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The importance of exercise: Increased water velocity improves growth of Atlantic salmon in closed cages

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ABSTRACT

There is increasing concern about Norwegian salmon farming and the possible environmental impacts from sea lice, escaped fish and release of toxic chemicals and organic emissions to the coastal waters. Closed containment systems (CCS) have the potential to eliminate the problems with sea lice and to reduce escapes and emissions. When closing the cages, water volumes and velocity are regulated and the identification of optimal current velocities for growth and fish welfare from sea transfer to harvest size becomes necessary. This study describes two trials with LOW (0.10–0.27 BL/s) and MODERATE (0.36–0.63 BL/s) water velocity on performance of post-smolt Atlantic salmon in CCS. In trial 1 (168 days, 10.9 °C, fish size: 884–3007 g and 41.5–59.0 cm), round weight increased with 219 g ($p = .012$) and condition factor with 0.11 ($p = .016$) in the MODERATE group compared with LOW group. The MODERATE group obtained specific growth rate (SGR) of 0.76 and thermal growth coefficient (TGC) of 2.75, compared to 0.72 and 2.56 in the LOW group. MODERATE water velocity was also associated with higher relative heart size (RHS) ($p = .016$), higher liver index (HSI) ($p = .005$), increased fillet yield ($p \leq .001$) and lower levels of cathepsin activity in muscle tissue. In trial 2 (46 days, 7.1 °C, fish size: 327–482 g and 29.9–33.7 cm), round weight increased with 52 g ($p = .019$) and condition factor with 0.05 ($p = .009$) in the MODERATE group compared with LOW group. The MODERATE group obtained SGR of 0.77 and TGC of 2.68, compared to SGR of 0.60 and TGC of 2.02 in the LOW group. No significant difference was observed in white muscle cell hyperplasia, measured as the proportion of small (< 20 µm diameter) muscle fibres ($p = .145$). Both trials showed only minor differences in slaughter yield, fillet quality (protein, fat, water) and mortality. The present study shows that moderate water velocity (0.36–0.63 BL/s) is favourable for growth rates for Atlantic salmon during the entire on-growing period in CCS. Effects on a broader range of metabolic variables and welfare indicators were also documented.

1. Introduction

Production of post-smolt Atlantic salmon (*Salmo salar*) primarily occurs in netpen cages in coastal areas. With Norwegian salmon farming's rapid growth in the last few decades, the environmental impact of salmon production has received more public attention. Negative effects on wild salmon populations by spread of diseases (Garseth et al., 2013) and escaped fish (Naylor et al., 2000; Naylor et al., 2005) and the potential negative effects of nutrient overloading in the coastal areas are important controversies to solve if Norwegian aquaculture should continue to grow. Problems with salmon lice (*L.salmonis*) on both farmed salmon and wild salmonids and the emergence and rapid spread of drug-resistant lice have forced farms to abandon chemical treatments and to develop non-medicinal treatments or alternative farming strategies (Hjeltnes et al., 2018).

One possible solution to prevent salmon lice infestation in salmon farms is to use more closed cage technologies. In closed containment systems (CCS) (Calabrese, 2017) intake water can be pumped from deeper water layers avoiding infective salmon lice copepodites (Nilsen et al., 2017a). Using a rigid, closed cage design or tarpaulin bags with surrounding safety nets and using sites better sheltered from extreme wind and waves could reduce the risk of escaped fish. In addition, the local environmental impact can be reduced by collecting and reusing settleable particles from faeces and surplus feed.

Water velocity is an environmental parameter with a profound impact on fish metabolism, growth, behaviour and welfare (Palstra and Planas, 2011). First of all, higher water velocity can boost the growth of farmed fish (Leon, 1986; Christiansen et al., 1989; Jobling et al., 1993; Jørgensen and Jobling, 1993; Young and Cech, 1993; Davison, 1997;

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Castro et al., 2011; Li et al., 2016; Ytrestøyl et al., 2017). Higher growth rates have been linked to increased feed intake and more effective feed-conversion ratio (Jobling et al., 1993; Davison, 1997; Castro et al., 2011). Higher water velocity also improves flesh texture (Totland et al., 1987; Tachibana et al., 1988; Bugeon et al., 2003; Li et al., 2016) and general robustness (Takle et al., 2010) and may lower aggression (Kalleberg, 1958; Jobling et al.; 1993; Solstorm et al., 2016) and lead to a reduced stress response (Woodward and Smith, 1985; Young and Cech 1993; Huntingford, 2010; Solstorm et al., 2015). On the other hand, too high water velocities will lead to increased oxygen need and anaerobic metabolism with increased levels of lactate (Davison, 1997; Palstra et al., 2010) and finally to exhaustion, reduced growth and impaired fish welfare (Solstorm et al., 2015; Solstorm et al., 2016).

CCS operation requires pumping large volumes of water with a continuous oxygen supply (Nilsen et al., 2017b; Summerfelt et al., 2016; Sveen et al., 2016). With intensified CCS production, reduced specific water consumption (SWC) could reduce both water quality and water velocity. If the water is oxygenated but not aerated, the build-up of fish metabolites, especially carbon dioxide (CO_2) and total ammonia nitrogen (TAN) will set the limit for lowest acceptable water flow (Sanni and Forsberg, 1996; Bergheim and Fivelstad, 2014). Regulating CCS water flow facilitates the regulation of water velocity to optimise fish growth and welfare. Most studies on different water velocities have been performed over shorter periods with small fish in freshwater. While there are some studies on how salmon adapt to different water velocities in open sea cages (Oppedal et al., 2011; Johansson et al., 2014), there is a need for more specific knowledge about the effect of continuous water velocity in CCS on growth, flesh quality and welfare in Atlantic salmon grown from post-smolt to harvest size.

The present study's main aim was to investigate how two different water velocities on post-smolt Atlantic salmon in a CCS affected growth, chemical composition of fillet, myotomal cathepsin activity and muscle cell development. We also observed fish behaviour and evaluated the results from a fish welfare perspective.

2. Materials and methods

2.1. Cages and water quality

Circular CCS of 40 m^3 volume were used in the present experiment (AkvaDesign AS, Brønnøysund, Norway). The CCS consisted of tarpaulin bags with water pumped from 25 m depth (Nilsen et al., 2017a). Water was pumped (5.5 kW, Xylem Norway AS) into a floating tank (4.5m^3) and then distributed to each cage securing identical temperature and quality of inlet water to all cages. Water flow was 250–275 L/min for each cage, with retention time of approximately 2.5 h. The current in the three LOW velocity cages (6–8 cm/s) was created by incoming water alone. In the three MODERATE velocity cages (19–21 cm/s), an extra current booster (D-Icer 1, 6228D 2HPw, Taylor Made Products, 65 Harrison street, Gloversville, NY 12078) was located opposite to the water inlet pipe. The current boosters were installed at 1 m depth, 0.5–0.75 m from the cage wall (Fig. 1). Water velocity was kept constant throughout the experiment and, consequently, the relative swimming velocity (BL/s) decreased as the fish grew.

The cages were circular with a diameter of 4.8 m and a total depth of 4 m (ratio of 1:1.2), with a short 60° open-ended inlet located at 0.5 m depth creating a circular, primary current, see Fig. 1. Each cage was supplied with external light mounted on the floating ring supporting the tarpaulin bags (LED 2x50W 230 V IP65, Etman Distribusjon AS, Egersund, Norway). Oxygen was supplied by a diffusor net (AkvaDesign AS), oxygen and temperature were logged with 10-min intervals at 2 m depth (FDO 700 IQ SW, WTW/Xylem). Mean oxygen saturation was regulated to 80–95% in all cages. Water quality parameters such as pH, temperature, dissolved oxygen and salinity were measured with SmarTroll MP handheld sensor (Tormatic AS, Norway). Carbon dioxide was measured with OxyGuard CO_2 portable meter (OxyGuard AS, Denmark). Water samples for laboratory analysis of pH (NS-EN ISO 10523), total ammonia nitrogen (TAN, NS-EN ISO 14911) and total suspended solids (TSS, NS-EN 872) were collected at 1 m depth with Ruttner type water sampler (Fybikon AS, Norway), and stored at + 4 °C in sterile plastic bottles for chemical water analysis until delivery to the laboratory (Kystlab Prebio, Brønnøysund, Norway).

2.2. Fish and rearing conditions

Trial 1 lasted from June to November 2015, and trial 2 from February to April 2017. The fish were fed to satiation every day, using Betten feeders S1–125 automatic feeding system (Betten Maskinstasjon AS, Vågland, Norway). All experimental procedures were in accordance with the regulations controlling experiments/procedures on live animals in Norway, and the study complies with the policies relating to animal ethics. Due to the experiment's nature, permission from the Norwegian Research Authority was not required. All fish were returned to one of the commercial cages after the experiment.

Dead fish were collected two to five times per week with the lift-up integrated in the water outlet. Injured or weak fish were netted, killed and recorded as mortality. All dead or killed fish were inspected, weighed and autopsied. Kidneys were examined for macroscopic signs of nephrocalcinosis, a typical lesion when levels of CO_2 exceeds 10–15 mg/L (Fivelstad et al., 2003; Fivelstad et al., 2018) and gills were examined for macroscopic signs of gill diseases. Cause-specific mortality was scored in five categories: (1) ulcers and fin lesions, (2) physical trauma, (3) infectious diseases, (4) runts or (5) unknown.

Trial 1: Atlantic salmon (*S. salar*, Salmo breed) from Bindalssmolt AS, 7982 Bindalseidet, Norway. From sea transfer in November 2014 until May 2015 the fish were reared in commercial-scale CCS (2870 m^3 volume) at site Møllebogen (65°N 12° E), and then moved to Norsk Havbrukssenter, Brønnøysund, Norway (65.5°N 12.1°E) where the trial took place from June 10th 2015 to November 17th 2015. On May 27th 2015, 1800 salmon (mean ± SE weight 894 ± 4.6 g, length 41.5 ± 0.06 cm) were evenly distributed into the six closed cages. Each cage was stocked with 300 salmon, 250 fish untagged and 50 fish PIT tagged intraperitoneally with GPT12 Pre-load tags (12.5 mm, 134.2 kHz) (Biomark, Boise, USA), using MK25™ Implant Gun (Biomark, Boise, USA). Biomark 601™ Reader (Biomark, Boise, USA) was used to read the PIT tag ID. Water velocity was adjusted to LOW (6 ± 0.4 cm/s) and MODERATE (21 ± 0.7 cm/s) June 10th. This corresponded to an initial water velocity of 0.14 and 0.5 body lengths per second (BL/s) and final water velocity of 0.10 and 0.36 BL/s respectively. The last feeding day was November 15th with final sampling on November 17th and 18th. During the trial, one cage in each group was excluded due to large variations in oxygen levels and/or water velocity, and the presented data are therefore based on the four remaining cages. Feeding was Spirit S600-50A 7 mm from 10.6 to 29.7, Premium 1200-50A 9 mm from 30.7–14.10, and Premium 2500-50A 9 mm from 15.10 to 17.11 (Skretting AS, Stavanger, Norway). Stocking density was between 7 kg/m^3 and 20 kg/m^3. The trial lasted for 168 days.

Trial 2: Atlantic salmon (*S. salar*, AquaGen) from Grytåga settefisk, 8860 Tjøtta, Norway. From sea transfer in October 2016 until January 2017 the fish were reared in commercial scale CCS (6000 m^3 volume) at site Sæterosen (65.3°N 12.3°E), AkvaFuture AS, 8900 Brønnøysund, Norway. On January 31st, 7200 post-smolt salmon with an average weight of 300 g were evenly distributed into six closed cages. After one month with acclimatisation to low speed in all cages, mean weight and length in each cage were determined on February 27th by randomised sampling of 150 fish from each cage. Water velocity was adjusted to LOW (8 ± 0.6 cm/s) and MODERATE (19 ± 0.7 cm/s), corresponding to initial water velocity of 0.27 and 0.63 BL/s and final water velocity of 0.24 and 0.57 BL/s respectively. The trial started on March 1st 2017. The last feeding day was April 17th, the trial ended on April 18th, and the final samples were taken April 19th to 20th. Feeding was Intro 200 HH 50 mg Q 5 mm (Biomar AS, Myre, Norway). Stocking density was

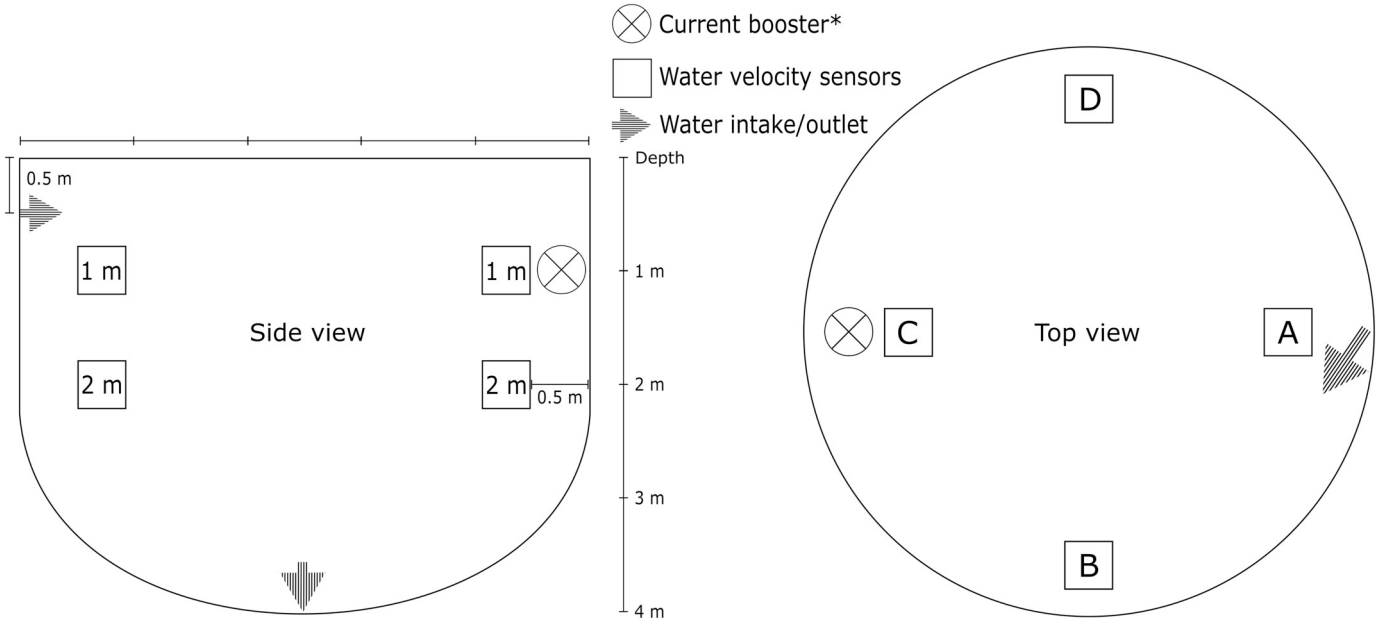

Fig. 1. Schematic presentation of the test cages. Left: side view with location of inlet, outlet, current booster (* only in the MODERATE group) and the positions where water velocity was measured. Right: top view with location of water inlet, current booster and the positions where water velocity was measured.

between 10 kg/m^3 and 13 kg/m^3 and the trial lasted for 46 days.

2.3. Water velocity and fish behaviour

Trial 1: The circular, horizontal water velocity was measured weekly (1 to 3 min' duration in each position, continuous logging) with Flow Rate Sensor LQ2-LE with the software Labquest 2 (Vernier Software and Technology, USA, www.Vernier.com). These measurements were validated with an SD6000 current sensor (Nortek AS) in October 2015. Three parallel measures in LOW and MODERATE cages with Vernier Flow Rate sensor and Nortek SD6000 showed no significant difference between the two methods, and the results from these methods in trial 1 are reported in the same tables and figures. Water velocity was measured at position A, C and D (1 and 2 m depth, 0.5 to 0.75 m from the cage wall, see Fig. 1), and reported at group level as mean velocity (SE) of all positions and both depths from the two remaining cages in each group. Careful observations of the fish were conducted once a week on the presence of individuals with visible lesions and on behaviour: schooling behaviour, vertical positioning, abnormal behaviours, feeding activity and swimming speed relative to water velocity.

Trial 2: Water velocity was measured weekly (0.1 to 70.4 h duration in each position, 10 min logging intervals) with two SD6000 current sensors (Nortek AS) at 1 and 2 m depths in positions A and C (Fig. 1), and reported at group level as mean velocity (SE) of both positions and depths from all three cages in each group. Fish behaviour and lesions were observed as in trial 1.

2.4. Sampling procedures

Trial 1: Weight and length of all fish were recorded on May 27th, of 50 fish in each cage on August 26th, and of 200 fish in each cage on November 17th. The fish were collected by purse seine and taken using scoop net and anesthetised with tricaine methanesulfonate (Tricaine Pharmaq 1000 mg/L, Pharmaq AS) 30–80 mg/L, before measuring. At each of the three sampling dates, 15 untagged fish from each cage were randomly sampled for the evaluation of biometrics and chemical fillet analysis. This fish were stunned by a sharp blow to the head, gill arches on both sides were cut and the fish bled for half an hour in cold seawater and then individually labelled. Sampling at the site included round weight, fork length, gutted weight, liver weight and heart weight (including the bulbus arteriosus) of each individual. The fish were then iced in polystyrene boxes and sent by boat to the Faculty of Biosciences and Aquaculture, Nord University (Bodø, Norway) and stored at +4 °C awaiting further analysis of fillet chemical content and analysis of Cathepsin B, B + L and H.

Trial 2: Weight and length of 100 fish in each cage were recorded on February 27th and 150 fish in each cage on April 18th. Fish were anaesthetised with benzocaine (Benzoac Vet 200 mg/mL, ACD Pharmaceuticals AS) 0.2–0.25 mL/L. Sampling included the same parameters and procedures as in trial 1, except from the enzyme analysis and with additional sampling for muscle cell analysis.

2.5. Biometrics

Weight (W) was recorded as round body weight in g (± 1 g), length (L) as fork length (± 0.5 cm) and reported as group mean and standard deviation (SD). Condition factor was calculated as:

$$CF = 100 \cdot (W/L^3)$$

Specific growth rate (SGR) was calculated as (Houde and Scheckter, 1981):

$$SGR = 100 \cdot (\ln(W_1) - \ln(W_0))/(t_1 - t_0)$$

where W_1 and W_0 are weights on days t_1 and t_0, respectively. Thermal growth coefficient (TGC) was calculated as (Alanärä et al., 1994):

$$TGC = 1000 \cdot (W_1^{1/3} - W_0^{1/3})/(T \cdot t)$$

where T is temperature in °C and t is time in days.

The SGR and TGC models are limited to fish size between 50 and 3000 g and temperatures between 4 °C and 14 °C (Alanärä et al., 2001).

Slaughter yield was calculated as: 100·(gutted weight/round body weight). Fillet yield was calculated as: 100·(weight of both fillets/round body weight). Hepasomatic index (HSI) was calculated as: 100·(liver weight/ round body weight). Relative heart size (RHS) was calculated as: 100·(heart weight/round body weight). Water velocity relative to fish size was calculated as: body lengths per second (BL/s). Production intensity in the cages was calculated as: specific water consumption (SWC) in litres of water used per kg fish per minute (L/kg/min), feed load (FL) in g feed/m^3 water flow (g/m^3) and density as kg fish per m^3

cage volume (kg/m^3).

2.6. Fillet chemical composition and cathepsin activity

At day 5 post mortem, the fish was filleted and both fillets were weighed. The Norwegian quality cut (NQC) (NS 9401, 1994) from the right fillet was homogenised in a Braun MR530 Turbo-Accesorios homogeniser (Braun, Germany), and the mince stored at −40 °C before chemical analysis (both trials) of protein, fat, water content, and cathepsin (only trial 1) B, B + L and H activity, in duplicates. In trial 1, all homogenised samples from sample one were analysed with the following chemical reference methods: water content was determined after drying at 104 °C for 20 h, protein analysed as Kjeldahl-nitrogen using factor 6.25 (Kjeltec 1030 Auto analyzer; Foss Tecator AB, Höganäs, Sweden), and fat was determined by extraction in ethyl acetate (Norwegian Standard NS-9402E, 1994E). In sample two and three, a representative sample of 50% of the fish were analysed with chemical methods and all homogenised samples were consecutively scanned by Near Infrared Spectroscopy (NIR) before freezing, using DA 7200 Diode Array High Speed analyzer (Perten Instruments AB, Hägersten, Sweden). The instrument was operating in the wavelength range of 950–1650 nm. In trial 2, all samples were scanned by NIR and 50% of the samples were analysed with chemical methods. A representative selection of fish from the last sampling in trial 2 were used for calibration of NIR data to chemical analysed data of water (six factors, $R^2 = 0.984$), fat (five factors, $R^2 = 0.978$) and protein (nine factors, $R^2 = 0.976$) respectively, using PLS regression in the Unscrambler® X software (version 10.4, CAMO Software AS, Oslo, Norway). The PLS models were configured with full cross-validation and SD-1 weighting of the Y-variable, further used for prediction of individual content of water, protein and fat for all fish in trail 2, according to previously described methods (Solberg, 1992, 1997). Cathepsin B, B + L and H acitivity (trial 1) were analysed as described by Hagen et al. (2008).

2.7. Muscle cell analysis

In trial 2, two fish from each cage at initial sampling, and five fish from each cage at final sampling were analysed for muscle cell histology according to Johnston et al. (1999). A 5 mm thick cross-sectional slice was taken directly behind the dorsal fin, and three muscle blocks (0.5 × 0.5 × 0.5 cm) were cut out (Fig. 2) covered with Shandon™ Cryomatrix™ (Thermo Fisher Scientific, MA, USA) and frozen in isopentane cooled in liquid nitrogen (−159 °C) for 60 s, wrapped in aluminium foil and stored at −80 °C until preparation. Transverse muscle sections were cut at 8 µm in a cryostat (CryoStar NX50, Thermo Fisher Scientific, MA, USA) and stained with hematoxylin solution (Papanicolaou's solution 1a Harris' hematoxylin solution, Merck, Germany). Slides were examined with light microscopy (Axioskop 2 mot plus, Zeiss, Germany) and photographed with a digital camera (Axiocam HRc, Zeiss, Germany) mounted directly on the microscope with 10× magnification. Using Axiovision 4.8 (Zeiss, Germany) the circumference of a minimum of 450 white muscle cells (fast cells) were measured for each fish, and cell density, diameter and cell area was calculated.

2.8. Statistical analysis

Weight, length, condition factor and all qualitative outcome variables are reported as group means with standard deviation (SD). Statistical analysis of the effect of water velocity on growth data, chemical content and cathepsin activity were performed using the IBM SPSS Statistics v. 22.0 (IBM Corporation, NY, US). Weight, length, condition factor (CF), RHS, HSI, slaughter yield and fillet yield were analysed with a mixed linear regression model (maximum likelihood) with group as fixed effect and cage as random effect. The effects of water velocity on chemical analysis and enzyme activity were analysed with group as fixed effect and both cage and gutted weight as random effects. The effect of gutted weight on the model was from low to moderate and the results from the analysis with both cage and gutted weight as random effects are reported. Residuals were plotted with a P—P plot, the effect of extreme outliers on the models was evaluated and if necessary they were removed before the final analysis. The statistical analysis is reported as the differences between the MODERATE and LOW groups with 95% confidence intervals and *p*-values.

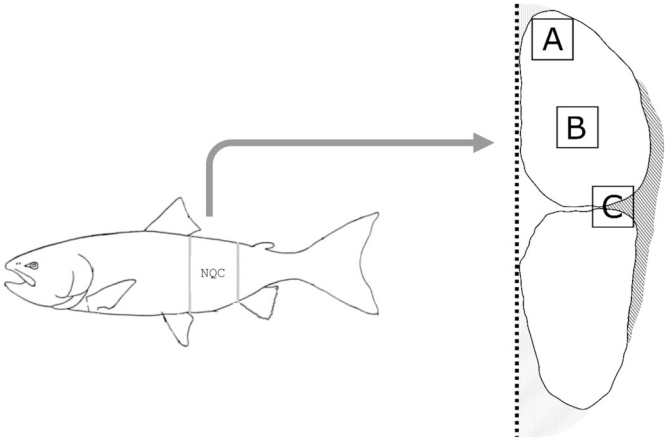

Fig. 2. Schematic view of Norwegian quality cut (left), and the sample sites for muscle fibre analysis (right). The right panel shows the 5 mm thick cross-sectional slice of the fish, taken directly behind the dorsal fin, with the location of three muscle blocks from each fish: A and B from epaxial white muscle fibres, C from white and red muscle fibres at the lateral line.

The muscle cell distribution was analysed using R (3.3.1). Distribution of muscle cell diameter was evaluated using smooth non-parametric distributions where 450 measurements of cell diameter were fitted using a kernel function (Johnston et al., 1999). Groups compared had similar body mass and length ($n = 9$ LOW group, $n = 13$ MODERATE group). A Kolmogorov–Smirnov two-sample test was used to test the null hypothesis that the probability density functions (PDFs) of groups were equal over all diameters. Density curves for each treatment were also compared graphically by constructing a variability band around the density estimate for the combined populations using the mean smoothing parameter h, varying between 0.17 and 0.19 for the different groups (Bowman and Azzalini, 2003). This can be used to distinguish the underlying structure in the distributions from random variation providing an indicator of which part(s) of the distribution of diameters contributed to any significant differences.

3. Results

3.1. Growth

The mean weight and condition factor was higher in the MODERATE velocity group at the end of both trials (Tables 1 and 4, Fig. 3). In trial 1, the MODERATE group had 7.9% increased weight, in trial 2, 12.1% increased weight compared with LOW. There were no differences in start weight, length and condition factor between the two groups. At mid-evaluation (trial 1) the groups were also equal.

In trial 1, SGR increased by 5.9% from LOW (0.68) to MODERATE (0.72), TGC increased by 7.4% from LOW (2.56) to MODERATE (2.75). In the individually tagged fish in trial 1 (total $n = 120$), mean SGR (SD) in the LOW group was 0.63 (0.13), in the MODERATE group 0.67 (0.10), an increase of 6.3%. In trial 2 SGR increased with 28% from LOW (0.60) to MODERATE (0.77), and TGC with 33% from LOW (2.02) to MODERATE (2.68) (Table 2).

Table 1
Number of sampled fish (n), mean (SD) weight (g), length (cm) and condition factor (CF) in Atlantic salmon exposed for either LOW or MODERATE water velocities in two separate trials (168 days in trial 1, 46 days in trial 2).

Trial		Sample	LOW			MODERATE		
			n	Mean	SD	n	Mean	SD
1	Weight (g)	1	600	884	112	600	894	115
		2	100	1392	279	92	1435	252
		3	425	2782*	546	438	3003*	548
	Length (cm)	1	600	41.5	1.6	600	41.7	1.6
		2	100	48.7	2.7	98	49.1	2.3
		3	425	58.9	3.7	438	58.9	3.6
	CF	1	599	1.24	0.08	600	1.23	0.07
		2	100	1.19	0.10	89	1.21	0.10
		3	425	1.34*	0.12	433	1.45*	0.13
2	Weight (g)	1	292	327	111	296	338	127
		2	449	430*	161	447	482*	194
	Length (cm)	1	292	29.9	3.5	296	30.2	4.0
		2	449	33.0	3.9	447	33.7	4.2
	CF	1	292	1.19	0.09	296	1.17	0.10
		2	449	1.14**	0.08	447	1.19**	0.08

Significant differences between groups are indicated with: *:$p \leq .05$, **:$p \leq .01$.

3.2. Biometry

In trial 1, moderate water velocity increased the relative heart size (RHS) with 0.008%, the liver index (HIS) with 0.05% and fillet yield with 1.96% (Table 4). In trial 2, there were no differences in RHS or HSI and fillet yield was not recorded.

3.3. Fillet chemical content and cathepsin activity

There were only small differences in chemical composition of fillets in both trials (Table 3, Table 4). Fat (and thus also water) content was highly affected by body weight and using both cage and gutted body weight as random effects in the linear regression model removed most of the effect of water velocity on water, fat and protein content.

Cathepsin levels in both groups (only trial 1) decreased during the trial (Table 3). At the end of trial 1, mean activity levels of all three cathepsins (B, B + L, H) were lower in the MODERATE group compared with LOW when analysed with cage and gutted weight as random effects, but with a significant level of effect only in cathepsin B ($p = .008$) and cathepsin H ($p = .044$). (Table 4).

3.4. Muscle cellularity

At the end of trial 2, white muscle hyperplasia measured as the proportion of small fibres (< 20 µm diameter) showed no significant difference between MODERATE and LOW. The overall fibre distribution after removing 11 outliers due to extreme size or extremely different fibre diameter distribution did show a significant difference between groups (Kruskal-Wallis, $p = .005$), but this was not consistent with the probability density distribution in Fig. 4 which shows no deviation from the probability density area.

3.5. Water velocity and fish behaviour

The mean (SE) measured horizontal water velocity in trial 1 was 6 cm/s (0.4) in the LOW group and 21 cm/s (0.7) in the MODERATE group. In trial 2, water velocity was 8 cm/s (0.6) and 19 cm/s (0.7), see Table 5. The formation of a free vortex or irrotational zone with poorer mixing or lower velocities close to the centre drain, as described by Timmons et al. (1998), was not observed. The majority of observations in all cages and both trials showed a schooling behaviour where the fish swam counter-current with swimming speed slightly faster than the water velocity, and most (typically > 90%) fish formed a "doughnut" distribution at 0.5 to 2.5 m depth, with detours to the surface during feeding cycles and as part of the usual rolling and jumping behaviour and to refill the swim bladder. If current velocity dropped below 2 cm/s (LOW group) the schooling activity tended to disintegrate, with fish starting to swim in all directions. Immediately after transfer to the research cages, we observed fish with loss of scales in all cages. Some of these developed into skin lesions and ulcers, as described under 3.7 Mortality.

3.6. Temperature and water quality

The temperature profiles for both trials are shown in Table 6. The mean oxygen levels in all cages were between 82.8 and 94.7% DO (Table 7). The measured level of CO_2 in trial 1 was ≤2 mg/L. In both trials pH was between 7.4 and 7.9 in all cages, corresponding to CO_2 levels < 8 mg/L (Nilsen et al., 2017b). Total ammonia Nitrogen (TAN) values were ≤ 0.7 mg/L, with salinity 32.0 ppm and pH ≥ 7.4 corresponding to levels of toxic ammonia (NH_3) < 0.004 mg/L (Fivelstad et al., 1995). Levels of suspended solids (TSS) were < 20 mg/L. The specific water consumption (L/kg/min) was, in trial 1, between 0.31 and 0.94, and, in trial 2, between 0.31 and 0.42. The feed load (g/m^3) in trial 1 was between 6.1 and 17.4, and, in trial 2, between 11.3 and 15 (Table 8).

3.7. Mortality

Mortality in trial 1 was 7.7% in the LOW group and 6.5% in MODERATE group (Table 9). Cause-specific mortality was classified as «Unknown» (58%), «Ulcer and fin lesions» (32%) and «Other trauma» (10%). Mortality in trial 2 was 1.9% in LOW group and 2.3% in MODERATE group. Cause-specific mortality was classified as «Ulcer and fin lesions» (91%), «Runts» (7%) and «Unknown» (2%). No signs of other infectious diseases, gill lesions or kidney lesions (nephrocalcinosis) were detected.

4. Discussion

The trials' aim was to determine the effect of two different water velocities on growth, muscle cellularity, chemical composition and enzyme cathepsin activity of muscle in post-smolt Atlantic salmon in closed cages (CCS). The main finding in both trials is enhanced growth with increased water velocity.

4.1. Growth and muscle cell hyperplasia

In the MODERATE group (0.4–0.6 BL/s), the salmon had 7.9–12.1% increased weight (1.1–1.3 g/day) compared with the LOW group (0.1–0.3 BL/s). The relationship between growth and water velocity is demonstrated in several studies covering salmonids and other farmed species (Leon, 1986; Totland et al., 1987; Christiansen et al., 1989; Jørgensen and Jobling, 1993; Martin and Johnston, 2005; Palstra and Planas, 2011; Davison and Herbert, 2014). Other studies also show minor differences in length with increasing water velocity (Martin and Johnston, 2005; Davison and Herbert, 2014). In one of the few studies on large salmon in seawater, Totland et al. (1987) compared adult salmon (2 kg) in a swimming raceway for 8 months at 0.45–0.40 BL/s with fish in standard cages, with 38% higher weight gain in the raceway system. The durations of our trials were 168 and 46 days. Further studies with longer trial periods on large fish, and trials in commercial scale cages are necessary to establish the optimal water velocity for growth and fish welfare in CCS.

The SGR in our trials were between 0.60 and 0.77 and lower than common industry standards. Expected SGR for salmon of 300–500 g at 6–7 °C is 0.77–1.06 and 0.49–1.31 for salmon of 800–3000 g at 9–14 °C (Skretting, 2012). The TGC between 2.02 and 2.68 was also lower than

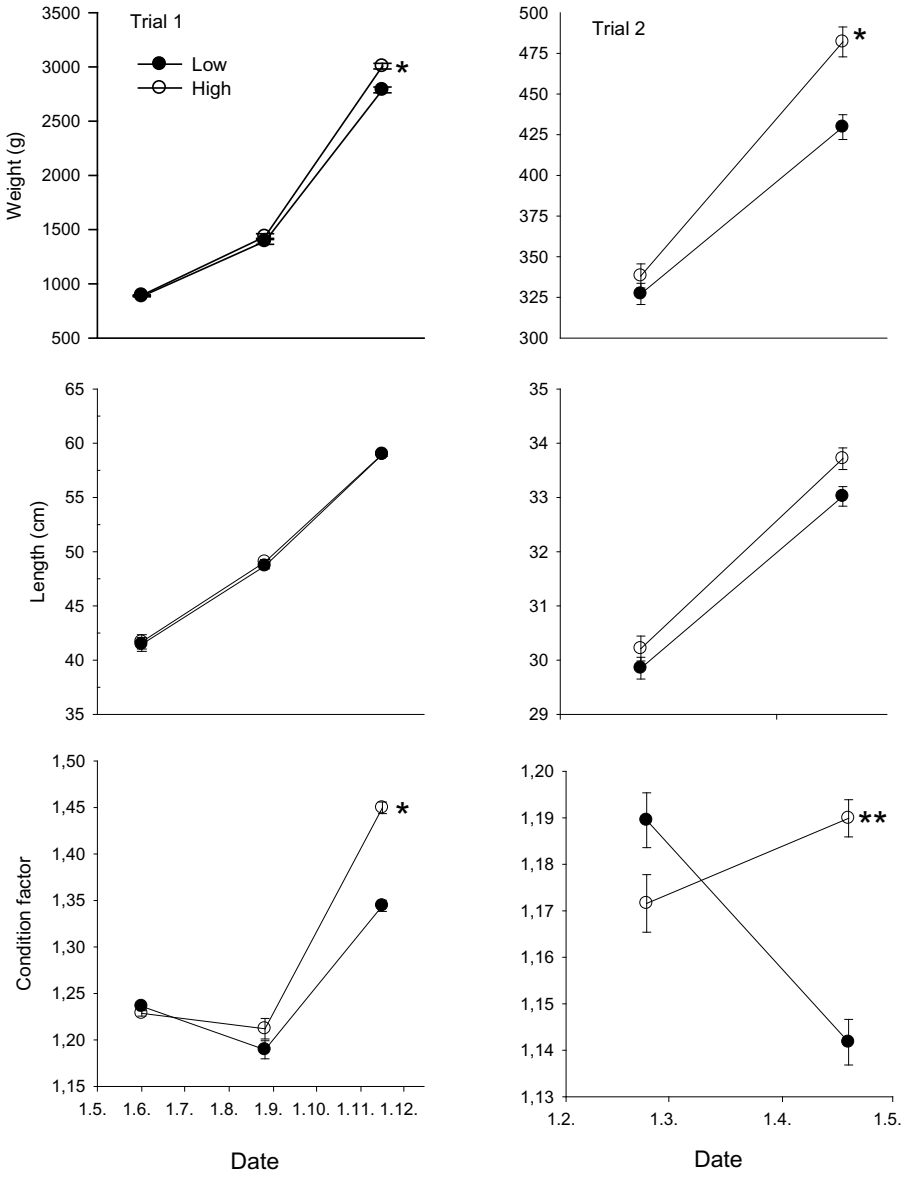

Fig. 3. Mean (SE) weight (g), length (cm) and condition factor in Atlantic salmon exposed for either LOW or MODERATE water velocities in two separate trials (168 days in trial 1, 46 days in trial 2). Significant differences between groups are indicated with: *: $p \leq .05$, **: $p \leq .01$.

Table 2
Number of sampled fish (n), thermal growth coefficient (TGC) and specific growth rate (SGR) in Atlantic salmon exposed for either LOW or MODERATE water velocities in two separate trials (168 days in trial 1, 46 days in trial 2). Data from total samples of fish in both trials, and from an individually tagged subsample of fish (mean and SE) in trial 1.

		LOW			MODERATE		
		n	TGC	SGR	n	TGC	SGR
Trial 1	Total	425	2.56	0.72	438	2.75	0.76
	Tagged	52		0.63 (0.13)	68		0.67 (0.10)
Trial 2	Total	449	2.02	0.60	427	2.68	0.77

expected growth rates (2.7–3.0) in commercial closed cages (Thorarensen and Farrell, 2011; Nilsen et al., 2017a). These moderate growth rates could be caused by stress from handling procedures prior to the experiment and the environmental change caused by transfer to smaller research cages. Longer periods of acclimatisation could have improved the growth during the trials. Growth, measured as mean body weight and CF, stagnated for both groups at mid-sampling in trial 1. In trial 2, the condition factor in the LOW group was even reduced from 1.19 to 1.14 during a trial period of 46 days. Furthermore, TGC of 2.02 in the LOW group in trial 2 indicates suppressed growth in this group. In trial 1, two cages were excluded, and the study's statistical strength reduced. Nevertheless, the group differences in weight and condition factor, after adjusting for cage effects, were significant in both trials. The differences in growth rates between the LOW and MODERATE groups of tagged fish and total population in trial 1 were similar to the total group data, supporting the overall results. The reduced SGR in the tagged fish compared to the whole group could have been caused by adverse reactions to the tagging.

The increased growth is principally muscle growth, as the salmon in the MODERATE group in both trials had increased weight, but no or

Table 3
Number of sampled fish (n), mean (SD) relative heart size (RHS), hepatosomatic index (HSI), slaughter yield (% of round body weight), fillet yield (% of round body weight, only trial 1), fillet content of protein, fat and water (% of total fillet weight) and activity of cathepsin enzymes (cathepsin B, B + L and H, only trial 1, measured as mmol AMC/min/g) in muscle tissue in Atlantic salmon exposed to either LOW or MODERATE water velocity (168 days in trial 1, 46 days in trial 2).

Trial	Parameter	Start			LOW			MODERATE		
		n	Mean	SD	n	Mean	SD	n	Mean	SD
1	Relative heart size (RHS %)	53	0.10	0.02	30	0.11*	0.01	30	0.12*	0.01
	Liver index (HSI %)	53	0.86	0.28	30	0.99**	0.07	30	1.04**	0.06
	Fillet yield (%)	53	66.8	3.2	30	64.1***	1.5	30	66.0***	2.0
	Slaughter yield (%)	53	90.2	1.5	30	87.9	1.2	30	88.1	1.0
	Water (%)	60	69.0	1.2	30	65.0	1.1	30	64.3	1.1
	Fat (%)	60	10.5	1.3	30	15.7	1.6	30	16.2	1.3
	Protein (%)	59	20.2	0.4	30	19.1	0.4	30	18.9	0.4
	Cat B (mmol AMC/min/g)	60	1077.1	192.4	30	898.2**	204.8	30	679.8**	130.4
	Cat B + L (mmol AMC/min/g)	60	562.2	109.8	30	414.2	90.0	30	339.6	80.7
	Cat H (mmol AMC/min/g)	60	340.1	147.4	30	278.9*	125.6	30	181.4*	119.9
2	Relative heart size (RHS %)	59	0.11	0.01	23	0.11	0.02	30	0.11	0.01
	Liver index (HSI %)	58	1.13	0.21	25	1.00	0.11	30	1.08	0.25
	Slaughter yield (%)	58	84.6	2.5	24	88.5	1.4	30	87.3	1.8
	Water (%)	60	68.0	1.2	30	69.6*	1.6	30	68.6*	1.4
	Fat (%)	60	10.9	1.5	30	9.4	1.8	30	10.4	1.8
	Protein (%)	60	19.1	0.8	30	20.5**	0.2	30	20.3**	0.2

Table 4
The effect of MODERATE water velocity compared with LOW water velocity on growth, fish quality and cathepsin enzyme activity (mmol AMC/min/g) analysed with a mixed model ML linear regression. The differences between MODERATE and LOW velocity groups are reported with 95% confidence intervals and p-values.

Trial	Parameter	Effect of MODERATE water velocity			
		Diff.	Low 95% CI	High 95% CI	p-Value
1	Weight (g)	219	79	358	0.012*
	Condition factor (CF)	0.11	0.03	0.18	0.016*
	Relative heart size (%)	0.008	0.001	0.014	0.016*
	Liver index (%)	0.05	0.02	0.08	0.005**
	Fillet yield (%)	1.96	1.06	2.86	< 0.001***
	Cat B (mmol AMC/min/g)	−218.4	−342.1	−94.7	0.008**
	Cat B + L (mmol AMC/min/g)	−77.7	−176.5	21.1	0.094
	Cat H (mmol AMC/min/g)	−97.6	−191.4	−4.3	0.044*
2	Weight (g)	52	12	92	0.019*
	Condition factor (CF)	0.05	0.02	0.08	0.009**
	Water (%)	−0.8	−1.4	−0.1	0.030*
	Protein (%)	−0.16	−0.28	−0.04	0.009**

Table 5
Water velocity as cm/s (mean and SE). Relative water velocity as body lengths per second (BL/s) is reported from the start (BL/s_0) and end (BL/s_1) of each trial. Current velocity was measured at 1 and 2 m depth, during 15.07.15 to 16.11.2015 (trial 1) and 28.02.2017 to 10.04.2017 (trial 2).

	LOW				MODERATE			
	cm/s	SE	BL/s_0	BL/s_1	cm/s	SE	BL/s_0	BL/s_1
Trial 1	6	0.4	0.14	0.10	21	0.7	0.50	0.36
Trial 2	8	0.6	0.27	0.24	19	0.7	0.63	0.57

Table 6
Trial length in days and temperature (°C) mean, maximum and minimum. Trial 1: June to November 2015, trial 2: March to April 2017. In each trial, the temperatures were identical in all cages.

	Days	T (°C)		
		Mean	Min	Max
Trial 1	168	10.9	8.7	14.2
Trial 2	46	7.1	6.2	7.6

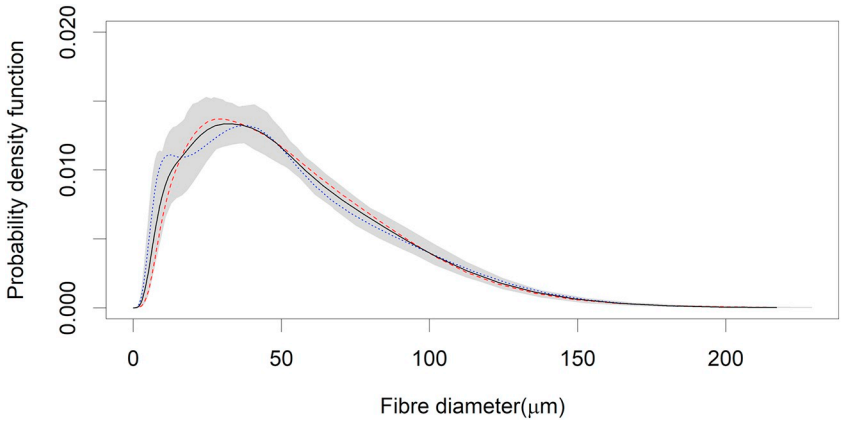

Fig. 4. White muscle fibre distribution in Atlantic salmon exposed for either LOW or MODERATE water velocities (Trial 2). Plot of distribution of fibre size data in μm. Dotted line: LOW group, dashed line: MODERATE group, solid line: total mean and with the 95% probability distribution as the grey area. Samples from 5 fish in each cage, three muscle samples from each fish, a minimum of 150 fibres from each muscle sample.

Table 7
Levels of dissolved oxygen (DO %) in all cages in trial 1 and trial 2; mean, SD, minimum and maximum levels, excluding data from Trial 2, cage L1, in the period from 09.03 to 12.03.

	DO %	LOW			MODERATE		
		L1	L2	L3	M1	M2	M3
Trial 1	Mean	93.8	95.8		94.0	87.6	
	SD	16.2	16.5		9.8	2.7	
	Min	72.3	85.2		82.6	85.3	
	Max	131.7	139.9		113.3	93.2	
Trial 2	Mean	94.7	85.1	82.8	83.1	83.0	83.9
	SD	4.9	4.5	2.3	2.0	2.2	2.7
	Min	82.3	76.7	78.9	79.9	79.4	78.7
	Max	126.6	113.8	106.0	93.6	88.4	143.5

Table 8
SWC = specific water consumption (L/kg/min), FL = feed load (g feed/m^3 water) at start and end of each trial. Density was between 7 and 20 kg/m^3 in trial 1, between 10 and 13 kg/m^3 in trial 2.

	SWC (L/kg/min)		FL (g/m^3)	
	Start	End	Start	End
Trial 1	0.94	0.31	6.1	17.4
Trial 2	0.42	0.31	11.3	15

Table 9
Number of fish (cages) in each group, total accumulated mortality (CM_{total}%) at cage level in LOW and MODERATE groups.

Trial	n	LOW				MODERATE		
		Days	CM_{total}%	Min	Max	CM_{total}%	Min	Max
1	600 (2)	168	7.7	6.0	9.3	6.5	5.0	8.0
2	3600 (3)	46	1.9	1.4	2.9	2.3	1.4	2.8

small differences in length compared to the LOW group. Thus, the condition factor increases with water velocity. This agrees with other studies on Atlantic salmon (Totland et al., 1987; Kiessling et al., 1994; Castro et al., 2011; Solstorm et al., 2015). Histology of muscle in trial 2 showed no clear effect on white fibres. Other studies have shown that higher swimming speed increases the size of white muscle cells (Greer Walker, 1971; Greer Walker and Pull, 1973; Davison and Goldspink, 1977; Davison and Goldspink, 1978). It is possible that the difference in water velocity was too small and the test period too short to produce significant differences between the groups.

4.2. Biometry, fillet chemical composition and cathepsin activity

Increased relative liver and heart size in trial 1 indicates increased metabolism and improved cardiac output in the MODERATE group. This is supported by previous studies on both pre-smolt (Castro et al., 2011) and post-smolt Atlantic salmon (Solstorm et al., 2015). Higher swimming speed is also shown to increase the stroke volume, cardiac output and maximum power output in rainbow trout (Farrell et al., 1991). Increased HSI might reflect an enhanced metabolic activity normally associated with increased nutrient utilisation (Jobling, 1985). The difference in fillet yield in trial 1 is most likely an effect of a larger fish with increased CF.

Differences in body composition (% of water, fat and protein in the fillet) between the two velocity groups were insignificant or of minor biological relevance. Muscle composition in smaller fish is, in several studies, reported to be affected by swimming exercise. Solstorm et al. (2015), found that juvenile salmon (98 g) at fast and moderate water velocity (1.5 and 0.8 BL/s) had lower fat content in the muscle compared with fish at slow velocity. Christiansen et al. (1989) observed that exercise (2.3–1.1 BL/s) was linked to decreased fat content and increased protein content in arctic char fry (1 g). In a study on larger Atlantic salmon post-smolt (1168 g) no differences in body composition of water, protein and fat were found with exercise (0.3–1.06 BL/s) (Grisdale-Helland et al., 2013). As a general observation, experiments with small fish use higher water velocity relative to body size compared to experiments with large fish, and this could be part of the explanation to different results. Studies for longer time periods with more sequential sampling would most likely provide more precise data on the relationship between water velocity, muscle cell recruitment, growth and fillet composition.

In trial 1, the MODERATE group had lower mean cathepsin activity than the LOW group. Significant differences was measured for cathepsin B and H, but not for cathepsin B + L. But with only two cages in each group, the cage effect reduced the strength of the study, and we evaluate the overall cathepsin activity (B, B + L, H) in the muscle tissue as reduced in the MODERATE group. Cathepsin is a large family of proteases that participate in protein degradation in lysosomes and endosomes, as well as in cytosol and the nucleus. Stress could be the cause of increased proteolytic activity, possibly mediated by elevated plasma cortisol (Mommsen et al., 1999). Environmental stress involving high fish density ($\geq$125 kg/m^3) or low water flow ($\leq$0.3 L/kg/min) increases cathepsin activity in salmon (Bahuaud et al., 2010; Sveen et al., 2016). The levels of cathepsin enzymes in the skin and muscle are therefore suggested as a possible stress indicator. If up-regulation of cathepsins indicates an adaptation to environmental stress, the results of this study indicate reduced stress in the MODERATE group.

4.3. Water velocity, swimming behaviour and fish welfare

The MODERATE groups were established with water velocity comparable with water velocity measured in commercial CCS (2780–6000 m^3 volumes, 20–25 cm/s, data not shown). The LOW groups were designed as a reference with approximately one-third of the velocity in the MODERATE groups. In all observations of the MODERATE groups and in a majority of observations of LOW groups, swimming activity was organised as circular schooling with a slow forward advancement. The true swimming speed in most observations for both groups was therefore slightly faster than the measured water velocity, with the exception of occasional bursts of activity connected to eating or rolling/jumping at the surface. The true swimming velocity was difficult to evaluate when the swimming activity in the LOW group broke down to more individual movement patterns. One possible bias is that the fish in the circular research cages could have been exposed to different water velocities. We observed that swimming speed was principally determined by water velocity, and the fish generally avoided the extreme velocities close to inlet or current boosters and close to the cage wall or the centre.

Swimming performance of salmonids is described in many studies. The salmon in this study were exposed to water velocities between 0.1 and 0.67 BL/s, and with an actual swimming speed somewhat faster than the water velocity. Critical swimming speed (U_{crit}) is defined as the maximum swimming speed before the fish reaches exhaustion (Brett, 1964; Tudorache et al., 2007). The U_{crit} of Atlantic salmon (408–491 g, 34.0–36.9 cm) across temperatures from 3 °C to 23 °C was determined by Hvas et al. (2017), with highest U_{crit} at 18 °C (93.1 ± 1.2 cm/s, 2.7 BL/s) and U_{crit} at 8 and 13 °C (the temperatures closest to our trials) of 2.3 and 2.6 BL/s respectively. Optimal swimming speed or U_{opt} is defined as the speed at which the cost of transport (COT) is lowest (Tucker, 1970; Beamish, 1978; Tudorache et al., 2007). A raceway study with post-smolt Atlantic salmon (98.6 g, 22.3 cm, 10 °C) at 0.2, 0.8 and 1.5 BL/s showed reduced performance and welfare at the highest swimming speed and best welfare at 0.8 BL/s (Solstorm et al., 2015). According to other authors the U_{opt} for salmonids is close to 1.0 BL/s (Thorarensen and Farrell, 2011; Drenner et al., 2012). In a study of

brook char Tudorache et al. (2007) defined a new measure for swimming performance, the preferred swimming speed or U_{pref}. For brook char (26.2 ± 0.6 cm, 12.2 ± 0.9 °C) the U_{opt} = 1.02 ± 0.47 BL/s and U_{pref} = 0.78–0.95 ± 0.03 BL/s were closely related, but data also showed that, during much of their time spent in a tilted raceway, the char preferred to swim at speeds ≤0.76 BL/s, and the authors suggest that a study of preferred swimming speed (U_{pref}) could be a way to determine welfare-friendly swimming speeds in aquaculture systems. A larger difference in water velocity between the groups in our trial could probably have increased the differences of some of the outcome variables in this study. Regulation of water flow and water velocity in such circular CCS systems is more complicated than in a raceway. In the LOW group, water velocity sometimes dropped towards levels where the schooling behaviour started to disintegrate. In the MODERATE group, the actual swimming speed was below the reported U_{pref} and U_{opt}, and could have been increased. It was technically difficult to increase the water velocity in this group, and more effort was invested in stabilising the velocities between the cages in each group. When comparing the environment inside netpen cages with CCS, there are several differences. In open sea cages the fish have to adapt to several important environmental factors such as light, oxygen levels and access to feed in a situation with temperature differences in the vertical water column and regular fluctuations of water velocity (Oppedal et al., 2011, Johansson et al., 2014). This forces the fish to adopt multiple behavioural trade-offs. In CCS the temperature gradient inside the cage is generally negligible (Nilsen et al., 2017a), and oxygen levels are controlled automatically through the built-in oxygenation systems. With stabilised temperatures and oxygen levels within the cage, water velocity could be adjusted to fish size, fish health and seasonal fluctuation of water temperatures. Spatial differences in water velocities inside the CCS could also be possible to utilise in order to provide an environment suitable for different individual behavioural needs or coping styles. In commercial scale CCS it will be necessary to determine the variation of water velocities and swimming speeds throughout the whole cage volume, how fish respond to this variation and how this again affects welfare, growth, muscle development and chemical composition.

In both trials it was necessary to transfer fish from the large cages to the research cages, but probably with a negative effect on the growth rates in both groups. The change of environment and more restricted volume could have a negative impact on behaviour and feed intake in both groups. Another factor contributing to the reduced growth in the LOW groups could be increased stress (Solstorm et al., 2015), possibly mediated by an increase in negative social interaction between the fish when water velocities are too low to support a stable, circular schooling behaviour. There were no differences in total mortality or mortality causes between the LOW and MODERATE groups. To transfer fish to the research cages, it was necessary to use netting and handling with the accompanying stress and risk of injuries such as loss of scales, even with precautions to minimise the negative impact of the research procedures. This could also have been the main cause of ulcer-related mortality in both trials. A trial with salmon of harvest size (4.8 kg, 67.3 cm) showed that exercise (35 to 70 cm/s) for periods between 1.5 and 12 h accelerated the recovery after crowding stress, compared to fish exposed to 0 cm/s (Veiseth et al., 2006). Applying higher water velocity to our closed cages during the acclimatisation period could have reduced the immediate stressful effect of handling and sampling.

The water quality throughout the study was good, and should not represent any risk of reduced growth performance or fish welfare. Long-term exposure to levels of CO_2 above 10–15 mg/L has been shown to cause reduced growth rate, increased feed conversion ratio (FCR) and nephrocalcinosis (Thorarensen and Farrell, 2011, Fivelstad et al., 2018). Recommended maximum concentration of CO_2 is 10 to 15 mg/L (Thorarensen and Farrell, 2011; Fivelstad et al., 2003; Fivelstad et al., 2018), for NH_3 0.012 to 0.025 mg/L (Fivelstad et al., 1995; Knoph and Thorud, 1995). For suspended solids, suggested maximum levels for long-term exposure are from 15 mg/L (Chen et al., 1993) to 80–100 mg/L (Wedemeyer, 1996). The trials were also designed to balance biomass, feed and water flow without exceeding recommended maximum levels of density, SWC and feed load.

5. Conclusion

Increase of water velocity from 0.1–0.2 BL/s (LOW) to 0.3–0.6 BL/s (MODERATE) enhances growth rates and muscle development in Atlantic salmon (300–3000 g, 7–11 °C). The main effects in two trials of 168 (trial 1) and 46 days' (trial 2) duration are increased body weight and condition factor (both trials), increased relative heart and liver size (trial 1), increased fillet yield (trial 1) and reduced levels of cathepsin activity in muscle tissue (trial 1). MODERATE water velocity had little impact on the chemical composition (protein, fat, water) of fillets (both trials) and the size distribution of muscle cells (trial 2). This study shows that a MODERATE water velocity is favourable for growth rates for Atlantic salmon during the entire on-growing period in CCS (300–3000 g). Indications of an effect on a broader range of metabolic variables and welfare indicators were also documented. These results should be tested with studies on a commercial scale CCS for longer periods (≥120 days), with more detailed sequential sampling procedures. Individual tagging of fish and parallel studies of fish behaviour and preferences would also add valuable information when interpreting such growth studies.

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