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Characterization of Senegalese sole, *Solea senegalensis*, male broodstock in terms of sperm production and quality

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Abstract

Sperm quality and production have never been characterized in *Solea senegalensis* males. Reproduction in captivity in this species has been obtained mostly with wild-captured animals, because it is common that the F1 generation fails to reproduce. However, there is no information on sperm quality from both types of broodstocks. The aim of the present study was to characterize sperm production and to describe the profiles of spermiation in individual wild-captured males. Also, sperm quality and production were determined in two types of broodstocks established in our facilities; wild-captured and F1 individuals. The males were analyzed for their fluency and identified as fluent or non-fluent. The sperm volume, cell concentration, sperm production and motility were recorded from mid February until mid November in both broodstocks. Results showed that *S. senegalensis* males can produce motile sperm during all this period, with specific peaks of high spermiation and a high percentage of fluent males. This fact was observed in both male broodstocks. There was a large variability in terms of sperm profiles in males maintained under the same conditions. Sperm volume collected in this species was very small and ranged from 5 to 20 μl in F1 broodstock and 10 to 80 μl in wild-captured broodstock. Cell density ranged from 0.7 to 1.2×10^9 spermatozoa/ml in F1 males to values of $1–2\times10^9$ spermatozoa/ml for the wild-captured males. Sperm production (total spermatozoa per stripping) was also very low and ranged from 20×10^6 spermatozoa for F1 broodstock to $40–60\times10^6$ spermatozoa for wild-captured broodstock. Our results demonstrated that sperm production in this species is very low and variable according to the type of males. These results suggest that a previous selection of males according to their fluency, sperm production and provenience (wild-captured or F1) should be taken into account in the establishment of a *S. senegalensis* broodstock.

Keywords: Senegalese sole; Male broodstock; Sperm quality; Motility; Sperm concentration

1. Introduction

The Senegalese sole, *Solea senegalensis* has been considered a new species for aquaculture diversification, a means of avoiding the present problems of aquaculture saturated markets. This flatfish is very common in Mediterranean and Southern Atlantic waters and, due to its wide distribution, is a potential culture species. Sole aquaculture interest started some decades ago, with pioneer work by Ramos (1982) and Dinis (1986). Since then, several studies have been made on larval rearing and nutritional requirements as well as on metabolism (Aragão et al., 2004; Morais et al., 2005). Other studies on genetics, pathologies and skeletal malformations have also contributed to the knowledge of the species biology and requirements for production (Gavaia et al., 2002; Soares et al., 2002; Porta et al., 2006). Presently,

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there is a large amount of information on sole rearing, but broodstock reproduction problems (failure in reproduction, poor egg quality and low fertilization rates) are still a bottleneck for commercial culture. The reproduction of *S. senegalensis* in captivity has been a focus of research in Spain and Portugal since the early 1980's (Ramos, 1982; Rodriguez and Pascual, 1982; Dinis, 1986; Anguis and Cañavate, 2005). There is not much information on gamete quality in this species, mostly because fish spawn naturally. Most of the problems encountered in the reproduction of this species has been attributed to females (low oocyte production and quality), and little is known about sperm production and quality. Until recently, the male reproduction system, spermatozoon morphology and male strategies have not received much attention (Medina et al., 2000; García-López et al., 2005). Other aspects that could influence reproduction in this species, such as sperm production and quality, have never been studied.

Several reproductive dysfunctions in males maintained in captivity have been identified in flatfish species, such as yellowtail flounder and turbot (Zohar and Mylonas, 2001). Males from broodstocks captured from the wild during the reproductive season can produce milt with non-mobile sperm (Berlinsky et al., 1997) or milt with high viscosity which does not mix with water during egg fertilization (Vermeirssen et al., 1998, 2000). Presently, Senegalese sole reproduction is obtained using breeders captured from the wild (Dinis et al., 1999), since reproduction of F1 broodstocks has failed (Porta et al., 2006; Dinis, personal comment). However, there is no information as to whether these reproductive dysfunctions are associated with male or female gametes.

The fact that sperm quality has never been characterized in Senegalese sole makes it impossible to know whether males contribute to the main reproductive problems in this species, both in wild-captured and F1 broodstocks.

The aim of this study was to characterize Senegalese sole sperm quality and production from individual males during the spermiation period and from two types of broodstock, wild-captured and F1 males.

2. Materials and methods

2.1. Broodstock management

Eleven Senegalese sole males captured from the wild (mean weight 1.190±0.300 kg), acclimatised for a period of 6 months were stocked in our facilities at the Ramalhete Experimental Station, Faro, Portugal in a 3000 l round fiberglass tank with sand substrate. Water exchange was kept at the 500 l/h and aeration was provided. Photoperiod was the natural for the season and was adapted each month, simulating environmental conditions in the area (January-9 h light/15 h dark; July-14 h light/10 h dark). Eleven individuals raised in the facilities (F1, 4-years old, mean weight 1.080± 0.220 kg) from a previous broodstock were also acclimatised under the same conditions. Both broodstocks were tagged with individual chips in order to identify each specimen. Temperature and salinity were normal for the season and varied according to Fig. 1. Broodstocks were fed 3% of biomass each day consisting of polychaete, mussel and squid. All individuals were maintained under these conditions 3 months before the experiments started and during the experimental period (mid February to mid November).

2.2. Male analysis and sperm collection

Each individual was sampled fortnightly and classified as fluent or non-fluent. Fluent males were

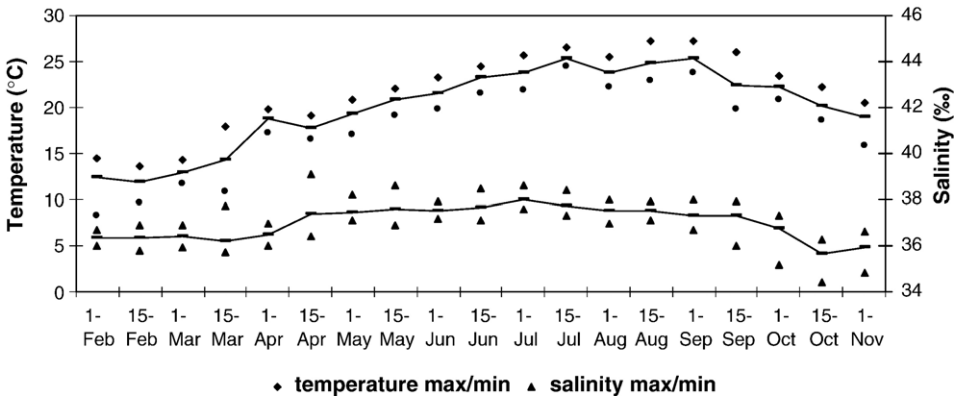

Fig. 1. Mean values of water temperature and salinity in the broodstock tanks. Minimum and maximum values are also presented for each parameter.