-progressive motility was observed in a range of 6–8.

4. Discussion

In present study, we assessed impacts of the pH of the activation media on the motility percentage and duration of spermatozoa. Overall, the period of motility of *O. mykiss* and *S. coruhensis* sperm cells is longest at pH 9.0 in the examined range of 6.0 to 9.0. In addition, non-progressive motility was observed at a pH of 6.

The activation of fish sperm cells is influenced by pH as direct and indirect [10, 33]. Hence, movement and longevity of sperm cells is related with pH of the activation medium. In addition, sperm motility parameters change based on intracellular and external pH [33]. Sea urchin [34] and mammals [22, 35, 36] are substantially influenced by intracellular pH in contrast with salmonids, cyprinids and sturgeons. Hypertonic solutions are required in order to become motile of sperm in marine fish species [22, 30, 37, 38] due to fact that a rapid flow of ions (influx) and water (efflux) [30, 39, 40-43].

In Salmonids, several studies have been conducted at pH sensitivity of sperm motility [11, 18, 31, 32]. Ingermann et al. [11] studied in the chinook salmon *Oncorhynchus tshawytscha* and steelhead *Oncorhynchus mykiss* and determined very low levels of motility at pH values at or below 7.5 prior to water exposure while maximum motility was at pH values of about 8.0 and above. Ingermann et al. [31] stated that activation at high pH caused to the rapid onset of sperm motility. Ciereszko et al. [32] determined that no motility was at pH 7.0 and below in *O. mykiss*. In agreement with previous studies, our data demonstrated that movement and longevity of motile sperm cells of *O. mykiss* and *S. coruhensis* present their maximum value in diluted activation solutions buffered at a pH of 9. The low pH negatively affected the sperm motility.

5. Conclusions

Based on the results, this present study illustrates that sperm motility and duration was negatively affected by low pH values. The best results were obtained from activation solution including NaCl, CaCl₂, Glucose, Tris and a pH of 9. Additionally, high pH value (9.0) can be effectively used in activation media.

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