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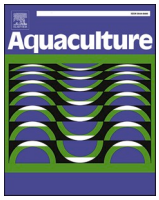

Interaction of temperature and photoperiod on male postsmolt maturation of Atlantic salmon (*Salmo salar* L.)

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ABSTRACT

Maturation of Atlantic salmon male postsmolts is a concern in aquaculture due to its increasing occurrence under intensive rearing conditions and its negative impact on growth, welfare and seawater readiness. The effect of temperature and photoperiod on maturation was assessed in male postsmolts kept in freshwater. We used a 2 × 2 factorial design with two temperatures (12.5 and 15 °C) and two photoperiods (a group in continuous light LD24:0 or LL, and another receiving a 5-week LD12:12 winter signal or WS). Salmon in the four resulting treatments (1000 parr, initial mean weight 52.1 ± 5.2 g) were reared in a flow-through system from 28 October 2019 to 30 May 2020. Morphology (body weight, condition factor), maturation indicators including gonado-somatic index (GSI), plasma 11-Ketotestosterone (11-KT), pituitary follicle-stimulating hormone β-subunit (*fshb*) and luteinizing hormone β-subunit (*lhb*) transcript levels, as well as smoltification markers (Na^+, K^+- ATPase activity) were assessed. Results revealed that rearing salmon at 15 °C was the most important factor promoting early maturation, leading to 100% of males maturing in late May in 15-WS, and 75% in 15-LL. However, the groups receiving a winter signal (WS) displayed a highly synchronized onset and progression of maturation specially at 15 °C, revealed by the low variability observed among individuals in GSI and *fshb* transcription after the WS. This evidences the role of the photoperiod switch from short to long day as *zeitgeber* for sexual maturation. On the contrary, under constant light (LL), entry into maturation was not synchronized among individuals, and onset of maturation occurred spontaneously in a proportion of males highly dependent upon temperature (75% in 15-LL, 25% in 12.5-LL). Signs of smoltification were poor at both temperatures, and the WS did not induce development of hypo-osmoregulatory abilities in any case. This suggests that a winter signal may not induce smoltification if introduced at high temperature or when fish have reached large size, and instead may increase the risk of a sexual maturation response. These findings are relevant for the aquaculture industry, since similar rearing conditions are currently used in the industry, including constant high water temperature and winter signal regimes. The use of such conditions can increase the risk of early maturation, as well as of poor hypo-osmoregulatory performance.

1. Introduction

In recent years, early maturation of male Atlantic salmon postsmolts has become a concern for aquaculture producers, since larger proportions of mature fish have sometimes been observed under intensive conditions (Fjelldal et al., 2018; Good and Davidson, 2016; Melo et al., 2014). This phenomenon occurs primarily in males due to the lower energetic investments required for testis development in comparison to female egg production (Adams and Thorpe, 1989; Simpson, 1992). Early maturation can represent high economic costs for salmon producers, due to physiological and behavioral changes that lead to poor growth and food conversion ratio (FCR) (Fraser et al., 2019; Good and Davidson, 2016; McClure et al., 2007), issues with osmoregulatory performance and reduced welfare (Taranger et al., 2010), and possibility of higher mortality rates (Schulz et al., 2006).

Atlantic salmon commence maturation as a result of complex

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interactions between environmental and internal factors (Good and Davidson, 2016; Taranger et al., 2010) as well as genetic background (Fjelldal et al., 2020). External factors such as water temperature, photoperiod (Adams and Thorpe, 1989; Fjelldal et al., 2011; Imsland et al., 2014) or diet (Herbinger and Friars, 1992; Kadri et al., 1996; Rowe and Thorpe, 1990a; Thorpe et al., 1990) influence fish developmental rate, growth, and thus the availability of resources for maturation. Fast-growing salmon are more likely to prioritize maturation from an early stage, as suggested by studies linking early maturation and energy-related parameters like growth (Berglund, 1995, 1992; Rowe and Thorpe, 1990b), lipid levels (Rowe et al., 1991; Simpson, 1992), or condition factor (Peterson and Harmon, 2005). In addition, seasonal variations in environmental factors such as photoperiod act as a *zeitgeber* or entraining cue that signals the right timing for initiation of maturation in order to ensure maximal survival of the offspring (Berrill et al., 2006, 2003; Bromage et al., 2001; Fjelldal et al., 2011; Fraser et al., 2022). If energy levels are assessed as sufficient by a "critical time window", signaled by the photoperiod change towards increasing day length occurring in nature in spring, then sexual maturation can commence (Taranger et al., 2010; Thorpe, 1994, 2007).

In modern aquaculture settings such as Recirculation Aquaculture Systems (RAS) for smolt/postsmolt production, intensive conditions such as constant high temperature and continuous light can accelerate growth and energy acquisition, thus advancing the time at which salmon accrue resources for maturation (Good and Davidson, 2016). In high-energy juvenile salmon, the photoperiod regime introduced to induce smoltification (a period of short day length that mimics winter light conditions for 5–6 weeks, followed by constant light) can instead act as a synchronizing cue that leads to early sexual maturation (Fjelldal et al., 2020; Fraser et al., 2022). However, if salmon have not yet adopted sexual maturation as their priority, they are more likely to respond to a photoperiod switch from short to long day undergoing the parr-smolt transformation (Fraser et al., 2022; Thorpe, 1994; Thorpe et al., 1998).

Onset of maturation requires the activation of the Brain-Pituitary-Gonad (BPG) axis (Schulz et al., 2010; Taranger et al., 2010), which is characterized by a rise in gonadotropic activity of the pituitary as a result of activation signals sent from the hypothalamus (Zohar et al., 2010). In response, the pituitary increases the production of follicle-stimulating hormone (Fsh), a hormone that exerts its action binding to testis receptors and causing an increase in 11-Ketotestosterone (11-KT) production. These hormonal changes support the first stages of spermatogenesis (Maugars and Schmitz, 2008; Schulz et al., 2010). Subsequently, following stages (spermiogenesis and final spermiation) are distinguished by a reduction in Fsh and an increase in production of luteinizing hormone (Lh) by the pituitary gland, which occurs together with an increase in progestins and other steroids before the release of mature spermatozoa (Schulz et al., 2010). These endocrine changes occur alongside a reduction of hypo-osmoregulatory abilities and specifically in gill Na^+, K^+-ATPase (NKA) activity in preparation for the pre-spawning upstream migration (Schulz et al., 2006; Shrimpton, 2013; Taranger et al., 2010). Conversely, smoltification is characterized among others by a rise in gill NKA activity in preparation for a downstream migration to seawater (Björnsson et al., 2011; McCormick et al., 1995; Stefansson et al., 2007). Thorpe (1986) considered these two processes in developmental conflict, which entails that early-maturing salmon may have osmoregulatory problems in seawater (Fjelldal et al., 2020, 2018; Taranger et al., 2010). However, under intensive rearing conditions, maturation and smoltification have also been found to commence simultaneously thus suggesting their compatibility (Fjelldal et al., 2011, 2018), but it was concluded that progressing maturation is likely to disrupt full development of hypo-osmoregulatory abilities (Fjelldal et al., 2018; Fraser et al., 2022).

Previous research has investigated the effects of temperature and photoperiod, separately and together, on male postsmolt maturation of Atlantic salmon. In a recent study, Pino Martinez et al. (2023) reported 100% of male postsmolts maturing early at 18 °C, displaying an early activation of the BPG in response to high temperature. In contrast, at 12.5 °C, early maturation had lower prevalence (~40%) and at 8 °C it did not occur. These findings are consistent with previous studies reporting higher levels of early maturation in fish reared at high temperatures (Fjelldal et al., 2018, 2011; Imsland et al., 2014; Melo et al., 2014), and with others that found links between high temperature and early maturation using a multivariable approach (McClure et al., 2007; Pino Martinez et al., 2021). High temperature can exert its influence on maturation not only by direct stimulation of the BPG, but also through promoting fast growth, developmental rate or energy accumulation (Jonsson et al., 2013). Rearing salmon at high temperature up to an optimum of around 14 °C leads to higher growth and appetite (Handeland et al., 2008), and thus, at high temperature salmon can accrue resources for maturation earlier (Jonsson et al., 2013). The influence of photoperiod on early maturation in freshwater has been investigated on salmon parr (Berrill et al., 2006, 2003; Skilbrei and Heino, 2011) and postsmolts (Fjelldal et al., 2018, 2011; Fraser et al., 2022). In these studies, salmon experiencing a period of winter light conditions (either an artificial 6-week LD12:12 winter signal, or during a simulated natural photoperiod) followed by a switch to constant light conditions, displayed higher proportion of maturing males. Furthermore, results from Fjelldal et al. (2011) evidence the crucial role of the photoperiod change from winter to spring as a signaling cue for maturation in fish kept under stimulatory conditions for development (at high temperature). In their study, male salmon kept at 16 °C that experienced a change to continuous light (from LD12:12 to LD24:0) matured at remarkable percentages (47%). On the contrary, those exposed to a switch from LD12:12 to LD18:6 and 16 °C, and those experiencing LD12:12 to LD24:0 but at lower temperature (5 or 10 °C), did not mature. Recently, Fraser et al. (2022) have reported similar findings, concluding that using a 3-h scotophase (LD21:3) instead of constant light (LD24:0) after the winter signal is sufficient to significantly reduce the proportion of maturing males from 62% to 19%.

The previous studies evidence the relevance of water temperature and photoperiod for early maturation of Atlantic salmon. However, further investigation is needed to provide more clarity on how intensive manipulation of both variables together influence the occurrence and endocrine regulation of postsmolt maturation and their osmoregulatory performance. The aim of this study was thus to examine the presence of male postsmolt maturation and the evaluation of physiological markers of maturation and smoltification over time under different constant temperatures (12.5 °C and 15 °C) and photoperiod regimes (constant light LD24:0, use of LD12:12 winter signal) commonly used in modern aquaculture.

2. Materials and methods

2.1. Ethic statement

The authors confirm that the ethical policies of the journal, as per the journal's author guidelines page, have been adhered to. This study was approved by the local representative of Animal Welfare at the Department of Biological Sciences, University of Bergen (Norway) as an aquaculture study with standard rearing conditions (FOTS application ID26676). Samplings were performed as established by the Norwegian Animal Research Authority.

2.2. Experimental setup

The study was carried out from 28 October 2019 to 7 May 2020 at the flow-through facilities of the Department of Biological Sciences (BIO, Bergen, Norway). It took place in freshwater, and consisted on a 2 × 2 factorial design with two temperatures (12.5 and 15 °C) and two photoperiods (one group in continuous light LD24:0 or LL, and another that received a 5-week LD12:12 winter signal or WS). This produced four experimental groups (15-LL, 15-WS, 12.5-LL and 12.5-WS, see Fig. 1)

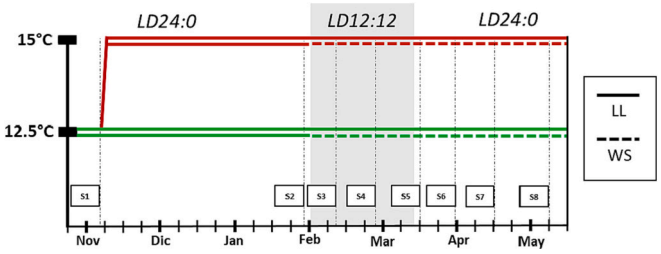

Fig. 1. Diagram displaying experimental conditions in the four experimental groups, including temperature, photoperiods and timing of the eight samplings performed. The experiment started in late October 2019 when fish mean weight was 52.1 ± 5.2 g, and finished with the last sampling on early May 2020. The samplings performed are serially labeled from 1 to 8. The timing at which the winter signal was introduced in a pair of tanks of each temperature is represented by the shaded area.

that were reared in duplicate tanks.

On 28 October 2019, 1000 Atlantic salmon parr (mean weight 52.1 ± 5.2 g) of Salmobreed strain (Erfjord Stamfisk AS) and mixed sex, were transferred from a commercial RAS facility (Bremnes Seashore AS, Trovåg, Rogaland, Norway) and randomly distributed among 8 tanks of 0.5 m^3 ($n = 125$/tank). Until transfer, fish had been kept under constant light (LD24:0) from first feeding following standard production protocols in the RAS. After transfer, initial conditions included water temperature 12.5 °C, specific flow rate 0.5 L × kg^{-1} × min^{-1} and LD24:0 photoperiod. Fish were allowed to acclimate for a week, after which temperature was changed to 15 °C in four tanks, keeping the rest at 12.5 °C. From 1 February to 8 March 2020, a 5-week LD12:12 winter signal was introduced in two tanks at 12.5 °C and two tanks at 15 °C, with the other four kept under LD24:0 until the end of the experiment in May. Light in the tanks was provided by BLV halogen tungsten bulbs (12 V, 35 W) that produce warm white light but with higher relative presence of yellow and red wavelengths. All tanks were covered with opaque lids that impaired any external light penetrating inside, and bulbs were attached to an opening in the center of the lid to equally distribute the light in the tank. The room lights were switched off during the dark hours of the winter signal period. The experimental room was always closed to prevent disturbance to fish and light penetration from the external corridors. All groups were fed a full ration throughout the experiment, using Biomar CPK 40® and CPK 100® feeds provided by Bremnes Seashore AS. Feed was supplied using conveyor-belt feeders over a 24 h cycle except during the winter signal, when feed was supplied over a 12 h cycle in all treatments.

2.3. Sampling regime

Eight samplings were performed during this experiment (Fig. 1). In sampling 1, when all tanks were at 12.5 °C, only 12 males were collected. In sampling two, before any photoperiod change, 12 males were collected from tanks at 12.5 °C and 12 males from tanks at 15 °C. In the remaining six samplings, we aimed to collect 6 males per tank (12 per treatment). However, the final N varied between 10 and 16 males per treatment and sampling depending on the ratio male/female obtained, in order to maintain a similar stocking density. The exact number of males and females extracted per treatment in each sampling is displayed in Table 1. Fish were sacrificed with an overdose of benzocaine (Benzoak vet.® 20%, ACD Pharma AS, Norway) higher than 50 mg/L by bath. Blood was collected from the caudal vein using heparinized syringes, and centrifuged 4 min at 5000 rpm and 4 °C. Plasma was immediately frozen in dry ice and used for 11-KT analyses. Fork length and body weight were measured to the nearest 0.1 cm and g respectively. Fish were dissected and gonads examined to determine sex and degree of maturation, keeping only the males. Testes were excised and weighed to the nearest 0.001 g. The first gill arch from the right side was kept in SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) to analyze Na^+, K^+, ATPase activity (NKA). The pituitary gland was excised, kept in RNAlater® and incubated for 24 h at 4 °C. All tissues were kept at −80 °C until analysis. Condition factor (K) was calculated as $K = W \times 100/L^3$ (W = body weight in grams, L = fork length in cm). Gonadosomatic index was calculated as $GSI(\%) = W_{gonad} \times 100/W_{body}$ and used as an index of maturity status. All samples ($n = 320$) were used for morphometric and lab analyses.

2.4. Lab analyses

2.4.1. 11-Ketotestosterone analysis

Sex steroids were first extracted in a two-step process following a method modified from Pankhurst and Carragher (1992), and then determined by enzyme-linked immunosorbent assay (ELISA) as in Cuisset et al. (1994). ED80 and ED20 standard values were 0.04 ng × mL^{-1} and 1.00 ng × mL^{-1} respectively, while the detection limit of the assay was 0.005 ng × mL^{-1}. Samples with concentrations above the highest standard were diluted and re-run. Internal 11-KT standards were prepared using plasma from mature male Atlantic salmon previously extracted. Assays with a CV > 10% for 11-KT were repeated. Standard steroids were purchased from Sigma Aldrich®, while acetylcholine esterase-labeled tracers and microplates precoated with monoclonal mouse antirabbit IgG were obtained from Cayman Chemicals (USA).

Table 1
Number of total male individuals, maturing males, and females collected from each treatment in the different samplings.

		15-WS			15-LL			12.5-WS			12.5-LL		
Sampling	Date	N male	N maturing	N female	N male	N maturing	N female	N male	N maturing	N female	N male	N maturing	N female
S1	2019-11-11	–	–	–	–	–	–	–	–	–	**12**	**0**	**10**
S2	2020-01-28	–	–	–	**12**	**0**	**11**	–	–	–	**12**	**0**	**8**
S3	2020-02-11	**12**	**0**	**9**	**12**	**0**	**7**	**12**	**0**	**11**	**11**	**0**	**12**
S4	2020-02-25	**12**	**0**	**9**	**11**	**1**	**16**	**12**	**0**	**10**	**13**	**0**	**9**
S5	2020-03-15	**13**	**1**	**11**	**10**	**4**	**20**	**12**	**0**	**12**	**13**	**1**	**8**
S6	2020-03-29	**11**	**0**	**26**	**12**	**4**	**9**	**10**	**1**	**13**	**12**	**0**	**12**
S7	2020-04-16	**16**	**13**	**8**	**12**	**7**	**11**	**12**	**4**	**9**	**12**	**1**	**9**
S8	2020-05-07	**12**	**12**	**16**	**12**	**9**	**11**	**12**	**6**	**25**	**12**	**3**	**15**

2.4.2. Gene transcription of pituitary fshb and lhb

Real-time PCR (RT-PCR) was used to analyze relative transcription of pituitary genes *fshb and lhb*, calculated with the efficiency-corrected method using *ef1a* as reference gene. Pituitary total RNA extraction, cDNA synthesis, and RT-PCR were performed exactly as in Pino Martinez et al. (2023), using the same oligos, however in this case 385 ng of total RNA were reverse-transcribed to cDNA.

2.4.3. Na^+, K^+, ATPase activity in gills

Na^+, K^+, ATPase activity (NKA) was determined following the method from McCormick (1993). This assay is based on the hydrolysis of ATP to ADP in an ouabain-sensitive protein fraction obtained from gills. The reaction is enzymatically linked to the oxidation of NADH to NAD^+ by pyruvate kinase and lactic dehydrogenase, and is performed with and without ouabain, a potent NKA inhibitor. The difference in ATP hydrolysis in presence and absence of ouabain is measured for 10 min at 25 °C and 340 nm in a Tecan Spark® multimode microplate reader, and expressed as μmol ADP × mg protein^{-1} × hour^{-1}.

2.5. Statistical analyses

Fisher's Exact Test for count data was used to find significant differences in proportion of maturation between temperatures and photoperiods in the whole experiment and at each sampling point. For statistical models, distribution of response variables was checked with Shapiro-Wilks test and outliers were explored using boxplots. Linear Mixed Effects models (LME) were fitted between response variables and the predictors "temperature", "photoperiod", "time" and their two-way interactions as fixed effects, and "tank" as random effect, in order to account for random variance between tank replicates (see Supplementary Table 1). Model residuals were checked graphically to assess assumptions such as normality (with q-q plots), linearity (residuals vs fitted plots), homogeneity of variance (scale-location plots) and influential outliers (residuals vs Leverage plots with Cook's distance). Homogeneity of variance was also checked with Levene's test. When assumptions were not met, the response variable was log-, square-root- or inverse-transformed (Supplementary Table 1), the model repeated, and assumptions re-checked. Significant models were followed by Tukey HSD post-hoc tests to find significant differences in the response variable between pairwise groups (those sharing one experimental condition, either temperature or photoperiod) at each sampling (explained in the results section and summarized in Table 2), and within groups over time (explained in the results section and displayed in the graphs with asterisks). Plots of response variables over time for each treatment display mean ± standard error. A significance level $\alpha = 0.05$ was used. All statistical analyses were performed in R and Rstudio, using the packages "car" (Fox and Weisberg, 2019), "ggplot2" (Wickham, 2016), "ggpubr" (Kassambara, 2017), "Rmisc" (Hope, 2013), "emmeans" (Lenth et al., 2018), "lme4" (Bates et al., 2015), and "nlme" (Pinheiro et al., 2017).

GSI > 0.06% was set as threshold for maturation exactly as in Pino Martinez et al. (2023). Ilustration of the two maturity categories based on GSI distribution and outliers is displayed in Supplementary Fig. 1. Individuals from different treatments and with different GSI (mature and immature) are displayed in Supplementary Fig. 2.

3. Results

3.1. Percentage of maturation

The percentage of maturing males in each group over time is displayed in Fig. 2. Overall proportion of maturation in the experiment was significantly dependent on temperature ($p < 0.001$) but not on photoperiod. After pooling by temperature, the proportion of maturation was significantly higher at 15 °C than at 12.5 °C in the last two samplings (both $p < 0.001$). No significant differences in proportion of maturing males occurred between photoperiods at any sampling. Sexual maturation appeared first in males from 15-LL in late February (9% of males), and continued increasing until early May (75%). In this group the process was not synchronized among individuals, with males displaying either mid-advanced maturity or totally immature testes. In 15-WS, the process commenced later (in mid-April after the winter signal) but very synchronized among individuals, reaching a 100% of males maturing in May which all displayed a similar advanced degree of testis development. In 12.5-WS, male maturation appeared in a similar trend to 15-WS but in a lower proportion (50% of males in May). Finally, the lowest percentage of maturation was found in 12.5-LL, with 25% of the males maturing in early May. In this group as in 15-LL, those males engaged in maturation displayed mid-advanced testis development, however the majority were totally immature. No maturing individual was found producing milt. Percentage of maturation in the two replicate tanks of each treatment is presented in Supplementary Table 2. Maturation was present in males over 250 g at both temperatures, but salmon at 15 °C reached higher GSI at smaller size than fish at 12.5 °C (Supplementary Fig. 3).

3.2. Morphometrics and GSI

Body weight (Fig. 3A) was significantly dependent only on time ($p < 0.001$) and there were no significant differences between any experimental group at any time point (Table 2).

Condition factor (Fig. 3B) was also significantly dependent only upon time ($p < 0.001$). Significant differences between pairwise groups occurred in late March among 15-LL and 15-WS ($p < 0.01$), and in mid-April between both 15-WS and 12.5-LL versus 12.5-WS (both $p < 0.05$) (see Table 2). Over time, significant changes occurred in all groups (described in caption Fig. 3B but not presented in graph to improve visualization). A comparison of external appearance (size, body condition, coloration) between individuals from the four treatments in early May is shown in Supplementary Fig. 4.

GSI (Fig. 3C) was significantly dependent upon temperature, time and the interaction temperature×time (all $p < 0.001$). Significant differences between paired groups occurred in mid-April and early May. In both sampling dates, GSI was larger in 15-LL than in 12.5-LL (both $p < 0.05$), and larger in 15-WS than in 12.5-WS (both $p < 0.05$). GSI did not differ in any of these two samplings between LL and WS groups at each temperature (see Table 2). Large variability (SE) of GSI was observed in both LL groups when maturation commenced, while in both WS groups GSI variability was small even after onset of maturation. Over time, significant variations in GSI occurred in all groups. In 15-LL, a first increase in GSI occurred from early February to mid-March ($p < 0.05$), continuing with an increase until early May ($p < 0.05$). The increases in 15-LL occurred with large intra-sampling variability. In contrast, in 15-WS, the large increase in GSI took place after the winter signal, from late March to mid-April ($p < 0.001$) and then to early May ($p < 0.05$), with little intra-sampling variability. In 12.5-WS, GSI displayed a similar but less pronounced increase than the one in 15-WS, with a significant increase from late March to early May ($p < 0.01$). Finally, in 12.5-LL a significant increase in GSI was only apparent from late February to early May ($p < 0.01$) and, as in 15-LL, with large intra-sampling variability.

3.3. Plasma 11-Ketotestosterone concentration and gene transcription of pituitary fshb and lhb

Plasma 11-KT concentration (Fig. 4A) was significantly dependent on photoperiod ($p < 0.05$), time ($p < 0.001$) and the interactions photoperiod×time ($p < 0.01$) and temperature×time ($p < 0.001$). Significant differences between paired groups occurred in mid-March and mid-April (Table 2). In mid-March, plasma 11-KT was higher in 15-LL than 15-WS ($p < 0.05$), while in mid-April, 11-KT levels were higher in 15-LL than in 12.5-LL ($p < 0.05$). Over time, significant differences occurred in all treatments except 12.5-LL. In 15-LL, mean plasma 11-KT levels increased gradually, but the only significant rise occurred from late

Table 2
P-values resulting from the pairwise Tukey HSD post-hoc tests after each of the seven statistical models performed. Significant effects ($p < 0.05$) are displayed in red, while in brown are shown values $0.05 < p < 0.1$ that may suggest certain biological trends.

Weight

Sampling	WS 12.5°C - 15°C	LL 12.5°C - 15°C	12.5°C LL - WS	15°C LL - WS
2	0.885	0.244	0.514	0.452
3	0.124	0.701	0.984	0.198
4	0.473	0.407	0.737	0.655
5	0.220	0.692	0.803	0.514
6	0.729	0.947	0.458	0.266
7	0.140	0.857	0.132	0.897
8	0.389	0.500	0.195	0.887

Condition factor

Sampling	WS 12.5°C - 15°C	LL 12.5°C - 15°C	12.5°C LL - WS	15°C LL - WS
2	0.309	0.893	0.856	0.291
3	0.145	0.889	0.428	0.491
4	0.435	0.663	0.575	0.509
5	0.639	0.520	0.484	0.675
6	0.111	0.527	0.544	0.025
7	0.038	0.234	0.044	0.198
8	0.813	0.150	0.217	0.601

GSI

Sampling	WS 12.5°C - 15°C	LL 12.5°C - 15°C	12.5°C LL - WS	15°C LL - WS
2	0.939	0.700	0.951	0.773
3	0.216	0.557	0.207	0.590
4	0.624	0.826	0.469	0.205
5	0.499	0.142	0.868	0.056
6	0.465	0.173	0.852	0.056
7	0.020	0.016	0.720	0.923
8	0.016	0.027	0.155	0.078

11-KT

Sampling	WS 12.5°C - 15°C	LL 12.5°C - 15°C	12.5°C LL - WS	15°C LL - WS
2	0.675	0.067	0.083	0.811
3	0.199	0.924	0.541	0.392
4	0.180	0.631	0.327	0.098
5	0.278	0.869	0.060	0.025
6	0.506	0.499	0.311	0.779
7	0.331	0.036	0.167	0.764
8	0.133	0.153	0.539	0.472

fshb

Sampling	WS 12.5°C - 15°C	LL 12.5°C - 15°C	12.5°C LL - WS	15°C LL - WS
2	0.371	0.575	0.944	0.820
3	0.965	0.268	0.423	0.677
4	0.629	0.235	0.670	0.081
5	0.939	0.140	0.792	0.094
6	0.767	0.062	0.853	0.119
7	0.096	0.012	0.173	0.628
8	0.026	0.065	0.518	0.175

lhb

Sampling	WS 12.5°C - 15°C	LL 12.5°C - 15°C	12.5°C LL - WS	15°C LL - WS
2	0.854	0.308	0.295	0.823
3	0.980	0.465	0.965	0.419
4	0.231	0.906	0.945	0.302
5	0.798	0.901	0.746	0.849
6	0.147	0.948	0.762	0.099
7	0.558	0.053	0.933	0.118
8	0.124	0.178	0.901	0.702

NKA activity

Sampling	WS 12.5°C - 15°C	LL 12.5°C - 15°C	12.5°C LL - WS	15°C LL - WS
2	0.149	0.105	0.362	0.254
3	0.604	0.029	0.688	0.074
4	0.042	0.018	0.556	0.798
5	0.056	0.022	0.679	0.536
6	0.224	0.086	0.845	0.403
7	0.125	0.182	0.685	0.914
8	0.163	0.706	0.420	0.707

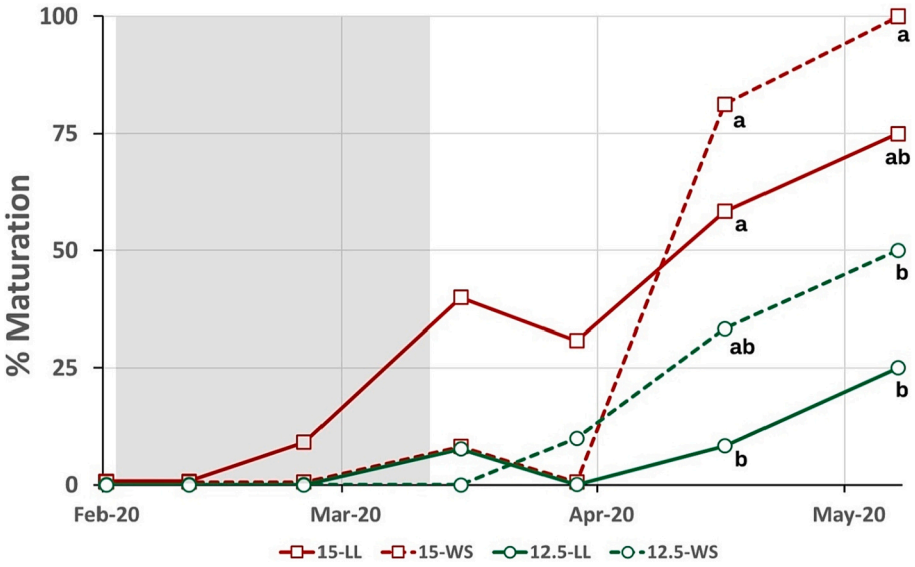

Fig. 2. Percentage of males maturing found in each treatment over time for all samplings except the first. GSI > 0.06% was the threshold value used to determine the proportion of maturing males. The shaded area represents the 5-week period (from 1 February to 8 March) in which LD12:12 was introduced in the WS groups. Significant differences ($p < 0.05$) in proportion of maturing males between treatments at given samplings are displayed with letters "a" and "b".

March to mid-April ($p < 0.05$). In the two WS groups this increase occurred after the winter signal from mid-March to mid-April ($p < 0.001$ for 15-WS and $p < 0.05$ for 12.5-WS).

Pituitary *fshb* transcript levels (Fig. 4B) were significantly dependent on temperature ($p < 0.001$), photoperiod ($p < 0.05$), time ($p < 0.001$) and the interactions temperature×photoperiod ($p < 0.05$) and temperature×time ($p < 0.01$). Significant differences between paired groups occurred in mid-April, when *fshb* transcript levels were higher in 15-LL than in 12.5-LL ($p < 0.05$), and early May, with higher *fshb* levels in 15-WS than in 12.5-WS ($p < 0.05$) (Table 2). Variability of this marker was similar to the one of GSI, high in both LL groups when maturation was first observed, and smaller in both WS groups. Over time, significant changes were present in the four treatments, but at different times and intensity. In 15-LL, there was a gradual increase significant from late February until mid-April LL ($p < 0.05$). In 12.5-LL, the rise was only significant from early February to early May ($p < 0.05$). In 15-WS, *fshb* transcript levels increased abruptly and synchronized among individuals after the winter signal from late March to early May ($p < 0.001$), while in 12.5-WS this occurred from late February to early May ($p < 0.01$).

Pituitary *lhb* transcription (Fig. 4C) was significantly dependent on time ($p < 0.001$) and interaction temperature×time ($p < 0.01$). No significant differences occurred between treatments at any sampling (Table 2). An over time increase occurred gradually in 15-LL from late February to mid-April ($p < 0.05$), and after the winter signal in 15-WS, from late March to early May ($p < 0.001$).

3.4. Gill NKA activity

Gill NKA (Fig. 5) activity was significantly dependent on temperature ($p < 0.001$), time ($p < 0.001$) and the interaction temperature×time ($p < 0.05$). No significant differences in NKA activity occurred between different photoperiods at the same temperature, but large variability (SE) in NKA activity occurred at 12.5 °C in late January. Significant differences among groups were observed between temperatures at three samplings. In early February, gill NKA was higher in 12.5-LL than 15-LL ($p < 0.05$). In late February, NKA activity was higher in the two 12.5 °C groups than in the two 15 °C (both $p < 0.05$). Finally, in mid-March NKA activity was higher in 12.5-LL than in 15-LL ($p < 0.05$), but not different in the two WS groups (Table 2). Over time, significant decreases occurred in 12.5-LL from mid-March to early May ($p < 0.05$), in 12.5-WS from late January to early May ($p < 0.05$) and in 15-WS from early February to mid-March ($p < 0.05$). All early maturing individuals (GSI > 0.06%) displayed consistently low NKA activity (Supplementary Fig. 5 (GSI (%) vs NKA activity)).

4. Discussion

Both high temperature and the use of a winter signal photoperiod contributed to male maturation at postsmolt size, but they played different roles. According to our results, high temperature was the main factor promoting early sexual maturation, as previously reported in Pino Martinez et al. (2023), Imsland et al. (2014) or Melo et al. (2014), while the switch from short to long day acted as the entraining environmental cue triggering the process. The promoting effect of high temperature was first evident by the higher proportion of maturation and higher GSI in both 15 °C groups than in both 12.5 °C groups throughout the experiment. The entraining effect of the photoperiod switch was revealed by the different patterns of testis development observed in both WS groups (synchronized among individuals), compared to the LL groups (diverging over time between fish highly mature or totally immature). The synchronized onset of maturation observed in both WS groups after the photoperiod switch explains the greater proportion of maturing fish found in these groups in contrast to their corresponding temperature groups kept in LL.

The high individual variability observed in GSI (large SE) in 15-LL in every sampling from late February, reveals a diverging sexual development response in that group, with males found at either mid-advanced maturity stages (high GSI) or totally immature (very low GSI). Additionally, mean GSI of maturing fish in 15-LL was greater than in 15-WS, despite the larger proportion of individuals engaged in maturation in 15-WS from mid-April (see Fig. 2A). This reveals that gonad development in individuals already maturing at 15 °C was more advanced under constant light than after receiving a winter signal. Similar findings were reported by Schulz et al. (2006), who observed a lower proportion of maturing individuals under LL than under natural photoperiod, but those maturing in LL had higher GSI. This study also showed a dichotomical response in testis development in LL, with fish either very mature vs totally immature, evidencing lack of synchrony in initiation of maturation. This suggests that exposure to high temperature alone, in absence of photoperiod cue, can lead to a "spontaneous" or non-synchronized onset of maturation in many male individuals (as high as 75% in our study in 15-LL in May), as previous authors have also

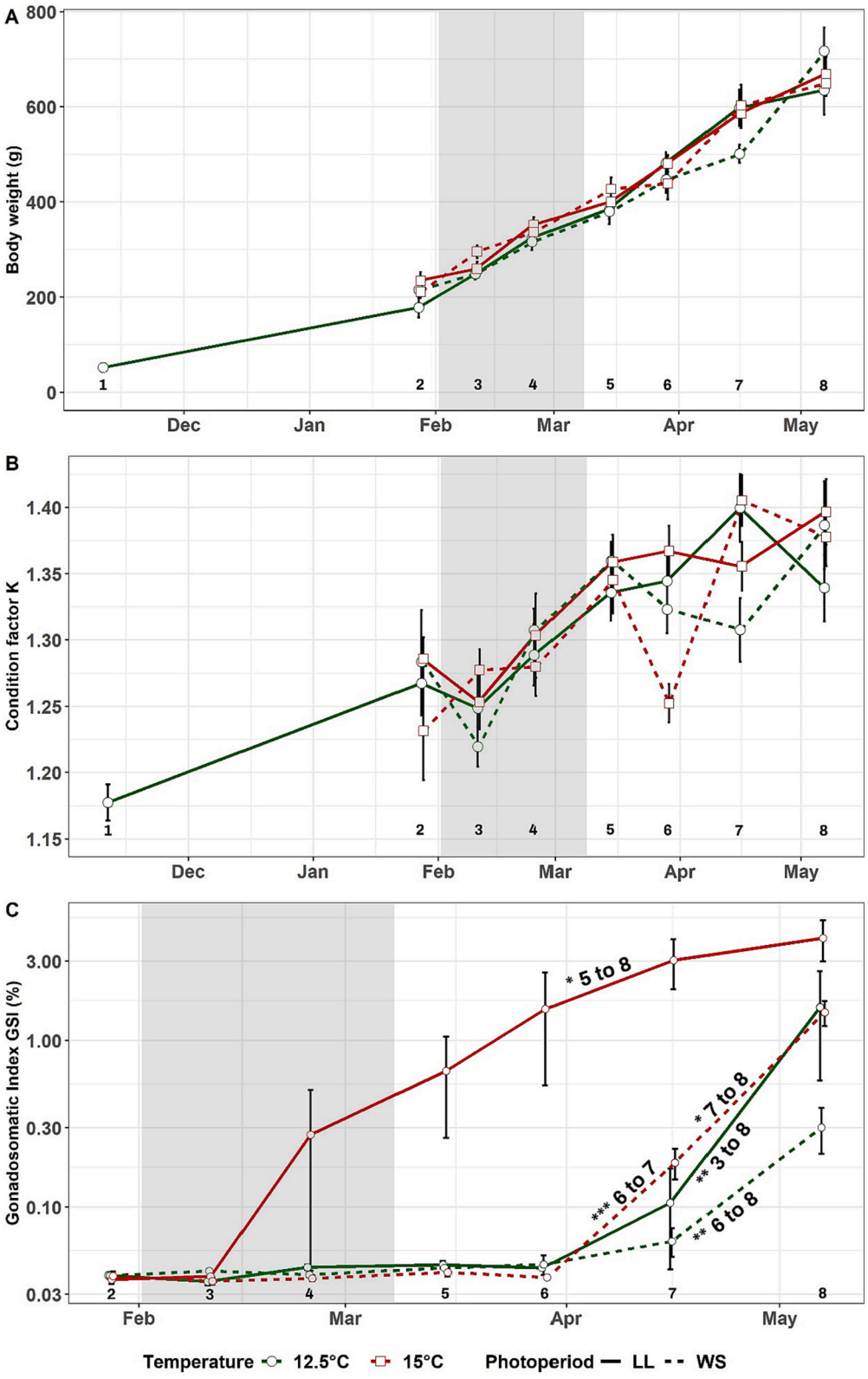

Fig. 3. Body weight (A), condition factor (B) and gonadosomatic index (C) over time in the four experimental groups. In (A) and (B), the response is displayed for the eight samplings, while in (C) the first sampling has been removed for better visualization. The "Y" axis in the GSI figure (C) is displayed in logarithmic scale. Numbers from 1 to 8 at the bottom of the three graphs represent the sampling number and aid with explanation of significant differences over time within each group. Significant differences between experimental groups at given samplings are explained in the results section, and presented in Table 2. Significant differences over time are displayed with asterisks as follows: (*) p-value<0.05, (**) p-value <0.01, (***) p-value<0.001. This significance code is located next to the corresponding line, and is followed by the sampling numbers between which such difference occurred. In plot (B), significant changes over time are not displayed in the graph, and are described here: ** in 12.5-LL from 4 to 7; ** in 12.5-WS from 3 to 5; * in 15-LL from 3 to 5; * in 15-WS from 2 to 5, and *** from 6 to 7. The shaded area in all graphs represents the 5-week period (from 1 February to 8 March) in which LD12:12 was introduced in the WS groups.

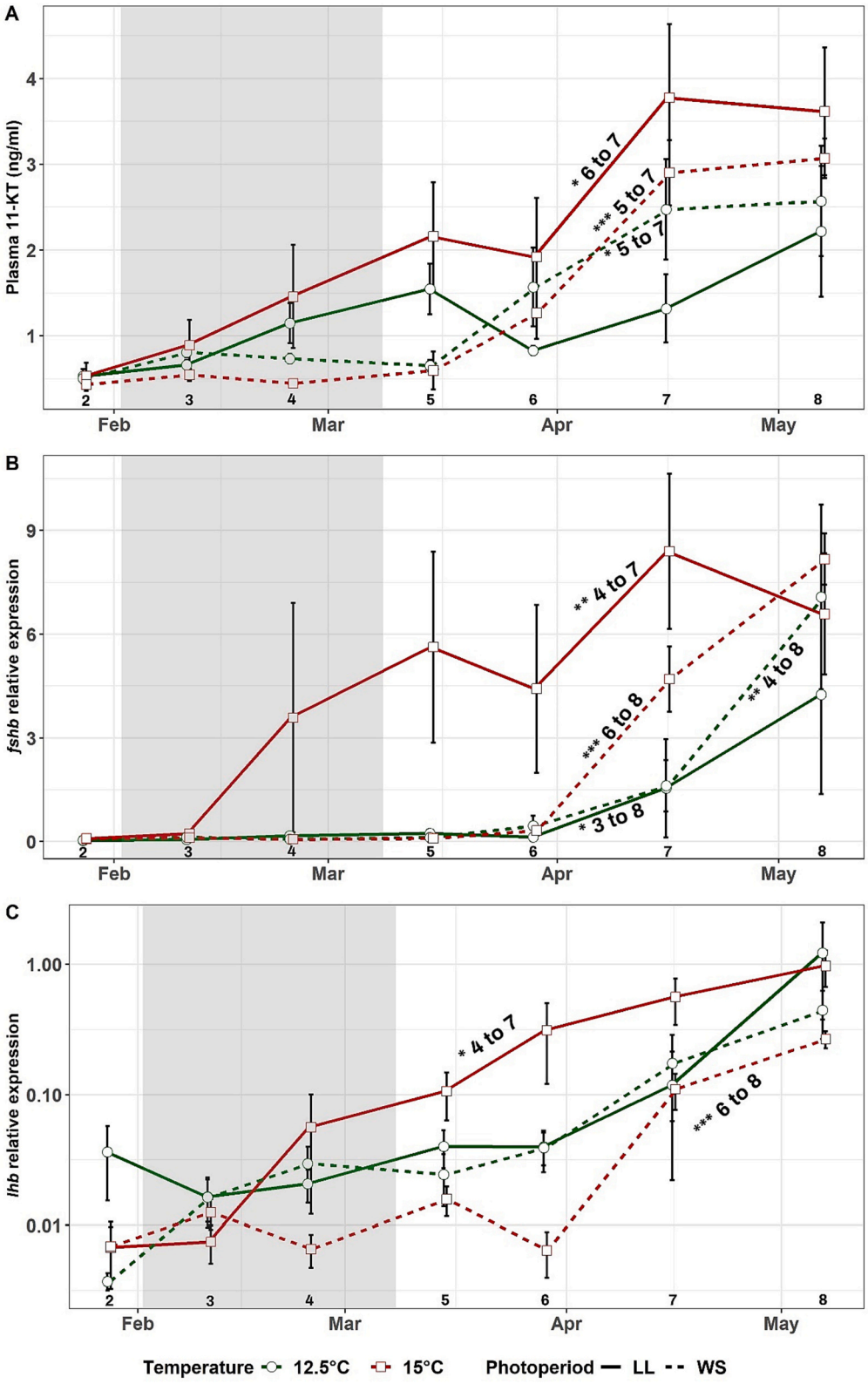

Fig. 4. Plasma 11-KT concentrations (A), *fshb* relative expression (B) and *lhb* relative expression (C) over time in the four experimental groups. Only samplings 2–8 are displayed for better visualization. The "Y" axis in the *lhb* (C) figure is displayed in logarithmic scale. Numbers from 2 to 8 at the bottom of the three graphs represent the sampling number and aid with explanation of significant differences over time within each group. Significant differences between experimental groups at given samplings are explained in the results section, and presented in Table 2. Significant differences over time are displayed with asterisks as follows: (*) p-value<0.05, (**) p-value <0.01, (***) p-value<0.001. This significance code is located next to the corresponding line, and is followed by the sampling numbers between which such difference occurred. The shaded area in all graphs represents the 5-week period (from 1 February to 8 March) in which LD12:12 was introduced in the WS groups.

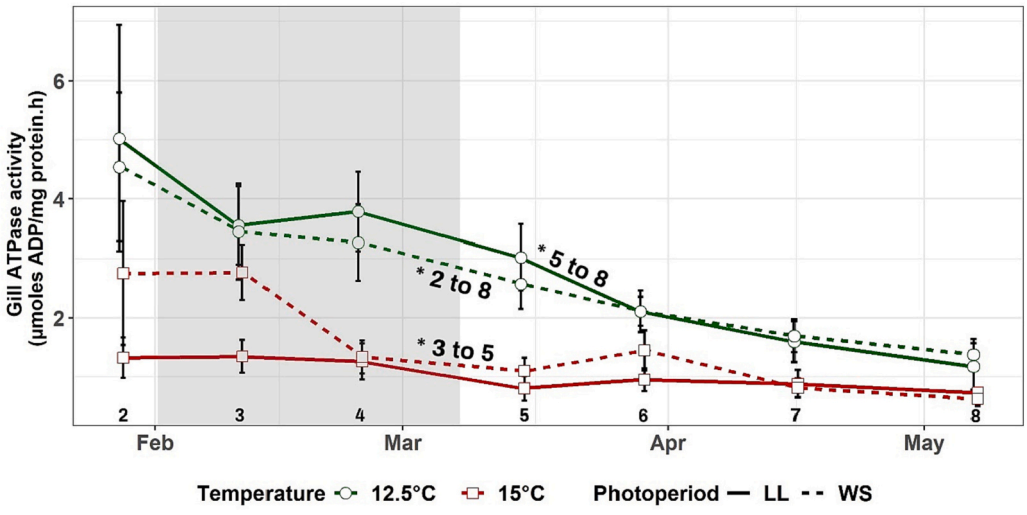

Fig. 5. Gill NKA activity over time in the four experimental groups. Only samplings from 2 to 8 are displayed since gill samples were not collected in sampling 1. Numbers from 2 to 8 at the bottom of the graph represents the sampling number and aid with explanation of significant differences over time within each group. Significant differences between experimental groups at given samplings are explained in the results section, and presented in Table 2. Significant differences over time are displayed with asterisks as follows: (*) p-value<0.05, (**) p-value <0.01, (***) p-value<0.001. This significance code is located next to the corresponding line, and is followed by the sampling numbers between which such difference occurred. The shaded area represents the 5-week period (from 1 February to 8 March) in which LD12:12 was introduced in the WS groups.

stated (Fjelldal et al., 2018; Imsland et al., 2014). In contrast, the low GSI variability (low SE) observed in 15-WS in April suggests a highly synchronized onset of maturation in response to the switch in photoperiod from LD12:12 to LD24:0. In terms of light regime, this group could be considered "equivalent" to the one under natural photoperiod in the mentioned study of Schulz et al. (2006), where all males matured but displaying slower testis development than those that matured in LL because they had commenced later in response to the photoperiod cue. Similar trends were observed respectively in 12.5-LL (spontaneous maturation in some males and high variability of GSI) and 12.5-WS (synchronized testis development), but proportion of maturation in these two groups was overall lower due to the lower temperature. This synchronized patterns of gonad development in the WS groups in contrast to the LL groups clearly evidence the role of the winter signal as an entraining environmental cue for maturation, as suggested by previous authors (Fjelldal et al., 2011; Fraser et al., 2022; Taranger et al., 2010).

Endocrine markers of sexual maturation (gene transcription of pituitary *fshb* and plasma 11-KT levels) were clearly aligned with patterns described for GSI and percentage of maturation in each treatment. Onset of maturation is characterized by increases in production of Fsh and 11-KT (Maugars and Schmitz, 2008; Melo et al., 2014; Nóbrega et al., 2015; Schulz et al., 2010, 2019), and these occurred in the four treatments but differently. In 15-LL, mean levels of pituitary *fshb* expression and plasma 11-KT highly resembled the GSI figure, increasing the earliest and displaying the highest levels of all groups but with the largest intra-sampling variability (see Fig. 4A and B). This reflects that in 15-LL, the neuroendocrine activation triggering the onset of maturation in many males was asynchronous and only driven by internal signals in maturing individuals, not the response to any specific environmental cue. There were males in 15-LL in each sampling that remained fully immature (as in Schulz et al., 2006), evidencing that high temperature alone was not sufficient to promote "spontaneous" maturation in all individuals. This suggests that in the absence of entraining cue, genetic determination may play a crucial role (Ayllon et al., 2015; Fjelldal et al., 2020), an aspect discussed in following paragraphs. A similar response in terms of high individual variability in GSI and *fshb* transcription at onset of maturation was observed in 12.5-LL, evidencing an asynchronous activation of the BPG only in some males of this group, but delayed in comparison to 15-LL. Collectively, the observed patterns in the two LL groups reveal, first, the strong effect of high temperature on activating the physiological onset of sexual development, and second, that a switch to a longer daylength is not necessary to trigger this process (Fjelldal et al., 2011; Imsland et al., 2014), and internal signs can be sufficient. In contrast to the LL groups, pituitary *fshb* transcription and plasma 11-KT in both WS groups displayed highly synchronized increases (smaller intra-sampling variability), with higher intensity at 15 °C than at 12.5 °C. These findings are aligned to those described above for GSI and add support to the role of the photoperiod switch as a *zeitgeber* (Bromage et al., 2001; Fjelldal et al., 2011; Fraser et al., 2022; Taranger et al., 2010) that triggers the neuroendocrine cascade leading to initiation of spermatogenesis (Schulz et al., 2010; Zohar et al., 2010).

The reason why onset of maturation in LL occurred spontaneously only in some individuals and not in others, despite having experienced the same environmental conditions, might be linked to genetic differences (Ayllon et al., 2015; Fjelldal et al., 2020) that can cause differences in physiological thresholds for maturation within a salmon population (Herbinger and Newkirk, 1990; Thorpe et al., 1998). Ayllon et al. (2015) reported the existence of the gene *vgll3* in Atlantic salmon that to a high extent controls age at maturation in wild and domesticated salmon. Based on this, Fjelldal et al. (2020) established early and late maturing genotypes of *vgll3*, and showed higher porportion of postsmolt maturation in homozygous fish for the early maturing genotype than the in the other two genotypes (heterozygous and homozygous for the late maturing genotype). This suggests that within salmon populations, even if they are domestic and selected for late maturation, there may always be a percentage of individuals that genetically are more determined to mature early than the rest. This genetic determination to mature early is likely modulated by the environment (temperature, photoperiod, feed) via growth (Thorpe et al., 1998), which means that under stimulating rearing conditions, even salmon with lower genetic determination may initiate maturation. This is supported by conclusions in Ayllon et al. (2019), who stated that the impact of the *vgll3* locus on age at maturation is most likely modulated by the rearing conditions. Thus, intensive aquaculture conditions that promote fast development can stimulate the early initiation of maturation in fish that are not that highly determined according to their genetic background. Our results in both LL groups, where a "spontaneous" onset of maturation occurred, are aligned with this view. Despite these two groups reaching similar weight/size, the proportion of maturing males was lower in 12.5-LL (25%) than in 15-LL (75%). This suggests that in 12.5-LL early maturation must have commenced only in individuals with the lowest thresholds for maturation (this is, in those highly determined to mature). On the contrary, in 15-LL, the strong stimulatory effect of high temperature most likely

modulated the influence of genetic determination on early maturation, resulting in a larger proportion of males maturing, some of which may have similar or even lower genetic determination to mature than counterparts in 12.5-LL. Overall this indicates that environmental conditions can modulate the effects of genetic background on early maturation, and thus the use in intensive aquaculture of late-maturation strains cannot guarantee that early maturation will not occur. The genetic background and physiology of salmon not maturing in 15-LL despite the highly stimulating conditions experienced could be worth further investigation, to gain deeper understanding of the role of genetics on early maturation, and with the aim to implement selective breeeding programs for late maturation strains.

The absence of running milt in maturing fish and the lack of differences in *lhb* expression between treatments at any time, indicates that maturation was not completed during the study, a finding also reported in Pino Martinez et al. (2023). Final stages of maturation are physiologically characterized among others by a peak in production of Lh (Maugars and Schmitz, 2008; Schulz et al., 2010). In our experiment, an increasing trend of *lhb* expression occurred in all groups, but it was only signficant in the two treatments at 15 °C. This suggests that fish reared at 15 °C may have entered late stages of maturation, but still required more time to complete the process. However, given that in nature salmon spawning occurs in fall when water temperature has commenced to decrease, high or moderate sustained temperature may represent an inappropriate environmental cue for final testis maturation. This is supported by findings from Taranger et al. (2003) and Vikingstad et al. (2016), who have reported that elevated water temperature can disrupt production of pituitary Lh and synthesis of sex steroid 17,20βP (DHP), both crucial to complete last stages of spermatogenesis and final release of spermatozoa (Schulz et al., 2010). Consequently, maturing salmon in this study may have experienced a disruption in the final stages of the maturation process, although this would not mitigate the potential harm that early maturation may cause in aquaculture settings due to decreased growth and higher tendency to complete maturation at the next opportunity (Fraser et al., 2019).

The specific physiological mechanism by which high temperature promoted early maturation is unclear from our results. However, we suggest that endocrine changes promoted by high temperature (Pankhurst and King, 2010) most likely led to higher stimulation of the BPG axis (Adams and Thorpe, 1989; Imsland et al., 2014; Pino Martinez et al., 2023) and higher availability of energy (Good and Davidson, 2016; Imsland et al., 2014; Jonsson et al., 2013). In regard to early stimulation of the BPG axis, Fleming et al. (unpublished data) have performed analyses on hypothalamus of males from the present study, observing that exposure to 15 °C resulted in a clear upregulation throughout the experiment of neuroendocrine factors controlling sexual maturation. This included significantly higher transcription of the kisspeptin receptor *gpr54* and of gonadotropin-releasing hormone *gnrha* at high temperature, irrespective of the photoperiod regime experienced (Fleming et al., unpublished data). Such findings reveal a temperature-induced activation of the brain areas that integrate internal and external signals influencing maturation (Taranger et al., 2010; Yaron and Levavi-Sivan, 2011; Zohar et al., 2010), and possibly evidence a mechanism by which high temperature could promote an early commitment to sexual maturation. In respect to growth or availability of energy, in the present study we found no differences in body weight among treatments and no differences in condition factor until late March (the main period for energy accummulation before initiation of maturation). Consequently the differences in proportion of maturation between treatments cannot be explained by differential somatic growth (as also suggested by Imsland et al., 2014), but to some extent by different energy availability at different temperatures. According to Handeland et al. (2008), salmon reared at 14–18 °C up to 120 g tend to display the highest appetite, and therefore tend to ingest more feed. Then, at higher temperature, it has been suggested that acquisition of surplus resources from feed is more efficient (Jonsson et al., 2013). As a result, salmon reared at higher temperature (15 °C) may have more available resources for maturation earlier, despite having acquired similar somatic growth or body size as those reared at 12.5 °C. This, combined with the stimulatory effect of high temperature on the reproductive axis, would lead to the high percentages of early maturation observed.

In the present study we could not explore if body size plays a role on early maturation as an absolute factor, first due to the lack of differences in weight betweent treatments, and second because the WS was introduced at similar size in all groups (at ~200 g). However, reaching a certain size when a switch in photoperiod to long day takes place is considered important for onset of maturation (Berrill et al., 2003; Fraser et al., 2022; Skilbrei and Heino, 2011). According to Fraser et al. (2019, based on unpublished data from T. Hansen), salmon that reached larger size after the winter signal showed higher tendency to mature during the smoltification period. Body size at a given time is the result of the developmental process undergone by the fish under the specific conditions of temperature, photoperiod and access to feed experienced. Consequently, reaching a certain size may act as a proxy for fulfillment of required energy thresholds for maturation as defined by Thorpe (2007), although such size threshold would necessarily be dependent upon the specific rearing conditions (specially temperature). In our study, size at introduction of the winter signal was around 200 g in all groups (see Fig. 2A). At this point, many salmon in the four treatments may have already fulfilled physiological thresholds for maturation. As a result, the photoperiod manipulation specially at high temperature simply triggered and synchronized its onset in many males. Assessing the existence of size thresholds for maturation under given rearing conditions could help further understand salmon requirements for early maturation to help avoid this issue.

Introducing a winter signal is a standard practice in salmon aquaculture to induce hypo-osmoregulatory abilities and prepare fish for seawater (Björnsson et al., 2000; Handeland et al., 2003; McCormick et al., 1995). However, signs of smoltification (higher values of gill NKA) were observed in some individuals of around 200 g at 12.5 °C in late January when all groups were still under continuous light. This indicates a "spontaneous" preparation for seawater in those individuals as part of their continuous development, without the entrainment of a photoperiod signal. Signs of smoltification such as increased NKA activity and reduced condition factor have been previously reported in Atlantic salmon as fish grow (Metcalfe, 1998; Pino Martinez et al., 2021; Saunders et al., 1994) regardless of photoperiod manipulation (Fjelldal et al., 2018). In fact, Imsland et al. (2014) stated that salmon can acquire smolt features such as elevated gill NKA activity before reaching 200 g regardless of the photoperiod regime experienced. However, despite the signs of increased salinity tolerance observed at 12.5 °C in late January, those groups afterwards displayed a clear decline in NKA activity under both photoperiod regimes, and showed a tendency to sexually mature; groups at 15 °C displayed instead very low NKA throughout the trial, and matured early in very high proportion. Based on this evidence, we suggest that development of seawater tolerance was impaired in all our groups by a combination of factors including sustained exposure to moderate/high water temperature, large size reached before the winter signal, and physiological conflict with ongoing endocrine regulation of early maturation. Discussion on how these factors may have acted is attempted following.

Relatively high temperature advances the parr-smolt tansformation (Handeland et al., 2004) but also induces a quick loss of smolt characteristics (Handeland et al., 2004; McCormick and Saunders, 1987); if too high, temperature can even impair normal development of seawater tolerance (McCormick et al., 1999; Stefansson et al., 2008). Based on this, we suggest that the mechanisms by which water temperature affected development of hypo-osmoregulatory abilities must have been different at 12.5 °C and at 15 °C. At 12.5 °C, the decreasing trend observed in NKA activity suggests that these fish may have been already out of their optimum "smolt window" (200–250 day-degrees after peak NKA activity) when they received the winter signal (Handeland et al.,

2004; McCormick et al., 1999). In addition, continuous exposure to 12.5 °C must have accelerated the loss of smolt characteristics (Handeland et al., 2004; McCormick et al., 1999; Stefansson et al., 1998), most likely enhanced by the lack of exposure to seawater (McCormick et al., 1987). As a result, salmon that had commenced smoltification by size at 12.5 °C subsequently de-smoltified (Duston et al., 1991; Fjelldal et al., 2018; Shrimpton et al., 2000), showing very low NKA activity by the end of the trial. According to Stefansson et al. (2008), de-smolted salmon typically lose hypo-osmoregulatory abilities as well as some external characteristics of smolts, but do not experience a full reversion to parr state. These de-smolted salmon have shown a rapid capacity to adapt their osmoregulatory functions if exposed to seawater (Fjelldal et al., 2018), but further research on their adaptability to salinity changes could be of interest, since the conditions producing most of these individuals (12.5-LL) also led to the lowest proportion of maturing males. In contrast, at 15 °C, the consistently low NKA activity observed under both photoperiods suggests a direct disruption of osmoregulatory functions by high temperature. Very high water temperatures can limit or impair the parr-smolt transformation and development of seawater tolerance in Atlantic salmon and other species (Stefansson et al., 2008). For example, in Chinook salmon (*Oncorhynchus tshawytscha*), suppresion of NKA activity has been found above 17 °C (Marine and Cech, 2004), while in steelhead trout (*Salmo gairdneri*) this has occurred above 13–15 °C (Zaugg, 1981). A recent study (Pino Martinez et al., 2023) has shown that Atlantic salmon reared at 18 °C displayed a very similar and low NKA activity profile as fish reared at 15 °C in the present study, thus adding support to the impairing effect of high temperature on smoltification. High temperature may cause direct decrease in NKA activity by reducing the number of gill ionocytes (through higher cell death or less renewal), or indirectly by altering endocrine regulation of this enzyme's functions (McCormick et al., 1999).

However, the endocrine processes regulating sexual maturation most likely also contributed to the poor development of hypo-osmoregulatory abilities observed particularly at 15 °C. Physiology of maturation can interfere with osmoregulatory changes during smoltification (Schulz et al., 2006, 2019; Taranger et al., 2010), due to high increase in circulating androgens (Lundqvist et al., 1990) and estrogens (Madsen et al., 2004; McCormick et al., 2005). In addition, the disruptive effects of maturation on the physiology of smoltification can extend for long periods of time (Lundqvist et al., 1990; Saunders et al., 1994). Consequently, initiation and development of sexual maturation most likely contributed to the low gill NKA activity observed at high temperature. However, smoltification and maturation have been observed sequentially (Berrill et al., 2003; Saunders et al., 1994), or even simultaneously (Fjelldal et al., 2018, 2011), which suggests that despite incurring in some type of developmental conflict, both processes can to some extent co-exist. This may appear contradictory to our results, since in our study, as in Pino Martinez et al. (2023), there seemed to be a mutually exclusive choice between smoltification and maturation as a life strategy. It might be that under some circumstances, both processes can commence simultaneously, for example when the rearing conditions experienced during the pre-smolt phase have not caused an early clear choice of life-history strategy. For example, conditions in Fjelldal et al. (2018) (early period at 12 or 5 °C, followed by an increase in temperature to 16 °C simultaneous with an increase in daylenght) could represent an environment that can result in the simultaneous onset of maturation and smoltification. On the contrary, conditions in our present study and in Pino Martinez et al. (2023) (sustained high temperature for months before the change in photoperiod) may illustrate those leading to a mutually-exclusive choice of life strategy. This early period at sustained high temperature may promote such a high stimulation of the BPG axis and faster accumulation of energy that maturation would gain priority over smoltification (Policansky, 1983; Thorpe, 1994; Thorpe et al., 1998). Conversely, a pre-smolt phase at lower temperature (for example 12 °C or 5 °C as in Fjelldal et al., 2018) may not represent such stimulatory conditions for sexual development, thus not inducing such a clear early commitment to a life-history strategy. However, even if under some conditions salmon may respond to the change in photoperiod conditions with an initial simultaneous onset of both processes, progressing maturation is likely to end up disrupting development of seawater tolerance (Fjelldal et al., 2018; Fraser et al., 2022).

Regardless of whether smoltification and maturation may co-develop, the environmental manipulation performed in this study led to onset of early maturation while apparently failed to produce high-quality smolts. This raises questions about the convenience of using these type of intensive conditions to maximize growth in the aquaculture industry, given the potential risk of economic losses caused by early maturation and poor hypo-osmoregulatory peformance.

5. Conclusion

Sustained high temperature was the factor most clearly linked to early maturation, irrespective of the presence or absence of an entraining photoperiod cue. In the absence of such cue, the males most genetically determined to mature might be those initiating maturation "spontaneously", but with a remarkable modulatory effect of high temperature. On the contrary, introducing a winter signal and the subsequent switch to LD24:0 acted as a entraining cue and triggered a highly synchronized maturation response among individuals, which was also very dependent upon temperature. This evidence is highly relevant for industry in the current context of postsmolt production especially in RAS, where salmon is raised to larger size and temperatures used are high and sustained over time. In this study we have demonstrated that accelerated development (at high temperature) by itself can trigger maturation, but a combination with a photoperiod cue to induce smoltification can remarkably increase the risk of postsmolt maturation. In addition, a winter signal appears not to induce smoltification if introduced at high temperature or when salmon have reached large size, and instead can trigger a sexual maturation response after returning to constant light. In this regard, it might be of interest for research and the salmon industry to further investigate if the size reached when the winter signal is introduced influences early maturation and smoltification.

Author contribution

SH and AKDI established the projects and gathered the funding. EPM designed the experiment. EPM, PB, MSF and SH carried out the samplings. EPM performed gene transcription and NKA analyses and PB revised and guaranteed quality of results. BN contributed with plasma 11-KT analyses. EPM carried out data analysis, drafted and wrote the manuscript. MSF, SOS and AKDI provided editorial assistance and helped writing the document. All authors critically revised the manuscript and approved the final version.

CRediT authorship contribution statement

Enrique Pino Martinez: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Pablo Balseiro:** Conceptualization, Methodology, Validation, Writing – review & editing. **Mitchell S. Fleming:** Conceptualization, Writing – review & editing. **Sigurd O. Stefansson:** Conceptualization, Writing – review & editing, Resources. **Birgitta Norberg:** Formal analysis, Resources, Writing – review & editing. **Albert Kjartan Dagbjartarson Imsland:** Conceptualization, Writing – review & editing, Project administration, Funding acquisition. **Sigurd O. Handeland:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data supporting the findings of this study are available from the corresponding author, upon reasonable request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2023.739325.

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