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Quantitative characteristics of Atlantic halibut, *Hippoglossus hippoglossus* L., semen throughout the reproductive season

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Abstract

The overall objective of the study was to investigate changes in quantitative parameters of Atlantic halibut (*Hippoglossus hippoglossus* L.) semen throughout the reproductive season in order to systematize the knowledge about biology of Atlantic halibut spermatozoa. Semen samples were collected from February to May from broodstock males kept under either a natural or 3-month advanced photoperiod regime. Spermatozoa concentration, semen pH and osmolality, as well as spermatozoa motility parameters were investigated. The use of catheterization of sperm was examined. Also, fertilization tests were performed. We found that spermatozoa concentration increases in a linear-like mode towards the end of the spawning season, which correlated with a decrease in a number of spermatozoa motility parameters, including actual percentage of motile spermatozoa (MOT), curvilinear velocity (VCL) and straight-line velocity (VSL) of spermatozoa. A breakpoint in MOT occurred when spermatozoa reached a concentration in the range of 17–20×10^9 spermatozoa/mL. The fertilization ability of sperm from males kept under natural photoperiod decreased in April. Survival of embryos at 80° days produced by fertilizing eggs of single female with sperm from natural photoperiod males was 88, 76 and 41% on April 09 and 17, and May 01, respectively, whereas using sperm from 3-month delayed photoperiod males for fertilizing eggs from the same female on April 27 resulted in 80% of surviving embryos, not differing significantly from the data from April 09. Physical decomposition of spermatozoa was observed towards the end of

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the season and it was related to an increase in the whole semen osmolality. Catheterization of semen did not improve spermatozoa motility parameters, however, it reduced the variation in recorded values, especially in the case of pH, caused by contamination with feces or urine. Post-seasonal decrease in spermatozoa concentration was likely related to intensive ageing processes. Based on the present study and available data by other researchers, a model of changes of quantitative parameters in Atlantic halibut semen throughout the reproductive season is proposed.

Keywords: Atlantic halibut; CASA; Fish; Reproduction; Sperm

1. Introduction

Atlantic halibut, *Hippoglossus hippoglossus* L., is the largest flatfish, with individual fish of 300 kg or more being reported in the wild [1]. For the last two decades, this species has been considered promising for coldwater marine aquaculture. Despite intensive efforts, halibut farming is not yet well established and its production yield varies yearly [2]. High mortality and developmental abnormalities at early developmental stages (embryos, larvae and post-larvae) are among the key issues that hamper the development of halibut aquaculture. Better understanding of reproductive biology in this species seems to be one the most important ways to overcome these problems. In the wild, Atlantic halibut spawns in deep water. For example, in Norwegian coastal waters, the halibut spawns at depths of between 300 and 700 m, where temperature and salinity are stable within narrow ranges of 5–7 °C and 34.5–34.9 ppt, respectively [3]. Halibut show a discontinuous reproductive pattern reaching reproductive readiness in the springtime [4]. Females are batch spawners and males show prenuptial spermatogenesis with spawning readiness at the peak of gonadal activity [5]. Endocrine regulation and testes organization during the reproductive cycle in Atlantic halibut have been described [5], providing the base knowledge of spermatogenesis in this species. Recently, it has been demonstrated that manipulation by implanting halibut males with gonadotrophin-releasing hormone (GnRH) agonist may induce an earlier start of spermiation and increase semen hydration [6].

The main goal of the present study was to learn more about temporal changes in the quantitative characteristics of Atlantic halibut semen over the reproductive season. Towards the end of the reproductive season, halibut milt becomes very viscous, difficult to express, and spermatozoa motility decreases [6–8]. To date, no systematic studies have been reported on quantifying these changes. The data on Atlantic halibut semen characteristics are limited to specific experimental approaches or result in inconsistent information, as in the case of spermatozoa concentrations [7,8]. In the present study, we collected sperm samples from two farmed broodstocks from February to May, during the reproductive season. One broodstock was kept under a natural photoperiod regime, with spawning readiness corresponding to the sampling period, whereas the other broodstock was kept under an advanced photoperiod regime, with spermiation in November, 2–3 months before the onset of the study. Therefore, characteristics of semen samples from the advanced-photoperiod group only provided information about post-seasonal effects. We monitored spermatozoa concentration, motility parameters and semen osmolality. Also, we