Canadian Journal of Zoology

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Journal:	<i>Canadian Journal of Zoology</i>
Manuscript ID	cjz-2020-0288.R2
Manuscript Type:	Article
Date Submitted by the Author:	21-May-2021
Complete List of Authors:	Fang, Yuanchang; The University of British Columbia, Department of Zoology Emerman, Joshua; The University of British Columbia, Department of Zoology Chan, Victor; The University of British Columbia, Department of Zoology Stiller, Kevin; The University of British Columbia, Department of Zoology; Nofima AS Brauner, Colin; The University of British Columbia, Department of Zoology Richards, Jeffrey; The University of British Columbia, Department of Zoology
Is your manuscript invited for consideration in a Special Issue?:	Not applicable (regular submission)
Keyword:	Closed containment aquaculture, precocious maturation, RAS, OSMOREGULATION < Discipline, gonadosomatic index, optimal environmental conditions

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Growth, feed conversion, sexual maturation and cataract formation in coho and Atlantic salmon post-smolts reared at different salinities in recirculating aquaculture systems for over one year

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Abstract

There is an increasing interest in defining optimal conditions to rear salmon to market size in recirculating aquaculture systems (RAS). Salinity however, as a parameter that can be potentially manipulated, has been poorly studied. To address this knowledge gap, we reared coho (*Oncorhynchus kisutch* Walbaum, 1792) and Atlantic (*Salmo salar* Linnaeus, 1758) salmon from smolt to market size over ~460 days at five different salinities (0, 5, 10, 20 and 30 ppt) and examined their growth performance. We found that both species reared at intermediate salinities (5-10 ppt) started to show significantly larger body mass than fish reared either in freshwater or full-strength seawater at around day 250-300. These growth-enhancing effects were maintained until day 460. The higher growth in Atlantic salmon at intermediate salinities was associated with reduced economic feed conversion ratio, but this was not observed in coho salmon. The all-female coho salmon showed no incidence of sexual maturation and negligible cataract formation, while the mixed-sex Atlantic salmon showed high levels of sexual maturation (up to 50%) and presence of cataracts (up to 60%). Our results indicate that all-female coho salmon may be better suited to RAS than Atlantic salmon in some aspects. However, in both species, long-term rearing at intermediate salinities improves growth in RAS.

Keywords

Atlantic salmon (*Salmo salar*), Closed containment aquaculture, coho salmon (*Oncorhynchus kisutch*), precocious maturation, RAS, osmoregulation, gonadosomatic index, optimal environmental conditions

Introduction

The production of seafood from capture fisheries has remained largely unchanged over the past four decades, yet the demand for seafood continues to grow (FAO, 2020). To make up for the deficit in seafood capture, there has been tremendous growth in the aquaculture sector (FAO, 2020). Salmonid aquaculture is a particularly fast-growing sector with a production of ~3.5 million tonnes in 2017, while Atlantic salmon (*Salmo salar* Linnaeus, 1758) are the most widely cultured salmonid with ~2.3 million tonnes farmed in 2017 (FAO, 2019), but there is interest in expanding the number of species used in aquaculture. For example, coho salmon (*Oncorhynchus kisutch* Walbaum, 1792), a native species in British Columbia, Canada (DFO, Salmon Facts) are increasingly being used in aquaculture, with a 2017 global farmed production of 180,000 tonnes (FAO, 2019). Traditional Atlantic salmon aquaculture typically involves rearing salmon in freshwater hatcheries from hatch to smoltification at which point they are transferred to open net pens in marine coastal environments for grow out to market size. In recent years, however, the use of open net pens has been criticized due to some of their potentially negative impacts on coastal environments, farmed to wild fish disease transmission, and escapements (Folke et al. 1994; Naylor et al. 2005; Kutti et al. 2008).

In response to environmental concerns related to open net-pen farming, and in an effort to diversify salmon aquaculture, there has been growing interest in using land-based recirculating aquaculture systems (RAS) for full life-cycle grow out of farmed salmon. The use of land-based RAS for salmon aquaculture allows farms to be placed near urban centers to reduce transportation costs, which eliminates the risk of escapements and farmed-to-wild disease transfer, and it allows the

operators to control environmental conditions to optimize fish growth and welfare. However, land-based RAS facilities have higher construction and operation costs compared with open-net pens, which impacts profitability. In addition, the aquaculture industry has observed high levels of early sexual maturation and eye cataracts in salmon reared in RAS. Sexually mature fish reallocate energy from somatic tissues to reproduction, which subsequently slows growth, impairs feed conversion and deteriorates flesh quality (Good et al. 2016), while cataract formation results in reduced sight or blindness and reduces food consumption in Atlantic salmon (Breck and Sveier 2001; Ersdal et al. 2001). Rearing fish under conditions that reduce sexual maturation and cataracts, but yield optimal growth and welfare is one of the ways to maximize production output in RAS; however, truly optimal conditions have rarely been defined for rearing of fish in RAS (Brauner and Richards, 2020).

Salinity is one of many environmental parameters that can be manipulated in RAS and given that osmoregulation requires the input of energy, there is the potential that differences in rearing salinity could affect growth in salmon. In freshwater, fish gain water by osmosis and passively lose ions by diffusion across gills and skin. To compensate for passive ion movement, freshwater fish excrete large quantities of diluted urine (Duffy et al. 2011), reabsorb ions in the kidney (Nishimura and Fan 2003; Marshall and Grosell 2006), and actively take up ions from the water at gills (Laurent et al. 1985; Perry 1998; McCormick et al. 2009) and from the diet across the intestine (Flik and Verboost 1993; Bucking and Wood 2007). In contrast, in seawater, fish passively gain ions and lose water. To compensate, fish drink seawater (Nobata et al. 2013), excrete excessive ions at the kidney (Duffy et al. 2011) and at gills (McCormick et al. 2009; Hwang et al. 2011), and precipitate divalent cations in the intestine to prevent their absorption (Grosell et al. 2001; Brauner et al. 2013). The metabolic costs associated with osmoregulation in freshwater and seawater are debated, but in

general it is thought that the metabolic costs could be as high as between 10 and 50% of the resting metabolic rate (Bœuf and Payan, 2001), although much lower estimates have been reported (e.g., Christensen et al. (2019) suggested 5% in European Perch (*Perca fluviatilis* Linnaeus, 1758)). In theory, if the metabolic costs of osmoregulation are high, then fish reared under conditions where the salinity is iso-osmotic with internal fluids (~10 ppt) would have lower osmotic gradients and lower metabolic costs associated with osmoregulation. Under these conditions, the alleviated metabolic scope could be allocated to other physiological processes including growth (Brauner and Richards, 2020). In line with this prediction, previous studies have observed improved growth rates in some fish species reared at intermediate salinities (Otto 1971; Lambert et al. 1994; Gaumet et al. 1995). It remains unknown however, whether salinity can influence growth of post-smolt salmon during prolonged growout from smolt to market size in RAS, which is of interest to the aquaculture industry.

The goal of this study was to determine the effects of salinity on growth, feed conversion, incidence of early maturation and cataracts in coho and Atlantic salmon reared in RAS from smolt to market-sized adults. Smolts of two species were reared continuously at salinities of 0, 5, 10, 20 and 30 ppt for up to 460 days in RAS. Body mass was measured every ~3 months to evaluate growth of fish and the amount of feed provided was recorded daily for the calculation of economic feed conversion ratio (eFCR), while sexual maturation and cataract formation were determined near the end of the growth trial.

Materials and Methods

Recirculating Aquaculture Systems

The growth trials were conducted in the *Initiative for the Study of the Environment and its Aquatic systems (InSEAS)* at The University of British Columbia (UBC), Vancouver, BC, Canada. In this study, we used five 15,000 L RAS systems, each equipped with two 5 m³ tanks. Each system is equipped with a radial flow separator and a micro-bead filter to remove solid waste, and a foam fractionator to remove suspended organic compounds. Each RAS is also equipped with a degasser for carbon dioxide removal, two bio-filters for nitrification and denitrification, an oxygenator, a pH controller for stabilizing pH *via* sodium bicarbonate (NaHCO₃) delivery, an ultraviolet (UV) sterilizer, and a heat exchanger for regulating temperature. In each RAS, oxygen tension, pH and salinity are measured and monitored continuously by probes and recorded on a data acquisition computer. Suspended above each 5 m³ tank in each RAS is a T Drum 2,000 programmable feeder (Arvo-Tec, Finland) controlled by the Arvo-Tec Professional Feeding Control System (Arvo-Tec, Finland). Tank illumination is provided by fluorescent lighting on the ceiling of the fish rooms.

Experiment 1 - System Validation

In order to determine if fish growth characteristics were similar across the replicate tanks of our independent RAS, we reared coho salmon in our RAS under the same environmental conditions and monitored growth. Approximately 3,800 female coho salmon smolts were obtained from Target Marine (Sechelt, BC, Canada), transported to the *InSEAS* facility at UBC, and temporarily held in two 10,000 L flow-through freshwater tanks for recovery. All fish were then anesthetized in an aqueous solution of 0.1 g L⁻¹ tricaine methanesulfonate (MS222) buffered with 0.2 g L⁻¹

NaHCO₃, measured for body mass, and then placed in well-oxygenated freshwater for recovery. After fish had fully recovered, they were randomly and evenly distributed into five RAS, each with two 5 m³ fish tanks (ten 5 m³ tanks in total). Fish were reared in recirculated freshwater and under 24 h of light, while water temperature, pH and oxygen tensions were maintained at around 9.5 °C, 6.5 and >82% air saturation, respectively. Water ammonia, nitrite (NO₂-N) and nitrate (NO₃-N) levels were measured at least twice a week and were maintained below 6, 4 and 160 ppm, respectively, by adding freshwater when the levels were too high. Feed (Skretting Canada West, BC, Canada) was delivered by the automatic feeders and gradually increased until fish were fed to satiation, which was determined by monitoring for uneaten feed on the bottom of fish tanks and in the radial flow separators. During this time feeding rates in all tanks were similar. Fish were maintained under these conditions for 150 days, at which point all fish were terminally sampled by euthanizing with an overdose of MS222 (0.2 g L⁻¹ MS222 buffered with 0.4 g L⁻¹ NaHCO₃) and were measured for body mass.

Experiment 2 - Salinity Growth Trial

In order to study the effects of salinity on growth of salmon, we then reared coho and Atlantic salmon at different salinities in RAS from smolt through to market-sized adults, and the overall experimental design is shown in Figure 1. Approximately 3,000 female coho salmon smolts were obtained from Target Marine (Sechelt, BC, Canada) and held in two 10,000 L flow-through freshwater tanks temporarily for recovery from transport. All fish were then randomly and evenly distributed into 5 independent RAS. Each RAS had two 5 m³ fish tanks and coho salmon were placed into one of the two tanks in each RAS. The salinity of each RAS was gradually increased

to one of 0, 5, 10, 20 and 30 ppt within a week by adding sea salt (Instant Ocean® Sea Salt, Blacksburg, VA) into the systems, and once the final salinity was achieved it was maintained at that level throughout the trial.

Fish were reared under 24 h of light (a condition commonly used in industry) while water temperature, pH and oxygen tensions were maintained at around 12.5 °C, 6.7 and >90% air saturation, respectively. Within each RAS, about 200-400 L of water per day was added to replace water lost due to micro-bead filter back-flushing which was associated with the appropriate addition of sea salt to maintain target salinity. Water ammonia, NO₂-N and NO₃-N levels were measured at least twice a week and maintained below 6, 4 and 160 ppm, respectively, by adding freshwater when the levels were too high. Initially for the first 6 months, fish were fed (Skretting feed) a maintenance diet of 0.2% of body mass per day, in order to keep ammonia levels low as denitrifying bacteria were populating the biological filters.

Once the biological filters began to efficiently remove nitrogenous wastes, these coho salmon were fed to satiation which was determined by monitoring the tank bottom and the radial flow separator for uneaten feed. At this time, 100 coho from each tank were randomly selected, anesthetized with a buffered solution of MS222 and measured for fork length and body mass. Fish were then transferred to a temporary holding tank (100 L) of well-oxygenated water at their respective rearing salinities for recovery, after which they were returned to their rearing tanks. This first measurement is referred to as day 0 for the growth trial, as this refers to the time that feeding rates were increased to satiation (about 2% body mass per day), but it is important to note that the coho salmon had already been in their salinity treatments at a lower ration for 6 months. Beyond day 0, fork length

and body mass were measured every two to three months, on days 59, 156, 257, 355 and 460 of the growth trial. Around day 156, fish feed was switched from Skretting to Taplow (BC, Canada) in all tanks. Our RAS systems are semi-commercial scale, so the commercial fish stocking density of $>100 \text{ kg m}^{-3}$ exceeds the capacities of our waste processing units. For the normal operation of the systems and the health of fish, we tried to maintain the stocking density at $\sim 40 \text{ kg m}^{-3}$ which based on our prior knowledge, was the maximum stocking density where desired water quality parameters could be ensured. During the entire growth trial, stocking density was calculated after each sampling. If the stocking density exceeded 40 kg m^{-3} , fish were randomly selected and euthanized with an overdose of MS222 in order to reduce the stocking density back to 40 kg m^{-3} . Fish were also periodically removed from tanks and used in other experiments, but the amount of fish used was small relative to the total fish number, which posed a minimal effect on the stocking density.

At around day 30 for coho salmon, $\sim 3,000$ Atlantic salmon smolts were obtained from Omega Pacific Hatchery (Great Central Lake, Port Alberni, BC, Canada), held in two large flow-through freshwater tanks and treated with formalin to prevent *Saprolegnia* spp. infections. A month later (day 59 for coho salmon), 200 Atlantic salmon from each of the two flow-through tanks were randomly selected, anesthetized and fork length and body mass measured as described above. These data are referred to as day 0 for Atlantic salmon. The 200 sampled fish, along with the rest of the Atlantic salmon held in the flow-through freshwater tanks, were then randomly distributed into the second 5 m^3 tank (600 fish per tank) in each of the 5 RAS systems (the first tank contained coho salmon). Atlantic salmon were transferred directly into the respective salinity of each RAS and were fed to satiation (about 2% body mass per day) by the automatic feeders as described

above. Fork length and body mass were measured every two to three months thereafter, on days 96, 197, 295 and 405. Similar to coho salmon, the stocking density of Atlantic salmon was maintained at around 40 kg m^{-3} through culling following each sampling period. Similarly, fish were also periodically removed from tanks and used in other experiments, but the amount of fish used was small relative to the total fish number, which posed a minimal effect on the stocking density.

To measure growth rate over time in individual fish, pit tags (Biomark HPT12, ID, USA) were implanted in ~100 coho salmon in each tank on day 156 and ~100 Atlantic salmon in each tank on day 96. The pit tag was inserted into the body cavity posterior to the tips of the pectoral fins. Fish tag IDs were scanned by a reader (Biomark 601 Handheld Reader, ID, USA) and corresponding fork length and body mass of tagged fish were recorded in a computer program (Tag Manager Software, Biomark, ID, USA). At later sampling times for measurements of fork length and body mass, all randomly sampled fish were scanned for the presence of tags. When tags were found, the length and mass of the tagged fish were recorded in the program and used to calculate individual growth rates.

For coho salmon at day 355 and 460 and Atlantic salmon at day 295 and 405, sexual maturation and cataracts were identified and quantified. Sexual maturation was defined as the presence of a dark brown skin color, hooked jaw, and a soft belly, while cataracts were defined as the presence of white precipitate in the eyes. Also, at the conclusion of the study, up to 50 fish (or all fish in the tank if there were less than 50) in each salinity treatment were terminally sampled and measured for body mass and gonad mass to calculate gonadosomatic index (GSI).

Ethics approval

All the experimental protocols involved with animals were approved by Animal Care Committee at the University of British Columbia (certificate number: A17-0011).

Calculations

Growth rate (GR) was calculated as:

$$GR (g \text{ day}^{-1}) = \frac{Mass_2 (g) - Mass_1 (g)}{Time_2 (day) - Time_1 (day)}$$

where $Mass_1$ and $Mass_2$ are the mean body mass (or body mass of individual tagged fish) from sampling $Time_1$ and $Time_2$, respectively.

In order to understand the growth pattern and health of the fish, condition factor was calculated for each fish sampled:

$$Condition \ Factor = \frac{Mass (g)}{Fork \ Length (mm)^3} \times 10^5$$

Economic feed conversion ratio (eFCR) was calculated to estimate the efficiency of feed being converted into fish biomass, which accounts for biomass gain in mortalities to avoid overestimation:

$$eFCR = \frac{Total \ Feed \ Fed (kg)}{Total \ Live \ Biomass \ Gained (kg) + Total \ Mortality \ Biomass \ Gained (kg)}$$

Feeding amount and mortalities were recorded daily.

Gonadosomatic index (GSI) was calculated as:

$$GSI = \frac{\text{Gonad Mass (g)}}{\text{Body Mass (g)}} \times 100\%$$

Statistical Analysis

All statistical analysis was conducted in R (v 3.6.1). Kruskal-Wallis one-way analysis of variance (ANOVA) was used to determine whether the levels of ammonia, NO₂-N, NO₃-N, pH, oxygen tension, temperature and salinity differed between the RAS as these data were not normally distributed despite attempt of transformation. For the analysis of growth, each fish was considered to be a single replicate even though they were all sampled from the same tank. No statistical analysis could be conducted for whole-tank GR and eFCR because their calculations were based on the difference between two means for the population sampled from a given tank (i.e., $n=1$). Two-way ANOVA was conducted to examine the effects of salinity (or tank ID in the system validation trial) and exposure time on body mass, condition factor, GSI and pit tagged fish GR. If significant differences were detected, Tukey post hoc multiple comparison was conducted to reveal where the significant differences occurred. An α of 0.05 was used for the threshold of significant difference for all the analysis.

Results

Experiment 1 - System Validation

In order to determine if fish grow at similar rates in our different RAS when held under identical conditions, we reared coho salmon in freshwater in both 5 m³ tanks of 5 of the 7 RAS for 5 months. Although we attempted to maintain similar water chemistry across all RAS, there was statistically significant variation in water quality between systems, including ammonia, pH, oxygen tension and temperature (Table 1); however, the magnitude of variation was small and unlikely to have measurable biological effects. A failure in one system (RAS 2) early in the freshwater growth trial resulted in mortality, therefore this system was removed from the analysis (Table 2). At the start of the growth trial, the coho salmon in each RAS tank were all of similar size ($P=0.105$). The coho salmon grew well in all RAS tanks over the 5-month growth period and there were no significant differences in the final mean body mass between tanks ($P=0.529$). As a result, we conclude that when fish are reared under similar conditions in our RAS, they show similar growth rates.

Experiment 2 - Salinity Growth Trial

Coho Salmon

Growth

In coho salmon, a two-way ANOVA revealed significant effects of salinity ($P<0.001$) and time ($P<0.001$) on body mass, with a significant interaction between factors ($P=0.002$) (Figure 2). There

was no significant effect of salinity on body mass at day 0, 59 or 156. At day 257 however, coho reared at 10 ppt were significantly larger than those reared at 0 and 30 ppt, with those reared at 5 and 20 ppt showing intermediate sizes. At day 355, coho reared at 10 ppt continued to have the highest mean body mass, but at this sampling time, these fish were only significantly larger than those reared at 0 ppt. At day 460, fish at 10 ppt were the largest and were significantly larger than those reared at 0 and 20 ppt. The 30 ppt treatment was terminated before sampling on day 460 due to a system failure.

There were significant effects of salinity ($P=0.019$) and time ($P<0.001$) on condition factor, with a significant interaction term ($P<0.001$) (Table 3). Condition factor in general increased over time, but varied across salinities at different sampling time points. Calculated growth rates (GR) were generally higher in coho salmon reared at 10 ppt compared with other salinities during the day 0 to 59 and day 156 to 257 sampling periods, whereas during the other sampling periods there were no obvious and consistent differences between salinity treatments. Of the fish that were recaptured at every sampling time point, two-way ANOVA revealed significant effects of salinity ($P=0.001$) and time ($P=0.002$) on GR, with no significant interaction ($P=0.064$). In general, GR of these fish increased between day 156 and 460, with relatively higher GR at intermediate salinities, which is in agreement with changes in body mass and calculated growth rate at the whole-tank level. Over the first 156 days, there were no clear effects of salinity on economic feed conversion ratio (eFCR), but from day 156 onward, eFCR appeared to be higher in coho reared at 10 ppt compared with other salinities.

Sexual maturation and cataracts

There was no evidence of sexual maturation in coho salmon at day 355 and 460 and there were no differences in GSI between the salinities at day 460 (one-way ANOVA; $P=0.083$) (Table 4). Very few cataracts (<10%) were observed in coho salmon reared at 0 and 20 ppt at day 355 and day 460, and the rest of salinities did not show any sign of cataract incidences near the end of growth trial.

Atlantic Salmon

Growth

In Atlantic salmon, two-way ANOVA revealed significant effects of salinity ($P<0.001$) and time ($P<0.001$) on body mass, with a significant interaction between the two factors ($P<0.001$) (Figure 3). Post-hoc analysis revealed that there were no differences in body mass between salinities at day 96 and 197; however, at day 295, fish reared at 5 and 10 ppt were significantly larger than those at 0 ppt, while fish reared at 20 and 30 ppt were intermediate. At day 400, fish reared at 5 ppt were significantly larger than all other salinities, while no difference was found in body mass of fish between 10 and 20 ppt, and fish in these two treatments were significantly larger than 0 and 30 ppt.

There were significant effects of salinity ($P<0.001$) and time ($P<0.001$) on condition factor, with a significant interaction term ($P<0.001$) (Table 5). Condition factor in general increased over time, but varied across salinities at different sampling time points. Calculated GR of Atlantic salmon

was highest at 10 ppt from day 197 to 295 and at 5 ppt from day 295 to 400. Similarly, in an attempt to examine GR on individual Atlantic salmon, we pit tagged ~100 of the total ~500 Atlantic salmon in each tank at day 96. Of the fish that were recaptured at each time point, two-way ANOVA revealed significant effects of salinity and time on GR, with significant interaction terms (all $P < 0.001$). From day 96 to 295, no statistically significant differences were found in tagged fish GR between salinities within each sampling time; however, from day 295 to 400, this measurement was significantly higher at 5 ppt relative to other salinities, and lowest at 0 and 30 ppt, which showed a very similar trend as the calculated GR. Fish reared at 0 ppt always had the highest eFCR at all sampling points between day 96 and 400. Accordingly, from day 197 to 295, eFCR was lowest at 10 ppt, while from day 295 to 400, eFCR was lowest at 5 ppt.

Sexual maturation and cataracts

At day 295, the rates of sexual maturation in Atlantic salmon reared at 0 and 30 ppt were about twice those of fish reared at 5 and 10 ppt (Table 6). At day 400, around 50% of Atlantic salmon were sexually mature at 0, 10 and 20 ppt, while <30% of the fish were mature at 5 and 30 ppt. Salinity significantly influenced GSI of fish at day 400 (one-way ANOVA; $P < 0.001$) when an increase (up to 60%) was seen in GSI at 0, 10 and 20 ppt, compared with 5 and 30 ppt, consistent with sexual maturation observations. At day 295 and 400, there were 30% to 60% of fish showing cataracts at 5, 10 and 20 ppt, while <15% of fish showed cataracts at 0 and 30 ppt.

Discussion

The current study clearly demonstrates that both coho and Atlantic salmon can be grown in RAS from smolt through to market-sized adults and that rearing environment can significantly affect growth rates in both species. Indeed, when rearing environment is similar across replicate RAS, coho salmon showed similar growth rates. In response to changes in salinity, we found that both coho and Atlantic salmon grew well, but around day 200 to 300 the fish reared at intermediate salinities (5-10 ppt) were generally larger than fish reared at other salinities including freshwater and full-strength seawater. There were negligible levels of sexual maturation or cataracts observed in coho salmon in all treatments, while up to 50% of the Atlantic salmon sexually matured at 0, 10 and 20 ppt and up to 60% displayed cataracts at 5, 10 and 20 ppt.

Experiment 1 - System Validation

In order to demonstrate that there were no systematic effects of tank and RAS on growth, we reared fish under relatively consistent conditions across two tanks in several of our RAS systems and monitored growth. As expected, we found that there were no differences in growth of coho salmon from different tanks and systems. There were small, but significant differences in water chemistry between RAS (Table 1), but all the variation was within what is deemed as acceptable levels for farmed fish: unionized ammonia ($\leq 0.012\text{--}0.025\text{ mg L}^{-1}$), $\text{NO}_2\text{-N}$ ($< 0.1\text{ mg L}^{-1}$), $\text{NO}_3\text{-N}$ ($< 1\text{--}400\text{ mg L}^{-1}$) and oxygen tension (80-100%) (Berg et al. 1993; Fivelstad et al. 1995; Wedemeyer 1996; Lygren et al. 2000; Bergheim et al. 2006; Thorarensen and Farrell 2011; Tidwell 2012). As a result, the slight variation in water chemistry is unlikely to be of biological significance. Overall, the validation study demonstrates that fish grow equally well across our RAS when water conditions are similar, which then affords us the opportunity to intentionally manipulate important aspects of

environmental condition (i.e., salinity) and examine the effects on growth. Due to the laboratory limitation of 5 RAS, replication of a given treatment is very limited; however, we have demonstrated that our systems introduced no systematic bias, so by selecting a gradient of environmental conditions (e.g., a range of salinities from freshwater to full-strength seawater), we can evaluate the effects of the environment on fish growth by using regression-style and curve fitting approaches, which further improves our ability to draw meaningful conclusions of optimal environmental conditions for growth in RAS where there is limited opportunity for replication. Further replicated studies can then be done on specific levels of a given parameter to permit a more quantitative estimate of the effect of that parameter on growth. Bhujel (2008) also indicates high constructional costs associated with using aquaculture systems to conduct research, and suggests that individual fish can be tagged within a tank and used as replicates for research purposes. In the Experiment 2 of our study, a number of fish were pit-tagged for this reason to permit statistical analysis of growth rates which were compared with whole-tank measurements.

Experiment 2 - Salinity Growth Trial

The growth rates of both species increased from ~ 1 to 10 g day^{-1} over the 400-day growth trial. These growth rates are lower than the reported values in the salmon aquaculture industry (Thorarensen and Farrell 2011). This is in part due to a lower feeding rate of 2% body mass per day used in this study compared to a feeding rate of $>3\%$ in the industry, as well as tank effects due to our relatively small tank size (5m^3) relative to industry sized tanks. Although the absolute growth rates are lower than those often observed in the industry, the relative differences among treatments are informative. In theory, fish reared at intermediate salinities (~ 10 ppt) should have

lower costs of osmoregulation due to the decreased osmotic gradients between the environment and blood. This theory is supported by Morgan and Iwama (1998) who found that gill Na^+ , K^+ -ATPase activity from coho salmon smolts reared at 10 ppt was significantly lower than coho salmon reared at 0 and 28 ppt. If the lower gill Na^+ , K^+ -ATPase activity measured at intermediate salinities equates to an overall reduction in the costs of osmoregulation, these lower costs should alleviate metabolic scope that could then be re-allocated toward other physiological functions including greater somatic growth (Brauner and Richards, 2020). Indeed, our growth data in both coho and Atlantic salmon are consistent with this hypothesis and both species attained a larger size at intermediate salinities (5-10 ppt) compared with those reared in freshwater or full-strength seawater. In addition, subsequent studies in coho and Atlantic salmon suggest that there were no effects of salinity on other performance variables including metabolic rate, hypoxia tolerance, swimming performance (Fang et al. 2019), or thermal tolerance (Hines et al. 2019). However, in coho salmon, osmoregulation appeared to be most challenged at 30 relative to 2.5, 5 and 10 ppt (Damsgaard et al. 2020). Although both species showed higher growth at intermediate salinities in the present study, the timing of the effects differed between species. In coho salmon, we observed significantly higher body mass at 10 ppt at day 257, but the calculated GR of this group was already higher than other salinities as early as day 59. These data suggest that growth in coho salmon reared at 10 ppt was enhanced immediately after the onset of the trial and that the early effects of salinity on growth were carried forward throughout the entire growth trial. In Atlantic salmon, we first observed a larger body mass at intermediate salinities at day 295, which corresponded to timeframe where calculated GR and GR of tagged fish was higher. In contrast to coho salmon however, the largest increase in body mass in Atlantic salmon was observed at 5 ppt (rather than 10 ppt) late in the study period (between day 295 and 400).

The growth-enhancing effects of intermediate salinities observed in the present study is in general agreement with the results of Otto (1971) who conducted an 8-month growth trial on coho salmon fry at salinities ranging from 0 to 25 ppt and found that fish reared at 5 to 10 ppt had the highest growth rate. Lambert et al. (1994) similarly reported that Atlantic cod (*Gadus morhua* Linnaeus, 1758) reared at ~14 ppt had up to a 63% higher specific growth rate than cod reared at 28 ppt. Juvenile turbot (*Scophthalmus maximus* Linnaeus, 1758) were also found to have higher growth rates at intermediate salinities between 10 and 19 ppt, compared with full-strength seawater (Gaumet et al. 1995). In contrast, other studies suggest that fish reared at intermediate salinities do not always have the highest growth rate. In particular, studies on rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) (McKay and Gjerde 1985; Morgan and Iwama 1991), steelhead trout (*Oncorhynchus mykiss* Walbaum, 1792; Morgan and Iwama 1991) and Abant trout (*Salmo trutta abanticus* Tortonese, 1954; Kocabas et al. 2011) revealed that these species generally have the highest growth rate in freshwater and that growth rate decreased with increasing salinity up to seawater. Furthermore, Arnesen et al. (1993) found no effect of salinity (10-35 ppt) on the growth of Arctic charr (*Salvelinus alpinus* Linnaeus, 1758). McCormick et al. (1989) investigated the interactive effects of salinity (0, 10 and 30 ppt) and feeding ration on the growth of Atlantic salmon post-smolts (23-70 g) over 6 weeks and they found that salinity had no effect on growth when feeding ration was below 3.1% body mass per day, but as ration was increased to 5.1% body mass per day, fish reared at 10 ppt had the lowest growth. Collectively, these data imply that there may not be a consistent effect of salinity on growth in fish and salinity might interact with other factors, such as species, feeding ration, and life stage. In addition, the length of the growth trials differs across studies (ranging from 20 to 240 days) and our study is the longest duration at over 400 days.

Indeed, for Atlantic salmon, we would not have observed an impact of salinity had we chosen any rearing duration of less than 200 days post-smolt. Therefore, by employing a long and industry relevant duration for our growth trials, we have revealed that salinity is an important environmental parameter that can enhance growth in both coho and Atlantic salmon.

Several studies have suggested that higher growth of fish at intermediate salinities may be due to increased feed conversion efficiency (decreased eFCR). Indeed, Gracia-López et al. (2006) demonstrated in common snook (*Centropomus undecimalis* Bloch, 1792) that rearing at an iso-osmotic salinity resulted in a low apparent heat increment of feeding, which translates into lower overall cost of digestion. Thus, with the same amount of energy, fish at intermediate salinities are able to process more food, which could eventually result in faster growth. Similarly, Tsuzuki et al. (2007) reared juvenile fat snook (*Centropomus parallelus* Poey, 1860) at 5, 15 and 35 ppt, and found that fish reared at 15 ppt had the lowest FCR which was associated with the highest gut alkaline proteinase and amylase activities, indicating that intermediate salinities may improve feed digestion by increasing digestive enzyme activities. Indeed, our data in Atlantic salmon are consistent with these previously published reports whereby the large changes in body mass observed at 10 ppt between day 197 to 295 and at 5 ppt between day 295 to 400 were associated with lower eFCR. In contrast, the higher growth rates observed in coho salmon at 10 ppt were not directly associated with a lower eFCR, which suggests that the higher growth at 10 ppt was not induced *via* an increase in feed conversion. In general, our study indicates that the effect of salinity on feed conversion may be species-specific in salmon, and salinity might influence other factors that affect growth.

Precocious maturation and cataracts

Precocious sexual maturation in salmon is undesirable in aquaculture, as the high metabolic costs associated with gonadal development result in impaired somatic growth, reduced flesh quality, and changes in coloration (Good et al. 2016). Higher rates of precocious maturation, particularly in males, have been reported in Atlantic salmon reared in RAS. For example, Berg et al. (1996) and Summerfelt et al. (2013) observed up to 25% and 80% sexual maturation of ~3 and 4 kg male Atlantic salmon reared in closed RAS, respectively. The cause of the early maturation is unknown, but it has been suggested that the accumulation of excreted sex hormones in RAS may be an underlying cause (Good et al. 2014). In our study, we did not observe precocious maturation in coho salmon, but in Atlantic salmon we observed very high rates of maturation rates (pp to 50%), which were associated with high GSI. The coho salmon used in the current study were an all-female strain, while our Atlantic salmon were from a mix sexed population (male:female = 1:1) and indeed, we found that most of the mature Atlantic salmon were male. The current aquaculture industry has widely adopted all-female strain salmon in production, so our study again validates the feasibility of using female salmon to reduce precocious sexual maturation, of obvious benefit to the industry. At the end of our study, Atlantic salmon reared at 5 and 30 ppt had the lowest incidence of sexual maturation, and fish reared at these two groups had the highest and lowest body mass, respectively. Fish reared at 5 ppt may allocate spare energy to somatic growth, while fish reared at 30 ppt may consume a large amount of energy on osmoregulation, both of which could potentially leave little energy for gonadal development. The effects of salinity on precocious maturation in Atlantic salmon are novel and warrant further investigation as this is a major issue facing the wide-spread adoption of closed-containment RAS for rearing Atlantic salmon.

Cataract formation in the eyes is another serious issue in aquaculture production, as cataracts can lead to visual disturbance and blindness, which in turn reduces food consumption and growth (Breck and Sveier 2001; Ersdal et al. 2001). We observed nearly no cataracts in coho salmon at any salinity. This finding contrasts with a study in coho salmon smolts (Iwata et al. 1987) where incidence of cataracts was low in freshwater but increased following transfer to seawater. The incidence of cataracts was further elevated in smolts that were less able to regulate the osmolality of their plasma and aqueous humor (presumably the basis of the cataracts). Two studies conducted by Breck and Sveier (2001) and Ersdal et al. (2001) found a negative association between the degree of cataract formation and growth rates in Atlantic salmon. In the current study, however Atlantic salmon reared at 5, 10 and 20 ppt had relatively higher incidences of cataract formation (30-60%) compared with freshwater and seawater (0-15%), but these were the salinities that yielded higher growth. It is also worth noting that although coho and Atlantic salmon were reared in separate tanks, these were housed within the same RAS, and thus the two species were exposed to identical water quality. Thus, the differences in the incidence of cataracts may be species specific.

Conclusions

Our long-term study of the effects of salinity on the growth of coho and Atlantic salmon in RAS demonstrates that fish reared at intermediate salinities (5-10 ppt) generally grew to a larger body mass than fish reared in freshwater or seawater, although there were species-specific differences

in the timing at which higher growth rates were observed. Furthermore, we demonstrated that the higher growth at intermediate salinities was associated with improved feed conversion in Atlantic salmon, but not in coho salmon. Thus, the growth-enhancing effects of intermediate salinities in coho salmon might be caused by other factors that remain to be investigated. We also demonstrated that our all-female strain of coho salmon had a negligible incidence of precocious maturation and cataracts, compared with our mixed-sex population of Atlantic salmon. The all-female strain of coho salmon performed well in our study and may represent a better candidate species for full-lifecycle rearing of salmon in RAS until an all-female strain of Atlantic salmon are available for investigation. Overall, in both species, we clearly demonstrated that rearing at intermediate salinities improves growth in our experimental RAS, which should be replicated on an industrially relevant scale.

Acknowledgements

Funding was provided from the Natural Sciences and Engineering Research Council (NSERC) of Canada, Strategic Partnership Grants for Projects (STPGP 462933-14) to CJB and JGR. We would like to thank the undergraduate and graduate volunteers from Brauner's and Richards' Labs, especially Naomi Pleizier, Jacelyn Shu and Derek Somo. We also acknowledge the irreplaceable contributions of our Aquatic Facility Manager, Patrick Tamkee, and other facility staff, including Eric Lotto, Bruce Gillespie, Vincent Grant and Neil Fowler in maintaining functions of the systems. We thank Taplow Feeds Ltd for the generous donation of fish food in this study.

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Figure Captions

Figure 1. The experimental design and timeline of the salinity growth trial for coho (*Oncorhynchus kisutch*) and Atlantic (*Salmo salar*) salmon.

Figure 2. Mean body mass of coho salmon (*Oncorhynchus kisutch*) reared at salinities of 0, 5, 10, 20 and 30 ppt sampled throughout the growth trial. About 100 fish were sampled from each salinity during each sampling time period, except day 355 ($n=66-102$) and day 460 ($n=57-131$), due to lower numbers of fish in a few systems caused by cumulative mortalities. All data are mean $\pm$ SEM. Letters that differ indicate statistically significant differences in body mass between salinities within the respective sampling time period ($P<0.05$).

Figure 3. Mean body mass of Atlantic salmon (*Salmo salar*) reared at salinities of 0, 5, 10, 20 and 30 ppt sampled throughout the growth trial. Initial mean body mass (day 0) was 70.58 g $\pm$ 1.11 ($n=400$). About 100 fish were sampled from each salinity during each sampling time period, except day 400 ($n=21-132$), due to lower numbers of fish at 0 and 30 ppt caused by fungus and pump failure. All data are mean $\pm$ SEM. Letters that differ indicate statistically significant differences in body mass between salinities within the respective sampling time period ($P<0.05$).

Table 1. Water parameters of the five recirculating aquaculture systems (RAS) in Experiment 1; System Validation, where coho salmon (*Oncorhynchus kisutch*) were reared for 5 months in freshwater. All data are mean ± SEM (*n*=91-143). Superscript letters that differ indicate mean values that are significantly different from one another (*P*<0.05).

RAS #	Unionized Ammonia (µg L ⁻¹)	NO ₂ -N (mg L ⁻¹)	NO ₃ -N (mg L ⁻¹)	pH	Dissolved Oxygen (%)	Temperature (°C)
1	0.2±0.02 ^a	0.00±0.00 ^a	3.0±0.6 ^a	6.4±0.03 ^{ab}	84.9±0.05 ^a	9.1±0.05 ^a
2	0.2±0.02 ^{ab}	0.05±0.10 ^a	3.2±0.6 ^a	6.4±0.03 ^a	91.9±0.05 ^b	9.5±0.1 ^{bc}
3	0.4±0.04 ^b	0.13±0.14 ^a	2.7±0.4 ^a	6.6±0.02 ^c	82.3±0.06 ^c	10.0±0.1 ^{bd}
4	0.3±0.03 ^{ab}	0.01±0.02 ^a	2.5±0.4 ^a	6.5±0.02 ^{ab}	88.4±0.05 ^d	9.6±0.08 ^{bcd}
5	0.3±0.02 ^{ab}	0.02±0.03 ^a	2.6±0.4 ^a	6.5±0.03 ^b	82.3±0.09 ^{ce}	9.9±0.2 ^b

Table 2. Mean body mass of coho salmon (*Oncorhynchus kisutch*) ($n=338-404$) measured before and after 5 months of rearing in freshwater RAS in Experiment 1; System Validation. Systems are numbered 1-5 with replicate tanks indicated by A or B. Data from system 2 was removed due to a mortality event early on in the growth trial. All data are mean $\pm$ SEM. No statistical differences were detected between tanks for initial or final body mass (one-way ANOVA, $P>0.05$).

Tank	Initial body mass (g)	Final body mass (g)
1A	191.4 $\pm$ 2.8	521.8 $\pm$ 12.0
1B	200.5 $\pm$ 3.2	541.5 $\pm$ 11.3
3A	190.3 $\pm$ 3.2	524.5 $\pm$ 11.5
3B	190.0 $\pm$ 3.0	537.4 $\pm$ 11.7
4A	195.1 $\pm$ 3.0	536.7 $\pm$ 10.0
4B	191.3 $\pm$ 2.9	542.3 $\pm$ 10.6
5A	196.7 $\pm$ 3.0	545.7 $\pm$ 9.8
5B	189.9 $\pm$ 2.8	533.2 $\pm$ 9.8

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Table 3. Changes in condition factor ($n=57-131$), growth rate (GR) and economic feed conversion ratio (eFCR) of coho salmon (*Oncorhynchus kisutch*) reared at 0, 5, 10, 20 and 30 ppt for 460 days in Experiment 2; Salinity Growth Trial. GR was calculated based upon changes in body mass of whole tank means ($n=1$), or in individually pit-tagged fish ($n=8-48$). Data are mean $\pm$ SEM. At each sampling time, values with superscript letters that differ indicate statistically significant differences ($P<0.05$).

		Salinity (ppt)				
		0	5	10	20	30
Condition Factor	Day 0	1.11 $\pm$ 0.01 ^a	1.09 $\pm$ 0.01 ^a	1.08 $\pm$ 0.01 ^a	1.07 $\pm$ 0.01 ^a	0.98 $\pm$ 0.02 ^b
	Day 59	1.19 $\pm$ 0.01 ^a	1.18 $\pm$ 0.02 ^a	1.22 $\pm$ 0.02 ^a	1.23 $\pm$ 0.02 ^a	1.18 $\pm$ 0.03 ^a
	Day 156	1.32 $\pm$ 0.03 ^a	1.32 $\pm$ 0.02 ^a	1.34 $\pm$ 0.02 ^a	1.35 $\pm$ 0.01 ^a	1.35 $\pm$ 0.01 ^a
	Day 257	1.38 $\pm$ 0.01 ^a	1.39 $\pm$ 0.02 ^a	1.41 $\pm$ 0.01 ^a	1.41 $\pm$ 0.02 ^a	1.45 $\pm$ 0.01 ^a
	Day 355	1.40 $\pm$ 0.01 ^{ab}	1.34 $\pm$ 0.02 ^a	1.36 $\pm$ 0.01 ^a	1.33 $\pm$ 0.01 ^a	1.47 $\pm$ 0.01 ^b
	Day 460	1.50 $\pm$ 0.02 ^a	1.44 $\pm$ 0.02 ^{ab}	1.51 $\pm$ 0.02 ^{ab}	1.42 $\pm$ 0.02 ^b	NA
Whole tank Growth Rate (g day ⁻¹)	Day 0-59	1.73	2.03	3.04	2.34	1.65
	Day 59-156	2.57	2.93	3.01	3.04	2.73
	Day 156-257	3.45	4.25	4.96	4.14	4.27
	Day 257-355	5.71	4.92	5.10	5.42	6.37
	Day 355-460	6.82	7.96	6.88	5.21	NA
Pit Tagged Fish Growth Rate (g day ⁻¹)	Day 156-257	3.05 $\pm$ 0.27 ^a	3.95 $\pm$ 0.29 ^a	4.15 $\pm$ 0.29 ^a	3.22 $\pm$ 0.30 ^a	4.19 $\pm$ 0.43 ^a
	Day 257-355	4.69 $\pm$ 0.59 ^a	5.02 $\pm$ 0.40 ^a	4.96 $\pm$ 0.55 ^a	4.42 $\pm$ 0.42 ^a	5.70 $\pm$ 0.70 ^a
	Day 355-460	6.85 $\pm$ 0.88 ^b	7.62 $\pm$ 0.55 ^b	7.35 $\pm$ 0.61 ^b	4.34 $\pm$ 0.55 ^a	NA
Economic Feed Conversion Ratio	Day 0-59	0.76	0.72	0.70	0.81	0.83
	Day 59-156	1.20	1.09	1.07	1.15	1.04
	Day 156-257	0.64	1.44	1.96	1.49	1.68
	Day 257-355	0.84	1.48	1.95	1.27	1.62
	Day 355-460	0.85	0.48	3.06	2.35	NA

Table 4. Incidence of sexual maturation, gonadosomatic index (GSI) ($n=50-59$) and presence of cataracts in coho salmon (*Oncorhynchus kisutch*) reared at 0, 5, 10, 20 and 30 ppt for 460 days in Experiment 2; Salinity Growth Trial. Data are mean $\pm$ SEM. Letters that differ indicate statistically significant differences in GSI between salinities within each respective sampling time period ($P<0.05$).

		Salinity (ppt)				
		0	5	10	20	30
Sexual Maturation (%)	Day 355	0	0	0	0	0
	Day 460	0	0	0	0	NA
Gonadosomatic Index (%)	Day 460	0.37 $\pm$ 0.03a	0.35 $\pm$ 0.03a	0.36 $\pm$ 0.02a	0.28 $\pm$ 0.02a	NA
Cataract (%)	Day 355	8	0	0	7	0
	Day 460	0	0	0	4	NA

Table 5. Condition factor ($n=21-132$), growth rate (GR) and economic feed conversion ratio (eFCR) of Atlantic salmon (*Salmo salar*) reared at 0, 5, 10, 20 and 30 ppt for 400 days in Experiment 2; Salinity Growth Trial. GR was calculated based upon changes in body mass of whole tank means ($n=1$), or in individually pit-tagged fish ($n=2-19$). Data are mean $\pm$ SEM. At each sampling time, values with superscript letters that differ indicate statistically significant differences ($P<0.05$).

		Salinity (ppt)				
		0	5	10	20	30
Condition Factor	Day 96	0.99 $\pm$ 0.01 ^a	1.06 $\pm$ 0.01 ^{ab}	1.08 $\pm$ 0.01 ^b	1.05 $\pm$ 0.01 ^{ab}	1.11 $\pm$ 0.01 ^b
	Day 197	1.15 $\pm$ 0.01 ^b	1.15 $\pm$ 0.01 ^b	1.20 $\pm$ 0.03 ^{ab}	1.14 $\pm$ 0.01 ^b	1.26 $\pm$ 0.03 ^a
	Day 295	1.17 $\pm$ 0.01 ^a	1.16 $\pm$ 0.01 ^a	1.13 $\pm$ 0.01 ^a	1.14 $\pm$ 0.01 ^a	1.13 $\pm$ 0.01 ^a
	Day 400	1.15 $\pm$ 0.03 ^{ac}	1.31 $\pm$ 0.01 ^b	1.24 $\pm$ 0.01 ^c	1.21 $\pm$ 0.01 ^c	1.05 $\pm$ 0.02 ^a
Whole tank Growth Rate (g day ⁻¹)	Day 0-96	1.21	1.84	1.79	1.83	1.83
	Day 96-197	3.69	3.65	4.20	3.99	4.26
	Day 197-295	3.23	4.32	5.10	3.68	3.51
	Day 295-400	3.17	9.37	4.51	5.77	1.77
Tagged Fish Growth Rate (g day ⁻¹)	Day 96-197	NA	3.94 $\pm$ 0.26 ^a	4.69 $\pm$ 0.28 ^a	3.80 $\pm$ 0.37 ^a	4.14 $\pm$ 0.22 ^a
	Day 197-295	NA	4.06 $\pm$ 0.81 ^a	4.51 $\pm$ 2.00 ^a	3.45 $\pm$ 0.28 ^a	2.74 $\pm$ 0.68 ^a
	Day 295-400	3.04 $\pm$ 1.01 ^a	10.52 $\pm$ 0.83 ^c	6.31 $\pm$ 0.84 ^b	5.41 $\pm$ 0.50 ^{ab}	0.07 $\pm$ 1.02 ^a
Economic Feed Conversion Ratio	Day 0-96	0.63	0.69	0.71	0.70	0.69
	Day 96-197	2.94	0.87	0.84	1.80	0.81
	Day 197-295	3.19	1.54	1.19	2.43	1.95
	Day 295-400	20.81	0.38	2.80	2.61	1.80

Table 6. Incidence of sexual maturation, gonadosomatic index (GSI) ($n=21-57$) and presence of cataracts in Atlantic salmon (*Salmo salar*) reared at 0, 5, 10, 20 and 30 ppt for 460 days in Experiment 2; Salinity Growth Trial. Data are mean $\pm$ SEM. Letters that differ indicate statistically significant differences in GSI between salinities within each respective sampling time period ($P<0.05$).

		Salinity (ppt)				
		0	5	10	20	30
Sexual Maturation (%)	Day 295	22	11	11	18	23
	Day 400	52	29	48	46	15
Gonadosomatic Index (%)	Day 400	4.38 $\pm$ 1.07a	2.18 $\pm$ 0.42bc	3.75 $\pm$ 0.5ab	3.16 $\pm$ 0.48ab	1.63 $\pm$ 0.49c
Cataract (%)	Day 295	3	39	49	66	8
	Day 400	0	39	37	34	15

Figure 1. The experimental design and timeline of the salinity growth trial for coho (*Oncorhynchus kisutch*) and Atlantic (*Salmo salar*) salmon.

Figure 2. Mean body mass of coho salmon (*Oncorhynchus kisutch*) reared at salinities of 0, 5, 10, 20 and 30 ppt sampled throughout the growth trial. About 100 fish were sampled from each salinity during each sampling time period, except day 355 ($n=66-102$) and day 460 ($n=57-131$), due to lower numbers of fish in a few systems caused by cumulative mortalities. All data are mean $\pm$ SEM. Letters that differ indicate statistically significant differences in body mass between salinities within the respective sampling time period ($P < 0.05$).

Figure 3. Mean body mass of Atlantic salmon (*Salmo salar*) reared at salinities of 0, 5, 10, 20 and 30 ppt sampled throughout the growth trial. Initial mean body mass (day 0) was $70.58 \text{ g} \pm 1.11$ ($n=400$). About 100 fish were sampled from each salinity during each sampling time period, except day 400 ($n=21\text{--}132$), due to lower numbers of fish at 0 and 30 ppt caused by fungus and pump failure. All data are mean $\pm$ SEM. Letters that differ indicate statistically significant differences in body mass between salinities within the respective sampling time period ($P<0.05$).