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Quantitative characteristics of Atlantic halibut (*Hippoglossus hippoglossus* L.) egg quality throughout the reproductive season

Tormod H. Skaalsvik [a], Sylvie L. Bolla [a], Per-Ove Thornqvist [b], Igor Babiak [a,*]

[a] *Faculty of Biosciences and Aquaculture, University of Nordland, Bodø, Norway*
[b] *Faculty of Medicine, Uppsala University, Uppsala, Sweden*

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ABSTRACT

Assessment of egg quality is an important aspect in finfish hatchery management, but guidelines for such assessment are scarce, especially for marine fish production. In the present work, potential indicators of egg and larval quality were measured in 39 batches of eggs of Atlantic halibut (*Hippoglossus hippoglossus* L.) throughout the reproductive season. The paternal influence on offspring was minimized by using the same cryopreserved semen for all fertilizations; consequently, maternal effects were emphasized. The progression of the spawning season and ovarian fluid parameters, including pH, electrical conductivity (EC), osmolality, and the amount of fluid were registered. The behavior of the broodfish at egg collection was registered and the eggs were analyzed for cortisol content. Fertilization and hatching rates as well as larvae survival were calculated, and larval standard length and myotome height were measured. Furthermore, the occurrence of major types of larval deformities was registered. The myotome height was significantly ($P < 0.05$) affected by the spawning season progress. Cortisol content in the eggs was decreasing with the progressing spawning season and correlated positively with the occurrence of yolk-sac edema. The ovarian fluid pH and EC were significantly related to fertilization and hatching rates. High fertilization and hatching success was associated with pH greater than 7.9 and EC less than 2.5 mS/cm. Low fertilization rates (<50%) resulted in further low hatchability from such egg batches. Ovarian fluid EC was significantly and positively related to increased occurrence of yolk-sac edema. High quantity of ovarian fluid in egg batches was associated with reduced egg quality in terms of fertilization and hatching rates and occurrence of yolk-sac edema. A cumulative effect of ovarian fluid pH, EC, osmolality, and quantity explained up to 62% of the total variation in fertilization rates. The findings from the present study indicate that parameters measurable at the initial phase of production, in particular ovarian fluid pH and EC, might have a potential for future use as egg quality indicators in hatchery management.

1. Introduction

Atlantic halibut (*Hippoglossus hippoglossus* L.) is an interesting species for aquaculture because of its high filet outcome, high market value, low annual catches, rapid growth, and low mortalities during the ongrowing stage [1–3]. Despite three decades of research, the species has not yet been successfully implemented into sustainable aquaculture. Problems related to egg and larval quality are among the primary factors slowing down the development of halibut aquaculture. It is well known that in marine batch spawners, such as the Atlantic halibut, egg and larval

* Corresponding author. Tel.: +47 75517922; fax: +47 755 17457.
E-mail address: igor.babiak@uin.no (I. Babiak).

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quality are highly variable, especially among individuals held in captivity [4,5]. Atlantic halibut belongs to fishes most difficult to reproduce in captivity as they usually do not spawn spontaneously and synchronously, hence collecting fertilizable eggs requires substantial expertise. In addition, as it reaches considerably big size at sexual maturity the handling is labor-intensive and broodstock maintenance is costly [4].

Assessing the quality of the eggs at an early stage is an important aspect of hatchery management, and the need for such procedures has previously been highlighted by the International Council for the Exploration of the Sea [5]. Till now, laborious operations are frequently carried out on batches of eggs that later will be lost or discarded because of insufficient quality. As suggested by Bromage et al. [6], to be of practical benefit assessment of egg quality should be simple to perform and not require lengthy or sophisticated laboratory procedures. In addition, it should be possible to carry out the assessments as soon as possible after gamete collection, so that low quality batches could be discarded at early stage. In a study on Atlantic cod (*Gadus morhua*), another coldwater marine batch spawner, it was concluded that differences in quality between egg batches from a single female are more important than differences between females; therefore, egg batch quality should be monitored continuously throughout the spawning season [7].

Numerous factors have been suggested to affect egg quality in fish, including egg aging, maternal condition and age, broodstock diet, endocrine status of females during oocyte growth, genetic factors, husbandry practices, stripping procedures, chemical composition, size of the egg, and hygiene [6,8,9]. Egg quality in Atlantic halibut is associated with expression levels of certain maternal genes [10] and its offspring development can be affected also by paternal factors, although maternal ones play a more important role [11].

In the present study, the main objective was to investigate maternal factors affecting offspring quality, and to establish reliable quality indicators throughout the spawning season of Atlantic halibut. Eggs were collected after close observation of the first egg release, as it has previously been reported that establishing ovulatory rhythms of each individual fish can optimize timing of stripping and hence improve egg yield and quality of the subsequent batches [12].

The eggs were collected over a period of 2 months, and seasonal effects at both individual and group levels were investigated as well as the behavior of the broodfish at egg collection. Measurements of ovarian fluid quantity, pH, electrical conductivity (EC), and osmolality of the ovarian fluid were performed, and eggs were analyzed for cortisol content. Offspring quality was assessed through measurements of fertilization and hatching rates as well as larval size, survival, and morphology.

2. Materials and methods

2.1. Experimental setup

The design of the study is given in Figure 1. In total, 39 batches of eggs were collected from 17 females of Atlantic

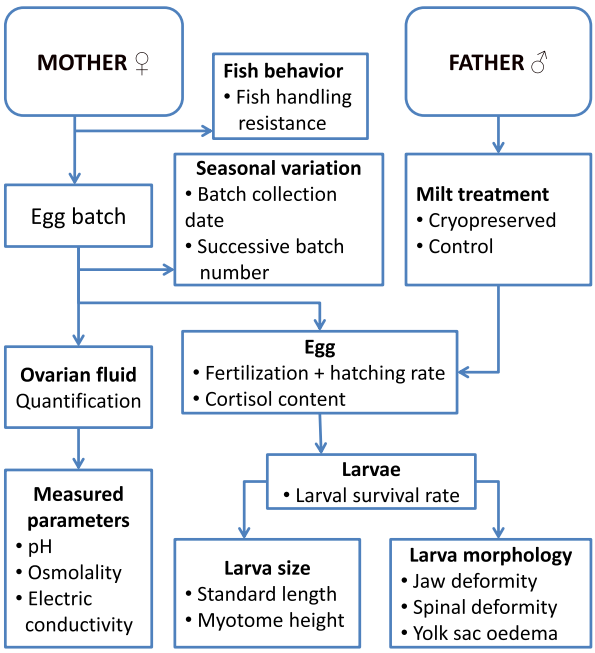

Fig. 1. Design of the study. Overview of the quantified parameters and measure timing of egg quality parameters throughout the reproductive season of Atlantic halibut. Detailed explanations are given in Section 2. (For interpretation of the references to color in this Figure, the reader is referred to the web version of this article.)

halibut (26–95 kg) in the course of the study, from March 11 to May 20, 2007. Five subsamples of approximately 100 eggs each were collected from each batch and inseminated separately with cryopreserved semen. To minimize the variation caused by paternal factors, the semen was cryopreserved from a single sample collected from a single male being at the peak of the reproductive season in the preceding reproductive season; the high quality of this semen was thoroughly examined as described by Babiak et al. [13]. As a control to check for any effects of using cryopreserved semen, five additional subsamples of eggs from each egg batch were inseminated with fresh semen from different males. After hatching, larvae were collected and set for further incubation and subsequent examination of larval performance. In addition to assessments of egg and larvae performance parameters, seasonality was registered and ovarian fluid parameters were measured.

2.2. Broodstock management and collection of gametes

Gametes were collected from Atlantic halibut broodstock held at the Mørkvedbukta Research Station of University of Nordland, Bodø, Norway (67°18′20″N 14°32′57″E). The circular holding tanks (≈157 m^3) with continuous flow-through water were covered with lightproof tents, and the photoperiod was advanced for 1 month. Before each collection of gametes, the water level was lowered from 200 to 90 cm. The water was pumped from 50 m depth and flow rate of the water was adjusted to keep the oxygen level greater than 80% saturation. Water temperature was measured every day and averaged 7.2 °C