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The effect of photoperiod manipulation on Atlantic salmon growth, smoltification and sexual maturation: A case study of a commercial RAS

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Abstract

The effect of photoperiod manipulation on growth, smoltification and maturation was assessed in Atlantic salmon through an observational study performed in a commercial RAS facility from June (25.0 ± 11.0 g) to October 2018 (151.1 ± 25.5 g). Half of the commercial cohort 721 was raised in continuous light LD24:0 (LL) while the other received a 6-week LD12:12 winter signal (WS) for smoltification from 12 August to 21 September. Parameters related to growth (body weight, gene expression of pituitary *gh1* and *gh2*, and liver *ghr1*, *igf1* and *igfbp1a*), smoltification (condition factor, plasma sodium and cortisol, gill NKA activity and *nka1a*, *nka1b* and *nkcc1a* expression) and maturation (GSI and pituitary *fshb* and *lhb* expression) were analysed. Afterwards, a multivariate analysis was performed on production data from five commercial cohorts raised in the facility (including 721) to identify variables potentially linked to early maturation in RAS. Results from the observational study indicated weak compensatory growth and slightly better smoltification in WS, although signs of size-induced smoltification were present in LL. Smoltification indicators were poor in both treatments, suggesting that smolts may not be yet ready for seawater. No maturation was observed in any photoperiod treatment; however, the multivariate assessment suggested that such lack of maturation might be rather linked to the low mean temperature and SGR experienced by our cohort.

KEYWORDS
compensatory growth, early puberty, postsmolt, Recirculation Aquaculture Systems, temperature, winter signal

1 | INTRODUCTION

Salmon aquaculture is currently experiencing a shift towards intensification and the use of closed-containment systems (CCS), which allows more control over rearing conditions and may help solve sustainability issues associated with traditional salmon farming (Good & Davidson, 2016). The freshwater phase of this strategy entails the production of larger and more robust postsmolts (up to 500 g) in Recirculation Aquaculture Systems (RAS), resulting in the extension of the freshwater phase inland and the reduction in the time spent at sea (Bergheim et al., 2009; Dalsgaard et al., 2013). By

using this approach, the industry aims to improve postsmolt performance, robustness and survival, as well as significantly reduce the impact of sustainability problems such as sea lice infestations and other disease outbreaks (Fjelldal et al., 2018), escapees, waste input in ecosystems or non-disease-related mortality of postsmolts (Davidson et al., 2016; Summerfelt & Christianson, 2014).

However, intensification of rearing conditions in RAS has increased the incidence of male early sexual maturation (Good & Davidson, 2016). Recent communications from Bremnes Seashore AS (Norway) have reported percentages of early maturation ranging between 10% and 20% in some salmon groups raised in their new commercial RAS facility for postsmolt production at Trovåg, Norway (Knutsen, pers. comm., 2017). This represents a serious challenge to the success of the postsmolt production strategy. During early sexual maturation, salmon reduce feed intake and divert existing resources for testis development, resulting in poor food conversion ratio (FCR) and reduced growth rate (McClure et al., 2007). In addition, mature postsmolts cannot be easily identified and culled in freshwater and often end up being transferred to the sea (Good & Davidson, 2016), where they can suffer high mortality, reduced welfare or increased susceptibility to disease most likely due to their poor osmoregulatory ability (Taranger et al., 2010). This considered the occurrence of early sexual maturation represents high economic losses for the industry (McClure et al., 2007) as well as a fish welfare concern (Taranger et al., 2010) and may compromise the economic feasibility of the postsmolt production strategy.

Various external and internal factors are considered main causes of early maturation. Many studies have found clear links between maturation and environmental variables such as photoperiod manipulation (Berrill et al., 2003, 2006; Fjelldal et al., 2011; Good et al., 2016; Peterson & Harmon, 2005; Skilbrei & Heino, 2011; Taranger et al., 1999) or high water temperature (Fjelldal et al., 2011, 2018; Imsland et al., 2014; McClure et al., 2007). Internal factors concomitant to fish development such as high growth rate, size or lipid levels, and external factors that directly influence all the previous such as intensive diets are also causes of early maturation (Herbinger & Friars, 1992; Kadri et al., 1996; Peterson & Harmon, 2005; Rowe & Thorpe, 1990a, 1990b; Rowe et al., 1991; Shearer et al., 2006; Simpson, 1992; Thorpe, 1994). However, how these factors influence the physiological and endocrine mechanisms that control sexual maturation in Atlantic salmon is not well understood.

To undergo sexual maturation, salmon must first meet a certain energetic or physiological threshold. Then, an appropriate environmental cue such as the photoperiod shift to increasing day length occurring during a 'critical time window' after winter will signal the upcoming spring and trigger puberty in high-energy fish (Berrill et al., 2006; Thorpe, 1994). If fish did not accrue enough resources (by reaching a certain size, growth or lipid levels) by such 'critical time window', maturation will be postponed one year (Duston & Saunders, 1992; Taranger et al., 1999, 2010; Thorpe, 1994). Water temperature will influence whether such energy or size thresholds are met by the time at which photoperiodic cue is introduced, as salmon raised in higher water temperature generally display higher growth and maturity rates (Adams & Thorpe, 1989; Fjelldal et al., 2011; Imsland et al., 2014). In the context of commercial postsmolt production in RAS where intensive rearing conditions (high temperature and intensive feeding) accelerate developmental rates, and where an artificial winter signal is introduced to induce smoltification (6–8 weeks LD12:12 followed by 6 weeks in LD24:0), the risk of early maturation increases dramatically.

Physiologically, the onset of maturation is controlled by the activation of the brain–pituitary–gonad (BPG) axis in response to, among others, the switch in light conditions from winter to spring (Taranger et al., 2010). This activation is characterized by an initial peak in gene expression of pituitary follicle-stimulating hormone (*fshb*), which promotes initial stages of gonad development, followed by a high expression of luteinizing hormone (*lhb*) responsible for the latest stages of testis development and spermiogenesis (Maugars & Schmitz, 2008; Schulz et al., 2010). In addition, photoperiod manipulation affects smoltification and growth (Björnsson et al., 2000; McCormick et al., 1987, 1995).

In commercial facilities, salmon are normally raised in constant light (LD24:0) from first feeding to accelerate growth. After a period, an artificial winter signal (LD12:12) is introduced for six to eight weeks followed by six more weeks in LD24:0. This photoperiod change induces an increase in Na^+, K^+-ATPase (NKA) activity in gills that reflects the development of hypo-osmoregulatory ability and seawater tolerance indicative of successful smoltification (Handeland et al., 2003; McCormick et al., 1987; Stefansson et al., 1991). Along with the higher NKA activity, gills display increasing mRNA transcripts of the seawater isoform of this enzyme (*nka1b*) and the Na^+, K^+, $2Cl^-$ (*nkcc1a*) cotransporter, and a reduction in expression of the freshwater isoform (*nka1a*) (Nilsen et al., 2007). These osmoregulatory changes in gills allow salmon to switch from active uptake of NaCl in freshwater (to counteract passive loss of ions) to actively excrete NaCl in seawater in order to avoid dehydration (Urke et al., 2014). Thus, development of hypo-osmoregulatory abilities is reflected in plasma concentration of ions like sodium, with slightly lower levels expected in freshwater (Piironen et al., 2013) and a large increase observed after transfer to seawater (Handeland et al., 2014).

Other endocrine changes in response to spring light conditions and associated with smoltification include an increase in plasma growth hormone (Björnsson et al., 2011; Dickhoff et al., 1997; Handeland et al., 2003; Nordgarden et al., 2007; Stefansson et al., 1991), in plasma cortisol levels (Björnsson et al., 2011; Dickhoff et al., 1997), a peak in gene expression of pituitary growth hormone (Ágústsson et al., 2001, 2003) and higher expression of liver insulin-like growth factor-1. Levels of this liver hormone are also regarded as a reliable indicator of somatic growth (Beckman, 2011; Reinecke, 2010).

Several authors have aimed at understanding the effects of photoperiod manipulation on early maturation and smoltification of Atlantic salmon during the freshwater phase (Berrill et al., 2003, 2006; Fjelldal et al., 2011, 2018; Melo et al., 2014; Skilbrei & Heino, 2011). Despite

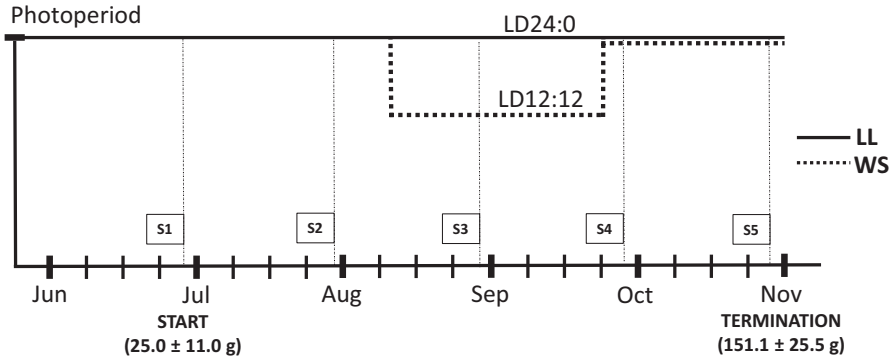

FIGURE 1 Photoperiod and samplings regimes during the trial performed in the commercial Recirculation Aquaculture Systems (RAS). Two treatments were tested, one (LL) maintained under 24 hours light the whole trial and other (winter signal [WS]) that received a winter signal (LD12:12) during 6 weeks from 12 August to 21 September. The mean weight of the fish at the beginning (June) and end (October) of the trial was 25.0 ± 11.0 g and 151.1 ± 25.5 g respectively. The sampling points are serially labelled (S1–5)

the differences in photoperiodic conditions and timing of their introduction in these studies, they all found higher incidence of early maturation in groups of salmon exposed to winter light conditions followed by a switch to summer conditions. Additionally, some of these studies (Berrill et al., 2003; Fjelldal et al., 2011, 2018) reported the occurrence of some mature fish that displayed certain morphological and physiological traits related to smoltification. This suggests that maturation and smoltification, although in developmental conflict (Thorpe, 1986), may in part occur in response to the same photoperiod change.

However, none of the studies mentioned took place in RAS systems, and thus, a research gap exists on how conditions during RAS production can affect salmon performance and early maturation (Good & Davidson, 2016). Fast development under RAS conditions can lead to salmon quickly meeting the nutritional/physiological thresholds required for early maturation, which may be triggered by the LD12:12 winter signal classically used to induce smoltification. Based on this, we established two main objectives for our research. First, we investigated how the introduction of a LD12:12 winter signal during commercial production in RAS affects growth, smoltification and maturation of male Atlantic salmon, by assessing a series of endocrine and osmoregulatory markers. Second, in order to identify variables that might be most linked to early maturation during postsmolt production in RAS, we performed a basic multivariate assessment on production data from five commercial cohorts reared in the same RAS facility.

2 | MATERIALS AND METHODS

2.1 | Ethic statement

The authors confirm that ethical policies of the journal, as per the journal's author guidelines page, have been adhered to. The study was approved by the local representative of Animal Welfare at the Department of Biological Sciences, University of Bergen (Norway), as a field study in a commercial facility, and samplings were performed as established by the Norwegian Animal Research Authority.

2.2 | Study setup

This study consisted on two parts. The first was a case study trial that followed the commercial batch 721 in a large-scale RAS facility under two different light regimes, in order to find out differences in performance and maturation caused by photoperiod manipulation. The second part collected data recorded by the company from the last five cohorts produced in the facility (717–721) including the one followed in the trial, and using a multivariate approach, aimed to find out variables most linked with early maturation.

2.2.1 | Trial conditions

The trial was an observational study during commercial production of Atlantic salmon postsmolts in large-scale RAS (Bremnes Seashore AS, Trovåg, Rogaland, Norway) and was carried out in freshwater from late June 2018 (mean weight 25.0 ± 11.0 g) to late October 2018 (mean weight 151.1 ± 25.5 g, Figure 1). We followed the commercial batch 721, hatched in late November 2017, of Bolaks strain and mixed sex, which was reared from first feeding under constant photoperiod (LD24:0). Fish were fed appropriate commercial feed (Biomar) in a 24-hour cycle according to standard rearing protocols. On 12 August 2018, the batch 721 was divided into two subgroups, one (LL) that was maintained in LD24:0 and another (WS) that received 6 weeks winter signal (LD12:12) to induce smoltification. During this period, the WS group was fed a full ration over a 12-hour cycle. On 21 September, this group was returned to LD24:0 for six more weeks, until both groups were transferred to seawater in early November 2018.

2.2.2 | RAS facility and operations during the trial

At the time of this study, the RAS facility had four fully operational production units, including hatchery, first-feeding hall, freshwater rearing hall 0 (H0) and freshwater rearing hall 1 (H1). After hatching, the group 721 spent approximately 90 days in flow-through hatching cabinets

(4–5 L × min^{-1}, average temperature 3.9°C) until yolk sac was absorbed. Salmon were then transferred to the first-feeding hall, consisting of 60 tanks of 2 m^3 and 1.5 m diameter, each of them holding approximately 15000 juveniles. Here, they spent 75 days at 10.4°C on average until they reached 4–5 grams. Already at parr stage, the group was moved to H0, a RAS freshwater unit consisting of 15 tanks of 100 m^3 and 8 m diameter that held a maximum stocking density of 50 kg × m^{-3}. In this unit, the group spent 100 days at 15.1°C until they reached 40 g. Finally, they were transferred to H1, a RAS freshwater unit with 12 tanks of 320 m^3 and 12 m diameter, each holding a maximum density of 50 kg × m^{-3}. Here, the group was vaccinated when they reached about 50 g, and the winter signal for smoltification was introduced for 6 weeks in half of the group at 60 g. After approximately 90 days at 13.8°C on average, the batch was moved to seawater.

The freshwater used in the RAS is collected from Malasætvatnet (5568 Vikebygd, Rogaland, Norway, UTM 59.537960, 5.554396), and undergoes mechanical filtration and O_3 treatment before entering the RAS circuit. Water is heated or cooled depending on external temperature. RAS water treatment is performed in a Mixed Bed Biofilm Reactor (MBBR) that uses bacteria to degrade ammonia and in a Fixed Bed Reactor that supports its action by treating one third of the water mass. Finally, CO_2 is removed in stripping pools, and O_2 is later injected before the water is recirculated into the tanks. Overall, 95%–98% of the water is recirculated in the system (depending on temperature rather than water quality), while average time of water spent in tanks is 45 minutes.

2.2.3 | Basic multivariate analysis of factors related to maturation in RAS

In order to find out variables most linked to early sexual maturation during salmon production in RAS, a simple multivariate analysis was carried out using production data from the last five commercial batches reared in the facility (717–721). These batches were different cohorts of approximately 1 M individuals raised in the facility subsequently over time. They all were mixed sex populations raised under LD24:0 from first feeding and received a LD12:12 winter signal to induce smoltification. They all followed a similar rearing protocol and steps as described for the cohort 721 in the previous section, although experienced different conditions from 721. Raw data used for the multivariate analyses were routinely collected by staff at the facility as part of their monitoring protocols and included mean temperature per month, mean weight per month and percentage of maturation estimated in each group at the end of the freshwater phase. The way the working dataset was built and how it was analysed are described in the statistics section.

2.3 | Trial samplings

During the 5-month trial in the RAS, we performed one sampling at the end of each month (Figure 1), collecting $n = 30$ male individuals per treatment except in Samplings 1 and 2 where $n = 60$ males were collected since only LL group was present (Total $n = 300$). Fish were collected from the rearing tanks in batches of 12 individuals using long dip nets and were immediately euthanized to limit induced stress. Euthanasia was performed with an overdose of benzocaine (Benzoak vet.® 20%; ACD Pharma AS) higher than 50 mg/L (>20 ml Benzoak/100 L water). Blood samples were collected from the caudal vein with heparinized syringes and centrifuged 3 minutes at 5000 rpm to separate plasma from blood cells. Plasma was immediately frozen in dry ice for cortisol and sodium analysis. Fork length and body weight were measured to the nearest 0.1 cm or g respectively. Fish were opened with a lateral incision in the intraperitoneal cavity and gonads examined to visually determine sex, keeping males and discarding females. Testes were excised and weighed to the nearest 0.1 mg. Liver was removed, weighed to the nearest 0.1 mg and a sample of approximately 0.5 g immediately frozen in dry ice. The first gill arch from the left side of the fish was excised, the cartilage removed, and the tissue kept in RNAlater® for gene expression analysis. 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) and immediately frozen in dry ice for subsequent measurement of Na^+, K^+, ATPase activity (NKA). Finally, the pituitary gland was excised and kept in RNAlater®. Samples in RNA later® were maintained at 4°C for 24 hours to allow the solution to penetrate the tissue. Afterwards, all samples were kept at −80°C until analysis. Condition factor (K) was calculated as $K = W/L^3$ where W is fish weight in grams and L fork length in cm. Gonadosomatic index was calculated as GSI (%) = $W_{gonad} \times 100/W_{body}$ (both in grams) and used as an index of maturity status. A subsample of 103 fish, including a similar number of individuals from all samplings and photoperiod groups, were randomly selected for gene expression analyses in pituitary, while 82 of those were selected for liver and gill gene expression analyses as well as for NKA activity and sodium analyses. Cortisol was measured in a subsample of 65 fish from all samplings and photoperiods. Morphometric data were studied in all 300 male individuals sampled.

2.4 | Lab analyses of trial samples

2.4.1 | Na^+, K^+, ATPase activity in gills

Na^+, K^+, ATPase activity (NKA) was determined in gills using the method published by McCormick (1993). This method estimates NKA activity based on the hydrolysis of ATP to ADP from oubain-sensitive protein fraction, in a reaction enzymatically linked to the oxidation of NADH to NAD^+ by pyruvate kinase and lactic dehydrogenase. This reaction was measured for 10 minutes at 25°C and 340 nm in a Tecan Spark® multimode microplate reader. NKA was then determined as the difference of ATP hydrolysis in presence and absence of ouabain, a specific NKA inhibitor, and expressed as μmol ADP × mg $protein^{-1}$ × $hour^{-1}$.

2.4.2 | Sodium in plasma

Sodium concentration in plasma was measured by potentiometry using the Pentra c400 with ion-selective electrode (ISE)

module clinical chemistry analyser (HORIBA). The ISE module was calibrated using the ABX Pentra Standards 1 and 2, and ABX Pentra Reference. Sodium was measured in a plasma volume higher than 100 µl using specific electrodes and membrane as well as a solution with a known concentration of the ion, following the manufacturer recommendations.

2.4.3 | Cortisol concentration in plasma

Commercial competitive enzyme-linked immunosorbent assay (ELISA) was used to determine plasma cortisol following manufacturer instructions (Demeditec Diagnostics GmbH, DEH3388). Assays were carried out in 96-well plates that included standards of known concentration and controls for quality check. Plasma samples (10 µl) were analysed in triplicate, and cortisol concentration was determined by competition for binding the coating anti-cortisol antibody with a known concentration of horseradish peroxidase-labelled cortisol. Colour developed by TMB (3,3′, 5,5′-Tetramethylbenzidine) was measured at 450 nm in a Tecan Spark® multimode microplate reader and compared to standards. Cortisol concentration was calculated using 4-Parameters Marquardt logistic regression with an extrapolation factor of 1.

2.4.4 | Gene expression analyses in pituitary, liver and gill

Real-time PCR (RT-PCR) was used to analyse the expression of select genes in pituitary (*gh1*, *gh2*, *fshb*, *lhb*), liver (*ghr1*, *igf1*, *igf1bp1a*) and gills (*nka1a*, *nka1b*, *nkcc1a*). Total RNA from pituitaries was isolated with the RNeasy® Plus Micro Kit (Quiagen) using β-mercaptoethanol (Bio-Rad, USA) and following manufacturer instructions. Liver and gill total RNA isolation was carried out from 20 mg of tissue in the QIAsymphony SP automatic nucleic acid isolation system using the QIAsymphony RNA Kit (Quiagen) as described by manufacturer. Total RNA concentration was measured in a Qubit® 3.0 Fluorometer using the Qubit® RNA BR Assay Kit (Invitrogen™; ThermoFisher Scientific Inc.). RNA purity was assessed with the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). Purity was confirmed with 260/280 and 260/230 ratios above 1.8. Total RNA (500 ng for liver and gill; 150 ng for pituitary) was reverse-transcribed to cDNA using SuperscriptTM III Reverse Transcriptase and Oligo(dT)20 Primer (Invitrogen™; ThermoFisher Scientific Inc.) following manufacturer instructions. Gene expression was analysed in a Bio-Rad CFX96 Touch Real-Time PCR system (Bio-Rad Laboratories) using 2.5 µl diluted cDNA (1:40 for pituitary transcription, 1:50 for gill transcription and 1:80 for liver transcription), 0.25 µl of each primer (200 nmol × L^{-1}), 6.25 µl iTaq™ Universal SYBR® Green Supermix (Bio-Rad) and 3.25 µl ultrapure water, in a total reaction volume of 12.5 µl per well. The RT-PCR protocol consisted on 3 minutes at 95°C followed by 35 cycles (40 cycles for *lhb* gene) at 95°C for 15 seconds and 60°C for 1 minute. Samples were run in duplicate with the oligos listed in Table 1. Duplicates with a CV > 1.25% were repeated or not considered for analysis. Before RT-PCR reactions, seven 2-fold dilutions of pooled samples for each tissue were run in triplicate to build a standard curve and calculate primer efficiency for each primer set. The efficiency (E) was estimated with the formula $E = 10^{(-1/slope)}$, with the slope obtained from the plot of log (RNA concentration of the pool) versus threshold cycle (Ct) values. Specificity of the amplicon was confirmed by running a melting curve. After running the RT-PCR, the relative transcription levels of the genes were calculated using the efficiency-corrected method with *ef1α* as reference gene (Pfaffl, 2001). To verify the sequence identity of the RT-PCR products, the amplicons were amplified in a 25 µl volume reaction including 1X Standard Taq reaction buffer, 200 µM of dNTPs and 0.2 µM of the respective primers and 25 U × ml^{-1} of Taq DNA polymerase (New England Biolabs). This PCR was conducted on a C1000 TM Thermocycler (Bio-Rad) with an initial denaturation at 95°C for 30 seconds, 35 cycles of 95°C for 15 seconds, 60°C for 20 seconds and 68°C for 20 seconds, and a final extension step of 5 minutes at 68°C. PCR products were purified using the QIAquick PCR purification kit (Qiagen). The sequencing reaction of purified PCR product was accomplished using Big Dye® Terminator Cycle Sequencing Kit v.3.1 in an Applied Biosystem 3730XL capillary sequencer (ThermoFisher Scientific Inc.) in the University of Bergen sequencing facility. The sequences obtained were aligned in MEGA5.2 (Tamura et al., 2011) together with genomic and RNA sequences obtained from GenBank database to visually confirm the PCR product identity.

2.5 | Statistical analyses

2.5.1 | Trial data

Distribution of all response variables was graphically checked with histograms, and normality was tested with Shapiro–Wilk test, while outliers were first explored using boxplots. Linear models (two-way ANOVA or ANCOVA) were fitted between each response variable and the predictors' photoperiod, time and their interaction. Model residuals were checked graphically to assess normality (q-q plots), non-linear patterns (residuals vs fitted plots), homogeneity of variance (scale-location plots) or influential outliers (residuals vs Leverage plots with Cook's distance). Homogeneity of variance over time and among groups was also checked with Levene's test. When model assumptions were not met, the response variable was log- or square root-transformed, the model re-run and residual checks performed again. When no transformation helped, a generalized linear model was fitted using a Gamma distribution with an 'inverse' link since most response variables were highly right-skewed. Significant models were followed by Tukey HSD post hoc tests to find significant differences between groups within each time point and within groups over time. All plots of response variables over time for the two photoperiod treatments display mean values and standard error bars.

TABLE 1 Oligo sequences used for RT-PCR

Gene	Primer	Sequence (5′ > 3′)	GenBank Accession Number	Reference
ghr1	ss_ghr1-F	TGGACACCCAGTGCTTGATG	AF403539	Hevrøy et al. 2015
	ss_ghr1-R	TCCCTGAAGCCAATGGTGAT		
igf1	ss_igf1-F	GATGTCTTCAAGAGTGCGATGTG	—	Pierce et al. 2004
	ss_igf1-R	CGCCGAAGTCAGGGTTAGG		
igf1 bp	ss_igfbp1a-F	AGCTCTGGAGAAGATTGCTAAG	NM_001279140.1	Present work
	ss_igfbp1a-R	GCTTTGTAGAGTCCGTGTTTG		
gh1	ss_gh1-F	GGTTTCCCAGATACAGATTAG	NM_001123676.1	Present work
	ss_gh1-R	GCTCAGAGTAATAGTCAATATAG		
gh2	ss_gh2-F	GGGTGAAATGGGAACTTGTAGAG	XM_014204437.1	Present work
	ss_gh2-R	CCATCTGTGGACATACCAAAAGC		
fsh	ss_fshb-F	GCGAAACGACGGATCTGAA	—	Maugars & Schmitz, 2008
	ss_fshb-R	GGCAACGGGTATGAAGAAGG		
lh	ss_lhb-F	CCCAACGTGCTTAGTCATTC	NM_001173671.1	Present work
	ss_lhb-R	AAACCGGCTCCTTGGTG		
nkaα1a	ss_nkaα1a-F	CCAGGATCACTCAATGTCACTCT	AY692142	Nilsen et al., 2007/ Modified after Nilsen et al., 2007
	ss_nkaα1a-R	CAAAGGCAAATGAGTTTAATATC		
nkaα1b	ss_nkaα1b-F	GCTACATCTCAACCAACAACATTACAC	AY692143	Nilsen et al., 2007
	ss_nkaα1b-R	TGCAGCTGAGTGCACCAT		
nkacc1a	ss_nkcc1a-F	GATGATCTGCGGCCATGTTC	AJ417890	Nilsen et al., 2007/ Esbaugh et al. 2014
	ss_nkcc1a-R	TCTGGTCATTGGACAGCTCTTTG		
ef1a	ss_ef1α-F	CCCCTCCAGGACGTTTACAAA	AF321836	Olsvik et al. 2005
	ss_ef1α-R	CACACGGCCCACAGGTACA		

A significance level α = 0.05 was used in all cases. All statistical analyses were performed in R and Rstudio, using the packages 'car' (Fox & Weisberg, 2019), 'ggplot2' (Wickham, 2016), 'ggpubr' (Kassambara, 2017), 'Rmisc' (Hope, 2013) and 'emmeans' (Lenth et al., 2018).

2.5.2 | Basic multivariate analysis of factors related to maturation in RAS

Data from five commercial cohorts reared in RAS were analysed using a basic multivariate approach that included a Pearson correlation matrix and a principal component analysis (PCA). First, production data from each of the cohorts were retrieved from the company logs. This included average temperatures per month, mean weight per month and the estimated percentage of maturation in each group before transfer to seawater. The specific growth ratio (SGR) per month for each group was calculated using monthly mean weights as SGR (%) = (ln(W2) − ln(W1) × 100)/t (in days). Photoperiod data were not introduced in this analysis since all groups had been reared under similar conditions. Second, using these variables, a new set of summary variables related to size, growth and temperature was calculated for each cohort, including 'temperature sum', 'average temperature', 'temperature range', 'average SGR' and 'total weight gain'. Since conditions during early development may also influence the decision to mature as parr or postsmolt, the variables 'average temperature of the first 5 months' and 'average SGR of the first 5 months' were also calculated. Finally, the basic multivariate analysis was performed on this dataset first by building a Pearson correlation matrix that included all variables, followed by a principal component analysis (PCA) on the scaled dataset (real values minus the mean and divided by the standard deviation). Variables showing Pearson correlation coefficients equal or smaller to 0.25 (positive or negative) were considered not relevant for maturation and were not included in the subsequent PCA. This analysis was carried out in R and Rstudio, using the packages 'psych' (Revelle, 2011), 'FactoMiner' (Lê et al., 2008) and 'factoextra' (Kassambara & Mundt, 2017).

3 | RESULTS

3.1 | Trial data

3.1.1 | Growth-related parameters

Figure 2 displays mean body weight and mean temperature per month for the full cohort 721 as estimated by the staff at the

RAS facility. Growth was slow during the first 5 months of rearing (December to April) in correspondence with the low temperature measured in the facility. After that, fish started to grow faster corresponding with an increase in water temperature.

In the sample from the 5-month trial, body weight was significantly dependent on the interaction between photoperiod and time (log-transformed ANCOVA, $p < 0.05$), with fish in WS displaying compensatory growth and overtaking the LL group in September and October (Figure 3A). However, there were no significant differences in weight between treatments at any sampling. Pituitary gene expression of *gh1* and *gh2* (Figure 3B,C) was generally consistent with this trend. A log-transformed two-way ANOVA for *gh1* expression showed no significance of any of the model terms; however, a log-transformed one-way ANOVA displayed overall significantly higher transcript levels of *gh1* in the WS group ($p < 0.01$). In addition, levels of *gh1* in the WS group in late August after introduction of the LD12:12 were significantly higher than those measured in late July in LL (Tukey post hoc test, $p < 0.05$). For *gh2*, a log-transformed two-way ANOVA showed that expression of this gene was only dependent on photoperiod ($p < 0.01$), with overall significantly higher levels in the WS group. Similar to *gh1*, expression levels of *gh2* in late August in the WS group were significantly higher than those measured in late July in LL before introduction of the winter signal (Tukey post hoc test, $p < 0.01$). Expression of the receptor *ghr1* (Figure 3D) was significantly dependent upon photoperiod and the interaction between photoperiod and time (log-transformed two-way ANOVA, both model terms $p < 0.01$). Thus, individuals in the WS group displayed a peak in *ghr1* expression in late September significantly higher than levels in the LL group (Tukey post hoc test, $p < 0.001$). Liver *igf1* expression (Figure 3E) was significantly dependent on the interaction between photoperiod and time (log-transformed two-way ANOVA, $p < 0.05$), with transcript levels in the WS group showing a sudden but not significant peak in late September that decreased significantly in late October (Tukey post hoc test, $p < 0.05$), but not significant differences between treatments at any time. Finally, a log-transformed two-way ANOVA showed that liver *igf1bp1a* expression (Figure 3F) was significantly dependent on photoperiod ($p < 0.0001$), time ($p < 0.01$) and their interaction ($p < 0.05$). Liver transcripts of this gene did not change over time in the LL group, while displayed significantly higher levels in the WS group in late August and September compared to the LL group (Tukey post hoc tests, $p < 0.001$ and $p < 0.05$ respectively). Additionally, *igf1bp1a* expression significantly decreased in the WS group from August to October (Tukey post hoc test, $p < 0.01$).

3.1.2 | Smoltification-related parameters

A generalized linear model (GLM) with Gamma distribution with an 'inverse' link showed significantly lower values of K (Figure 4A) in the WS group compared to LL ($p < 0.001$), with such differences occurring in August and October (Tukey post hoc tests, $p < 0.001$ and $p < 0.01$ respectively). Over time, we observed a significant decrease in K from August to October that was similar in both photoperiods (no significant interaction photoperiod×time). A square-rooted two-way ANOVA did not show any significant effect of photoperiod or time on gill NKA activity (Figure 4B), although an increasing but not significant trend in NKA was observed in the WS group from late September in contrast with a decreasing trend in the LL group. A log-transformed two-way ANOVA showed that gill gene expression of the freshwater isoform of this enzyme (*nka1a*, Figure 4C) was significantly dependent only on time ($p < 0.001$) with no difference between photoperiod groups. Thus, transcript levels of *nka1a* showed an increasing trend in the first 2 months, followed by a significant decrease in both treatments from late August to late September (Tukey post hoc tests, both $p < 0.001$). Gill transcript levels of the seawater isoform (*nka1b*, Figure 4D) were significantly dependent (log-transformed two-way ANOVA) on photoperiod ($p < 0.001$) and the interaction photoperiod×time ($p < 0.05$), with a significant increase in expression observed only in the WS group from August to

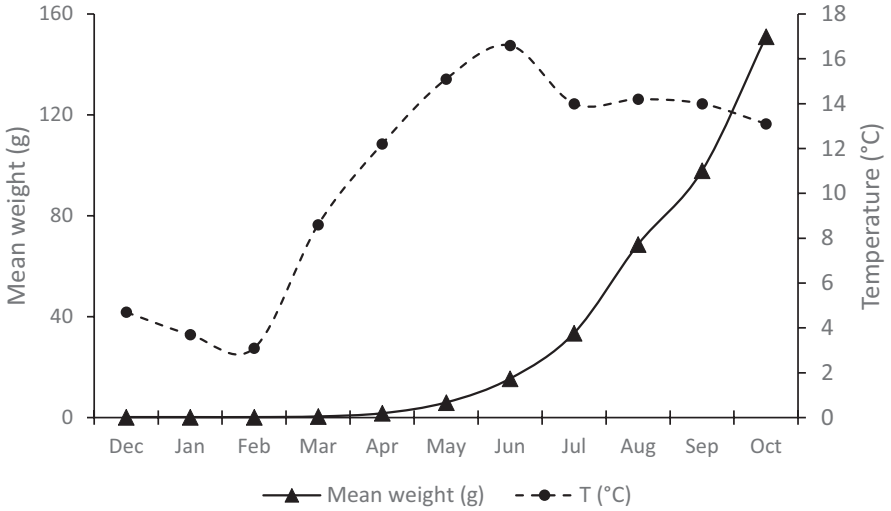

FIGURE 2 Mean body weight and temperature profile over time for the whole commercial group 721 estimated by staff at the facility.

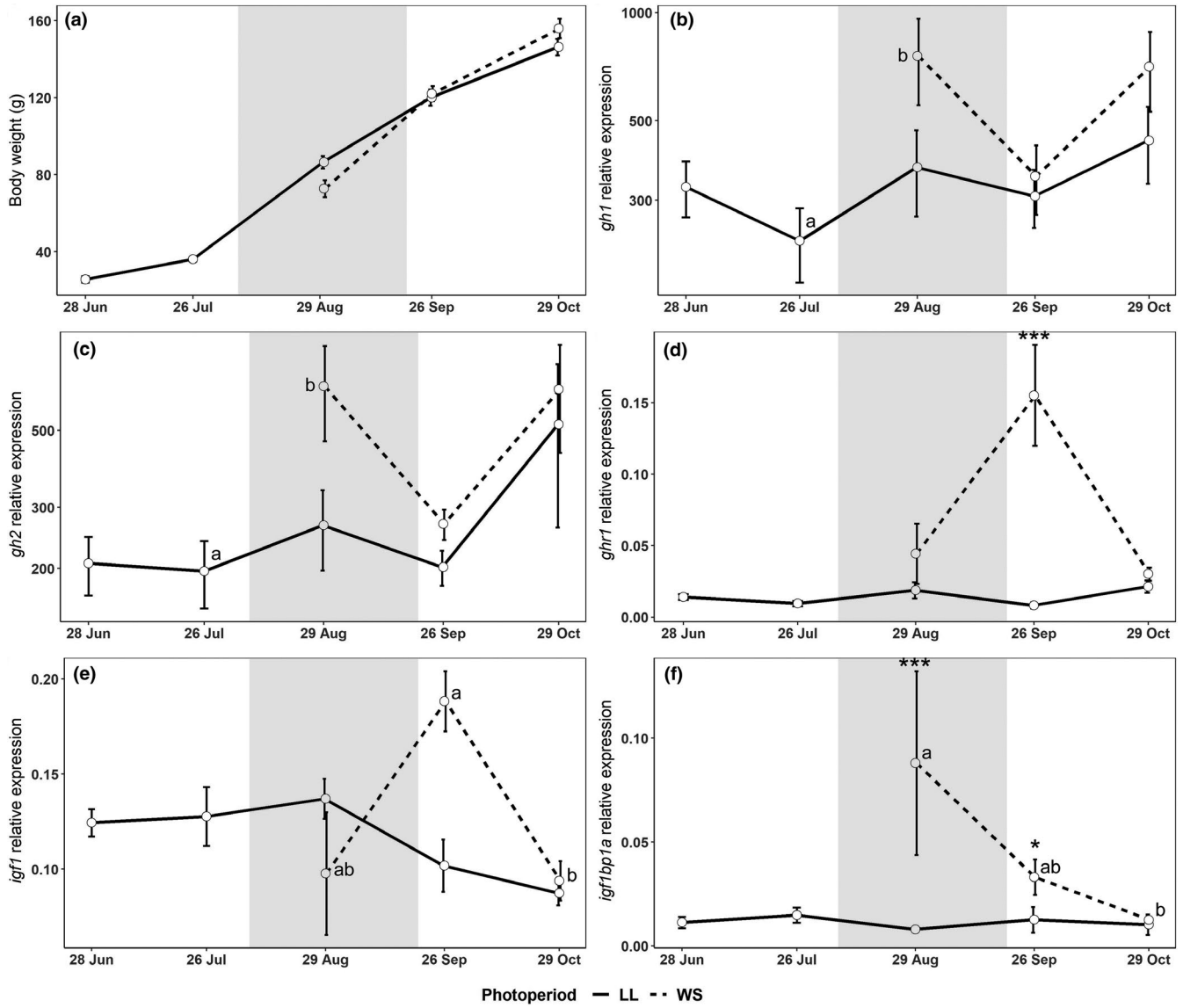

FIGURE 3 Over time response of growth-related parameters measured for the two photoperiod groups followed in the Recirculation Aquaculture Systems (RAS) facility. Variables include body weight in grams (A), relative expression of pituitary gh1 (B) and gh2 (C), and liver relative expression of ghr1 (D), igf1 (E) and igf1bp1a (F). Significant differences between photoperiod groups at a given time are displayed with asterisks as follows: (*) *p*-value <0.05, (**) *p*-value <0.01 and (***) *p*-value <0.001. Significant differences over time within the winter signal (WS) group are displayed with 'a' or 'b'. In graphs (B) and (C), 'a' and 'b' have been used to indicate a significant difference between different photoperiod groups. Note that the *Y* axis of gh1 and gh2 graphs is displayed in logarithmic scale. The shaded area in all graphs represents the 6-week period (from 12 August to 21 September) in which LD12:12 was introduced in the WS group to induce smoltification

September (Tukey post hoc test $p < 0.001$) that did not occur in LL. Gill *nkcc1a* (Figure 4E) followed a similar expression pattern to the one of the *nka1b* isoform, displaying increasing levels over time in both photoperiod groups, although none of the model terms were significant (log-transformed two-way ANOVA). Sodium concentration in plasma (Figure S1) showed a decreasing trend in the WS group, but no significant differences occurred (square-rooted two-way ANOVA). Finally, a log-transformed two-way ANOVA showed that plasma cortisol levels (Figure 4F) were significantly dependent on photoperiod ($p < 0.05$) and time ($p < 0.01$), with fish in the WS group displaying overall higher levels of cortisol than the in LL group, and a general increase in cortisol levels over time in both groups. The greatest change in plasma levels of this hormone was observed in the WS group in late August after introduction of the LD12:12, when we observed significantly higher levels than those measured in late July (Tukey post hoc test, $p < 0.01$).

3.1.3 | Maturation-related parameters

No obvious signs of sexual maturation were observed across the trial, with an overall mean GSI (±SD) of 0.041 ± 0.016%. A GLM with Gamma distribution and an 'inverse' link showed that photoperiod manipulation did not have any significant effect on gonad

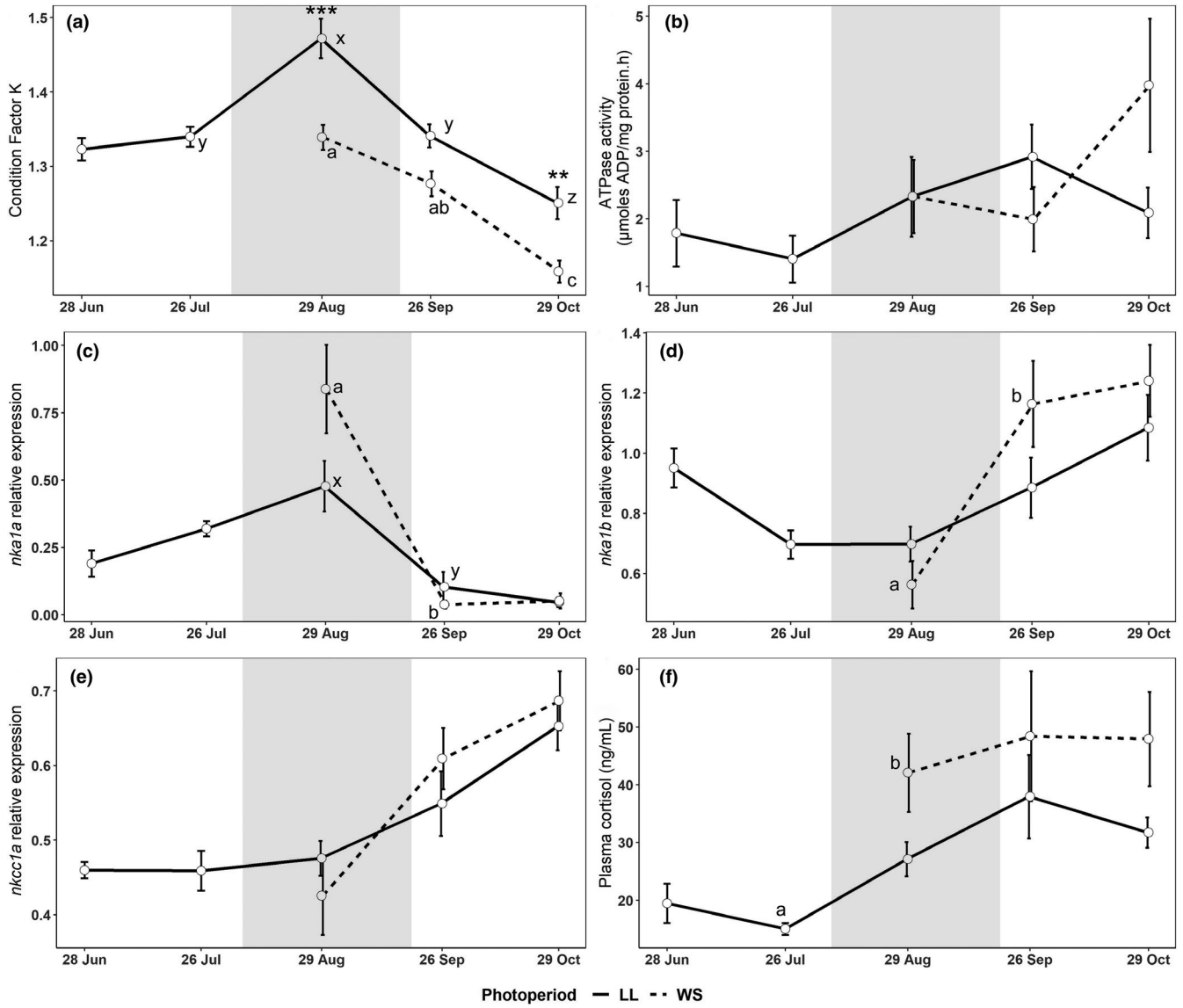

FIGURE 4 Over time response of smoltification-related parameters measured in the two photoperiod groups followed in the Recirculation Aquaculture Systems (RAS) facility, including condition factor K (A), gill NKA activity (B), relative expression of gill nka1a (C), nka1b (D) and nkcc1a (E), and plasma cortisol levels (F). Significant differences between photoperiod groups at a given time are displayed with asterisks as follows: (*) *p*-value <0.05, (**) *p*-value <0.01 and (***) *p*-value <0.001. Significant differences over time within photoperiod groups are shown with 'a', 'b' and 'c' in the winter signal (WS) group and 'x', 'y' or 'z' in the LL group. Only in graph (F), 'a' and 'b' have been used to indicate a significant difference between different photoperiod groups. The shaded area in all graphs represents the 6-week period (from 12 August to 21 September) in which LD12:12 was introduced in the WS group to induce smoltification

development and therefore on sexual maturation, but that there was a significant effect of time ($p < 0.001$). Indeed, significantly higher values of GSI and high individual variation in those values were observed in June and July (Figure 5A), in contrast with the rest of the samplings (Tukey post hoc tests, June and July vs rest $p < 0.001$). Gene expression analyses of pituitary gonadotropins showed that mRNA transcript levels of *fshb* (Figure 5B) were significantly dependent only on time (log-transformed two-way ANOVA, $p < 0.001$) but not photoperiod, with significantly higher levels observed in June compared to the rest of samplings (Tukey post hoc test, $p < 0.001$). Relative expression of *lhb* (Figure 5C) was low throughout the trial, with no significant differences observed between photoperiod groups or over time (log-transformed two-way ANOVA).

3.2 | Basic multivariate analysis of factors related with maturation in RAS

The basic multivariate analysis consisted on a Pearson correlation matrix followed by a PCA and was performed on production data from the last five cohorts raised in the facility. Dates of hatching and seawater transfer, mean weight at seawater transfer and percentage of maturation for each group are presented in Table 2. The Pearson

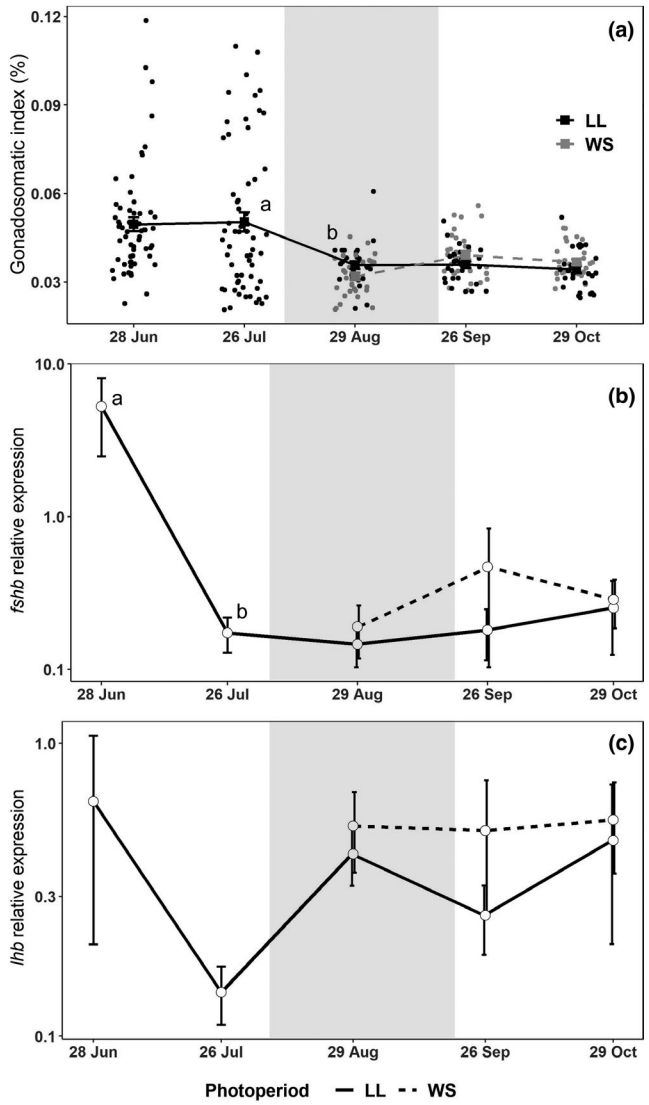

FIGURE 5 GSI (%) (A) and pituitary expression of gonadotropins fshb (B) and lhb (C) over time in both photoperiod groups followed in the Recirculation Aquaculture Systems (RAS) facility. Significant differences over time are displayed with 'a' and 'b'. Note that in fshb and lhb graphs, logarithmic scale is used in the *Y* axis. The shaded area in all graphs represents the 6-week period (from 12 August to 21 September) in which LD12:12 was introduced in the winter signal (WS) group to induce smoltification

correlation coefficient matrix (Table 3) showed that '% Maturation' was highly correlated with 'Mean Temperature' (Pearson = 0.90) and 'Mean SGR' (Pearson = 0.80). Other variables showing a relevant degree of correlation with maturation were 'SGR first 5 months', 'Mean T first 5 months' and 'T range', although not as high as 'Mean Temperature' and 'Mean SGR'. The remaining two variables 'T sum' and 'Weight gain' showed low correlation with maturation. Among predictors, there was a remarkable degree of collinearity between 'mean Temperature' and 'mean SGR' (Pearson = 0.66). There was also high collinearity between 'mean Temperature' and 'mean T first 5 months' (0.77), and between 'mean T first 5 months' and 'mean SGR first 5 months' (0.82), but not between 'mean SGR' and 'mean SGR first 5 months' (0.32).

The principal component analysis (PCA) on data from the five groups clearly revealed the association between '% Maturation' with 'Mean T' and 'Mean SGR'. The PCA biplot (Figure 6) shows that cohorts with highest percentage of maturation (717 and 718, both 10% maturity), also had highest mean temperature and SGR. On the contrary, groups with no incidence of maturation had either low mean SGR (719) or low mean temperature during the production cycle (721, the group we followed). Finally, the group 720 (5% of early maturation) had intermediate mean temperature and SGR compared to the others. The rest of variables were not as associated with percentage of maturation, although temperature range showed a clear negative correlation.

4 | DISCUSSION

Findings from the 5-month trial revealed that introduction of the LD12:12 winter signal only promoted compensatory growth and slightly better smoltification indicators, while it had no effect on early maturation for the duration of the study. Results from the multivariate assessment suggested that such lack of maturation in the cohort followed (721) might be linked to the low mean temperature and comparatively low SGR experienced, which impaired fish to accrue required resources for maturation during the freshwater phase. These findings are discussed in detail in the following lines.

4.1 | Compensatory growth in WS and endocrine mechanisms controlling growth

The growth-related parameters analysed indicated that introduction of LD12:12 winter signal had no negative effect on fish growth. Fish in the WS group experienced a compensatory response in growth after an initial small delay that was most likely caused by the introduction of the shorter day length. It could be argued that the change in feeding regime experienced by this group (from 24 to 12 hours feeding cycle) may also be involved in this effect. However, according to Imsland

TABLE 2 Hatching and seawater transfer dates, mean weight at sea water transfer and percentage of sexual maturation for each of the five production cohorts included in the multivariable analysis

	Trovåg production groups				
	717	718	719	720	721
Hatching date	Feb 17	Apr 17	Jun 17	Sep 17	Nov 17
Seawater transfer date	Nov 17	Feb 18	Jul 18	Oct 18	Nov 18
Mean weight (g) at transfer	110	204	215	237	150
Maturation (%)	10	10	0	5	0

TABLE 3 Pearson correlation coefficient matrix for all pairs of variables studied

	Response	Predictors					
	% Maturation	Weight gain	Mean SGR	SGR first 5 m	T sum	Mean T	T first 5 m
Weight gain	−0.25	1.00					
Mean SGR	0.80	−0.74	1.00				
SGR first 5 m	0.52	−0.22	0.32	1.00			
T sum	−0.24	0.91	−0.72	0.09	1.00		
Mean T	0.90	−0.12	0.66	0.68	0.03	1.00	
T first 5 m	0.57	0.31	0.09	0.82	0.53	0.77	1.00
T range	−0.53	−0.39	−0.10	−0.62	−0.60	−0.81	−0.92

Note: Coefficients between % maturation and the rest of the variables reveal the degree and direction of the relationship between each of them and early maturation. Coefficients between 'predictors' describe the degree of collinearity between variables if they were to be used in a predictive model for early maturation.

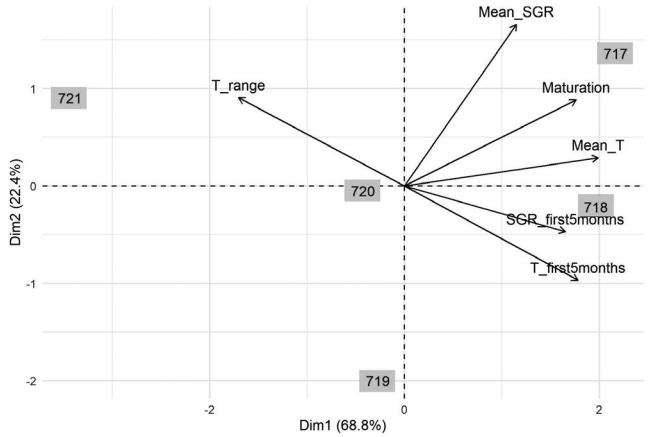

FIGURE 6 Principal component analysis (PCA) biplot displaying variables and production groups included in the analysis. The first two principal components accounted for 91.2% of the dataset variability. Variables are represented as arrows, with directions indicating patterns of increase. Close arrows in the same direction indicate highly correlated variables. Production groups are displayed in the biplot with their number framed within a grey rectangle, and located in the graph depending on the scaled value that they score for each of the variables

et al. (2014), the enhancing effects of constant light on growth are so large that the photoperiod change rather than feeding is most likely responsible for the compensatory effect observed. Compensatory growth is a well-known mechanism in Atlantic salmon, used by the species to offset the effects of periods with poor conditions for growth (low temperature, reduced light and low food availability) and restore energy reserves when there is good opportunity (Morgan & Metcalfe, 2001). Compensatory growth has been previously observed in salmon subjected to short-day photoperiods (Mortensen & Damsgård, 1993), or food deprivation among others (Stefansson et al., 2009), with such compensatory effect being pronounced at high temperatures such as those used in RAS (Mortensen & Damsgård, 1993). The occurrence of compensatory growth in WS and the lack of significant differences in body weight among treatments suggests that introducing a 6-week LD12:12 regime to induce smoltification in RAS should not concern the industry in terms of growth performance.

The patterns of variation observed in pituitary *gh1* and *gh2* expression, and in liver receptor *gh1r*, *igf1* and *igf1bp1a* expression in the WS group collectively reflect the weak but significant compensatory growth effect. Increased levels of growth hormone cause a rise in IGF-I to induce somatic growth (Beckman, 2011). This effect is modulated by plasma IGFBP-1, which binds to circulating IGF-I and modulates the endocrine growth-promoting activity of this liver hormone on muscle and skeleton, by limiting its bioavailability and increasing its half-life (Beckman, 2011; Beckman et al., 2004). High levels of plasma IGFBP-1 are inducible under a variety of catabolic conditions including changes in temperature, food deprivation or stress among others (Kajimura & Duan, 2007). The consequence is often a delay in somatic growth as for example occurs during periods with poor environmental conditions for development (Kawaguchi et al., 2013; Pierce et al., 2006; Shimizu et al., 2006).

In our case, liver expression of *igf1* in WS in late August was still very low while binding protein *igf1bp1a* expression displayed the highest levels. Together they reflect the restraining effect on growth as a result of introducing the LD12:12 winter signal photoperiod. According to some authors, this effect is mediated by an increase in glucocorticoids like cortisol (Kajimura & Duan, 2007; Pierce et al., 2006), which is consistent with the overall significantly higher levels of cortisol we observed in the WS group. Such higher levels of plasma cortisol in WS most likely reflect two processes, namely an initial adaptive period of higher stress after photoperiod reduction, as well as physiological changes mediated by cortisol occurring during preparation for smoltification (Stefansson et al., 2007). On the contrary, in late September, (a week after returning to 24 hours light), changes in various markers (significant peak in *gh1r*, increase in *igf1* and reduction in *igf1bp1a* expression) most likely reflect the peak in compensatory growth. Finally, the significant decrease in *igf1* from September and the ongoing significant decrease in *igf1bp1a* from August to late October (with similar levels of both markers in WS and LL) may reveal the end of the compensatory growth effect in WS.

4.2 | Endocrine mechanisms of osmoregulation and occurrence of size-induced smoltification

Introduction of the LD12:12 winter signal had positive effects on morphological and physiological changes associated with smoltification, although differences between the two treatments were not highly remarkable. We observed lower condition factor in the WS group, reflecting morphological changes associated with smoltification (Berrill et al., 2006; Björnsson et al., 1989; Stefansson et al., 2007). Physiological parameters measured in gills also reflected better signs of smoltification in the WS group. First, the large individual variability of NKA in WS in late October may indicate the development of hypo-osmoregulatory abilities related to smoltification (Björnsson et al., 2011; Handeland et al., 2003; McCormick et al., 1987), but only in some individuals. The slight increase in NKA observed in the WS group was preceded by a series of physiological changes in gill (simultaneous reduction in *nka1a* expression and rise in *nka1b*) that also reflect osmoregulatory changes occurring during smoltification (Nilsen et al., 2007; Stefansson et al., 2007). Additionally, our results in *gh1*, *gh2* and *igf1* expression are consistent with previous studies that reported higher growth hormone (Björnsson et al., 2000, 2011; Dickhoff et al., 1997; Handeland et al., 2003) and higher IGF-I (Beckman, 2011; Björnsson et al., 2011; Reinecke, 2010) under spring conditions and during smoltification. Finally, plasma cortisol levels observed in WS further support that fish in that treatment underwent seawater adaptations better than those in the LL group, since higher cortisol levels are observed during smoltification (Björnsson et al., 2011; Dickhoff et al., 1997).

Despite the slightly better smoltification indicators in the WS group, no significant differences in any smoltification marker occurred between treatments at any sampling, with similar patterns of variation over time observed in gill *nka1a*, *nka1b* and *nkcc1a* expression, plasma sodium and cortisol levels. The reported signs of smoltification occurred spontaneously in LL at similar times as in WS, including significant reductions in condition factor and *nka1a* expression, and increasing (but not significant) trends in *nka1b*, *nkcc1a* and cortisol after returning to LD24:0. This suggests that seawater adaptations may have started in the whole group 721 regardless of light regime as a result of reaching a size threshold for smoltification (Metcalfe, 1998; Saunders et al., 1994). These results are in agreement with Fjelldal et al. (2018), who suggested that a change in photoperiod may not be required for domesticated Atlantic salmon to commence smoltification.

However, whether such degree of size-induced smoltification changes as those observed in LL would be enough to perform well in seawater is unclear, especially considering the poor levels of NKA activity displayed. Furthermore, the lack of significant differences in NKA activity between WS and LL suggest poor seawater adaptations even in WS despite having received a LD12:12 artificial winter, thus implying that this group may not be ready for seawater at the end of the trial. According to Knutsen (pers. comm.), NKA activity measured in smolts produced in Norway seems to be decreasing over the years, and values as low as 5–6 μmol ADP × mg protein^{-1} × hour^{-1} are not uncommon even in salmon that received a winter signal. Factors that may explain these poor smoltification indicators include high water temperatures (McCormick et al., 1999) which are often reached in RAS, and low pH/high aluminium present in Norwegian water inlets (Kristensen et al., 2009; Nilsen et al., 2010). The consequence is that some postsmolts raised in RAS may not be fully prepared for seawater transfer, risking poor performance, welfare issues and higher mortalities. The use of an intermediate brackish water step in RAS may help induce full seawater tolerance before final transfer to sea pens, an approach currently gaining interest in commercial RAS facilities in Norway (Mota et al., 2019).

4.3 | Lack of sexual maturation during the trial

Introduction of the LD12:12 winter signal did not promote sexual maturation for the duration of the trial. However, we observed early signs of gonad development (higher GSI) in some parr in June and July before any photoperiod manipulation, corresponding with elevated expression of pituitary *fshb* only in June. Since FSH is responsible for promoting initial gonadal growth and early stages of spermatogenesis (Maugars & Schmitz, 2008; Schulz et al., 2010), an option could be that the higher expression of *fshb* in June was reflecting the initiation of reproductive development in those fish. As part of the highly diverse reproductive strategy of Atlantic salmon, a small percentage of fast-growing 0+ individuals may naturally undergo sexual maturation as parr in freshwater (Myers et al., 1986; Saunders et al., 1982; Thorpe, 1994). This is a process that appears to be highly dependent on genetics and fast growth after first feeding (Berrill et al., 2003) and that develops quickly under spring and summer light conditions, thus occurring under continuous light (Saunders & Henderson, 1988). However, if parr sexual maturation were a generally ongoing process in our group, we would expect more individuals with high GSI and *fshb* expression in following samplings after June, which did not occur.

Instead, we observed a significant reduction in *fshb* expression from July and in GSI from August, which rather suggests an 'unsuccessful attempt' of sexually maturing. The higher levels of *fshb* observed in June may reflect a physiological response to the only environmental cue experienced by the full group 721 at that time (since they all were under 24 hours constant photoperiod), a steady increase in temperature from February (see Figure 2). However, by June, fish in the cohort 721 may have not accrued enough energy levels to undergo sexual development as parr, and therefore, the process did not continue. This would explain the decrease in *fshb* expression from July and the reduction in GSI observed afterwards. Support for this can be found in Maugars and Schmitz (2008), who observed a peak in *fhsb* transcript levels in non-maturing parr in response to environmental parameters such as increasing temperature and daylight. According to this, activation of the BPG axis may always be initiated in salmon in response to environmental cues, regardless of the energetic status of the fish. However, such energy reserves or developmental status will determine whether that reproductive activation fully develops into a complete sexual maturation process, or if this process is postponed one more year (Taranger

et al., 2010; Thorpe, 1994). Additionally, although a photoperiod switch from winter to spring is considered the entraining environmental cue that triggers the onset of maturation, this process can also be initiated under constant photoperiod without introducing a shift in day length (Fjelldal et al., 2018; Imsland et al., 2014; Saunders & Henderson, 1988). This supports the idea that other cues such as a temperature increase (Fjelldal et al., 2011; Good & Davidson, 2016; Melo et al., 2014) or inner developmental signs can also activate the BPG axis to start maturation. All considered, higher focus is put on other variables more related to fish developmental rate such as temperature, size or growth, as risk factors for early puberty.

4.4 | Basic multivariate analysis of factors related with maturation in RAS

Indeed, results from the basic multivariate assessment pointed at temperature as the variable most linked to early maturation, closely followed by growth. Thus, commercial cohorts with higher percentage of early maturation (717, 718) were those reared under higher mean temperature and with higher mean SGR. Discriminating the individual influence of each variable on maturation is difficult given the intrinsic link between the two (Handeland et al., 2008); however, it is likely that high temperature acted as the main driving factor for maturation by increasing the rate of development (McCormick et al., 2002). Higher maturation seemed also linked to stability of these two variables, evidenced by the fact that conditions during early development (represented by variables calculated only for the first 5 months) were not as relevant for early maturation as sustained conditions. Further support for the link between higher maturation and stability of stimulating conditions is inferred from the negative correlation found between temperature range and early maturation, which reveals that maturation was higher when temperature was more stable. This is in agreement with Policansky (1983), who highlighted that under stable conditions for development fish should grow fast and mature as soon as possible.

Considering this, the lack of maturation in our trial might be related to the low average temperature experienced by the cohort followed (721) during their period in the facility (which was the lowest among all cohorts in the study), and to a lesser extent, by their relatively low SGR and high temperature range. Supporting evidence can be found in Imsland et al. (2014), who reported the highest percentage of maturation in salmon reared at higher temperature that also displayed the highest growth. Additionally, the low temperature and SGR experienced by the cohort 721 until June may also help explain the unsuccessful parr maturation attempt we noticed at that time. According to Good and Davidson (2016), many physiological effects concomitant with increased temperature (high growth and lipid levels) are also linked to early maturation. In this context, the cohort 721 may have not accrued enough energy resources or size for sexual maturation by any time point, due to the low developmental rates experienced as a result of low mean temperature.

However, conclusions derived from this basic multivariate assessment must be observed with caution, since the sample size was small (n = 5 cohorts), and important variables for maturation such as photoperiod or diet were not available to be included. A full multivariate approach might be highly useful to explore and find patterns within large datasets from aquaculture production (as for seawater farms in McClure et al., 2007), but a larger sample size would produce stronger and more reliable conclusions. However, the patterns observed in this analysis seem clear enough to at least point at potential risks for maturation to be investigated further. A future and more comprehensive study that included large and standardized production data collected from RAS facilities through several years and from different companies may help understand much better early maturation in the context of commercial postsmolt production, and even help build predictive models.

5 | CONCLUSION

Introduction of a 5-week LD12:12 winter signal caused compensatory growth and positive effects on smoltification in Atlantic salmon raised in RAS, although signs of size-induced smoltification were also present to a lower extent under LD24:0. While this may indicate that there is no need of a winter signal to induce smoltification in RAS, the poor indicators observed in fish under constant light suggest otherwise. Additionally, the winter signal had no effect on promoting early maturation, at least for the duration of the trial. Based only on these findings, an artificial winter signal would be generally recommended during postsmolt production in RAS, but its potential to cause issues with early maturation remains unclear and must not be overlooked. To conclude with certainty that the winter signal had no effect on maturation, a longer postsmolt observation period before seawater transfer would be desirable (up to approximately 300 g). Additionally, according to results from the basic analysis of five cohorts raised in the facility, the lack of maturation in our cohort might be linked to the temperature profile and growth pattern experienced. This may have prevented sexual maturation from occurring due to fish not reaching the required physiological threshold, thus undermining the entraining effects of photoperiod manipulation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

SH established the projects and gathered the funding. SH and EPM designed the study together with Bremnes Seashore AS. EPM, PB, CP and SH performed samplings. EPM, TH, CP and PB performed laboratory analyses. PB and CP revised and guaranteed quality of laboratory results. EPM carried out data analysis, drafted and wrote the manuscript. MSF provided thorough editorial assistance and helped writing the document. All authors critically revised the manuscript and approved the final version.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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