



Freshwater history influences farmed Atlantic salmon (*Salmo salar*) performance in seawater

Floriana Lai^{a,*}, Ivar Rønnestad^a, Sergey Budaev^a, Pablo Balseiro^a, Virginie Gelebart^a, Cindy Pedrosa^a, Anita Stevnebø^b, Erlend Haugarvoll^b, Øyvind J. Korsøen^c, Kasper Løberg Tangen^c, Ole Folkedal^d, Sigurd Handeland^a

^a Department of Biological Sciences, University of Bergen, Bergen, Norway

^b Lingalaks AS, Norheimsund, Norway

^c Bluegrove AS, Oslo, Norway

^d Institute of Marine Research, Bergen, Norway

ARTICLE INFO

Keywords:

Recirculating aquaculture system (RAS)
Flow through system (FTS)
Smolt quality
Seawater performance
Growth performance
Net pens
Environmental variation
Unpredictability

ABSTRACT

The quality of smolts is one of the key factors for successful salmon production. We compared growth performance, physiological traits, and environmental adaptation in Atlantic salmon (*Salmo salar*) after transfer into a sea cages farm site from recirculating aquaculture system (RAS) to a similar sized group of the same genetic lineage and produced in the same husbandry facility but under flow through system (FTS) conditions. Fish vertical distribution within the sea cages and water environment were continuously recorded, while biometry data and biological samples were collected monthly. No significant difference in size and smolt development between the FTS and RAS-produced fish were observed at the end of the freshwater phase. However, after transfer into seawater, the RAS fish showed an array of physiological and molecular differences that were maintained and resulted in significantly increased mortality and lower growth over the full production cycle. The RAS smolts were characterized by lower body weight, length, K factor, HSI (indicator of energy reserve), NKA activity, plasma levels of lactate, triacylglycerol, sodium, calcium, and phosphorus than FTS fish in seawater. This implicated osmoregulatory and allostatic maladaptation for the RAS fish during the first months in seawater. Both FTS and RAS fish showed physiological and behavioral adjustments during the seawater production linked with predictable (e.g., seasonal, diurnal) and short-term unpredictable variation in water temperature and oxygen. However, the RAS fish were characterized by lower physiological response to stressful effects of unpredictable environmental variability.

Based on our data, the freshwater history sets the basis for the performance success of the FTS and RAS smolts in seawater. FTS fish showed faster adaptation to the seawater environment, with higher osmoregulatory capacity, and higher physiological robustness to seasonal changes than RAS fish, which reflected in their difference in growth and harvest size. We emphasize the crucial role of the freshwater rearing conditions as the foundation for successful physiological adaptation to seawater, growth, and the success of salmon farming production.

List of abbreviations

AEO	average experienced oxygen
APD	average preferred depth
APT	average preferred temperature
FTS	flow through system
GLS	generalized least squares (estimation)
HSI	hepatosomatic index

(continued on next column)

(continued)

K	condition factor
OLS	ordinary least squares (estimation)
RAS	recirculating aquaculture systems
RGR	relative growth rate
SFR	specific feeding rate
SGR	specific growth rate

* Corresponding author at: University of Bergen, Department of Biological Sciences, Pb 7803, 5020 Bergen, Norway.

E-mail address: floriana.lai@uib.no (F. Lai).

1. Introduction

Norwegian aquaculture of Atlantic salmon (*Salmo salar*) has expanded rapidly over the last 40 years, persisting as the world's leading farmed salmon producer, and accounting for an annual production of >1.5 million tons of fish in 2021 (Fiskeridirektoratet, 2022). As part of the continuous development, the Norwegian aquaculture industry has been at the forefront of developing novel technologies to support increased capacity and sustainability, resulting in an increase in the use of recirculating aquaculture systems (RAS).

In comparison to the more conventional flow through system (FTS), the RAS provides a more stable and controlled rearing environment, improved hygiene and disease management, and decreased water usage and environmental impact (Dalsgaard et al., 2013). On the other hand, RAS includes high investment and operating costs, and high technical complexity in comparison to the traditional FTS (Ahmed and Turchini, 2021). A wide range of studies support the use of RAS for salmon production, focusing on water salinity (Ytrestøyl et al., 2020), water flow (Kolarevic et al., 2014; Ytrestøyl et al., 2020), levels of dissolved oxygen (Kolarevic et al., 2014), carbon dioxide (Good et al., 2018), stocking densities (Liu et al., 2017; Wang et al., 2019) and feeding rates (Sun et al., 2016). There have also been studies conducted to determine the significance of temperature in the production of salmon smolts in RAS. Crouse et al. (2022), for example, recommend a temperature of 12 °C to achieve good growth performance while avoiding sexual maturation, whereas Ignatz et al. (2020) suggested a temperature of 10.5 °C or 13.5 °C for achieving good growth performance and nutrient utilization in triploid females of Atlantic salmon. As of yet, there have been only a limited number of studies investigating and comparing the production of Atlantic salmon in FTS and RAS. Kolarevic et al. (2014), for example, showed that fish reared in RAS or FTS at a constant 13 °C water temperature exhibited no difference in growth performance during the freshwater production phase and up to four months in seawater, however the FTS showed higher prevalence of fin damage and operculum shortage. Meanwhile, in the Tasmanian strain of Atlantic salmon, fish from FTS systems raised under an average temperature of 6 °C showed higher post-transfer growth performance and physiological ability to adapt to seawater than RAS fish farmed at constant 14 °C (van Rijn et al., 2020).

To date, the RAS have been largely used for production of juvenile of Atlantic salmon, with RAS fish accounting for the majority of smolts stocked in Norwegian sea cages (Meriac, 2019). However, the grow-out phase as post-smolts reared in sea cages remains challenging for the fish, where the mean accumulated mortality in Norway has been ~15% over the recent years (Grefsrud et al., 2023). Fish farmers have indeed listed "smolt quality" as a major underpinning factor for mortality (Bleie and Skrudland, 2014), implying that intensive freshwater production commonly produces fish that are unfit for thriving in sea cages. The quality of smolts is evidently one of the key factors for successful salmon production. The smolts are transferred from a controllable and relatively stable freshwater tank environment with high fish density and into sea cages where multiple environmental factors naturally fluctuate in time and space (Oppedal et al., 2011). The transfer success of Atlantic salmon to sea cages is largely dependent on the smolt's physiological ability to cope with environmental challenges as well as exposure to pathogens and parasites. In particular, early life experiences, as a result of different rearing strategies, are known to differentially influence physiological responses and plasticity later in the production cycle (Jonsson and Jonsson, 2014; Tang et al., 2022). Poor environmental conditions, for example, can result in low growth, small adult size, and low levels of energy storage (Taborsky, 2006).

As salmon are ectotherms, temperature determines the rates of virtually all biochemical reactions and thus the pace of physiological processes (Volkoff and Rønnestad, 2020). In contrast to FTS, where the freshwater temperature follows ambient seasonal variations, the RAS is typically characterized by a stable and controlled rearing environment.

We hypothesize that prolonged exposure to a stable environment, including stable temperature for growth optimization in RAS, can affect the fish's physiological plasticity and ability to adapt to and cope with the abrupt and seasonal changes in environmental factors in sea cages, potentially affecting the fish growth, welfare and survival of smolts. Thus, the purpose of this study was to evaluate and compare the performance, physiological traits, and environmental adaptability of Atlantic salmon smolts transferred to sea cages from a RAS controlled and stable environment to a similar sized group of the same genetic lineage and husbandry facility but under FTS variable conditions. The two groups were monitored during a Norwegian commercial production, from freshwater to harvest, under the same standardized management protocols. Biometry and physiological parameters associated with growth, seawater adaptation and energy balance were examined monthly, whereas fish vertical distribution in sea cages and the water environment was monitored daily.

2. Material and methods

2.1. Ethics statement

The study was approved as a field study in a commercial farming facility by the local representative of Sævareid Fiskeanlegg AS and Lingalaks AS, and samplings were carried out in accordance with the Norwegian Animal Research Authority guidelines.

2.2. Experimental design

2.2.1. Freshwater phase

Two groups of Atlantic salmon from the same SalmoBreed Bolaks strain (Eikelandososen, Norway), were monitored during commercial production from freshwater to harvest.

Batches of eggs from the same breeding stock were hatched in January 2020 and April 2020 (later spawning) at Sævareid Fiskeanlegg AS in the Hardangerfjord (60°). Both groups were reared under the same facility's standardized management protocols and maintained at a constant temperature of 8 °C for the initial 60 days after hatching, following which they were transferred to flow through tanks and subjected to seasonal fluctuations in environmental temperature and a 24-h simulated photoperiod regime (LD24:0) until they reached ~ 35 g fish size (Fig. 1).

The first hatched batch was then relocated and divided among three new FTS, which included two 12-m-diameter and one 15-m-diameter indoor tank supplied with water from the lake outside the facility. The water was not treated but oxygenated before going into the fish tanks. Temperature, oxygen CO₂, and pH were continuously measured from the inlet water (sensors Oxyguard). The second group of hatched fish was moved to two circular RAS tanks, each with a diameter of 14 m tanks where water from the outlets was filtered with mechanical drum filters (Hydrotech, Veolia water technologies) and biological treatment (Biofilm Chip P, Kaldnes MBBR, KrügerKaldnes). The water was then degassed and oxygenated within pressure cones before returning to the culture tanks via sump pumps. Temperature, oxygen CO₂, and pH were continuously measured in the sump pumps, in addition two oxygen sensors were placed inside every tank. Water parameters and oxidation reduction potential were measured continuously with different sensors stations in the system (Blue Unit A/S). In addition, water samples were taken and manually analysed in the facility lab every second day. As per routine of the facility, both groups were vaccinated (Pentium Forte Plus and Clynav, Elanco Denmark ApS, Norway Branch) at the end of September 2020.

Upon body weight of 77.8 ± 5.95 g and 75.43 ± 1.01 g for FTS and RAS respectively, the fish were subjected to a photoperiod with 12 h of light and 12 h of darkness (LD12:12), followed by 4–5 weeks (RAS-FTS) on LD24:0 to induce smoltification (Fig. 1). During this phase, trained personnel from the Sævareid facility monitored the fish smoltification

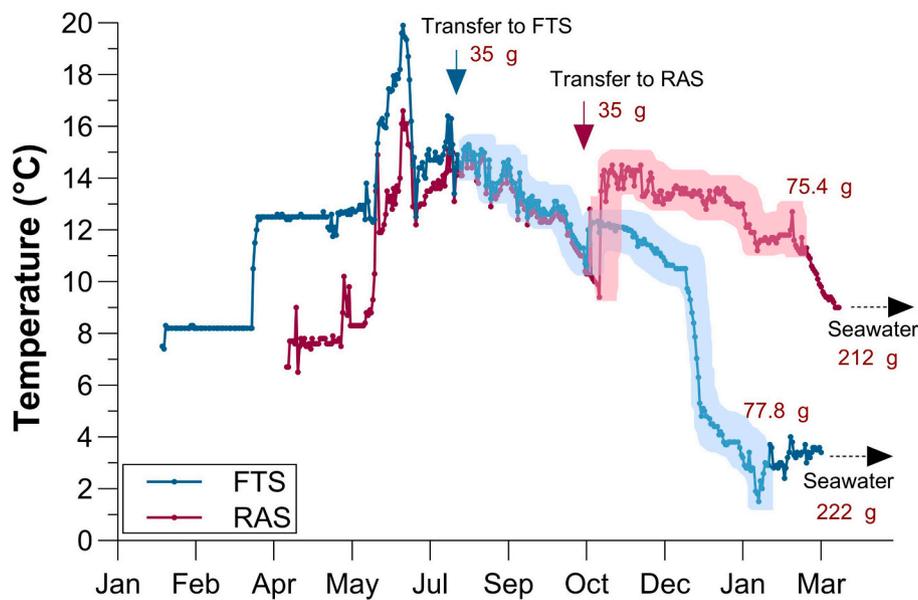


Fig. 1. Freshwater temperature history of fish reared in either flow through systems (FTS) or intensive recirculation aquaculture systems (RAS) before seawater transfer. Two batches of eggs from the same genetic lineage, one hatched in January 2020 (FTS) and one in April 2020 (RAS), were reared at LD24:0 light regime under the same facility’s standardized management protocols until they reached ~ 35 g fish size. Fish were then relocated in either new FTS or RAS tanks and subjected to LD12:12 (shown as blue highlight for FTS and in red for RAS), followed by LD24:0 to induce smoltification. Average fish body weight at start, during and at the end of FTS and RAS production is indicated in red font. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

development through the visual assessment of external characteristics of the fish (parr marks, silver coloration, and fin edges) for smolt index scoring (Table S1). All fish were fed in surplus using an automatic feeding systems and a commercial diet (EWOS and Skretting) and salt-fed prior seawater transfer (Nutra Supreme Ionic RC, 4). The fish were transferred to sea upon smoltification index of 4, where FTS and RAS showed a similar body weight (FTS 222 ± 6.4 g, 107 degree day (dd) from reset of LD24:0; 212 ± 1.0 g RAS, 271 dd from reset of LD24:0).

2.2.2. Seawater phase

Approximately 300 k fish from the FTS tanks and the same number from the RAS tanks (Table 1) were transported by well boat from the Sævareid facility to a single farm site of Lingalaks AS in the Hardangerfjord in Norway. For logistics reasons, the transport of the two groups was done 10 days apart in March 2021 (Fig. 1). Both groups were released and randomly distributed into 3 cages of FTS fish and 3 cages of RAS fish (25 × 25 m, and 40 m deep) (Fig. S1). The fish were fed a commercial diet (SG feed, Salmon group) and pellets were dispersed pneumatically over a circular (10 m in diameter) surface area in the cage

center by an automatic feeding system (Feedstation, Scale AQ). Feed control and fed amount was based on appetite per meal as observed in live video-streams from a remote-controlled sub-surface camera (Neo-Vision, Scale AQ) with pan and tilt and connected to a winch for vertical profiling in each cage. Moreover, for feed control purposes an echosounder system (CageEye, Bluegrove AS, Oslo, Norway), showing online fish density per depth in the central feeding volume, was used. Fish were harvested between February and April of 2022.

2.3. Sampling

One sampling was conducted in the freshwater phase, three (FTS) and four (RAS) days prior the fish were transferred to the sea cages at Lingalaks facility in March 2021. Then, monthly samplings were taken during seawater phase from April 2021 to January 2022. 10 fish per tank/cage were collected at each sampling time. Fish in the sea cages were captured by submerging a casting net (5 m × 5 m × 5 m) into the cage and subsequently pulling it up to the surface. Fish were crowded and randomly collected using a dip net. In both freshwater and seawater phases, fish were euthanized with a lethal overdose (over 250 mg/l) of

Table 1

Growth performance and accumulated mortality of Atlantic salmon over a full commercial scale sea cage production cycle. Data are from Lingalaks AS. *SGR (Specific growth rate), TGC (Thermal growth rate) and SFR (Specific feeding rate). The high mortality count registered for the FTS accounts the longer stay in seawater compared to RAS fish that were started harvesting 4 weeks earlier.

Cage	Freshwater rearing system	Transfer date	Average weight at transfer	Number of smolt at transfer	Average weight at slaughter	Closing first day of harvest	Closing last date of harvest	Closing SGR	Closing TGC	Closing SFR	Mortality count
2	RAS	29.03.2021	214	99,096	3661.67	29.01.2022	23.02.2022	0.89	2.46	1.00	11,930
5	RAS	28.03.2021	211	100,120	3639.81	03.02.2022	04.03.2022	0.86	2.42	0.96	11,418
6	RAS	28.03.2021	211	98,881	3634.67	04.02.2022	11.02.2022	0.90	2.46	1.00	13,718
AVERAGE RAS			212 ± 1.0	99,366	3645.4 ± 8.3			0.88 ± 0.01	2.45 ± 0.01	0.99 ± 0.01	12,355 ± 697.2
1	FTS	17.03.2021	210	101,313	4114.20	05.03.2022	26.03.2022	0.81	2.45	0.92	17,417
3	FTS	18.03.2021	232	101,342	4356.87	10.03.2022	17.03.2022	0.81	2.50	0.91	12,377
4	FTS	17.03.2021	224	100,825	4215.42	11.03.2022	04.04.2022	0.78	2.43	0.88	15,883
AVERAGE FTS			222 ± 6.4	101,160	4228.8 ± 70.4			0.80 ± 0.01	2.46 ± 0.02	0.90 ± 0.01	15,226 ± 1492

Benzocaine (ACD Pharmaceuticals AS) and blood was collected from the fish caudal vein using 2 ml syringes (BD Plastipak, VWR, Norway) with 23G heparinized needles (Microlance TM³, VWR, Norway). The blood was stored on ice until centrifuged (Eppendorf SE, Germany) at 5000 rpm for 3 min. Subsequently, plasma was collected and stored at -80°C . Fork length and weight were recorded, and liver, heart and gonads were weighed using an electronic precision balance with an accuracy of three significant numbers (VWR International AS, Norway). The second gill arch from the right side of the fish was excised, and dipped in SEI buffer (250 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) and immediately frozen in dry ice for subsequent measurement of Na^{+} , K^{+} , ATPase activity (NKA).

Production data of body weight at sea cage transfer and harvest, growth performance and mortality data from Lingalaks AS were used to validate our data set and are presented in Table 1.

2.4. Fish distribution and behavior

Fish vertical distribution from 0 to 25 m depth was continuously recorded by echo sound from an upwards-facing transducer (50 kHz, 44° opening angle) positioned centrally within each cage at 26 m depth (CageEye, Bluegrove, Oslo, Norway). Echo data of fish distribution were processed in 1 m depth layers every 15 min throughout the whole experiment, excluding periods of down time. The longitudinal echosounder data of fish density per depth helped in characterizing the patterns of fish distribution and thus the environmental preference of the fish over depth in the cages. To obtain a single measure of fish distribution, we calculated the depth of the maximum fish density at each time point, which was assumed to represent the fish average preferred swimming depth (APD) horizon (Oppedal et al., 2007).

2.5. Environmental measurements

During the seawater phase, temperature and oxygen level were measured every 2 h using an automatic vertical profiling buoy (APB5, SAIV AS, Bergen, Norway) from May 2021 to February 2022 (Fig. 2) (Fig. S1). Because temperature is a major factor affecting fish physiology and performance (Volkoff and Rønnestad, 2020), we calculated the temperature at the APD by linear interpolation of the temperature values recorded at three different depths (5, 10, 20 m) for each time point. This was assumed to be the average preferred available temperature (APT) of the fish in trade-off with light gradients (Oppedal et al., 2011). The oxygen level at the APT was interpolated at each time point over the grid of all depth (5, 10, 15, 20, 25 m) and is hereafter referred to as average experienced oxygen saturation (AEO).

2.6. Fish growth and physiological performance

2.6.1. Growth rate, condition factor, hepatosomatic index and cardiosomatic index

Fish weight and fork length were obtained from the monthly fish sampling. To determine the fitness of the fish, the condition factor (K) was calculated using the following equation:

$$K = 100 \times w/l^3 \quad (1)$$

where w is the weight (g) and l is the length of the fish (cm) (Froese, 2006).

Additionally, averaged specific growth rate (SGR) was calculated from the monthly sampling data following the Houde and Sचेckter (1981) formula:

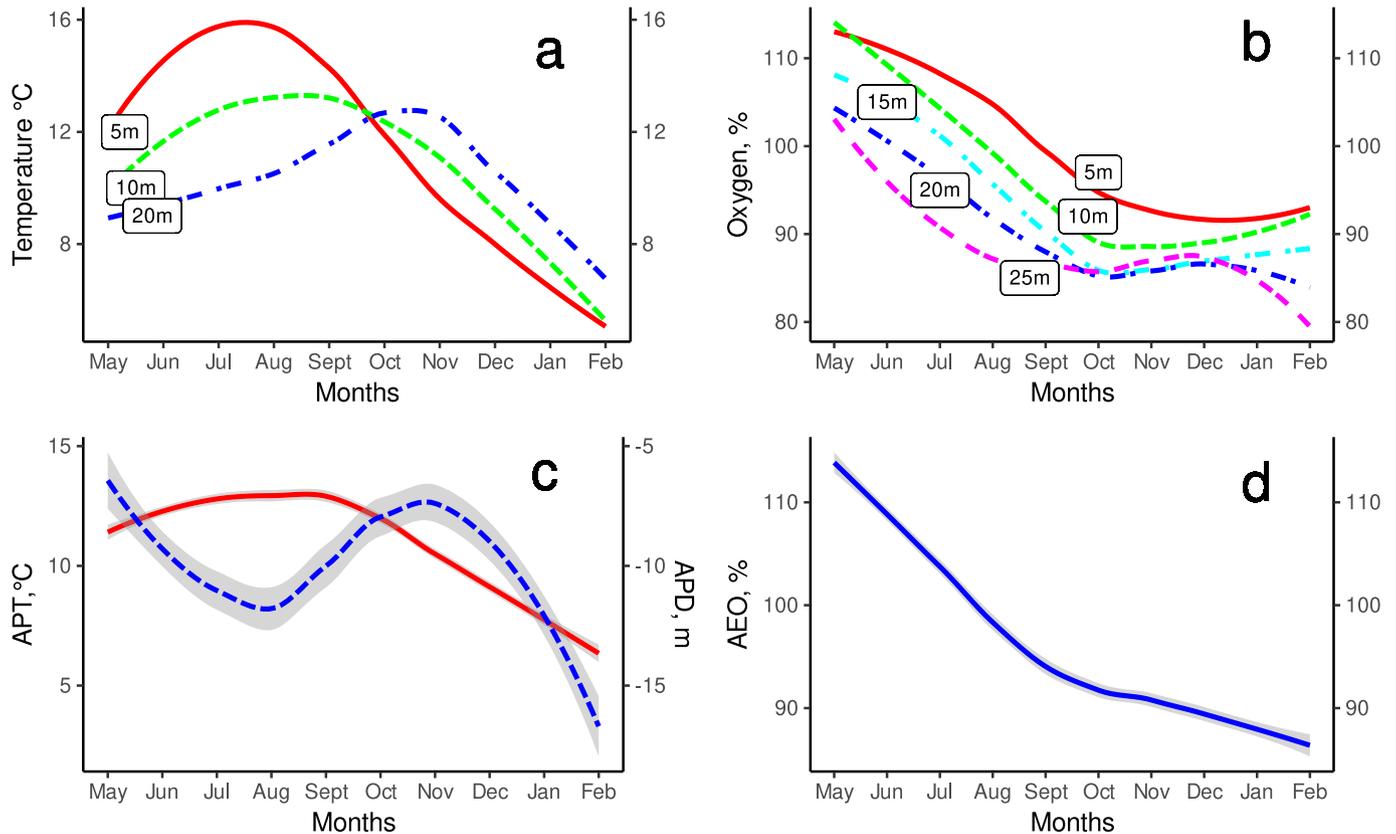


Fig. 2. Seawater environmental measurements. a-b) Seawater temperature ($^{\circ}\text{C}$) and oxygen (%) during the post-smolt production in the large-scale breeding facility in Bergadalen; c) average preferred depth (APD, dashed line) and temperature (APT); d) average experienced oxygen saturation (AEO). Due to some technicalities, measurements were only taken from May 2021 to February 2022. Data represent local-polynomial-regression (loess) smoothed values, shades represent SEM.

$$\text{SGR} = \left(e^{\frac{\ln w_2 - \ln w_1}{t_2 - t_1}} - 1 \right) 100 \quad (2)$$

where w_1 and w_2 are successive measures of the fish weight (g) and $t_2 - t_1$ is the time interval (days) between the measurements.

To provide an indication of the energy status and the metabolic activity of the fish, the hepatosomatic index (HSI) was calculated using the equation cited by [Chellappa et al. \(1995\)](#):

$$\text{HSI} = w_l/w \times 100 \quad (3)$$

in which w_l is the liver weight (g), and w is the weight of the whole fish (g).

While the cardiosomatic index (CSI) was calculated as follow:

$$\text{CSI} = w_h/w \times 100 \quad (4)$$

in which w_h is the heart weight (g).

Gonadosomatic index (GSI) was calculated as the ratio of the gonad weight to the whole fish weight (g):

$$\text{GSI} = w_g/w \times 100 \quad (5)$$

2.6.2. Feed amount and conversion rate

The specific feeding rate (%) was calculated as

$$\text{SFR} = 100 \times \frac{\sum F}{\sum B} \quad (6)$$

where $\sum F$ is the total feed mass (kg) per day and $\sum B$ is the total fish biomass (kg) for that day. The feed conversion rate (FCR) was calculated as

$$\text{FCR} = \frac{F}{w_2 - w_1} \quad (7)$$

where $w_2 - w_1$ is the weight increment and F is the mass of the feed provided over a specific time period. In this study, the statistical analysis was based on daily data.

2.6.3. Hematological traits

Plasma analyses were conducted using the Pentra C400 clinical chemistry analyzer (HORIBA, Japan). Chloride, sodium and potassium were analysed by potentiometry using the ion-selective electrode (ISE) module. The remaining parameters were determined by spectrophotometry using the appropriate HORIBA kit (ABX Pentra Calcium AS CP for calcium, ABX Pentra Magnesium RTU for magnesium, ABX Pentra Glucose HK CP for glucose, ABX Pentra Lactic Acid reagent for lactic acid, ABX Pentra Phosphorus CP for inorganic phosphorus, ABX Pentra Cholesterol CP for cholesterol and ABX Pentra Triglycerides CP for Triglycerides). At least 150 μl of plasma per sample was in the instrument to perform the analyses in parallel. All the analyses were calibrated, and quality controlled checked following manufacturer instructions.

2.6.4. Na^+ , K^+ - ATPase activity in gills

Smoltification was monitored using a combination of morphological and physiological traits, such as dark fin margins, silvery scales and high Na^+ , K^+ - ATPase (NKA) activity in the gills. The NKA activity was determined using the method described by [McCormick \(1993\)](#). Measurements were based on the hydrolysis of ATP to ADP from the ouabain-sensitive protein fraction, in a reaction enzymatically linked to the oxidation of NADH to NAD^+ by pyruvate kinase and lactic dehydrogenase. This reaction was measured for 10 min at 25 °C and 340 nm in a Tecan Spark® multi-mode microplate reader. The total amount of protein in the homogenate was determined by the bicinchoninic acid (BCA) protein assay kit (Thermo Scientific, IL, USA). NKA activity was then determined as the difference of ATP hydrolysis in presence and absence of ouabain, a specific NKA inhibitor, and expressed as μmol

ADP \times mg protein⁻¹ \times h⁻¹. 10 fish per tank/cage from March 2021 to January 2022 were used for the NKA analysis.

2.7. Statistical analysis

Statistical analyses were performed using R (R Core Team, 2018) and GraphPad Prism (Graph-Pad Software, version 9.3.1, San Diego, USA). Variables were checked for skewness and normality using the D'Agostino-Pearson omnibus test and Shapiro-Wilk's tests. When significant deviations from normality were detected, we used normalizing and linearizing transformations—log or square root as appropriate—with the minimum value adjustment as described by [Osborne \(2002\)](#). For multiple comparisons, the p values were adjusted by Benjamini, Krieger and Yekutieli procedure controlling false discovery rate. We used 'emmeans' R package for post-hoc comparisons. Data are normally presented as mean \pm SEM, unless otherwise stated. Confidence intervals (CI) presented are at 95% level. Significance levels † $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

2.7.1. Environmental unpredictability

Unpredictability of the environmental variables (temperature and dissolved oxygen) was assessed using the ratio of the mean square successive difference to variance ([von Neumann, 1941](#)):

$$A = \frac{\frac{1}{n-1} \sum_{i=1}^{n-1} (x_{i+1} - x_i)^2}{\frac{2}{n} \sum_{i=1}^n (x_i - \bar{x})^2} \quad (8)$$

Here n is the length of the time series, x_i is the i -th data value and \bar{x} is the mean. This index provides an estimate of the degree of smoothness and predictability for a time series data. For example, if there is a monotonic trend without turning points $A = 0$, but when the pattern of ups and downs in the data is random unpredictable, $A = 1$.

2.7.2. Statistical models

Fish characteristics from the monthly fish sampling during the seawater period represented time series data with the values close in time being more similar than data remote in time. Such data are expected to violate the assumption of independence for errors ([Fox, 2016](#); [Houde and Scheckter, 1981](#)). We therefore constructed linear models using generalized least squares (GLS) with restricted maximum likelihood (REML) and maximum likelihood (ML) estimation ([Faraway, 2009](#); [Fox, 2016](#)). Unlike common least squares or analysis of variance, GLS can be used when the assumptions of balanced design and independence of the errors are violated. Our strategy in model building was as follow: (a) construct a preliminary linear model $y = X\beta + \epsilon$ with ordinary least squares (OLS), investigate the distribution of errors (ϵ) and their serial correlations; (b) fit a GLS model $y = X\beta + \epsilon_t$ with the error term ϵ_t accounting for serial correlation using n -order autoregressive ARMA(n) process $\epsilon_n = \sum_{i=1}^n (\phi_i \epsilon_{t-i}) + \epsilon_t$, where n represent the lags that show significant autocorrelations and partial autocorrelations in the OLS model. For example, for a second order ARMA (2) errors $\epsilon_2 = \phi_1 \epsilon_{t-1} + \phi_2 \epsilon_{t-2} + \epsilon_t$ ([Fox, 2016](#)). This was computed using the R 'gls' function from the 'nlme' package ([Pinheiro et al., 2023](#)). In certain cases, we fitted the generalized linear models (GLM) with Gaussian response ([Fox, 2016](#)). For nonlinear analysis, we also computed orthogonal polynomial GLS models; then R 'poly' function was used. To account for breakpoint in linear relations, we fitted segmented models with a changepoint using the method of ([Muggeo, 2003](#)) implemented in the 'segmented' package of R ([Muggeo, 2008](#)).

2.7.3. Model selection

Several models were usually constructed in each case. We selected the best and most parsimonious inferential model using the Akaike weights (weights of evidence) over all models in a series. The 'MuMIn' R package ([Barton, 2023](#)) was used for such calculations. The Akaike weight of a specific model can be interpreted as the probability that this

model provides the best statistical approximation within the given set of models considered. Therefore, the higher the Akaike weight is, the more plausible is the model. Ideally, a plausible model should have the evidence weight over 0.8–0.9. The benefit of this approach is that it can be used with multiple models that are not necessarily nested. To compare the fit of nested models using analysis of variance, we (re)fitted their parameters using the maximum likelihood (ML) because REML applies transformation depending on the fixed effect, thereby making models incomparable (see Fox (2016) but note that there were no differences between ML and REML solutions). When more than one candidate model showed relatively high Akaike weights, we tried to be inclusive and selected a model with more predictors. This helped not overlook weaker effects, which is adequate for exploratory analysis.

3. Results

3.1. Fish rearing condition

During the later part of the freshwater phase, the FTS group was reared at lower water temperature compared to the RAS system. As shown in Fig. 1, in the five months prior to seawater transfer, the rearing water in the FTS system ranged from 12.0 ± 1.2 °C to 3.1 ± 0.0 °C, while in the RAS group ranged from 14.2 ± 0.00 °C to 9 ± 0.00 °C, exposing the two groups to different rearing water temperatures prior to seawater transfer. Oxygen levels were similar and within the range of 70–95% in the outlet of both FTS and RAS systems. At the time of transfer into the sea cages, the seawater temperature measured around 7°C in the uppermost 10-m seawater layer (value provided by the production site) then the water temperature registered pronounced seasonal changes that were stronger in the top layer, reaching as high as 15.8 ± 0.74 °C at 5 m depth in July 2021 (Fig. 2a). Oxygen saturation was also characterized by significant variability (Fig. 2b), but it never fell below 70.1%.

3.2. Feed amount during the seawater period

Detailed data from the feeding system was available for the seawater period. We analysed the effects of *Time* (month), *System* (RAS/FTS) and *Cage* (nested within *System*) on the specific feeding rate (SFR, eq. 6) and the feed conversion rate (FCR, eq. 7) using an autoregressive GLS models of the form:

M0: Full model, all interactions:

$$\sqrt{\text{SFR}} = \beta_0 + \beta_1 T + \beta_2 r + \beta_3 (r/c) + \sum (\beta_i, T, r) + \epsilon_i$$

$$\text{FCR} = \beta_0 + \beta_1 T + \beta_2 r + \beta_3 (r/c) + \sum (\beta_i, T, r) + \epsilon_i$$

M1: No interactions:

$$\sqrt{\text{SFR}} = \beta_0 + \beta_1 T + \beta_2 r + \beta_3 (r/c) + \epsilon_i$$

$$\text{FCR} = \beta_0 + \beta_1 T + \beta_2 r + \beta_3 (r/c) + \epsilon_i$$

with the raw data points representing daily time series values. Here *T* is *Time* (months), *r* is *System* (FTS/RAS) *c* is cage, $\sum (\beta_i, T, r)$ denotes all interactions and ϵ_i is *t*-order serially correlated error.

The full model M0 provided higher Akaike weights (0.85) and significantly better fit (*L*-ratio = 9.5, *df* = 11.8, *p* = 0.023) for SFR. For FCR, the simpler M1 provided better Akaike weight (1.0) with no significant difference in fit (*L*-ratio = 2.79, *df* = 12.9, *p* = 0.425). However, for all models, only the *Time* effect was significant for both SFR and FCR (Tables S2 and S3). This indicates that FTS and RAS groups were consistently fed equally (biomass-adjusted) during the seawater stage and there were no differences in the overall feeding efficiency. SFR first increased and then reduced over the successive months of the study ($1.53 \pm 0.06\%$ to $2.30 \pm 0.04\%$ and then $0.42 \pm 0.08\%$, Fig. S2) while FCR raised from 0.86 ± 0.02 to 1.42 ± 0.05 , (Fig. S3).

3.3. Fish distribution in sea cages, temperature and oxygen

The profiles of the fish density over the depth levels were typically very dynamic and variable. Although there was often one clear center of mass on the echograms (most individuals occupying one horizon), it could quickly change to periods of more even distribution (fish spread widely) or bi- or multi-modal distribution (fish formed several groups). In spite of variability, we believe the depth of maximum fish density (APD) represents a good proxy for the overall fish distribution. APD showed seasonal changes (Fig. S5) according to the seawater temperature as did dissolved oxygen at this depth (Fig. 2). APT—the temperature at APD—significantly correlated with the water temperature recorded at 5 m (FTS: $\rho = 0.92$, *p* < 0.0001, RAS: $\rho = 0.97$, *p* < 0.0001), 10 m (FTS: $\rho = 0.98$, *p* < 0.001; RAS: $\rho = 0.80$, *p* = 0.014) but not so at 20 m (FTS: $\rho = 0.56$, *p* < 0.095, RAS: $\rho = 0.16$, *p* > 0.68). Its pattern suggests that the fish tended to avoid low (<5 °C) as well as high (>16 °C) surface temperatures (Fig. S4) throughout the whole study period.

The pattern of the APD changes over the whole duration of the study was well approximated by an orthogonal polynomial GLS model of the 6th order with serially correlated residuals (Table S4, Fig. S5):

$$\log_{10}(\text{APD}) = \beta_0 + \beta_1 T + \beta_2 T^2 + \beta_3 T^3 + \beta_4 T^4 + \beta_5 T^5 + \beta_6 T^6 + \beta_7 r + \epsilon_i$$

It provided a significantly better inference (Akaike evidence weight 1.0, AIC = -3.5) than a simple linear GLS model (AIC = 19.6). The effect of the *System* factor was not significant in either model (nonlinear: *t* = 0.96, *p* > 0.33; linear *t* = 0.94, *p* > 0.34) suggesting that the pattern of the depth preference did not differ between FTS and RAS fish.

A nonlinear GLS model for APT approximating it with second-order orthogonal polynomials (Table S5, Fig. S6)

$$\sqrt{\text{APT}} = \beta_0 + \beta_1 T + \beta_2 T^2 + \beta_3 r + \epsilon_i$$

should be preferred (Akaike evidence weight 1.0, AIC = -61.7) over a linear GSL model (AIC = 5.5). For APT, the effect of the *System* factor was not significant (nonlinear: *t* = -0.84 *p* > 0.40; linear *t* = -0.70, *p* > 0.48). This suggests that the FTS and RAS groups did not differ in their average pattern of temperature preference.

A similar statistical modelling for the AEO (oxygen at APD) revealed that a second-order polynomial model (AIC = 254.9)

$$\sqrt{\text{AEO}} = \beta_0 + \beta_1 T + \beta_2 T^2 + \beta_3 r + \sum (\beta_i, T, r) + \epsilon_i$$

(including the interaction term $\sum (\beta_i, T, r)$) should be chosen over a linear (AIC = 259.8) with the Akaike evidence weight 0.92. However, for oxygen all effects were significant including *Time*, *System* and their interaction (Table S6). This points out that the FTS and RAS fish still displayed certain differences in their preferred depth, but this was not detected in our analysis of APD and APT. However, the FTS and RAS fish experienced some differences in the dissolved oxygen: lower level in RAS fish than in FT with the difference increasing from December (Fig. S7). This might suggest that the RAS group could prefer to be a little deeper than FTS in winter due to the higher temperature despite the reduced dissolved oxygen levels. RAS and FTS cages may also have somewhat different oxygen regimes due to spatial position of the cages (Fig. S1).

3.4. Atlantic salmon performance

3.4.1. Length and weight growth over the freshwater period

Prior to seawater transfer in March 2021, both groups showed very similar average body weight (FTS, 218.7 ± 6.8 g; RAS 213.4 ± 8.5 g), fork length (FTS, 26.8 ± 0.3 cm; RAS 26.6 ± 0.4 cm) and K (FTS 1.1 ± 0.0 ; RAS, 1.1 ± 0.0). No significant differences were found in these measures (body weight: $t_{620} = 0.03$, *p* > 0.9; fork length: $t_{620} = 0.17$, *p* > 0.8; K: $t_{620} = 0.02$, *p* > 0.9) at this period (Fig. 3).

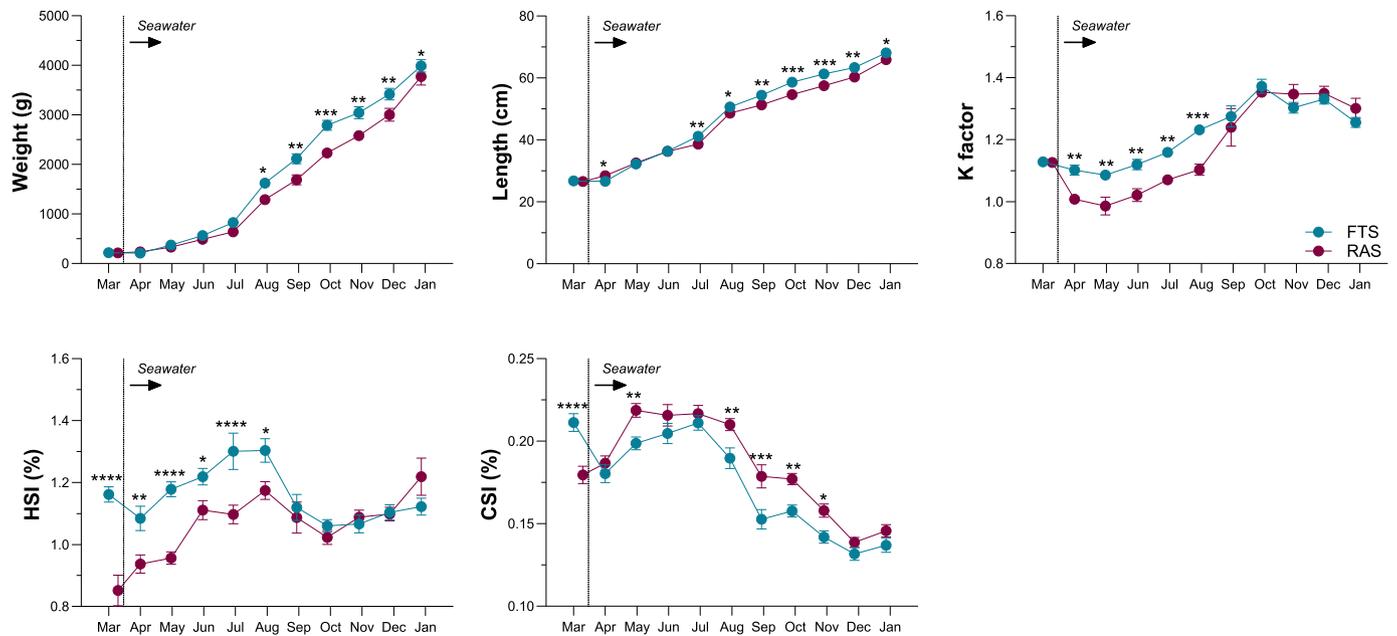


Fig. 3. Growth performance of Atlantic salmon reared in FTS or RAS system prior and after seawater transfer. For logistics reasons, in March 2021 the sampling and transfer of the two groups to sea was done 10 days apart. Data represent mean \pm SEM. $n = 30$ /group with the exception of March which have $n = 20$ in RAS.

3.4.2. Length and weight growth over the seawater period

During the first period in the sea cages, both FTS and RAS groups showed similar body weight and length (Fig. 3). However, significant growth differences between the two groups were observed 4 months after seawater transfer (Fig. 3, Table S7 and S8). From late summer (August 2021), fish coming from the FTS system showed a statistically higher increase in both weight and length compared to the fish raised in the RAS system ($p < 0.0001$) (Table S7 and Table S8). The RAS group experienced a notable mortality rate during the concluding phase of sea production (see further discussion below). Consequently, the fish in the RAS group were harvested four weeks before the FTS group (Table 1), with the farmer reporting an average closing gross weight and SGR of 4229 ± 70.48 g and 0.80 ± 0.01 g for the FTS group and 3646 ± 8.29 g and 0.88 ± 0.01 g for the RAS group (Table 1).

To understand how the different factors affected the growth and underlying physiological processes in the two groups we conducted a series of statistical modelling. We included the following predictors into the GLS models using the fish fork length and weight as the response variables (Tables S7 and S8): *Time* (months), *Sex* (male/female), *System* (RAS/FTS), *APT* and *AEO*. The full GLS models (including all interactions) showed that all β weights were non-significant (non-intercept $ps > 0.1$). This might have been due to over parametrization and excessive variance in the full model (particularly, many interaction terms). We suspected that a better focused model can be built with reduced error variation. Therefore, we conducted a model selection procedure for a range of GLS models. Because our primary objective was to understand the causes of the differences between FTS and RAS fish, we were mainly interested in *System* and its interaction with other predictors (“targeted interactions”).

M0: full model with all possible interactions:

$$\log_{10}(L) = \beta_0 + \beta_1 T + \beta_2 s + \beta_3 r + \beta_4 t + \beta_5 o + \sum \beta_i(T, s, r, t, o) + \epsilon_t$$

M1: No environmental factors but interactions.

$$\log_{10}(L) = \beta_0 + \beta_1 T + \beta_2 s + \beta_3 r + \beta_4 T \times s + \beta_5 T \times r + \beta_6 s \times r + \epsilon_t$$

M2: Minimal model:

$$\log_{10}(L) = \beta_0 + \beta_1 T + \beta_2 s + \beta_3 r + \epsilon_t$$

M3: Minimal model, no environmental factors, no targeted

interactions.

$$\log_{10}(L) = \beta_0 + \beta_1 T + \beta_2 s + \beta_3 r + \beta_4 T \times r + \epsilon_t$$

M4: Model with environmental factors but no interactions.

$$\log_{10}(L) = \beta_0 + \beta_1 T + \beta_2 s + \beta_3 r + \beta_4 t + \beta_5 o + \epsilon_t$$

M5: Model with environmental factors and targeted interaction.

$$\log_{10}(L) = \beta_0 + \beta_1 T + \beta_2 s + \beta_3 r + \beta_4 t + \beta_5 o + \beta_6 r \times T + \beta_7 r \times t + \beta_8 r \times o + \epsilon_t$$

Here L denotes the fork length (equations are identical for weight), T is time (months), s is sex, r is system (RAS/FTS), t is APT, o is AEO, $\sum \beta_i(T, s, r, t, o)$ denotes all combinations of interactions and ϵ_t , t -order serially correlated errors.

For both fork length and weight, the model M4 showed perfect Akaike evidence weight (fork length: 1.00, AIC = -657.4; body weight: 1.00, AIC = -181.7) with the next model having zero Akaike weight (length: AIC = -617.4; weight: AIC = -141.7). We may therefore be confident that M4 is the best inferential model among those considered. The difference between M4 and the full model M0 was not significant (length: L -ratio = 30.57, $df = 9.35, p \geq 0.24$; weight: L -ratio = 17.82, $df = 9.35, p \geq 0.882$) pointing that additional parameters do not contribute to any reduction of residual variation. In the M4 model, most effects (*Time*, *Sex*, *System*, *APT*) were significant for both length and weight (Tables S7 and S8). The effect of AEO was close to the significance level for the fish length but not significant for weight. Inspection of the contrasts show that, overall, the FTS group was characterized by higher length and weight than RAS. Males tended to have higher length and weight than females. Additionally, both temperature and dissolved oxygen (when significant) tended to have a positive link with the length and weight.

3.4.3. K-factor

The RAS and FTS groups showed difference in K factor during the seawater production as indicated by the timeline slopes; RAS: 0.050 ± 0.007 , CI -0.004 to 0.006; FTS: 0.026 ± 0.006 , CI 0.014 to 0.040 (Fig. 3). From the first month in the sea cages, the two groups showed distinct significant K differences, with the RAS group having a lower K factor than the FTS group in the first 5 months in seawater ($p < 0.0001$) (Table S9). The differences in K factor between the two groups of fish

disappeared in the late summer (September 2021) and similarly increased in both groups during the fall-winter season (Fig. 3).

The full GLS model (including all interactions) for *K-factor* showed that all β weights were non-significant (non-intercept $ps > 0.1$). This might have been due to excessive variance and large number of parameters in the full model (particularly, many interaction terms). We suspected that a better focused model can be built with reduced error variation. Therefore, we conducted a model selection procedure for a range of GLS models using the same workflow as with the length and weight.

M0:

$$K = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \beta_4 t + \beta_5 o + \sum \beta_i (T, s, r, t, o) + \epsilon_i$$

M1:

$$K = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \beta_4 T \times s + \beta_5 T \times r + \beta_6 S \times r + \epsilon_i$$

M2:

$$K = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \epsilon_i$$

M3:

$$K = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \beta_4 T \times r + \epsilon_i$$

M4:

$$K = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \beta_4 t + \beta_5 o + \epsilon_i$$

M5:

$$K = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \beta_4 t + \beta_5 o + \beta_6 r \times T + \beta_7 r \times t + \beta_8 r \times o + \epsilon_i$$

The models M2 and M3 showed both the best AIC weights with minimum difference (M2: 0.51, AIC = -541.64; M3: 0.49, AIC = -541.59) with the next models weighting essentially zero (next AIC = -520.3). The difference between the best M3 model and the full model M0 was not significant (L -ratio = 33.78, $df = 10.37$, $p = 0.17$) whereas M3 provided a better fit than M2 (log-likelihood, respectively, 299.03 and 293.03; L -ratio = 10.48, $df = 9.10$, $p = 0.001$). The more inclusive M3 (Table S9) was presented for interpreting.

We also tested the hypothesis that there was a breakpoint in the *K-factor* pattern over time. To do this, we fitted a gaussian GLM (without *Sex* factor that did not have significant link) with the best model structure M3 and then added a segmented relationship to the *Time* factor. The fitted model revealed a significant changepoint corresponding to October (Davies test for changepoint, $p < 0.001$). The resulting consecutive regression slopes were 0.053 ± 0.005 (CI 0.044 to 0.063) before and -0.032 ± 0.009 (CI -0.051 to -0.014) after the breakpoint. Thus, *K-factor* change over time can be split into two distinct periods: increase to October and slight reduction afterwards.

3.4.4. HSI

When analysing the HSI as indicator of energy status and the metabolic activity, the FTS and RAS fish displayed quite different patterns. The fish reared in the FTS system showed a significantly higher HSI compared to those in the RAS system (FDR-corrected $t_{620} = 6.66$, $p < 0.0001$) at the freshwater stage. For the seawater stage, the full GLS model (Table S10) with all interactions showed a complicated pattern with nearly all effects non-significant. Therefore, we conducted a model selection procedure for a range of GLS models using the same workflow as with the other measures above.

M0:

$$\sqrt{HSI} = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \beta_4 t + \beta_5 o + \sum \beta_i (T, s, r, t, o) + \epsilon_i$$

M1:

$$\sqrt{HSI} = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \beta_4 T \times s + \beta_5 T \times r + \beta_6 S \times r + \epsilon_i$$

M2:

$$\sqrt{HSI} = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \epsilon_i$$

M3:

$$\sqrt{HSI} = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \beta_4 T \times r + \epsilon_i$$

M4:

$$\sqrt{HSI} = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \beta_4 t + \beta_5 o + \epsilon_i$$

M5:

$$\sqrt{HSI} = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \beta_4 t + \beta_5 o + \beta_6 T \times r + \epsilon_i$$

The models M2 and M3 showed both the best AIC weights with minimum difference (M2: 0.51, AIC = -1025.6; M3: 0.49, AIC = -1025.1) with the next models weighting essentially zero (next AIC = -1001.5). There was also significant difference in fit between M3 and the full model M0 (log likelihood values respectively 541.71 and 594.92, L -ratio = 106.40 $df = 6.33$, $p \leq 0.001$; note that the opposite differences in fit between REML and ML estimation, suggesting that the single linear model was poorly specified), M3 also had better fit than M2 (log-likelihood values 541.72 and 536.00; L -ratio = 11.43, $df = 5.6$, $p < 0.001$). The more inclusive M3 (Table S10) was therefore considered the best model. The FTS group scored higher on *HSI* than RAS. Importantly, the RAS and FTS groups had the opposite dynamics over time as indicated by the slopes. RAS: 0.004 ± 0.002 , CI -0.0004 to 0.008; FTS: -0.006 ± 0.002 , CI -0.010 to 0.002. The plot (Fig. 3) reveals that RAS tended to increase the score and finally caught up FTS.

The pattern of HSI changed over time (Fig. 3) indicating that there may have been a breakpoint. To test this hypothesis, we fitted a gaussian GLM with square root link (excluding nonsignificant *Sex* factor) and then added a segmented relationship to the *Time* factor. The fitted model indicated a changepoint corresponding to June which was significant (Davies test for changepoint, $p = 0.009$). The resulting consecutive regression slopes were 0.041 ± 0.016 (CI 0.009 to 0.073) before and -0.010 ± 0.002 (CI -0.015 to -0.005) after the breakpoint.

We can summarize these patterns as follows. RAS fish started from a significantly lower HSI than FTS during the freshwater period. The two groups showed dissimilar dynamics over time (not affected by the environmental factors) before and after June. The FTS group overall had higher HSI scores than RAS during the first stage. However, RAS fish had a higher rate of HSI increase (higher positive slope) resulting in no differences between the two groups subsequently.

3.4.5. CSI

The fish reared in the RAS system showed a significantly lower CSI than FTS (FDR-corrected $t_{620} = 4.38$, $p = 0.003$) at the freshwater stage. To find the optimal inferential GLS model accounting for the effects of different factors at the seawater stage, the following models were considered with the same workflow as with the other measures above.

M0:

$$CSI = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \beta_4 t + \beta_5 o + \sum \beta_i (T, s, r, t, o) + \epsilon_i$$

M1:

$$CSI = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \beta_4 T \times s + \beta_5 T \times r + \beta_6 S \times r + \epsilon_i$$

M2:

$$CSI = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \epsilon_i$$

M3:

$$CSI = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \beta_4 T \times r + \epsilon_i$$

M4:

$$CSI = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \beta_4 t + \beta_5 o + \epsilon_i$$

M5:

$$CSI = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 r + \beta_4 t + \beta_5 o + \beta_6 T \times r + \epsilon_t$$

The models M2 was clearly the best, with AIC weights (0.99, AIC = -2196.3) with the next models weighting essentially zero (next AIC = -2185.5). The differences in fit between M2 and the full model M0 were significant ($L\text{-ratio} = 107.58$, $df = 33.5$, $p \leq 0.001$). Males had significantly higher CSI scores than females and RAS fish scored higher on CSI than FTS. The results are presented in Table S11.

3.4.6. GSI

RAS fish had higher GSI values than FTS fish during the freshwater stage (0.13 ± 0.008 versus 0.09 ± 0.006 , FDR-corrected $t_{620} = 3.30$, $p = 0.006$), with females also scoring significantly higher (0.14 ± 0.007) than males (0.08 ± 0.007 ; FDR-corrected $t_{620} = 7.07$, $p < 0.0001$). The following candidate models were considered for describing the GSI data at the seawater stage:

M0:

$$\sqrt{GSI} = \beta_0 + \beta_1 T + \beta_2 s + \beta_3 r + \beta_4 t + \beta_5 o + \sum \beta_i (T, s, r, t, o) + \epsilon_t$$

M1:

$$\sqrt{GSI} = \beta_0 + \beta_1 T + \beta_2 s + \beta_3 r + \beta_4 T \times s + \beta_5 T \times r + \beta_6 s \times r + \epsilon_t$$

M2:

$$\sqrt{GSI} = \beta_0 + \beta_1 T + \beta_2 s + \beta_3 r + \epsilon_t$$

M3:

$$\sqrt{GSI} = \beta_0 + \beta_1 T + \beta_2 s + \beta_3 r + \beta_4 T \times r + \epsilon_t$$

M4:

$$\sqrt{GSI} = \beta_0 + \beta_1 T + \beta_2 s + \beta_3 r + \beta_4 t + \beta_5 o + \epsilon_t$$

M5:

$$\sqrt{GSI} = \beta_0 + \beta_1 T + \beta_2 s + \beta_3 r + \beta_4 t + \beta_5 o + \beta_6 T \times r + \epsilon_t$$

The models M2 and M4 showed both the best AIC weights ($\Delta AIC < 2$) (M2: 0.69, AIC = -1506.6; M4: 0.28, AIC = -1504.8) with the next models weighting essentially zero (next AIC = -1500.0). Also, M4 provided a better fit than M2 (log-likelihood, respectively, 797.57 and 778.38; $L\text{-ratio} = 38.38$, $df = 5.7$, $p < 0.001$). While M2 was a better candidate for a prediction model, M4 suggested two potentially interesting environmental effects (significantly reducing residual variation) that may have affected GSI: APT (temperature) and AEO (oxygen) which were close to the standard significance (Table S12). This led us to prefer M4 with caution. The possible environmental effects (although $0.1 < p < 0.05$) include a negative link between temperature and GSI and positive, between oxygen and GSI.

3.4.7. Mortality

Mortalities that were registered during the seawater phase showed a higher incidence in the RAS group than in the FTS right after seawater transfer and in the later stage of the production (Fig. S9). For instance, in the RAS group 1.2% of fish were lost in the first month in seawater, compared to 0.6% loss from the FTS group. In addition, both groups experienced 8.1% for the RAS and 6.7% mortality for the FTS in the last 2 months in seawater, which rates were primarily attributed to treatments for sea lice, Amoebic gill disease (AGD), Pasteurella and cardiomyopathy syndrome (CMS), for which fish were treated periodically (Table S13 and Table S14). Due to higher mortality in the RAS group, these fish had to be harvested four weeks earlier than the FTS group. Overall, at the end of the production, the mortality during the seawater phase amounted to 11.2% for the RAS group, while 9.2% for the FTS group.

To quantify mortality, we calculated weekly ratio of the number of dead fish to the actual total group size N_{dead}/N_{total} . Because the pattern of mortality seemed to be U-shaped, an orthogonal polynomial GLS model

with serially correlated errors was fitted in addition to the linear models:

M1 (linear with interaction)

$$\log_{10}(N_{dead}/N_{total}) = \beta_0 + \beta_1 T + \beta_2 s + \beta_3 T \times s + \epsilon_t$$

M2 (linear, no interaction)

$$\log_{10}(N_{dead}/N_{total}) = \beta_0 + \beta_1 T + \beta_2 s + \epsilon_t$$

M3 (orthogonal polynomial)

$$\log_{10}(N_{dead}/N_{total}) = \beta_0 + \beta_1 T + \beta_2 T^2 + \beta_3 s + \epsilon_t$$

Here we investigated the effects of two predictors: *Time* and *System* (RAS/FTS). They were highly significant in **M1** (*Time*: $\beta_1 t = 5.66$, $p < 0.001$, *System*: $\beta_2 t = 3.43$, $p < 0.001$; but interaction $\beta_3 t = 0.25$, $p > 0.80$), **M2** (*Time*: $\beta_1 t = 5.72$, $p < 0.001$; *System*: $\beta_2 t = 8.98$, $p < 0.001$) and **M3** (Table S15). To compare the quality of the fit provided by the alternative models we calculated their respective Akaike weights. The polynomial model **M3** was an unquestionably better model: **M3** AIC = 1655.9, weight = 1.0 (**M2** AIC = 1745.3; **M1** AIC = 1756.1). The difference in fit between **M3** and the next **M2** was significant (log-likelihoods -805.97 and -857.56; $L\text{-ratio} = 82.64$, $df = 9.8$, $p < 0.001$). This confirms the U-pattern with heightened mortality scores during the freshwater stage and spring-summer (start of seawater trials) and in winter (end of trials). Importantly, RAS fish overall had higher mortality than FTS (main effect significant, Table S15, Fig. S9).

3.4.8. Na^+ , K^+ - ATPase activity in gills

Gill NKA activity levels related to osmoregulatory adaptation to seawater were observed both in the FTS and RAS groups at the end of the freshwater phase, with no statistically significant differences between the two (7.1 ± 0.65 and 6.05 ± 0.87 respectively) (Fig. 4). The NKA levels in the FTS group peaked once in seawater ($p < 0.0046$), being twice as high as the NKA levels observed in the RAS fish under the same environmental conditions ($p < 0.0001$). Contrarily, an increase in NKA activity in the RAS group was observed only two months after seawater transfer in May 2021 ($p < 0.0001$), yet its level remained significantly lower than NKA activity levels in FTS fish ($p = 0.0309$). No differences were observed 3 months after seawater transfer in June 2021, where NKA levels in FTS and RAS measured 7.79 ± 0.57 and 7.24 ± 0.34 respectively.

3.4.9. Hematological traits

There were no differences in freshwater phase plasma ions between the FTS and RAS groups, except for potassium and phosphorus, which were significantly higher in the FTS than in the RAS ($p = 0.0022$ and $p =$

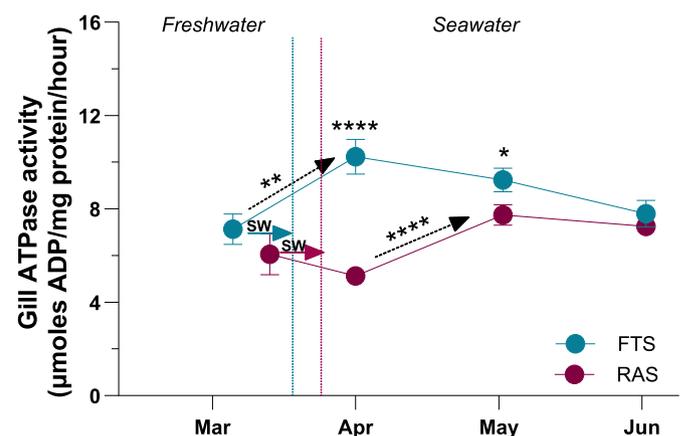


Fig. 4. Gill Na^+ , K^+ - ATPase activity before and after seawater transfer in Atlantic salmon produced in FTS or in intensive RAS systems. Data represent mean \pm SEM. $n = 30$ /group, except for March where RAS $n = 20$. For logistics reasons, the sampling and transfer of the two groups to sea was done 10 days apart in March 2021.

0.0019). Before seawater transfer, the fish reared in the FTS systems also demonstrated higher triacylglycerol and lactate levels than the RAS fish ($p < 0.001$ and $p < 0.0213$ respectively, Fig. S10).

To investigate the effects of the different factors on plasma ions following seawater transfer, we analysed the data using the same statistical modelling approach as the growth performance. Specifically, we considered the same six models (M0: full model, reduced models M1–M5) with the same model selection procedure. Overall, we observed significant effect of the *System* factor on sodium (Table S17), glucose (Table S18), calcium (Table S19), phosphorus (Table S20), cholesterol (Table S21) and triacylglycerol (Table S22) levels. Overall, the FTS group tended to display higher level of each of these ions than RAS fish (all β s for *System* r coded as RAS vs FTS in all models negative). For lactate, the effect of AEO (Tables S24) was significant. Significant breakpoints in the linear relationship over time were detected for sodium, glucose, lactate, calcium, magnesium, phosphorus (all at the time point corresponding to August with the highest temperature) suggesting that August was characterized by the peak values. Triacylglycerol showed an opposite pattern with a significant breakpoint between June–July, when its level reached minimum (Table S26).

3.5. Effect of environmental unpredictability

To investigate the effects of environmental unpredictability on fish performance during the seawater stage, we added the the ratio of the mean square successive difference to variance A (eq. 8) calculated for temperature (AT) and oxygen (AO) to the linear model that was deemed the best in out model selection procedure (i.e., best within the set of models M0 to M5, see above). We first fitted the models including the targeted interactions *System* \times A . When these interactions proved nonsignificant, we refitted the simplified models with only the additive terms. The fitting procedure was GLS with serially correlated errors.

Neither AT nor AO had significant effect on the body length (Table S27). But unpredictability of the oxygen level AO demonstrated significant negative effect (Table S28) on the body weight (higher AO linked with lower weight). For K-factor, both temperature (AT) and oxygen (AO) unpredictability index provided significant terms in the model (Table S29). Both higher AT and AO were linked with lower K factor.

HSI showed an interesting pattern of relationships differing between the FTS and RAS groups. While, on the whole, AO but not AT had significant effect on HSI, interaction between AT and *System* proved highly significant (Table S30). Whereas for the FTS group AT predicted slightly higher HSI (slope 0.036 ± 0.027 , CI -0.018 to 0.090), for RAS fish, AT was linked with significant reduction of HSI (slope -0.093 ± 0.042 , CI -0.174 to -0.011).

For CSI, AO but not AT effect was significant, but again, interaction terms with *System* were both significant (Table S31). Specifically, temperature unpredictability AT resulted in significant reduction of CSI in RAS fish (slope -0.03 ± 0.013 , CI -0.056 to -0.004) that was not characteristic of the FTS group (slope 0.009 ± 0.009 , CI -0.008 to 0.026). For AO interaction, unpredictability of the oxygen was linked with a higher increase of CSI in FTS group (slope 0.039 ± 0.008 , CI 0.025 to 0.054) than in RAS group (slope 0.013 ± 0.009 , CI -0.004 to 0.031). Finally, for GSI, AT but not AO showed a significant main effect as well as interaction with *System* (Table S32). Here temperature unpredictability tended to result in higher GSI in FTS (slope 0.064 ± 0.017 , CI 0.030 to 0.098) but not in RAS fish (slope -0.001 ± 0.024 , CI -0.052 to 0.051).

Overall, these results suggest that environmental unpredictability may have a negative effect on the fish growth (body weight and K factor) and HSI during the seawater stage. Furthermore, RAS fish tended to display reduced robustness to possible stressful effects of environmental unpredictability.

4. Discussion

In the current study we compared the growth performance, physiological traits, and environmental adaptation of two groups of smolt from the same genetic lineage reared in a RAS controlled and stable freshwater environment to smolt reared in a traditional FTS at natural and variable water temperature housed in the same husbandry facility. Once smoltified, fish were then transferred to a sea cages farm site and farmed under common commercial husbandry practices until harvest.

Atlantic salmon smolts produced in the FTS were characterized by higher body weight and length, and higher physiological robustness to seasonal effects and potentially stressful environmental unpredictability in seawater than the smolt produced in RAS. The early experience of rearing conditions in RAS (stable environment) and FTS (variable environment) may have set the basis for a successful seawater transfer, acclimation, physiological adaptation, and growth of smolts under seasonal environmental changes and unpredictable variability in the sea cages. Using statistical modelling we have identified the environmental factors that may have contributed to the marked differences between FTS and RAS fish: temperature, dissolved oxygen and their unpredictability. Yet, our results must be interpreted with some caution because the effects of temperature, oxygen and unpredictability followed their natural patterns and were not experimentally manipulated.

4.1. Growth and physiological performance

Fish produced in FTS and RAS had similar body size and smolt development by the conclusion of the freshwater phase. The FTS and RAS smolt presented comparable weight, length and K factor, as well as comparable osmoregulatory ability with sodium, chloride and potassium plasma levels, and gills NKA activity within the range of smoltified Atlantic salmon of similar size in freshwater (Handeland et al., 2003b; Pino Martinez et al., 2021). Few significant differences in physiological traits related to energy storage and mobilization were observed. HSI, plasma lactate and triacylglycerol levels were higher in the FTS smolt than in the RAS, suggesting a different metabolic status and/or energy homeostasis (Chellappa et al., 1995; Sheridan, 1994). Such variations in physiological parameters associated with energy balance can be ascribed to the contrasting rearing settings encountered by the two groups of fish throughout the smoltification phase. Specifically, the FTS group were subjected to a broader and colder temperature range in the rearing water, whereas the RAS smolts experienced a narrower and warmer temperature. Temperature has a crucial role in controlling the physiological processes of ectothermic organisms, and fish are generally able to cope with gradual temperature changes that occur in natural environments (e.g., daily variation, currents, and seasonal cooling) (Donaldson et al., 2008). Hence, the differences in the physiological factors related to energy balance observed between the FTS and RAS may be attributed to homeostatic mechanisms that fish actively trigger during their acclimation to thermal fluctuations (Crawshaw, 1979; Volkoff and Rønnestad, 2020). Moreover, it is crucial to acknowledge that the two groups were exposed to distinct LD12:12 photoperiod durations during freshwater production, and we cannot exclude that the photoperiod might have taken part in setting the homeostatic differences between the two groups of smolts. Nevertheless, the comparable smoltification index and NKA activity seen in both groups of smolts indicate that there was no discernible impact on the smoltifying process of the groups.

The first weeks in seawater represent a physiological critical period for salmon, with a common decrease in K factor and high mortality caused by both osmoregulatory challenges and adaptation into the new seawater environment (Grefsrud et al., 2023). In our study, we observed a detrimental impact of seawater transfer on growth in both FTS and RAS fish. Within the initial four months in seawater, both groups experienced a decrease in K factor and a slow gain in weight. However, the transfer into seawater appeared to be more challenging for the RAS

group, as they demonstrated a more pronounced decrease in K factor compared to the FTS, which only levelled 6 months after transfer. Concomitantly, the higher HSI already present in the freshwater phase of the FTS, was also observed up to 6 months after seawater transfer, supporting the different energy homeostasis between the two groups already observed at the end of the freshwater phase. Differences in hypo-osmoregulatory capacities between FTS and RAS were also observed. Once transferred into seawater, the FTS fish showed an increase in NKA activity, as a sign of osmoregulatory adaptation into the seawater environment (Handeland et al., 2003a). In contrast, the smolt from the RAS system showed a delay in the increase in NKA activity, which was observed only two months after seawater transfer. Transfer to seawater induced increase in plasma level of ions like sodium, chloride, calcium and phosphorus in both groups as hypo-osmoregulatory adaptation to the new sea environment (Handeland et al., 2014). However, some osmoregulatory differences were observed in RAS fish, which had significantly lower plasma sodium, calcium, and phosphorus levels than FTS fish. This finding could point to physiological challenges for RAS fish. Adaptation to increased salinity is demanding for the fish and can last for relatively long time. The RAS fish therefore seemed to display impaired osmoregulation as it illustrated by much higher mortality in this group in the first weeks after seawater transfer.

In comparison to other published studies with a shorter duration, we followed the fish for an entire production cycle, from freshwater to harvest based on commercial protocols. The growth performance of the two groups diverged from six months post-transfer until harvest. The FTS smolt showed a higher increase in weight and length compared to the RAS fish, which could be attributed to higher stored energy available, as indicated by higher HSI, cholesterol, and triacylglycerol levels in the FTS group. Indeed, it appears that the physiological differences observed between FTS and RAS fish during the first months after transfer established differences in growth performance in the months following transfer until harvest.

The total mortality registered during seawater production was 11.2% for RAS and 9.2% for FTS. In general, despite the differences between the two groups, the registered mortalities are below the reported national mean mortality of 15% per generation over the last five years, and the 19–20% in the current area of the study (Grefsrud et al., 2023). Both FTS and RAS experienced high mortality rates before harvest, which is a common welfare concern and financial burden in aquaculture. In our study, the major cause of mortalities was due to diseases, which affected the RAS fish more with 8.1% mortalities than the 6.7% mortalities in FTS two months prior harvest. In addition, due to the higher incidence of disease in one group than the other, the farmer started to harvest the RAS group four weeks earlier than the FTS. Interestingly, the RAS group showed a higher CSI compared to the FTS during the seawater phase. Such differences in the cardio index might be linked to the higher mortality registered in this group compared to the FTS. The higher CSI reported in the RAS fish during the seawater phase might have been at the base of the impairment of cardiac health, premature mortality and production loss as also reported in other studies of salmonids reared under intensive conditions (Brijs et al., 2020; Frisk et al., 2020). In particular, Atlantic salmon reared under intensive conditions showed slower growth rates at sea, distinct pathological cardiac morphological alterations, and CMS-related cardiac rupture at a different production facility (Frisk et al., 2020), supporting our findings and differences between FTS and RAS smolts.

The disparities in performance and physiological traits between the FTS and RAS fish during the seawater phase can be ascribed to differences in their rearing system and thermal history the fish experienced in the freshwater phase. Specifically, raising RAS fish in a more controlled and warmer setting, as opposed to the FTS group, may have resulted in the development of a different rate of adaptation to a fluctuating seawater environment, to which the RAS may not have had the necessary acclimation history as the FTS. Specifically, both groups were transferred to seawater in March 2021, where the water temperature in

the uppermost 10-m layer was 7 °C. Given the thermal history of the FTS and RAS in the freshwater phase, the change in temperature to a seawater environment may have presented the two groups with distinct thermal challenges. For instance, the fact that the FTS smolts experienced a warmer temperature (increasing from 3 °C in freshwater), while the RAS encountered a colder temperature (decreasing from 9 °C), may have resulted in an adaptation process that appeared to be more difficult for the RAS smolts. In addition, as the severity of thermal acclimation is known to dampen stress responses (Madaro et al., 2018; Tang et al., 2022), the differences in physiological adaptation and responses observed in RAS once in seawater could relate to an accumulation of challenges (Crawshaw, 1979; Madaro et al., 2018).

4.2. Temperature and oxygen as key factors in plasticity modulation

The growth difference observed between the FTS and RAS fish during sea production may be also related to their physiological response to predictable and unpredictable environmental variation. Fjord farming sites, as in the case of the sea cages in this study, experience relatively large seasonal changes in environmental conditions, with temperature being positively correlated with depth in winter and negatively correlated with depth in summer (Oppedal et al., 2011). Such variability in water temperature is of great physiological significance for ectotherm fish as salmon, as it influences fish metabolic pathways, feed intake, digestion and growth (Oppedal et al., 2011). For instance, in Atlantic salmon acclimated to SW at 4.3° C versus to 9.4° C, the physiological disturbance was greater, and the recovery was slower at low temperature (Handeland et al., 2003a), and appropriate physiological adaptation are at the base to promote stress resilience during this critical lifecycle period (Tang et al., 2022).

In our study, the fish regulated their average preferred depth to maintain some optimal temperature level: warmer at low winter temperatures and colder at high summer temperatures. In addition to smooth predictable seasonal change in the temperature and oxygen level, fish living in sea cages also experience unpredictable chaotic fluctuations that are presumably linked to short term local weather and sea conditions. The range and extent of such unpredictable fluctuations can depend on the season. Our statistical modelling results indicate that such unpredictability is stressful for the fish, and it is linked with poorer growth performance. While ongoing seasonal changes are acknowledged as a critical element in aquaculture, the significance of unpredictable and chaotic environmental fluctuations has not received any attention. One reason for this might be that they are not easy to quantify. Furthermore, little is known how fish perceive and physiologically respond to such random fluctuations. Salmon responds behaviourally to environmental fluctuations by changing their swimming depth which commonly affect the local fish density (Oppedal et al., 2011), and controlled lab studies show that Atlantic salmon post-smolt are sensitive towards sudden temperature fluctuations (Folkedal et al., 2012). However, our data provide one of the first evidence that sustained unpredictability of environmental fluctuations is stressful for caged fish.

The index we used in this study (A, eq. 8) is simple, but it quantifies unpredictability proper rather than just variability: variability can be predictable or unpredictable. A is linked with the Hurst exponent, a metric of the random Bronian process (Tarnopolski, 2016). Another advantage is that, unlike advanced measures of nonlinear dynamics (e.g. Tang et al., 2015), A involves sampling of successive values that can be a realistic task for fish. Fish may therefore detect environmental unpredictability using a similar subjective computational proxy and use it for making physiological and cognitive decisions (Budaev et al., 2019). We suggest that the von Neumann's unpredictability measure should be more routinely monitored in aquaculture.

A good physiological adaptation to the water environment, as well as the proper stress resilience, will determine the smolts' success in transferring to new rearing conditions. In our study, the RAS fish were reared in freshwater environment that followed the more standard

procedure used by Norwegian farmers, with a stable and controlled environment, while the FTS fish were reared in water that followed natural environmental variability of temperature and dissolved oxygen, including unpredictable fluctuations. These radical differences in early experience of environmental conditions may have had prolonged effects on the FTS and RAS fish performance once in sea cages, and the rearing temperature in freshwater might have been an important component in determining the physiological difference rate of success of between the two groups. According to our findings, FTS fish developed a higher degree of physiological plasticity to both predictable and unforeseen environmental changes, making them more resilient. Our statistical modelling showed temperature to be the key factor in the growth performance of both groups. During the sea production phase both groups were exposed to natural seasonal variations, with warm water in the summer and cold water in the winter. The water temperature peaked in August and was lowest in January the following year. Physiological adjustment, with evidence for plasma energy metabolites as glucose, lactate, triacylglycerol, and cholesterol, increased in the warmer seasons, reaching their peak levels in August. This period is however characterized by a lower response of the same physiological parameters in the RAS fish compared to the FTS fish, suggesting different physiological plasticity and response between the two groups to the same environmental challenge. In addition, the FTS fish increased their energy storage during the warm months of the year, as indicated by higher K factor and HSI, which was then allocated to growth during the colder season. The RAS, on the other hand, had a massive catch-up of condition factor to the level of the FTS group in August, which could indicate adaptation and a compensatory effect but were unable to fully compensate for the lost growth during the first 6 months at sea.

Our data suggest that unpredictable fluctuations in temperature and oxygen level negatively affect the fish and these effects may be more stressful for the RAS fish. As a result, the FTS appeared to have greater physiological plasticity towards environmental variability than the RAS. The different freshwater history of the two groups may be at the root of these physiological differences and responses to water temperature. Thus, exposure to a controlled and highly stable environment (e.g. temperature) in the RAS system might not have given the fish the ability to develop a good capacity to adapt to the sea cage environment. The multifactorial environmental variations, also including for salinity, turbidity and water current, and exposure to pathogens and parasites (not accounted for here), might have imposed high allostatic load and compromised welfare in the RAS fish (Korte et al., 2007). The use of RAS systems allows for controlled production and rapid fish growth in a relatively short period of time. However, prolonged experience of unchanging environment may make the fish develop an internal expectation of stability through allostatic mechanisms (McEwen and Wingfield, 2003; Sterling, 2012). When these fish suddenly encounter significant unexpected changes and fluctuations, their allostatic regulation seems to become impaired. This reduces their ability to adapt to changing environments, induces stress and increases susceptibility to disease in sea cages. Evolved adaptive mechanisms involving life history trade-off (Stearns, 1992) can also contribute to the reduced robustness of the RAS fish. Sudden change and subsequent uncertainty would signal poor future fitness prospects. This would make the fish follow the conservative tactics avoiding long-term fitness risks. Investments in expensive osmoregulatory mechanisms of sea migration and fast somatic growth could then be retarded. In our view, the results of this study emphasize two primary points: a) The disparity in fresh water temperatures at the time of seawater transfer (lower for FTS and higher and for RAS) likely played a significant role in the acclimation and adaptation in the new seawater environment in the sea cages; b) Subjecting fish to a wider range of seasonal temperatures in FTS, as opposed to stable temperatures in RAS, likely contributed to the development of increased physiological resilience and adaptability once exposed to varying seasonal conditions in seawater.

5. Conclusion

Atlantic salmon smolt produced in spring from parallel FTS and RAS systems showed several physiological and molecular differences, as well as differences in survival rate in seawater. The use of two distinct production systems, RAS and FTS, resulted in different growth performance of Atlantic salmon smolts during the production and harvest period in seawater. Based on our data, the FTS fish showed a faster adaptation to seawater environment, with an higher osmoregulatory capacity than RAS fish once in sea cages, and higher physiological robustness to seasonal changes than the RAS fish, which we regard as crucial for their difference in growth rate and harvest size. We hypothesize that these physiological differences are linked to the water temperature and its variability during the freshwater phase, which may have exposed the two groups to different environmental challenges, allowing the FTS to develop a more physiologically plastic response to environmental changes than the RAS fish once in seawater. Thus, our study shows that raising salmon in a highly stable RAS environment may negatively affect their robustness towards coping in sea cages. This may induce an array of physiological characteristics and impair their growth performance. Even though further experimental research is needed to determine the causal links, a potential mitigation strategy would be to increase environmental variability in RAS systems to stimulate fish capacity for coping with their future sea environment. In addition, the present study emphasises the significant role that history of freshwater rearing conditions played in establishing the groundwork for the fish's successful physiological adaptation to seawater, growth performance, and ultimately the success of production of Atlantic salmon.

Funding

This study was supported by the Research Council of Norway (RCN), NoFood2Waste grant No. 317770.

Author contributions

The study was conceived and designed by FL, IR, PB, AS, EH, ØK, KLT, OF and SH. Experiment and sampling were executed by FL, VG, CP, and AP. FL, VG and CP performed preparatory lab work and NKA analysis. PB analysed plasma. Statistical analysis and related graphs done by FL and SB. FL and SB drafted the manuscript. All authors read, revised, and approved the submitted version.

CRedit authorship contribution statement

Floriana Lai: Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Ivar Rønnestad:** Project administration, Writing – original draft, Writing – review & editing. **Sergey Budaev:** Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Pablo Balseiro:** Methodology, Project administration, Writing – original draft, Writing – review & editing. **Virginie Gelebart:** Methodology, Writing – original draft, Writing – review & editing. **Cindy Pedrosa:** Methodology, Writing – original draft, Writing – review & editing. **Anita Stevnebo:** Methodology, Project administration, Writing – original draft, Writing – review & editing. **Erlend Haugarvoll:** Project administration, Writing – original draft, Writing – review & editing. **Øyvind J. Korsøen:** Project administration, Writing – original draft, Writing – review & editing. **Kasper Løberg Tangen:** Project administration, Writing – original draft, Writing – review & editing. **Ole Folkedal:** Project administration, Writing – original draft, Writing – review & editing. **Sigurd Handeland:** Project administration, Writing – original draft, Writing – review & editing.

Declaration of competing interest

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

Data availability

All data generated or analysed during this study are included in the Supplementary Material.

Acknowledgments

The authors thank Suliman Elsadin, Helene Bjørnevik Bjelland, and the personnel from Sævaried AS, and Gaute Femsteinevik, Roald Kåreid, Leif Aksnes from Lingalaks AS for their assistance during samplings, data collection for water parameters, fish feeding and production.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2024.740750>.

References

- Ahmed, N., Turchini, G.M., 2021. Recirculating aquaculture systems (RAS): environmental solution and climate change adaptation. *J. Clean. Prod.* <https://doi.org/10.1016/j.jclepro.2021.126604>.
- Barton, K., 2023. MuMIn: Multi-Model Inference. R Package Version 1.47.5.
- Bleie, H., Skrudland, A., 2014. Tap av Laksefisk i Sjø Rapport fra Mattilsynet 1–36.
- Brijs, J., Hjeltnest, P., Berg, C., Johansen, I.B., Sundh, H., Roques, J.A.C., Ekström, A., Sandblom, E., Sundell, K., Olsson, C., Axelsson, M., Gräns, A., 2020. Prevalence and severity of cardiac abnormalities and arteriosclerosis in farmed rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 526, 735417. <https://doi.org/10.1016/j.aquaculture.2020.735417>.
- Budaev, S., Jørgensen, C., Mangel, M., Eliassen, S., Giske, J., 2019. Decision-making from the animal perspective: bridging ecology and subjective cognition. *Front. Ecol. Evol.* 7, 164. <https://doi.org/10.3389/fevo.2019.00164>.
- Chellappa, S., Huntingford, F.A., Strang, R.H.C., Thomson, R.Y., 1995. Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. *J. Fish Biol.* 47, 775–787.
- Crawshaw, L.I., 1979. Responses to rapid temperature change in vertebrate ectotherms. *Integr. Comp. Biol.* 19, 225–237. <https://doi.org/10.1093/icb/19.1.225>.
- Crouse, C., Davidson, J., Good, C., 2022. The effects of two water temperature regimes on Atlantic salmon (*Salmo salar*) growth performance and maturation in freshwater recirculating aquaculture systems. *Aquaculture* 553. <https://doi.org/10.1016/j.aquaculture.2022.738063>.
- Dalsgaard, J., Lund, I., Thorarinnottir, R., Drenngstig, A., Arvonen, K., Pedersen, P.B., 2013. Farming different species in RAS in Nordic countries: current status and future perspectives. *Aquac. Eng.* 53, 2–13. <https://doi.org/10.1016/j.aquaeng.2012.11.008>.
- Donaldson, M.R., Cooke, S.J., Patterson, D.A., Macdonald, J.S., 2008. Cold shock and fish. *J. Fish Biol.* 73, 1491–1530. <https://doi.org/10.1111/j.1095-8649.2008.02061.x>.
- Faraway, J.J., 2009. Linear Models with R, Chapman and Hall/CRF. <https://doi.org/10.4324/9780203507278>.
- Fiskeridirektoratet, 2022. Akvakulturstatistikk: settefiskproduksjon av laks, regnbueørret og orret.
- Folkedal, O., Torgersen, T., Olsen, R.E., Fernö, Anders, Nilsson, J., Oppedal, F., Stien, L. H., Kristiansen, T.S., Fernö, A., 2012. Duration of effects of acute environmental changes on food anticipatory behaviour, feed intake, oxygen consumption, and cortisol release in Atlantic salmon parr. *Physiol. Behav.* 105, 283–291. <https://doi.org/10.1016/j.physbeh.2011.07.015>.
- Fox, J., 2016. Applied Regression Analysis and Generalized Linear Models, Third Ed. Sage, Thousand Oaks CA.
- Frisk, M., Høyland, M., Zhang, L., Vindas, M.A., Øverli, Ø., Johansen, I.B., 2020. Intensive smolt production is associated with deviating cardiac morphology in Atlantic salmon (*Salmo salar* L.). *Aquaculture* 529, 735615. <https://doi.org/10.1016/j.aquaculture.2020.735615>.
- Froese, R., 2006. Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. *J. Appl. Ichthyol.* <https://doi.org/10.1111/j.1439-0426.2006.00805.x>.
- Good, C., Davidson, J., Terjesen, B.F., Takle, H., Kolarevic, J., Bæverfjord, G., Summerfelt, S., 2018. The effects of long-term 20 mg/L carbon dioxide exposure on the health and performance of Atlantic salmon *Salmo salar* post-smolts in water recirculation aquaculture systems. *Aquac. Eng.* 81, 1–9. <https://doi.org/10.1016/j.aquaeng.2018.01.003>.
- Grefsrud, E.S., Andersen, L.B., Grøsvik, B.E., Karlsen, Ø., Kvamme, B.O., Hansen, P.K., Husa, V., Sandlund, N., Stien, L.H., Solberg, M.F., 2023. Risikorapport Norsk Fiskeoppdrett Produksjonsdødelighet hos oppdrettsfisk og miljøeffekter. Rapp. fra Havforsknings nr.2023–6.
- Handeland, S.O., Björnsson, B.T., Arnesen, A.M., Stefansson, S.O., 2003a. Seawater adaptation and growth of post-smolt Atlantic salmon (*Salmo salar*) of wild and farmed strains. *Aquaculture* 220, 367–384. [https://doi.org/10.1016/S0044-8486\(02\)00508-2](https://doi.org/10.1016/S0044-8486(02)00508-2).
- Handeland, S.O., Porter, M., Björnsson, B.T., Stefansson, S.O., 2003b. Osmoregulation and growth in a wild and a selected strain of Atlantic salmon smolts on two photoperiod regimes. *Aquaculture* 222, 29–43. [https://doi.org/10.1016/S0044-8486\(03\)00100-5](https://doi.org/10.1016/S0044-8486(03)00100-5).
- Handeland, S.O., Imsland, A.K., Nilsen, T.O., Ebbesson, L.O.E., Hofield, C.D., Pedrosa, C., Toften, H., Stefansson, S.O., 2014. Osmoregulation in Atlantic salmon *Salmo salar* smolts transferred to seawater at different temperatures. *J. Fish Biol.* 85, 1163–1176. <https://doi.org/10.1111/jfb.12481>.
- Houde, E.D., Scheckter, R.C., 1981. Growth rates, rations and cohort consumption of marine fish larvae in relation to prey concentrations. *Rapp. P.-V. Reun. Cons. Int. Explor. Mer.* 178, 441–453.
- Ignatz, E.H., Dumas, A., Benfey, T.J., Hori, T.S., Braden, L.M., Runnighan, C.D., Rise, M.L., Westcott, J.D., 2020. Growth performance and nutrient utilization of growth hormone transgenic female triploid Atlantic salmon (*Salmo salar*) reared at three temperatures in a land-based freshwater recirculating aquaculture system (RAS). *Aquaculture* 519. <https://doi.org/10.1016/j.aquaculture.2019.734896>.
- Jonsson, B., Jonsson, N., 2014. Early environment influences later performance in fishes. *J. Fish Biol.* 85, 151–188. <https://doi.org/10.1111/jfb.12432>.
- Kolarevic, J., Bæverfjord, G., Takle, H., Ytteborg, E., Reiten, B.K.M., Nergård, S., Terjesen, B.F., 2014. Performance and welfare of Atlantic salmon smolt reared in recirculating or flow through aquaculture systems. *Aquaculture* 432, 15–25. <https://doi.org/10.1016/j.aquaculture.2014.03.033>.
- Korte, S.M., Olivier, B., Koolhaas, J.M., 2007. A new animal welfare concept based on allostasis. *Physiol. Behav.* 92, 422–428. <https://doi.org/10.1016/j.physbeh.2006.10.018>.
- Liu, B., Liu, Y., Sun, G., 2017. Effects of stocking density on growth performance and welfare-related physiological parameters of Atlantic salmon *Salmo salar* L. in recirculating aquaculture system. *Aquac. Res.* 48, 2133–2144. <https://doi.org/10.1111/are.13050>.
- Madaro, A., Folkedal, O., Maiolo, S., Alvanopoulou, M., Olsen, R.E., 2018. Effects of acclimation temperature on cortisol and oxygen consumption in Atlantic salmon (*Salmo salar*) post-smolt exposed to acute stress. *Aquaculture* 497, 331–335. <https://doi.org/10.1016/j.aquaculture.2018.07.056>.
- McCormick, S.D., 1993. Methods for nonlethal gill biopsy and measurement of Na⁺, K⁺ + -ATPase activity. *Can. J. Fish. Aquat. Sci.* 50, 656–658. <https://doi.org/10.1139/f93-075>.
- McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. *Horm. Behav.* 43, 2–15. [https://doi.org/10.1016/S0018-506X\(02\)00024-7](https://doi.org/10.1016/S0018-506X(02)00024-7).
- Meriac, A., 2019. Smolt Production and the Potential for Solid Waste Collection in Norway. *Nofima Rep. nr 25/2019*.
- Muggeo, V., 2003. Estimating regression models with unknown break-points. *Stat. Med.* 22, 3055–3071. <https://doi.org/10.1002/sim.1545>.
- Muggeo, V., 2008. Segmented: An R Package to Fit Regression Models with Broken-line Relationships, vol. 8. R News, pp. 20–25.
- von Neumann, J., 1941. Distribution of the ratio of the mean square successive difference to the variance. *Ann. Math. Stat.* 12, 367–395.
- Oppedal, F., Juell, J.E., Johansson, D., 2007. Thermo- and photoregulatory swimming behaviour of aged Atlantic salmon: implications for photoperiod management and fish welfare. *Aquaculture* 265, 70–81. <https://doi.org/10.1016/j.aquaculture.2007.01.050>.
- Oppedal, F., Dempster, T., Stien, L.H., 2011. Environmental drivers of Atlantic salmon behaviour in sea-cages: a review. *Aquaculture*. <https://doi.org/10.1016/j.aquaculture.2010.11.020>.
- Osborne, J.W., 2002. Notes on the use of data transformations. *Pract. Assess. Res. Eval.* 8 <https://doi.org/10.7275/4vng-5608>.
- Pinheiro, J., Bates, D., Team, R.C., 2023. Linear and Nonlinear Mixed Effects Models. R Packag. Version 3.1-162.
- Pino Martinez, E., Balseiro, P., Pedrosa, C., Haugen, T.S., Fleming, M.S., Handeland, S.O., 2021. The effect of photoperiod manipulation on Atlantic salmon growth, smoltification and sexual maturation: a case study of a commercial RAS. *Aquac. Res.* 52, 2593–2608. <https://doi.org/10.1111/are.15107>.
- van Rijn, C.A., Jones, P.L., Evans, B.S., Huynh, C., McCormick, S.D., Afonso, L.O.B., 2020. Characterization of smoltification in the Tasmanian strain of Atlantic salmon (*Salmo salar*) in recirculation and flow-through systems. *Aquaculture* 516, 734603. <https://doi.org/10.1016/j.aquaculture.2019.734603>.
- Sheridan, M.A., 1994. Regulation of lipid metabolism in poikilothermic vertebrates. *Comp. Biochem. Physiol. – Part B Biochem.* 107, 495–508. [https://doi.org/10.1016/0305-0491\(94\)90176-7](https://doi.org/10.1016/0305-0491(94)90176-7).
- Stearns, S.C., 1992. *The Evolution of Life-Histories*. Oxford University Press, Oxford.
- Sterling, P., 2012. Allostasis: a model of predictive regulation. *Physiol. Behav.* 106, 5–15. <https://doi.org/10.1016/j.physbeh.2011.06.004>.
- Sun, G., Liu, Y., Qiu, D., Yi, M., Li, X., Li, Y., 2016. Effects of feeding rate and frequency on growth performance, digestion and nutrients balances of Atlantic salmon (*Salmo salar*) in recirculating aquaculture systems (RAS). *Aquac. Res.* 47, 176–188. <https://doi.org/10.1111/are.12480>.
- Taborsky, B., 2006. Mothers determine offspring size in response to own juvenile growth conditions. *Biol. Lett.* 2, 225–228. <https://doi.org/10.1098/rsbl.2005.0422>.

- Tang, L., Lv, H., Yang, F., Yu, L., 2015. Complexity testing techniques for time series data: a comprehensive literature review. *Chaos, Solitons Fractals* 81, 117–135. <https://doi.org/10.1016/j.chaos.2015.09.002>.
- Tang, P.A., Stefansson, S.O., Nilsen, T.O., Gharbi, N., Lai, F., Tronci, V., Balseiro, P., Marnix, M., Ebbesson, L.O.E., 2022. Exposure to cold temperatures differentially modulates neural plasticity and stress responses in post-smolt Atlantic salmon (*Salmo salar*). *Aquaculture* 560. <https://doi.org/10.1016/j.aquaculture.2022.738458>.
- Tarnopolski, M., 2016. On the relationship between the Hurst exponent, the ratio of the mean square successive difference to the variance, and the number of turning points. *Phys. A Stat. Mech. Appl.* 461, 662–673. <https://doi.org/10.1016/j.physa.2016.06.004>.
- Volkoff, H., Rønnestad, I., 2020. Effects of temperature on feeding and digestive processes in fish. *Temperature*. <https://doi.org/10.1080/23328940.2020.1765950>.
- Wang, Y., Chi, L., Liu, Q., Xiao, Y., Ma, D., Xiao, Z., Xu, S., Li, J., 2019. Effects of stocking density on the growth and immunity of Atlantic salmon *Salmo salar* reared in recirculating aquaculture system (RAS). *J. Oceanol. Limnol.* 37, 350–360. <https://doi.org/10.1007/s00343-019-7350-7>.
- Ytrestøyl, T., Takle, H., Kolarevic, J., Calabrese, S., Timmerhaus, G., Rosseland, B.O., Teien, H.C., Nilsen, T.O., Handeland, S.O., Stefansson, S.O., Ebbesson, L.O.E., Terjesen, B.F., 2020. Performance and welfare of Atlantic salmon, *Salmo salar* L. post-smolts in recirculating aquaculture systems: importance of salinity and water velocity. *J. World Aquacult. Soc.* 51, 373–392. <https://doi.org/10.1111/jwas.12682>.