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APPLIED RESEARCH

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Photoperiod in recirculation aquaculture systems and timing of seawater transfer affect seawater growth performance of Atlantic salmon (*Salmo salar*)

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Abstract

Production of Atlantic salmon smolts in recirculation aquaculture systems (RAS) is growing, and novel production protocols using continuous light in RAS are being implemented in the industry. In the present study, Atlantic Salmon parr were exposed to either a traditional protocol (short-day winter signal [12:12 L:D] for 6 weeks) or to continuous light. Both photoperiods were applied in freshwater (FW) and brackish water RAS. Salmon from all treatments were transferred to seawater pens at 200 and 600 g and grown until slaughter size. A control group was smoltified with a 6-week short-day winter signal and kept in FW until sea transfer at 100 g. Continuous light gave a higher growth rate in RAS but reduced feed intake and growth and increased feed conversion ratio during the first 8 weeks in seawater. However, at slaughter, fish exposed to continuous light was bigger than fish given a winter signal because of the higher growth rate in RAS. Slaughter weight was lowest in fish transferred to sea at 600 g, despite having the highest day-degree sum during their life span. The best performing group was the control group transferred at 100 g.

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All treatments handled transfer to seawater and survival and maturation were not affected by the treatments in RAS. The immune status was examined with a multigene expression assay on BioMark HD platform from parr stage to 5–7 months after seawater transfer. Overall, there was no significant effect of photoperiod or salinity on the expression of the selected immune genes. In sum, the results from this study indicate that using continuous light in RAS may have negative effects on performance shortly after transfer in fish transferred to sea at 200 g, whereas at 600 g, all treatments had reduced growth after transfer irrespective of treatment in RAS.

KEYWORDS
growth, osmoregulation, recirculating aquaculture systems, salmon production, survival

1 | INTRODUCTION

Wild Atlantic salmon (*Salmo salar*) live in freshwater (FW) for a period of 2–5 years before the juvenile parr undergo smoltification triggered by the natural photoperiod, with short days during winter followed by increasing day lengths in spring. The smoltification process reverses the osmoregulatory machinery enabling the salmon to live in seawater but also induces changes in metabolism and behavior preparing for life as a marine pelagic fish (Björnsson, Einarsdottir, & Power, 2012; Hoar, 1988; McCormick, Hansen, Quinn, & Saunders, 1998; McCormick & Saunders, 1987; Nilsen et al., 2008). A loss of body lipid reserves during smoltification and increased length growth make the smolt slimmer and reduce the condition factor (CF). In seawater, the osmolarity of the fish internal fluids is around one-third of the surrounding water (Evans, Piermarini, & Choe, 2005), hence they become hypoosmotic relative to the external medium. Salmon drink seawater and actively secrete salts across specialized mitochondria-rich cells in the gills (McCormick, Regish, Christensen, & Björnsson, 2013). Hypo-osmoregulatory ability is developed during smoltification in salmonids through increase of Na^+ K^+ ATPase (NKA), activity, and the number and size of ionocytes (chloride cells) in the gills combined with enhanced water permeability in the intestine (Boeuf, 1993; Ura et al., 1997). Two NKA isoforms are present in chloride cells, one characteristic for FW adapted fish, NKAα1a, and the other, NKAα1b, predominantly expressed in seawater adapted fish (McCormick et al., 2013; McCormick, Regish, & Christensen, 2009; Nilsen et al., 2007). The ratio and abundance of these isoforms may thus be used as an indicator of seawater adaptation in smolts. McCormick et al. (2013) showed that the increase in NKA during smoltification is primarily because of an increased abundance of NKAα1b, whereas there was almost no change in the abundance of gill NKAα1a during smolt development in FW.

Of the smolts transferred to sea cages in Norway, an average mortality of 13–19% is recorded before they reach harvest size (www.Barentswatch.no). A large part of the losses takes place shortly after seawater transfer. High occurrence of infectious diseases during this period can be associated with increased pathogens pressure and systemic immune suppression during smoltification indicated by down-regulation of multiple genes (Johansson, Timmerhaus, Afanasyev, Jorgensen, & Krasnov, 2016), which persists for several months after seawater transfer (Karlsen et al., 2018). The character and magnitude of these changes vary from study to study and can be largely determined by the farm conditions and production protocols. A multigene expression assay for evaluation of immune

competence in salmon smolts and growers (ImCom) was recently developed and validated (Bakke et al., 2021; Krasnov, Afanasyev, Nylund, & Rebl, 2020; Krasnov et al., 2021). The first version of the assay included 92 genes that showed stable expression changes in 115 challenge trials with pathogens and inflammatory agents. The genes were selected to represent key pathways of the innate and adaptive immune system. Extensive testing of ImCom on experimental and field material showed that 46 immune and stress genes were sufficient for diagnostics. A reference gill data set (RGDS) representing good health status was established and the normal ranges of gene expression were defined.

In addition to diseases, infection with salmon lice, frequent handling associated with delousing operations and other stressors also contribute to the mortality in seawater. Reducing the time spent in open sea cages by moving parts of the production cycle into land-based recirculating aquaculture systems (RAS) can ease the problems with infectious diseases and salmon lice and improve survival in the sea. The average smolt size in Norwegian aquaculture has increased in recent years, and farmers are testing novel protocols for production of smolts without the use of a photoperiod manipulated short-day winter signal to induce smoltification. A winter signal has been shown to be necessary for the development of a seawater adaptation in Atlantic salmon parr (Stefansson et al., 2007). Earlier studies have shown a reduced growth in the first weeks in seawater using continuous light throughout the FW period (Imsland, Handeland, & Stefansson, 2014; Saunders, Henderson, & Harmon, 1985; Sigholt, Staurnes, Jakobsen, & Åsgård, 1995; Solbakken, Hansen, & Stefansson, 1994; Striberny et al., 2021). Most early studies were however done on small fish, and it is known that Atlantic salmon and other salmonids show an increase in NKA and growth hormone and develop seawater tolerance and morphology of a seawater adapted fish when they reach a size of 100–150 g, independent of photoperiod (Handeland, Imsland, Björnsson, & Stefansson, 2013; Sigholt et al., 1995). The effects of using continuous light in the FW phase in larger salmon on performance in seawater has not been tested in long-term experiments. Most studies are also done in flow-through (FT) systems, although RAS is the production systems that are most common in commercial aquaculture today. Few studies on the development of seawater tolerance of Atlantic salmon produced in RAS are available, but there are indications that production system may influence the NKA activity and expression ratio of the NKAα1b and NKAα1a isoforms (Kolarevic et al., 2014; van Rijn et al., 2020). Here we report an experiment where photoperiod manipulation used for induction of smoltification was compared with a novel protocol using constant light during the entire RAS period. Both photoperiods were tested in FW RAS and brackish water RAS (12 ppt) from 100 g until seawater transfer at 200 and 600 g. Survival, growth performance, expression of immune genes, maturation and weight, and chemical composition were followed in RAS and in sea cages until slaughter at around 4.5 kg.

2 | MATERIALS AND METHODS

2.1 | Experimental conditions and design

This trial was carried out at the Nofima Centre for Recirculation in Aquaculture at Sunndalsøra, Norway, and the seawater location was Gildeskål Research station (Gifas) at Inndyr, Norway. The experiment was conducted in accordance with guidelines provided in Norwegian and European legislations related to animal research, and a formal approval of the experimental protocol was given by the Norwegian Food Safety Authority (FOTS ID 8586).

Two identical RAS used in this study were described in Terjesen et al. (2013). The % recirculation in the three RAS was 98–99%, with a daily average water exchange of 25%. The oxygen saturation was kept above 80% in all tanks by automatic control (programmable logic controller NSJ8, Omron, Kyoto, Japan) and temperature was set to 12°C. All tanks were equipped with individual oxygenation down-flow bubble contactors. The water velocity in the tanks was adjusted during the trial to 1.0 body length per second (bl/s). The water velocity in the tanks was measured regularly at four positions in the tank (at water depths of 20 and 80 cm, 40 and 60 cm from the tank wall) using a Höntch propeller with HLOG software (Waiblingen, Germany). The set pH point of 7.5 was maintained in both

systems using pH online probe connected to Walchem WDP 320 (Holliston, MA) control system that was controlling the addition of bicarbonate using a dosing system (IWAKI EW, Tokyo, Japan).

Atlantic salmon parr (Salmo breed strain) were hatched at Nofima and kept in an FW FT system until they were 32 (±3) g. All fish were tagged with passive integrated transponders (PIT) and 5,400 fish were distributed in 18 randomly assigned 3.2 m^3 tanks (300 fish per tank, stocking density 3 kg/m^3) in an FW RAS. The fish were then subjected to two different photoperiod treatments: (a) a 6-week period of short-day length (12 hr light, 12 hr darkness, 12:12 LD) followed by exposure to 24 hr light for a minimum of 4 weeks to induce smoltification (Handeland & Stefansson, 2001), or (b) exposure to continuous light (24:0 LD) during the entire trial period in RAS (Figure 1). Until the fish were 100 g, both RAS provided only FW to the experimental tanks. Water from two RAS was mixed to quality conditions to create similar water for all tanks in the experiment. Subsequently, two systems were separated, and one RAS was gradually shifted from FW to 12 ppt during a 2-week period (August 23 to September 6) to allow the MBBR to acclimate to higher salinity (maximum 1% change in salinity per day). The 12 ppt salinity was obtained by mixing approximately one-third of make-up flow from seawater taken from a depth of 40 m, and the remainder of the make-up flow from FW groundwater wells. Water was filtered and UV-treated before it was pumped into the RAS. When the RAS had reached 12 ppt the water was distributed to the tanks with fish on brackish water.

On August 23, when the average weight was 100 g, the control group was transferred to sea cages at Gifas located in Northern Norway. The second batch of fish was transferred to sea cages on the October 6, when the fish

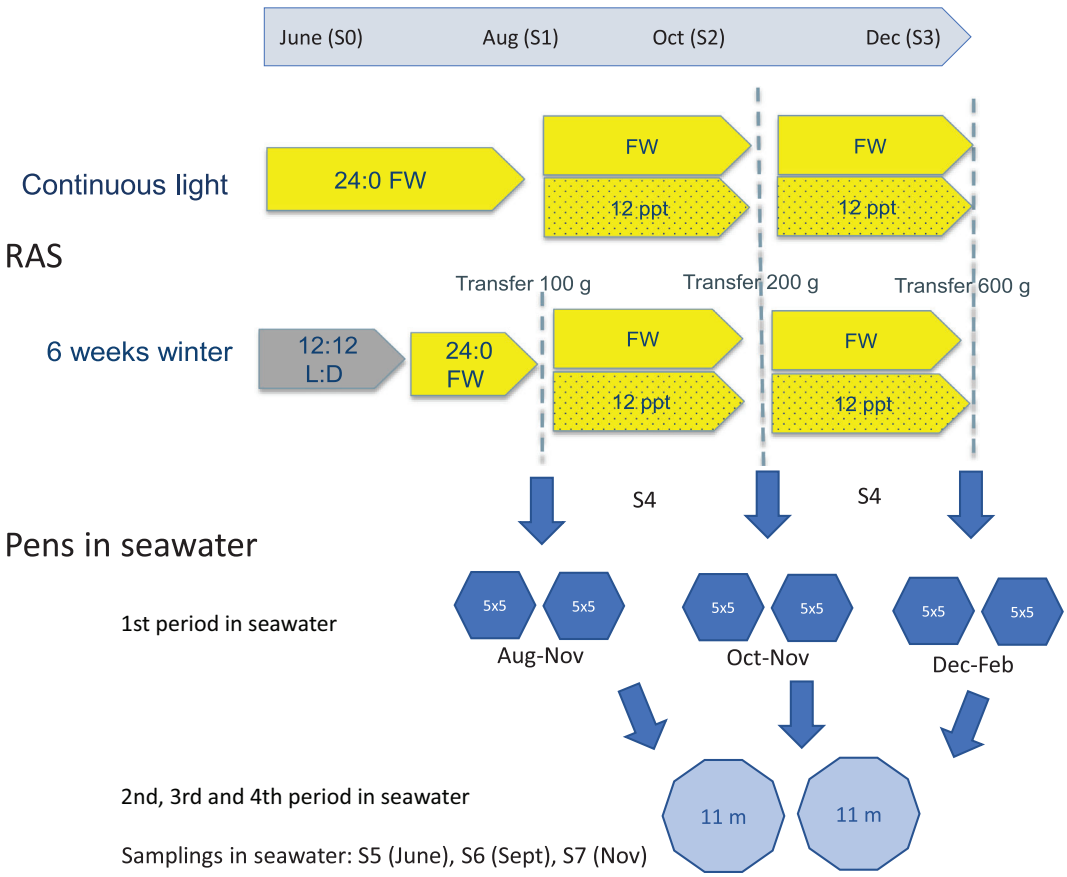

FIGURE 1 Experimental design and timeline of the trial. Samplings are indicated by S0–S7

was around 200 g, and the third batch was transferred on December 10, when the average weight was close to 600 g. A total of 500 fish per treatment were transferred at each stocking.

Seawater tolerance tests (72 hr exposure to 34 ppt) according to Kolarevic et al. (2014) were carried out before transfer of fish to seawater pens to ensure that the fish were able to regulate plasma osmolarity in seawater. On the day of seawater transfer, the water level in the tanks was reduced to one-third, and the fish were sedated in the tank with AQUI-S at the dose level recommended by the producer (2.7 mg/L, AQUI-S® New Zealand Ltd., Lower Hutt, New Zealand). The fish were carefully netted out of the tanks, bulk weighed, and lifted into the tanks of the truck. A product containing polyvinylpyrrolidone (PVP, 60 mg/L) and EDTA (Vidalife, Syndel Laboratories Ltd, Canada) was used when netting and handling the fish to reduce friction during netting and prevent skin damage. The transport of the fish to the Gifas station took 13–15 hr. When unloading the fish, a tube was connected to the tanks on the truck and the fish were transferred directly into the pens by gravity, thereby avoiding pumping. The first 5–8 weeks after transfer, the fish were kept in 125 m^3 pens (5 × 5 × 5 m) equipped with a system for collection of excess feed, to measure feed intake after seawater transfer. After this period, the treatments were pooled in duplicate pens (11 m, 100 m^2) until slaughter in November the following year. The 11 m cages were equipped with automatic feeders and temperature and oxygen were measured daily at 1, 3, and 5 m depth.

The fish were fed commercial diets with recommended pellet size and composition according to fish size during the trial. The feed used in RAS was developed for RAS facilities (Skretting RC, 3 and 4 mm pellet) that is suitable for a size interval of 40–600 g. It contained 22–25% fat, 45–51% protein, crude fiber 0.6–4.5%, pigment 70 mg/kg, ash 9–11%, and an energy content of 22–23 MJ/kg according to the manufacturer's declaration. After the first batch of fish was transferred to seawater, fish in RAS and seawater were fed the same feed. After all fish had been transferred to seawater, a standard seawater diet was fed to all treatment groups (Skretting Spirit and Premium).

2.2 | Water quality measurements

Temperature (PT100, Hyptech, Drammen, Norway) was measured continuously in each RAS during the experiment. Salinity was measured daily in all systems after systems were separated and salinity was increased in one RAS using a portable Multi 3410 meter and TetraCon® 925–3 conductivity probe (WTW GmbH, Weilheim, Germany).

Water samples were collected at the tank outlets ($n = 3$ during FW phase and $n = 4$ during BW phase) and prior to entry to the tanks on four occasions: at the start of the experiment (17.06), prior to transfer of 100 g (17.08), 200 g (29.09), and 600 g (05.12) Atlantic salmon to sea cages. Samples were analyzed for total ammonium nitrogen, NO_2—N, NO_3—N, alkalinity, and turbidity. Nitrogen compounds were analyzed as described in Terjesen et al. (2013). Alkalinity was measured according to APHA (1999) and turbidity was measured with Turbiquant 1500 IR (Merck, Darmstadt, Germany).

2.3 | Sampling and analysis of fish

Fish was sampled on four occasions in RAS; before the short-day photoperiod treatment (S0) (at 40 g) and before transfer of the fish to seawater pens at 100 g (S1), 200 g (S2), and 600 g (S3) (Figure 1). In addition, blood samples were collected after seawater challenge tests run between July and November (seven tests in total).

Samples of gills for gene expression analysis were collected from 10 fish per tank before the short-day treatment (at 40 g) and before transfer of fish to seawater pens at 100, 200, and 600 g, respectively. All sampled fish were anesthetized with AQUI-S before they were killed with a blow to the head, and bodyweight and length were recorded. The gonads were dissected out and weighed for calculation of gonadosomatic index (GSI). A pooled sample of 10 fish per treatment was analyzed for whole-body content of dry matter (DM; 105°C, until constant weight), crude lipid after HCl hydrolysis (Soxtec HT6, Tecator, Höganäs, Sweden), crude protein (N × 6.25; Kjeltec Auto

System, Tecator, Höganäs, Sweden), ash (550°C, overnight), and energy (Parr 1271 adiabatic bomb calorimeter, Parr Instrument Company, Moline, IL).

After seawater transfer, the fish were weighed 5–8 weeks after transfer (S4, end first period in seawater) and in June 5–7 months after transfer (S5), in September, 8–10 months after transfer (S6), and at slaughter in November (S7, 11–14 months after sea transfer). Norwegian quality cut (NQC) samples were collected from 10 fish per treatment and analyzed for fat content. Gonads were weighed as described above. Gill samples were collected in June (S5) for immunological analysis.

2.4 | Evaluation of seawater tolerance

Seawater tolerance was assessed by testing the ability of the fish to regulate serum chloride, sodium, and magnesium levels to less than 140, 170, and 1.5 mmol/L, respectively (Arnesen et al., 2003) during a 72 hr exposure to full-strength sea water (34 ppt) according to Kolarevic et al. (2014). Blood samples were taken from 10 fish at the end of the 72 hr seawater challenge tests using heparinized vacutainers (Terumo Europe, Leuven Belgium). Blood samples were centrifuged (3,000*g*, 10 min, 4°C) and the serum was frozen at −20°C for analysis of chloride, sodium, and magnesium (Horiba Pentra C400). The activity of the enzyme Na^+, K^+ ATPase (NKA) in the gills of fish in the different was determined at the same time as fish were subjected to seawater challenge tests using the method of McCormick (1993) and activity expressed as μmol ADP mg protein hr^{-1}. Samples were taken between July and the end of November (seven samplings in total). The gene expression of the isoforms typical for FW and seawater tolerant fish was quantified using a qPCR method commercialized by Pharmaq Analytiq. Smolt characteristics were also measured using a commercial standard visual scoring system (Smolt index, scale 1–4) that evaluates body silver color (4 = most silvery), parr marks (4 is no parr marks), and darkening of the fins (0 = no darkening).

2.5 | Multigene expression analyses in gills

Development of the assay on BioMark HD platform (Fluidigm), the gene composition, and PCR primers and methods are presented in Krasnov et al. (2020). Analyses included S1, S2, S3, S4, and finally, S5 5 or 9 months in the sea, at around 1 kg ($n = 380$). Tissue pieces were placed in tubes with steel beads containing 400 μl lysis buffer (Qiagen, Hilden, Germany) and 20 μl proteinase K (50 mg/ml), homogenized in FastPrep 96 (MP Biomedicals) for 120 s at maximum speed, centrifuged, and incubated for 30 min at 37°C. RNA was extracted on Biomek 4000 robot using Agencourt RNAdvance Tissue kit (Qiagen, Hilden, Germany). The extracted RNA (1 μl) was reverse-transcribed using the master mix (Fluidigm, South San Franciso, CA). After addition of primers (100 μM) and the PreAmp master mix (Fluidigm, South San Franciso, CA), 12 preamplification cycles were run in a TAdvanced thermocycler, Biometra (Jena, Germany). The products were treated with exonuclease I (New England BioLabs, Ipswich, MA) and diluted in an SsoFast EvaGreen supermix with Low ROX (Bio-Rad, Hercules, CA) and ×20 DNA-binding dye sample loading reagent. The samples and primer mixes were loaded to the respective inlets of dynamic array IFC chips, primed in the BioMark IFC controller MX (48.48) or HX (96.96) (Fluidigm, South San Franciso, CA), and placed in the BioMark HD system (Fluidigm, South San Franciso, CA). The qPCR was performed according to the array-specific cycling programs. The raw qPCR results were retrieved with Fluidigm RealTime PCR analysis software v. 3.0.2 and results were transferred in a relational database. The geometric means of *ef1a* and *rps20*, the two reference genes with stable expression were used for calculation of ΔΔCt values. Mean deviation from the RGDS with 645 samples from different projects (Krasnov et al., 2020) was calculated as $\sum(x_i - \bar{x}_I)/n$, where x_i and $\bar{x}_I$ are respectively values in a sample and reference set, and n is the number of genes.

2.6 | Calculations and statistical analysis

Specific growth rate (% day^{-1}) between two sampling points was calculated as follows:

$$\mathbf{SGR} = (\mathbf{\ln BW_2} - \mathbf{\ln BW_1}) \times \mathbf{100}/\mathbf{d}\ (\mathrm{BW} = \text{bodyweight}, d = \text{number of days}).$$

The thermal growth coefficient (TGC), was calculated as follows:

$$\mathrm{TGC} = 1000 * \left(\mathrm{BW_2}^{1/3} - \mathrm{BW_1}^{1/3}\right) \times (\text{number of day degrees})^{-1}.$$

The feed intake per pen was calculated by taking the difference between the amount of feed fed to each pen and the amount of uneaten pellet collected. Individual daily and cumulative feed intake was calculated by dividing the feed intake per pen with the number of fish in the pen.

The feed conversion ratio (FCR) was calculated as follows: feed eaten (kg)/weight gain (kg) (as is).

Gonadosomatic index (GSI) was calculated from bodyweight (BW) and the gonad weight (W_o):

$$\mathrm{GSI} = 100 \times W_o/\mathrm{BW}.$$

Individual weight and fork length (L) were recorded to calculate the condition factor (CF):

$$\mathrm{CF} = \mathrm{BW} \times L^{-3} \times 100.$$

Survival was calculated as follows:

$$\%\text{survival} = \left[100 * \left(\text{number of fish}_{(\text{end})}/\text{number of fish}_{(\text{start})}\right)\right].$$

Statistical analysis on growth, survival, organ indexes, and blood parameters were performed in SAS Jmp. A two-way mixed model ANOVA with salinity and photoperiod as the fixed factors was performed for each trial period or sampling point. *p*-values <.05 were considered significant. Response variables given in percent were arcsin transformed before analysis by ANOVA. The gene expression data were analyzed with ANOVA followed with LSD test using Statistica 13. Pen or tank was the statistical unit for all parameters.

3 | RESULTS

3.1 | Water quality

The mean temperatures during the experiment were 12.45 ± 0.56 and 12.50 ± 0.49°C in RAS 1 and RAS 2 during the trial. In RAS 1 (FW) the temperature ranged from 10.3 and 13.5°C, while in RAS 2 (BW) temperature ranged from 10.9 to 13.9°C, respectively. After salinity in RAS 2 was gradually increased over a period of 2 weeks, the average measured salinity in the system was 12.2 ± 1.2 ppt, while in RAS 1 it was 0.3 ± 0.3 ppt.

Water quality parameters measured at the tank level were similar during FW phase as expected, because of the mixing of RAS water between two systems (Table 1). After water quality in RAS2 was changed to BW, all measured water quality parameters in tanks were significantly different both at 200 and 600 g transfer (Table 1). This can be partly explained by the higher feed loads in BW RAS because of additional biomass that was added in this system as a part of another experiment. However, all measured water quality parameters in both systems were within recommendation for Atlantic salmon.

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TABLE 1 Water quality measured prior to tank inlet and at tank outlets in two RAS during experiment: at the start of experiment (17.06), prior to transfer of 100 g (17.08), 200 g (29.09), and 600 g (05.12) Atlantic salmon to sea cages

		RAS id/date							
		Start		100 g		200 g		600 g	
Water quality parameter	Sampling location	RAS 1 (FW)	RAS 2 (FW)	RAS 1 (FW)	RAS 2 (FW)	RAS 1 (FW)[a]	RAS 2 (BW)	RAS 1 (FW)[a]	RAS 2 (BW)
Turbidity (NTU)	Tank inlet	0.8	0.9	3.0	2.7	1.4	3.9	0.3	4.0
	Tank outlet	1.0 ± 0.1	0.8 ± 0.1	2.7 ± 0.2	2.5 ± 0.2	1.2 ± 0.3	3.4 ± 0.9	0.5 ± 0.1	4.4 ± 1.2
Alkalinity (mg/L as $CaCO_3$)	Tank inlet	25	22	35	37	43	296	38	128
	Tank outlet	25.1 ± 0.8	24.4 ± 1.8	35.8 ± 1.2	36.6 ± 0.4	45.5 ± 4.4	291.5 ± 5.3	40.0 ± 2.8	125.5 ± 3.0
NO_2—N (mg/L)	Tank inlet	<0.01	<0.01	0.05	0.04	0.11	0.09	0.09	0.1
	Tank outlet	<0.01	<0.01	0.06 ± 0.00	0.05 ± 0.01	0.11 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.10 ± 0.00
NO_3—N (mg/L)	Tank inlet	4.47	4.34	4.56	4.39	5.78	7.84	8.8	13.6
	Tank outlet	4.39 ± 0.06	4.39 ± 0.05	4.56 ± 0.02	4.41 ± 0.11	5.88 ± 0.04	7.71 ± 0.45	8.83 ± 0.34	14.48 ± 0.52
TAN (mg/L)	Tank inlet	0.04	0.03	0.05	0.05	0.03	0.08	0.02	0.16
	Tank outlet	0.06 ± 0.00	0.06 ± 0.01	0.09 ± 0.00	0.1 ± 0.11	0.11 ± 0.02	0.18 ± 0.02	0.10 ± 0.03	0.27 ± 0.04

Note: Values for tank outlets are averages of three ($n = 3$) tanks (June 17 and August 17) or four ($n = 4$) tanks (September 29 and December 5) ± SD. NO_2—N <0.01 indicate that measured values were below detection level of used equipment. RAS 1 and RAS 2 were running with fresh water (FW) during June 17 and August 17. During September 29 and December 5 RAS 2 was operated with brackish water (BW).

Abbreviations: RAS, recirculation aquaculture systems; TAN, total ammonium nitrogen.

[a]All water quality parameters in RAS 1 on 200 and 600 g transfer were significantly different compared with RAS 2 ($p \leq .05$).

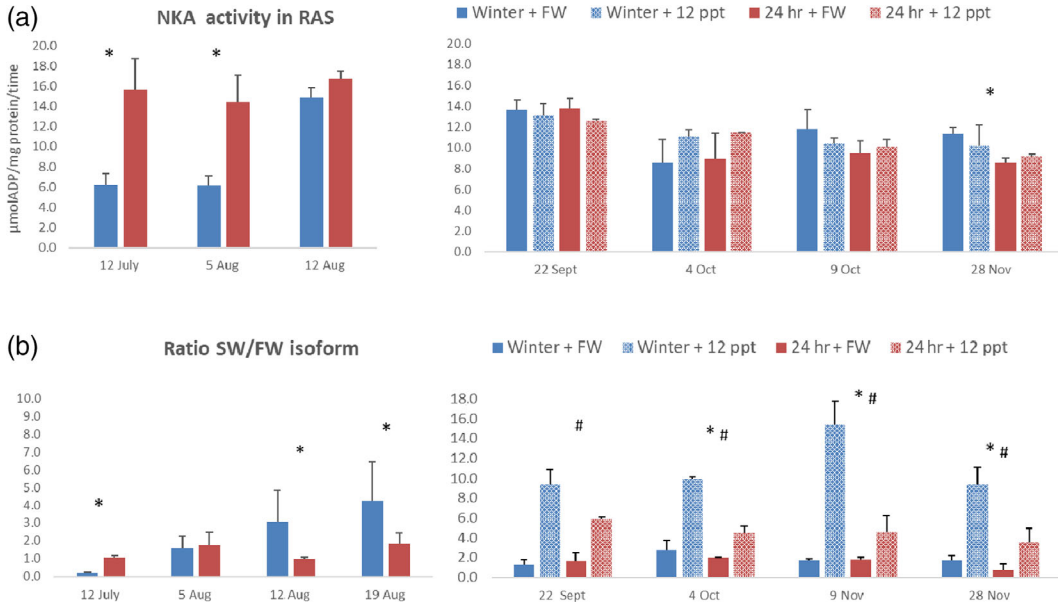

FIGURE 2 (a) Activity of the enzyme Sodium Potassium ATPase (NKA) in gills from fish given a winter signal (12:12 L:D) and fish on continuous light (24 hr) in recirculation aquaculture systems (RAS). (b) Relative gene expression of the seawater (SW) and freshwater (FW) isoforms of the enzyme Sodium Potassium ATPase (NKA) fish given a winter signal (12:12 L:D) and fish on continuous light (24 hr) in RAS. Ratios are based on mean values per treatment ± SD ($n = 3$). Eighteen fish were sampled per treatment. Values are means per treatment ± SEM. (*) significant effects of photoperiod, (#) significant effect of salinity ($p < .05$)

3.2 | Seawater tolerance and smolt status

In fish given a 6-week 12:12 L:D winter signal (from June 13 to July 25), the NKA activity was low (6.2) on the first samplings (July 12 and August 5) but increased to 14.8 µmol ADP/mg protein/hr on August 19 (Figure 2a). In fish kept on continuous light, the NKA activity was close to 15 µmol ADP/mg protein/hr on the first three samplings. From the end of August, salinity was increased to 12 ppt in two treatments. However, salinity did not have a significant effect on the total NKA activity between September 22 and November 28 (Figure 2a). The total NKA activity decreased during fall in all treatments ($p < .0001$), but there was no effect of photoperiod in September and October. In November, NKA activity was significantly higher in salmon given a short-day winter signal.

Photoperiod also had a significant effect on the gene expression of the seawater (NKAα1b) and FW (NKAα1a) isoforms of NKA ($p < .001$, Figure 2b). The ratio of the SW/FW isoform of NKA in fish given a winter signal increased from July to August ($p < .0001$), whereas in fish exposed to 24 hr light the ratio of the FW and SW isoform did not change significantly in the same period. Salinity had the largest effect on the isomer composition, increasing the expression of the SW isoform relative to the FW isoform ($p < .0001$). A winter signal also increased the ratio of SW/FW isoform and there was also a significant positive interaction between a winter signal and 12 ppt salinity ($p < .05$).

Smolt status was also assessed from August 5 based on the fish morphology (CF, silver color, parr marks, darkening of the fins). Data are given in Table S1. No fish had visible parr marks during the trial period, and score for silver color was above 3.1 for all treatment groups in RAS. There were no significant effects of salinity on silver color score in RAS. The score for silver color was initially higher in fish at 24 hr light compared with fish given a winter signal in early August (score 3.9 and 3.5 respectively, $p < .001$), but by mid-August, there was no difference between the treatments. At transfer of 100 g control fish (S1), fish given a winter signal was more silver colored than fish on continuous light (3.8 versus 3.5, $p < .05$). This was also the case at the transfer of 200 g fish (S2). On the sampling before transfer of 600 g fish (S3), there was no effect of photoperiod treatment on silver color (mean of 3.9). Darkening of

the fins increased from the beginning of August (no effect of photoperiod) to transfer of 100 g fish in late August when fish given a winter signal had darker fins (3.2) compared with fish on continuous light (2.4) ($p < .01$). For the rest of the RAS period, there was no effect of photoperiod or salinity on fin color (Table S1).

No mortality was observed in the seven seawater challenge tests performed between August 8 and December 5. Serum ion concentrations of Cl, Na, and Mg after sea water challenge tests are given in Figure S1. In the first seawater challenge test (August 8), fish given a winter signal had higher serum Cl (155 mmol/L) concentration compared with fish on continuous light (141 mmol/L) ($p < .05$). After the seawater challenge tests in late August, September, and November, there was no significant difference between treatments in serum Cl concentrations. In December, at transfer of 600 g fish, salmon in FW had higher serum Cl concentrations (138 ± 1 mmol/L) after the sea water challenge test compared with fish at 12 ppt (134 ± 1 mmol/L) ($p < .05$). This was also the case for Na (174 ± 3 and 170 ± 2 mmol/L, respectively). Serum Mg concentrations after sea water challenge tests were higher in salmon in FW than in 12 ppt in September (1.35 ± 0.4 and 1.12 ± 0.4 mmol/L respectively) ($p < .05$), whereas there was no significant effect of treatment in November. In December, Mg concentrations were higher after seawater challenge in salmon kept in FW than in salmon in 12 ppt (1.47 ± 0.22 and 1.30 ± 0.13) and in salmon given a winter signal treatment (1.46 ± 0.14) compared with 24 hr light (1.29 ± 0.10) ($p < .01$).

3.3 | Expression of immune genes

Comparison with the gill reference data set showed overall lower expression of immune genes in the present study, gradual increase with time, and eventually high level after several months in the sea (Figure S2A). Most part of the analyzed immune genes (31 of 46) changed expression with time and 25 genes were up-regulated (Figure S2B). The greatest changes were observed in the genes of innate antiviral and inflammatory responses including *serum amyloid a5*, *cathelicidin*, *igfc receptor*, *tnf decoy receptor*, and *mmp 9*. Both 200 and 600 g fish (S3 and S4) reared in fresh water at constant light showed slightly lower expression. However, the difference between the treatment groups had completely disappeared at the last time-point in seawater (June, S5). No significant effect of photoperiod and salinity in RAS was found in the entire data set.

3.4 | Performance in RAS

The mortality in RAS was low for all treatments (<0.5%) and there were no effects of photoperiod treatment or salinity on mortality in RAS. There was a positive effect of continuous light ($p < .001$) and 12 ppt ($p < .05$) salinity on growth rate up to 200 g in RAS (Table 2), whereas only continuous light in RAS had a significant positive effect on TGC up to 600 g ($p < .05$). The difference in bodyweight was 25% when the fish were 200 and only 7% when the fish were 600 g. Fish on continuous light and 12 ppt were largest at seawater transfer both at 200 and 600 g (Table 2). A winter signal reduced the CF up to 200 g compared to fish on 24 hr light, whereas there was no significant effect of photoperiod on CF when the fish were 600 g. Brackish water also reduced CF in 200 g fish compared with the group in FW, but the effect was no longer significant when the fish was reared up to 600 g in RAS (Table 2).

3.5 | Performance in seawater

No mortality occurred during transport to the seawater facility. After seawater transfer, the mortality was highest during the first 5–8 weeks after seawater transfer, and the cumulative mortality ranged between 1.1 and 3.8% for all treatments, or 0.03–0.1% per day (Figure 3, Table 3). However, mortality in seawater was not significantly affected by treatment in RAS.

The control group (100 g) was transferred to seawater on August 23 when the water temperature was 13.2°C (-Figure S3). The fish started to feed within a few days after transfer, and the mean feed intake for the first 35 days

TABLE 2 Bodyweight (BW), condition factor (CF), male gonadosomatic index (GSI, % og bodyweight) and growth rate (specific growth rate, SGR), and thermal growth coefficient, (TGC) in RAS

Sampling	Treatment	BW	CF	Male GSI	SGR	TGC
S1 (100 g)	Winter + FW	106 ± 8	1.21 ± 0.02	0.11 ± 0.03	1.80 ± 0.06	1.82 ± 0.07
	24:0 + FW	151 ± 8	1.34 ± 0.02	0.05 ± 0.01	2.33 ± 0.08	2.54 ± 0.11
	Photoperiod	$p < .0001$	$p < .0001$	$p < .01$	$p < .0001$	$p < .0001$
S2 (200 g)	Winter + FW	208 ± 2	1.25 ± 0.05	0.77 ± 0.84	1.76 ± 0.01	2.02 ± 0.01
	Winter +12 ppt	214 ± 7	1.18 ± 0.00	0.11 ± 0.03	1.79 ± 0.03	2.07 ± 0.05
	24:0 + FW	263 ± 8	1.36 ± 0.01	0.05 ± 0.00	1.94 ± 0.02	2.34 ± 0.04
	24:0 + 12 ppt	280 ± 12	1.33 ± 0.02	0.05 ± 0.01	2.01 ± 0.03	2.45 ± 0.06
	Photoperiod	$p < .0001$	$p < .0001$	Ns	$p < .001$	$p < .001$
	Salinity	$p < .05$	$p < .05$	Ns	$p < .05$	$p < .05$
S3 (600 g)	Winter + FW	557 ± 9	1.37 ± 0.06	1.51 ± 0.22	1.69 ± 0.01	2.35 ± 0.02
	Winter +12 ppt	543 ± 21	1.36 ± 0.05	0.14 ± 0.10	1.67 ± 0.02	2.32 ± 0.05
	24:0 + FW	574 ± 24	1.37 ± 0.00	0.25 ± 0.30	1.69 ± 0.02	2.36 ± 0.05
	24:0 + 12 ppt	605 ± 10	1.40 ± 0.01	0.71 ± 1.38	1.72 ± 0.00	2.45 ± 0.02
	Photoperiod	$p < .05$	Ns	Ns	Ns	$p < .05$
	Salinity	$p < .01$	Ns	Ns	Ns	Ns

Note: Data from male and female fish are pooled for BW and CF. Values are means per treatment ± SEM ($n = 2$ tanks). Twenty individuals from each treatment were sampled.
Abbreviations: FW, fresh water; RAS, recirculation aquaculture systems.

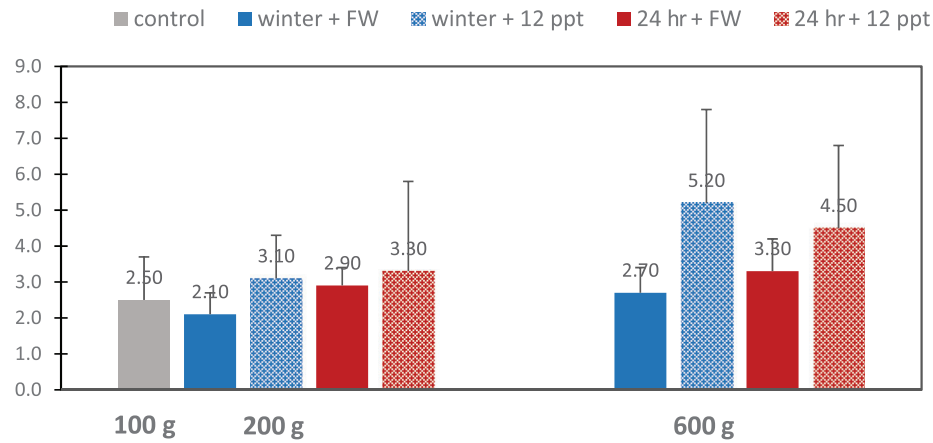

FIGURE 3 Cumulative mortality (in % of total) from February to November in seawater. Values are means per treatment ± SEM ($n = 2$)

was 1% of biomass per day (Table 3). The TGC and SGR of the control fish for the first 5 weeks in seawater were 1.98 ± 0.06 and 1.17 ± 0.05 respectively, and the FCR was 0.96 ± 0.0 (Table 3). The CF was reduced after 5 weeks in seawater compared with the end of the RAS period ($p < .01$). The cumulative mortality was 2.6% and the mortality per day was 0.03%.

At transfer of the 200 g fish (October 10), the water temperature was close to 10°C and the fish started feeding within a week after transfer. The body weight increased during the first 5 weeks in seawater and the % feed intake, CF, FCR, TGC, and SGR for 200 g fish given a winter signal was not significantly different from the 100 g

TABLE 3 Body weight (BW) and condition factor (CF) at the end of the period in 5 × 5 m pens, and feed intake (FI, % of biomass/day), feed conversion ratio (FCR), growth (SGR, TGC) and % daily mortality during the period in 5 × 5 m pens after seawater transfer

Transfer size	Treatment	BW (g)	CF	FI	FCR	SGR	TGC	Mortality (%/day)	Cumulative mortality (%)
100 g	Control	263 ± 1	1.14 ± 0.07	1.03 ± 0.02	0.96 ± 0.0	1.17 ± 0.05	1.98 ± 0.06	0.03 ± 0.00	2.6 ± 0.3
200 g	Winter + FW	297 ± 13	1.14 ± 0.02	0.96 ± 0.04	1.01 ± 0.05	0.93 ± 0.09	2.03 ± 0.21	0.03 ± 0.00	1.3 ± 0.0
	Winter + 12 ppt	300 ± 3	1.11 ± 0.03	0.92 ± 0.02	1.03 ± 0.08	0.88 ± 0.06	1.93 ± 0.11	0.10 ± 0.03	3.8 ± 1.1
	24:0 + FW	328 ± 18	1.13 ± 0.01	0.77 ± 0.02	1.29 ± 0.10	0.58 ± 0.07	1.32 ± 0.19	0.03 ± 0.01	1.1 ± 0.2
	24:0 + 12 ppt	353 ± 20	1.16 ± 0.03	0.73 ± 0.03	1.20 ± 0.16	0.59 ± 0.03	1.38 ± 0.09	0.04 ± 0.02	1.5 ± 0.5
	Photoperiod	$p < .001$	Ns	$p < .0001$	$p < .05$	$p < .001$	$p < .001$	Ns	Ns
	Salinity	Ns	Ns	Ns	Ns	Ns	Ns	Ns	$p = .09$
600 g	Winter + FW	657 ± 9	1.08 ± 0.01	0.26 ± 0.00	1.03 ± 0.08	0.26 ± 0.01	1.59 ± 0.01	0.03 ± 0.00	2.6 ± 0.0
	Winter + 12 ppt	646 ± 24	1.07 ± 0.03	0.27 ± 0.01	0.93 ± 0.06	0.28 ± 0.01	1.68 ± 0.03	0.04 ± 0.03	2.7 ± 0.6
	24:0 + FW	668 ± 12	1.16 ± 0.06	0.24 ± 0.01	0.99 ± 0.01	0.24 ± 0.00	1.47 ± 0.01	0.04 ± 0.00	2.7 ± 0.2
	24:0 + 12 ppt	714 ± 11	1.11 ± 0.05	0.25 ± 0.00	0.98 ± 0.19	0.26 ± 0.04	1.63 ± 0.04	0.02 ± 0.01	1.6 ± 1.0
	Photoperiod	$p < .05$	Ns	$p < .05$	Ns	Ns	Ns	Ns	Ns
	Salinity	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns

Note: Values are means per treatment ± SEM, $N = 2$ pens per treatment.
Abbreviations: FW, fresh water; SGR, specific growth rate; TGC, thermal growth coefficient.

control (Table 3). However, there was a significant negative effect of continuous light in RAS on the feed intake, FCR, and growth at the first 5 weeks after transfer ($p < .01$) compared with fish given a winter signal, but there was no significant effect of salinity in RAS (Table 3). Despite the poorer performance initially in seawater, fish on continuous light in RAS were still larger than fish given a winter signal ($p < .001$) 5 weeks after transfer, but there were no longer effects of treatment in RAS on CF. The CF was reduced after 5 weeks in seawater in all treatments compared with the CF at seawater transfer ($p < .001$). The fish transferred to seawater at 200 g had a daily mortality of 0.03–0.1% during the period in 5 × 5 m pens (Table 3), but there was no significant effect of treatment in RAS on mortality.

The water temperature had dropped to around 7°C when the 600 g fish were transferred to seawater on December 10. It took around 3 weeks before the fish started to feed, and the feed intake and growth were very low after seawater transfer compared with fish transferred as 100 and 200 g (Table 3). Therefore, this group was kept 8 weeks in 5 × 5 cages before being moved to 11 m cages in February. There were no effects of photoperiod or salinity in RAS on SGR, TGC, FCR, the first 8 weeks in seawater. However, fish on 24 hr light in RAS was larger than fish given a winter signal at the end of the period in 5 × 5 m pens ($p < .05$). The fish transferred at 600 g had a daily mortality of 0.2–0.4% and cumulative mortality between 1.6 and 2.7% in the 5 × 5 m pens, with no significant effects of salinity or photoperiod treatment in RAS.

The second period in the sea was from transfer to 11 m cages and until June 1. Control fish and 200 g fish were transferred to 11 m cages in November, and 600 g fish were transferred in early February the following year. Thus, the timing and length of period 2 are not directly comparable for the different transfer sizes. The control group and fish transferred at 200 g were both transferred to 11 m cages in November and had the same day-degrees sum in 11 m cages in period 2 (176 days, 893 day-degrees) and can be directly compared, whereas for the 600 g fish transferred to 11 m cages in February period 2 lasted for 90 days (450 day-degrees). TGC is therefore the most relevant growth parameter to use for comparison with this group since it accounts for both temperature and size differences. The length of period 3 (June–September) and period 4 (September–November) was the same for all treatments.

At the sampling on June 1, there was no significant effect of light treatment or salinity in RAS on bodyweight. A total of 50 fish per treatment was weighed and measured. The body weight, CF, and GSI on June 1 are given in Table 4. Fish on continuous light and 12 ppt in RAS was still slightly larger than the other treatments, both for fish transferred at 200 and 600 g, but the differences were not significant. There was however an effect of size at transfer, fish transferred to sea as 600 g had the highest bodyweight, whereas fish transferred to sea at 200 g had the lowest ($p < .001$) (Table 4). There were no differences in CF between treatments. No significant effects of photoperiod or salinity in RAS on TGC for the 200 and 600 g fish in period 2 in the sea pens were found (Table 5), but SGR was higher in fish given a winter signal ($p < .05$). SGR was also affected by size/time of transfer, control fish had the highest SGR and 600 g fish the lowest ($p < .05$). However, the 100 g control fish (TGC 3.81) performed better than fish transferred to sea at 200 and 600 g (TGC 3.22 and 3.27 respectively, $p < .05$). The next sampling (S3) was on September 20–21. In total, 60 fish per treatment were weighed. Fish transferred at 600 g were now on average 2,600 g, whereas fish transferred at 100 and 200 g were 3,250 and 3,091 g, respectively. Thus, fish transferred at 600 g had lower growth rates compared with fish transferred at 100 and 200 g during summer ($p < .0001$, Table 5). For fish transferred at 200 g there was a tendency for a lower bodyweight in fish given a winter signal ($p = .06$), whereas there was no effect of light treatment in RAS for the 600 g transfer. Salinity in RAS had no effect on bodyweight in September and CF was not affected by photoperiod or salinity in RAS (Table 4). However, CF was lower in fish transferred at 600 g ($p < .05$). When the trial was terminated on November 27–28, the control group was significantly larger (4,872 g) than fish transferred at 200 g (4,671 g) and fish transferred at 600 g (4,120 g) ($p < .0001$, Table 4). There was also a significant effect of photoperiod treatment in RAS, fish on continuous light in RAS were on average 163 g heavier at harvest (mean of all fish transferred at 200 and 600 g) than fish given a winter signal in RAS ($p < .05$). However, the effect of photoperiod in RAS was larger for the 200 g transfer (238 g) than the 600 g transfer (90 g). Fish in all treatments grew well from September to November, with TGCs between 3.48 and

TABLE 4 Body weight (BW), condition factor (CF), and gonadosomatic index (GSI) during seawater grow out

		BW (g)			CF			GSI		
Transfer size	**Treatment**	**June**	**September**	**November**	**June**	**September**	**November**	**June**	**September**	**November**
100 g	Winter + FW	945 ± 11	3,251 ± 45	4,872 ± 93	1.15 ± 0.01	1.37 ± 0.08	1.47 ± 0.16	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
200 g	Winter +FW	896 ± 68	3,049 ± 3	4,532 ± 123	1.14 ± 0.06	1.35 ± 0.11	1.42 ± 0.12	0.06 ± 0.00	0.06 ± 0.02	0.06 ± 0.00
	Winter +12 ppt	918 ± 5	3,036 ± 73	4,572 ± 27	1.17 ± 0.03	1.34 ± 0.10	1.44 ± 0.12	0.05 ± 0.02	0.06 ± 0.01	0.06 ± 0.00
	24:0 + FW	906 ± 10	3,103 ± 11	4,809 ± 150	1.16 ± 0.00	1.36 ± 0.08	1.43 ± 0.09	0.06 ± 0.02	0.05 ± 0.01	0.06 ± 0.00
	24:0 + 12 ppt	950 ± 44	3,176 ± 80	4,771 ± 70	1.17 ± 0.03	1.35 ± 0.08	1.43 ± 0.10	0.04 ± 0.00	0.05 ± 0.01	0.08 ± 0.02
	Photoperiod	Ns	Ns	$p < .01$	Ns	Ns	Ns	Ns	Ns	Ns
	Salinity	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
600 g	Winter +FW	1,048 ± 33	2,553 ± 137	4,173 ± 247	1.15 ± 0.02	1.28 ± 0.09	1.41 ± 0.10	0.10 ± 0.04	0.07 ± 0.04	0.06 ± 0.00
	Winter +12 ppt	1,044 ± 15	2,607 ± 135	3,981 ± 49	1.16 ± 0.05	1.29 ± 0.11	1.40 ± 0.09	0.11 ± 0.05	0.07 ± 0.03	0.06 ± 0.01
	24:0 + FW	1,049 ± 22	2,694 ± 149	4,156 ± 197	1.17 ± 0.01	1.31 ± 0.10	1.43 ± 0.14	0.05 ± 0.00	0.06 ± 0.01	0.06 ± 0.01
	24:0 + 12 ppt	1,139 ± 4	2,554 ± 230	4,170 ± 20	1.16 ± 0.01	1.29 ± 0.08	1.42 ± 0.11	0.07 ± 0.04	0.07 ± 0.03	0.06 ± 0.06
	Photoperiod	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
	Salinity	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
	Transfer size	$p < .0001$	$p < .0001$	$p < .0001$	Ns	$p < .05$	Ns	$p < .05$	Ns	Ns

Note: Values are means per treatment ± SEM, $N = 2$ pens per treatment.
Abbreviation: FW = fresh water.

4.16 (Table 5). There was no significant effect of treatment in RAS or size at transfer on TGC in September–November, but there was a tendency for a higher SGR during fall for the 600 g transfer ($p = .09$).

When comparing the growth during the entire seawater phase, the experimental treatment factor with the largest effect on seawater growth was the timing of seawater transfer ($p < .0001$). There was no significant difference between 100 and 200 g transfer in TGC in seawater (mean TGC of 3.33 and 3.31 respectively), but the TGC in seawater was lower for the 600 g transfer (mean TGC 2.93). Fish given a winter signal in RAS were smaller at transfer to seawater and had slightly higher growth rate in seawater compared with fish on continuous light in RAS ($p < .05$). However, fish on continuous light in RAS were slightly bigger at harvest ($p < .05$) because of their higher growth rate in RAS and higher bodyweight at sea transfer. When comparing growth during the whole trial, from 30 g in RAS to harvest in November the following year, salmon transferred at 100 g had the highest SGR and TGC (Table 5) and the lowest day-degree sum (4,587 day-degrees). Salmon transferred at 200 g had a mean TGC of 2.97, and SGR of 1.04, and a total of 4,605 day-degrees, whereas the 600 g transfer had the lowest overall growth (mean TGC of 2.68 and SGR of 1.01) and the highest number of day-degrees (4858). There was no significant effect of salinity in RAS on total growth rate during the trial, but there was a small but significant positive effect of continuous light in RAS on TGC during the whole trial ($p < .05$).

For the entire period in seawater, the cumulative mortality was 5.1% in control fish and the daily mortality was 0.011%. For the other treatments, the cumulative mortality during the whole seawater phase was between 3.4 and 7.8% and was not affected by photoperiod and salinity treatment in RAS. There was however a tendency for a higher % daily mortality in fish transferred at 600 g ($p = .09$) compared with fish transferred at 100 and 200 g.

3.6 | Body composition

Fish on continuous light in RAS had a higher fat and energy content than fish given a winter signal when the fish was 200 g (Table S2) ($p < .01$). The nutrient composition was not affected by water salinity. From 200 to 600 g, the fat and energy content increased in all treatments, and the ash content decreased ($p < .001$). At 600 g, there was no significant effect of photoperiod or salinity on the body composition.

The body content of ash, fat, and energy decreased during the first weeks in seawater. After the first 5–8 weeks in seawater pens, there was still a higher energy content in fish exposed to continuous light in RAS ($p < .05$, Table S3), whereas salinity in RAS did not have a significant effect on the nutrient composition. At harvest, there were still significant effects of treatment in RAS and time of transfer on the body content of fat and energy (Table S3). Fish transferred at 600 g and given a winter signal had slightly lower fat and energy content compared to fish on continuous light in RAS ($p < .01$) and fish in FW RAS had higher fat and energy content compared with fish on 12 ppt ($p < .001$). There was also an effect of size at transfer, the energy and fat content at slaughter were highest in fish transferred at 100 g and lowest in fish transferred as 600 g ($p < .001$). The fat content of fillet (NQC) was measured in June, September, and at harvest in November. In June, there was no effect of previous treatment in RAS on % fat content in fish transferred as 200 g, but there was a significantly lower fat content in NQC of fish transferred at 200 g (6.4–7%) compared with fish transferred as 100 and 600 g (8.5–9.4%) (Table S3, $p < .0001$). In fish transferred at 600 g there was a lower fat content in NQC in fish exposed to 12 ppt in RAS, but no effect of photoperiod. In September and November, there were still highest fat content in NQC of fish transferred as 100 g, but now the fat content was lowest in fish transferred as 600 g (Table S3). There were no effects of treatment in RAS on fat content in NQC in September and November.

3.7 | Male maturation

Maturation was assessed by weighing the gonads and calculating the gomatosomatic index (GSI). Maturing fish have gonads that are starting to develop, but are still not fully mature. Fish were defined as mature when sperm was

TABLE 5 Growth rates (SGR, TGC) during seawater grow out in 11 m cages

		SGR				TGC			
Transfer size	**Treatment**	**Period 2**	**Period 3**	**Period 4**	**Whole trial**	**Period 2**	**Period 3**	**Period 4**	**Whole trial**
100 g	Winter + FW	0.73 ± 0.00	1.15 ± 0.00	0.71 ± 0.03	1.05 ± 0.00	3.81 ± 0.01	3.82 ± 0.02	3.64 ± 0.16	3.04 ± 0.02
200 g	Winter + FW	0.63 ± 0.07	1.15 ± 0.07	0.69 ± 0.06	1.04 ± 0.01	3.32 ± 0.36	3.71 ± 0.19	3.48 ± 0.34	2.94 ± 0.03
	Winter + 12 ppt	0.64 ± 0.00	1.12 ± 0.02	0.72 ± 0.05	1.04 ± 0.00	3.39 ± 0.01	3.63 ± 0.08	3.61 ± 0.23	2.95 ± 0.00
	24:0 + FW	0.58 ± 0.04	1.15 ± 0.11	0.77 ± 0.07	1.04 ± 0.01	3.11 ± 0.28	3.75 ± 0.29	3.91 ± 0.37	3.00 ± 0.03
	24:0 + 12 ppt	0.56 ± 0.06	1.13 ± 0.07	0.71 ± 0.04	1.04 ± 0.00	3.09 ± 0.30	3.71 ± 0.21	3.63 ± 0.18	3.00 ± 0.00
600 g	Winter + FW	0.52 ± 0.02	0.83 ± 0.02	0.86 ± 0.01	1.02 ± 0.01	3.25 ± 0.19	2.68 ± 0.11	4.14 ± 0.02	2.69 ± 0.00
	Winter + 12 ppt	0.53 ± 0.05	0.85 ± 0.06	0.73 ± 0.07	1.01 ± 0.00	3.34 ± 0.31	2.76 ± 0.22	3.51 ± 0.31	2.64 ± 0.01
	24:0 + FW	0.50 ± 0.00	0.88 ± 0.07	0.74 ± 0.01	1.01 ± 0.01	3.15 ± 0.00	2.86 ± 0.25	3.60 ± 0.01	2.68 ± 0.03
	24:0 + 12 ppt	0.52 ± 0.02	0.75 ± 0.09	0.87 ± 0.17	1.01 ± 0.00	3.34 ± 0.09	2.46 ± 0.32	4.16 ± 0.36	2.69 ± 0.00
	Photoperiod	$p < .05$	Ns	Ns	Ns	Ns	Ns	Ns	$p = .0520$
	Salinity	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
	Transfer size	$p < .0001$	$p < .0001$	Ns	$p < .0001$	$p < .05$	$p < .0001$	Ns	$p < .0001$

Note: Period 2: November–June for 100 and 200 g transfer, February–June for 600 g transfer. Period 3: June–September, Period 4: September–November. Values are mean ± SEM per treatment. $N = 2$ pens per treatment.

Abbreviations: FW, fresh water; SGR, specific growth rate; TGC, thermal growth coefficient.

released when the abdomen was gently squeezed. No mature fish were found when fish were examined at 100 g in RAS. The GSI was however higher in male fish given a winter signal (Table 2). When the fish were 200 g, mature males were found only in the 12:12 FW treatment where 11% of the males were mature or maturing. In total, 3.8% of the sampled fish were mature. When the fish were 600 g, 6.3% of the sampled fish were mature males. The mature males were found in the 12:12 FW treatment (11% of males were mature) and in the 24 h 12 ppt treatment (5.6% of the males were mature). There was a tendency for a higher GSI in the 12:12 FW treatment compared with the other treatments ($p = 0.10$) when the fish were 600 g. There was also an increasing GSI with increasing size in RAS ($p < 0.05$). In seawater, gonads were weighed in June (S4), September (S5), and at slaughter in November (S6). In June, the GSI had decreased compared with in RAS, but fish transferred as 600 g still had a higher GSI compared with fish transferred as 100 and 200 g (Table 4, $p < .05$). Of a total of 180 examined fish, only five mature males were found at the end of May (2.7%). Of these, four were transferred to sea at 600 g. In September and November, no mature fish were found and the GSI was lower than in June. There were no significant effects of treatment in RAS on GSI or frequency of maturation in seawater. No mature females were found during the trial.

4 | DISCUSSION

In this study, underyearling (0+) salmon were produced in RAS using two photoperiod protocols: (a) using a winter signal to induce smoltification or (b) using a novel protocol with continuous light during the whole period in RAS. The use of continuous light in RAS has become popular because feed intake and growth is reduced during the short-day winter period, and it is also easier to manage feeding in RAS on continuous light when feed can be distributed throughout 24 hr. The salmon were transferred to seawater at different sizes from late summer to early winter and such a strategy could potentially give farmers more flexibility in production if the fish can handle the transfer to seawater. Whether these strategies will be successful depends on the effects on fish performance, not only in RAS, but also after sea transfer. In the present study, growth rate and energy content of salmon, both in RAS and after seawater transfer, were influenced by photoperiod treatment. Salmon on continuous light in RAS were larger and had higher CF, lipid, and energy content compared with fish given a winter signal. A positive effect of brackish water on growth rate was observed in RAS, especially in fish kept on continuous light. Positive effects of salinities between 8 and 20 ppt on growth and feed utilization compared with full-strength seawater has been reported in previous studies and in other species (Boeuf & Payan, 2001; Dietz, Stiller, Griese, Schulz, & Susenbeth, 2013; Gutt, 1985; Imsland et al., 2001; Ytrestøyl et al., 2020). Lower metabolic costs associated with osmoregulation at an isotonic salinity may explain the higher growth potential found in several marine teleost species at salinities close to 10‰. In the present study, 12 ppt in RAS had a positive effect on growth up to 200 g, whereas at 600 g, the effect was no longer significant. Benefits of isotonic salinity in RAS at lower body weight but not in larger fish can be associated with the lower surface-to-volume ratio of larger fish and may thus lower costs of osmoregulation.

The overall survival during the first weeks in seawater in the present study was comparable to controlled studies with stocking small-sized smolts in the sea (Kolarevic et al., 2014). Induction of smoltification process with a period of winter light treatment has been considered essential for seawater tolerance in small A. salmon (Stefansson et al., 2007). However, the present study did not show any beneficial effect of a winter signal on seawater tolerance and survival after seawater transfer for the 200 and 600 g groups. A 100 g fish on continuous light was not transferred to seawater, thus it is possible that a winter signal could have had a positive effect on seawater tolerance in a 100 g salmon. In the present study, fish on continuous light developed the morphology of a seawater tolerant Atlantic salmon, with silvery skin and darkening of the fins (Björnsson et al., 2012; McCormick et al., 1998; Mizuno, Misaka, Ando, & Kitamura, 2004). Thus, it seems that Atlantic salmon is capable of developing seawater tolerance as it grows larger, even in the absence of a winter signal. This is supported by previous work on Atlantic salmon (Handeland et al., 2013; Imsland et al., 2014; Sigholt et al., 1995). Handeland et al. (2013) found a peak in NKA activity at a size range of 113–162 g in salmon exposed to continuous light, suggesting that seawater tolerance in

A. salmon is to some extent driven by size and that salmon will develop smolt characteristics at a certain size. A growth reduction after transfer to seawater of fish exposed to continuous light in RAS was observed in the present study, and this has also been shown in previous studies (Imsland et al., 2014; Saunders et al., 1985; Sigholt et al., 1995; Solbakken et al., 1994; Striberny et al., 2021). The difference in appetite and growth in the sea after transfer could be related to osmoregulatory problems. Striberny et al. (2021) observed reduced growth after sea transfer of small (40 g) and larger (130 g) salmon on continuous light despite an equally good hypo-osmoregulatory ability in seawater challenge tests as salmon given a winter signal in both size groups. Striberny et al. (2021) also found a higher CF in salmon on continuous light and low feed intake and high FCR after sea water transfer, similar to what was found in the present study for salmon transferred at both 200 and 600 g fish. In the present study, serum ion concentrations after the seawater challenge tests and the NKA activity during fall were similar among treatments, except for higher values in fish given a winter signal at the final sampling in late November. Gill NKA activity is generally accepted as an indicator of good smolt quality and is correlated with hypo-osmoregulatory ability and high seawater survival rates in salmonids (Handeland, Björnsson, Arnesen, & Stefansson, 2003). In this context, it should be noted that the survival in seawater after transfer in the present study was not related to photoperiod and salinity in RAS or fish size at the time of transfer. Seawater tolerance is also known to be higher in large fish, immature large Atlantic salmon (~3 kg) in FT systems display relatively low levels of gill NKA activity levels following transfer to both fresh- and sea water, but they are still able to regulate ion homeostasis (Bystriansky & Schulte, 2011). Thus, lower NKA activity can meet requirements of large post-smolts in the marine phase because of the lower relationship between surface and volume in larger fish (Nilsen et al., 2007; Stefansson et al., 2012). The ratio and abundance of the NKA isoforms were affected by photoperiod and salinity in the present study. The ratio of the NKAα1b/ NKAα1a increased during smoltification in fish given a winter signal as expected. Later in the trial, when the fish was larger, the main effect on the ratio was salinity, 12 ppt increased the ratio of NKAα1b/ NKAα1a significantly compared with FW treatment. However, this did not correlate with fish performance in seawater challenge tests or growth and survival after seawater transfer.

The reduced growth in fish exposed to continous light after transfer could be related to the higher CF and fat content compared with fish given a winter signal. Energy status and changes in photoperiod are important cues for endocrine regulation of appetite and growth (Ali, Nicieza, & Wootton, 2003; Frøiland et al., 2012; Salmerón et al., 2015; Trombley, Mustafa, & Schmitz, 2014). The size of smolts at sea transfer and the seasonal variations of water temperature and photoperiod along the Norwegian coast influence the growth rate. Both endogenous and exogenous factors affect proximate composition of salmon, and muscle fat content usually increases during fall together with the increase of body weight (Dessen, Weihe, Hatlen, Thomassen, & Rørvik, 2017; Mørkøre & Rørvik, 2001; Nordgarden, Ørnsrud, Hansen, & Hemre, 2003). The high fat diets used in commercial farming today (Aas, Ytrestøyl, & Åsgård, 2019; Ytrestøyl, Aas, & Åsgård, 2015) may promote increased lipid deposition in both muscle and in the abdominal cavity (Bendiksen, Arnesen, & Jobling, 2003; Einen & Roem, 1997; Hillestad, Johnsen, Austreng, & Asgard, 1998; Jobling, Andreassen, Larsen, & Olsen, 2002). Increased lipid deposition has been shown to cause a reduction in feed intake in salmonids (Jobling & Johansen, 1999; Jobling, Larsen, Andreassen, & Olsen, 2002; Johansen, Sveier, & Jobling, 2003; Shearer, Silverstein, & Dickhoff, 1997; Shearer, Silverstein, Plisetskaya, 1997; Silverstein, Shearer, Dickhoff, & Plisetskaya, 1999). This is consistent with the lipostatic regulatory hypothesis, which suggests that the amount of stored fat contributes to regulation of the energy intake and the homeostasis of adiposity through a negative feedback control on appetite and feed consumption in fish (Jobling & Johansen, 1999; Johansen et al., 2003). In the present study, fish grown to 200 g on continuous light in RAS had higher fat content in whole body at seawater transfer compared with salmon given a winter signal, and they had lower growth the first weeks in seawater. Fish raised to 600 g in RAS had accumulated more lipid in whole body compared with the 200 g fish, and all treatments performed poorly the first period in seawater. However, the growth improved during winter, and the fish transferred as 600 g were the largest on the onset of summer. Because of low growth during summer, this group was the smallest in September. The reason for the reduced growth during summer cannot be fully explained, the CF in June was similar for all groups, and fat content in NQC was similar in June in fish transferred as

100 and 600 g, but the TGC during summer was very different, 3.8 and between 2.6 and 2.9 respectively. Whole-body fat and energy was not measured during seawater grow out, and there might be differences between treatments in visceral and liver fat content, which may have an impact on growth. In September, fish transferred at 100 and 200 g had higher lipid content in NQC than fish transferred as 600 g, and there was a tendency for å higher growth rate in the latter group during fall. However, the bodyweight and fat content in NQC of 600 g fish at slaughter was lower than for fish transferred as 100 and 200 g. The total day-degree sum during the trial was highest for the 600 g transfer, so temperature alone cannot explain the differences in growth between transfer sizes. Thus, there is a need to develop a deeper understanding of how energy status and environmental factors such as photoperiod and temperature interact and control the endocrine regulation of growth rate in salmon transferred to seawater at different times of the year.

Early maturation can become a problem in RAS (Davidson et al., 2016; Good, Davidson, Earley, Lee, & Summerfelt, 2014; Good, Weber, May, Davidson, & Summerfelt, 2016), and understanding factors regulating the onset of sexual maturation in RAS is important when developing production strategies for large post-smolts. Both photoperiod and water temperature are important cues that can affect maturation (Fjelldal, Hansen, & Huang, 2011; Good & Davidson, 2016), but to initiate sexual maturation, the fish must also have sufficient energy stores (Thorpe, 1994). In the present study, there were clear indications of increasing maturation among males the longer the fish stayed in RAS. The numbers of mature or maturing males were higher when the fish were 600 g compared with when they were 200 g, and at 100 g no maturing males were found. The mature males were unlike those maturing as parr, they were quite silvery in color and without obvious external sign of maturation except for a slightly higher CF. Such mature males are difficult to recognize unless they are dissected. The energy status is known to affect the decision whether to mature or not (Good & Davidson, 2016), and the higher energy level in the fish on continuous light in RAS could potentially trigger maturation. However, in 100 g fish, the GSI was higher in fish given a winter signal, whereas in both the 200 and 600 g fish, there was no significant effect of photoperiod or salinity on male maturation in RAS. The whole-body energy level increased with time in RAS, and was higher in 600 g fish than in 200 g fish, and the frequency of mature males was also higher at 600 g. Thus, it was suspected that maturation would continue in seawater in this group. At sea transfer of the 600 g fish, 6.3% were mature or maturing males. The cumulative mortality in the first 8 weeks in seawater in this group was between 1.6 and 2.7%, and only a few dead fish were mature. In June, only five mature fish were found among 180 fish examined, four of them were transferred at 600 g. No mature fish were found in September and at slaughter. Thus, it seems that the fish that were maturing in RAS and transferred to seawater at low temperature and short days in December have reversed the maturation process, a phenomenon also described in small Atlantic salmon maturing as pre-smolts (jacks) that was transferred from warm water and continuous light to cold water and short days (Fraser, Fjelldal, Schultz, Norberg, & Hansen, 2019).

The smoltification process affect the expression changes of immune genes in salmon, which has been shown to persist several months after seawater transfer in several independent studies, and this finding stimulated the development of ImCom, a multigene test for assessment of immune competence in salmon. Comparison of smolt batches with good, intermediate, and poor performance in the field has revealed three types of problems: massive down-regulation (immune suppression), unhealthy upregulation because of infection and/or inflammation, and imbalance—concurrent increase and decrease of expression of a large part of genes in the assay (Krasnov et al., 2020). The immune status is diagnosed by the mean and square deviations of expression levels (ΔΔCt) from RGDS, two sigmas were set as a threshold. ImCom was used in the reported study taking into account vulnerability of the immune system during smoltification and first months in the sea. The temporal changes were large and in accordance with expectations; upregulation was observed after several months. The highest increase was shown by genes of innate immunity involved in antiviral and inflammatory responses. These genes take part in defense from one side, but too high expression of inflammatory markers (*drtp*, *cathelicidin*, *c1q-like adipose protein*, *lect2* and *mmp9*) may indicate pathology (Krasnov et al., 2020). However, comparions with RGDS showed that expression changes remained well below the safety thresholds. Differences between the treatment groups were much smaller in scale: they were

observed for a short period and completely disappeared after a few months in the sea. Immune genes showed no response to the light regimens and salinity in RAS. Hence the changes in production protocols did not seem to have an effect on the immune system.

5 | CONCLUSION

The development of new production protocols in Atlantic salmon aquaculture may face biological constraints that offset the technical and economic benefits. Intriguingly, the conventionally produced 100 g smolt had the best performance with largest average harvest weight, low mortality, and no mature fish. However, this study has demonstrated the high adaptability of juvenile salmon, and that it is possible to use continuous light in RAS without adverse effects on mortality and growth in seawater. Performance of smolt stocked at 200 g was good and indicates the potential to increase the smolt size to reduce production time in sea. Nevertheless, changes toward production of larger post-smolts should be done with caution and be based on scientific data to support decisions. Ultimately, the availability of different protocols and transfer sizes without compromising fish performance will add flexibility to salmon production and potentially increase the total production.

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SUPPORTING INFORMATION

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