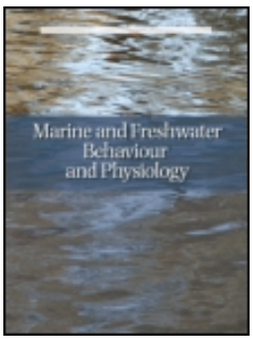

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SHORT COMMUNICATION

Combined effects of physicochemical variables (pH and salinity) on sperm motility: characterization of sperm motility in European sea bass *Dicentrarchus labrax*

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ABSTRACT

Gamete activation in fish is an important step in terms of artificial fertilization of oocytes, cryopreservation studies and other experimental manipulations. Salinity and pH differences in activation media affect to sperm motility and fertilizing ability. These experiments were therefore designed to investigate the combined effects of pH (range 5.0–9.0) and salinity (20, 30, 37, and 45‰) of activation media on sperm motility of European sea bass *Dicentrarchus labrax*. The best results were obtained at salinity 37‰ and a pH of 9.0. Our results also demonstrated that non-progressive motility at salinity 45‰ was observed in the range of 5.0–9.0 pH. In conclusion, spermatozoa can be motile at a wide range of pH and salinity values although the percent of motile spermatozoa and motility duration are negatively affected by low pH values.

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European sea bass; *Dicentrarchus labrax*; sperm; medium; pH; salinity; sperm; activation

Sperm motility is an important functional parameter for successful fertilization in fish (Islam & Akhter 2011). Sperm cells in most fish species are immotile in the seminal plasma and have to be released into the water to trigger motility and become metabolically active (Dzyuba et al. 2010). Characteristics of activation media are therefore important in terms of initiation and progression of sperm motility. Sperm motility and duration are influenced by various determinants providing activation of axonemal movement such as pH, temperature, ions, salinity, and osmolality (Cosson et al. 1999; Morisawa et al. 1999; Alavı & Cosson 2006; Islam & Akhter 2011; İnanan & Ögretmen 2015). In particular, the pH of activating solution is an important factor in fish due to the initiation of sperm motility and its duration (Marian et al. 1997). Some studies reported that activation of spermatozoa depends on the intracellular pH in sea urchin (Lee et al. 1983) and mammals (Wong et al. 1981; Babcock et al. 1983; Cosson 2004). Intracellular proton concentration is moreover affected by external pH and, changes occur in the membrane potential and motility behavior (Boitano & Omoto 1991, 1992). In contrast, Oda and Morisawa (1993) suggested that an increase in intracellular pH caused sperm activation in several marine fish species.

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A great deal of past research has focused on the effects of pH on the sperm motility of various finfish species, namely, mullet *Mugil capito* (Hines & Yashouv 1971), rainbow trout *Oncorhynchus mykiss* (Billard 1983; Billard & Cosson 1988; Gatti et al. 1990; İnanan & Ögretmen 2015), halibut *Hippoglossus hippoglossus* (Billard et al. 1993), sea bass *Dicentrarchus labrax* (Billard 1980; Chambeyron & Zohar 1990), paddlefish *Polyodon spathula* (Linhart et al. 1995; Cosson & Linhart 1996), turbot (Chauvaud et al. 1995), white sturgeon *Acipenser transmontanus* (Ingermann et al. 2002), Siberian sturgeon *A. baeri* (Gallis et al. 1991), steelhead trout (Ingermann et al. 2008), *Salvelinus fontinalis, Salmo trutta, Salmo salar, Thymallus thymallus* (Ciereszko et al. 2010), *Larimichthys polyactis* (Le et al. 2011), shabut *Barbus grypus* (Öğretmen et al. 2014), *Merluccius australis* (Effer et al. 2013), and European eel *Anguilla anguilla* (Gallego et al. 2014). European sea bass is an economically important fish species and cultivated worldwide. Sperm quality studies of this species are therefore important for aquaculture. To our knowledge, no information is available regarding the combined effect of pH and salinity of activation media on sperm motility of European sea bass although the effect of pH on activation of European sea bass spermatozoa in seawater has been studied (Cosson 2004). Within this context, the present study investigated the combined effect of pH (range 5.0–9.0) and salinity (20, 30, 37, and 45‰) in the activation media on the percent of motile spermatozoa and the duration of motility in European sea bass.

European sea bass *D. labrax* were obtained from the hatchery of a commercial fish farm in Muğla, Turkey. Fish were reared in fiberglass tanks with a rearing volume of 10 m^3. Each tank contained 50 fish. The water flow was 10 L/min per tank. The temperature was measured with a digital thermometer twice a day (8:00–9:00, 16:00–17:00). The temperature and salinity of the incoming water was 17.0 ± 0.1 °C and ‰37, respectively. The sperm was collected from individuals of a broodstock with 553 ± 96 g (mean ± SD) mean weight. Experiments were conducted with gametes of + 2 and + 3-year-old specimens (n = 12) during the natural reproductive season in wild stock in December 2014. Only males producing more than 4 ml of sperm were used and fish sperm from males was pooled. This study was performed in accordance with the ethical guidelines stipulated by the ethical committee of the University of Muğla Sıtkı Koçman. The males were anesthetized with 2-Phenoxiethanol (0.3 ml L^{-1}) before stripping. The sperm was collected by a gentle abdominal massage, collected into glass vials and stored on ice (2–4 °C) until used. Caution was exercised to prevent contamination of the semen with urine, feces, blood, mucus, or water. Soon after collection, tubes containing undiluted sperm were placed in a polystyrene box and kept at 3 ± 1 °C for a maximum duration of one hour. Each sample was evaluated for its motility parameters at 40 × magnification using a light microscope with a digital image processing software connected to the computer (Zeiss Axio Scope with AxioVision) to evaluate the percentage of spermatozoa motility and duration at room temperature (20 °C) immediately recorded for 1 min post-activation using a CCD video camera mounted on a phase-contrast microscope. The video records obtained were scanned to determine the percentages of progressive motility (%) and the durations of progressive motility (s). The percentage of motile sperm was estimated as the cells performing progressive forward movement, while the duration of motility was determined as the time until forward movement stopped. The percentage of sperm motility was assessed using an arbitrary scale with 10% interval increments in which non motilility represented 0%. Hatchery water was used as activating solution (37‰ salinity and pH 7.8) for evaluating freshly collected semen. The effects of pH

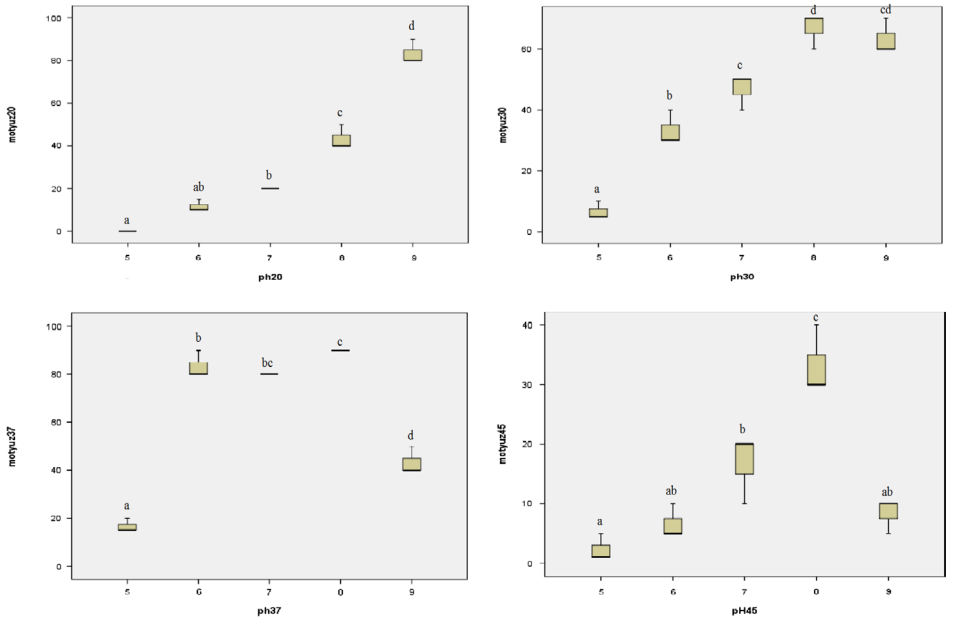

Figure 1. (Colour online) Effects of pH (5, 6, 7, 8 and 9) on percentage of spermatozoa motility of European seabass *D. labrax* at different salinity concentrations (a) 20‰, (b) 30‰, (c) 37‰, (d) 45‰ (Non-progressive motility) ($p < 0.05$).

were tested at a range of 5.0–9.0. In addition, different salinity concentrations (20, 30, 37, and 45‰) were arranged with natural sea salt and tested in the activation medium. Sperm was activated at the dilution ratio of 1:50 with activation solution. The percent of motile spermatozoa and motility duration was immediately recorded for 1 min post-activation. All values were expressed as mean ± SD and analyzed by SPSS for Win 14.0 software. A one-way ANOVA with a Duncan test was used to determine whether results of treatments were significantly different from the control group ($p < 0.05$).

The percentage and duration of motile spermatozoa are presented in Figure 1 and Figure 2, respectively. At salinity 20‰, the highest motility (>85%) was observed at pH 9.0 ($F = 28.125$, $p = 0.000$; $p < 0.05$) while the highest duration of motility (20–24 s) was obtained at pH range 8–9 ($F = 61.219$, $p = 0.000$; $p < 0.05$). At salinity 30‰, the highest motility (60–70%) was at pH 8.0 and 9.0 ($F = 59.038$, $p = 0.000$; $p < 0.05$) while the motility duration (28 s) was observed at pH 8.0 ($F = 62.952$, $p = 0.000$; $p < 0.05$). At salinity 37‰, the highest motility (80–90%) was at pH range 6.0–8.0 ($F = 29.037$, $p = 0.000$; $p < 0.05$) while the highest motility duration (25–30 s) was observed at pH 8.0 ($F = 30.038$, $p = 0.000$; $p < 0.05$). Non-progressive motility at salinity 45‰ was observed at a pH between 5.0 and 9.0 ($F = 280.236$, $p = 0.000$; $p < 0.05$).

The pH has direct and indirect effects on the activation of fish spermatozoa (Alavi and Cosson 2005) and the duration and percentage of sperm motility in fish depend on the pH of the activation medium. Influences of intracellular and external pH on sperm motility parameters change depend on species (Alavi and Cosson 2005). The intracellular pH in sea urchin (Lee et al. 1983) and mammals (Wong et al. 1981; Babcock et al. 1983; Cosson 2004) is important for sperm motility parameters, while its effect is lower in salmonids,

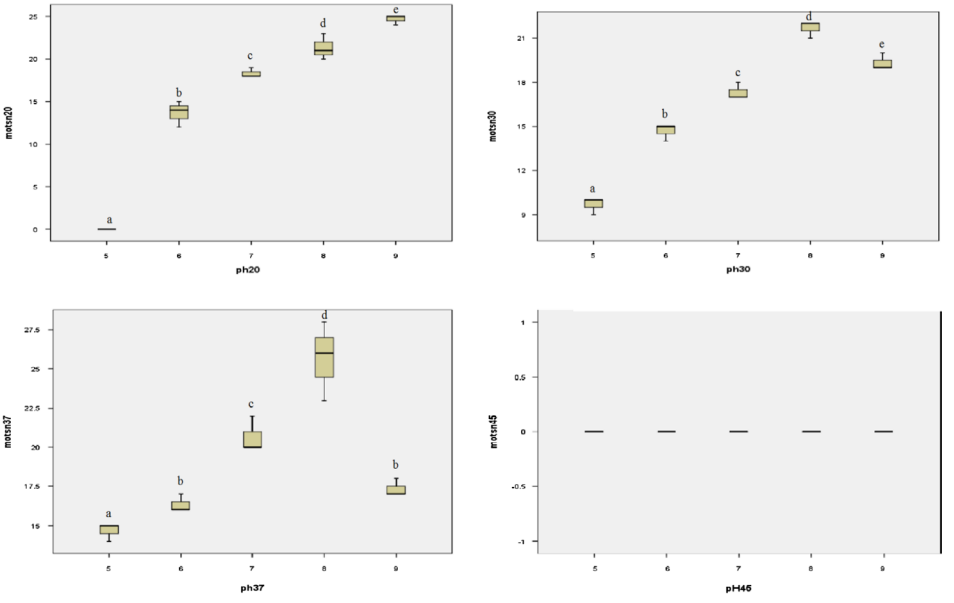

Figure 2. (Colour online) Effects of pH (5, 6, 7, 8 and 9) on duration of spermatozoa motility of European seabass *D. labrax* at different salinity concentrations (a) 20‰, (b) 30‰, (c) 37‰, (d) 45‰ (Non-progressive motility) ($p < 0.05$).

cyprinids, and sturgeons. Hypertonic solutions are needed for the sperm of marine fish species to become motile (Morisawa & Suzuki 1980; Cosson 2004; Morisawa 2008; Gallego et al. 2014) due to the rapid flow of ions (influx) and water (efflux) when the sperm cells are released into marine water (Oda & Morisawa 1993; Zilli et al. 2009; Gallego et al. 2014). Activation of European sea bass spermatozoa occurs between pH 5 and 10 in seawater (Cosson 2004). In agreement with this study, our results suggest that the maximum value of motility duration and the percentage of motile spermatozoa cells were in diluted seawater buffered at pH 9.0. The sperm motility was negatively affected by the low pH. In addition, an increase in sperm motility was found at low salinity (‰ 20) with the increase of pH.

In conclusion, the present study indicated that the percent of motile spermatozoa and motility duration were negatively affected by low pH values. Our results indicate that for European sea bass spermatozoa, the best results were obtained at a salinity of 37‰ and pH of 9.0. Our results suggested additionally that good results could be obtained at a low salinity (20‰) and high pH value (9.0). This study will be of value for fertilization and sperm cryopreservation studies of the species.

Disclosure statement

No potential conflict of interest was reported by the authors.

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