Production of market-size North American strain Atlantic salmon *Salmo salar* in a land
based recirculation aquaculture system using freshwater

John Davidson 1, Travis May 1, Christopher Good 1, Thomas Waldrop 2, Brett Kenney 3, Bendik Fyhn Terjesen 4, and Steven Summerfelt * 1

1 The Conservation Fund’s Freshwater Institute, 1098 Turner Road, Shepherdstown, WV 25443, United States
2 Healthy Earth - Sarasota, 13200 Fruitville Road, Sarasota, FL 34202, United States
3 West Virginia University, Division of Animal and Nutritional Sciences, P.O. Box 6108,
Morgantown, WV 26506-6108, United States
4 Nofima, NO-6600 Sunndalsøra, Norway

*Corresponding author’s contact info: s.summerfelt@freshwaterinstitute.org
Ph. 304-876-2815 xt.211; fax: 304-870-2208

Abstract

There is interest in culturing Atlantic salmon *Salmo salar* to market-size in land-based,
closed containment systems that use recirculation aquaculture systems (RAS), as this technology
often enables facilities to locate near major markets, obtain permits, exclude obligate pathogens,
and/or reduce environmental impacts. Use of land-based RAS to intensively culture market-size
Atlantic salmon is a relatively new frontier and little information is available. Three trials were
conducted to evaluate the performance of two North American strains of Atlantic salmon raised
from post-smolt to market-size (4-5 kg) in a near-commercial scale (260 m³), land-based RAS
using only freshwater. St. John River (SJR) salmon were reared during the first trial, and
Cascade salmon (CS1 and CS2) were evaluated during two subsequent trials. Salmon were
received as fertilized “eyed” eggs and cultured on-site through the entire production cycle. The
grow-out period began at 14-16 months post-hatch when salmon post-smolt weighed 0.34 - 0.75
kg on average. CS1 and SJR salmon grew 386-393 g/month to a mean size of 4.1-4.2 kg and CS2
salmon grew 413 g/month to a mean size of 4.9 kg prior to first harvest. Thereafter, weekly
salmon harvests commenced for the next 6-19 weeks. The grow-out period, excluding harvest,
lasted 9-10 months for each trial. Average water temperature was maintained at 15-16 °C.

Consistently linear growth rates were achieved by each population suggesting that growth was relatively independent of fish cohort/genetic strain, fish size, and maximum biomass density, which was 35, 100, and 118 kg/m³ for SJR, CS1, and CS2, respectively. Feed conversion ratios ranged from 1.07-1.10. Fish mortality (including culls) for SJR, CS1, and CS2 was 9.5, 6.6, and 7.5 % of the original number of stocked fish, respectively. No obligate fish pathogens, kudoa, sea lice, or pervasive parasites were detected. Salmon were not vaccinated against specific pathogens; and no antibiotics, pesticides, or harsh chemotherapeutants were used. Hydrogen peroxide (50-100 ppm) and salt (10 ppt) were occasionally used to treat fungus during pre-smolt production, and salt (2-3 ppt) was used to treat fungus or ameliorate stress after handling events.

No salmon escaped the facility due to built-in fish exclusion barriers. Early male maturation was observed during each trial. Male salmon began to exhibit maturation traits (kype, darkened skin coloration) at a mean weight of 1.5-2 kg and were removed from the grow-out system when they weighed 2-3 kg. SJR, CS1, and CS2 populations exhibited 37.0, 38.5, and 17.0 % maturity, respectively. Fillet yield and product quality of immature, market-size salmon were comparable to reported measurements for commercially available salmon reared in net pens. This research suggests that it is biologically and technologically feasible to culture Atlantic salmon from post-smolt to market-size in a land-based RAS of suitable commercial scale; however, early male maturation could represent a production barrier. As of 2016, all-female Atlantic salmon eggs are commercially available and could provide an expedient solution to the problem of early male maturation in RAS.

Keywords: Atlantic salmon; recirculation aquaculture systems; land-based; closed containment
1. Introduction

There is interest in culturing Atlantic salmon *Salmo salar* to market-size in land-based, closed containment systems using water recirculation technology (Summerfelt and Christianson, 2014). These culture systems provide an alternate approach that isolates fish from potentially sensitive marine ecosystems, while supplying built-in measures to prevent environmental impacts such as the discharge of nutrients and particulates, escapees, and fish pathogens.

Recirculation aquaculture systems (RAS) provide advantages such as: 1) reduced water use in the face of diminishing resources (Kristensen et al., 2009); 2) small-volume, concentrated effluents that can be effectively treated to minimize pollution (Sharrer et al., 2010); 3) optimized culture environment that can be tuned to meet the biological requirements of fish (Summerfelt et al., 2001); 4) enhanced biosecurity and disease control (Bebak-Williams et al., 2001); 5) containment of non-native fish to prevent interaction with wild populations (Summerfelt and Vinci, 2008); and 6) opportunity for vertical integration and increased revenue through the recovery or value-added use of waste stream nitrogen and phosphorus for practices such as aquaponics (Adler et al., 2000). Recirculation aquaculture systems also provide increased potential for siting where energy and other resources are affordable or near major seafood markets, which could lead to enhanced product quality and reduced carbon footprint of the shipped product (Martins et al., 2010; Liu et al., 2016).

Use of RAS to culture Atlantic salmon is not a novel practice. Some commercial salmon companies are now producing smolts using RAS (Bergheim et al., 2009). However, juvenile Atlantic salmon are typically cultured in single-pass, land-based aquaculture systems using freshwater until the fish undergo smoltification. Salmon smolts are then transported from
onshore tanks to marine net cages for continued culture to market-size. In recent years, salmon smolts have been cultured to approximately 140-170 g in land-based systems prior to relocation to net cages for grow-out (Bergheim et al., 2009). However, an increasing number of Norwegian and Faroe Islands facilities are planning to culture salmon smolts and post-smolts to larger sizes (250-1000 g) in land-based RAS and partial reuse systems (with some already in operation), in order to reduce sea lice susceptibility, increase fish robustness, reduce mortality during the sea phase, and to decrease overall production time (Bergheim et al., 2009; Dalsgaard et al., 2013; Ytrestøyl et al., 2013).

Although Atlantic salmon smolt production in RAS is becoming more common, very little published data is available that describes the production and performance metrics of salmon raised to market-size in land-based RAS. Thus, three trials were conducted evaluating the performance of several groups of North American-strain Atlantic salmon cultured in freshwater from fertilized “eyed” egg to market-size food fish, primarily in RAS. The grow-out culture period from post-smolt to market-size in a near-commercial-scale RAS was the primary focus of this research. Results from these trials will provide information on the technical and biological feasibility of commercial production of market-size Atlantic salmon in land-based recirculation aquaculture systems, which will facilitate decision-making by the salmon farming industry, investors, aquaculture researchers, and engineers.

2. Materials and Methods

2.1. Atlantic salmon

Three groups of mixed-sex, North American-strain Atlantic salmon were evaluated including: a St. John River (SJR) strain from Cooke Aquaculture (Bingham, ME, USA); and two
cohorts of Cascade salmon (CS1 and CS2) from Icicle Seafoods, Inc. (Seattle, WA, USA). All procured egg lots were specific-pathogen-free-certified and each germplasm was diploid. Salmon were raised entirely in freshwater from fertilized “eyed” egg to market-size food fish (4-5 kg).

2.2. Incubation

Eyed eggs were iodine disinfected upon arrival and placed in an 8-stack Heath-Tecna incubator (Marisource, Tacoma, WA, USA) within a RAS equipped with two chillers, an ultraviolet irradiation unit, two pumps to recirculate the water, and a water aeration column. SJR, CS1, and CS2 egg lots were received at 356, 330, and 349 accumulated temperature units (ATUs), respectively. Each egg lot was incubated at an average water temperature of 7-8 °C. Designation of Day 1 of the growth cycle corresponded with 50 % egg hatch. After hatching, alevins remained in the system until the majority of the yolk sac was absorbed. Prior to complete yolk-sac absorption, water temperature was gradually increased to 10 °C to acclimate fish for transfer to a nursery system. Atlantic salmon eggs/ alevins were kept in the incubation system for an average of 48 days or 440 ATUs. Survival for SJR, CS1, and CS2 during this phase was 84, 85, and 90 % respectively.

2.3. Nursery system

Juvenile Atlantic salmon were transferred from the incubation system to a single-pass, flow-through nursery system with twelve 0.5 m³ circular tanks, maintained at 12-14 °C. The system was enclosed by a tent constructed with opaque plastic to omit natural light. Artificial light was provided by overhead, full-spectrum incandescent bulbs. Atlantic salmon fry were fed commercially available diets from Bio-Oregon (Westbrook, ME, USA) and Zeigler Brothers Inc.
(Gardners, PA, USA) using a computer operated feeding program (Freshwater Institute, Shepherdstown, WV, USA) integrated with automated feeders (Model 907, Sterner Fish Tech AS feeders, Ski, Norway) to deliver precise feed amounts at set intervals. Feed was provided hourly during the “lights-on” phase of specific photoperiod regimens. Feeding rates were determined using standardized feeding charts provided by feed suppliers and industry and by observations of feeding response and wasted feed. Daily feeding rates for first-feeding salmon began near 4.5% of tank biomass and declined to approximately 2% by the end of this period.

When juvenile salmon were stocked in the nursery system, a 24-h continuous light photoperiod was used, with one exception; half of the CS2 cohort was reared under an 18-h light: 6-h dark photoperiod (Good et al., 2015a). After 7-9 months of culture (approximately 40-60 g mean weight; Table 1), all fish were subjected to a photoperiod described by industry as an “S0 winter”, a lighting regimen designed to provide a short day length and thereby trigger smoltification in the first year of the salmon life cycle. During the artificial winter, each cohort was exposed to a 12-h light: 12-h dark photoperiod lasting about 6 weeks. At the end of this period, 24-h continuous light was generally reinstituted. The only exception was for CS2; these fish were returned to their original photoperiod treatments, either 24-h light or 18-h light: 6-h dark. These photoperiod regimens continued when CS2 was transferred to a partial reuse system in order to evaluate the long-term effect of light treatments on early maturation (Good et al., 2015a). Additional detail regarding the timing and length of photoperiod manipulation and other pre-smolt milestones is provided in Table 1.

2.4. Post-smolt production
CS1 and CS2 salmon were transferred to a partial reuse system when they reached 250 and 270 days post-hatch or 70 and 107 g, respectively. The partial reuse system was equipped with three 10 m³ dual-drain culture tanks, a microscreen drum filter, a pump sump, a forced ventilation cascade aeration column, and a low head oxygenator (LHO) and sump (Summerfelt et al., 2004). The system recycled approximately 85% of water relative to the recycle flow. Solids laden water (15% of recycle flow) discharged through the bottom center drain of each tank and was replaced with an equal volume of spring water, which provided enough dilution to limit ammonia accumulation in the absence of a biofilter. Water temperature ranged from 12-14 °C, depending on season. Lighting was mainly provided by overhead, metal halide bulbs; however, full-spectrum compact fluorescent bulbs were used when tanks were enclosed for photoperiod treatment of the CS2 salmon.

Each group of salmon was fed commercially available diets from Bio-Oregon (Westbrook, ME, USA) and EWOS (Surrey, British Columbia, Canada) at a rate of 1-2% of the tank biomass, depending on mean fish weight. Diets contained 43-47% protein and 24% fat and were supplemented with 30 ppm astaxanthin and 30 ppm canthaxanthin. Feed was distributed by individual screw-auger-style feeders with 20 kg hoppers (Pentair Aquatic Ecosystems, Apopka, FL, USA). Nine equally spaced feeding events occurred each day around-the-clock or, in the case of specific photoperiod treatments, only when lights were on. Time and duration of feeding events were set using a timer control system that was integrated with the feeders.

2.5. Grow-out

SJR salmon remained in the nursery system until they were 15.5 months old and weighed 340 g; at this time 2,052 fish were moved to a near-commercial scale (260 m³) grow-out system.
with a 150 m³ culture tank (Davidson and Summerfelt, 2005). CS1 and CS2 salmon (5,651 and 6,906 fish, respectively) were transferred from the post-smolt system to the grow-out system at 13.9 and 14.3 months post-hatch when they weighed 750 and 510 g, respectively (Table 2). Due to tank space limitations, SJR and CS1 were comingled for 1-2 months with separate groups of market-size Atlantic salmon that were nearing the end of production. CS2 salmon were stocked into an empty grow-out system after fish from the previous cohort had been harvested. CS2 salmon from each of two photoperiod treatments were fin clipped for future identification.

The grow-out system used two 5-HP centrifugal pumps to move 4,900 L/min of water from the lowest hydraulic grade-line (a pump sump) to the highest elevation, the top of a cyclonic fluidized-sand biofilter. Water exiting the biofilter gravity flowed through a forced-ventilation cascade aeration column, a LHO and LHO sump, and entered the culture tank through a water distribution manifold (Fig. 1). The majority of recycled water (90 %) was discharged from the culture tank at a side-box drain and gravity flowed through a microscreen drum filter equipped with 90-μm sieve panels and into a pump sump, where the water recycling process began again. The remaining 10 % of flow flushed through the tank’s bottom center drain to a radial flow settler equipped with an automated valve that opened approximately once an hour to flush settleable solids collected in the cone-bottom. Biosolids backwashed from the drum filter and settler were collected and dewatered on-site using gravity thickening settlers. Some of the overtopping flow leaving the radial flow settler was released from the system and replaced with cool, 12-13 °C spring water. This discharge rate varied from 0-178 L/min depending on season and was regulated to maintain the system water temperature at 15-17 °C; it was controlled by directing more or less flow back into the system via manual valve adjustment. Discharged system water was replaced with spring water at an average makeup flow rate of 55 L/min or 80
m³/day. System hydraulic retention time ranged from 1-15 days and feed loading rate was 1-2 kg feed/ m³ daily makeup water. The water flow rate through the culture tank created a mean tank hydraulic retention time of 30 min. Ozone was generated from a 99.5 % pure oxygen feed gas by a System GM-2 generator (Primozone, Löddeköpinge, Sweden) and injected into the LHO. Oxidation reduction potential (ORP) was measured by an SC100 Universal Controller (Hach Company, Loveland, CO, USA) during the first trial and a YSI 5500D unit (YSI Inc., Yellow Springs, OH, USA) for the last two trials. An on/off feedback loop between the ORP monitoring systems and the ozone generator was used to maintain ORP between 280-320 mV to control water clarity while maintaining ozone residual at safe levels (Summerfelt et al., 2009a). A 24-h photoperiod was provided with overhead metal halide lights (400 Watt; 23500 lumens; 4000 K color temperature). Each cohort was fed a commercially available diet (Dynamic Red™, EWOS, Surrey, British Columbia, Canada). Diets contained 40-45 % protein and 29-30 % fat and were supplemented with 30 ppm astaxanthin and 30 ppm canthaxanthin. Feed was distributed by two screw-auger feeders with 60 kg hoppers (Pentair Aquatic Ecosystems, Inc., Apopka, FL, USA) equipped with spreaders. Nine equally spaced feeding events occurred each day around-the-clock. Time and duration of feeding events were set using a timer control system integrated with the feeders. Fish were fed to near-satiation by adjusting feeding rates based on feeding activity and daily observations of wasted feed flushed from the tank through a bottom center-drain standpipe. Maximum daily feed delivered to the grow-out tank was approximately 100 kg for each cohort.

2.6. Fish sampling procedures

Performance metrics
Weight samples of 10-60 randomly selected fish, usually measured as bulk weights, were collected at monthly intervals for assessment of mean weights and calculation of growth performance metrics. Fish were crowded using a clam shell grader and then netted from the tank for each sampling event. Thermal growth coefficients (TGC), economic feed conversion ratio (FCR), condition factor (CF), and gonadosomatic index (GSI) were calculated as follows:

\[
TGC = \frac{(End \ Weight^{\frac{1}{3}} - Start \ Weight^{\frac{1}{3}})}{(Days \ Between \ * \ Avg. \ Temp.) \ * \ 1000}
\]

Where weight is in grams and temperature is in °C.

\[
FCR = \frac{Cumulative \ Feed \ Delivered}{Fish \ Biomass \ Gain}
\]

\[
CF = 100,000 \ * \ \frac{Weight}{(Length)^3}
\]

\[
GSI (\%) = \frac{(Gonad \ Weight/ \ Whole \ Fish \ Body \ Weight)}{100}
\]

**Fish Health**

Fish populations were observed daily and, when practical, individuals with external lesions (e.g. skin ulceration, severely eroded fins, or significant external *Saprolegnia* spp. infections) were removed and humanely euthanized. During performance sampling events, each sampled fish was likewise inspected, and those demonstrating external lesions were removed from the population. Following American Fisheries Society Fish Health Section guidelines (AFS-FHS, 2014), sixty fish from each cohort were euthanized near the end of each production cycle and screened for listed bacterial, viral, and parasitic fish pathogens. Tissue samples were tested by Kennebec River Biosciences (Richmond, ME, USA) for the following pathogens: infectious salmon anemia virus (ISAV), infectious pancreatic necrosis virus (IPNV), viral hemorrhagic septicemia virus (VHSV), Oncorhynchus masou virus (OMV), spring viremia of carp virus
Aeromonas salmonicida (causative agent of furunculosis), Renibacterium salmoninarum (bacterial kidney disease), Yersinia ruckeri (enteric redmouth disease), Myxobolus cerebralis (whirling disease), and Ceratomyxa shasta (ceratomyxosis). Additionally, PCR was used to screen for the myxosporean parasite Kudoa thyrsites due to concerns in the Atlantic salmon industry in British Columbia, Canada regarding product downgrading as a consequence of fillet infiltration and consequent post-mortem myoliquification (Dawson-Coates et al., 2003).

Kudoa screening was carried out for CS1 and CS2, but not for SJR salmon, on muscle samples taken from a standardized fillet section from 60 fish and tested in pooled 5-fish batches.

2.7. Culling and harvesting

Precocious males

Early maturing males are undesirable due to potentially reduced growth and decreased feed conversion efficiency (McClure et al., 2007), as well as reduced product quality (Aksnes et al., 1986); and were therefore removed at various points in the production cycle (Table 1, 2). Mature males were identified and removed as precocious parr during the pre-smolt phase and as “grilse” during the grow-out phase. Identification was based on colorimetric and/or morphometric changes; precocious parr demonstrated a bronze or yellow color, often with red spots and free-flowing milt, and grilse demonstrated darker coloration and developing kype. Grilse were observed in substantial numbers during the grow-out phase and were specifically harvested when mean fish weight was ≥ 2 kg, by crowding the population using a clam-shell grader (Summerfelt et al., 2009b) and subsequently netting individuals that displayed the aforementioned characteristics. Time post-hatch and mean weight of maturing males upon removal are described for each cohort (Tables 1 and 2).
Premium salmon harvest

Harvest of market-size salmon began when the mean population weights were $\geq 4$ kg. Salmon were crowded using a custom aluminum and polyethylene clam-shell grader (Emperor Aquatics Inc., Pottstown, PA, USA). Average size fish were harvested weekly from the SJR and CS1 cohorts; while the largest salmon were selectively top-graded from the CS2 population during each harvest. Salmon from each cohort were harvested at different rates (Table 2) depending on arrangements with processors and distributors. Harvest methods included hand-netting of crowded fish, as well as use of the tank’s side-wall-box that was designed to collect fish and dewater the flow for ease of fish handling and sorting (Summerfelt et al., 2009b). Salmon were then transported to two partial reuse depuration systems, each equipped with a single 11 m³ culture tank. Hydraulic retention time of the depuration systems averaged 2-3 hours. Salmon were kept off feed and typically purged for 6 days according to standard operating procedures described in Davidson et al. (2014). Following purging, salmon were euthanized and bled using a model SI-7C percussive stunner (Baader Seafood Innovations, Cleveland, Australia) and held in an ice slurry for approximately 30 mins to quickly cool the fish prior to packing on ice. Harvested salmon were then sent to a local processing facility where they were filleted and sold into various markets in Canada and the United States.

2.8. Product quality

A representative number of harvested salmon were filleted and evaluated for the following product quality attributes: head-on-gutted yield, butterfly fillet yield, trimmed skin-on and skinless fillet yield, belly flap and fillet thickness, fillet color, fillet proximate composition, and
fillet fatty acid content. Proximate composition of skinless fillets was measured (AOAC, 1990) once for SJR when the salmon were close to 4 kg; and twice for CS1 at approximately 4 and 5 kg. Fillet proximate composition was not assessed for CS2 salmon. Fillet color was analyzed using a chromameter (Model CR-300; Minolta Camera Co. Ltd., Osaka, Japan) calibrated with a standard white plate No.21333180 (CIE L* 93.1; a* 0.3135; b* 0.3198). Visual fillet color assessment was made using the Roche SalmoFan™ Lineal, generally at monthly intervals after fish reached 1 kg and continuing to harvest. Texture of cooked fillets was measured by placing fillets skin-side up, in a Kramer Shear Cell. Shear force was measured using a Texture Analyzer (model TA-Hdi, Texture Technologies Corp., Scarsdale, NY, USA) equipped with a 5-blade Kramer shear attachment at a cross speed of 127 mm/min. Values were expressed as peak force generated per gram of sample. For subsequent fatty acid analyses, total lipids were extracted with a chloroform: methanol mixture (2:1, v/v) using the method of Bligh and Dyer (1959).

2.9. Water quality analyses

Water samples were collected weekly and tested on-site for total ammonia nitrogen (TAN), nitrite nitrogen (NO$_2$-N), nitrate nitrogen (NO$_3$-N), carbon dioxide (CO$_2$), alkalinity, total suspended solids (TSS), and total phosphorus (TP). All parameters were analyzed according to methods described in APHA (2005, 2012) and HACH (2003). Dissolved oxygen and temperature of the grow-out system were recorded daily from continuous monitoring systems, including a PT4 unit (Point Four Systems, Inc., British Columbia, CA) equipped with Oxyguard probes (Oxyguard International, Farum, Denmark) during the first trial and a YSI 5500D with optical probes (YSI Inc., Yellow Springs, OH, USA) for the last two trials. Oxidative reduction potential was measured at the culture tank inlet and sidewall box outlet by differential ORP digital sensors.
with platinum electrode (Model DRD1R5, Hach Company, Loveland, CO, USA) and displayed by an SC100 Universal Controller (Hach Company, Loveland, CO, USA) for the first trial. ORP and pH were monitored with the YSI 5500D system for the last two trials.

3. Results

3.1. Growth and survival (Grow-Out)

St. John River salmon

SJR salmon grew from 0.34 to 4.2 kg in 9.8 months (393 g/month) in the near-commercial-scale RAS (Fig. 2). At this time, harvest of market size (4.2 kg mean weight) salmon began. Salmon continued to grow during the 10-week harvest period. By the end of the harvest cycle, mean fish weight was 4.7 kg (Fig. 2) and corresponding CF was 1.83 ± 0.03. Thermal growth coefficient from time of stocking to first harvest was 2.01. Economic FCR, which accounted for all feed delivered to the fish over the grow-out trial duration, was 1.09. Maximum biomass density was 35 kg/m³, which is relatively low, but was intentionally kept at < 40 kg/m³ because this was the first attempt to culture Atlantic salmon on-site under these conditions. The total production period including approximately 2.5 months of harvesting lasted just over 12 months. Total mortality including culls (fish with fungus, unthrifty fish, or fish removed for quality assurance at harvest) and jumpers (salmon that perished by leaping over surrounding jump screens) was 11.4 % of fish stocked. Of this total, 3.9 % represented in-tank mortalities, 5.6 % were culls, and 1.9 % were jumpers (Table 3).

Cascade I salmon
CS1 salmon grew from 0.75 to 4.1 kg in 8.7 months (386 g/month) in the commercial-scale RAS (Fig. 2). Harvests began when CS1 salmon reached a mean weight of 4.1 kg and continued thereafter for the next 4 months. Remaining salmon continued to grow during the harvest period and achieved a mean weight of 5.7 kg with a CF of 1.84 ± 0.06 by the end of the trial. Thermal growth coefficient from time of stocking to first harvest was 1.65; economic FCR was 1.07; and maximum biomass density reached 100 kg/m³. The total production period, including approximately 4 months of harvesting, lasted just over 13 months. Total mortality, including culls and jumpers, was 7.0 % of fish stocked. Of this total, 2.7 % were in-tank mortalities, 3.9 % were culls, and 0.4 % were jumpers (Table 3).

Cascade II salmon

CS2 salmon grew from 0.51 to 4.9 kg in 10.6 months (413 g/month). At this time, a selective harvest of the largest salmon (4.9 kg mean weight) began. Thermal growth coefficient averaged 1.86 and economic FCR was 1.10 over the grow-out trial duration. Maximum biomass density was 118 kg/m³. Salmon were harvested at a faster rate during this trial, approximately 2,000 kg per event over a 6-wk period. The largest fish were removed during each harvest; therefore, average fish weight diminished from 4.9 kg at initial harvest to approximately 3.5 kg at final harvest. Condition factor at the onset of harvesting was 1.69 ± 0.10. Total production period including approximately 1.5 months of harvesting lasted exactly 12 months. Total mortality, including culls and jumpers, was 8.2 % of fish stocked. Of this total, 2.6 % were in-tank mortalities, 4.9 % were culls, and 0.7% were jumpers (Table 3).

3.2. Early maturing males (grilse)
Removal of grilse from the SJR, CS1, and CS2 cohorts began when fish were 19-21 months old and weighed 2.7, 2.6, and 2.1 kg, respectively (Table 2). Initial grilse culling events took place after approximately 5-6 months of production in the grow-out system (Table 2). A second culling event was conducted for the SJR salmon to remove the remainder of early maturing males at approximately 24 months post-hatch when the mean population weight was 3.7 kg (Table 2, 4). Several additional culling events were conducted within 20 days of the initial for CS1 and CS2 to remove the remaining mature males from the tank. A total of 1,800, 5,442, and 2,657 kg of grilse biomass (whole, uncut body weight) was harvested for the SJR, CS1, and CS2 cohorts, respectively (Table 4). Early maturing males made up approximately 37, 38.5, and 17 % of the SJR, CS1, and CS2 populations, respectively (Table 4). Fillets of early maturing males were generally leaner and contained less pigmentation compared to fillets of immature fish at final harvest. Various product forms were tested including standard fillets and hot and cold smoked product (Table 4).

3.3. Gonadosomatic index

GSI assessment of maturing males from the SJR and CS2 cohorts indicated that grilse were present in these populations at 17-19 months of age when the mean population weight was 1.3-1.4 kg (Fig. 3). The majority of female salmon did not mature during these trials. The average GSI of immature salmon (mostly females) from each cohort did not increase beyond 0.5 % (Fig. 3). A few maturing females were sampled during the SJR and CS1 trials, but only one maturing female was noted during sampling events for the CS2 trial (Fig. 3). Approximately ≤ 2-3 % of the female population had a GSI > 1 %.
3.4. Premium salmon harvests

Harvesting began when each cohort reached a mean weight ≥ 4 kg. SJR, CS1, and CS2 weighed 4.2, 4.1, and 4.9 kg and were 25.3, 22.6, and 24.9 months old, respectively, when harvests commenced. At this stage of production, each group had been in the grow-out system for 8.5-10.5 months. Total premium biomass (whole, uncut fish) of 5,200 kg (435 fish), 13,382 kg (2,752 fish), and 12,695 kg (2,952 fish) was harvested for the SJR, CS1, and CS2 cohorts, respectively (Table 3). The amount of biomass removed during each harvest was generally related to the rate at which value-chain sectors could process and distribute the product. SJR salmon were used for test marketing or distributed to local food banks, while CS1 and CS2 salmon were sold through various seafood distribution companies. SJR, CS1, and CS2 salmon were harvested throughout 5, 16, and 7 events, over 10, 19, and 6 weeks, respectively (Table 2). After the first two trials, the process had been streamlined for CS2, resulting in faster salmon removal and a substantially shorter harvesting window.

3.5. Fish health

No major fish health events occurred during any of the grow-out trials, and no listed fish pathogens were detected. In addition, no kudoa (*Kudoa thyrsites*) was detected and no sea lice were observed; although detection of these salt water parasites was not expected in a land-based, freshwater environment. The only occasional fish health issue, affecting each cohort to varying degrees, was the occurrence of external *Saprolegnia* spp., i.e. “fungal” infections, or Saprolegniasis. No vaccines were administered at any stage of production. In addition, no formalin, chemicals, antibiotics, pesticides, or other chemotherapeutants were used to treat salmon during these grow-out trials, with the exception of low concentrations (2-3 ppt) of
sodium chloride which was used to relieve handling stress related to harvesting and fish sampling events and to control Saprolegniasis. Hydrogen peroxide (50-100 ppm) and salt bath treatments (10 ppt) were used to ameliorate Saprolegniasis during egg incubation and fry production in the flow-through nursery system.

3.6. Water quality

Average water temperature for the SJR, CS1, and CS2 grow-out trials was 15.6, 15.6, and 15.2 °C, respectively; and dissolved oxygen was maintained at or above 90% saturation (Table 5). Average TAN was ≤ 0.3 mg/L and max TAN was ≤ 0.6 mg/L; while mean NO₂-N was ≤ 0.02 mg/L and max NO₂-N never exceeded 0.21 mg/L, indicating efficient biofilter performance. Mean NO₃-N for the SJR, CS1, and CS2 trials was 19-24 mg/L and max NO₃-N was 60, 32, and 65 mg/L, respectively. Mean TSS was < 3 mg/L and max TSS never exceeded 5 mg/L. Average CO₂ ranged from 9-14 mg/L during the three trials; while max CO₂ for SJR, CS1, and CS2 salmon was 16, 24, and 20 mg/L, respectively. Mean TP ranged from 0.7-0.9 mg/L with max levels reaching 1.3-2.2 mg/L, and average alkalinity was maintained at > 200 mg/L (as CaCO₃) primarily through addition of highly alkaline makeup water. Water hardness, an indicator of calcium, magnesium, and other low-level cation levels in fish culture systems, was not assessed; however, previous on-site studies have shown that make-up and RAS water hardness are typically near 300 mg/L as CaCO₃ (Davidson et al., 2009; 2013).

3.7. Fillet and product quality attributes

Processing yield was similar for each cohort despite slight differences in average harvest weight (Table 6). Head-on-gutted yield for CS1 and CS2 was 91.1 ± 0.4 and 90.5 ± 0.6 %,
respectively. Butterfly fillet yield for SJR, CS1, and CS2 was 74.8 ± 0.5, 74.8 ± 0.4, and 74.7 ± 0.9 %, respectively. Skin-on fillet yield for CS1 and CS2 was 61.2 ± 0.6 and 61.9 ± 0.9 %, respectively; and skinless fillet yield for SJR, CS1, and CS2 was 57.8 ± 0.5, 57.0 ± 0.6, and 57.3 ± 0.9 %, respectively (Table 6). Head-on-gutted and skin-on fillet yield were not assessed for SJR salmon.

Fillet composition was measured for SJR and CS1 salmon of similar size (approximately 4 kg). SJR and CS1 fillets had moisture content of 63.8 ± 0.6 and 63.1 ± 0.6 %; protein content of 15.9 ± 0.7 and 20.0 ± 0.2 %; lipid content of 20.4 ± 0.2 and 15.2 ± 0.7 %; and ash content of 1.5 ± 0.1 and 1.2 ± 0.0 %, respectively. As CS1 salmon grew from 4 to ≥ 5 kg, fillet protein content remained about the same, but fillet fat increased from 15.2 ± 0.7 to 17.0 ± 0.3 % (Table 7).

The sum of the following omega-3 fatty acids was designated total n-3 content in fillets: 1) α-linolenic acid - ALA - 18:3, n-3; 2) eicosatrienoic acid - ETE - 20:3, n-3; 3) eicosapentaenoic acid - EPA - 20:5, n-3; and 4) decosahexaeonic acid - DHA - 22:6, n-3. Omega-3 fatty acid levels for SJR, CS1, and CS2 salmon were 17.6 ± 0.7, 21.6 ± 2.8, and 23.6 ± 0.5 mg/g, respectively. The omega-3 fatty acids, EPA and DHA, which are desired for a variety of human health and nutrition benefits (Simopoulos, 1991) were available in fillets for each cohort (Table 8). Four detectable omega-6 fatty acids, 1) calendic - CA - 18:3, n-6; 2) linoleic - LA - 18:2, n-6c; 3) dihomo-gamma-linoleic - DGLA - 20:3, n-6; and 4) arachidonic acids - AA - 20:4, n-6, were used to calculate total omega-6 fatty acid levels in fillets. Omega-6 fatty acids for SJR, CS1, and CS2 were 11.3 ± 0.4, 10.4 ± 1.3, and 14.8 ± 0.7 mg/g, respectively; resulting in omega 6: 3 ratios of 0.63 ± 0.01, 0.45 ± 0.03, and 0.60 ± 0.06 (Table 8), respectively.

Average SalmoFan™ Lineal fillet color for 4-5 kg salmon from each cohort was 26-29 (Table 6; Fig. 4). Fillet color increased with fish size/age and maximum fillet color was generally
measured at harvest (Fig. 4). The mean SalmoFan™ Lineal score for mature salmon (4-5 kg) was 22-24 and was therefore much paler than fillet color of immature salmon. Fillets of some mature male salmon were completely devoid of red/orange pigmentation and were therefore not quantifiable using SalmoFan™ Lineal score. Fillet color measurements obtained using the Minolta colorimeter for immature market size salmon from SJR (≤ 4 kg), CS1 (~ 4 kg), and CS2 (≥ 5 kg) were: L* (lightness) was 35.3 ± 0.5, 39.1 ± 1.1, and 38.5 ± 0.79, respectively; a* (red) was 8.7 ± 0.4, 12.9 ± 0.6, and 9.2 ± 0.6, respectively; and b* (yellow) was 9.5 ± 0.3, 15.5 ± 0.5, and 11.9 ± 1.0, respectively (Table 7). Minolta fillet color scores were not collected for CS2 salmon.

4. Discussion

4.1. Fish performance metrics

4.1.1. Growth

Each cohort reached market size approximately 9-10 months after stocking in the near-commercial scale RAS. Post-smolt Atlantic salmon (CS1 and SJR) grew an average of 386-393 grams per month to a mean size of 4.1 to 4.2 kg at a mean water temperature of 15-16 °C. CS2 grew at a similar rate (413 g/month) to first harvest at which time the fish were selectively top-graded at 4.9 kg. Thereafter, comparable growth data was not as readily available for CS2 as a result of the top grading harvest technique. Growth data during the grow-out period was approximately linear (Fig. 2) for all three cohorts, suggesting that cumulative weight gain (grams/day) was relatively independent of fish cohort/strain, fish size, and maximum biomass density, which ranged from 35 kg/m³ for SJR to as high as 118 kg/m³ for CS2. Atlantic salmon cultured in the commercial-scale RAS achieved harvest size approximately 2 years from hatch.
The primary separating factors governing age at which the three cohorts reached market-size were: 1) targeted size at harvest and 2) growth rate from approximately 300 to 450 days post hatch, the period immediately following smoltification. Post-smolt salmon appeared to be more fragile during this period, possibly due to lack of salt water exposure; and growth was relatively inconsistent (Fig. 2). CS1 salmon did not encounter a significant growth delay, but the growth rates of SJR and CS2 salmon appeared to be inhibited during this period. CS2 and SJR salmon encountered a relatively mild bacterial gill disease infection and fungus, respectively, during this production phase which likely contributed to reduced growth performance.

During these trials, 2-4 additional months were generally required to reach final harvest, depending on: 1) how much biomass could be moved to market each week, 2) number of fish originally stocked, and 3) final target biomass/ density. Overall, the complete Atlantic salmon grow-out period, including harvest, was generally accomplished in 12 months. Marine Harvest (2015) reported that net-pen-reared Atlantic salmon grow from 0.1 to 4-5 kg in 14-24 months. Use of continuous 24-hr light and consistent water temperature averaging 15-16 °C contributed to the rapid growth to harvest size in the present study. Imsland et al. (2014) reported that the growth-enhancing effect of continuous light alone is equivalent to a 4.5 °C temperature increase towards optimum for Atlantic salmon post-smolt in comparison to a simulated natural photoperiod.

Thermal growth coefficients (1.65-2.01), however, were slightly lower in the present study compared to those reported by the net-pen industry. For example, in 2003, the Scottish and Chilean salmon industries reported average TGCs of 2.36 and 2.37, respectively (Neuman et al., 2004). Thorarensen and Farrell (2011) suggested that under optimal conditions post-smolt Atlantic salmon cultured in closed containment systems should achieve TGCs of 2.7-3.0. A
variety of factors could have contributed to the lower-than-projected TGCs. First, a portion of the population was handled monthly to assess mean weight and other performance metrics. During these sampling events, a large clam shell grader was placed in the tank, which affected the entire population; therefore, feeding was reduced or paused entirely for 1-2 days surrounding each event to alleviate stress. Early maturing males could also have contributed to reduced TGC by transitioning energy typically used for growth toward reproductive development. Additionally, North American-strain Atlantic salmon were evaluated; therefore generalized comparisons should consider the genetic source. Furthermore, freshwater was used exclusively and the growth implications of culturing market-size Atlantic salmon in freshwater versus seawater are not well documented. Lastly, these trials were the first attempts to culture Atlantic salmon on-site in a commercial scale RAS.

4.1.2. Feed conversion

Feed conversion ratios for each grow-out trial (1.07-1.10) were comparable to FCR’s reported by the net cage industry. During an indoor tank study designed to simulate seasonal variation in post-smolt Atlantic salmon growth and FCR of local net cage operations, Nordgarden et al. (2003) found that FCR fluctuated from 0.7-1.7 based on seawater temperature. Johnston et al. (2002) reported FCR’s of 0.90-0.92 for net-cage reared Atlantic salmon fed diets with various protein levels and grown from 0.057-5.36 kg. In contrast, Thorarersen and Farrell (2011) reported an average FCR of approximately 1.26 for commercial farms. Improvements to FCR’s measured during the present trials are expected as feeding protocols are refined and as new diets for post-smolt Atlantic salmon are developed and utilized. Diets fed in RAS must: 1) accommodate the rapid metabolism of the fish raised in RAS environments, 2) produce intact
and settleable fecal matter that can be effectively removed from the culture system, and 3) should minimize excretion of excess phosphorous and nitrogen, in order to minimize environmental impacts and maintain compliance with effluent discharge standards (Davidson et al., 2013).

4.1.3. **Condition factor**

Market-size salmon from each cohort were robust, with deep bodies and relatively high average CFs ranging from 1.69-1.84. Rørå et al. (1998) measured a CF of 1.40 for net-cage-reared Norwegian Atlantic salmon with a mean weight of 4.2 kg. Mørkøre and Rørvik (2001) reported a CF of approximately 1.5 for 4-5+ kg salmon from Norwegian sea cages; and Acharya (2011) measured an average CF of 1.38 for Norwegian salmon > 5 kg. This limited data suggests that RAS-produced salmon could exhibit slightly greater CF compared to net cage-reared salmon. Increased CF could be advantageous, because higher CF generally correlates with a greater percentage of flesh present on the fish body and increased fillet yield (Rørå et al., 1998).

Rasmussen (2001) stated that broader and deeper-bodied fish are considered more desirable by processors due to higher yields. However, elevated CF has also been described as a physiological trigger for maturation. For example, Herbinger and Friars (1991) linked the initiation of Atlantic salmon grilsing with specific levels of lipid storage and a corresponding increase in CF. It is unclear whether the swimming velocity provided in the RAS grow-out system used in the present trials provided optimal exercise and therefore contributed to increased muscle growth and a correspondingly high CF or if the swimming velocity was suboptimal. Davidson and Summerfelt (2004) found that the maximum rotational water velocity in the grow-out tank used in the present trials was approximately 30 cm/sec, which equates to a swimming velocity for market size salmon that is slightly lower than the 1-2 body lengths/sec recommended as optimal by Davison.
Research is needed to determine an optimal swimming velocity for market-size Atlantic salmon cultured in commercial-scale RAS, as measured by effects on growth, condition factor, maturation rate, and other performance metrics.

4.1.4. Fish health

Survival during the grow-out phase was >90% for each trial (Table 4). Fish health screening indicated that all cohorts were free of listed pathogens such as ISAV and IPNV that have historically been a problem for the commercial salmon industry (Mardones et al., 2009; Roberts and Pearson, 2005). In addition, parasites that are common in marine net pen operations, such as kudoa (Dawson-Coates et al., 2003) and sea lice (Revie et al., 2002) were not detected. This was not surprising given the inland location of the operation in West Virginia and exclusive use of freshwater, but nonetheless indicates that these harmful and costly parasites can be excluded when culturing salmon in land-based RAS. The general good health and lack of significant disease events during these trials is particularly noteworthy in the absence of vaccination against specific pathogens, a practice that is normally required in the traditional salmon industry to prevent major losses from disease. Strict biosecurity was maintained, including procurement of specific-pathogen-free eggs and use of: contained systems within enclosed buildings, disinfectant footbaths, hand sanitizer, net and equipment disinfection stations, and an underground spring source that is free of listed pathogens. These biosecurity practices would likely result in cost savings relative to eliminated use of vaccines, chemotherapeutants, and sea lice treatments, as well as the subsequent benefits of general good health such as increased growth performance, reduced stress, and enhanced survival. One anomalous fish health issue, noted only during the second Cascade strain growout trial, was the occurrence of a condition known as systemic
granuloma (fully described by Good et al., 2015b); this pathology was first observed grossly
during end-of-study pathogen screening, at a prevalence of approximately 10-20% of sampled
fish. Further investigation at harvest indicated that this condition was most likely metabolic in
nature, and not the result of an infectious agent (Good et al., 2015b). Systemic granuloma has not
been observed in subsequent salmon growout trials, and the reason(s) for its occurrence in the
CS2 trial remain unclear. Otherwise, the main fish health concern during all grow-out trials was
the occasional bout with *Saprolegnia* spp., i.e. external oomycete infections, typically referred to
as “fungus”. External fungus was treated in the grow-out system with occasional low dose (2-3
ppt) salt treatments, which did not impact biofilter function. Monthly fish handling to obtain
mean weight and growth data likely contributed to external fungal infections, as the protective
slime and scale layers of sampled fish were disturbed during each sampling event. In addition,
during juvenile production, salmon that were smolting or desmoltifying were more susceptible to
fungal infections. Therefore, a longer window should likely be permitted to allow salmon to
transition through these sensitive life stages prior to handling. We estimate that fungus
contributed to about half of the mortalities and culls noted for each production trial; therefore,
more research is necessary to evaluate best methods to avoid and to treat external fungal
infections for Atlantic salmon cultured in freshwater RAS. Recent research indicates that RAS
operations with access to brackish water with 10 to 20 ppt salinity might have an inherent
advantage compared to freshwater operations. During a study evaluating post-smolt Atlantic
salmon performance at various salinities, Ytrestøyl et al. (2013) found that growth performance
and survival was greatest at 12 ppt, and health issues related to fungus were not noted.

4.1.5. Fish density
CS1 and CS2 salmon were cultured at maximum densities of 100 and 118 kg/m$^3$, respectively; these biomass densities did not appear to negatively impact growth (Fig. 2), survival, and other key performance metrics. Turnbull et al. (2008) considered a range of parameters, such as the aforementioned performance variables, as best indicators for fish welfare. While it is difficult to assess welfare in relation to stocking density based on behavioral indicators alone (Turnbull et al., 2008), the Atlantic salmon cultured in the commercial scale RAS during the present trials did not exhibit behavior indicative of compromised welfare. For example, fish were relatively docile, did not compete aggressively for food or exhibit excessive agonistic fish-to-fish interaction, and distributed evenly throughout the grow-out tank.

The amount of fish biomass that can be supported in an intensive aquaculture system is typically defined first by fish metabolism and the resulting rates of oxygen consumption and waste production and second by fish behavior (Wedemeyer, 1996). Recirculating aquaculture systems that use pure oxygen injection and efficient gas transfer devices such as LHO’s, like those utilized during this study, can supersaturate the culture water with oxygen and thereby support high oxygen consumption rates (Colt and Watten, 1988), ultimately allowing safe culture of fish up to 120 kg/m$^3$ (Timmons et al., 2001). In contrast, net cages are generally oxygen limited due to fluctuating environmental conditions (Davis, 1975; Johansson et al., 2006); oxygen saturation as low as 30% has been reported (Oppedal et al., 2011). Therefore, Atlantic salmon densities are typically maintained at 15-25 kg/m$^3$ in net pens (Turnbull et al., 2005; Johansson et al., 2006). Thorarensen and Farrell (2011) reviewed the literature associated with Atlantic salmon rearing density and concluded that post-smolt Atlantic salmon can be cultured up to at least 80 kg/m$^3$ in closed containment systems. Physiological welfare indicators related to density, such as fin erosion and cataracts, were also monitored during sampling events; based on
these qualitative assessments welfare did not appear to be compromised at the selected rearing densities in the closed containment environment; however, additional research designed to measure stress and welfare indicators for post-smolt Atlantic salmon cultured in RAS to \( \geq 100 \) kg/m\(^3\) would be useful. Meanwhile, these trials provide preliminary evidence that 4-5 kg salmon can be effectively cultured in RAS to \( \geq 100 \) kg/m\(^3\) when optimal water quality is maintained.

4.2. Early maturation

The onset of reproductive development in Atlantic salmon is impacted by many factors and is a highly flexible process. The timing and degree of maturation in Atlantic salmon can be influenced by photoperiod (Taranger et al., 1998; Imsland et al., 2014; Melo et al., 2014), water temperature (Adams and Thorpe, 1989; Fjelldal et al., 2011; Imsland et al., 2014), water salinity (Melo et al., 2014), feed intake (Kadri, 2003), vaccination (Fjelldal et al., 2012); nutrition (Alne et al., 2009; Fjelldal et al., 2012), lipid reserves (Rowe and Thorpe, 1990), growth rate (Duston and Saunders, 1999), and stock genetics (Wolters, 2010; Barson et al., 2015). Many of these factors likely interact to influence reproductive development. For example, Imsland et al. (2014) observed increased maturation and much faster growth of male Atlantic salmon cultured with 24-h lighting and warmer water temperatures (12.7 °C v. 8.3 °C) and concluded that photoperiod was the primary directive for the onset of maturation, but temperature likely controlled the magnitude of the photoperiod effect. Melo et al. (2014) found that saltwater, more so than freshwater, stimulated the onset of spermatogenesis in post-smolt Atlantic salmon. In addition, a 12-h light: 12-h dark photoperiod hastened the completion of post-smolt spermatogenesis compared to a continuous 24-h light regime, irrespective of salinity (Melo et al., 2014). Exposure
to 16 °C water and long day lengths at the end of the smoltification regime can also stimulate male maturation (Melo et al., 2014).

The results of the present grow-out trials indicate that early maturation of post-smolt male Atlantic salmon may be more frequent in freshwater RAS (at least under the study conditions) compared to net cage culture; however, the exact cause is unclear. High percentages of early maturing males were removed during each grow-out trial, particularly from the SJR and CS1 cohorts. Coincidentally, both groups were comingled upon stocking in the commercial scale RAS with adult salmon (some of which were maturing) that were awaiting harvest. This is noteworthy because several studies (Good et al., 2014; Mota et al., 2014) have found that steroid hormones produced by maturing fish, including Atlantic salmon, can accumulate in RAS operated at relatively low water exchange rates. Therefore, it is reasonable to hypothesize that hormones released by older, maturing salmon possibly acted to stimulate maturation in younger post-smolt salmon from the SJR and CS1 groups. This hypothesis is supported by the lower percentage of early maturation observed for CS2 salmon which were not comingled with adult salmon, but instead stocked into an empty grow-out system. Albeit, the presence of hormones in the water was not assessed during these trials and differences in other variables such as fish density, water temperature, and pre-smolt culture conditions are confounding and prevent definitive conclusions regarding the cause of grilsing.

Regarding early maturation, some interesting findings resulted from photomanipulation of the CS2 salmon, which were divided amongst a 24-h photoperiod and an 18-h light: 6-h dark regime (Good et al., 2015a). The use of an 18:6 photoperiod during first-year-rearing was associated with increased male maturation. These results are contrary to research by Fjelldal et al. (2011) that showed that fewer male salmon matured early when photoperiod was
manipulated to an 18-hr day versus salmon exposed to continuous 24-hr light. Salmon cultured during the present trials were reared exclusively in freshwater; while salmon described in Fjelldal et al. (2011) were cultured in brackish water during first-year rearing and in seawater for the grow-out phase. Many factors could have interacted to cause the difference in observations between studies.

The prevalence of early maturing males observed during these trials could represent a significant challenge for commercial grow-out of market-size Atlantic salmon in land-based RAS when culturing mixed-sex populations. Early maturing males generally had lighter fillet color and inconsistent fillet texture, and were perceived by seafood distributors as a less than premium product that warranted a reduced market price. Development of strategies to reduce the prevalence of early maturing males in RAS would certainly result in economic benefit. Therefore, research is needed to identify parameters responsible for triggering early maturation in RAS. However, a more expedient approach to resolve the problem is likely the use of an all-female germplasm. As of 2016, all-female eggs are commercially available from an Icelandic egg supplier. All-female eggs have also been produced by a Tasmanian Atlantic salmon company and are currently being evaluated at the Freshwater Institute. Production of all-female salmon would likely eliminate the previously described incidence of grilsing, because the majority of early maturing fish observed during these trials were males. In addition, production of all-female salmon would eliminate aggressive inter-sex behavior that could lead to stress, reduced growth, and increased prevalence of fungus associated with fin-nipping or biting. Selective breeding for reduced early maturation also shows promise, as Barson et al. (2015) recently identified early and late variants of an Atlantic salmon gene that influences age at maturity. In addition, new non-GMO technologies for production of non-maturing fish are
available (Wong and Zohar, 2015) and can potentially be used to produce a mixed-sex population of Atlantic salmon that will not grilse.

4.3. RAS water quality and system performance

The engineering design and unit process efficiencies of the commercial-scale RAS maintained all key water quality concentrations within previously reported safe limits for salmonids (Davidson et al., 2009). The water use metrics employed during these trials can be referenced by system design engineers and RAS production managers. Most importantly, feed loading rate typically ranged from 1-2 kg feed/ m$^3$ of makeup water and daily makeup water addition averaged 80 m$^3$/day.

4.3.1. Water temperature

Average water temperature was maintained between 15-16 °C for each trial, near the reported optimal temperature for post-smolt Atlantic salmon growth (Handeland et al., 2008). Temperature control appears to be an inherent advantage of RAS that can lead to growth optimization. During these grow-out trials, water temperature was controlled by adding more or less cool spring water depending on season to maintain relatively constant temperature.

However, more research is needed to determine the optimal water temperature for post-smolt Atlantic salmon production in freshwater RAS that maximizes growth performance, while limiting early maturation.

4.3.2. Nitrogen
Nitrification across the fluidized-sand biofilter was efficient and reliable. Over almost three years of nearly continuous operation, maximum TAN and NO$_2$-N concentrations measured in the culture tank reached only 0.56 mg/L and 0.21 mg/L, respectively, with mean concentrations less than or equal to 0.30 mg/L and 0.02 mg/L, respectively (Table 5). Nitrate nitrogen, a measure of the intensity of water reuse in the RAS, was intentionally maintained at < 75-100 mg/L based on the findings of Davidson et al. (2014), who evaluated the effects of nitrate on rainbow trout cultured in low exchange RAS. Maximum NO$_3$-N levels reached 60-65 mg/L during the SJR and CS2 trials. Available literature on Atlantic salmon tolerance to nitrate is limited, with the exception of a study by Freitag et al. (2015) who concluded that pre-smolt Atlantic salmon were not negatively affected by NO$_3$-N levels of 101.8 mg/L and were therefore a good candidate species for RAS. Unpublished on-site research indicates that Freitag’s conclusion is consistent for post-smolt Atlantic salmon production. Establishment of an upper nitrate threshold for Atlantic salmon is important for RAS production because it influences the system water exchange rate and the inclusion/exclusion of denitrification unit processes in the RAS design loop.

4.3.3. Alkalinity

The research site for these trials is located with access to an underground spring that supplies an average flow of nearly 4,000 L/min. The karst geology of the aquifer imparts high alkalinity (approximately 250 mg/L as CaCO$_3$) to the spring water, which provides increased buffering capacity to toxicants and other general advantages for fish production. Mean alkalinity was maintained at > 200 mg/L as CaCO$_3$ for each grow-out trial. During periods of maximum feed loading the biofilter consumed alkalinity at a faster rate than supplied by the make-up water.
Periodic addition of sodium bicarbonate was used to maintain alkalinity at > 100 mg/L to ensure optimal biofilter nitrification.

4.3.4. Carbon dioxide

Post-smolt Atlantic salmon were cultured at average CO₂ concentrations ≤ 14 mg/L; however, maximum CO₂ for the CS1 and CS2 trials reached 20 and 24 mg/L, respectively (Table 7). Despite exposure to slightly elevated CO₂ levels, the CS1 and CS2 cohorts grew at a faster rate and demonstrated greater survival compared to SJR salmon which were cultured at CO₂ concentrations ranging from 9 to 16 mg/L. Thus, the Cascade salmon were apparently not impacted by occasional CO₂ concentrations of 20-24 mg/L. These findings are consistent with those of another on-site study which reported no difference in post-smolt Atlantic salmon health, performance, or welfare in replicated RAS with mean CO₂ levels of 10 versus 20 mg/L when the systems were maintained at 12-13 °C and dissolved oxygen kept near saturation (Good et al., 2012). It is important to note that the alkaline culture water available during the present trials sharply contrasts the water quality in Norway, which is typically very soft with low alkalinity and pH (Bergheim et al., 2009). Factors, such as alkalinity, pH, water temperature, and dissolved oxygen interact to influence the CO₂ tolerance threshold of Atlantic salmon and other fish (Fivelstad et al., 1999; Wedemeyer, 1996; Good et al., 2010); therefore, CO₂ concentrations measured during this study appear to be acceptable under the tested conditions but might not be appropriate for commercial salmon grow-out in locations with differing water quality. For example, Fivelstad et al (2015) found that post-smolt salmon growth was suppressed when CO₂ exceeded 19 mg/L in flow through systems using full strength seawater, and nephrocalcinosis occurred at 16 mg/L. The recommended CO₂ concentration for salmon smolt farms in Norway is...
<15 mg/L (FOR, 2004; Bergheim, 2009). More research evaluating CO₂ thresholds for salmon reared in RAS environments is needed.

4.3.5. Total suspended solids

The RAS grow-out system was maintained with relatively low suspended solids by optimizing tank hydraulics, fractionating solids using a dual-drain tank design, and polishing the recycled flow with a microscreen drum filter. In addition, clarity of the culture water was maintained by nearly continuous low-dose ozone injection within the LHO. A culture tank with clearer water could enhance the ability of the fish to see, feed optimally, and grow (Sigler et al., 1984) and allows the farmer to observe fish health, behavior, and feeding activity (Christensen et al., 2000); thus clear water with low suspended solids is likely advantageous for Atlantic salmon production in RAS.

4.4. Waste discharge

Treatment, capture, or removal of phosphorus, nitrogen, and organic matter is seldom achieved in typical culture systems used to produce market-size Atlantic salmon. In contrast, the RAS grow-out system created two discrete effluents, which were treated to remove wastes prior to discharge. The larger of the two effluents was the system overflow, which was nearly equal in volume to the makeup water added to the pump sump. Water quality of the system overflow was similar to the mean tank water quality reported in Table 5; thus, this effluent contained mean concentrations of 0.7-0.9 mg/L total phosphorus, 20-25 mg/L of total inorganic nitrogen (nearly all NO₃-N), and 1-3 mg/L of TSS. These nutrients were concentrated in a relatively small flow that could be treated further, if required by a specific discharge permit. For example, woodchip
bioreactors could likely be used to remove the majority of inorganic nitrogen remaining in the effluent (Lepine et al., 2015). A much smaller volume discharge was created by the drum filter backwash and sediment trap flow flushed from the base of the radial flow settler (Fig. 1).

Reduced scale (1/12) research using replicated systems with the same technology (Davidson et al., 2013) has shown that combined drum filter and settler flushing flows generally average 0.5% of the total recycle flow; thus, the estimated grow-out system backwash volume was approximately 23 L/min. In addition, approximately 22% of the feed is converted to fecal matter, resulting in suspended solids waste that is flushed from the system in these two discrete discharges (Davidson and Summerfelt, 2005). The backwash and flushing flow was treated and dewatered on-site using gravity thickening settlers described by Sharrer et al. (2010) to produce a slurry of approximately 9% dry weight, which was removed by a contract hauler during the present study. Nitrogen and phosphorus contained in these biosolids could potentially be reclaimed as a soil amendment when applied at agronomic rates to row crops or hay fields.

However, in a RAS using brackish water or full-strength seawater, the captured biosolids would have to be further pressed or centrifuged to remove saltwater, producing a relatively dry cake before its use as a soil amendment. A mass balance indicates that approximately 90% of TSS was captured in the gravity thickening settlers, while approximately 10% of the TSS was contained in the supernatant overflowing the gravity thickening settlers and the grow-out system overflow. The combined gravity thickening settler and grow-out system overflows were treated along with other fish production system flows by a central microscreen drum filter, which is followed by a pair of fish exclusion barriers. The treated water leaving the filtration systems discharges to a tributary of the Chesapeake Bay watershed, and was therefore monitored monthly (and more recently weekly) under a pollution discharge permit.
4.5. Fish exclusion

Due to the closed containment design of the RAS grow-out system and the associated waste treatment systems, no salmon escaped the facility. Inherently, it is very difficult for fish to escape the tank and bypass the drain structures; however, in the rare occurrence that a fish passes through the piping, built-in fish exclusion barriers are in place to trap even the smallest fry. The ability of land-based RAS to effectively contain fish and prevent fish escapement to the wild is an added advantage of this technology (Summerfelt and Vinci, 2008).

4.6. Harvesting logistics

Important insight regarding harvesting logistics was gained during each trial. For example, when market-size salmon were harvested at a slower rate, as was the case for the SJR and CS1 groups, the mean weight of fish removed during subsequent harvests continued to increase incrementally. For example, harvesting of SJR and CS1 began when the salmon were just over 4 kg, but mean harvest weights of 4.7 and 5.7 kg were recorded at the conclusion of each respective trial. In contrast, when larger salmon biomasses were removed each week through selective top-grading (as was the case for CS2), the harvesting window was shortened; smaller fish representative of the lower size distribution did not have ample time to grow; and the average harvest weight decreased with time. These experiences demonstrate the importance of harvesting rate when designing a bioplan for market-size salmon production in land-based RAS.

A minimum depuration period of 6 days was used to effectively purge off-flavor compounds (MIB and geosmin) from the salmon flesh. Clean, biofilm-free partial reuse systems operated with rapid water exchange rates (2-3 hr HRT) were used to depurate the salmon. Required time
for depuration may vary among cohorts and production sites; therefore, it is important for each production facility to establish their own standard operating procedure (Davidson et al., 2014).

4.7. Product quality

In general, product quality measurements from salmon cultured to market-size during these trials were comparable to data reported for commercial net cage operations. Fillet fat content of 4-5 kg salmon harvested from RAS during the present trials ranged from approximately 14 to 20%. Mørkøre et al. (2001) reported average fillet fat content ranging from 14.5–21.8% for market-size Atlantic salmon harvested from four Norwegian cage farms where fish were fed standard diets containing 33% lipid and average starvation time prior to harvest was 17 days. Jensen et al. (2012) reported fillet fat content of approximately 12% from Norwegian farmed salmon weighing approximately 3.5 kg; and Acharya (2011) measured an average fillet fat content of 15–16% from Norwegian Atlantic salmon > 5 kg. Mørkøre and Rørvik (2001) found that fillet lipid content of net cage-reared salmon varied seasonally and was dependent on ocean water temperature. Many additional factors can influence the fillet lipid content of cultured salmonids, particularly dietary lipid (Einen and Skrede, 1998; Chaiyapechara et al., 2003). Based on this abbreviated literature review, salmon cultured during the present trials had fillet fat levels that were comparable to Atlantic salmon from the net cage industry.

During the present study, an omega-6:3 ratio of 0.48–0.63 was measured. Jensen et al (2012) assessed the fatty acid content of commercially farmed salmon from Norway and reported an omega 6:3 ratio of 0.44. A standard North American commercial salmon diet was used during the present trials; therefore, the fillet fatty acid content was likely similar to that of commercially
available salmon. Most importantly, the omega-3 fatty acids, EPA and DHA, which are known for consumer health benefits were maintained in Atlantic salmon fillets during these trials.

Average head-on-gutted yield (slaughter yield) of market-size salmon harvested during the present trials ranged from 87.8-91.1%. Similar results were described by Acharya (2011), which reported a slaughter yield of 90.7-90.8% for market-size salmon (> 5 kg) sampled from the Norwegian net-cage industry. Another study reported a slaughter yield for 4-5 kg Norwegian salmon of 90-93% depending on starvation period (Einen et al., 1998). The average butterfly fillet yield or boneless, untrimmed fillet yield measured during the present study was consistent between grow-out trails, ranging from 74.7-74.8%. Acharya (2011) reported an average untrimmed fillet yield from farmed Norwegian Atlantic salmon (> 5 kg mean weight) of 72.2%.

Fillet color gradually increased with time, as carotenoids contained in the feed were deposited in the fillet. When each cohort reached market-size, the red/orange color of fillets still tended to be increasing, indicating some potential for improvement. Optimal salmon fillet coloration is dependent on uptake and storage of astaxanthin and canthaxanthin in the flesh, which is largely affected by composition of these carotenoids in the feed and the feeding regimens employed (Nickell and Springate, 2001). During the present trials, 30 ppm astaxanthin and 30 ppm canthaxanthin were included in the diets. Inclusion of astaxanthin and canthaxanthin in salmon diets is allowable up to a total combined concentration of 80 ppm in the U.S.; therefore, subsequent on-site studies plan to evaluate the effect of maximum carotenoid inclusion.

Generalized comparisons of product quality between RAS-produced salmon and commercial salmon should be considered with perspective, as many variables such as genetics, feed composition, and depuration period, to name a few, can impact these metrics. The provided product quality information is meant to serve as a baseline that can be referenced by prospective...
fish farmers planning to culture salmon in RAS under similar conditions. Other product quality measurements, including fillet thickness, belly flap thickness, and fillet texture are also included as reference for industry.

5. Conclusions

This study suggests that land-based closed-containment systems can be used to produce market size Atlantic salmon in the face of water resource limitations, pollution restrictions, and mounting disease challenges common in open environments. As a proof of concept, the study also suggests that producing salmon in RAS is biologically and technically viable. Data from three successive cohorts of Atlantic salmon highlighted that the fish grow to market size in freshwater in approximately 2 years from hatch with high survival, efficient FCR, acceptable health and welfare, and almost no therapeutic treatment cost. Use of seawater or brackish water was not necessary.

This study did not evaluate production economics; however, Liu et al. (2016) recently provided a detailed assessment of the fixed and variable costs, market development, and potential sale price for market-size Atlantic salmon production in land-based RAS compared to traditional net pen production. The prevalence of early maturing male salmon encountered in the three trials would pose a serious constraint to the economics of production in land-based closed-containment systems. Fortunately, previous yet unpublished research at The Conservation Fund Freshwater Institute indicates that an all-female germplasm can be cultured to market-size to eliminate early maturing male salmon. An all-female source of Atlantic salmon was not available when these three trials were conducted, but at least one source is commercially available, as of 2016.
These findings significantly advance our understanding of Atlantic salmon performance in freshwater recirculation systems. They also inform the existing salmon farming industry, government officials, funders, and conservation advocates on the potential of land-based, freshwater, closed-containment systems for grow-out of Atlantic salmon to market size. If land-based, freshwater, closed-containment systems for producing market-size Atlantic salmon ultimately prove to be cost competitive, this could enable the salmon farming industry to expand production to inland areas adjacent to large markets, where fish escapes, disease, and/or genetic interactions between farmed and wild fish stocks would be less likely.

6. Acknowledgements

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**Tables**

Table 1. Pre-smolt salmon production milestones with corresponding days posthatch (age) and mean weight.
~ Indicates approximate weight, because average weight was not assessed at every milestone.

<table>
<thead>
<tr>
<th>Pre-Smolt Salmon Production Milestones</th>
<th>Days Posthatch</th>
<th>Mean Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>St. John River</td>
<td>Cascade I</td>
</tr>
<tr>
<td>Stocked in nursery system, first feeding</td>
<td>42</td>
<td>34</td>
</tr>
<tr>
<td>S₀ winter photoperiod begins</td>
<td>269</td>
<td>202</td>
</tr>
<tr>
<td>S₀ winter photoperiod ends, 24-h light resumed</td>
<td>312</td>
<td>239</td>
</tr>
<tr>
<td>Precocious males removed</td>
<td>439</td>
<td>249</td>
</tr>
<tr>
<td>Stocked in intermediate partial reuse system</td>
<td>-</td>
<td>250</td>
</tr>
</tbody>
</table>

Table 2. Post-smolt salmon production milestones with corresponding days posthatch (age), mean weight, and number of days of culture in the commercial-scale RAS grow-out system.

<table>
<thead>
<tr>
<th>Post-Smolt Salmon Production Milestones</th>
<th>Days Posthatch/ Days in Grow-out</th>
<th>Mean Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>St. John River</td>
<td>Cascade I</td>
</tr>
<tr>
<td>Stocked in commercial scale WRAS</td>
<td>465/1</td>
<td>417/1</td>
</tr>
<tr>
<td>First male (grilse) salmon harvest</td>
<td>616/152</td>
<td>564/148</td>
</tr>
<tr>
<td>Last male (grilse) salmon harvest</td>
<td>728/264</td>
<td>582/166</td>
</tr>
<tr>
<td>First premium (≥ 4 kg) salmon harvest</td>
<td>759/294</td>
<td>679/262</td>
</tr>
<tr>
<td>Last premium (≥ 4 kg) salmon harvest</td>
<td>831/367</td>
<td>812/396</td>
</tr>
<tr>
<td>Number of weekly premium harvests</td>
<td>5</td>
<td>16</td>
</tr>
</tbody>
</table>
Table 3. Summary of mortalities, culls, jumpers, harvest numbers, and use of fish for each cohort after stocking into the growout system at age 417-465 days posthatch until final harvest.

<table>
<thead>
<tr>
<th></th>
<th>Number of Fish</th>
<th>% of Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>St. John</td>
<td>Cascade I</td>
</tr>
<tr>
<td>Mortalities</td>
<td>82</td>
<td>155</td>
</tr>
<tr>
<td>Culls (Fungus, Unthrifty fish)</td>
<td>114</td>
<td>221</td>
</tr>
<tr>
<td>Jumpers</td>
<td>41</td>
<td>21</td>
</tr>
<tr>
<td>Premium Salmon harvested for market</td>
<td>435</td>
<td>2,752</td>
</tr>
<tr>
<td>Early Maturing Males harvested</td>
<td>751</td>
<td>2,178</td>
</tr>
<tr>
<td>Bottom cull to reduce biomass density</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Salmon harvested for other research</td>
<td>629</td>
<td>324</td>
</tr>
<tr>
<td>Total</td>
<td>2,052</td>
<td>5,651</td>
</tr>
</tbody>
</table>

* Of this total, 90 fish (1.3% of population) were culled directly from the grow-out system and 253 (3.6%) were removed during harvest for quality assurance.

Table 4. Grilse harvest size, harvest biomass, prevalence, and post-harvest use during each trial.

<table>
<thead>
<tr>
<th></th>
<th>St. John River</th>
<th>Cascade I</th>
<th>Cascade II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of weekly grilse harvests</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Grilse harvest mean size (kg)</td>
<td>2.7 &amp; 3.7</td>
<td>2.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Prevalence (% of total population)</td>
<td>36.6</td>
<td>38.5</td>
<td>17.1</td>
</tr>
<tr>
<td>Total grilse harvest (kg)</td>
<td>1,800</td>
<td>5,442</td>
<td>2,657 *</td>
</tr>
<tr>
<td>Other harvest – bottom cull (kg)</td>
<td>-</td>
<td>-</td>
<td>2,868 †</td>
</tr>
<tr>
<td>Post-harvest use</td>
<td>Hot smoked</td>
<td>Cold smoked</td>
<td>Fresh &amp; smoked fillets</td>
</tr>
</tbody>
</table>

* Of the 2,657 kg harvested, 2,330 kg were removed at a mean weight of 2.1 kg and 327 kg were removed for quality assurance during harvest events.
† Bottom cull, mostly immature fish from the bottom size distribution removed to balance end biomass and density objectives.
### Table 5. Average water quality and concentration range during grow-out. Measurements are in mg/L unless otherwise noted.

<table>
<thead>
<tr>
<th>Water Quality</th>
<th>Mean</th>
<th>Min/Max Range</th>
<th>Min/Max Range</th>
<th>Min/Max Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>St. John</td>
<td>Cascade I</td>
<td>Cascade II</td>
<td>St. John</td>
</tr>
<tr>
<td>Alkalinity (as CaCO₃)</td>
<td>212 ± 7</td>
<td>226 ± 3</td>
<td>209 ± 9</td>
<td>114 - 281</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>9 ± 0</td>
<td>14 ± 1</td>
<td>13 ± 1</td>
<td>4 - 16</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>10.9 ± 0.0</td>
<td>11.3 ± 0.1</td>
<td>11.9 ± 0.1</td>
<td>9.5 - 12.5</td>
</tr>
<tr>
<td>Hardness</td>
<td>~ 300</td>
<td>~ 300</td>
<td>~ 300</td>
<td>~ 300</td>
</tr>
<tr>
<td>Nitrite nitrogen</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.02 ± 0.01</td>
<td>0.00 - 0.13</td>
</tr>
<tr>
<td>Nitrate nitrogen</td>
<td>19 ± 2</td>
<td>19 ± 1</td>
<td>24 ± 3</td>
<td>3 - 60</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>15.6 ± 0.0</td>
<td>15.6 ± 0.1</td>
<td>15.2 ± 0.0</td>
<td>14.3 - 17.7</td>
</tr>
<tr>
<td>Total ammonia nitrogen</td>
<td>0.11 ± 0.01</td>
<td>0.22 ± 0.01</td>
<td>0.30 ± 0.03</td>
<td>0.01 - 0.56</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>0.9 ± 0.1</td>
<td>0.7 ± 0.0</td>
<td>0.9 ± 0.1</td>
<td>0.6 - 1.3</td>
</tr>
<tr>
<td>Total suspended solids</td>
<td>1.2 ± 0.1</td>
<td>2.3 ± 0.2</td>
<td>2.5 ± 0.1</td>
<td>0.3 - 4.5</td>
</tr>
</tbody>
</table>

### Table 6. Summary of processing attributes for premium market-size Atlantic salmon from each cohort.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>St. John River</th>
<th>Cascade I (4 kg)</th>
<th>Cascade I (&gt;5 kg)</th>
<th>Cascade II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Fish</td>
<td>21</td>
<td>6</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Days of Purging</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Initial Whole Body Weight (kg)</td>
<td>3.81 ± 0.13</td>
<td>4.21 ± 0.18</td>
<td>5.30 ± 0.14</td>
<td>4.75 ± 0.30</td>
</tr>
<tr>
<td>Head-On-Gutted Yield (%)</td>
<td>-</td>
<td>91.1 ± 0.4</td>
<td>90.5 ± 0.6</td>
<td>87.8 ± 1.3</td>
</tr>
<tr>
<td>Butterfly Fillet Yield (%)</td>
<td>74.8 ± 0.5</td>
<td>74.8 ± 0.4</td>
<td>74.7 ± 0.9</td>
<td>-</td>
</tr>
<tr>
<td>Skin-On Fillet Yield (%)</td>
<td>-</td>
<td>61.2 ± 0.6</td>
<td>61.9 ± 0.9</td>
<td>-</td>
</tr>
<tr>
<td>Skin-Off Fillet Yield (%)</td>
<td>57.8 ± 0.5</td>
<td>57.0 ± 0.6</td>
<td>57.3 ± 0.9</td>
<td>-</td>
</tr>
<tr>
<td>Belly Flap Thickness (mm)</td>
<td>-</td>
<td>-</td>
<td>15.8 ± 0.4</td>
<td>-</td>
</tr>
<tr>
<td>Fillet Thickness (mm)</td>
<td>30.3 ± 0.6</td>
<td>36.3 ± 0.8</td>
<td>34.3 ± 1.2</td>
<td>-</td>
</tr>
</tbody>
</table>

- Indicates data was not collected for specified parameter
Table 7. Summary of fillet quality attributes for premium market-size Atlantic salmon from each cohort.

<table>
<thead>
<tr>
<th></th>
<th>St. John River</th>
<th>Cascade I</th>
<th>Cascade II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(&lt; 4 kg)</td>
<td>(4 kg)</td>
<td>(&gt;5 kg)</td>
</tr>
<tr>
<td>Fillet Moisture (%)</td>
<td>63.8 ± 0.6</td>
<td>63.1 ± 0.6</td>
<td>62.0 ± 0.3</td>
</tr>
<tr>
<td>Fillet Protein (%)</td>
<td>15.9 ± 0.7</td>
<td>20.0 ± 0.2</td>
<td>19.8 ± 0.3</td>
</tr>
<tr>
<td>Fillet Fat (%)</td>
<td>20.4 ± 0.2</td>
<td>15.2 ± 0.7</td>
<td>17.0 ± 0.3</td>
</tr>
<tr>
<td>Fillet Ash (%)</td>
<td>1.5 ± 0.1</td>
<td>1.2 ± 0.0</td>
<td>1.1 ± 0.0</td>
</tr>
<tr>
<td>Total Omega-3 Fatty Acids (mg/g)</td>
<td>17.6 ± 0.7</td>
<td>21.6 ± 2.8</td>
<td>23.6 ± 0.5</td>
</tr>
<tr>
<td>Total Omega-6 Fatty Acids (mg/g)</td>
<td>11.3 ± 0.4</td>
<td>10.4 ± 1.3</td>
<td>14.8 ± 0.7</td>
</tr>
<tr>
<td>Fillet Color (L)</td>
<td>35.3 ± 0.5</td>
<td>39.1 ± 1.1</td>
<td>38.5 ± 0.8</td>
</tr>
<tr>
<td>Fillet Color (A)</td>
<td>8.9 ± 0.4</td>
<td>12.9 ± 0.6</td>
<td>9.2 ± 0.6</td>
</tr>
<tr>
<td>Fillet Color (B)</td>
<td>9.5 ± 0.3</td>
<td>15.5 ± 0.5</td>
<td>11.9 ± 1.0</td>
</tr>
<tr>
<td>Rouche Color Fan Score</td>
<td>28-29</td>
<td>26-27</td>
<td>27-28</td>
</tr>
<tr>
<td>Fillet Texture (g/g wt)</td>
<td>414 ± 13</td>
<td>387 ± 33</td>
<td>394 ± 21</td>
</tr>
</tbody>
</table>
Table 8. Fatty acid composition of market-size (4-5 kg) Atlantic salmon fillets for St. John River (n=18) and Cascade I (n=6) grow-out trials.

<table>
<thead>
<tr>
<th>Fatty acids (mg/g)</th>
<th>% of Total FA</th>
<th>mg/g</th>
<th>% of Total FA</th>
<th>mg/g</th>
<th>% of Total FA</th>
<th>mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>0.05 ± 0.00</td>
<td>0.05 ± 0.00</td>
<td>0.07 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.04 ± 0.02</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>14:0</td>
<td>5.06 ± 0.07</td>
<td>4.79 ± 0.26</td>
<td>5.63 ± 0.13</td>
<td>4.82 ± 0.54</td>
<td>4.54 ± 1.24</td>
<td>5.36 ± 1.81</td>
</tr>
<tr>
<td>14:1</td>
<td>0.06 ± 0.00</td>
<td>0.06 ± 0.00</td>
<td>0.13 ± 0.06</td>
<td>0.12 ± 0.06</td>
<td>0.05 ± 0.03</td>
<td>0.05 ± 0.04</td>
</tr>
<tr>
<td>15:0</td>
<td>0.39 ± 0.00</td>
<td>0.36 ± 0.02</td>
<td>0.28 ± 0.06</td>
<td>0.24 ± 0.06</td>
<td>0.38 ± 0.03</td>
<td>0.44 ± 0.05</td>
</tr>
<tr>
<td>16:0</td>
<td>17.6 ± 0.16</td>
<td>16.6 ± 0.86</td>
<td>16.1 ± 0.66</td>
<td>14.0 ± 1.96</td>
<td>18.3 ± 0.82</td>
<td>21.4 ± 2.40</td>
</tr>
<tr>
<td>16:1</td>
<td>8.17 ± 0.11</td>
<td>7.66 ± 0.35</td>
<td>8.14 ± 0.49</td>
<td>7.06 ± 0.94</td>
<td>8.97 ± 0.56</td>
<td>10.5 ± 1.42</td>
</tr>
<tr>
<td>17:0</td>
<td>0.34 ± 0.00</td>
<td>0.32 ± 0.02</td>
<td>0.29 ± 0.04</td>
<td>0.24 ± 0.05</td>
<td>0.31 ± 0.01</td>
<td>0.36 ± 0.03</td>
</tr>
<tr>
<td>18:0</td>
<td>4.08 ± 0.06</td>
<td>3.86 ± 0.22</td>
<td>5.74 ± 0.66</td>
<td>4.95 ± 0.90</td>
<td>3.97 ± 0.20</td>
<td>4.63 ± 0.26</td>
</tr>
<tr>
<td>18:1, n-9t</td>
<td>0.52 ± 0.12</td>
<td>0.53 ± 0.15</td>
<td>0.11 ± 0.05</td>
<td>0.09 ± 0.05</td>
<td>0.37 ± 0.23</td>
<td>0.43 ± 0.25</td>
</tr>
<tr>
<td>18:1, n-9c</td>
<td>29.3 ± 0.15</td>
<td>27.6 ± 1.38</td>
<td>23.7 ± 0.96</td>
<td>20.1 ± 2.16</td>
<td>28.4 ± 0.72</td>
<td>33.2 ± 3.10</td>
</tr>
<tr>
<td>18:2, n-6c (LA)</td>
<td>10.5 ± 0.11</td>
<td>9.73 ± 0.38</td>
<td>9.48 ± 0.19</td>
<td>8.18 ± 1.01</td>
<td>10.4 ± 0.51</td>
<td>12.1 ± 1.24</td>
</tr>
<tr>
<td>18:3, n-6</td>
<td>0.29 ± 0.01</td>
<td>0.27 ± 0.01</td>
<td>0.43 ± 0.05</td>
<td>0.36 ± 0.06</td>
<td>0.25 ± 0.02</td>
<td>0.29 ± 0.04</td>
</tr>
<tr>
<td>18:3, n-3 (ALA)</td>
<td>3.87 ± 0.04</td>
<td>3.62 ± 0.16</td>
<td>2.56 ± 0.13</td>
<td>2.23 ± 0.31</td>
<td>2.83 ± 0.12</td>
<td>3.31 ± 0.25</td>
</tr>
<tr>
<td>20:0</td>
<td>0.15 ± 0.00</td>
<td>0.15 ± 0.01</td>
<td>0.14 ± 0.01</td>
<td>0.12 ± 0.02</td>
<td>0.13 ± 0.03</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>20:1</td>
<td>2.69 ± 0.04</td>
<td>2.51 ± 0.12</td>
<td>1.98 ± 0.13</td>
<td>1.71 ± 0.23</td>
<td>2.08 ± 0.22</td>
<td>2.42 ± 0.24</td>
</tr>
<tr>
<td>20:2</td>
<td>0.52 ±0.01</td>
<td>0.49 ± 0.04</td>
<td>0.79 ± 0.17</td>
<td>0.69 ± 0.18</td>
<td>0.57 ± 0.11</td>
<td>0.67 ± 0.13</td>
</tr>
<tr>
<td>20:3, n-6</td>
<td>0.33 ± 0.01</td>
<td>0.31 ± 0.02</td>
<td>0.37 ± 0.02</td>
<td>0.31 ± 0.04</td>
<td>0.29 ± 0.04</td>
<td>0.34 ± 0.05</td>
</tr>
<tr>
<td>20:3, n-3</td>
<td>0.31 ± 0.01</td>
<td>0.30 ± 0.02</td>
<td>0.23 ± 0.01</td>
<td>0.20 ± 0.03</td>
<td>0.25 ± 0.06</td>
<td>0.29 ± 0.07</td>
</tr>
<tr>
<td>20:4, n-6</td>
<td>0.71 ± 0.01</td>
<td>0.66 ± 0.02</td>
<td>1.04 ± 0.03</td>
<td>0.89 ± 0.11</td>
<td>0.80 ± 0.05</td>
<td>0.93 ± 0.05</td>
</tr>
<tr>
<td>20:5, n-3 (EPA)</td>
<td>5.17 ± 0.08</td>
<td>4.81 ± 0.18</td>
<td>7.24 ± 0.25</td>
<td>6.19 ± 0.73</td>
<td>5.48 ± 0.44</td>
<td>6.38 ± 0.40</td>
</tr>
<tr>
<td>22:0</td>
<td>0.11 ± 0.01</td>
<td>0.11 ± 0.02</td>
<td>0.02 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>22:1, n-9</td>
<td>0.35 ± 0.01</td>
<td>0.33 ± 0.02</td>
<td>0.26 ± 0.02</td>
<td>0.23 ± 0.03</td>
<td>0.23 ± 0.08</td>
<td>0.27 ± 0.09</td>
</tr>
<tr>
<td>22:6, n-3 (DHA)</td>
<td>9.26 ± 0.19</td>
<td>8.56 ± 0.30</td>
<td>15.2 ± 1.20</td>
<td>13.0 ± 1.84</td>
<td>11.2 ± 1.09</td>
<td>13.0 ± 0.97</td>
</tr>
<tr>
<td>24:0</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>&lt; det</td>
<td>0.00 ± 0.00</td>
<td>&lt; det</td>
</tr>
<tr>
<td>24:1</td>
<td>0.18 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.14 ± 0.04</td>
<td>0.11 ± 0.03</td>
<td>0.16 ± 0.06</td>
<td>0.18 ± 0.06</td>
</tr>
</tbody>
</table>

< det = below detection limit
Figures

Fig. 1. Process flow drawing of the commercial scale recirculation aquaculture system used to culture post-smolt Atlantic salmon to market-size (Summerfelt et al., 2009a). Courtesy Kata Rishel, Freshwater Institute Engineering Services.

Fig. 2. Growth performance (average salmon weight) from fry stage to market-size for the three Atlantic salmon cohorts evaluated. Day 1 of the life cycle is equivalent with egg hatch.

Fig. 3. Gonadosomatic index as it relates to mean salmon weight for each salmon grow-out trial.

Fig. 4. Relationship of size and red/orange fillet color for immature Atlantic salmon (GSI > 1.0 %) from each cohort (at left) and for maturing Atlantic salmon (GSI > 1.0%), i.e. mostly males (at right).
Figure 1
Figure 2
Assessment of gonadosomatic index concluded when male salmon reached a mean weight of approximately 3.5 kg because all males were culled from the population and absent thereafter.

The two notations for GSI of “Maturing Females” indicate: the first time that GSI >1.0% was measured for any female over the sampling duration (n=3) and the GSI of several maturing females (n=7) sampled during the final harvest. Maturing females represented < 2-3% of the population.
Figure 4