Received: 30 October 2017 | Accepted: 6 May 2018

DOI: 10.1111/jfb.13659

JOURNAL OF FISH BIOLOGY fsbi

SPECIAL ISSUE REGULAR PAPER

Comparison between Atlantic salmon *Salmo salar* post-smolts reared in open sea cages and in the Preline raceway semi-closed containment aquaculture system

Pablo Balseiro[1] | Øyvind Moe[1] | Ingrid Gamlem[1] | Munetaka Shimizu[2] | Harald Sveier[3] | Tom O. Nilsen[1] | Nobuto Kaneko[2] | Lars Ebbesson[1] | Cindy Pedrosa[1] | Valentina Tronci[1] | Are Nylund[4] | Sigurd O. Handeland[1]

[1]Uni Research Environment, Uni Research AS, Bergen, Norway

[2]Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Japan

[3]Lerøy Seafood Group ASA, Bergen, Norway

[4]Department of Biology, University of Bergen, Bergen, Norway

Correspondence
Sigurd O. Handeland, Uni Research Environment, Uni Research AS, Nygårdsgaten 112, 5008 Bergen, Norway.
Email: sigurd.handeland@uni.no

Funding information
This work was funded by the Norges Forskningsråd (project #237856) and the CtrlAQUA partners.

The use of closed containment (CCS) or semi-closed containment systems (S-CCS) for Atlantic salmon *Salmo salar* aquaculture is under evaluation in Norway. One such system is the Preline S-CCS, a floating raceway system that pumps water from 35 m depth creating a constant current through the system. Exposing fish to moderate water currents is considered aerobic exercise and it is often perceived as positive for fish welfare, growth, food utilization, muscle development and cardiac health. The present study compared fish reared in the Preline S-CCS and in a reference open pen. Samples were taken in fresh water before being transferred to the seawater systems and after 1, 2 and 4 months in seawater and analysed for growth, mortality, muscle development and plasma insulin-like growth factor I (IGF-I) levels. Moreover, gene transcription were determined in the skeletal muscle [*igf-I*, insulin-like growth factor 1 receptor a (*igf1ra*) and insulin-like growth factor 1 binding protein 1a (*igf1bp1a*)] and cardiac transcription factors [myocyte-specific enhancer factor 2C (*mef2c*), *gata4* and vascular endothelial growth factor (*vegf*)]. While the results suggest that post-smolts in Preline S-CCS were smaller than reference fish, fish from Preline S-CCS have less accumulated mortality at the end of the experiment and showed 2.44 times more small muscle fibres than the reference group fish after 4 months in seawater. These results confirmed what was previously observed in the second generation of Preline. Similar levels of big muscle fibres between Preline S-CCS and reference suggest a similar hypertrophy of muscle fibres even with lower IGF-I expression in the Preline S-CCS. Cardiac gene transcription suggests cardiac hypertrophy was observed after 4 months in seawater in the Preline S-CCS group. Altogether, Preline S-CCS is a promising technology able to produce more robust *S. salar* with a faster growth and lower mortality in the subsequent standard open cage system growth period.

KEYWORDS

heart, IGF-I, muscle, Preline, *Salmo salar*, S-CCS, training

1 | INTRODUCTION

Atlantic salmon *Salmo salar* L. 1758 farming is one of the largest industries in Norway and production occurs in sea cages open to the environment along most of the Norwegian cost, with 990 active salmon farming sites in 2015 (Directorate of Fisheries, 2016). In Norway, a typical production cycle entails *S. salar* smolts being transferred to open sea cages during autumn (60–80 g) or early spring (100–150 g in mass), followed by a growth phase in open net sea cages located along the coast. Hjeltnes *et al*. (2017) reported that about 20% of fish transferred to sea are not harvested, in part due to poor smolt quality, diseases and disease treatment and escapees (Bleie & Skrudland,

2014; Gullestad *et al*., 2011). This has limited the Norwegian salmon industry to maintain a sustainable growth and motivated stakeholders to search for new production strategies. One approach has focused on production of larger and more robust smolts and post-smolts in closed (CCS) or semi-closed (S-CCS) containment system prior to transfer to open sea cages where the *S. salar* grow until market size. The post-smolt phase in these systems is often referred to as the period after seawater transfer and until the fish reach up to 1 kg. Thus, in recent years, several variants of semi-closed or closed farming technologies have been launched, placed either in land or in the sea and differing in size, shape and construction material. The use of CCS or S-CCS reduces the total time fish spend in open cage systems. Theoretically, these systems may optimize fish welfare, reduce pathogen interaction, reduce risk of escapees and allow treatment of discharge water, decreasing organic pollution. However, all these technologies have higher cost than traditional open cages and need to be carefully tested before implementing in a commercial scale.

Salmon lice *Lepeophtheirus salmonis* represents one of the biggest challenges in the *S. salar* aquaculture industry, inflicting heavy economic losses to producers, along with negative effects on the wild salmonid population (Costello, 2009). Since *L. salmonis* copepodids are present on the surface layers, pumping water from greater depths, as in the Preline S-CCS, would theoretically avoid infestation. Moreover, poor cardiac function is a factor that may contribute to high mortality rates after transfer to seawater and lack of sustained exercise may be one of the causes (Håstein *et al*., 2005; Poppe *et al*., 2003). One of the advantages of CCS and S-CCS is the possibility of controlling the water current. The Preline S-CCS used in the current study induces a constant water flow through the system. Since salmonids are mobile fish that can be motivated to swim against a constant current, this flow produces an aerobic training for fish. Cardiac health is one of the main factors that are influenced by the effects of aerobic exercise in fish. Aerobic training has been associated with increased growth (Castro *et al*., 2011; Solstorm *et al*., 2015; Totland *et al*., 1987), better feed conversion (Christiansen *et al*., 1992; Leon, 1986) and development of skeletal and cardiac muscle (Castro *et al*., 2013; Ibarz *et al*., 2011; Rasmussen *et al*., 2011; Totland *et al*., 1987). Another important advantage is reduced aggressive behaviour. Fish swimming towards a constant current tend to form schools and present fewer interactions, reducing hierarchies and resulting in more food available for subordinate fish (Adams *et al*., 1995; Brännäs, 2009; Solstorm *et al*., 2016).

Fish growth is commonly used as an indicator of animal performance since growth is highly influenced by environmental variables (Thorarensen & Farrell, 2011). Thermal growth coefficient (G_{TC}) summarizes fish growth taking temperature into account, allowing the comparison of fish influenced by different water temperatures (Iwama & Tautz, 1981; Thorarensen & Farrell, 2011). However, the G_{TC} model is inaccurate when temperatures exceed the growth optimum. Therefore, additional measurements are essential to evaluate fish somatic growth potential.

Fish muscle growth is based on continuous recruitment of muscle fibres through the life cycle (Stickland, 1983). In fish, skeletal muscle consists in white muscle fibres mainly used for rapid anaerobic movements and red muscle fibres for sustained aerobic swimming, which rarely exceeds 200 and 50 μm, respectively (Weatherley *et al*., 1988). In *S. salar*, skeletal muscle fibres per myotome develop from approximately 5,000 at hatching to 180,000 during smoltification and exceeding 1 million fibres when reaching a mass of 4 kg (Johnston, 1999). However, it seems that as the fish increases in size, the muscle fibre recruitment contribution decreases while the hypertrophy contribution increases (Stickland, 1983; Weatherley *et al*., 1980).

Several studies with teleosts suggest that insulin-like growth factor I (IGF-I) plasma concentrations are positively correlated with individual growth rates and can be a useful growth index (Beckman, 2011; Beckman *et al*., 2004a; Kaneko *et al*., 2015). Growth hormone is released from the anterior pituitary and stimulates the production of IGF-I in the liver, which is the principle source of plasma circulating IGF-I (Daughaday & Rotwein, 1989; Ohlsson *et al*., 2009). IGF-I effects include stimulation of cell proliferation, differentiation and protein synthesis (Fuentes *et al*., 2013), which are mediated through IGF-I cell surface receptors (Igf1ra) (Mendez *et al*., 2001), while IGF binding proteins (Igfbps) modulate the bioavailability and half-life of IGF-I in the extracellular environment (Kawaguchi *et al*., 2013; Wood *et al*., 2005). Moreover, other non-hepatic tissues produce IGF-I locally, including skeletal muscle (Mendez *et al*., 2001) with autocrine and paracrine effects. Evidence supports that both muscle-derived and liver-derived IGF-I promote the same effects in muscle growth although the main driver remains unclear (Fuentes *et al*., 2013). As the skeletal muscle, teleost cardiac muscle growth is driven by cardiomyocyte hypertrophy and hyperplasia and can also be stimulated by aerobic training (Castro *et al*., 2013). Hyperplasia in mammals is modulated by cardiac transcription factors such as transcription factor *gata4* and the Myocyte-specific enhancer factor 2C (*mef2c*) (Akazawa & Komuro, 2003; Kolodziejczyk *et al*., 1999). Role of vascular endothelial growth factor (*vegf*) is critical during vertebrate angiogenesis (Yancopoulos *et al*., 2000) and it is implicated in the formation of new capillaries following prolonged exercise in fish (Castro *et al*., 2013; Iemitsu *et al*., 2006).

The present study reports on post-smolt performance and welfare from the Preline S-CCS in comparison with a reference open pen. Despite the huge potential of S-CCS to reduce infections of pathogens and improve cardiac health and growth through aerobic training, little information on the effects on post-smolts rearing is available. In particular, since the huge environmental variations under industrial scale production, small-scale studies cannot always be extrapolated to an intensive large-scale production. Previous observations on post-smolts reared in the Preline S-CCS suggested a better disease resistance as well as a higher potential for growth (H. Sveier, pers. obs.). Hence, the present study aims to provide a more detailed focus on the effect of aerobic training and the mechanisms of muscle development between S-CCS and open-cage rearing systems and thus provide our knowledge in these new large-scale production strategies.

2 | MATERIALS AND METHODS

2.1 | Animals

Salmo salar smolts from the strain Salmobreed QTL duo (n = 321,412) were used in the experiment. Fish were kept indoors in 70 m^3 tanks at natural temperature water and constant light during the freshwater period. Fish were fed with a commercial dry diet (EWOS AS; www.ewos.com) according to water temperature and fish size.

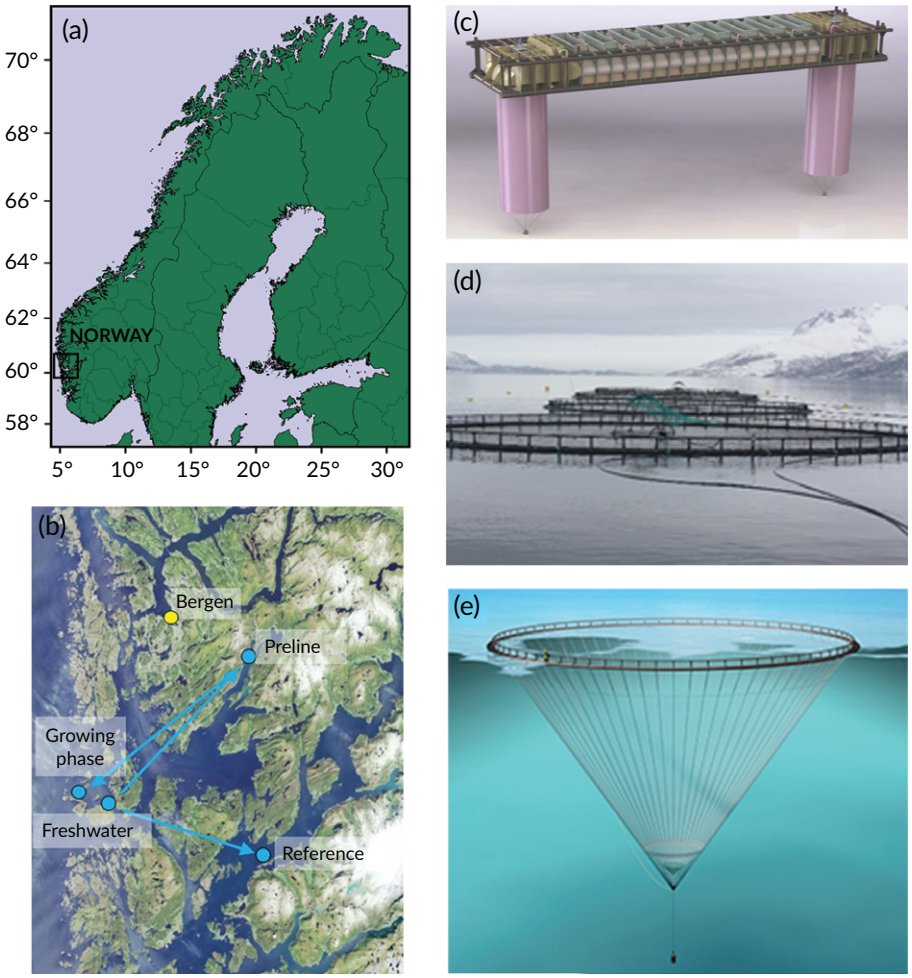

FIGURE 1 (a) Location of experiment area in Norway and (b) locations of the Preline semi-closed containment system (S-CCS), reference, freshwater and growing phase groups of *Salmo salar* post-smolts in Hordaland region; (c) schematic of the S-CCS; (d) standard open sea cages for *S. salar* production in Norway; (e) drawing of an open conical pen used to hold the reference group of fish

Standard protocol to induce smoltification (Handeland & Stefansson, 2001) were used beginning 11 January 2016 (Handeland & Stefansson, 2001). Smoltification was assessed using a combination of morphological and physiological traits: lowered condition, dark fin margins, silvery scales and high Na^+–K^+-ATPase (NKA) activity (Stefansson *et al*., 2008). After smoltification, the fish were transferred by well boat (Mowi Star) to their respective facilities.

2.2 | Preline semi-closed raceway

The Preline S-CCS is located at Sagen, in the Trengereid Fjord, Hordaland, south-west Norway in a sheltered location at 100 m depth (Figure 1(a), (b)).The Preline semi-closed raceway system has dimensions of 50 × 12 × 8 m, holding *c*. 2,000 m^3 of water (Figure 1(c)). The maximum water flow is 400 m^3 min^{-1}, with a total water exchange every 5–6 min. The water current measured 10–20 cm s^{-1}, using a Nortek Vector 3D acoustic velocimeter (NorTek; www.nortekgroup.com). The water is pumped from 35 m depth and circulate from inlet to outlet creating a constant one-way water current through the system.

2.3 | Experimental design

To compare post-smolt fitness parameters between the Preline and the standard open sea cage *S. salar* production protocol used in Norway (Figure 1(d)), *S. salar* smolts of the same cohort were transferred from fresh water (FW) to the Preline S-CCS (*n* = 157,126, 30 April 2016) and to a reference facility (*n* = 164,286, 5 May 2016). This reference facility is an open conical pen 60 m deep (Figure 1(e)) located in Skorpo, Hardanger, south-west Norway, in a location 250 m depth (Figure 1(a), (b)).

After 4 months in the sea, the Preline fish were transferred to a traditional sea cage facility at Buholmen on 31 August (Figure 1(b)). All husbandry practices at the farms were conducted in accordance with standard protocol for Lerøy Vest AS (www.leroy.no).

2.4 | Water quality

Oxygen concentrations, feeding, salinity and temperature were controlled and registered by automatic systems (OxyGuard Commander, Sterner AS; www.sterneras.no). Oxygen, temperature and salinity were registered at 3, 8 and 15 m in the open-cage systems at both Skorpo and Buholmen and in the inlet and outlet water in the Preline

S-CCS. All groups were checked twice per day, dead fish were removed and mortality was recorded. The fish in both treatments were fed commercial freshwater–seawater dry diets (EWOS).

2.5 | Sampling

Sampling was conducted in the freshwater phase (15 April) and three times during the seawater post-smolt growth phase: Preline (2 June, 29 June and 29 August) and reference (1 June, 30 June, 30 August). Thirty fish were randomly selected on each occasion and euthanized with a lethal dose of buffered MS222. Individual mass (M) and fork length (L_F) were measured for each individual before dissection. Heparinized 23G needle syringes were used to collect fish blood, which was centrifuged (3,000 g at 4° C) and plasma immediately frozen on dry ice and kept at −80° C until use. Two portions of muscle (3–5 mm thick) were obtained from the same area posterior to the dorsal fin on the left side of the fish and stored in buffered formalin for histological image analysis of muscle fibre size. An additional muscle sample was stored in RNAlater RNA stabilization solution (Thermo Fisher Scientific; www.thermofisher.com) for molecular analysis. In addition, the ventricle of the heart was divided in two parts by a sagittal cut, with one part fixed in buffered formalin and the other in RNAlater.

2.6 | Mass and growth

Mass estimations based on feed output were made by Lerøy Vest AS throughout the post-smolt phase using the FishTalk software (AkvaGroup; www.akvagroup.com) with a food conversion efficiency (FCE) input value of 1.1. Further, mass estimations (FCE = 1.1) were conducted from the start of the growing phase to 30 November 2016.

Thermal growth coefficient (G_{TC}) was used to reduce bias due to differences in growth related to differences in temperature between locations: $G_{TC} = (M_F^{1/3} - M_I^{1/3})1000(\Sigma T)^{-1}$, where M_F is the final mass, M_I is the initial mass and ΣT is the sum of daily temperatures. Fulton's condition factor (K; Fulton, 1904) was calculated as: $K = 100ML_F^{-3}$. The feed conversion ratio (C_{RF}) was determined for the post-smolt and growth phase using the equation: $C_{RF} = 100\Delta BF_C^{-1}$, where ΔB is the biomass gained and F_C is the food consumed.

2.7 | Gene transcription analysis

Before total RNA isolation of samples of tissue (20 mg heart or 60 mg muscle) were transferred to tubes containing 1.4 μm zirconium oxide beads and RLT buffer (Qiagen; www.qiagen.com). Muscle and heart tissue were homogenized for 15 min at 5,000 revolutions min^{-1} (Precellys 24, Bertin Technologies; www.bertin-instruments.com). Subsequent total RNA isolation from heart was carried out using the QIAsymphony SP system and the Qiasymphony RNA kit following manufacturer instructions (Qiagen). Total RNA from muscle was isolated using TRI-reagent (Sigma-Aldrich; www.sigmaaldrich.com) as described in the manufacturer's protocol.

The total RNA concentration and purity was measured using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Thermo Fisher). Purity was assessed with 260:280 and 260:230 ratios above 1.8. A selected number of samples were assessed for RNA integrity on RNA 6000 Nano LabChip kit using the Agilent 2100 Bioanalyzer (Agilent Technologies; www.agilent.com). Integrity was confirmed with RIN values higher than eight.

Complementary (c)DNA was reversely transcribed using 500 ng heart or 2 μg muscle total RNA (muscle) using oligo$(dt)_{20}$ primer and the Superscript III kit (Thermo Fisher). This step was carried out using a MicrolabSTARlet liquid handling workstation (Hamilton Robotics; www.hamiltoncompany.com). Gene transcription was measured using samples from freshwater smolts before transferring to seawater as well as after 1, 2 and 4 months in seawater (muscle) or after 4 months in seawater (heart).

Real Time PCR (rt-PCR) was carried out with a CFX96 Real-Time PCR detection system platform (Bio-Rad Laboratories; www.bio-rad.com) using the following PCR conditions: 3 min at 95° C, 37 repetitions of 15 s at 95° C and 1 min at 60° C and including a melting curve step at the end (10 s at 95° C, 5 s at 65° C and 5 s at 95° C). For each assay, triplicate two-fold cDNA dilution series from pooled samples were used to determine amplification efficiencies (E). Samples were run in 10 μl duplicates a using iTaq universal SYBR green supermix (Bio-Rad Laboratories), 0.3 μM of each primer and 3 μl of diluted cDNA (1:7.5 dilution for heart, 1:20 for muscle). Each plate included a negative control as well as a common pooled sampled used for the inter-calibration of assays among plates. The relative transcription levels of the genes were normalized following the efficiency corrected method (Pfaffl, 2001) using *ef1α* as a endogenous reference gene (Olsvik, *et al*., 2005). Primers used in this study are summarized in the Table 1.

2.8 | Pathogen screening

Heart samples from both sites, collected in the freshwater phase (n = 30) and last sampling of the post-smolt phase (n = 60) (all stored in RNAlater), were screened for piscine orthoreovirus (PRV) and salmonid alphavirus (SAV) targets (Andersen *et al*., 2007; Gunnarsson *et al*., 2017). Reactions were carried out using AgPt-ID one step rt-PCR kit (Thermo Fisher) and using *ef1α* as reference gene as previously described (Gunnarsson *et al*., 2017; Olsvik *et al*., 2005).

2.9 | IGF-I plasma determination

Time resolved competitive fluoro-immunoassay (TR-FIA) protocol was used to measure plasma IGF-I concentration (Small & Peterson, 2005). Prior to the assay, serum IGF-I was dissociated from the binding protein with acid-ethanol (Shimizu *et al*., 2000). Briefly, 96-well DELFIA pre-coated goat anti-rabbit IgG Microtitration plates (Perkin Elmer; www.perkinelmer.co.uk) were washed with 200 μl DELFIA wash buffer before each well received 20 μl anti-barramundi IGF-I rabbit antiserum (GroPep; www.gropep.com; diluted 1:8000) and 100 μl of standard-recombinant IGF-I (GroPep) or 35 μl plasma. Standards and samples were diluted in standard assay buffer. Plates were incubated overnight with shaking (600 revolutions min^{-1} at 4° C). After centrifugation (1 min at 3,000 g), europium labelled (0.05 ng μl^{-1}) IGF-I was added to each well and the plate incubated overnight under agitation (600 revolutions min^{-1} at 4° C). The plate was washed six times with 200 μl washing buffer before adding 200 μl DELFIA enhancement

TABLE 1 Primer sequences used for PCR in the present study of *Salmo salar* smolt performance in a Preline semi-closed containment system

Gene	Sequence (5′ > 3′)	Accession no.	Reference
ef1α	CCCCTCCAGGACGTTTACAAA	AF321836	Olsvik *et al*., 2005
	CACACGGCCCACAGGTACA		
igf-I	ATGTCTAGCGCTCATTTCTT	EF432852	Bower *et al*., 2008
	GAATTCTTACATTCGGTAGTTCCTT		
igf1bp1a	GGTCCCTGTCATGTGGAGTT	KC122927.1	Hevrøy *et al*., 2015
	TTCCAGAAGGACACACACCA		
igf1ra	TGCACAACTCCATCTTCACC	EU861008.1	Hevrøy *et al*., 2013
	GGGGCTCTCCTTCTGTCCTA		
mef2c	CACCGTAACTCGCCTGGTCT	GU252207	Castro *et al*., 2013
	GCTTGCGGTTGCTGTTCATA		
gata4	TCTCCATTCGACAGCTCCGT	HM475152	Castro *et al*., 2013
	CATCGCTCCACAGTTCACACA		
vegf	AGACAGCCCACATACCCAAG	NM_001124417	Castro *et al*., 2013
	GAAGACGTCCACCAGCATCT		

solution (PerkinElmer) to each well. After shaking at 600 revolutions min^{-1} for 10 min at room temperature Time-resolved fluorescence was measured by a fluorometer (ARVO *X*4; PerkinElmer) with emission and read wavelengths at 340 and 615 nm, respectively.

2.10 | Histology

Muscle and heart samples stored in buffered formalin were processed by an external laboratory (Fish Vet Group; www.fishvetgroup.com). Sections were scanned using a Zeiss Axio Scan.Z1 slide scanner and analysed using ZEN 2.3 (Zeiss; www.zeiss.com). A circular area of 1,000 μm in diameter in the epaxial white skeletal muscle was randomly chosen in approximately the same area for each section and muscle fibre size was measured for the widest diameter. Muscle fibre diameters measured were ranked for subsequent analysis into 12 groups by 20 μm size intervals up to fibres with >220 μm diameter (Fernandez *et al*., 2000). Histopathological signs of degeneration and necrosis were also examined.

2.11 | Statistical analysis

All statistical analyses were carried out using STATISTICA 13.2 (Tibco Software; www.tibco.com). Prior to statistical analysis, length, mass and condition factor were tested for normality using the Kolmogornov-Smirnov test and tested for homogeneity of variance using the Hartley *F*-max test. IGF-I plasma concentration and muscle transcription of *igf-I*, *igf1ra* and *igfbp1a* were tested for homogeneity of variance using the Levene's test.

One-way ANOVA was used to determine the level of significance for *M*, L_F, *K*, IGF-I plasma concentrations and gene transcription on muscle between the Preline S-CCS and reference open cage. Differences between fish showing symptoms of degeneration and necrosis were also explored using one-way ANOVA. Moreover, differences in plasma IGF-I concentration between SAV-infected and non-infected fish were observed using one-way ANOVA. Significant one-way ANOVA was followed by a Tukey honest significant dfifference post hoc test to determine differences among the experimental groups. A Kruskal-Wallis test was used to investigate the cardiac gene transcription of *mef2c*, *gata4* and *vegf* and muscle gene transcription through time. Significant results were followed by a Newman–Keuls test to test the differences. Differences were considered significant when $p < 0.05$. A one-way ANCOVA was conducted on the white skeletal muscle fibre size to determine the significance level between treatments, where L_F was used as a covariate factor. This was to remove the effect of size as a factor. Correlation between plasma IGF-I concentration and fish size (*M*, L_F and *K*) after 4 months in seawater was investigated using simple linear regression.

3 | RESULTS

3.1 | Water quality

The average water temperature measured at 3, 8 and 15 m depth for the open cage facilities (reference and Preline, Buholmen, growth phase) are shown in Figure 2(a). For the Preline S-CCS, the temperature was monitored in the outlet water. Temperature was higher during the first 4 months at the sea for post-smolts in the reference location than in the Preline S-CCS. In the growth phase after transferring the fish from the Preline S-CCS to open cage (in Buholmen), the temperature and the change through time were very similar on both systems.

The salinity (Figure 2(b)) was higher for the Preline group, both during the post-smolt and in the growth phases. In the Preline S-CCS, the salinity varied between 26.2 and 34.7, averaging 31.7 while in the reference open cage was in the range 21.6 and 27.5 and averaged 24.2. During the growing period, the range was slightly higher for the Preline (Buholmen) (26.9–30.5, average 28.8) than for the reference group (20.1–27.6, average 24.6). Variation in the oxygen concentration at the outlet water of Preline during the post-smolt phase was higher than in the reference. Oxygen variations both in Preline and reference were fast and short in time. Oxygen concentrations in the Preline ranged 72.2–131.56%, average 102.74%, while in the reference where 75.7–111.6%, average 97.21%. During the growth phase, oxygen values were similar in Preline (Buholmen) (83.8–99.4%, average 92.96%) and reference open cages (78–96%, average 86.63%).

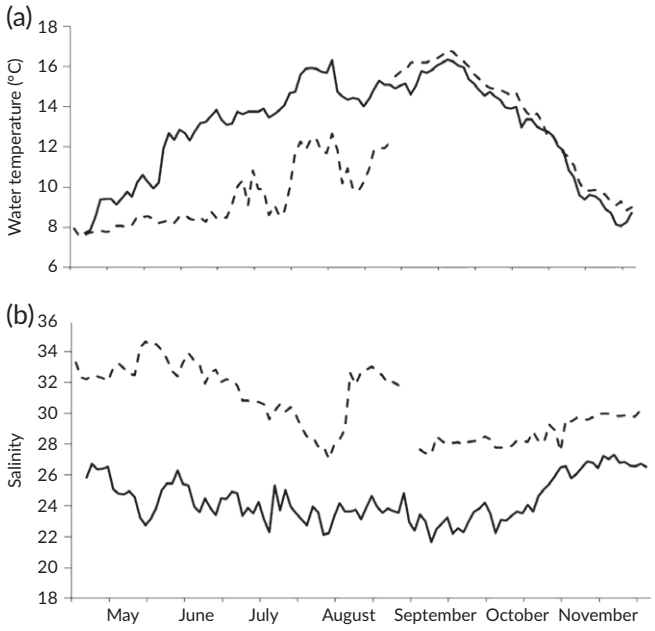

FIGURE 2 (a) Alternate day mean water temperature and (b) salinity at the *Salmo salar* post- smolt Preline semi-closed containment system (-----) and reference group (——) rearing systems between 5 May and 30 November 2016. Data from Preline S-CCS represents the Buholmen open-pen between 31 August and 30 November 2016

3.2 | Growth in mass and length

Mass (mean ± S.E.) from the FW sampling on 15 April was 101.0 ± 4.2 g. During post-smolt phase, mass gain was higher for the reference group (from 125.0 ± 4.4 g to 730.0 ± 57.2 g than for the Preline group (from 130.5 ± 10.9 g to 429.3 ± 15.4 g) Differences between groups were significant (one-way ANOVA, $F_{1,58}$ = 13.251; p < 0.001) after 2 months in seawater (Figure 3(a)).

Estimation on the mean mass of the fish in the tanks was done by Lerøy Vest AS based on feed output. After 1 month in seawater, mean mass increased from 132.0 to 443.6 g in the Preline group and from 136.7 to 733.5 g in the reference group (Figure 3(b)). On 30 November, 3 months after the post-smolt phase, the mean mass of Preline (Buholmen) group was estimated in 1475 g and in the reference group was 1,666 g (Figure 3(b)). Increase in mass during the growing period was 232% for the Preline (Buholmen) group and 123% for the reference group.

Fork length (mean ± S.E.) from FW was 20.6 ± 0.2 cm. During the post-smolt phase, increase in L_F was higher in the reference group (from 22.9 ± 0.2 to 38.7 ± 0.9 cm) than in the Preline group (from 23.2 ± 0.5 cm to 33.4 ± 0.3 cm) (Figure 3(c)). Differences between groups were significant (one way ANOVA, $F_{1,58}$ = 14.36, p < 0.001) after 2 months in seawater.

The condition factor (mean ± S.E.) was 1.15 ± 0.01 in FW. During the post-smolt phase, K declined in both groups before recovering (from 1.04 ± 0.01 to 1.12 ± 0.02 in the Preline group and from 1.03 ± 0.01 to 1.20 ± 0.02 in the reference group (Figure 3(d)). K was higher for the reference group than for Preline group after 4 months in seawater (one way ANOVA, $F_{1,58}$ = 6.138, p < 0.05).

The feed conversion ratio (C_{RF}) was lower for the Preline group both during post-smolt and growth phases (1.04 and 1.03, respectively) than in the reference group (1.08 in both phases; Table 2). Thermal growth coefficient (G_{TC}) was measured on the post-smolt phase being 2.778 for the Preline group and 3.149 for the reference

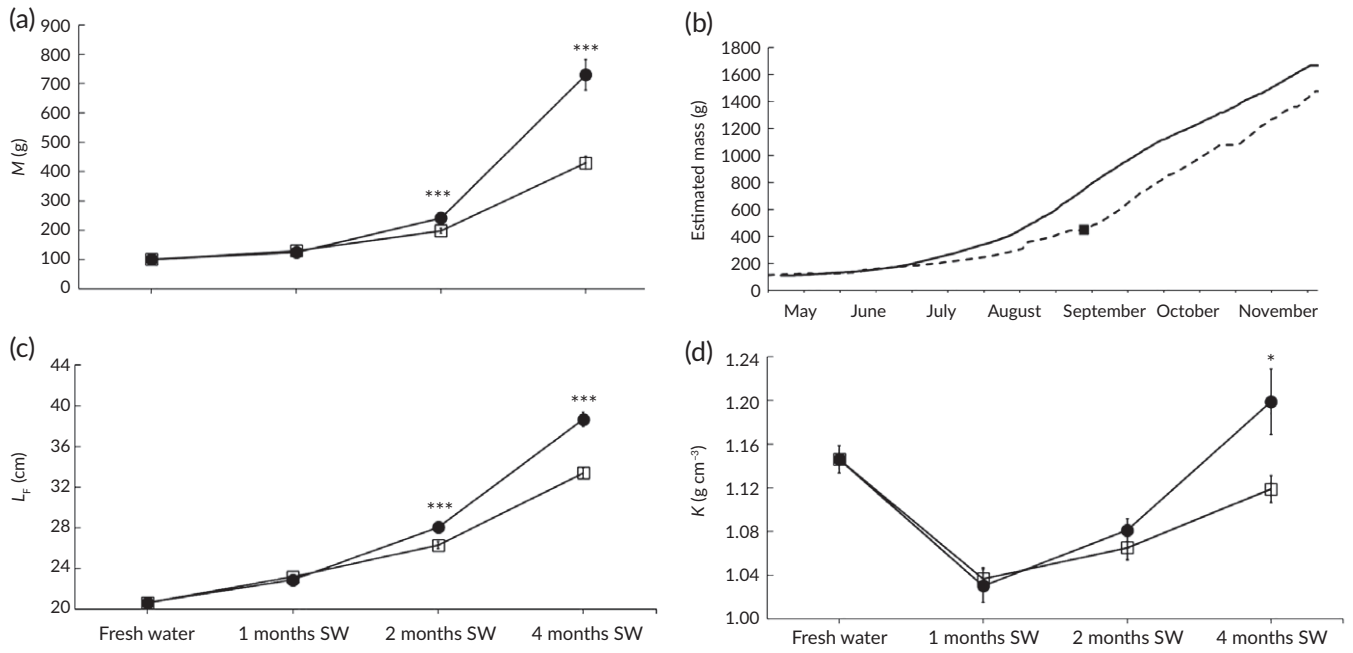

FIGURE 3 Mean [±S.E.; n = 30; (a), (c), (d)] *Salmo salar* growth in mass (M) fork length (L_F) and Fulton's condition factor (K) measured in freshwater (15 April 2016) and during the post-smolt phase (1–2 June; 1–2 June and 29–30 August 2016) (a) Measured mass (⊟) Preline, and (●) Reference, (b) estimated mean mass (Fishtalk calculations, C_{EF} = 1.1) (——) Reference, and (-----) Preline, (c) mean fork length (⊟) Preline, and (●) Reference and (d) condition factor (K) (⊟) Preline, and (●) Reference. Estimated mean mass covers both the post-smolt phase 5 May to 30 August, and the growth phase 31 August to 30 November. Changeover is indicated with a dot in the figure. SW, seawater. Significant difference between groups; *p < 0.05; ***p < 0.001

TABLE 2 Feed conversion ratio (C_{RF}) and thermal growth coefficient (G_{TC}, estimated for the growth phase) for *Salmo salar* kept in Preline semi-closed containment and reference rearing systems during the post-smolt and growth phases

Group	C_{RF}	G_{TC}	
		Measured	Estimated
Preline (post-smolt)	1.04	2.778	2.85
Reference (post-smolt)	1.08	3.149	3.041
Preline (growth)	1.03	–	3.001
Reference (growth)	1.08	–	2.318

group. Estimations predicted a G_{TC} of 3.001 and 2.318 in the growth phase for Preline and reference groups, respectively (Table 2).

3.3 | Pathological condition

During the post-smolt phase, the accumulated mortality for the Preline group increased from 0.54% after transferring to Preline system from freshwater facility until 1.34% after 4 months (Figure 4). During this post-smolt phase, the accumulated mortality in the reference cages was lower (0.98%). However, after 20 days in the growth phase, the accumulated mortality in the reference group surpassed the mortality in the Preline (Buholmen group). On 30 November, accumulative mortality was higher in the reference group (3.42%) than in the Preline (Buholmen) system (2.48%).

Fish from the reference group presented signs of degeneration and necrosis in the skeletal and heart muscle (26.7%). Using rt-PCR, SAV was detected in reference group fish after 4 months in seawater (16.67%), all of them presenting histopathological signs of disease. However, no SAV was detected on fish from fresh water or the Preline group. PRV was detected both in freshwater (80%), Preline (80%) and reference (73.33%) groups.

3.4 | Swimming behaviour

Fish in Preline S-CCS formed schools and swam constantly against the current (Supporting Information Video S1). Fish in the reference group presented a more erratic swimming, with more interactions among individuals.

3.5 | Histology

After 4 months in seawater, post-smolts kept in the Preline rearing system presented higher frequency of the smallest muscle fibres than fish in the reference group: 0–20 μm diameter (one way ANCOVA, $F_{1,56}$ = 5.297; p < 0.05) and 20–40 μm diameter (one way ANCOVA, $F_{1,56}$ = 5.790; p < 0.05. This equals 2.44 times higher frequency in the fibres in the 0–20 μm size class. The reference group had a higher frequency (one way ANCOVA, $F_{1,56}$ = 5.307; p < 0.001) on the 60–80 μm size class (Figure 5).

3.6 | Plasma IGF-I

At the end of the freshwater period, plasma IGF-I concentrations averaged 72.3 ± 7.5 ng ml^{-1}. During the first 2 months, plasma IGF-I concentrations were higher for the reference group. After 1 month, Preline group averaged 95.3 ± 7.2 ng ml^{-1} and the reference group averaged 127.5 ng ml^{-1} (one-way ANOVA, $F_{1,58}$ = 10.1626, p < 0.01). After 2 months, the averages were 129.5 + 8.0 and 204.8 + 12.7 ng ml^{-1} for Preline and reference groups, respectively (one-way ANOVA, $F_{1,58}$ = 25.2004, p < 0.001). After 4 months, plasma IGF-I concentration were the highest for both groups, but with no differences between them (Figure 6).

3.7 | Gene transcription

3.7.1 | Muscle gene transcription

In muscle samples, results indicated that there were no significant differences in the *igf-I* (Figure 7(a)) and *igf1ra* (Figure 7(b)) gene transcription between the Preline S-CCS and the reference open-cage system in any of the sampling points in the post-smolt phase. No significant differences were observed for *igfbp1a* after 1 and 2 months in seawater (Figure 7(c)). It was observed an increase in the transcription of *igfbp1a* over time but this was only significant after 4 months in seawater for the reference group (Kruskal-Wallis, $H_{3,36}$ = 4.090; p < 0.05). Moreover, no significant differences between rearing systems were observed at each sampling point.

3.7.2 | Heart gene transcription

Freshwater smolts had a significant higher transcription of *mef2c* (Kruskal-Wallis, $H_{2,86}$ = 41.599; p < 0.001), *gata4* (Kruskal-Wallis, $H_{2,81}$ = 23.897; p < 0.001) and *vegf* (Kruskal-Wallis, $H_{2,81}$ = 24.256; p < 0.001) than post-smolt groups after 4 months in seawater, independently of the rearing system (Figure 8). After 4 months, Preline fish expressed significantly higher *mef2c* (Kruskal-Wallis, $H_{1,56}$ = 8.094; p < 0.01) and *gata4* (Kruskal-Wallis, $H_{1,56}$ = 4.951; p < 0.05) than the reference group. No differences were found on the transcription of *vegf* between Preline and reference groups after 4 months in seawater. Since differences were found in the messenger (m)RNA transcription levels of *gata4* and *vegf* between SAV-infected and non-infected fish, all fish tested as SAV-positive were excluded from the analysis.

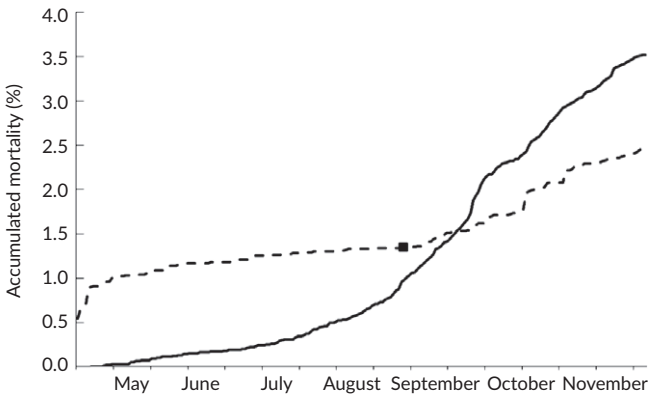

FIGURE 4 Accumulated mortality of *Salmo salar* in the Preline semi-closed containment system (S-CCS) 30 April to 30 August followed by the open pen growth phase (Buholmen) from 1 September to 30 November (-----; ■, changeover from S-CCS to open pen). The accumulated mortality in the reference group covers the period 5 May to 30 November (——)

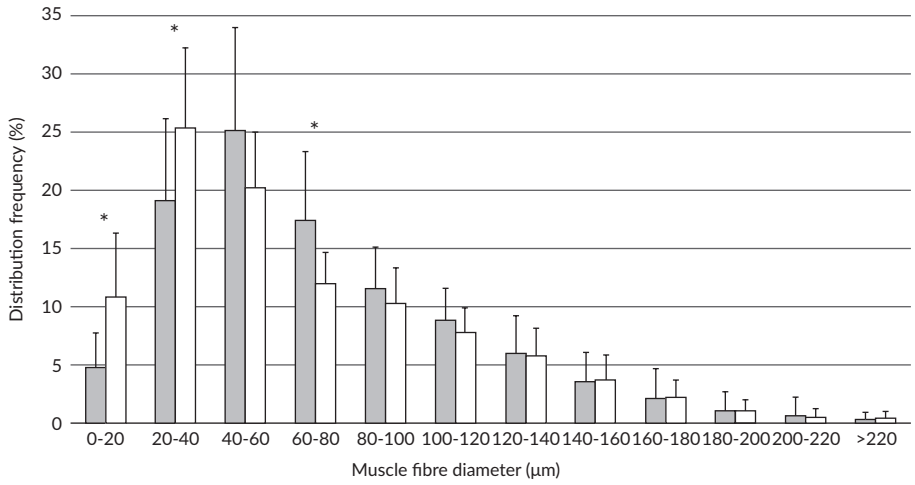

FIGURE 5 Mean (+S.E.) *Salmo salar* skeletal muscle fibre diameter frequency distribution reared in Preline semi-closed containment system (□) and reference *S. salar* (▨) after 4 months in seawater. Significant difference between groups; *$p < 0.05$; ***$p < 0.001$

4 | DISCUSSION

In the present study, post-smolts reared in the Preline S-CCS during spring and summer grew less both in length and mass than their counterparts in the reference group. Full-scale production systems permit the acquisition of reliable and valuable data for aquaculture management, but disadvantages come from the lack of replication and control of environmental variables that can also affect the experiment. Fish have the same genetic background and come from the same cohort, but differences between groups can be determined by environmental factors of any kind, location, presence of pathogens or feeding procedures in addition to the rearing systems itself. Therefore, results must be considered as indicative and future research on this platform must be carried out in order to confirm present results.

Environmental factors including temperature, salinity, photoperiod and oxygen affect growth and metabolism of teleosts (Boeuf & Payan, 2001; Brett, 1979), with temperature being regarded as one of the key factors influencing growth (Fry, 1971). Since Preline was pumping water from 35 m depth, fish in the reference group were exposed to higher temperatures than Preline fish during the post-smolt phase (12.9 *vs.* 9.5 on average); the difference started a few days after transfer (Figure 2(a)). In both systems, the temperature variation was similar, but always 3–4° C colder in the Preline. Moreover, transition from smolt to post-smolt includes a series of physiological adaptations of the fish to be able to survive in the marine environment. These adaptations are often associated with a lag phase in growth before being followed by an increase in growth rate (Stefansson *et al*., 2008), which can be reflected in the decrease in *K*. Differences in growth after 2 and especially 4 months could be conditioned by the higher Preline salinity as well as by lower temperature in this system (Figure 2(b)). However, it has been shown that *S. salar* post-smolt growth is independent of salinity in a range similar to the one observed in the present study (Duston, 1994). Optimal oxygen concentrations have been determined in the range 85–120% for *S. salar* at temperatures from 5–15° C (Thorarensen & Farrell, 2011). Oxygen concentration in the open cage fell into this range, but Preline oxygen concentration was more variable and fell outside these thresholds during short periods of time, which could potentially affect fish growth.

To account for differences in temperature between rearing system, but also from regional differences, a mass model incorporating growth rate per day dependent on the daily temperature was employed (G_{TC}). Due to the size of the experiment, G_{TC} was calculated over 30 fish sample instead of following individually tagged fish. It should be noted that during some parts of the growth phase, the water temperature exceeded 16° C, which could affect the G_{TC} value (Jobling, 2003). The G_{TC} was retained because the temperature profiles between the groups during the growth phase did not differ much and that both groups exceeded 16° C around the same time. Not surprisingly, Preline fish had a lower growth rate compared with the reference-group fish during the post-smolt phase. However, the G_{TC} in Preline (2.778) was still around the 2.71, a value considered to be average (Thorarensen & Farrell, 2011). After transferring to an open system for the growth phase (Buholmen), the G_{TC} increased in Preline fish, becoming higher than for the reference fish. It is important to note that estimated data based on feed output corresponded with the mass measurements conducted during the post-smolt sampling,

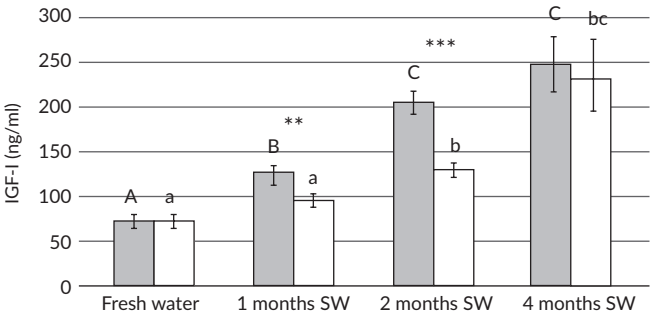

FIGURE 6 Mean (±S.E.; $n = 30$) plasma IGF-I concentration of *Salmo salar* in both fresh water and during rearing in Preline S- semi-closed containment system (S-CCS; □) and reference group (▨). Significant differences trough time are denoted with capital letters within the reference group, lower-case letters within the Preline S-CCS group and significant differences between rearing systems are shown: **$p < 0.01$; ***$p < 0.001$. SW, seawater

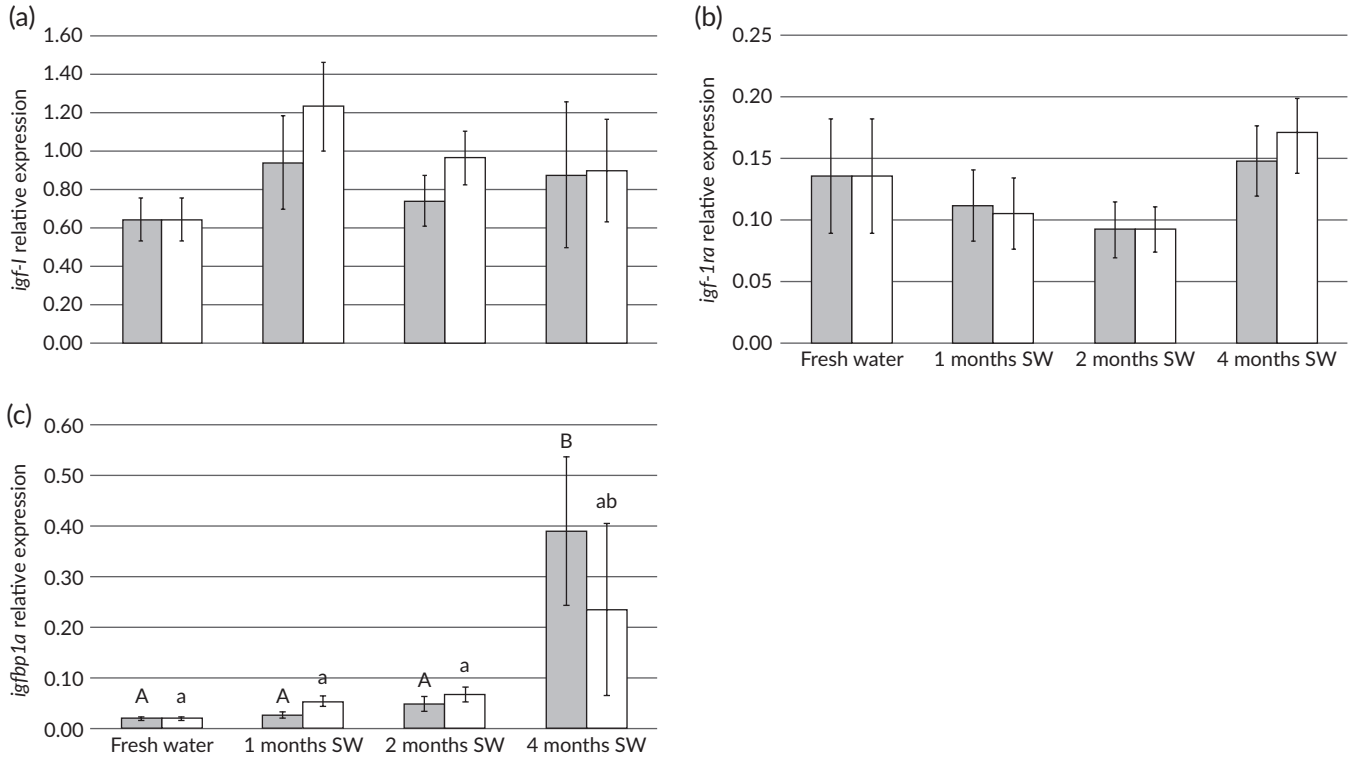

FIGURE 7 Mean (±S.E.; n = 30) relative gene transcription values for (a) *Igf-I* (■) Reference, and (□) Preline, (b) *igf1ra* (□) Reference, and (■) Preline, and (c) *igf1bp1a* (■) Reference, and (□) Preline using *ef1α* as standard in *Salmo salar* muscle, both in fresh water and during rearing in Preline semi-closed containment system (S-CCS; □) and reference group (■). Significant differences through time are denoted with capital letters within the reference group and lower-case letters within Preline S-CCS. SW, seawater

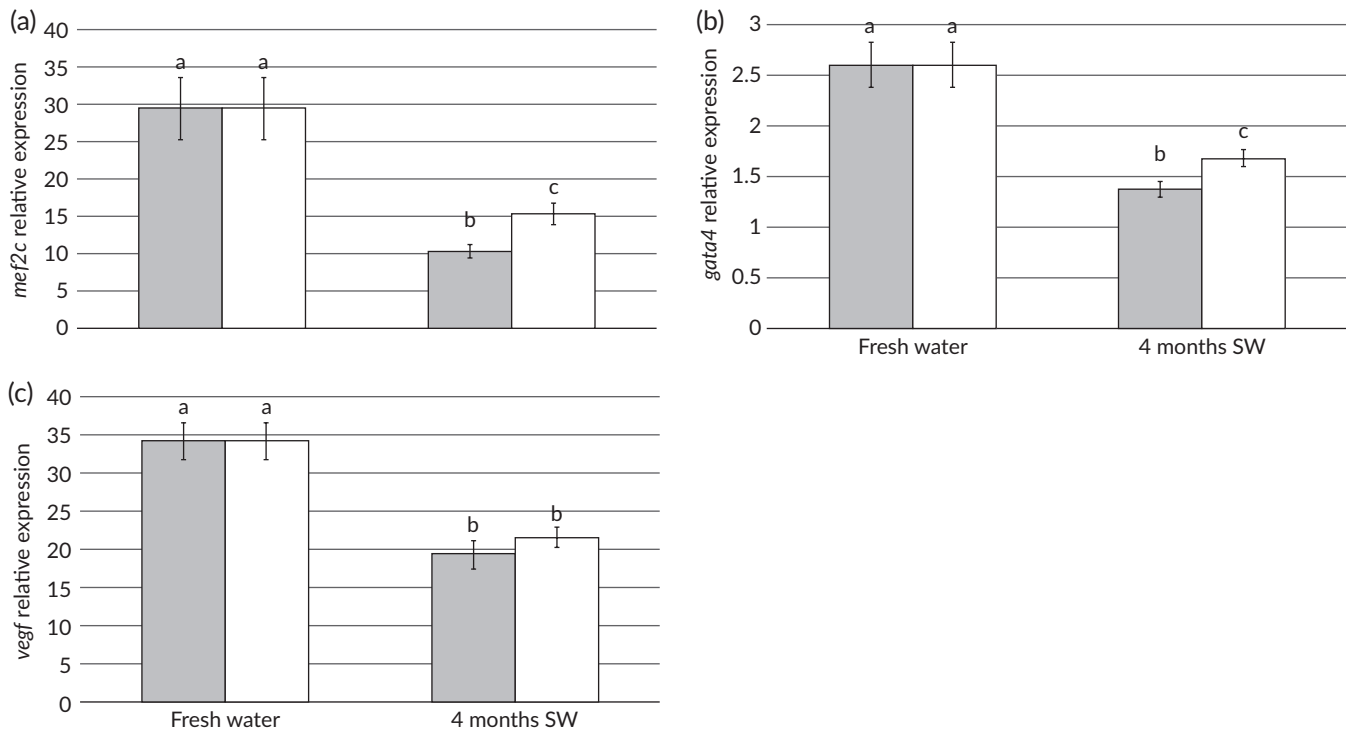

FIGURE 8 Mean (±S.E.; n = 30) relative gene transcription values for (a) *mef2c* (■) Reference, and (□) Preline, (b) *gata4* (■) Reference, and (□) Preline and (c) *vegf* (■) Reference, and (□) Preline using *ef1α* as standard in *Salmo salar* heart, both in fresh water and after rearing in Preline semi-closed containment system (S-CCS; □) and reference group open pen (■) for 4 months in seawater. Significant differences between groups are indicated by different lower-case letters

supporting validity of such estimations. At the end of the growth phase, the fish were still larger in the reference group system compared with the Preline (Buholmen) group, which nevertheless grew faster during the growth phase (Table 2 and Figure 3(b)). It cannot be concluded that this higher growth in the Preline (Buholmen) group was due to the Preline rearing, to the presence of SAV in the reference fish or simply to the fact that the fish coming from Preline were smaller when transferred to the open pen (Maclean & Metcalfe, 2001; Mortensen & Damsgård, 1993; Nicieza & Metcalfe, 1997). Moreover, C_{RF} was lower in the Preline than in the reference group. It has been observed that moderate exercise favours the food conversion through appetite stimulation (Christiansen *et al*., 1992; East & Magnan, 1987; Leon, 1986), although less drifting food in the Preline rearing system as result of a more precise feeding process may have played a role.

Swimming behaviour of fish in the Preline was quite different than in the open pen (Supporting Information Video S1). In Preline, fish swam against the current, showing fewer interactions and less aggressive behaviour, as it has been previously observed in fish exposed to constant currents (Christiansen *et al*., 1991; Jobling *et al*., 1993; Solstorm *et al*., 2016). In calm waters, salmonids tend to form dominant hierarchies, inducing differences in size within the fish population (Adams *et al*., 1995; Brännäs, 2009; Winberg *et al*., 1991). Aggressive behaviour can produce lesions, which can in turn facilitate infections. Furthermore, high aggression can lead to increased spontaneous activity with higher energetic costs and increment of the feed conversion ratio of the population (Christiansen *et al*., 1991; Solstorm *et al*., 2016).

Preline S-CCS showed a higher accumulated mortality than the reference group during the first period post transfer to seawater (Figure 4). A higher salinity in the Preline S-CCS would have been a greater ionoregulatory challenge for these fish, but also higher flow rate can play a role as it has been reported in salmonids during the first days of exposure to similar flow rates (Davison & Goldspink, 1977; Totland *et al*., 1987). Moreover, the mortality could be related to transportation problems. Mortality in the Preline S-CCS did reach a plateau, while the reference group increased gradually through time, especially during summer months. Shortly after transferring Preline fish to Buholmen for the growth phase, accumulated mortality became higher in the reference group. It is not clear if this effect was caused by more robust fish coming out of the Preline S-CCS or if other factors contributed, especially pathogens. No sea lice or signs of amoebic gill disease were observed in the third generation of Preline. Moreover, data from the Preline second generation showed lower prevalence in seawater pathogens in the Preline S-CCS than in the reference group (Supporting Information Figure S1). In the present generation, it has been reported SAV infection in fish from the reference group, but not from the Preline. SAV is the causative agent of pancreas disease, which can produce mortalities up to 50% linked to stress (Bang Jensen *et al*., 2012; Rodger & Mitchell, 2007). Although all muscle samples used in the study showed to be a carrier of PRV, PRV is common in farmed *S. salar* appearing often without any associated pathology (Kongtorp *et al*., 2004; Løvoll *et al*., 2012). Therefore, PRV most likely had little, if any, affect on fish growth.

Differences between SAV positive and negative fish were not observed for muscle fibre size. Therefore, all fish were included in the analysis. Muscle fibre size distribution changed through the ontogeny of fish from a higher percentage of small fibres at the beginning of the experiment to a greater proportion of large sizes after 4 months in seawater (Figure 5). This growth as result of hypertrophy with a low hyperplasia through development of the fish has been previously observed (Stickland, 1983; Weatherley *et al*., 1980). However, Preline fish presented a higher frequency of small muscle fibres (higher hyperplasia) than the reference group after 4 months in seawater, being also this phenomenon previously observed in the Preline second generation (Supporting Information Figure S2).

The majority of the fish muscle is white skeletal muscle, which is mainly used for anaerobic swimming (Videler, 1993), but also used for swimming under a critical current speed species and size-dependent (Burgetz *et al*., 1998; Johnston & Moon, 1980). Moreover, it has been observed that training can change the white muscle fibre profile (Rasmussen *et al*., 2011). Therefore, it is plausible to think that higher hyperplasia in white skeletal muscle could be an effect of aerobic training. Furthermore, several studies indicate that water speeds above 0.40 body lengths s^{-1} induce muscle hypertrophy (Bugeon *et al*., 2003; Castro *et al*., 2011; Ibarz *et al*., 2011; Martin & Johnston, 2005; Totland *et al*., 1987; Walker & Emerson, 1978). Although Preline fish were smaller, after 4 months in seawater they have a comparable frequency of big muscle fibres, suggesting muscle hypertrophy. Higher growth in the growth phase could be related to hypertrophy of small fibres recruited during training.

Plasma IGF-I concentration increased as a result of fish development in seawater, as it has been previously described (Beckman, 2011; McCormick, 2012). The reference group showed higher plasma IGF-I than Preline fish, reflecting the higher growth rate at this stage (Figure 6). Such differences are probably due to differences in environmental variables, mainly temperature, as it has been previously observed (Beckman, 2011; Brett, 1979; Gabillard *et al*., 2003; Larsen *et al*., 2001). Moreover, link between higher plasma IGF-I concentration and higher growth rate has been established for other teleost species (Beckman *et al*., 1998; Imsland *et al*., 2007; Mingarro *et al*., 2002; Pierce *et al*., 2001). The lack of correlation between plasma IGF-I and body size found after 4 months in seawater may reflect a difference between large and small-scale laboratory studies. Additionally, it is possible that elevated plasma IGF-I concentration in the Preline S-CCS reflected the training effect and the initiation of enhanced growth during the growth phase. No differences were found in plasma IGF-I concentration between SAV positive and negative.

Local *igf-I* and *igf1ra* transcription in muscle did not differ between the two rearing systems during the post-smolt period (Figure 7). Since muscle growth is mediated by the interaction of both IGF-I and its receptor (Beckman *et al*., 2004b), a higher transcription on the reference fish could be expected. This suggests that temperature was not an influencing factor on muscle transcription of *igf-I* and *igf1ra* as it has been previously observed in *Onchorhynchus mykiss* (Walbaum 1792) (Gabillard *et al*., 2003). Hence, the growth-promoting effects from temperature were probably mainly driven by liver derived IGF-I, rather than local muscle derived IGF-I. However, local muscle *igf-I* transcription should be interpreted with caution, as interactions with *igf1bps* may inhibit biological action (Beckman, 2011).

As differences between SAV positive and negative fish were observed for *gata4* and *vegf*, SAV positive fish were excluded from both analyses. When comparing heart gene transcription in fresh water and after 4 months in seawater, an ontogenic component in the three chosen markers was observed (Figure 8). After 4 months in seawater, no difference was observed between rearing systems for the *vegf*. However, significantly higher transcription of the transcription factors *mef2c* and *gata4* were found in the Preline fish than in the reference fish. Elevated *mef2c* and *gata4* transcription is an indication of increase in cardiomyocyte hypertrophy as an effect of training (Akazawa & Komuro, 2003; Castro *et al*., 2013; Kolodziejczyk *et al*., 1999). Since faster water currents seems to have higher magnitude effect of the markers considered (Castro *et al*., 2013), it seems that fish from Preline were not exposed to a water current strong enough to induce differences in *vegf* transcription.

In summary, fish in the Preline S-CCS exhibited lower growth during the post-smolt phase, as well as lower *igf-I* transcription. This was due to environmental differences between rearing systems, mainly temperature. However, Preline fish were forced to swim against a moderate current, causing mild aerobic training that fish in the reference group did not receive. The Preline fish had, at the end of the post-smolt phase, a 2.44 times higher frequency of muscle fibres in the smallest interval group (0–20 μm), compared with the reference fish, confirming previously observed results in Preline second generation. Furthermore, the number of large fibres remained constant, indicating both hyperplasia and hypertrophy of skeletal muscle. This was coupled with the increase in plasma IGF-I concentration as well as lower increment of *igf1bp1a* in muscle in the Preline group. Higher increase in growth rate for Preline fish during the growth phase reflected the hypertrophy of these newly recruited muscle fibres. Moreover, cardiomyocyte hypertrophy in response to aerobic training was demonstrated by higher transcription of *mef2c* and *gata4* in the heart, but training magnitude was not enough to elicit the *vegf* response. Taken together, the results show that Preline S-CCS fish did not grow as efficiently as in open pens due to lower temperatures, but training produces more robust fish with more potential growth and less mortalities in the growth phase than the reference group.

ACKNOWLEDGEMENTS

This research was part of the CtrlAQUA SFI, Centre for Closed-Containment Aquaculture programme. Lerøy Vest AS provided technical and economic support to the project. Norway map has been obtained from Maps-for-free (www.maps-for-free.com). Hordaland map has been obtained from Kartverket© database (www.kartverket.no). Fish pen picture was obtained from T. Bjørkan and it is deposited on Wikimedia Commons (www.commons.wikimedia.org). Fish pen draw has been provided by Aqva group (www.akvagroup.com).

Author contributions

S.H., L.E., T.O.N., H.S. and A.N. designed the experiment. P.B., Ø.M. and I.G. wrote the manuscript. Ø.M., I.G., T.O.N., C.P., V.T., P.B., L.E. and S.H. carried out the samplings. I.G., N.K. and M.S. performed plasma IGF-I analysis. V.T. and C.P. performed RNA isolation. H.S. supplied technology and monitored fish during experiment. A.N. performed pathological analysis. Ø.M. performed muscle histology analysis. P.B., Ø.M., I.G. and T.O.N. carried out qPCR analysis. All authors read, gave feedback and approved the manuscript.

REFERENCES

Adams, C. E., Huntingford, F. A., Krpal, J., Jobling, M., & Burnett, S. J. (1995). Exercise, agonistic behaviour and food acquisition in Arctic charr, *Salvelinus alpinus*. *Environmental Biology of Fishes*, *43*, 213–218.

Akazawa, H., & Komuro, I. (2003). Roles of cardiac transcription factors in cardiac hypertrophy. *Circulation Research*, *92*, 1079–1088.

Andersen, L., Bratland, A., Hodneland, K., & Nylund, A. (2007). Tissue tropism of salmonid alphaviruses (subtypes SAV1 and SAV3) in experimentally challenged Atlantic salmon (*Salmo salar* L.). *Archives of Virology*, *152*, 1871–1883.

Bang Jensen, B., Kristoffersen, A. B., Myr, C., & Brun, E. (2012). Cohort study of effect of vaccination on pancreas disease in Norwegian salmon aquaculture. *Diseases of Aquatic Organisms*, *102*, 23–31.

Beckman, B. R. (2011). Perspectives on concordant and discordant relations between insulin-like growth factor 1 (IGF1) and growth in fishes. *General and Comparative Endocrinology*, *170*, 233–252.

Beckman, B. R., Fairgrieve, W., Cooper, K. A., Mahnken, C. V., & Beamish, R. J. (2004a). Evaluation of endocrine indices of growth in individual postsmolt coho salmon. *Transactions of the American Fisheries Society*, *133*, 1057–1067.

Beckman, B. R., Larsen, D. A., Moriyama, S., Lee-Pawlak, B., & Dickhoff, W. W. (1998). Insulin-like growth factor-I and environmental modulation of growth during smoltification of spring chinook salmon (*Oncorhynchus tshawytscha*). *General and Comparative Endocrinology*, *109*, 325–335.

Beckman, B. R., Shimizu, M., Gadberry, B. A., Parkins, P. J., & Cooper, K. A. (2004b). The effect of temperature change on the relations among plasma IGF-I, 41-kDa IGFBP and growth rate in postsmolt coho salmon. *Aquaculture*, *241*, 601–619.

Bleie, H. & Skrudland, A. (2014). Tap av laksefisk i sjø. *Rapport fra Mattilsynet* (in Norwegian). Retrieved from www.mattilsynet.no/fisk_og_akvakultur/fiskevelferd/tap_av_laksefisk_i_sjo_rapport.15430/binary/Tap%20av%20laksefisk%20i%20sj%C3%B8%20rapport

Boeuf, G., & Payan, P. (2001). How should salinity influence fish growth? *Comparative Biochemistry and Physiology C*, *130*, 411–423.

Bower, N. I., Li, X., Taylor, R., & Johnston, I. A. (2008). Switching to fast growth: The insulin-like growth factor (IGF) system in skeletal muscle of Atlantic salmon. *Journal of Experimental Biology*, *211*, 3859–3870.

Brännäs, E. (2009). The effect of moderate exercise on growth and aggression depending on social rank in groups of Arctic charr (*Salvelinus alpinus* L.). *Applied Animal Behaviour Science*, *119*, 115–119.

Brett, J. (1979). Environmental factors and growth. In W. S. Hoar, D. J. Randall, & J. R. Brett (Eds.), *Bioenergetics and growth: Fish physiology* (Vol. VIII, pp. 599–675). New York, NY: Academic Press.

Bugeon, J., Lefevre, F., & Fauconneau, B. (2003). Fillet texture and muscle structure in brown trout (*Salmo trutta*) subjected to long-term exercise. *Aquaculture Research*, *34*, 1287–1295.

Burgetz, I. J., Rojas-Vargas, A., Hinch, S. G., & Randall, D. J. (1998). Initial recruitment of anaerobic metabolism during sub-maximal swimming in rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology*, *201*, 2711–2721.

Castro, V., Grisdale-Helland, B., Helland, S. J., Kristensen, T., Jørgensen, S. M., Helgerud, J., … Takle, H. (2011). Aerobic training stimulates growth and promotes disease resistance in Atlantic salmon (*Salmo salar*). *Comparative Biochemistry and Physiology A*, *160*, 278–290.

Castro, V., Grisdale-Helland, B., Helland, S. J., Torgersen, J., Kristensen, T., Claireaux, G., … Takle, H. (2013). Cardiac molecular-acclimation mechanisms in response to swimming-induced exercise in Atlantic salmon. *PLoS One*, *8*, e55056.

Christiansen, J. S., Jørgensen, E. H., & Jobling, M. (1991). Oxygen consumption in relation to sustained exercise and social stress in Arctic

charr (*Salvelinus alpinus* L.). *Journal of Experimental Zoology*, *260*, 149–156.

Christiansen, J. S., Svendsen, Y. S., & Jobling, M. (1992). The combined effects of stocking density and sustained exercise on the behaviour, food intake and growth of juvenile Arctic charr (*Salvelinus alpinus* L.). *Canadian Journal of Zoology*, *70*, 115–122.

Costello, M. J. (2009). The global economic cost of sea lice to the salmonid farming industry. *Journal of Fish Diseases*, *32*, 115–118.

Daughaday, W. H., & Rotwein, P. (1989). Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum and tissue concentrations. *Endocrine Reviews*, *10*, 68–91.

Davison, W., & Goldspink, G. (1977). The effect of prolonged exercise on the lateral musculature of the brown trout (*Salmo trutta*). *Journal of Experimental Biology*, *70*, 1–12.

Directorate of Fisheries (2016). *Laks, regnbueørret og ørret. Antall lokaliteter 2006–2016 (in Norwegian).* Retrieved from www.fiskeridir.no/content/download/7613/95478/version/17/file/sta-laks-mat-02-lokaliteter.xlsx

Duston, J. (1994). Effect of salinity on survival and growth of Atlantic salmon (*Salmo salar*) parr and smolts. *Aquaculture*, *121*, 115–124.

East, P., & Magnan, P. (1987). The effect of locomotor activity on the growth of brook charr, *Salvelinus fontinalis* Mitchill. *Canadian Journal of Zoology*, *65*, 843–846.

Fernandez, D. A., Calvo, J., Franklin, C. E., & Johnston, I. A. (2000). Muscle fibre types and size distribution in sub-antarctic notothenioid fishes. *Journal of Fish Biology*, *56*, 1295–1311.

Fry, F. (1971). The effect of environmental factors on the physiology of fish. *Fish Physiology*, *6*, 1–98.

Fuentes, E. N., Valdés, J. A., Molina, A., & Björnsson, B. T. (2013). Regulation of skeletal muscle growth in fish by the growth hormone–insulin-like growth factor system. *General and Comparative Endocrinology*, *192*, 136–148.

Fulton, T. W. (1904). *The rate of growth of fishes.* 22nd Annual Report of the Fisheries Board of Scotland 1903, 141–241.

Gabillard, J.-C., Weil, C., Rescan, P.-Y., Navarro, I., Gutiérrez, J., & Le Bail, P.-Y. (2003). Effects of environmental temperature on IGF1, IGF2 and IGF type I receptor expression in rainbow trout (*Oncorhynchus mykiss*). *General and Comparative Endocrinology*, *133*, 233–242.

Gullestad, P., Bjørgo, S., Eithun, I., Ervik, A., Gudding, R., Hansen, H., Johansen, R., Osland, A., Rødseth, M. & Røsvik, I. (2011). *Effektiv og bærekraftig arealbruk i havbruksnæringen – areal til begjær (in Norwegian).* (Technical Report No. xx). Oslo: The Royal Norwegian Ministry of Fisheries and Coastal Affairs. Retrieved from www.regjeringen.no/globalassets/upload/fkd/vedlegg/rapporter/2011/effektiv_og_baerekraftig_arealbruk_i_havbruksnaeringen.pdf

Gunnarsson, G., Karlsbakk, E., Blindheim, S., Plarre, H., Imsland, A., Handeland, S., … Nylund, A. (2017). Temporal changes in infections with some pathogens associated with gill disease in farmed Atlantic salmon (*Salmo salar* L). *Aquaculture*, *468*, 126–134.

Handeland, S. O., & Stefansson, S. O. (2001). Photoperiod control and influence of body size on off-season parr–smolt transformation and post-smolt growth. *Aquaculture*, *192*, 291–307.

Håstein, T., Scarfe, A. D., & Lund, V. L. (2005). Science-based assessment of welfare: Aquatic animals. *Revue Scientifique et Technique*, *24*, 529–547.

Hevrøy, E. M., Hunskår, C., de Gelder, S., Shimizu, M., Waagbø, R., Breck, O., … Hansen, T. (2013). GH–IGF system regulation of attenuated muscle growth and lipolysis in Atlantic salmon reared at elevated sea temperatures. *Journal of Comparative Physiology B*, *183*, 243–259.

Hevrøy, E. M., Tipsmark, C. K., Remø, S. C., Hansen, T., Fukuda, M., Torgersen, T., … Shimizu, M. (2015). Role of the GH-IGF-1 system in Atlantic salmon and rainbow trout postsmolts at elevated water temperature. *Comparative Biochemistry and Physiology A*, *188*, 127–138.

Hjeltnes, B., Bornø, G., Jansen, M. D., Haukaas, A. & Walde, C. (2017). *Fiskehelserapporten 2016 (in Norwegian).* Veterinærinstituttet. Retrieved from www.vetinst.no/rapporter-og-publikasjoner/rapporter/2017/fiskehelserapporten-2016

Ibarz, A., Felip, O., Fernández-Borràs, J., Martín-Pérez, M., Blasco, J., & Torrella, J. R. (2011). Sustained swimming improves muscle growth and cellularity in gilthead sea bream. *Journal of Comparative Physiology B*, *181*, 209–217.

Iemitsu, M., Maeda, S., Jesmin, S., Otsuki, T., & Miyauchi, T. (2006). Exercise training improves aging-induced downregulation of VEGF angiogenic signaling cascade in hearts. *American Journal of Physiology-Heart and Circulatory Physiology*, *291*, H1290–H1298.

Imsland, A. K., Björnsson, B. T., Gunnarsson, S., Foss, A., & Stefansson, S. O. (2007). Temperature and salinity effects on plasma insulin-like growth factor-I concentrations and growth in juvenile turbot (*Scophthalmus maximus*). *Aquaculture*, *271*, 546–552.

Iwama, G. K., & Tautz, A. F. (1981). A simple growth model for salmonids in hatcheries. *Canadian Journal of Fisheries and Aquatic Sciences*, *38*, 649–656.

Jobling, M. (2003). The thermal growth coefficient (TGC) model of fish growth: A cautionary note. *Aquaculture Research*, *34*, 581–584.

Jobling, M., Jørgensen, E. H., Arnesen, A. M., & Ringø, E. (1993). Feeding, growth and environmental requirements of Arctic charr: A review of aquaculture potential. *Aquaculture International*, *1*, 20–46.

Johnston, I. A. (1999). Muscle development and growth: Potential implications for flesh quality in fish. *Aquaculture*, *177*, 99–115.

Johnston, I. A., & Moon, T. W. (1980). Exercise training in skeletal muscle of brook trout (*Salvelinus fontinalis*). *Journal of Experimental Biology*, *87*, 177–194.

Kaneko, N., Taniyama, N., Inatani, Y., Nagano, Y., Fujiwara, M., Torao, M., … Shimizu, M. (2015). Circulating insulin-like growth factor I in juvenile chum salmon: Relationship with growth rate and changes during downstream and coastal migration in northeastern Hokkaido, Japan. *Fish Physiology and Biochemistry*, *41*, 991–1003.

Kawaguchi, K., Kaneko, N., Fukuda, M., Nakano, Y., Kimura, S., Hara, A., & Shimizu, M. (2013). Responses of insulin-like growth factor (IGF)-I and two IGF-binding protein-1 subtypes to fasting and re-feeding and their relationships with individual growth rates in yearling masu salmon (*Oncorhynchus masou*). *Comparative Biochemistry and Physiology A*, *165*, 191–198.

Kolodziejczyk, S. M., Wang, L., Balazsi, K., DeRepentigny, Y., Kothary, R., & Megeney, L. A. (1999). MEF2 is upregulated during cardiac hypertrophy and is required for normal post-natal growth of the myocardium. *Current Biology*, *9*, 1203–1206.

Kongtorp, R. T., Kjerstad, A., Taksdal, T., Guttvik, A., & Falk, K. (2004). Heart and skeletal muscle inflammation in Atlantic salmon, *Salmo salar* L.: A new infectious disease. *Journal of Fish Diseases*, *27*, 351–358.

Larsen, D. A., Beckman, B. R., & Dickhoff, W. W. (2001). The effect of low temperature and fasting during the winter on metabolic stores and endocrine physiology (insulin, insulin-like growth factor-I and thyroxine) of coho salmon, *Oncorhynchus kisutch*. *General and Comparative Endocrinology*, *123*, 308–323.

Leon, K. A. (1986). Effect of exercise on feed consumption, growth, food conversion and stamina of brook trout. *The Progressive Fish-Culturist*, *48*, 43–46.

Løvoll, M., Alarcón, M., Bang Jensen, B., Taksdal, T., Kristoffersen, A. B., & Tengs, T. (2012). Quantification of piscine reovirus (PRV) at different stages of Atlantic salmon *Salmo salar* production. *Diseases of Aquatic Organisms*, *99*, 7–12.

Maclean, A., & Metcalfe, N. (2001). Social status, access to food and compensatory growth in juvenile Atlantic salmon. *Journal of Fish Biology*, *58*, 1331–1346.

Martin, C., & Johnston, I. (2005). The role of myostatin and the calcineurin-signalling pathway in regulating muscle mass in response to exercise training in the rainbow trout *Oncorhynchus mykiss* Walbaum. *Journal of Experimental Biology*, *208*, 2083–2090.

McCormick, S. D. (2012). Smolt physiology and endocrinology. In S. D. McCormick, A. P. Farrell, & C. J. Brauner (Eds.), *Fish physiology* (pp. 199–251). Amsterdam, Netherland: Academic Press.

Mendez, E., Smith, A., Figueiredo-Garutti, M., Planas, J., Navarro, I., & Gutiérrez, J. (2001). Receptors for insulin-like growth factor-I (IGF-I) predominate over insulin receptors in skeletal muscle throughout the life cycle of brown trout, *Salmo trutta*. *General and Comparative Endocrinology*, *122*, 148–157.

Mingarro, M., de Celis, S. V.-R., Astola, A., Pendón, C., Valdivia, M. M. n., & Pérez-Sánchez, J. (2002). Endocrine mediators of seasonal growth in gilthead sea bream (*Sparus aurata*): The growth hormone and somatolactin paradigm. *General and Comparative Endocrinology*, *128*, 102–111.

Mortensen, A., & Damsgård, B. (1993). Compensatory growth and weight segregation following light and temperature manipulation of juvenile Atlantic salmon (*Salmo salar* L.) and Arctic charr (*Salvelinus alpinus* L.). *Aquaculture*, *114*, 261–272.

Nicieza, A. G., & Metcalfe, N. B. (1997). Growth compensation in juvenile Atlantic salmon: Responses to depressed temperature and food availability. *Ecology*, *78*, 2385–2400.

Ohlsson, C., Mohan, S., Sjogren, K., Tivesten, A., Isgaard, J., Isaksson, O., … Svensson, J. (2009). The role of liver-derived insulin-like growth factor-I. *Endocrine Reviews*, *30*, 494–535.

Olsvik, P. A., Lie, K. K., Jordal, A.-E. O., Nilsen, T. O., & Hordvik, I. (2005). Evaluation of potential reference genes in real-time RT-PCR studies of Atlantic salmon. *BMC Molecular Biology*, *6*, 21.

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research*, *29*, e45–e445.

Pierce, A. L., Beckman, B. R., Shearer, K. D., Larsen, D. A., & Dickhoff, W. W. (2001). Effects of ration on somatotropic hormones and growth in coho salmon. *Comparative Biochemistry and Physiology B*, *128*, 255–264.

Poppe, T. T., Johansen, R., Gunnes, G., & Tørud, B. (2003). Heart morphology in wild and farmed Atlantic salmon Salmo salar and rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms*, *57*, 103–108.

Rasmussen, R. S., Heinrich, M. T., Hyldig, G., Jacobsen, C., & Jokumsen, A. (2011). Moderate exercise of rainbow trout induces only minor differences in fatty acid profile, texture, white muscle fibres and proximate chemical composition of fillets. *Aquaculture*, *314*, 159–164.

Rodger, H., & Mitchell, S. (2007). Epidemiological observations of pancreas disease of farmed Atlantic salmon, *Salmo salar* L., in Ireland. *Journal of Fish Diseases*, *30*, 157–167.

Shimizu, M., Swanson, P., Fukada, H., Hara, A., & Dickhoff, W. W. (2000). Comparison of extraction methods and assay validation for salmon insulin-like growth factor-I using commercially available components. *General and Comparative Endocrinology*, *119*, 26–36.

Small, B. C., & Peterson, B. C. (2005). Establishment of a time-resolved fluoroimmunoassay for measuring plasma insulin-like growth factor I (IGF-I) in fish: Effect of fasting on plasma concentrations and tissue mRNA expression of IGF-I and growth hormone (GH) in channel catfish (*Ictalurus punctatus*). *Domestic Animal Endocrinology*, *28*, 202–215.

Solstorm, F., Solstorm, D., Oppedal, F., Fernö, A., Fraser, T., & Olsen, R. E. (2015). Fast currents reduce production performance of post-smolt Atlantic salmon. *Aquaculture Environment Interactions*, *7*, 125–134.

Solstorm, F., Solstorm, D., Oppedal, F., Olsen, R. E., Stien, L. H., & Fernö, A. (2016). Not too slow, not too fast: Water currents affect group structure, aggression and welfare in post-smolt Atlantic salmon *Salmo salar*. *Aquaculture Environment Interactions*, *8*, 339–347.

Stefansson, S., McCormick, S., Ebbesson, L. O., & Björnsson, B. T. (2008). Smoltification. In A. Finn & B. Kapoor (Eds.), *Fish larval physiology* (pp. 639–681). Enfield, NH: Science Publishers, Inc..

Stickland, N. (1983). Growth and development of muscle fibres in the rainbow trout (*Salmo gairdneri*). *Journal of Anatomy*, *137*, 323.

Thorarensen, H., & Farrell, A. P. (2011). The biological requirements for post-smolt Atlantic salmon in closed-containment systems. *Aquaculture*, *312*, 1–14.

Totland, G., Kryvi, H., Jødestøl, K., Christiansen, E., Tangerås, A., & Slinde, E. (1987). Growth and composition of the swimming muscle of adult Atlantic salmon (*Salmo salar* L.) during long-term sustained swimming. *Aquaculture*, *66*, 299–313.

Videler, J. J. (1993). *Fish swimming*. London, England: Chapman & Hall.

Walker, M. G., & Emerson, L. (1978). Sustained swimming speeds and myotomal muscle function in the trout, Salmo gairdneri. *Journal of Fish Biology*, *13*, 475–481.

Weatherley, A., Gill, H., & Lobo, A. (1988). Recruitment and maximal diameter of axial muscle fibres in teleosts and their relationship to somatic growth and ultimate size. *Journal of Fish Biology*, *33*, 851–859.

Weatherley, A., Gill, H., & Rogers, S. (1980). The relationship between mosaic muscle fibres and size in rainbow trout (*Salmo gairdneri*). *Journal of Fish Biology*, *17*, 603–610.

Winberg, S., Nilsson, G. E., & Olsén, K. H. (1991). Social rank and brain levels of monoamines and monoamine metabolites in Arctic charr, *Salvelinus alpinus* (L.). *Journal of Comparative Physiology A*, *168*, 241–246.

Wood, A. W., Duan, C., & Bern, H. A. (2005). Insulin-like growth factor signaling in fish. *International Review of Cytology*, *243*, 215–285.

Yancopoulos, G. D., Davis, S., Gale, N. W., Rudge, J. S., Wiegand, S. J., & Holash, J. (2000). Vascular-specific growth factors and blood vessel formation. *Nature*, *407*, 242–248.

SUPPORTING INFORMATION

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

How to cite this article: Balseiro P, Moe Ø, Gamlem I, et al. Comparison between Atlantic salmon *Salmo salar* post-smolts reared in open sea cages and in the Preline raceway semi-closed containment aquaculture system. *J Fish Biol*. 2018;93: 567–579. https://doi.org/10.1111/jfb.13659