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Parental effects on fertilization and hatching success and development of Atlantic halibut (*Hippoglossus hippoglossus* L.) embryos and larvae

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Abstract

The parental effects on fertilization and early life history traits were studied in Atlantic halibut, *Hippoglossus hippoglossus* L. Sperm from 12 different males were used to fertilize eggs of two females in separate crosses. The fertilization success were generally high, above 80% of developing embryos at 16-cell stage in 20 of 24 crosses with an average of 85.9 ± 17.6% and 87.2 ± 16.5% for female A and female B, respectively. Corresponding hatching success was 74.8 ± 17.7% and 41.6 ± 20.1%, respectively. The relationship between fertilization success and hatching success was positive. The parental influence on hatching was dominated by a strong and significant ($p < 0.001$) maternal effect; however, the paternal effect was also significant ($p < 0.001$). Furthermore, there was no relationship between fertilization success, hatching success and larvae viability as a high number of larvae developed locked jaws (i.e., were not functional). There was a significant ($p < 0.01$) difference in yield of functional larvae of female A (43%) and female B (56%), and also between crosses sired by different males. The standard length of offspring of female A (12.4 ± 0.5 mm) and B (12.6 ± 0.6 mm) were significantly ($p < 0.001$) different, and also significantly influenced by both the female ($p < 0.001$) and the male ($p < 0.001$). The present paper provides strong indications that not only the female, but also the male parent influences quantitative features of early development of their offspring.

Keywords: Atlantic halibut; Egg; Parental; Sperm; Viability

1. Introduction

Fertilization is the most commonly used criteria for egg viability, although there are contradicting reports on the relationship between fertilization success and viability of the offspring. Gamete quality is affected by several biotic and abiotic factors, such as size of the female, genetic influences, and broodstock nutrition and management [11]. For example, better timing of individual spawning rhythms and stripping of Atlantic halibut, *Hippoglossus hippoglossus* L., eggs at the correct time have demonstrated that both egg viability and production of eggs can be improved [1,2]. The viability of eggs and larvae are invariably linked to the quality of the unfertilized eggs and, hence, frequently considered to be almost solely under the influence of the female parent [3,16,32]. The quality of the male gametes is often neglected as an important factor affecting hatching and development of fish larvae [4]. Abnormal development of fish is often associated with unfavourable environmental stressors, and skeleton deformities often commence during early life stages [5,6]. Deformed jaws is a common deformity in halibut larvae. Even if the cause and relationship in many cases are unknown, it is

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frequently related to sub-optimal rearing conditions [7,8]. Moreover, skeleton deformities have been related to pollutions [9], sub- or super-optimal levels of essential feed ingredients [10], and genetic defects [11,12]. Abnormal development of fish larvae may affect the general welfare of the fish larvae and ultimately lead to a lower production yield in aquaculture. In the present study, single pair's crosses between Atlantic halibut males and females were conducted to study parental effects on fertilization, hatching success, hatching duration, survival, growth, and development of the jaws of halibut yolk sac larvae.

2. Materials and methods

2.1. Broodstock

Eggs and sperm for the experiment were stripped from broodstocks of Atlantic halibut, *H. hippoglossus*. Two wild caught (1990) females (female A, weight 82 kg and female B, weight 76 kg) and five wild caught (1990) males (male 1: 45 kg; male 2: 55 kg; male 3: 50 kg; male 4: 36.5 kg; male 5: 49 kg), were selected from a population at Norkveite AS in Bodø, held in a tank with a volume of 55 m^3 (depth 1.1 m). Further, 4 farmed (1993) males (male 6: 9.5 kg; male 7: 9 kg; male 8: 8 kg; male 9: 7 kg) were selected from a population at Norkveite AS, held in a tank with a volume of 55 m^3 (depth 1.1 m). Additionally, three wild caught (1991) males (male 10: 25 kg; male 11: 50 kg; and male 12: 60 kg) were selected from a population at Atlantic halibut AS in Rørvik, held in a tank with a volume of 50 m^3 (depth 1.0 m). The mass of males 10, 11, and 12 were estimated at the start of the experiment. The mass of the rest of the fish (females A and B, and males 1–9) were measured approximately a year prior to the experiment. All fish were hand fed three times a week outside the spawning season, and ad lib during the spawning season. At Norkveite AS the fish were fed a combination of whole herring and a moist pellet diet of commercial brood stock feed for marine fish (INVE, Fish Breed M, B-9200 Dendermonde, Belgium), and at Atlantic halibut AS, whole herring with vitamin capsules.

2.2. Temperature, salinity and dissolved oxygen

At Norkveite AS, temperature, salinity and dissolved oxygen of the sea water were measured using portable meters (Oxyguard-Handy Gama, OxyGuard International A/S, Birkeroed, Denmark; Aanderaa model 3210, Aandreaa, Bergen, Norway) regularly in the outlet water of the broodstock tanks through 12 months preceding the experiment. Temperature was 7.4 ± 1.3 °C ($n = 287$), minimum 4.7 °C in March and maximum 11.4 °C in September, while oxygen levels were maintained above 79% (average 90%, $n = 289$) by regulating the flow-through water in the tanks, salinity was around 34‰. At Atlantic halibut AS, average temperature, salinity and oxygen of intake water for the same period were 8 ± 0.5 °C, 34 ± 0.3‰ and 90 ± 3%, respectively.

2.3. Halibut eggs and sperm

Eggs of females A and B were stripped 4 h and 30 min and 1 h before fertilization, respectively, and stored in a temperature-controlled room at 5 °C until utilized [2]. All halibut males were stripped approximately 24 h before the start of the experiment. The sperm from Atlantic Halibut AS was stored in Zip lock bags on crushed ice and transported by air to Bodø. After stripping of fish at Norkveite AS, and after arrival of the sperm from Atlantic halibut AS, the sperm was diluted to 1:3 in HBSS (Hank's Balanced Salt Solutions, Sigma–Aldrich)[1], and stored in Zip lock bags on crushed ice in a temperature-controlled refrigerator at 2 °C until utilized [13]. Before stripping eggs and sperm, the urogenital pore was dried carefully to avoid polluting the gametes. To avoid or reduce potentially negative effects of urine [14] the sperm was carefully collected from the fish into a syringe. Eggs were stripped into 5-L plastic buckets. The diluent HBSS was added to the sperm to avoid possible negative effects of urine during cool storage [13,14].

2.4. Sperm osmolality, density and motility

The numbers of sperm cells from the males were estimated prior to fertilization by a cytometric method in a Bürker counting chamber using a microscope (400×) [15]. Prior to the fertilization, osmolalities were measured to 379 (male 1), 391 (male 2), 470 (male 3), 449 (male 4), 397 (male 5), 363 (male 6), 364 (male 7), 481 (male 8), 404 (male 9), 394 (male 10), 408 (male 11) and 385 (male 12) mOsm kg^{-1} with a Fiske One-Ten Osmometer (Model 110—Fiske Associates, Two Technology Way/781-320-5656, Norwood, Massachusetts 02062, USA) for sperm from male 1 to male 12, respectively. Computer-assisted sperm analysis (CASA)

[1] Modified Hanks' Balanced Salt Solution (Sigma–Aldrich Co., H 8264): NaCl 8 g/L; KCl 0.4 g/L; $CaCl_2 \cdot 2H_2O$ 0.185 g/L; $NaHCO_3$ 0.35 g/L; $MgSO_4$ 0.09767 g/L; KH_2PO_4 0.06 g/L; Na_2HPO_4 0.04788 g/L; D-glucose 1 g/L; pH 7.2. Osmolality 281 mOsm/kg, controlled by own measurements ($n = 3$) to 282 mOsm kg^{-1}.