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Sperm characteristics of wild and captive lebranche mullet *Mugil liza* (Valenciennes, 1836), subjected to sperm activation in different pH and salinity conditions

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ABSTRACT

In this article we describe basic aspects of the sperm biology of lebranche mullet (*Mugil liza*) in the wild and in captivity, in particular assessing the effects of salinity (0, 10, 20, 30, 35, 40, 50 and 60 g L^{-1}) and pH (6, 7, 8, 9 and 10) on sperm motility. Our results indicate that the highest percentage of motility was recorded with salinity 34.6 g L^{-1} (95 ± 10%) and the longest motility time was obtained with a salinity of 34.8 g L^{-1} (189 ± 15 s). Variations in the salinity between 30 and 35 g L^{-1} did not produce any significant alterations in sperm motility; however salinities of 20 and 50 g L^{-1} produced a significant loss of sperm motility. The highest percentage of motility was obtained at pH 8.5 (93 ± 12%), and the longest motility period at pH 8.7 (218 ± 13 s), while pH lower than or equal to 7 and equal to 10 both produced a significant loss in sperm motility. A positive correlation was found between pH/salinity and the motility percentage (R^2 = 0.94 and R^2 = 0.97) and motility time (R^2 = 0.86 and R^2 = 0.98). In seminal and morphometric parameters, statistically significant differences were observed in semen volume, sperm density, plasma membrane integrity and sperm morphometry between the groups studied, showing that the characteristics of the fish have a direct influence on sperm quality. The information generated in this research will be useful for developing biotechnology tools for the effective management of *Mugil liza* gametes.

1. Introduction

The Mugilidae family is represented by 66 species and 17 genera, distributed world-wide in tropical, subtropical and temperate zones (Monteiro-Ribas and Bonecker, 2001). The genus is of great importance for aquaculture around the world, with production of 12,360 tons in 2014; however the future expansion of mullet farming is limited because it depends mainly on wild fry (FAO, 2008). Egypt is by far the largest producer of farmed Mugilidae (Grey mullet, *Mugil cephalus*), however the Republic of Korea, Italy, Taiwan

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Province of China and Israel are also significant producers (FAO, 2008).

The lebranche mullet, *Mugil liza* (Valenciennes, 1836), is the species with the seventh highest capture volume in Brazil (MPA, 2011) and has been classified as overexploited since 2004 (IBAMA, 2007). The species presents characteristics that qualify it as an alternative for fish farming, such as: wide tolerance to salinity (0–90) and temperature (3–36 °C), high resistance to handling and easy food management (Cerqueira, 2004; Cerqueira et al., 2017).

Successful reproduction of any species in captivity requires due consideration of sperm quality to ensure efficient fertilisation (Rurangwa et al., 2004). Characteristics such as volume of semen, concentration of spermatozoa and motility are important parameters for assessing the reproductive capacity of an individual (Maria et al., 2010).

The only studies of the male gametes of lebranche mullet were performed by Eiras-Stofella and Gremski (1991), who described the spermatozoon ultrastructure; and by Serralheiro et al. (1992; 1999) and Otsubo (2010) who tested short-term and long-term cold-storage of semen. However, no studies have examined the sperm biology of this species, necessary for an understanding of reproductive aspects, in order to: 1) establish methods for gamete management in vitro (Cosson et al., 2008), both by short term conservation (Effer et al., 2013) and long term cryopreservation, so as to ensure the availability of gametes at any time of year (Figueroa et al., 2017); 2) identify a range of physiological parameters for sperm functionality (Figueroa et al., 2016a,b); 3) achieve effective management of breeding stock (Alavi et al., 2008; Mylonas et al., 2010); and 4) standardise protocols for the reproduction and rearing of this species in captivity.

In this study we assessed the effects of salinity and pH on the sperm motility of *Mugil liza*, as well as sperm structure and semen characteristics. We considered the effects of the parameters which govern sperm motility in fish (Billard and Cosson, 1989; Cosson, 2004, 2008; Morisawa, 2008) over the parameter ranges which might serve for the reproduction of *M. liza*, and compared gametes of captive males during first maturation with those of wild males during the breeding season. This preliminary information will be useful for future research into effective gamete management, allowing cultivation to be developed for commercial and/or research purposes.

2. Materials and methods

2.1. Fish groups

The individuals in captivity were produced in 2014 by hormone-induced spawning from wild specimens in the Laboratório de Piscicultura Marinha (LAPMAR) at Universidade Federal de Santa Santa Catarina (UFSC) (Brazil), as described by Cerqueira et al (2017). They were kept in special circular tanks in a hot-house, with a continuous water flow system providing a change rate of 100–300% per day with water pumped from the ocean. The water intake point was at Moçambique Beach, Florianópolis, Brazil (27°34′02″S, 48°25′44″W). The specimens were kept at natural temperature, ranging between 28 °C in summer and 17 °C in winter, with natural photoperiod. The fish were fed three times per day to satiation with a commercial diet containing 45% raw protein and 8% lipids. In the 2015 breeding season (June–July), the fish were aged 11 months and the males had reached maximum gonadal maturity, with semen released under slight abdominal pressure.

Semen samples were also taken from 15 wild fish for the purposes of comparison. The fish were captured with trawl nets by artisanal fishermen from the area of Barra da Lagoa, Florianópolis, Brazil (27°34′26″S, 48°25′27″W) on 29 June 2015 during the natural spawning season. Sperm was collected from the individuals by manipulation immediately after capture according to the modified protocol of Balamurugan and Munuswamy (2017).

2.2. Semen collection

A total of 15 captive fish (sexually mature) were anaesthetised with 50 ppm of Benzocaine. After anaesthesia, the abdominal and genital regions were cleaned with deionised water and dried with a paper towel. The semen was collected with 1 mL syringes (scale of 0.02 mL) under gentle abdominal pressure and immediately stored in an ice-cold container (without direct contact with the syringes) at 4 ± 1 °C protected from light; the samples were analysed no more than 30 min after storage. Samples contaminated with excrement or urine (recognizable by their colour and variation in viscosity) were discarded (Fauvel et al., 1999). The samples were identified by donor male and 15 of them were analysed individually for motility, viability, sperm density and spermatocrit; six males were used for morphometric evaluation and five to evaluate different sperm activators. All the individuals were weighed (g) with a digital scales and measured (cm). Collection and analysis of the semen from the wild fish followed the same procedure; the urogenital area was cleaned with a wet towel and the semen was collected with 1 mL syringes (scale of 0.02 mL) under gentle abdominal pressure. Contamination of sperm with seawater, blood, urine or faeces was carefully avoided. Sperm samples were placed on crushed ice immediately (without direct contact with the syringes) in absence of light, transported to the laboratory and stored at 4 °C until evaluation.

2.3. Sperm motility assessment

The semen from both groups of reproducers was activated in proportion 1:10 (v:v) (Agarwal et al., 2013) in a solution with salinity 35g L^{-1} and pH 9 (Lemos et al., 2014), using deionised water and artificial sea salt (Blue Treasure Reef Sea Salt, Quingdao Sea-Salt Aquarium Technology Co. Ltd., China) at a temperature of 4 ± 1 °C. The activator solution was previously prepared and maintained for 6h in a refrigerator coupled to an electronic temperature controller (Coel TLZ11, Manaus, Brazil). The temperature of