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Environmental impacts on the gonadotropic system in female Atlantic salmon (*Salmo salar*) during vitellogenesis: Photothermal effects on pituitary gonadotropins, ovarian gonadotropin receptor expression, plasma sex steroids and oocyte growth

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ABSTRACT

The gonadotropic system and ovarian growth and development were studied during vitellogenesis in female Atlantic salmon subjected to either simulated natural photoperiod and ambient water temperature (NL-amb), or an accelerating photoperiod (short day of LD8:16 from May 10) combined with either warmed (ca 2 °C above ambient; 8L-warm) or cooled water (ca 2 °C below ambient; 8L-cold) from May to September.

Monthly samples were collected from 10 females/group for determination of transcript levels of pituitary gonadotropin subunits (*fshb* and *lhb*) and ovarian gonadotropin receptors (*fshr* and *lhr*), plasma sex steroids (testosterone: T and estradiol-17β: E_2), gonadosomatic index (GSI) and oocyte size.

Short day in combination with either warmed or cooled water induced an earlier increase in pituitary *fshb* and *lhb* levels compared with NL-amb controls, and advanced ovarian growth and the seasonal profiles of T, E_2. By contrast only minor effects were seen of the photothermal treatments on ovarian *fshr* and *lhr*.

The 8L-cold had earlier increase in *fshb*, *lhb* and E_2, but similar oocyte and gonadal growth as 8L-warm, suggesting that the 8L-cold group tried to compensate for the lower water temperature during the period of rapid gonadal growth by increasing *fshb* and E_2 production.

Both the 8L-warm and 8L-cold groups showed incomplete ovulation in a proportion of the females, possibly due to the photoperiod advancement resulting in earlier readiness of spawning occurring at a higher ambient temperature, or due to some reproductive dysfunction caused by photothermal interference with normal neuroendocrine regulation of oocyte development and maturation.

1. Introduction

Photoperiod is regarded as a key factor controlling seasonal timing of gonadal development and spawning in Atlantic salmon (Bromage et al., 2001; Migaud et al., 2010; Taranger et al., 2010). A combination of long photoperiod early in the year, followed by short photoperiod during the summer months can advance ovarian growth and the timing of ovulation by several months (Taranger et al., 1998). However, elevated temperatures during the spawning season have been shown to compromise or inhibit final oocyte maturation and ovulation in Atlantic salmon (Taranger and Hansen, 1993; King and Pankhurst, 2004; Taranger et al., 2003; Vikingstad et al., 2008). Less is known about the effects of water temperature during the period of rapid gonadal growth of vitellogenesis prior to the spawning season (King and Pankhurst, 2003; King et al., 2003, 2007; Pankhurst and King, 2010; Pankhurst and Munday, 2011; Pankhurst et al., 2011; Anderson et al., 2012), and in particular how different water temperature can modulate the effects of photoperiod treatment during vitellogenesis.

Gonadal development, growth and maturation are under the control of the brain–pituitary–gonadal axis (BPG axis) by regulating the production and release of the two pituitary gonadotropins follicle stimulating hormone (Fsh) and luteinising hormone (Lh) into the blood circulation and their subsequent action on their cognate receptors follicle stimulating hormone receptor (*fshr*) and luteinising hormone receptor (*lhr*) in the gonads are regarded

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as key regulatory factors (reviewed by Zohar et al., 2010; Levavi-Sivan et al., 2010). The recent molecular characterization of the gonadotropin subunits *fshb* and *lhb* in Atlantic salmon, as well as the gonadotropins receptors *fshr* and *lhr* (Maugars and Schmitz, 2006) has enabled the development of specific and sensitive qPCR assays for these transcripts, allowing detailed investigation of how photoperiod and temperature can modulate the activation of the BPG axis during ovarian growth and spawning (Andersson et al., 2009, 2013). In order to study the effects of combined photothermal regime during the vitellogenic period of ovarian growth, we combined an accelerating photoperiod regime with either moderately cooled or warmed water during the summer months. To this end, we transferred female salmon broodstock to indoor tanks and subjected them to either control conditions with ambient photoperiod and water temperature of the site in Western Norway, or a short day photoperiod (LD8:16) from May 10 combined with either warmed or cooled water from May 31 to September 20. All groups were exposed to ambient water temperature from September 20 to allow final oocyte maturation and ovulation. The current paper reports the effects of these combined photothermal treatments on female pituitary gonadotropin subunits (*fshb* and *lhb*) and ovarian gonadotropin receptors (*fshr* and *lhr*), plasma sex steroids levels and ovarian development in Atlantic salmon broodstock.

2. Materials and methods

2.1. Fish material

Previously immature 2 sea-winter salmon (n = 482; 260 females and 222 males) of the AquaGen strain were maintained in sea cages (size 12 × 12 × 12 m) at IMR Matre Research Station in Western Norway (61°N) until May 2 under ambient light, when they were transferred into six 5 m diameter tanks (19.6 m^3 each) with controlled photoperiod and water temperature, two replicate tanks pr treatment. The tanks were supplied with freshwater with a small amount of seawater added to stabilize water quality and prevent fungal problems on the fish. The salmon broodstock were stocked with about 70 fish per tank and the mean body size at time of transfer to the tanks was 10.7 +/− 0.45 kg (mean +/− SEM). Salinity in the tanks ranged from 3.3 to 7.0 ppt during the study, and oxygen level were maintained over 90% saturation. There was no feeding after transfer to the tanks.

2.1.1. Experimental design

Following transfer to tanks, the salmon broodstock were subjected to three treatments from May 10; simulated natural photoperiod and temperature (NL-amb), short day (LD8:16) and warmed water (ca 2 °C above ambient; 8L-warm) and short day and cooled water (ca 2 °C below ambient; 8L-cold). The light was supplied by a single 150 W metal halide lamp placed above each tank. The temperature treatment started on May 31. The cooling and heating of the water was achieved by use of a heat pump and partial recirculation (around 50%) of the water at tank level combined with aeration. On September 20, all groups were transferred to ambient water temperature to allow ovulation and spermiation. This resulted in a day-degree sum during the temperature treatment period from May 10 to September 20 of 1337, 1619 and 1108 d °C in the NL-amb, 8L-warm and 8L-cold groups, respectively. The water temperature profiles for each experimental group are given in Fig. 1, and the water temperature range (min.–max.) per month is given in Table 1.

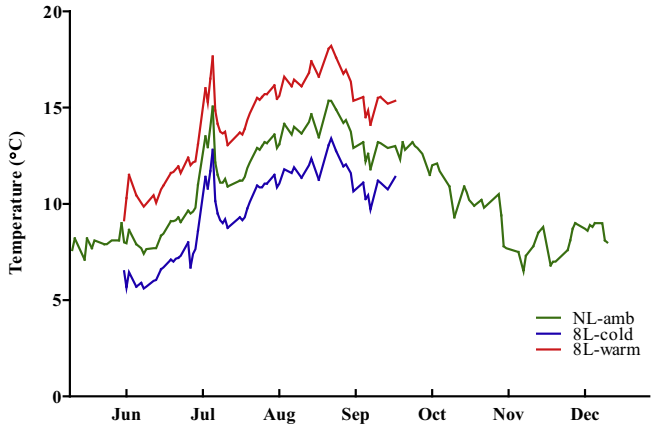

Fig. 1. Water temperature profile during the experiments three different photothermal treatments; NL-amb (▲), 8L-cold (■) and 8L-warm (●).

Table 1
Range (min.–max.) water temperature per month during the experiment in the different photothermal treatments; NL-amb (▲), 8L-cold (■) and 8L-warm (●).

Month	NL-amb	8L-cold	8L-warm
May (from May 31)	7.1–9.0	6.5	9.2
June	7.7–11.0	5.6–8.6	9.9–13.1
July	10.9–15.1	8.8–12.1	13.1–17.7
Aug	12.9–15.4	10.9–13.1	15.5–18.1
Sep (until Sep 18)	11.8–13.2	9.7–11.4	14.1–15.6

2.1.2. Sampling

Monthly samples were collected from 10 sacrificed females per group. The fish were randomly and rapidly collected by dip net from each tank after lowering the water level to 30 cm, and immediately anesthetised in 10 ppt metomidate (Syndel, Victoria, BC), measured for fork length and weighed, and 6 ml of blood was collected in heparinised syringes from the caudal veins. Subsequently, the fish were killed by cutting medulla oblongata, and pituitary and gonad tissues excised. The pituitaries were immediately snap-frozen in aluminum wraps in liquid N_2. Gonads were weighed to nearest 0.1 g, and one small transverse piece was snap-frozen in liquid N_2, and another was fixed in Bouins fixative for later determination of oocyte diameter.

2.2. Real-time, quantitative PCR assays for pituitary gonadotropin subunit mRNA expression and ovarian gonadotropin receptor mRNA expression

RNA isolation, and cDNA synthesis was performed as described in detail by Andersson et al. (2013). Sequences of primers and hydrolysis probes specific for Atlantic salmon *fshb*, *lhb* (Andersson et al., 2013), *fshr*, *lhr* (Andersson et al., 2009) and the reference gene *elongation factor 1α*, (*ef1 α*, Olsvik et al., 2005) are given in Table 2. All qPCR assays were performed in duplicate, using 96-well optical plates on an ABI Prism 7700 Sequence Detection System (Applied Biosystems) using default settings. For each 25 µl PCR reaction cDNA was mixed with 200 nM fluorogenic probe, 900 nM sense primer, 900 nM antisense primer in 1× TaqMan Universal PCR Master Mix (Applied Biosystems). For each PCR plate, no-template controls were run for each gene. The $\Delta\Delta C_t$ method was used to calculate relative gene expression levels, as described in detail previously (Bogerd et al., 2001). Expression data was then represented as fold change compared to the mean values of pituitary and ovary mRNA levels in the initial sampling in February.

2.3. Sex steroid analysis

Sex steroids (testosterone: T and estradiol-17β: E_2) were analyzed with ELISA on extracted plasma samples as detailed in Andersson et al. (2013).