New species for EU aquaculture

Deliverable Report

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Deliverable Title	Identification of potential disturbances in reproductive development in F1/F2 Atlantic halibut females		
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WP Title:	Reproduction and Genetics – Atlantic halibut		
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Other beneficiaries:	P3. IRTA		
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Objective: The objective of this deliverable was to document potential physiological differences between wild-captured females and F1/F2 female Atlantic halibut (*Hippoglossus hippoglossus*). Empirical data suggest a significant difference in spawning performance between wild-captured and hatchery-produced (F1/F2) Atlantic halibut females, but currently there is a lack of systematic documentation. Consequently, individual spawning cycles in cultured and wild-captured females and reproductive performance were documented. Plasma gonadotropin and sex steroid concentrations were monitored throughout almost a year in wild-caught and farmed females.

Description:

Identification of potential disturbances in reproductive development in F1/F2 Atlantic halibut females: This deliverable is a documentation of potential physiological differences between wild-captured females and F1/F2 female Atlantic halibut. The deliverable will describe differences between wild-captured and F1 spawners in sex steroid and gonadotropins plasma levels during almost a whole year.

Introduction

Work done in Task 5.1 showed some significant differences in spawning performance between wild-captured and farmed Atlantic halibut females. The Atlantic halibut is a group-synchronous, periodic spawner and in captivity wild-captured females will release 6-12 batches of eggs during a period of 2-4 weeks in the spawning season, which lasts from late February to late April in southwestern Norway. In order to obtain eggs with high viability, females have to be stripped according to their individual ovulatory rhythms, to prevent over-ripening and deterioration of the eggs (Norberg et al., 1991). While wild-caught females generally adapt well in captivity, displaying high fecundity with egg batches spawned at regular intervals, hatchery-produced F1/F2 females appear to suffer from a reproductive dysfunction, releasing small batches of eggs at irregular intervals. Reproductive performance of domesticated, wild-caught Atlantic halibut and farmed (F1) females was compared in task 5.1. Our results showed no differences in fecundity between wild-caught and farmed females, but ovulatory intervals seemed more irregular in the farmed broodstock (see **Table 5.3.1**).

Table 5.3.1. Biometric and spawning performance data of domesticated and farmed halibut breeders at IMR, Austevoll (from Deliverable D5.1).

	Domesticated females	Farmed (F1) females
n	3 (4[1])	5
length (cm)	150.7 ± 6.2	113.4 ± 3.9*
weight (kg)	48 ± 5.7	19.2 ± 2.3*
number of batches ·female^{-1}	7.3 ± 0.6	9.4 ± 1.7
spawning interval (hours)	82.2 ± 8.4	72.4 ± 22.9
batch volume (ml)	2300 ± 900	700 ± 300*
total fecundity (ml·female^{-1})	16700 ± 420	6800 ± 130*
relative fecundity (ml·kg^{-1})	347 ± 70	349 ± 84
average fertilization (%)	89 ± 7	61 ± 29

[1] One domesticated female was left undisturbed for most of the season, due to a large skin lesion, and was not included in calculations.

*=significant difference

Fertilization success values were similar, whereas hatching success was lower (**Fig. 5.3.1**) and egg diameter was slightly, but significantly, lower in farmed females (**Fig. 5.3.2**). To investigate possible differences in endocrine regulation of maturation, blood samples were taken at intervals of 3-5 weeks from September 2016 to July 2017. The samples were analysed for the sex steroids estradiol-17ß (E2) and testosterone (T), and the gonadotropins follicle stimulating hormone (Fsh) and luteinizing hormone (Lh). This is the first report of plasma concentrations of Fsh and Lh in Atlantic halibut.

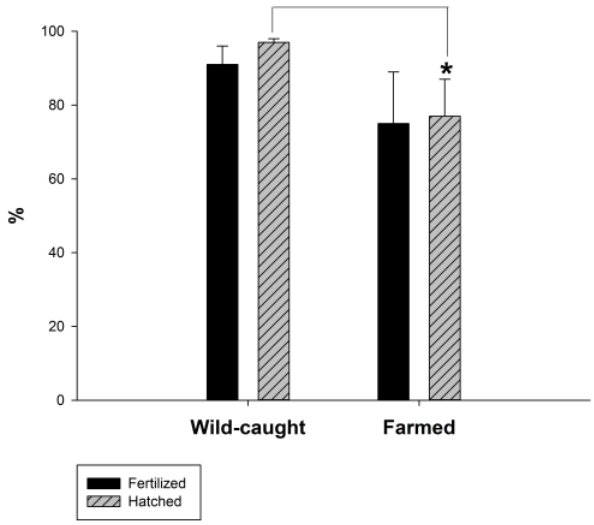

Figure 5.3.1 Fertilization and hatching rates in eggs from wild-caught and farmed female halibut broodstock.

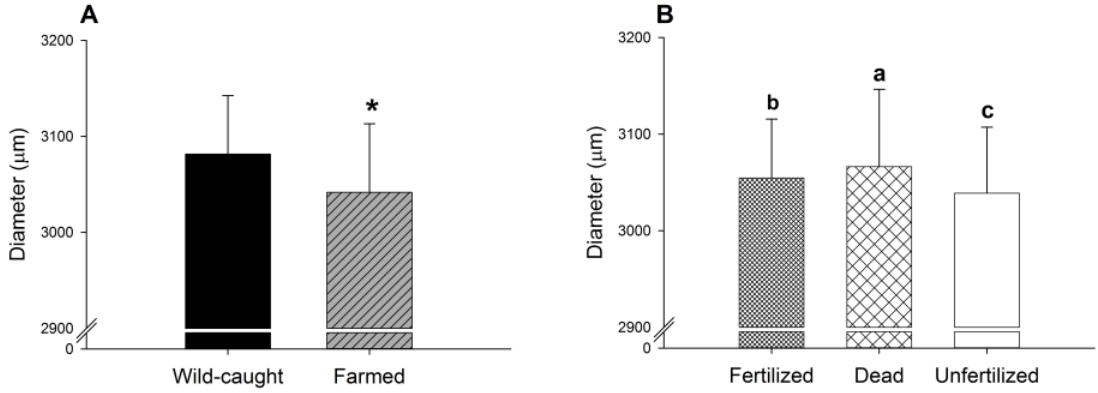

Figure 5.3.2 A. Egg diameter in wild-caught and farmed female halibut. **B**. Diameters of fertilized, dead and unfertilized halibut eggs.

Annual profiles of plasma hormone concentrations in wild-caught and farmed female Atlantic halibut

Methods

Blood samples were taken from the caudal vessels of 5 wild-caught and 5 farmed female Atlantic halibut at 3-6 week intervals, with the highest frequency during the spawning season. The sampled females were the same individuals as in task 5.1. Blood samples were centrifuged for 5 minutes at 12000 rpm and at 4°C. After

centrifugation, plasma was divided in aliquots, frozen immediately on dry ice and stored at -80°C until analysis.

Analysis of sex steroids was carried out by enzyme-linked immunosorbent assay (ELISA), validated for halibut as described previously (Cuisset et al., 1994; Weltzien et al., 2002). Steroids were extracted from blood plasma by a method modified from Pankhurst and Carragher (1992). Briefly, plasma samples (100 µl) were mixed with 1 ml ethyl acetate, vortexed for 20 sec and centrifuged for 3 minutes at 1800 rpm at 4 ˚C. The organic phase was collected by a Pasteur pipette and the hydrophilic phase was extracted once more with 1 ml of ethyl acetate. The extracts were evaporated in a Speed Vac centrifuge (Savant 1000, USA), and dissolved in 1 ml buffer (phosphate 0.1 M pH 7.4, 0.4 M NaCl, 1 mM EDTA) by heating (60 ˚C for 10 min). The extracted and dissolved steroids were stored at -20 ˚C until analysis by an ELISA (Cuisset et al., 1994). Extraction efficiency was >90% for all steroids. ED80 and ED20 were 0.004 ng ml^{-1} and 0.08 ng ml^{-1} for T and 0.006 ng ml^{-1} and 0.6 ng ml^{-1} for E2. Detection limits of the assays were 0.008 ng ml^{-1} for T and 0.015 ng ml^{-1} for E2, respectively. Internal standards were prepared from mature female Atlantic cod plasma extracted as described above. The accepted inter assay coefficient of variation was 10% for all steroids; assays with higher deviation of the internal standard were re-run. The intra-assay coefficient of variation was 6.8 % for E2 (n=10) and 5.6 % for T (n=10). Estradiol and T antisera, acetylcholine esterase-labelled tracers and microplates pre-coated with monoclonal mouse antirabbit IgG were supplied by Cayman Chemicals (USA). Standard steroids were purchased from Sigma Aldrich (Sigma reference standards). Cross reactivities for E2 and T antisera are described by the manufacturer.

Analyses of plasma Fsh and Lh concentrations were carried out by heterologous ELISAs, developed for Senegalese sole Fsh and Lh by Joan Cerdà's lab at IRTA, and validated for Atlantic halibut (Chauvigné et al., 2015, 2016). Plasma samples where hemolysis was detected were excluded from the analyses.

Results

Sex steroid concentrations

Mean plasma concentrations of E2 were 10-20 ng·ml^{-1} from October to December in both groups and 48.7±18 ng·ml^{-1} and 49.6±7.3 ng·ml^{-1} in February in wild-caught and farmed females, respectively. E2 remained high during the spawning period, decreased to basal levels <1 ng·ml^{-1} in May in all females and remained low for the remainder of the sampling period (**Fig. 5.3.3A**).

Mean plasma T concentrations remained low, <4.5 ng·ml^{-1}, in both groups until February when T reached 28.7±21.4 ng·ml^{-1} in wild-caught females, and March when T reached 32.9±12.5 ng·ml^{-1} in farmed females. Plasma T concentration was significantly higher in farmed than in wild-caught females in March, indicative of a later start of spawning. Post-spawning, mean plasma T concentrations dropped to <2.5 ng·ml^{-1} and remained low for the remaining sampling period (**Fig. 5.3.3B**).

Plasma gonadotropin concentrations

During gametogenesis, from September to January, mean plasma Fsh concentrations were 30-40 ng·ml^{-1} and 15-40 ng·ml^{-1} in wild-caught and farmed females, respectively. Individual variation was high, especially in wild-caught fish. Follicle-stimulating hormone showed a decreasing trend before the spawning period and was low during spawning in all fish. After spawning, from April onwards, mean plasma Fsh concentrations tended to increase in both groups (**Fig. 5.3.3C**).

Mean plasma Lh concentrations were relatively high, 20-60 ng·ml^{-1}, from September to December in both groups. Before spawning, mean Lh concentrations appeared to decrease. Highest plasma Lh concentrations were seen during the spawning period, in March, with peak levels of 58.4±14.3 ng·ml^{-1} and 69.5±40.2 ng·ml^{-1} in wild-caught and farmed fish, respectively. After spawning, Lh concentrations showed a decreasing trend in both wild-caught and farmed females and were <17 ng·ml^{-1} in July (**Fig. 5.3.3D**).

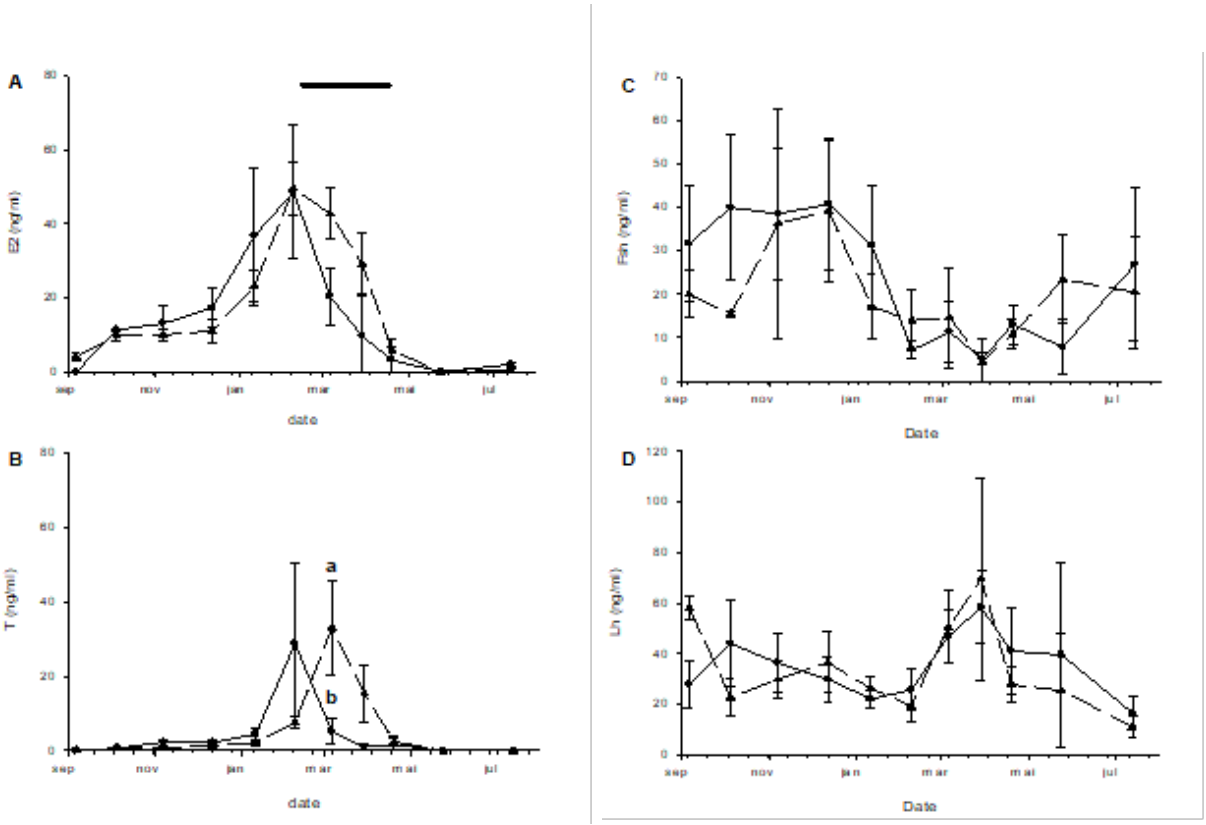

Figure 5.3.3. Annual plasma profiles of (A) Estradiol-17ß, (B) Testosterone, (C) Follicle-stimulating hormone and (D) Luteinising hormone in wild-caught (black circles, lines) and farmed (black triangles, stippled lines) halibut broodstock. Black bar indicates spawning period. Different letters indicate significant differences.

Discussion

Plasma concentrations of sex steroids were similar to what has been reported previously in Atlantic halibut (Methven et al., 1992), with annual profiles following ovarian growth and maturation. Highest E2 levels were recorded just prior to spawning, in the beginning of February, while both E2 and T remained elevated through the spawning period. No differences in average concentrations were seen between wild-caught and farmed females, although the highest individual E2 concentrations (80.4 and 95.8 $ng \cdot ml^{-1}$) were detected in wild-caught females. The wild-caught females were larger that the farmed ones, had a higher total egg production and hence a larger total ovary weight. This would result in a higher total capacity for steroid production which may explain the higher plasma concentrations in some individuals.

Plasma concentrations of the gonadotropins, Fsh and Lh, were documented for the first time in Atlantic halibut. Mean Fsh concentrations were relatively stable during vitellogenesis, from October to early February, consistent with a constitutive release of Fsh from the pituitary. Follicle-stimulating hormone exhibited low levels during spawning and an increasing trend after spawning was completed. This is consistent with previously reported results in other teleosts, including flatfish (cf. Levavi-Sivan et al., 2010; Chauvigné et al 2016). Mean Fsh concentrations were higher in wild-caught females than in farmed fish, but individual variations were high and further studies are needed to confirm if this result is consistent. Lh concentrations showed large individual variations through the reproductive cycle, but peak levels were apparent during spawning, in accordance with results in other teleost fish (Levavi-Sivan et al., 2010; Chauvigné et al 2016).

References

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Deviations:

Due to unexpected problems with sampling, biopsy samples could not be collected. Instead, plasma concentrations of the gonadotropins Fsh and Lh were documented through the reproductive cycle for the first time in Atlantic halibut. In addition, fecundity analyses carried out in task 5.1 revealed no differences between farmed and female halibut. Therefore, and in view of the scarcity and high value of individual wild-caught halibut breeders, it was decided not to carry out potential fecundity analyses which would have necessitated sacrifice of females.

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