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Performance and welfare of Atlantic salmon, *Salmo salar* L. post-smolts in recirculating aquaculture systems: Importance of salinity and water velocity

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Abstract

Producing a larger post-smolt in recirculating aquaculture systems (RAS) could shorten the production time in sea cages and potentially reduce mortality. Knowledge of the biological requirements of post-smolts in closed-containment systems is however lacking. In the present study, the effects of salinity and water velocity on growth, survival, health, and welfare of Atlantic salmon reared in RAS were examined. Salmon smolts were stocked in three separate RAS with salinities of 12, 22, and 32‰ and subjected to high (1.0 body lengths per s^{-1}) or low (0.3 body lengths $second^{-1}$) water velocity. Growth performance, survival, welfare, and physiological stress responses were monitored until the fish reached a bodyweight of around 450 g. Growth rate was higher at lower salinity and higher water velocity generally had a positive effect on growth in all salinities. Feed conversion ratio was lower at 12‰ compared to the 22 and 32‰ when the fish were between 250 and 450 g. Higher mortality, elevated plasma cortisol levels,

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higher incidence of cataract, and a higher expression of stress-induced genes in the skin (iNOS, Muc5ac-like) indicated a negative effect of higher salinity on fish welfare. Male maturation was low (<1%), and not affected by salinity or water velocity.

KEYWORDS

Atlantic salmon post-smolts, growth, recirculating aquaculture systems, salinity, water velocity, welfare

1 | INTRODUCTION

Of the Atlantic salmon (*Salmo salar*) smolts transferred to sea cages in Norway, an average of 16% is lost before they reach harvest size, and smolt quality and infections are the most important factors explaining the mortalities (Bleie & Skrudland, 2014). Most losses occur shortly after seawater transfer, so producing a larger and potentially more robust fish may improve survival and growth after sea transfer. Another major constraint for growth in the salmon farming industry is challenges related to parasites such as sea lice. The increased abundance of sea lice is a potential problem for wild salmon and sea trout stocks, and in farmed salmon, delousing operations cause increased stress, reduced growth, and increased mortality (Oppedal, Dempster, & Stien, 2011; Øverli et al., 2014; Stien et al., 2012). Reducing the time spent in open sea cages by moving parts of the production cycle into land-based recirculating aquaculture systems (RAS) may thus reduce problems associated with sea lice and diseases. Furthermore, shortening the production time in open sea cages could also add value and increase the total production volume of Atlantic salmon. However, a better understanding and control of the production environment prior to transfer to open cages in seawater is needed.

There are several challenges related to production of large Atlantic salmon post-smolts in land-based RAS. Seawater RAS may have higher operating costs compared to freshwater (FW) or brackish water RAS, because of the lower efficiency of carbon dioxide (Moran, 2010) and ammonia removal in seawater (Bakke et al., 2017; Chen, Ling, & Blancheton, 2006; Nijhof & Bovendeur, 1990). This will increase the scale of the biofilters and/or increase the need for pumping water in saltwater RAS compared to FW RAS. A solution may be to produce salmon in brackish water since some studies show an improved growth rate in teleost fish at salinities between 8–20‰ (Boeuf & Payan, 2001). The improved growth rate may be correlated with a lower standard metabolic rate because of reduced energy expenditure on osmoregulation, but also feed intake and feed conversion are affected by salinity (Árnason, Magnadóttir, Björnsson, Steinarsson, & Björnsson, 2013; Dietz, Stiller, Griese, Schulz, & Susenbeth, 2013; Imsland et al., 2001). Thus, producing post-smolts in RAS at a lower salinity could be a cost-efficient solution, if fish performance and health are not compromised. However, mixing of FW and seawater in brackish water RAS is a challenge if the FW contains high concentrations of humics (total organic carbon) or clay particles with bound aluminum (Al), as the increased ion strength will mobilize Al and create toxic "estuarine mixing zones" (Bjerknes et al., 2003; Teien, Standring, & Salbu, 2006).

The present study examined the effects and interactions of salinity and water velocity on performance and welfare of large post-smolts of Atlantic salmon reared in brackish (12 and 22 ppt) and seawater RAS (32 ppt). Land-based production of salmon in FW RAS has been associated with increased early male maturation (Davidson et al., 2016), so maturation was followed by measuring the gonadosomatoc index (GSI). The skin is one of the important barriers against pathogens and a healthy and intact skin is also vital for maintaining homeostasis. The status of the skin may thus be a good welfare indicator in fish. Skin morphology, mucus secretion, and expression genes indicative

of stress (*iNOS*, encoding the nitric oxide synthase enzyme that is the key driver for skin repair) and mucus secretion (*Muc5ac-like*) were therefore measured in skin. Sodium-potassium adenosine triphosphatase (NKA) activity in gills were determined to assess potential effects on osmoregulatory capacity, and plasma cortisol was measured as a stress indicator. Because metals may accumulate in the water in RAS facilities (Davidson, Good, Welsh, Brazil, & Summerfelt, 2009), accumulation of metals on the gill epithelium was measured.

2 | MATERIALS AND METHODS

2.1 | Experimental conditions and design

This trial was conducted with Atlantic salmon post-smolts at the Nofima Centre for Recirculation in Aquaculture at Sunndalsøra, Norway (Terjesen et al., 2013). Atlantic salmon parr (Bolaks strain) were hatched at Nofima and initially kept in a FW flow-through system in four separate tanks (tank size 3.2 m^3, diameter 2 m). When the fish reached a size of 20 g they were exposed to a 6-week period of short day length (12 hour light, 12 hour darkness, 12L:12D) followed by a 6 week period of 24-hour light to induce smoltification (Handeland & Stefansson, 2001). By the end of the light period, a subsample of fish (N = 10) were subjected to seawater challenge tests (72 hr exposure to 34‰ seawater, aerated and oxygenated water) (Kolarevic et al., 2014) to ensure sufficient seawater tolerance. Blood samples were taken at the end of the 72 hr exposure. Seawater tolerance was indicated by the ability of the fish to regulate plasma chloride levels to less than 150 mmol L^{-1} (Arnesen et al., 2003), during the seawater challenge test.

After the fish were fully smoltified, 7,200 fish (average 68 ± 1 g, *SD*), of which 3,000 were tagged with Passive Integrated Transponders (PIT), were distributed between twelve 3.2 m^3 tanks (600 fish per tank) in three separate RAS with salinities of 12, 22, and 32‰. Thus, there were four tanks with each salinity. At each salinity, the fish were subjected to high water velocity (1.0 ± 0.13 body lengths per second, bl s^{-1}) or low water velocity, (0.27 ± 0.05 bl s^{-1}) resulting in six different treatments, all in duplicate. Hence, each experimental factor was replicated in four tanks (water salinity) or six tanks (water velocity). To ensure efficient self-cleaning in the tanks, the velocity needed to be at least 0.27 bl s^{-1}. The flow rate and hence hydraulic retention time in the tanks was the same in the high- and low-velocity treatments. In order to achieve a stable water velocity in relation to fish body length, water velocity in all tanks was increased four times during the trial. The water velocity was manipulated by increasing the inlet water pressure and inlet angle, and in addition varying the number and diameter of the holes in the inlet pipes in the tanks. The water velocity in the tanks was measured five times during the study at four positions (at water depths of 20 and 80 cm, 40 and 60 cm from the tank wall, respectively) using a Höntch propeller with the HLOG software (Waiblingen, Germany). The water velocity was measured while the fish were present in the tanks. The water flow rate through the tanks was similar in all 12 tanks so that the hydraulic retention time in the tanks was comparable for the high- and low-velocity groups.

Individual bodyweight for the PIT-tagged fish as well as bulk weight per tank were recorded when the fish were around 250 and 450 g. The seawater, which had a salinity of 32‰, was taken from a depth of 40 m and filtered and UV treated. The 12 and 22‰ salinities were obtained by mixing fractions of ground-well FW with seawater in the RAS make-up water. The moving bed bioreactors (MBBR) in the three RAS were gradually shifted from FW to the intended salinity in periods of 2–4 weeks prior to the trial to allow the MBBR to acclimate to the higher salinity (maximum 15% change in salinity per day). To avoid high and fluctuating total ammonia nitrogen (TAN) and nitrite-nitrogen (NO_2-N) in the beginning of the experiment, the three RAS were stocked with seawater-acclimated salmon (bodyweight around 500 g) 3–4 weeks before the experiment started.

The mean biomass per tank at the start of the trial was 41.1 ± 0.7 (*SD*) kg resulting in a stocking density of 13.7 ± 0.2 kg m^{-3}. The density increased with fish growth but was reduced to ~35 kg m^{-3} and standardized in all tanks when fish were 250 g. Density never exceeded 70 kg m^{-3} in any of the tanks during the trial. The fish were

kept on 12-hr daylight (12L:12D) and were fed a commercial diet to satiation using automatic EX04 feeders with EX06 spreaders (Poro AB, Kåge, Sweden). Excess feed was collected, and the feed intake estimated based on the dry matter content of the waste feed and recovery tests of feed distributed in empty tanks (Helland, Grisdale-Helland, & Nerland, 1996).

The three RAS systems received a feed load between 5 and 20% (mean of 9–11% for the entire trial period, Table 1) of the total TAN removal capacity of the MBBRs. The RAS with 12 and 32‰ had a maximal feed load capacity of 63 kg per day, whereas the RAS running on 22‰ was a larger, but otherwise similar system with a capacity of 300 kg feed per day (Terjesen et al., 2013). Additional fish were held in extra tanks in the larger RAS (22‰) in order to maintain a similar daily feed load relative to the maximum feed load capacity in all three systems. Fish in the three RAS were fed the same commercial diet from Havsbrun (3 and 4.5 mm). The % recirculation in the three RAS was 98–99% with a daily water exchange of 25% (Table 1). The mean temperature was kept close to 12°C in all three systems and the oxygen levels were kept above 80% saturation in the tanks by automatic control (programmable logic controller NSJ8, Omron, Kyoto, Japan). All tanks were equipped with oxygenation down-flow bubble contactors (Terjesen et al., 2013). Water samples were taken on the system and tank level during the entire experiment (Section 2.2).

When the average bodyweight was approximately 250 and 450 g, all the fish in the tanks were counted and bodyweights of PIT-tagged fish registered. The fish that were not PIT tagged were bulk-weighed. Based on the measured bodyweight and total biomass per tank, the effect of salinity and water velocity on growth rate (TGC and SGR), feed conversion ratio (FCR), and survival was calculated. At the same time, biological samples (skin, gills, and blood) were collected from seven fish per tank. Gill NKA activity were measured in fish from the 12 and 32‰ groups to check the ion- and osmoregulatory status. Gill metal concentration and plasma cortisol were measured only at 450 g. Ten fish per tank were sampled for analysis of whole-body proximate composition. Liver, heart, and gonads were dissected out (14 fish per tank) and weighed for calculation of organ indices. All sampled fish were anesthetized with AQUI-S (AQUI-S® New Zealand Ltd., Lower Hutt, New Zealand), according to the producer's recommendations.

2.2 | Logging and analysis of water quality

Temperature (PT100, Hyptech, Drammen, Norway) and pH (Sensorex S8000CD-pH, Sensorex, Golden Grove) were measured continuously in the degasser sump of each of the three RAS. Dissolved oxygen saturation was measured continuously in all experimental tanks (Sensorex DO6442-T) in the middle of the water column opposite to the water inlet. To check for consistent biofilter function, the concentration of TAN and NO_2-N in the system was measured 4–5 times a week using a Hanna Instruments C203 2008 photometer (Hanna Instruments, Quebec, Canada) with reagents for TAN determination (method HI 93700) and for NO_2-N (method HI 93707). Salinity was measured daily in all three systems using a portable Multi 3,410 m with the TetraCon® 925-3 conductivity probe (WTW GmbH, Weilheim, Germany). Water quality at the tank level was recorded throughout the trial on six occasions. The water samples were analyzed for total inorganic carbon (TIC), TAN, NO_2-N, NO_3-N, alkalinity, total suspended solids (TSS), and turbidity. TIC was analyzed on fresh samples stored on ice (method 6/93 Rev. B, Perstorp Analytical, Sweden) using an FSIV autoanalyzer, with acidification of the sample and reaction with phenolphthalein (Summerfelt et al., 2015) and CO_2 concentration calculated according to (Summerfelt, Vinci, & Piedrahita, 2000) using pH and temperature measured at the time of sampling. TAN (sum of NH_3-N and NH_4-N), NO_2-N and NO_3-N concentrations in batch water samples were analyzed using an automated analyzer (Flow Solution IV, OI Analytical, College Station, TX), according to U.S. E.P.A Method 350.1 (U.S.EPA, 1983) for TAN and U.S. E.P.A Method 353.2 (U.S.EPA, 1983) for NO_3-N and NO_2-N. Water samples were analyzed for TSS according to standardized method 2,540 D (TSS dried at 103–105°C) (American Public Health Organization, APHA, 2005). Turbidity was measured on FW samples using a Turbiquant 1500 IR (Merck, Darmstadt, Germany), while alkalinity was measured according to APHA (1999).

TABLE 1 RAS conditions in the three systems

Salinity (‰)	Temp (°C)	pH	CO_2 (mg L^{-1})	TAN (mg L^{-1})	NO_2-N (mg L^{-1})	TSS (mg L^{-1})	Water exchange per day (% of RAS volume)	Feedload (% of MBBR capacity)	Feedload/water exchange (kg m^{-3} day^{-1})
11.6 ± 0.4[c]	12.4 ± 0.9	7.5 ± 0.2[b]	2.5 ± 0.8	0.2 ± 0.1	0.1 ± 0.1	5.4 ± 2.7	25 ± 7	11.0 ± 3.4	0.44 ± 0.30
22.1 ± 0.4[b]	12.1 ± 0.8	7.6 ± 0.1[ab]	2.9 ± 1.0	0.2 ± 0.1	0.6 ± 0.7	7.4 ± 3.5	24 ± 6	10.6 ± 3.9	0.49 ± 0.28
32.0 ± 0.8[a]	12.6 ± 0.9	7.8 ± 0.1[a]	1.6 ± 0.3	0.4 ± 0.1	0.1 ± 0.0	7.9 ± 1.5	25 ± 2	9.1 ± 3.9	0.46 ± 0.20

Note: Values are means for the entire trial with standard deviations (*SD*). Tanks with high and low water velocity are pooled. Samples for measuring water quality were collected at the tank outlet. Water quality data are based on six point measurements ($n = 6$) while nitrogen compounds are based on five point measurements ($n = 5$). Significant differences between treatments ($p < .05$) are marked with small letters.

Water was also analyzed for concentration of eight metals (Al, Zn, Cu, Mn, Cd, As, Ni, and Cr) from the three RAS. Water samples were acidified with ultrapure HNO_3 (5% volume) before analysis using ICP-MS (Agilent 8800 ICP Triple Quad). Analyses of certified seawater CASS 5 demonstrated good accuracy (Table 2).

2.3 | Analysis of fish samples

2.3.1 | Blood analysis and body composition

Blood samples were taken from the caudal vein using heparinized vacutainers (Terumo Europe, Leuven, Belgium). The blood was centrifuged and the plasma frozen at −80°C for later analysis of cortisol. Plasma cortisol levels were analyzed with a validated direct enzyme immunoassay (EIA) as outlined by Carey and McCormick (1998) and modified as described by Calabrese et al. (2017).

A pooled sample of 10 fish per tank 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), ash (550°C, overnight), and energy (Parr 1271 adiabatic bomb calorimeter, Parr Instrument Company, Moline, IL).

2.3.2 | Gill NKA activity and metal concentration

Gill NKA activity was determined in the 12 and 32‰ treatments using the method of McCormick (1993). Readings were taken at 340 nm for 10 min at 25°C. Protein in the homogenate was determined by a bicinchoninic acid method (Smith et al., 1985) and NKA activity expressed as μmol ADP mg protein hr^{-1}. To obtain information on metal concentrations in gills, the tissue was weighed and ultrapure HNO_3 (1 ml), milliQ water (0.5 ml), and internal standards (Rh, In) were added prior to digestion using an ultraclave (milestone Leutkirch, Germany). After digestion, samples were diluted to 10 ml before analysis using ICP-MS (Agilent 8800 ICP Triple Quad). Concentration of metals is presented as μg per dry weight of tissue (mean ± *SEM*). Following the same procedure, analyses of a standard reference material of dogfish liver (Dolt- 4) were within certified values (33.0 μg g^{-1}, 1945 μg g^{-1} and 25.4 μg g^{-1}, compared to the certified values 31 ± 1.1 μg Cu g^{-1}, 1833 ± 75 μg Fe g^{-1} and 24.3 ± 0.8 μg Cd g^{-1}, respectively).

2.3.3 | Skin surface morphology and gene expression

Skin samples were collected for determination of gene expression by qPCR and tissue staining. Skin samples (1 cm^2) were collected according to the Norwegian Quality Cut behind the dorsal fin and above the lateral line. The biopsies were fixed in 4% PFA overnight and then washed in 1 × PBST, before stepwise dehydration to 70% ethanol and storage at −20°C. To characterize skin surface morphology and density of mucus cells, staining with fluorescein-labeled lectins was used on whole-mount skin samples (N = 8 per treatment) (Sveen et al., 2016). Fluorescent cells were counted and adjusted to a rank between 0 (low cells numbers) and 3 (high cell numbers). Samples for gene expression were frozen directly in liquid nitrogen and stored at −80°C until analysis. Skin samples (N = 10 per treatment) were homogenized in TRIzol and total RNA was extracted using PureLink™ Pro 96 well purification kit (Thermo Fisher Scientific Inc., Waltham, MA) with on-column-DNase (Qiagen, MD) digestion according to the manufacturer's instructions and RNA concentrations were measured using NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific Inc.). cDNA synthesis and real-time quantitative PCR was conducted according to Sveen et al. (2016). Primers sequences were designed using ePrimer3 from the EMBOSS online package and synthesized by Invitrogen (Table 3). Cycle threshold values were calculated using the second derivative method in the LightCycler

TABLE 2 Concentrations of metals in each treatment, at different times of sampling

	Salinity (‰)	Al (μg L^{-1})	Cr (μg L^{-1})	Mn (μg L^{-1})	Fe (μg L^{-1})	Ni (μg L^{-1})	Cu (μg L^{-1})	As (μg L^{-1})	Cd (μg L^{-1})
Sampling 250 g	12	1.5 ± 0.5	<1	2.1 ± 1.8	20.4 ± 11.1	1.9 ± 1.2	2.1 ± 0.1	1.8 ± 0.7	0.14 ± 0.01
	22	2.1 ± 0.6	<1	1.6 ± 1.2	17.5 ± 5.6	0.9 ± 0.6	2 ± 0.5	1.8 ± 0.2	0.07 ± 0.05
	32	1.6 ± 0.2	<1	3.3 ± 0.5	30.3 ± 1.6[a]	4 ± 0.3[a,b]	2.1 ± 0.1	2.6 ± 0.1[a]	0.14 ± 0.01
Sampling 450 g	12	8.4 ± 11.4	<1	0.5 ± 0.7	6 ± 7.8	1 ± 0.1[b]	2.6 ± 2.1	1.3 ± 0.3	0.03 ± 0.01
	22[c]	25.5	<1	4.3[b]	19.4	2.9[b]	0.48	1.76	0.05
	32	13.2 ± 2.5	1.4 ± 0.3[b]	1.4 ± 0.2	20.8 ± 1.8[a]	7.2 ± 0.6[a,b]	0.7 ± 0.1	1.9 ± 0.2[a]	0.03 ± 0.01
CASS 5[d]		<1	<1	2.65	1.57	0.36	0.38	1.49	0.023
CASS 5 certified[e]				2.62 ± 0.20	1.44 ± 0.11	0.330 ± 0.023	0.380 ± 0.028	1.24 ± 0.09	0.0215 ± 0.0018

[a]Significantly difference from other treatment taken both sampling time into account.
[b]Significant difference from other treatment at same time of sampling.
[c]Only one of three replicate have been sampled.
[d]CASS 5 refers to analysis of a natural water reference material (National Research Council Canada, Canada) in the laboratory used in the present study.
[e]CASS 5 certified refers to the stated certified concentrations of these metals in the reference material, by the manufacturer (National Research Council Canada, Canada).
Note: Values are means per treatment ±*SD*, *n* = 3.

480 software (version 1.5.0.39). Relative expression ratios of test samples versus the average of the fish at 12‰ salinity and 0.3 bl/s and first sampling time point 250 g) were calculated according to the method described by Pfaffl (2004). The efficiency of the PCR reactions was estimated for all primer pairs by five times 1:5 dilution series of a cDNA mix of all used samples.

2.3.4 | Welfare scores

Before blood and tissue samples were taken, the fish were evaluated for external welfare indicators (cataract, skin lesions, operculum shortening and fin damage) by using a scoring system developed by Kolarevic et al. (2013). Each of the welfare indicators was given a score between 0 and 2, where 0 is good and 2 represents bad condition. Evaluation of fin condition (pectoral, pelvic, anal, dorsal and caudal fin) was performed by a scoring system from 0 to 5 developed by Hoyle et al. (2007), where 0 indicates no visible damage and 5 indicates total erosion of the fin. The welfare scores were done by the same person on all samplings.

2.4 | Calculations

The feed intake per tank was calculated from the difference between the amount of feed fed to each tank and the amount of uneaten feed collected on a grid at the tank outlet, corrected for dry matter content in feed and recovery of feed distributed in empty tanks (Helland et al., 1996). Individual daily and cumulative feed intake was calculated by dividing the feed intake per tank with the number of fish in the tank. Specific growth rate (% day^{-1}) between two sampling points was calculated as:

$$\mathrm{SGR} = (\ln \mathrm{BW}_2 - \ln \mathrm{BW}_1) \times 100/\mathrm{d}\ (\mathrm{BW} = \text{bodyweight}, \mathrm{d} = \text{number of days}).$$

The thermal growth coefficient, TGC, was calculated as:

$$\mathrm{TGC} = 1{,}000 * \left(\mathrm{BW_2}^{1/3} - \mathrm{BW_1}^{1/3}\right) \times (\text{number of day degrees})^{-1}.$$

The FCR was calculated as: feed eaten (kg)/weight gain (kg).

Gonado-somatic index (GSI), hepato-somatic index (HSI), and cardiomyo-somatic index (CSI) were calculated from bodyweight and the weight of heart, liver, and gonads (W_o):

TABLE 3 Forward and reverse primers for real-time PCR

Gene name	Symbol	Accession number		Primer sequence
Inducible nitric oxide synthase	iNOS	AF088999.1	F	GCTAAACTGTGCCTTCAACTCCA
			R	CTCCATTCCCAAAGGTGCTAGTTA
Mucin 5ac-like	MUC5ac-like	JT819124.1	F	AGGCGTCCTTGTCCAAATAA
			R	CCTCTGGAAACTGGATGGTC
Elongation factor 1 alfa (internal standard)	Elf1a	*AF321836*	F	CACCACCGGCCATCTGATCTACAA
			R	TCAGCAGCCTCCTTCTCGAACTTC
18S rRNA (internal standard)	18S rRNA	AJ427629	F	GCCCTATCAACTTTCGATGGTAC
			R	TTTGGATGTGGTAGCCGTTTCTC

$$\text{Organ index} = 100 \times W_o / BW.$$

Individual weight and fork length was recorded to calculate condition factor (CF):

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

Survival was calculated as:

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

2.5 | Statistical analysis

Statistical analysis on growth, survival, organ indexes, welfare scores, and blood parameters were performed in SAS Jmp. A two-way analysis of variance (ANOVA) with salinity and water velocity as 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.

For gill NKA and cortisol, all statistical analyses were performed in Statistica 12.0. (StatSoft, Inc.). A nested ANOVA (tanks nested within salinity and velocity) was used; significant ANOVAs were followed by Newman–keuls *post-hoc* tests to identify the effects of salinity, size, and water velocity. Homogeneity of variances and normality of distributions were tested using Levene's F-test and Shapiro–Wilk W-test, respectively (Zar, 1996).

For gene expression, statistical analyses were performed with R (www.r-project.org/, version 3.1.0). Gene expression data (relative fold changes) were $\log_2$ transformed and tested by Shapiro–Wilk's test (stats package v3.1.0) for normal distribution and Levene's test for equality of variance. Subsequently, two-way ANOVAs were performed to identify significant differences between groups (stats package). For ANOVA *p*-values <.05, *post-hoc* Tukey tests were performed (stats package) to detect which groups differed significantly from each other. Histological rank data were analyzed by Kruskal-Wallis rank tests and pairwise Wilcoxon rank tests with *p*-value correction according to Holm (stats package).

3 | RESULTS

3.1 | Water quality

The effect of water velocity on the water quality was not significant, and values from tanks with high and low velocity were therefore pooled in Table 1. Higher salinity resulted in higher pH and alkalinity in the tanks supplied with the 32‰ RAS water. The change in salinity was also reflected by dilution of trace metals such as Aluminum (Al) typically transported by FW and an increase in trace metals such as Arsen (As) typically present in saltwater (Table 2). The concentration of trace metals varied somewhat between sampling times, but the levels were generally low (in range: 2–25 μg Al L^{-1}, 1–4 μg Mn L^{-1}, 6–30 μg Fe L^{-1}, 0.9–7.2 μg Ni L^{-1}, 0.4–2.6 μg Cu L^{-1}, 1.8–2.6 μg As L^{-1}, 0.03–0.14 μg Cd L^{-1} and < 1–1.4 μg Cr L^{-1}). Calculated concentrations of CO_2 never exceeded 4 mg L^{-1} in the tank outlet level in any of the treatments (Table 1). Measured turbidity was below 3 NTU (Nephelometric Turbidity Units) for all treatments, and TSS ranged between 2 and 13 mg L^{-1}, but there were no significant effects of treatment. Mean values per treatment are given in Table 1. There were no significant differences between the three RAS in terms of TAN, NO_2 –N, TSS, and turbidity when the entire trial period was taken into account. NO_3-N concentrations ranged between 7–28 mg/L^{-1}.

3.2 | Survival and growth performance

Overall, higher survival was observed at lower salinity during the entire trial ($p < .05$, Table 4). In Period 1 (70–250 g), mortality was low, (less than 3% in all treatments) but there was a negative effect of higher salinity on survival (Table 4), whereas there was no significant effect of water velocity in Period 1. In Period 2 (250–450 g), mortality increased in the 32‰ treatment (6 and 14% for low and high water velocity, respectively), whereas the mortality in the 12 and 22‰ treatments ranged from 0.2 and 1.2%. In period 2, water velocity had no effect on the cumulative survival in 12 and 22‰, whereas the cumulative survival was lower at high velocity (85.7%) compared to low velocity (94%) in 32‰ seawater.

The development in bodyweight during the whole trial was significantly affected by both salinity and velocity. Bodyweight increased with decreasing salinity ($p < .01$), and higher water velocity had a positive effect on bodyweight ($p < .01$, Table 4). In Period 2 (250–450 g), there was a significant interaction between salinity and water velocity on growth rate because of a higher growth at low water velocity in fish at 22‰ compared to high water velocity (Table 4), whereas there were no such interactions in Period 1 (70–250 g). Fish at lower salinity utilized the feed more efficiently for growth in Period 2 (Table 4); the FCR increased with increasing salinity between 250 and 450 g. The CF was highest in the 250 g fish at 12‰ and higher water velocity increased CF in 250 g fish, but this was not the case in 450 g fish (Table 4). There was no significant increase in whole-body protein content with increasing fish size ($p = .07$) and protein content was not affected by salinity or water velocity (Table S1, supplementary material). The fat content of the fish was not affected by salinity or velocity, but there was a minor reduction from 14.4% fat in 250 g fish to 13.5% fat in 450 g fish ($p < .05$). The energy content of the fish was not affected by treatment but increased slightly with fish size from 9.2 to 9.4 MJ/kg in 250 and 450 g fish, respectively ($p < .05$). There were no significant effects of salinity or fish size on the content of ash or dry matter in the fish.

3.3 | Gill metal concentration and NKA activity

The concentration of trace metals in gills was low (<2 μg Al g^{-1} dry weight [dw] gill, 212–253 μg Fe g^{-1} dw gill, <1.5 μg Cu g^{-1} dw gill, 18–30 μg Mn g^{-1} dw gill, 0.04–0.10 μg Cd g^{-1} dw gill, <0.05 μg Ni g^{-1} dw gill, and < 0.1 μg Cr g^{-1} dw gill), (Table 5) and in agreement with the low trace metal concentration in the water. The concentration of Cd and Mn in gills of fish decreased significantly by increasing salinity, probably as a result of increased competition of ions such as Na, Ca, and Mg in the more saline water as the concentration of both Cd and Mn was relatively similar in the 12–32‰ RAS water.

Higher gill NKA activity levels were observed in 250 g ($p < .0001$) and 450 g ($p < .05$) sized post-smolts reared in 32‰ compared to at 12‰ (Figure 1). Gill NKA activity in 250 g post-smolts were higher at higher velocity when reared at 32‰. Water velocity did not affect gill NKA activity in 450 g post-smolt (Figure 1b).

3.4 | Organ indexes and maturation

In general, high water velocity increased the relative heart and liver size (Table 6). The CSI was lower at higher salinity, but this effect was most prominent at lower bodyweight ($p < .001$). Male fish had a higher CSI than female fish. The HSI was higher in 250 g fish compared to in the 450 g fish and was not affected by sex. There was no effect of salinity on HSI. Neither salinity nor water velocity had a significant effect on the GSI, but females had a higher GSI than male fish, being 0.09 and 0.03% at the final sampling at 450 g, respectively. The GSI did not change with size of the fish (Table 6) and the external appearance of the fish did not indicate sexual maturation in any of the treatments. A low incidence (<1%) of small mature (dwarf) males was found in the 12 and 22‰ treatments when the fish were bulk weighed. No dwarf males were found in 32‰, but a few were registered as dead fish.

TABLE 4 Bodyweight (BW), growth rate (specific growth rate, SGR, thermal growth coefficient, TGC), feed conversion ratio (FCR) and survival (%) in Period 1 and 2

Period	Salinity (‰)	Velocity	BW	CF	SGR	TGC	FCR	% survival
Period 1 ~250 g	12	Low	274 ± 7	1.37 ± 0.06	1.54 ± 0.01	2.28 ± 0.03	0.90 ± 0.01	99.9 ± 0.1
	22		251 ± 11	1.34 ± 0.07	1.47 ± 0.07	2.11 ± 0.11	0.94 ± 0.02	98.0 ± 0.7
	32		258 ± 15	1.33 ± 0.06	1.50 ± 0.07	2.18 ± 0.13	0.91 ± 0.09	98.2 ± 1.6
	12	High	299 ± 1	1.42 ± 0.07	1.65 ± 0.01	2.47 ± 0.01	0.80 ± 0.01	99.5 ± 0.0
	22		268 ± 12.1	1.35 ± 0.07	1.56 ± 0.07	2.26 ± 0.12	0.93 ± 0.01	98.2 ± 1.2
	32		260 ± 5.2	1.36 ± 0.07	1.50 ± 0.04	2.20 ± 0.06	0.86 ± 0.01	97.0 ± 0.7
	Salinity		$p < .01$	$p < .01$	$p < .01$	$p < .05$	$p = .06$	$p < .01$
	Velocity		$p < .01$	$p < .05$	$p < .05$	$p = .06$	$p = .06$	NS
	Salinity*velocity		NS	NS	NS	NS	NS	NS
Period 2 ~450 g	12	Low	420 ± 11	1.20 ± 0.06	0.84 ± 0.01	1.59 ± 0.02	0.90 ± 0.04	98.8 ± 0.2
	22		417 ± 5	1.20 ± 0.08	1.00 ± 0.11	1.85 ± 0.19	0.98 ± 0.02	99.7 ± 0.0
	32		371 ± 22	1.20 ± 0.07	0.71 ± 0.01	1.32 ± 0.02	1.14 ± 0.04	94.0 ± 0.8
	12	High	470 ± 7	1.19 ± 0.05	0.90 ± 0.04	1.76 ± 0.09	0.93 ± 0.02	99.8 ± 0.2
	22		429 ± 22	1.21 ± 0.07	0.92 ± 0.01	1.74 ± 0.05	0.95 ± 0.02	99.0 ± 1.4
	32		405 ± 2	1.19 ± 0.07	0.87 ± 0.05	1.63 ± 0.09	1.06 ± 0.09	85.7 ± 0.9
	Salinity		$p < .01$	$p < .05$	$p < .01$	$p < .01$	$p < .01$	$p < .05$
	Velocity		$p < .01$	$p < .0001$	NS	$p = .07$	$p = .07$	$p < .05$
	Salinity*velocity		NS	NS	$p < .05$	$p < .05$	NS	NS

Note: Values are means per treatment ±*SD* ($N = 2$ tanks).

TABLE 5 Concentrations of metals in gills

	Salinity (‰)	Al (μg g^{-1})	Cr (μg g^{-1})	Mn (μg g^{-1})	Fe (μg g^{-1})	Ni (μg g^{-1})	Cu (μg g^{-1})	Zn (μg g^{-1})	Cd (μg g^{-1})
Sampling 250 g	12	0.4 ± 0.5	<0.1	28 ± 6^{a,b}	220 ± 39	<0.05	1.4 ± 0.1	510 ± 93	0.07 ± 0.02
	22	0.5 ± 0.7	<0.1	23 ± 5	216 ± 43	<0.05	1.4 ± 0.1	506 ± 92	0.08 ± 0.11
	32	0.6 ± 0.8	<0.1	20 ± 5	253 ± 42^{a,b}	<0.05	1.5 ± 0.1	482 ± 78	0.06 ± 0.02
Sampling 450 g	12	0.4 ± 0.4	<0.1	30 ± 3^{a,b}	227 ± 28	<0.05	1.4 ± 0.1	523 ± 97	0.09 ± 0.02^{a}
	22	0.6 ± 0.4	<0.1	23 ± 4^{a}	212 ± 36	<0.05	1.4 ± 0.1	516 ± 104	0.06 ± 0.11^{a}
	32	0.5 ± 0.5	<0.1	18 ± 2^{a}	240±34^{b}	<0.05	1.5 ± 0.1	464 ± 58	0.04 ± 0.01^{a}

[a]Significantly difference from other treatment at same time of sampling.
[b]Significantly difference from other treatment taken both sampling time into account.
Note: Values are means per treatment ±*SD*.

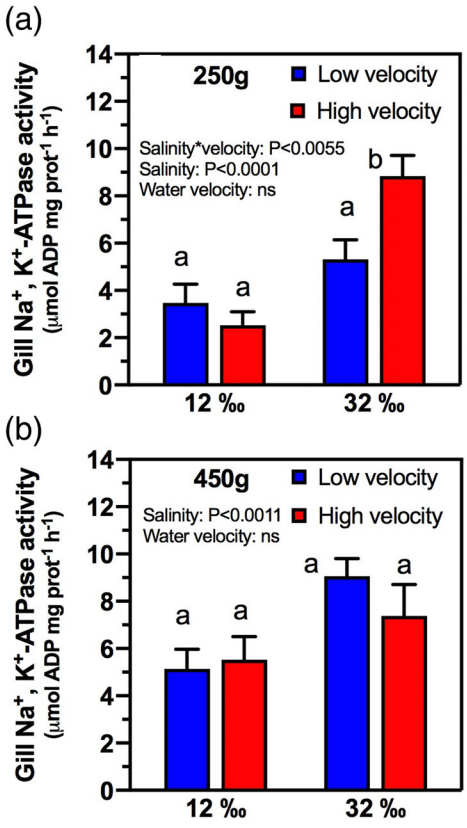

FIGURE 1 Gill NKA enzyme activity levels in (a) 250 g and (b) 450 g sized Atlantic salmon post-smolts reared at 12 and 32‰ in RAS. Data given are means per treatment ± *SEM* (*N* = 10–14). The significance of salinity, fish size, and water velocity on results are given. Significant differences between treatments (*p*-value <.05) are denoted by different letters A,B

3.5 | Welfare and stress indicators

Fin erosion scores increased with time in the experiment, with scores ranging from 0.5 to 2.8 at the end of the trial. The occurrence of skin lesions did not increase with time. Increased salinity led to more erosion of the caudal fin ($p < .05$) and high velocity led to more erosion of the anal fin at the end of the trial ($p < .01$). However, in general there were only marginal effects of salinity and water velocity on fin damage. The data on welfare scores are given in Table S2 in supplementary material. The effect of salinity on cataract scores was however significant; at 450 g, the cataract scores were 0.64 in exercised fish at 32‰, while they were lower than 0.07 in all the other treatments (Table S2). At the 450 g

TABLE 6 Conditionfactor (CF), Gonadosomatic index (GSI), Hematosomatic index (HSI), Cardiosomaticindex (CSI). F = females, M = males. Values are means per treatment ± SD (*N* = 2 tanks), 28 individuals from each treatment were sampled

			GSI		HSI		CSI	
Size	**Salinity (‰)**	**Velocity**	**F**	**M**	**F**	**M**	**F**	**M**
250 g	12	Low	0.09 ± 0.0	0.04 ± 0.0	1.06 ± 0.04	1.15 ± 0.18	0.081 ± 0.0	0.085 ± 0.0
	22		0.10 ± 0.0	0.03 ± 0.0	1.03 ± 0.02	1.05 ± 0.01	0.077 ± 0.0	0.077 ± 0.0
	32		0.10 ± 0.0	0.03 ± 0.0	1.09 0.03	1.10 ± 0.02	0.072 ± 0.0	0.074 ± 0.0
	12	High	0.08 ± 0.0	0.03 ± 0.0	1.15 ± 0.00	1.12 ± 0.09	0.084 ± 0.0	0.085 ± 0.0
	22		0.09 ± 0.0	0.03 ± 0.0	1.13 ± 0.04	1.12 ± 0.00	0.080 ± 0.0	0.086 ± 0.0
	32		0.09 ± 0.0	0.04 ± 0.0	1.15 ± 0.00	1.14 ± 0.03	0.077 ± 0.0	0.081 ± 0.0
		Salinity	NS		NS		*p* < .001	
		Velocity	NS		*p* < .05		*p* < .001	
		Sex	*p* < .0001		*NS*		*p* < .05	
		Salinity*velocity	NS		NS		NS	
450 g	12	Low	0.09 ± 0.0	0.03 ± 0.0	0.82 ± 0.01	0.82 ± 0.00	0.075 ± 0.00	0.077 ± 0.00
	22		0.10 ± 0.0	0.04 ± 0.0	0.84 ± 0.02	0.92 ± 0.05	0.070 ± 0.00	0.075 ± 0.00
	32		0.09 ± 0.0	0.03 ± 0.0	0.91 ± 0.01	0.90 ± 0.04	0.073 ± 0.00	0.072 ± 0.00
	12	High	0.09 ± 0.0	0.04 ± 0.0	0.89 ± 0.06	0.82 ± 0.02	0.081 ± 0.00	0.087 ± 0.00
	22		0.09 ± 0.0	0.03 ± 0.0	0.98 ± 0.09	0.95 ± 0.02	0.082 ± 0.00	0.086 ± 0.00
	32		0.09 ± 0.0	0.03 ± 0.0	0.94 ± 0.03	0.91 ± 0.08	0.077 ± 0.00	0.082 ± 0.00
		Salinity	NS		NS		*p* < .05	
		Velocity	NS		*p* < .05		*p* < .0001	
		Sex	*p* < .0001		*NS*		*p* < .05	
		Salinity*velocity	NS		NS		NS	

Abbreviations: F, females, M, males.

Note: Values are means per treatment ±*SD* (*N* = 2 tanks). Twenty-eight individuals from each treatment were sampled.

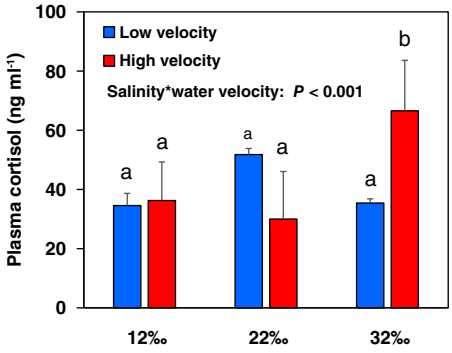

FIGURE 2 Plasma cortisol levels of 450 g post-smolt Atlantic salmon reared in RAS at 12, 22, and 32‰ at high and low water velocity. Data given are means per treatment ± *SEM* (N = 14). Significant differences between treatments (p-value <.05) are denoted by different letters A,B

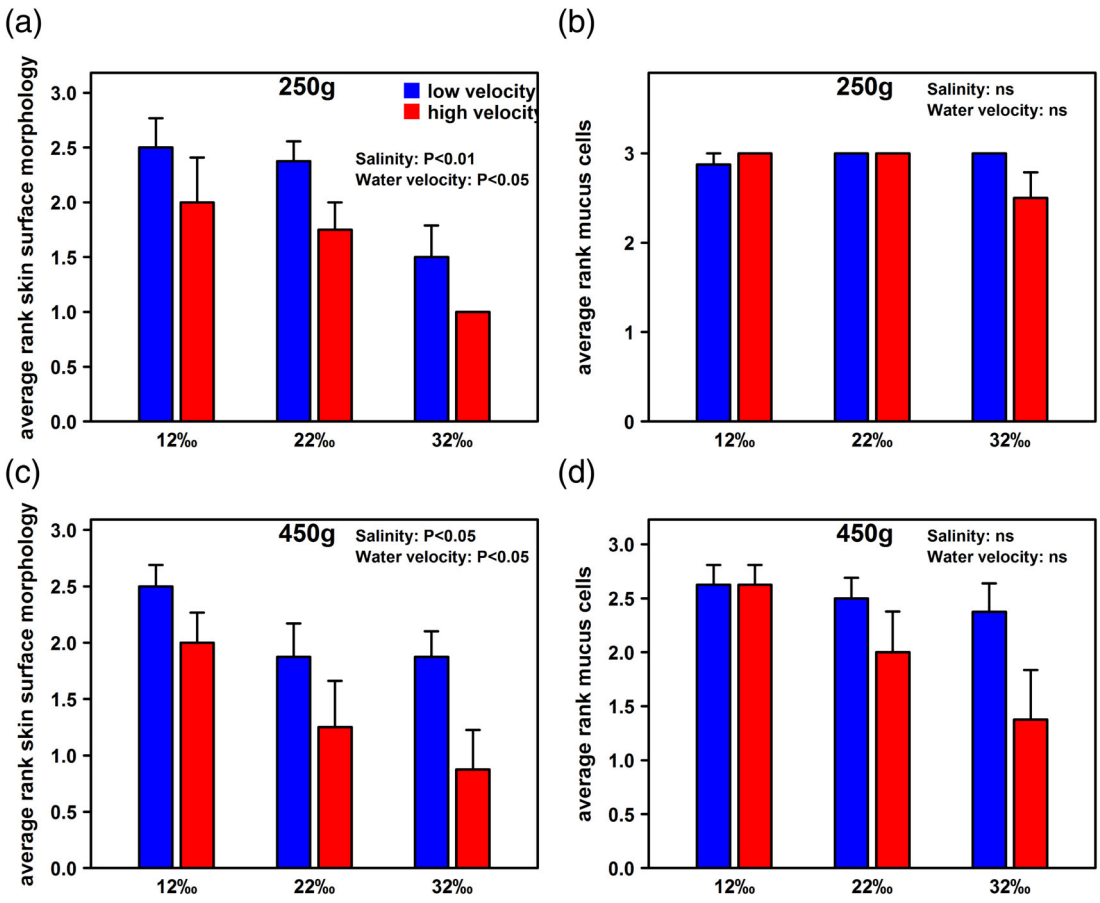

FIGURE 3 Ranks of skin surface morphology (left) and mucus cells (right) for fish grouped according to salinity and water velocity. Error bars indicate +/− standard errors. p-values of Kruskal-Wallis tests for salinity and water velocity are shown in the plots. Subsequent groupwise comparison (pairwise Wilcoxon-test with corrected p-values) did not result in significant differences between water velocity groups (p > .05)

sampling, exercised post-smolts in the 32‰ treatment had significantly increased plasma cortisol levels (67 ng·ml^{-1}) compared to the other treatments (Figure 2). Furthermore, the mean plasma cortisol levels were above 30 ng ml^{-1} in all treatments at 450 g.

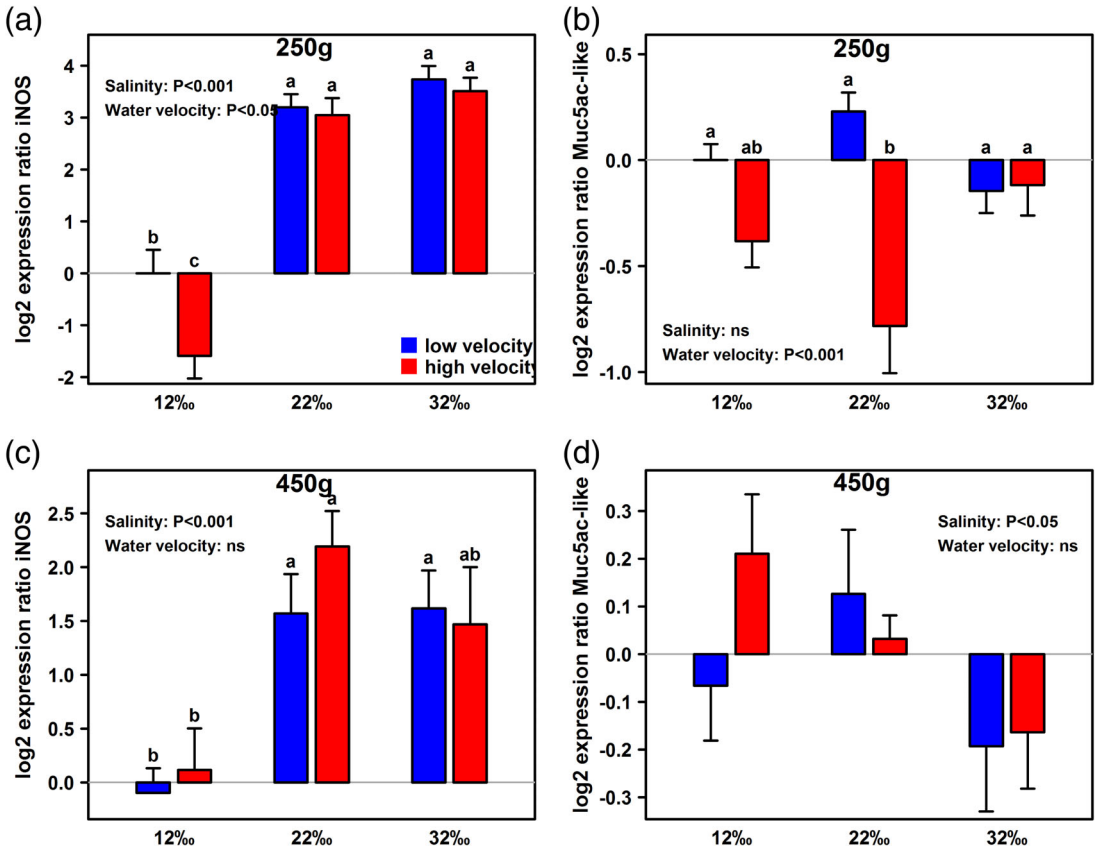

FIGURE 4 Relative gene expression of *iNOS* (left) and *Muc5ac-like* (right) in skin for fish grouped according to salinity and water velocity. Error bars indicate +/− standard errors. *p*-values of two-way ANOVA (salinity and water velocity) are shown in the plots. Significant differences (Tukey-test, *p*-value <.05) between water velocity groups are marked with letters A, B, and C for the different salinity and water velocity groups 12, 22, or 32‰

3.6 | Morphology and gene expression in skin

Skin surface morphology was negatively affected by increasing salinity and water velocity in Atlantic salmon post-smolts ($p < .05$, Figure 3a,c). Independent of post-smolt size, 32‰ salinity and higher water velocity resulted in the poorest skin surface morphology of all groups, while fish on 12‰ on low velocity had the best average rank. Semi-quantitative ranking of mucus cells on the skin surface was not significantly affected by water velocity or salinity. However, in 450 g fish at high velocity, decreasing mucus cell ranks were observed with increasing salinity ($p < .05$) (Figure 3d). There were no significant interactions between salinity and velocity.

The oxidative stress marker gene *inducible nitric oxide synthase* (*iNOS*) was significantly induced in skin of Atlantic salmon post-smolts reared in RAS at 22 and 32‰ salinity at 250 g (greater than sixfold) and 450 g (greater than threefold) compared to fish kept at 12‰ salinity (Figure 4a,c). High water velocity only had a significant effect on the analyzed genes at 250 g (ANOVA $p < .05$), *iNOS* mRNA expression was lower in the skin of fish on high velocity compared to fish at low velocity. The selected marker gene for mucus production, *Muc5AC-like*, showed a significantly lower mRNA expression in 250 g post-smolts at high velocity (ANOVA $p < .01$). Pairwise comparisons revealed that this effect was mainly because of the significant downregulation in high velocity fish, reared at 22‰ (Figure 4b,d). Different to 250 g fish, salinity had a significant effect on 450 g fish: The expression of *Muc5AC-like* mRNA was lowest for fish reared at 32‰ salinity ($p < .05$).

4 | DISCUSSION

Currently, there is a lack of information of what is an optimal water quality for Atlantic salmon post-smolts in RAS. The water quality in the three RAS in this experiment were well within the range regarded as safe levels of CO_2, TAN, NO_2-N, NH_3-N, TSS and turbidity for post-smolts in seawater (Knoph, 1995; Thorarensen & Farrell, 2011). Gill metal concentrations were low, and comparable with, for example, Al concentrations found in gills of Atlantic salmon in reference seawater (Bjerknes et al., 2003). Levels of other metals (Fe, Cu, Mn, Cd, Ni, and Cr) were similar to those found in the gills of brown trout (*Salmo trutta*) in FW (Rosseland et al., 2007). Thus, bioavailable metals from RAS production did not appear to cause problems in any of the salinities used in this experiment.

An important goal of the present study was to optimize the salinity and water velocity in land-based RAS with respect to fish welfare and growth. The results on fish performance show that using brackish water combined with moderate exercise in RAS is beneficial in terms of improved growth, feed utilization, and survival. The higher growth rate at the lowest salinity in RAS was established during the first months of the trial when the fish increased in weight from around 70–250 g, but there was also a significant positive effect of lower salinity on growth in Period 2 (250–450 g). Thus, at the end of the trial, fish at 32‰ weighed 13% less than fish at 12‰. The improved growth and feed utilization at a lower salinity in Atlantic salmon post-smolts is in accordance with results in several other teleost species, where salinities between 8 and 20% have a positive effect on growth and feed utilization compared to full strength seawater (Dietz et al., 2013; Gutt, 1985; Imsland et al., 2001; Lambert, Dutil, & Munro, 1994). 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‰. However, there may also be other mechanisms than reduced energy expenditure on osmoregulation that increase growth rate at lower salinities. The increased ingestion of water by salmon in a higher salinity (Evans, Piermarini, & Choe, 2005) may influence passage rate and/or digestibility of nutrients in a negative way, such as via effects on digestive enzymes as proposed by Boeuf & Payan, (2001). The present study shows a tendency for improved FCR at 12‰ compared to higher salinities, but the digestibility of nutrients was not measured in the present study.

Stress may increase the energy demand and reduce the energy available for growth, which may increase the FCR. The elevated cortisol levels in the 450-g trained fish in 32‰ compared to the other treatments may indicate a higher stress level at higher water velocity at 32‰ in RAS. This, in conjunction with the reduction in skin quality, and prevalence of cataracts further implies a reduced welfare in this treatment that may explain the lower survival seen in fish at 32‰ at higher velocity. Skin health may be a good indicator of environmental stress. The skin is in direct contact with the environment, and a healthy skin is vital for maintaining hydro-mineral balance and protection against pathogens in fish. In the present study, increasing salinity and water velocity negatively affected the skin surface morphology of Atlantic salmon. In support of the negative impact of high salinity on skin surface morphology, the oxidative stress marker gene iNOS, encoding the nitric oxide synthase enzyme that is the key driver for skin repair (Frank, Kämpfer, Wetzler, & Pfeilschifter, 2002), was highly upregulated in fish kept at salinities above 12‰. The poorer skin quality and thinner mucus layer at 32‰ would most likely have made the skin more vulnerable to damage when the fish were handled in connection with weighing at 250 g, and this may have contributed to the higher mortality observed in this treatment.

Exercise training increases the aerobic capacity of the fish through the combined effects on cardiac capacity and muscle morphology (Castro et al., 2011; Davison, 1997). Water velocities of 1.5 body lengths/second (bl s^{-1}) improved growth and feed conversion efficiencies in several salmonid species (Davison, 1997). As in earlier life stages of Atlantic salmon (Castro et al., 2011), the present study shows that increased water velocity improves growth rate and feed conversion in post-smolts, in the first period after seawater transfer. Increased water velocity has been found to increase the efficiency of energy and protein utilization for growth in 100 g Atlantic salmon post-smolts (Grisdale-Helland, Takle, & Helland, 2013). Higher water velocity also reduces agonistic behavior, and reduces stress levels, which is beneficial for fish welfare and robustness (Adams, Huntingford, Krpal, Jobling, & Burnett, 1995; Castro et al., 2011; Christiansen, Svendsen, & Jobling, 1992). Overall, the present study confirms that

increased water velocity can have a positive effect on post-smolt growth performance in RAS; fish at higher velocity were on average 8% bigger than fish at lower velocity at around 450 g. There was however not an effect of exercise on whole-body fat and protein levels in the present study, which supports the findings of Castro et al. (2011). In general, lipid content in fish is quite variable, and has been found to increase, decrease, and remain constant in response to exercise (Davison, 1997). During the salmon life cycle, lipid stores increase toward smoltification when they are mobilized for increased growth. After sea transfer, lipid concentration increases gradually until the fish starts to mature (Shearer, Åsgård, Andorsdöttir, & Aas, 1994). The proximate composition of the fish in the present study changed slightly from 250 to 450 g with a small increase in the protein/lipid ratio. The lipid level in the 250 g fish was however higher than what was reported by Shearer et al. (1994), whereas the protein levels were similar. In 450 g fish, the lipid level was still slightly higher compared to salmon of similar size in the study by Shearer et al. (1994). Thus, the reduction observed from 250 to 450 g could be a normalization of lipid levels, and not an indication of a negative development.

Atlantic salmon has the ability to mature already as parr in FW, during or shortly after smoltification in seawater (Fjelldal, Hansen, & Huang, 2011). The onset of maturation is governed by the interaction of genetic, physiological, and environmental factors recently reviewed by Good and Davidson (2016). Early maturation is often a problem in RAS (Davidson et al., 2016; Good, Davidson, Earley, Lee, & Summerfelt, 2014; Good, Weber, May, Davidson, & Summerfelt, 2015), and understanding the factors governing onset of sexual maturation in RAS is important when developing production strategies for large post-smolts. Energy status, photoperiod, and temperature are important signal cues for maturation (Good & Davidson, 2016). The effects of photoperiod regimes on maturation is conflicting; some authors report increased incidence of early maturation when salmon is exposed to continuous light (24:0) (Fjelldal et al., 2011), whereas other studies have found increased maturation after reduced photoperiod regimes (18:6 L:D) (Good et al., 2015). The reason may be the interaction between photoperiod and temperature, where water temperatures above 10°C have been found to increase early maturation in combination with 24 hr light (Fjelldal et al., 2011). Both low temperature and reduced period of daylight (12:12 L:D) reduce growth rate, CF, and fat deposition, which are important triggers of early maturation in salmon (Good & Davidson, 2016). The incidence of mature males was very low (<1%) in the present study and the GSI was below 0.35 in all treatments. The water temperature was also lower than in the trials reported by Good et al. (2014, 2015) and Davidson et al. (2016), and the interaction between these factors could be the reason for the low level of maturation found in the present trial. It is interesting that the fat level was initially quite high in the 250-g fish compared to salmon of similar size (Shearer et al., 1994). But even so, the male fish did not mature. In future studies, it would be of interest to follow maturation after transfer to seawater pens. Water temperature and the photoperiod that the fish experience after transfer to sea will most probably have an impact on growth and maturation in seawater. It is therefore important to transfer fish both in spring and autumn so the effect of increasing/ decreasing daylight and temperature on maturation can be assessed.

The ability to maintain ion homeostasis is reflected in a higher gill NKA enzyme activity in 250 and 450 g post-smolts reared at 32‰. This is consistent with an expected increase in ion secretory capacity when external salinity is high, despite enzyme levels in this study being somewhat lower than those previously reported in post-smolts (McCormick, 1993; Nilsen et al., 2007; Stefansson et al., 2012). Immature large Atlantic salmon (~3 kg) in flow-through systems display relatively low levels of gill NKA activity levels following transfer to both fresh- and seawater, while still being able to regulate ion homeostasis (Bystriansky & Schulte, 2011). This may be because of the smaller surface/volume ratio in larger fish, but fish skin also has thicker dermis in larger fish (Karlsen et al., 2018) that could decrease the permeability to ions. Thus, it could be speculated that an iso-osmotic environment is a bigger advantage for smaller fish than for larger fish. The positive effect of low salinity was most obvious in the first period of the trial (70–250 g). Traditionally, salmon smolts have been transferred to seawater pens at around 70 g; based on our results, it would be beneficial for growth performance and survival to keep them in brackish water RAS until 450 g if the fish are able to adapt to conditions in seawater pens. Whether brackish water is optimal for growth performance and reduces maturation if the fish are held in RAS until slaughter size is an area of ongoing research.

5 | CONCLUSIONS

The present study contributes to establishing a RAS production protocol that enables farmers to reduce the time salmon spend in open sea cages. Results from the study show that salinity below full-strength seawater combined with moderate exercise had a positive effect on salmon growth, survival, and welfare in RAS. Using water with salinity around 12‰ and a water velocity of minimum 1 bl s^{-1} may therefore be a cost-efficient production strategy provided that post-smolts can handle the subsequent transfer to open sea cages.

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Additional supporting information may be found online in the Supporting Information section at the end of this article.

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