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

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16

17  **Abstract**

18
19      There is interest in culturing Atlantic salmon *Salmo salar* to market-size in land-based,

20  closed containment systems that use recirculation aquaculture systems (RAS), as this technology

21  often enables facilities to locate near major markets, obtain permits, exclude obligate pathogens,

22  and/or reduce environmental impacts. Use of land-based RAS to intensively culture market-size

23  Atlantic salmon is a relatively new frontier and little information is available. Three trials were

24  conducted to evaluate the performance of two North American strains of Atlantic salmon raised

25  from post-smolt to market-size (4-5 kg) in a near-commercial scale (260 m$^3$), land-based RAS

26  using only freshwater. St. John River (SJR) salmon were reared during the first trial, and

27  Cascade salmon (CS1 and CS2) were evaluated during two subsequent trials. Salmon were

28  received as fertilized "eyed" eggs and cultured on-site through the entire production cycle. The

29  grow-out period began at 14-16 months post-hatch when salmon post-smolt weighed 0.34 - 0.75

30  kg on average. CS1 and SJR salmon grew 386-393 g/month to a mean size of 4.1-4.2 kg and CS2

31  salmon grew 413 g/month to a mean size of 4.9 kg prior to first harvest. Thereafter, weekly

32  salmon harvests commenced for the next 6-19 weeks. The grow-out period, excluding harvest,

33  lasted 9-10 months for each trial. Average water temperature was maintained at 15-16 $^{\circ}$ C.

34  Consistently linear growth rates were achieved by each population suggesting that growth was

35  relatively independent of fish cohort/genetic strain, fish size, and maximum biomass density,

36  which was 35, 100, and 118 kg/m$^3$ for SJR, CS1, and CS2, respectively. Feed conversion ratios

37  ranged from 1.07-1.10. Fish mortality (including culls) for SJR, CS1, and CS2 was 9.5, 6.6, and

38  7.5 % of the original number of stocked fish, respectively. No obligate fish pathogens, kudoa, sea

39  lice, or pervasive parasites were detected. Salmon were not vaccinated against specific

40  pathogens; and no antibiotics, pesticides, or harsh chemotherapeutants were used. Hydrogen

41  peroxide (50-100 ppm) and salt (10 ppt) were occasionally used to treat fungus during pre-smolt

42  production, and salt (2-3 ppt) was used to treat fungus or ameliorate stress after handling events.

43  No salmon escaped the facility due to built-in fish exclusion barriers. Early male maturation was

44  observed during each trial. Male salmon began to exhibit maturation traits (kype, darkened skin

45  coloration) at a mean weight of 1.5-2 kg and were removed from the grow-out system when they

46  weighed 2-3 kg. SJR, CS1, and CS2 populations exhibited 37.0, 38.5, and 17.0 % maturity,

47  respectively. Fillet yield and product quality of immature, market-size salmon were comparable

48  to reported measurements for commercially available salmon reared in net pens. This research

49  suggests that it is biologically and technologically feasible to culture Atlantic salmon from post-

50  smolt to market-size in a land-based RAS of suitable commercial scale; however, early male

51  maturation could represent a production barrier. As of 2016, all-female Atlantic salmon eggs are

52  commercially available and could provide an expedient solution to the problem of early male

53  maturation in RAS.

54

55  *Keywords*: Atlantic salmon; recirculation aquaculture systems; land-based; closed containment

56

## 1. Introduction

57

58

59    There is interest in culturing Atlantic salmon *Salmo salar* to market-size in land-based,

60    closed containment systems using water recirculation technology (Summerfelt and Christianson,

61    2014). These culture systems provide an alternate approach that isolates fish from potentially

62    sensitive marine ecosystems, while supplying built-in measures to prevent environmental

63    impacts such as the discharge of nutrients and particulates, escapees, and fish pathogens.

64    Recirculation aquaculture systems (RAS) provide advantages such as: 1) reduced water use in

65    the face of diminishing resources (Kristensen et al., 2009); 2) small-volume, concentrated

66    effluents that can be effectively treated to minimize pollution (Sharrer et al., 2010); 3) optimized

67    culture environment that can be tuned to meet the biological requirements of fish (Summerfelt et

68    al., 2001); 4) enhanced biosecurity and disease control (Bebak-Williams et al., 2001); 5)

69    containment of non-native fish to prevent interaction with wild populations (Summerfelt and

70    Vinci, 2008); and 6) opportunity for vertical integration and increased revenue through the

71    recovery or value-added use of waste stream nitrogen and phosphorus for practices such as

72    aquaponics (Adler et al., 2000). Recirculation aquaculture systems also provide increased

73    potential for siting where energy and other resources are affordable or near major seafood

74    markets, which could lead to enhanced product quality and reduced carbon footprint of the

75    shipped product (Martins et al., 2010; Liu et al., 2016).

76    Use of RAS to culture Atlantic salmon is not a novel practice. Some commercial salmon

77    companies are now producing smolts using RAS (Bergheim et al., 2009). However, juvenile

78    Atlantic salmon are typically cultured in single-pass, land-based aquaculture systems using

79    freshwater until the fish undergo smoltification. Salmon smolts are then transported from

80  onshore tanks to marine net cages for continued culture to market-size. In recent years, salmon

81  smolts have been cultured to approximately 140-170 g in land-based systems prior to relocation

82  to net cages for grow-out (Bergheim et al., 2009). However, an increasing number of Norwegian

83  and Faroe Islands facilities are planning to culture salmon smolts and post-smolts to larger sizes

84  (250-1000 g) in land-based RAS and partial reuse systems (with some already in operation), in

85  order to reduce sea lice susceptibility, increase fish robustness, reduce mortality during the sea

86  phase, and to decrease overall production time (Bergheim et al., 2009; Dalsgaard et al., 2013;

87  Ytrestøyl et al., 2013).

88      Although Atlantic salmon smolt production in RAS is becoming more common, very little

89  published data is available that describes the production and performance metrics of salmon

90  raised to market-size in land-based RAS. Thus, three trials were conducted evaluating the

91  performance of several groups of North American-strain Atlantic salmon cultured in freshwater

92  from fertilized "eyed" egg to market-size food fish, primarily in RAS. The grow-out culture

93  period from post-smolt to market-size in a near-commercial-scale RAS was the primary focus of

94  this research. Results from these trials will provide information on the technical and biological

95  feasibility of commercial production of market-size Atlantic salmon in land-based recirculation

96  aquaculture systems, which will facilitate decision-making by the salmon farming industry,

97  investors, aquaculture researchers, and engineers.

98
99
100  **2.  Materials and Methods**

101
102  *2.1. Atlantic salmon*

103      Three groups of mixed-sex, North American-strain Atlantic salmon were evaluated

104  including: a St. John River (SJR) strain from Cooke Aquaculture (Bingham, ME, USA); and two

105 cohorts of Cascade salmon (CS1 and CS2) from Icicle Seafoods, Inc. (Seattle, WA, USA). All

106 procured egg lots were specific-pathogen-free-certified and each germplasm was diploid. Salmon

107 were raised entirely in freshwater from fertilized "eyed" egg to market-size food fish (4-5 kg).

108

109 *2.2. Incubation*

110     Eyed eggs were iodine disinfected upon arrival and placed in an 8-stack Heath-Tecna

111 incubator (Marisource, Tacoma, WA, USA) within a RAS equipped with two chillers, an

112 ultraviolet irradiation unit, two pumps to recirculate the water, and a water aeration column. SJR,

113 CS1, and CS2 egg lots were received at 356, 330, and 349 accumulated temperature units

114 (ATUs), respectively. Each egg lot was incubated at an average water temperature of 7-8 $^{\circ}$C.

115 Designation of Day 1 of the growth cycle corresponded with 50 % egg hatch. After hatching,

116 alevins remained in the system until the majority of the yolk sac was absorbed. Prior to complete

117 yolk-sac absorption, water temperature was gradually increased to 10 $^{\circ}$C to acclimate fish for

118 transfer to a nursery system. Atlantic salmon eggs/ alevins were kept in the incubation system for

119 an average of 48 days or 440 ATUs. Survival for SJR, CS1, and CS2 during this phase was 84,

120 85, and 90 % respectively.

121

122 *2.3. Nursery system*

123     Juvenile Atlantic salmon were transferred from the incubation system to a single-pass, flow-

124 through nursery system with twelve 0.5 $m^3$ circular tanks, maintained at 12-14 $^{\circ}$C. The system

125 was enclosed by a tent constructed with opaque plastic to omit natural light. Artificial light was

126 provided by overhead, full-spectrum incandescent bulbs. Atlantic salmon fry were fed

127 commercially available diets from Bio-Oregon (Westbrook, ME, USA) and Zeigler Brothers Inc.

128    (Gardners, PA, USA) using a computer operated feeding program (Freshwater Institute,

129    Shepherdstown, WV, USA) integrated with automated feeders (Model 907, Sterner Fish Tech

130    AS feeders, Ski, Norway) to deliver precise feed amounts at set intervals. Feed was provided

131    hourly during the "lights-on" phase of specific photoperiod regimens. Feeding rates were

132    determined using standardized feeding charts provided by feed suppliers and industry and by

133    observations of feeding response and wasted feed. Daily feeding rates for first-feeding salmon

134    began near 4.5 % of tank biomass and declined to approximately 2 % by the end of this period.

135    When juvenile salmon were stocked in the nursery system, a 24-h continuous light

136    photoperiod was used, with one exception; half of the CS2 cohort was reared under an 18-h light:

137    6-h dark photoperiod (Good et al., 2015a). After 7-9 months of culture (approximately 40-60 g

138    mean weight; Table 1), all fish were subjected to a photoperiod described by industry as an "$S_0$

139    winter", a lighting regimen designed to provide a short day length and thereby trigger

140    smoltification in the first year of the salmon life cycle. During the artificial winter, each cohort

141    was exposed to a 12-h light: 12-h dark photoperiod lasting about 6 weeks. At the end of this

142    period, 24-h continuous light was generally reinstituted. The only exception was for CS2; these

143    fish were returned to their original photoperiod treatments, either 24-h light or 18-h light: 6-h

144    dark. These photoperiod regimens continued when CS2 was transferred to a partial reuse system

145    in order to evaluate the long-term effect of light treatments on early maturation (Good et al.,

146    2015a). Additional detail regarding the timing and length of photoperiod manipulation and other

147    pre-smolt milestones is provided in Table 1.

148

149    *2.4. Post-smolt production*

150   CS1 and CS2 salmon were transferred to a partial reuse system when they reached 250 and

151   270 days post-hatch or 70 and 107 g, respectively. The partial reuse system was equipped with

152   three 10 m$^3$ dual-drain culture tanks, a microscreen drum filter, a pump sump, a forced

153   ventilation cascade aeration column, and a low head oxygenator (LHO) and sump (Summerfelt et

154   al., 2004). The system recycled approximately 85 % of water relative to the recycle flow. Solids

155   laden water (15 % of recycle flow) discharged through the bottom center drain of each tank and

156   was replaced with an equal volume of spring water, which provided enough dilution to limit

157   ammonia accumulation in the absence of a biofilter. Water temperature ranged from 12-14 $^{\circ}$C,

158   depending on season. Lighting was mainly provided by overhead, metal halide bulbs; however,

159   full-spectrum compact fluorescent bulbs were used when tanks were enclosed for photoperiod

160   treatment of the CS2 salmon.

161   Each group of salmon was fed commercially available diets from Bio-Oregon (Westbrook,

162   ME, USA) and EWOS (Surrey, British Columbia, Canada) at a rate of 1-2 % of the tank

163   biomass, depending on mean fish weight. Diets contained 43-47 % protein and 24 % fat and

164   were supplemented with 30 ppm astaxanthin and 30 ppm canthaxanthin. Feed was distributed by

165   individual screw-auger-style feeders with 20 kg hoppers (Pentair Aquatic Ecosystems, Apopka,

166   FL, USA). Nine equally spaced feeding events occurred each day around-the-clock or, in the

167   case of specific photoperiod treatments, only when lights were on. Time and duration of feeding

168   events were set using a timer control system that was integrated with the feeders.

169

170   *2.5. Grow-out*

171   SJR salmon remained in the nursery system until they were 15.5 months old and weighed

172   340 g; at this time 2,052 fish were moved to a near-commercial scale (260 m$^3$) grow-out system

173    with a 150 m$^3$ culture tank (Davidson and Summerfelt, 2005). CS1 and CS2 salmon (5,651 and

174    6,906 fish, respectively) were transferred from the post-smolt system to the grow-out system at

175    13.9 and 14.3 months post-hatch when they weighed 750 and 510 g, respectively (Table 2). Due

176    to tank space limitations, SJR and CS1 were comingled for 1-2 months with separate groups of

177    market-size Atlantic salmon that were nearing the end of production. CS2 salmon were stocked

178    into an empty grow-out system after fish from the previous cohort had been harvested. CS2

179    salmon from each of two photoperiod treatments were fin clipped for future identification.

180        The grow-out system used two 5-HP centrifugal pumps to move 4,900 L/min of water from

181    the lowest hydraulic grade-line (a pump sump) to the highest elevation, the top of a cyclonic

182    fluidized-sand biofilter. Water exiting the biofilter gravity flowed through a forced-ventilation

183    cascade aeration column, a LHO and LHO sump, and entered the culture tank through a water

184    distribution manifold (Fig. 1). The majority of recycled water (90 %) was discharged from the

185    culture tank at a side-box drain and gravity flowed through a microscreen drum filter equipped

186    with 90-µm sieve panels and into a pump sump, where the water recycling process began again.

187    The remaining 10 % of flow flushed through the tank's bottom center drain to a radial flow

188    settler equipped with an automated valve that opened approximately once an hour to flush

189    settleable solids collected in the cone-bottom. Biosolids backwashed from the drum filter and

190    settler were collected and dewatered on-site using gravity thickening settlers. Some of the

191    overtopping flow leaving the radial flow settler was released from the system and replaced with

192    cool, 12-13 $^{\circ}$C spring water. This discharge rate varied from 0-178 L/min depending on season

193    and was regulated to maintain the system water temperature at 15-17 $^{\circ}$C; it was controlled by

194    directing more or less flow back into the system via manual valve adjustment. Discharged

195    system water was replaced with spring water at an average makeup flow rate of 55 L/min or 80

196    m$^3$/day. System hydraulic retention time ranged from 1-15 days and feed loading rate was 1-2 kg

197    feed/ m$^3$ daily makeup water. The water flow rate through the culture tank created a mean tank

198    hydraulic retention time of 30 min. Ozone was generated from a 99.5 % pure oxygen feed gas by

199    a System GM-2 generator (Primozone, Löddeköpinge, Sweden) and injected into the LHO.

200    Oxidation reduction potential (ORP) was measured by an SC100 Universal Controller (Hach

201    Company, Loveland, CO, USA) during the first trial and a YSI 5500D unit (YSI Inc., Yellow

202    Springs, OH, USA) for the last two trials. An on/off feedback loop between the ORP monitoring

203    systems and the ozone generator was used to maintain ORP between 280-320 mV to control

204    water clarity while maintaining ozone residual at safe levels (Summerfelt et al., 2009a). A 24-h

205    photoperiod was provided with overhead metal halide lights (400 Watt; 23500 lumens; 4000 K

206    color temperature). Each cohort was fed a commercially available diet (Dynamic Red ™,

207    EWOS, Surrey, British Columbia, Canada). Diets contained 40-45 % protein and 29-30 % fat

208    and were supplemented with 30 ppm astaxanthin and 30 ppm canthaxanthin. Feed was

209    distributed by two screw-auger feeders with 60 kg hoppers (Pentair Aquatic Ecosystems, Inc.,

210    Apopka, FL, USA) equipped with spreaders. Nine equally spaced feeding events occurred each

211    day around-the-clock. Time and duration of feeding events were set using a timer control system

212    integrated with the feeders. Fish were fed to near-satiation by adjusting feeding rates based on

213    feeding activity and daily observations of wasted feed flushed from the tank through a bottom

214    center-drain standpipe. Maximum daily feed delivered to the grow-out tank was approximately

215    100 kg for each cohort.

216

217    *2.6. Fish sampling procedures*

218    *Performance metrics*

219     Weight samples of 10-60 randomly selected fish, usually measured as bulk weights, were

220     collected at monthly intervals for assessment of mean weights and calculation of growth

221     performance metrics. Fish were crowded using a clam shell grader and then netted from the tank

222     for each sampling event. Thermal growth coefficients (TGC), economic feed conversion ratio

223     (FCR), condition factor (CF), and gonadosomatic index (GSI) were calculated as follows:

224

225     TGC = (End Weight $^{(1/3)}$ – Start Weight $^{(1/3)}$) / ((Days Between $*$ Avg. Temp.) $*$ 1000)

226     Where weight is in grams and temperature is in $^{\circ}$ C.

227     FCR = Cumulative Feed Delivered / Fish Biomass Gain

228     CF = 100,000 $*$ Weight/ (Length) $^{3}$

229     GSI (%) = (Gonad Weight/ Whole Fish Body Weight) $*$100

230

231     *Fish Health*

232     Fish populations were observed daily and, when practical, individuals with external lesions

233     (e.g. skin ulceration, severely eroded fins, or significant external *Saprolegnia* spp. infections)

234     were removed and humanely euthanized. During performance sampling events, each sampled

235     fish was likewise inspected, and those demonstrating external lesions were removed from the

236     population. Following American Fisheries Society Fish Health Section guidelines (AFS-FHS,

237     2014), sixty fish from each cohort were euthanized near the end of each production cycle and

238     screened for listed bacterial, viral, and parasitic fish pathogens. Tissue samples were tested by

239     Kennebec River Biosciences (Richmond, ME, USA) for the following pathogens: infectious

240     salmon anemia virus (ISAV), infectious pancreatic necrosis virus (IPNV), viral hemorrhagic

241     septicemia virus (VHSV), Oncorhynchus masou virus (OMV), spring viremia of carp virus

242   (SVCV); *Aeromonas salmonicida* (causative agent of furunculosis), *Renibacterium*

243   *salmoninarum* (bacterial kidney disease), *Yersinia ruckeri* (enteric redmouth disease), *Myxobolus*

244   *cerebralis* (whirling disease), and *Ceratomyxa shasta* (ceratomyxosis). Additionally, PCR was

245   used to screen for the myxosporean parasite *Kudoa thyrsites* due to concerns in the Atlantic

246   salmon industry in British Columbia, Canada regarding product downgrading as a consequence

247   of fillet infiltration and consequent post-mortem myoliquification (Dawson-Coates et al., 2003).

248   *Kudoa* screening was carried out for CS1 and CS2, but not for SJR salmon, on muscle samples

249   taken from a standardized fillet section from 60 fish and tested in pooled 5-fish batches.

250

251   *2.7. Culling and harvesting*

252   <u>*Precocious males*</u>

253   Early maturing males are undesirable due to potentially reduced growth and decreased feed

254   conversion efficiency (McClure et al., 2007), as well as reduced product quality (Aksnes et al.,

255   1986); and were therefore removed at various points in the production cycle (Table 1, 2). Mature

256   males were identified and removed as precocious parr during the pre-smolt phase and as "grilse"

257   during the grow-out phase. Identification was based on colorimetric and/or morphometric

258   changes; precocious parr demonstrated a bronze or yellow color, often with red spots and free-

259   flowing milt, and grilse demonstrated darker coloration and developing kype. Grilse were

260   observed in substantial numbers during the grow-out phase and were specifically harvested when

261   mean fish weight was $\geq 2$ kg, by crowding the population using a clam-shell grader (Summerfelt

262   et al., 2009b) and subsequently netting individuals that displayed the aforementioned

263   characteristics. Time post-hatch and mean weight of maturing males upon removal are described

264   for each cohort (Tables 1 and 2).

265

*Premium salmon harvest*

267    Harvest of market-size salmon began when the mean population weights were $\geq 4$ kg.

268  Salmon were crowded using a custom aluminum and polyethylene clam-shell grader (Emperor

269  Aquatics Inc., Pottstown, PA, USA). Average size fish were harvested weekly from the SJR and

270  CS1 cohorts; while the largest salmon were selectively top-graded from the CS2 population

271  during each harvest. Salmon from each cohort were harvested at different rates (Table 2)

272  depending on arrangements with processors and distributors. Harvest methods included hand-

273  netting of crowded fish, as well as use of the tank's side-wall-box that was designed to collect

274  fish and dewater the flow for ease of fish handling and sorting (Summerfelt et al., 2009b).

275  Salmon were then transported to two partial reuse depuration systems, each equipped with a

276  single 11 $m^3$ culture tank. Hydraulic retention time of the depuration systems averaged 2-3 hours.

277  Salmon were kept off feed and typically purged for 6 days according to standard operating

278  procedures described in Davidson et al. (2014). Following purging, salmon were euthanized and

279  bled using a model SI-7C percussive stunner (Baader Seafood Innovations, Cleveland, Australia)

280  and held in an ice slurry for approximately 30 mins to quickly cool the fish prior to packing on

281  ice. Harvested salmon were then sent to a local processing facility where they were filleted and

282  sold into various markets in Canada and the United States.

283

284  *2.8. Product quality*

285    A representative number of harvested salmon were filleted and evaluated for the following

286  product quality attributes: head-on-gutted yield, butterfly fillet yield, trimmed skin-on and

287  skinless fillet yield, belly flap and fillet thickness, fillet color, fillet proximate composition, and

288  fillet fatty acid content. Proximate composition of skinless fillets was measured (AOAC, 1990)

289  once for SJR when the salmon were close to 4 kg; and twice for CS1 at approximately 4 and 5

290  kg. Fillet proximate composition was not assessed for CS2 salmon. Fillet color was analyzed

291  using a chromameter (Model CR-300; Minolta Camera Co. Ltd., Osaka, Japan) calibrated with a

292  standard white plate No.21333180 (CIE L* 93.1; a* 0.3135; b* 0.3198). Visual fillet color

293  assessment was made using the Roche SalmoFan™ Lineal, generally at monthly intervals after

294  fish reached 1 kg and continuing to harvest. Texture of cooked fillets was measured by placing

295  fillets skin-side up, in a Kramer Shear Cell. Shear force was measured using a Texture Analyzer

296  (model TA-Hdi, Texture Technologies Corp., Scarsdale, NY, USA) equipped with a 5-blade

297  Kramer shear attachment at a cross speed of 127 mm/min. Values were expressed as peak force

298  generated per gram of sample. For subsequent fatty acid analyses, total lipids were extracted with

299  a chloroform: methanol mixture (2:1, v/v) using the method of Bligh and Dyer (1959).

300

301  *2.9. Water quality analyses*

302  Water samples were collected weekly and tested on-site for total ammonia nitrogen (TAN),

303  nitrite nitrogen ($NO_2$-N), nitrate nitrogen ($NO_3$-N), carbon dioxide ($CO_2$), alkalinity, total

304  suspended solids (TSS), and total phosphorus (TP). All parameters were analyzed according to

305  methods described in APHA (2005, 2012) and HACH (2003). Dissolved oxygen and temperature

306  of the grow-out system were recorded daily from continuous monitoring systems, including a

307  PT4 unit (Point Four Systems, Inc., British Columbia, CA) equipped with Oxyguard probes

308  (Oxyguard International, Farum, Denmark) during the first trial and a YSI 5500D with optical

309  probes (YSI Inc., Yellow Springs, OH, USA) for the last two trials. Oxidative reduction potential

310  was measured at the culture tank inlet and sidewall box outlet by differential ORP digital sensors

311 with platinum electrode (Model DRD1R5, Hach Company, Loveland, CO, USA) and displayed

312 by an SC100 Universal Controller (Hach Company, Loveland, CO, USA) for the first trial. ORP

313 and pH were monitored with the YSI 5500D system for the last two trials.

314

315 **3. Results**

316 *3.1. Growth and survival  (Grow-Out)*

317 <u>*St. John River salmon*</u>

318    SJR salmon grew from 0.34 to 4.2 kg in 9.8 months (393 g/month) in the near-commercial-

319 scale RAS (Fig. 2). At this time, harvest of market size (4.2 kg mean weight) salmon began.

320 Salmon continued to grow during the 10-week harvest period. By the end of the harvest cycle,

321 mean fish weight was 4.7 kg (Fig. 2) and corresponding CF was $1.83 \pm 0.03$. Thermal growth

322 coefficient from time of stocking to first harvest was 2.01. Economic FCR, which accounted for

323 all feed delivered to the fish over the grow-out trial duration, was 1.09. Maximum biomass

324 density was 35 kg/m$^3$, which is relatively low, but was intentionally kept at $< 40$ kg/m$^3$ because

325 this was the first attempt to culture Atlantic salmon on-site under these conditions. The total

326 production period including approximately 2.5 months of harvesting lasted just over 12 months.

327 Total mortality including culls (fish with fungus, unthrifty fish, or fish removed for quality

328 assurance at harvest) and jumpers (salmon that perished by leaping over surrounding jump

329 screens) was 11.4 % of fish stocked. Of this total, 3.9 % represented in-tank mortalities, 5.6 %

330 were culls, and 1.9 % were jumpers (Table 3).

331

332 <u>*Cascade I salmon*</u>

14

333    CS1 salmon grew from 0.75 to 4.1 kg in 8.7 months (386 g/month) in the commercial-scale

334    RAS (Fig. 2). Harvests began when CS1 salmon reached a mean weight of 4.1 kg and continued

335    thereafter for the next 4 months. Remaining salmon continued to grow during the harvest period

336    and achieved a mean weight of 5.7 kg with a CF of $1.84 \pm 0.06$ by the end of the trial. Thermal

337    growth coefficient from time of stocking to first harvest was 1.65;  economic FCR was 1.07; and

338    maximum biomass density reached 100 kg/m$^3$. The total production period, including

339    approximately 4 months of harvesting, lasted just over 13 months. Total mortality, including

340    culls and jumpers, was 7.0 % of fish stocked. Of this total, 2.7 % were in-tank mortalities, 3.9 %

341    were culls, and 0.4 % were jumpers (Table 3).

342

343    *Cascade II salmon*

344    CS2 salmon grew from 0.51 to 4.9 kg in 10.6 months (413 g/month). At this time, a selective

345    harvest of the largest salmon (4.9 kg mean weight) began. Thermal growth coefficient averaged

346    1.86 and economic FCR was 1.10 over the grow-out trial duration. Maximum biomass density

347    was 118 kg/m$^3$. Salmon were harvested at a faster rate during this trial,  approximately 2,000 kg

348    per event over a 6-wk period. The largest fish were removed during each harvest; therefore,

349    average fish weight diminished from 4.9 kg at initial harvest to approximately 3.5 kg at final

350    harvest. Condition factor at the onset of harvesting was $1.69 \pm 0.10$. Total production period

351    including approximately 1.5 months of harvesting lasted exactly 12 months. Total mortality,

352    including culls and jumpers, was 8.2 % of fish stocked. Of this total, 2.6 % were in-tank

353    mortalities, 4.9 % were culls, and 0.7% were jumpers (Table 3).

354

355    *3.2. Early maturing males (grilse)*

356       Removal of grilse from the SJR, CS1, and CS2 cohorts began when fish were 19-21 months

357    old and weighed 2.7, 2.6, and 2.1 kg, respectively (Table 2). Initial grilse culling events took

358    place after approximately 5-6 months of production in the grow-out system (Table 2). A second

359    culling event was conducted for the SJR salmon to remove the remainder of early maturing

360    males at approximately 24 months post-hatch when the mean population weight was 3.7 kg

361    (Table 2, 4). Several additional culling events were conducted within 20 days of the initial for

362    CS1 and CS2 to remove the remaining mature males from the tank. A total of 1,800, 5,442, and

363    2,657 kg of grilse biomass (whole, uncut body weight) was harvested for the SJR, CS1, and CS2

364    cohorts, respectively (Table 4). Early maturing males made up approximately 37, 38.5, and 17 %

365    of the SJR, CS1, and CS2 populations, respectively (Table 4). Fillets of early maturing males

366    were generally leaner and contained less pigmentation compared to fillets of immature fish at

367    final harvest. Various product forms were tested including standard fillets and hot and cold

368    smoked product (Table 4).

369

370    *3.3. Gonadosomatic index*

371      GSI assessment of maturing males from the SJR and CS2 cohorts indicated that grilse were

372    present in these populations at 17-19 months of age when the mean population weight was 1.3-

373    1.4 kg (Fig. 3). The majority of female salmon did not mature during these trials. The average

374    GSI of immature salmon (mostly females) from each cohort  did not increase beyond 0.5 % (Fig.

375    3). A few maturing females were sampled during the SJR and CS1 trials, but only one maturing

376    female was noted during sampling events for the CS2 trial (Fig. 3). Approximately $\leq$ 2-3 % of

377    the female population had a GSI $> 1$ %.

378

379     *3.4. Premium salmon harvests*

380       Harvesting began when each cohort reached a mean weight $\geq$ 4 kg. SJR, CS1, and CS2

381     weighed 4.2, 4.1, and 4.9 kg and were 25.3, 22.6, and 24.9 months old, respectively, when

382     harvests commenced. At this stage of production, each group had been in the grow-out system

383     for 8.5-10.5 months. Total premium biomass (whole, uncut fish) of 5,200 kg (435 fish), 13,382

384     kg (2,752 fish), and 12,695 kg (2,952 fish) was harvested for the SJR, CS1, and CS2 cohorts,

385     respectively (Table 3). The amount of biomass removed during each harvest was generally

386     related to the rate at which value-chain sectors could process and distribute the product. SJR

387     salmon were used for test marketing or distributed to local food banks, while CS1 and CS2

388     salmon were sold through various seafood distribution companies. SJR, CS1, and CS2 salmon

389     were harvested throughout 5, 16, and 7 events, over 10, 19, and 6 weeks, respectively (Table 2).

390     After the first two trials, the process had been streamlined for CS2, resulting in faster salmon

391     removal and a substantially shorter harvesting window.

392

393     *3.5. Fish health*

394       No major fish health events occurred during any of the grow-out trials, and no listed fish

395     pathogens were detected. In addition, no kudoa *(Kudoa thyrsites)* was detected and no sea lice

396     were observed; although detection of these salt water parasites was not expected in a land-based,

397     freshwater environment. The only occasional fish health issue, affecting each cohort to varying

398     degrees, was the occurrence of external *Saprolegnia* spp., i.e. "fungal" infections, or

399     Saprolegniasis. No vaccines were administered at any stage of production. In addition, no

400     formalin, chemicals, antibiotics, pesticides, or other chemotherapeutants were used to treat

401     salmon during these grow-out trials, with the exception of low concentrations (2-3 ppt) of

402    sodium chloride which was used to relieve handling stress related to harvesting and fish

403    sampling events and to control Saprolegniasis. Hydrogen peroxide (50-100 ppm) and salt bath

404    treatments (10 ppt) were used to ameliorate Saprolegniasis during egg incubation and fry

405    production in the flow-through nursery system.

406

407    *3.6. Water quality*

408        Average water temperature for the SJR, CS1, and CS2 grow-out trials was 15.6, 15.6, and

409    15.2 $^{\circ}$ C, respectively; and dissolved oxygen was maintained at or above 90 % saturation (Table

410    5). Average TAN was $\leq 0.3$ mg/L and max TAN was $\leq 0.6$ mg/L; while mean $NO_2$-N was $\leq 0.02$

411    mg/L and max $NO_2$-N never exceeded 0.21 mg/L, indicating efficient biofilter performance.

412    Mean $NO_3$-N for the SJR, CS1, and CS2 trials was 19-24 mg/L and max $NO_3$-N was 60, 32, and

413    65 mg/L, respectively. Mean TSS was $< 3$ mg/L and max TSS never exceeded 5 mg/L. Average

414    $CO_2$ ranged from 9-14 mg/L during the three trials; while max $CO_2$ for SJR, CS1, and CS2

415    salmon was 16, 24, and 20 mg/L, respectively. Mean TP ranged from 0.7-0.9 mg/L with max

416    levels reaching 1.3-2.2 mg/L, and average alkalinity was maintained at $> 200$ mg/L (as $CaCO_3$)

417    primarily through addition of highly alkaline makeup water. Water hardness, an indicator of

418    calcium, magnesium, and other low-level cation levels in fish culture systems, was not assessed;

419    however, previous on-site studies have shown that make-up and RAS water hardness are

420    typically near 300 mg/L as $CaCO_3$ (Davidson et al., 2009; 2013).

421

422    *3.7. Fillet and product quality attributes*

423        Processing yield was similar for each cohort despite slight differences in average harvest

424    weight (Table 6). Head-on-gutted yield for CS1 and CS2 was $91.1 \pm 0.4$ and $90.5 \pm 0.6$ %,

425    respectively. Butterfly fillet yield for SJR, CS1, and CS2 was 74.8 ± 0.5, 74.8 ± 0.4, and 74.7 ±

426    0.9 %, respectively. Skin-on fillet yield for CS1 and CS2 was 61.2 ± 0.6 and 61.9 ± 0.9 %,

427    respectively; and skinless fillet yield for SJR, CS1, and CS2 was 57.8 ± 0.5, 57.0 ± 0.6, and 57.3

428    ± 0.9 %, respectively (Table 6). Head-on-gutted and skin-on fillet yield were not assessed for

429    SJR salmon.

430        Fillet composition was measured for SJR and CS1 salmon of similar size (approximately 4

431    kg). SJR and CS1fillets had moisture content of 63.8 ± 0.6 and 63.1 ± 0.6 %; protein content of

432    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

433    ± 0.1 and 1.2 ± 0.0 %, respectively. As CS1 salmon grew from 4 to ≥ 5 kg, fillet protein content

434    remained about the same, but fillet fat increased from 15.2 ± 0.7 to 17.0 ± 0.3 % (Table 7).

435        The sum of the following omega-3 fatty acids was designated total n-3 content in fillets: 1) α-

436    linolenic acid - ALA - 18:3, n-3; 2) eicosatrienoic acid - ETE - 20:3, n-3; 3) eicosapentaeonic

437    acid - EPA - 20:5, n-3; and 4) decosahexaeonic acid - DHA - 22:6, n-3. Omega-3 fatty acid

438    levels for SJR, CS1, and CS2 salmon were 17.6 ± 0.7, 21.6 ± 2.8, and 23.6 ± 0.5 mg/g,

439    respectively. The omega-3 fatty acids, EPA and DHA, which are desired for a variety of human

440    health and nutrition benefits (Simopoulus, 1991) were available in fillets for each cohort (Table

441    8). Four detectable omega-6 fatty acids, 1) calendic - CA - 18:3, n-6; 2) linoleic - LA - 18:2, n-

442    6c; 3) dihomo-gamma-linoleic - DGLA - 20:3, n-6; and 4) arachidonic acids - AA - 20:4, n-6,

443    were used to calculate total omega-6 fatty acid levels in fillets. Omega-6 fatty acids for SJR,

444    CS1, and CS2 were 11.3 ± 0.4, 10.4 ± 1.3, and 14.8 ± 0.7 mg/g, respectively; resulting in omega

445    6: 3 ratios of 0.63 ± 0.01, 0.45 ± 0.03, and 0.60 ± 0.06 (Table 8), respectively.

446        Average SalmoFan™ Lineal fillet color for 4-5 kg salmon from each cohort was 26-29

447    (Table 6; Fig. 4). Fillet color increased with fish size/age and maximum fillet color was generally

448    measured at harvest (Fig. 4). The mean SalmoFan™ Lineal score for mature salmon (4-5 kg) was

449    22-24 and was therefore much paler than fillet color of immature salmon. Fillets of some mature

450    male salmon were completely devoid of red/orange pigmentation and were therefore not

451    quantifiable using SalmoFan™ Lineal score. Fillet color measurements obtained using the

452    Minolta colorimeter for immature market size salmon from SJR ($\leq$ 4 kg), CS1 (~ 4 kg), and CS2

453    ($\geq$ 5 kg) were: L* (lightness) was 35.3 ± 0.5, 39.1 ± 1.1, and 38.5 ± 0.79, respectively; a* (red)

454    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,

455    and 11.9 ± 1.0, respectively (Table 7). Minolta fillet color scores were not collected for CS2

456    salmon.

457

458    **4.  Discussion**

459    *4.1. Fish performance metrics*

460    *4.1.1.  Growth*

461         Each cohort reached market size approximately 9-10 months after stocking in the near-

462    commercial scale RAS. Post-smolt Atlantic salmon (CS1 and SJR) grew an average of 386-393

463    grams per month to a mean size of 4.1 to 4.2 kg at a mean water temperature of 15-16 $^{\circ}$ C. CS2

464    grew at a similar rate (413 g/month) to first harvest at which time the fish were selectively top-

465    graded at 4.9 kg. Thereafter, comparable growth data was not as readily available for CS2 as a

466    result of the top grading harvest technique. Growth data during the grow-out period was

467    approximately linear (Fig. 2) for all three cohorts, suggesting that cumulative weight gain

468    (grams/day) was relatively independent of fish cohort/strain, fish size, and maximum biomass

469    density, which ranged from 35 kg/m$^3$ for SJR to as high as 118 kg/m$^3$ for CS2. Atlantic salmon

470    cultured in the commercial-scale RAS achieved harvest size approximately 2 years from hatch.

471    The primary separating factors governing age at which the three cohorts reached market-size

472    were: 1) targeted size at harvest and 2) growth rate from approximately 300 to 450 days post

473    hatch, the period immediately following smoltification. Post-smolt salmon appeared to be more

474    fragile during this period, possibly due to lack of salt water exposure; and growth was relatively

475    inconsistent (Fig. 2). CS1 salmon did not encounter a significant growth delay, but the growth

476    rates of SJR and CS2 salmon appeared to be inhibited during this period. CS2 and SJR salmon

477    encountered a relatively mild bacterial gill disease infection and fungus, respectively, during this

478    production phase which likely contributed to reduced growth performance.

479    During these trials, 2-4 additional months were generally required to reach final harvest,

480    depending on: 1) how much biomass could be moved to market each week, 2) number of fish

481    originally stocked, and 3) final target biomass/ density. Overall, the complete Atlantic salmon

482    grow-out period, including harvest, was generally accomplished in 12 months. Marine Harvest

483    (2015) reported that net-pen-reared Atlantic salmon grow from 0.1 to 4-5 kg in 14-24 months.

484    Use of continuous 24-hr light and consistent water temperature averaging 15-16 $^{o}$C  contributed

485    to the rapid growth to harvest size in the present study. Imsland et al. (2014) reported that the

486    growth-enhancing effect of continuous light alone is equivalent to a 4.5 $^{\circ}$ C temperature increase

487    towards optimum for Atlantic salmon post-smolt in comparison to a simulated natural

488    photoperiod.

489    Thermal growth coefficients (1.65-2.01), however, were slightly lower in the present study

490    compared to those reported by the net-pen industry. For example, in 2003, the Scottish and

491    Chilean salmon industries reported average TGCs of 2.36 and 2.37, respectively (Neuman et al.,

492    2004). Thorarensen and Farrell (2011) suggested that under optimal conditions post-smolt

493    Atlantic salmon cultured in closed containment systems should achieve TGCs of 2.7-3.0. A

494    variety of factors could have contributed to the lower-than-projected TGCs. First, a portion of the

495    population was handled monthly to assess mean weight and other performance metrics. During

496    these sampling events, a large clam shell grader was placed in the tank, which affected the entire

497    population; therefore, feeding was reduced or paused entirely for 1-2 days surrounding each

498    event to alleviate stress. Early maturing males could also have contributed to reduced TGC by

499    transitioning energy typically used for growth toward reproductive development. Additionally,

500    North American-strain Atlantic salmon were evaluated; therefore generalized comparisons

501    should consider the genetic source. Furthermore, freshwater was used exclusively and the growth

502    implications of culturing market-size Atlantic salmon in freshwater versus seawater are not well

503    documented. Lastly, these trials were the first attempts to culture Atlantic salmon on-site in a

504    commercial scale RAS.

505

506    *4.1.2.  Feed conversion*

507    Feed conversion ratios for each grow-out trial (1.07-1.10) were comparable to FCR's

508    reported by the net cage industry. During an indoor tank study designed to simulate seasonal

509    variation in post-smolt Atlantic salmon growth and FCR of local net cage operations,

510    Nordgarden et al. (2003) found that FCR fluctuated from 0.7-1.7 based on seawater temperature.

511    Johnston et al. (2002) reported FCR's of 0.90-0.92 for net-cage reared Atlantic salmon fed diets

512    with various protein levels and grown from 0.057-5.36 kg. In contrast, Thorarensen and Farrell

513    (2011) reported an average FCR of approximately 1.26 for commercial farms. Improvements to

514    FCR's measured during the present trials are expected as feeding protocols are refined and as

515    new diets for post-smolt Atlantic salmon are developed and utilized. Diets fed in RAS must: 1)

516    accommodate the rapid metabolism of the fish raised in RAS environments, 2) produce intact

517 and settleable fecal matter that can be effectively removed from the culture system, and 3) should

518 minimize excretion of excess phosphorous and nitrogen, in order to minimize environmental

519 impacts and maintain compliance with effluent discharge standards (Davidson et al., 2013)

520

521 *4.1.3. Condition factor*

522     Market-size salmon from each cohort were robust, with deep bodies and relatively high

523 average CFs ranging from 1.69-1.84. Rørå et al. (1998) measured a CF of 1.40 for net-cage-

524 reared Norwegian Atlantic salmon with a mean weight of 4.2 kg. Mørkøre and Rørvik (2001)

525 reported a CF of approximately 1.5 for 4-5+ kg salmon from Norwegian sea cages; and Acharya

526 (2011) measured an average CF of 1.38 for Norwegian salmon > 5 kg. This limited data suggests

527 that RAS-produced salmon could exhibit slightly greater CF compared to net cage-reared

528 salmon. Increased CF could be advantageous, because higher CF generally correlates with a

529 greater percentage of flesh present on the fish body and increased fillet yield (Rørå et al., 1998).

530 Rasmussen (2001) stated that broader and deeper-bodied fish are considered more desirable by

531 processors due to higher yields. However, elevated CF has also been described as a physiological

532 trigger for maturation. For example, Herbinger and Friars (1991) linked the initiation of Atlantic

533 salmon grilsing with specific levels of lipid storage and a corresponding increase in CF. It is

534 unclear whether the swimming velocity provided in the RAS grow-out system used in the present

535 trials provided optimal exercise and therefore contributed to increased muscle growth and a

536 correspondingly high CF or if the swimming velocity was suboptimal. Davidson and Summerfelt

537 (2004) found that the maximum rotational water velocity in the grow-out tank used in the present

538 trials was approximately 30 cm/sec, which equates to a swimming velocity for market size

539 salmon that is slightly lower than the 1-2 body lengths/sec recommended as optimal by Davison

540    (1997). Research is needed to determine an optimal swimming velocity for market-size Atlantic

541    salmon cultured in commercial-scale RAS, as measured by effects on growth, condition factor,

542    maturation rate, and other performance metrics.

543

544    *4.1.4.  Fish health*

545    Survival during the grow-out phase was >90 % for each trial (Table 4). Fish health screening

546    indicated that all cohorts were free of listed pathogens such as ISAV and IPNV that have

547    historically been a problem for the commercial salmon industry (Mardones et al., 2009; Roberts

548    and Pearson, 2005). In addition, parasites that are common in marine net pen operations, such as

549    kudoa (Dawson-Coates et al., 2003) and sea lice (Revie et al., 2002) were not detected. This was

550    not surprising given the inland location of the operation in West Virginia and exclusive use of

551    freshwater, but nonetheless indicates that these harmful and costly parasites can be excluded

552    when culturing salmon in land-based RAS. The general good health and lack of significant

553    disease events during these trials is particularly noteworthy in the absence of vaccination against

554    specific pathogens, a practice that is normally required in the traditional salmon industry to

555    prevent major losses from disease. Strict biosecurity was maintained, including procurement of

556    specific-pathogen-free eggs and use of: contained systems within enclosed buildings, disinfectant

557    footbaths, hand sanitizer, net and equipment disinfection stations, and an underground spring

558    source that is free of listed pathogens. These biosecurity practices would likely result in cost

559    savings relative to eliminated use of vaccines, chemotherapeutants, and sea lice treatments, as

560    well as the subsequent benefits of general good health such as increased growth performance,

561    reduced stress, and enhanced survival. One anomalous fish health issue, noted only during the

562    second Cascade strain growout trial, was the occurrence of a condition known as systemic

24

563    granuloma (fully described by Good et al., 2015b); this pathology was first observed grossly

564    during end-of-study pathogen screening, at a prevalence of approximately 10-20 % of sampled

565    fish. Further investigation at harvest indicated that this condition was most likely metabolic in

566    nature, and not the result of an infectious agent (Good et al., 2015b). Systemic granuloma has not

567    been observed in subsequent salmon growout trials, and the reason(s) for its occurrence in the

568    CS2 trial remain unclear. Otherwise, the main fish health concern during all grow-out trials was

569    the occasional bout with *Saprolegnia* spp., i.e. external oomycete infections, typically referred to

570    as "fungus". External fungus was treated in the grow-out system with occasional low dose (2-3

571    ppt) salt treatments, which did not impact biofilter function. Monthly fish handling to obtain

572    mean weight and growth data likely contributed to external fungal infections, as the protective

573    slime and scale layers of sampled fish were disturbed during each sampling event. In addition,

574    during juvenile production, salmon that were smolting or desmoltifying were more susceptible to

575    fungal infections. Therefore, a longer window should likely be permitted to allow salmon to

576    transition through these sensitive life stages prior to handling. We estimate that fungus

577    contributed to about half of the mortalities and culls noted for each production trial; therefore,

578    more research is necessary to evaluate best methods to avoid and to treat external fungal

579    infections for Atlantic salmon cultured in freshwater RAS. Recent research indicates that RAS

580    operations with access to brackish water with 10 to 20 ppt salinity might have an inherent

581    advantage compared to freshwater operations. During a study evaluating post-smolt Atlantic

582    salmon performance at various salinities, Ytrestøyl et al. (2013) found that growth performance

583    and survival was greatest at12 ppt, and health issues related to fungus were not noted.

584

585    *4.1.5. Fish density*

586    CS1 and CS2 salmon were cultured at maximum densities of 100 and118 kg/m$^3$,

587    respectively; these biomass densities did not appear to negatively impact growth (Fig. 2),

588    survival, and other key performance metrics. Turnbull et al. (2008) considered a range of

589    parameters, such as the aforementioned performance variables, as best indicators for fish

590    welfare. While it is difficult to assess welfare in relation to stocking density based on behavioral

591    indicators alone (Turnbull et al., 2008), the Atlantic salmon cultured in the commercial scale

592    RAS during the present trials did not exhibit behavior indicative of compromised welfare. For

593    example, fish were relatively docile, did not compete aggressively for food or exhibit excessive

594    agonistic fish-to-fish interaction, and distributed evenly throughout the grow-out tank.

595    The amount of fish biomass that can be supported in an intensive aquaculture system is

596    typically defined first by fish metabolism and the resulting rates of oxygen consumption and

597    waste production and second by fish behavior (Wedemeyer, 1996). Recirculating aquaculture

598    systems that use pure oxygen injection and efficient gas transfer devices such as LHO's, like

599    those utilized during this study, can supersaturate the culture water with oxygen and thereby

600    support high oxygen consumption rates (Colt and Watten, 1988), ultimately allowing safe culture

601    of fish up to 120 kg/m$^3$ (Timmons et al., 2001). In contrast, net cages are generally oxygen

602    limited due to fluctuating environmental conditions (Davis, 1975; Johansson et al., 2006);

603    oxygen saturation as low as 30 % has been reported (Oppedal et al., 2011). Therefore, Atlantic

604    salmon densities are typically maintained at 15-25 kg/m$^3$ in net pens (Turnbull et al., 2005;

605    Johansson et al., 2006). Thorarensen and Farrell (2011) reviewed the literature associated with

606    Atlantic salmon rearing density and concluded that post-smolt Atlantic salmon can be cultured

607    up to at least 80 kg/m$^3$ in closed containment systems. Physiological welfare indicators related to

608    density, such as fin erosion and cataracts, were also monitored during sampling events; based on

609    these qualitative assessments welfare did not appear to be compromised at the selected rearing

610    densities in the closed containment environment; however, additional research designed to

611    measure stress and welfare indicators for post-smolt Atlantic salmon cultured in RAS to $\geq$ 100

612    kg/m$^3$ would be useful. Meanwhile, these trials provide preliminary evidence that 4-5 kg salmon

613    can be effectively cultured in RAS to $\geq$ 100 kg/m$^3$ when optimal water quality is maintained.

614

*4.2. Early maturation*

616    The onset of reproductive development in Atlantic salmon is impacted by many factors and is

617    a highly flexible process. The timing and degree of maturation in Atlantic salmon can be

618    influenced by photoperiod (Taranger et al., 1998; Imsland et al., 2014; Melo et al., 2014), water

619    temperature (Adams and Thorpe, 1989; Fjelldal et al., 2011; Imsland et al., 2014), water salinity

620    (Melo et al., 2014), feed intake (Kadri, 2003), vaccination (Fjelldal et al., 2012); nutrition (Alne

621    et al., 2009; Fjelldal et al., 2012), lipid reserves (Rowe and Thorpe, 1990), growth rate (Duston

622    and Saunders, 1999), and stock genetics (Wolters, 2010; Barson et al., 2015). Many of these

623    factors likely interact to influence reproductive development. For example, Imsland et al. (2014)

624    observed increased maturation and much faster growth of male Atlantic salmon cultured with 24-

625    h lighting and warmer water temperatures (12.7 ° C v. 8.3 ° C) and concluded that photoperiod

626    was the primary directive for the onset of maturation, but temperature likely controlled the

627    magnitude of the photoperiod effect. Melo et al. (2014) found that saltwater, more so than

628    freshwater, stimulated the onset of spermatogenesis in post-smolt Atlantic salmon. In addition, a

629    12-h light: 12-h dark photoperiod hastened the completion of post-smolt spermatogenesis

630    compared to a continuous 24-h light regime, irrespective of salinity (Melo et al., 2014). Exposure

631  to 16 ° C water and long day lengths at the end of the smoltification regime can also stimulate

632  male maturation (Melo et al., 2014).

633      The results of the present grow-out trials indicate that early maturation of post-smolt male

634  Atlantic salmon may be more frequent in freshwater RAS (at least under the study conditions)

635  compared to net cage culture; however, the exact cause is unclear. High percentages of early

636  maturing males were removed during each grow-out trial, particularly from the SJR and CS1

637  cohorts. Coincidentally, both groups were comingled upon stocking in the commercial scale

638  RAS with adult salmon (some of which were maturing) that were awaiting harvest. This is

639  noteworthy because several studies (Good et al., 2014; Mota et al., 2014) have found that steroid

640  hormones produced by maturing fish, including Atlantic salmon, can accumulate in RAS

641  operated at relatively low water exchange rates. Therefore, it is reasonable to hypothesize that

642  hormones released by older, maturing salmon possibly acted to stimulate maturation in younger

643  post-smolt salmon from the SJR and CS1 groups. This hypothesis is supported by the lower

644  percentage of early maturation observed for CS2 salmon which were not comingled with adult

645  salmon, but instead stocked into an empty grow-out system. Albeit, the presence of hormones in

646  the water was not assessed during these trials and differences in other variables such as fish

647  density, water temperature, and pre-smolt culture conditions are confounding and prevent

648  definitive conclusions regarding the cause of grilsing.

649      Regarding early maturation, some interesting findings resulted from photomanipulation of

650  the CS2 salmon, which were divided amongst a 24-h photoperiod and an 18-h light: 6-h dark

651  regime (Good et al., 2015a). The use of an18:6 photoperiod during first-year-rearing was

652  associated with increased male maturation. These results are contrary to research by Fjelldal et

653  al. (2011)  that showed that fewer male salmon matured early when photoperiod was

654 manipulated to an 18-hr day versus salmon exposed to continuous 24-hr light. Salmon cultured

655 during the present trials were reared exclusively in freshwater; while salmon described in

656 Fjelldal et al. (2011) were cultured in brackish water during first-year rearing and in seawater for

657 the grow-out phase. Many factors could have interacted to cause the difference in observations

658 between studies.

659      The prevalence of early maturing males observed during these trials could represent a

660 significant challenge for commercial grow-out of market-size Atlantic salmon in land-based

661 RAS when culturing mixed-sex populations. Early maturing males generally had lighter fillet

662 color and inconsistent fillet texture, and were perceived by seafood distributors as a less than

663 premium product that warranted a reduced market price. Development of strategies to reduce the

664 prevalence of early maturing males in RAS would certainly result in economic benefit.

665 Therefore, research is needed to identify parameters responsible for triggering early maturation

666 in RAS. However, a more expedient approach to resolve the problem is likely the use of an all-

667 female germplasm. As of 2016, all-female eggs are commercially available from an Icelandic

668 egg supplier.  All-female eggs have also been produced by a Tasmanian Atlantic salmon

669 company and are currently being evaluated at the Freshwater Institute. Production of all-female

670 salmon would likely eliminate the previously described incidence of grilsing, because the

671 majority of early maturing fish observed during these trials were males. In addition, production

672 of all-female salmon would eliminate aggressive inter-sex behavior that could lead to stress,

673 reduced growth, and increased prevalence of fungus associated with fin-nipping or biting.

674 Selective breeding for reduced early maturation also shows promise, as Barson et al. (2015)

675 recently identified early and late variants of an Atlantic salmon gene that influences age at

676 maturity. In addition, new non-GMO technologies for production of non-maturing fish are

677 available (Wong and Zohar, 2015) and can potentially be used to produce a mixed-sex

678 population of Atlantic salmon that will not grilse.

679

680 *4.3. RAS water quality and system performance*

681 The engineering design and unit process efficiencies of the commercial-scale RAS

682 maintained all key water quality concentrations within previously reported safe limits for

683 salmonids (Davidson et al., 2009). The water use metrics employed during these trials can be

684 referenced by system design engineers and RAS production managers. Most importantly, feed

685 loading rate typically ranged from 1-2 kg feed/ $m^3$ of makeup water and daily makeup water

686 addition averaged 80 $m^3$/day.

687

688 *4.3.1.  Water temperature*

689 Average water temperature was maintained between 15-16 $^o$C for each trial, near the

690 reported optimal temperature for post-smolt Atlantic salmon growth (Handeland et al., 2008).

691 Temperature control appears to be an inherent advantage of RAS that can lead to growth

692 optimization. During these grow-out trials, water temperature was controlled by adding more or

693 less cool spring water depending on season to maintain relatively constant temperature.

694 However, more research is needed to determine the optimal water temperature for post-smolt

695 Atlantic salmon production in freshwater RAS that maximizes growth performance, while

696 limiting early maturation.

697

698 *4.3.2.  Nitrogen*

699    Nitrification across the fluidized-sand biofilter was efficient and reliable. Over almost three

700    years of nearly continuous operation, maximum TAN and $NO_2$-N concentrations measured in the

701    culture tank reached only 0.56 mg/L and 0.21 mg/L, respectively, with mean concentrations less

702    than or equal to 0.30 mg/L and 0.02 mg/L, respectively (Table 5). Nitrate nitrogen, a measure of

703    the intensity of water reuse in the RAS, was intentionally maintained at $< 75\text{-}100$ mg/L based on

704    the findings of Davidson et al. (2014), who evaluated the effects of nitrate on rainbow trout

705    cultured in low exchange RAS. Maximum $NO_3$-N levels reached 60-65 mg/L during the SJR and

706    CS2 trials. Available literature on Atlantic salmon tolerance to nitrate is limited, with the

707    exception of a study by Freitag et al. (2015) who concluded that pre-smolt Atlantic salmon were

708    not negatively affected by $NO_3$-N levels of 101.8 mg/L and were therefore a good candidate

709    species for RAS. Unpublished on-site research indicates that Freitag's conclusion is consistent

710    for post-smolt Atlantic salmon production. Establishment of an upper nitrate threshold for

711    Atlantic salmon is important for RAS production because it influences the system water

712    exchange rate and the inclusion/exclusion of denitrification unit processes in the RAS design

713    loop.

714

715    *4.3.3.  Alkalinity*

716    The research site for these trials is located with access to an underground spring that supplies

717    an average flow of nearly 4,000 L/min. The karst geology of the aquifer imparts high alkalinity

718    (approximately 250 mg/L as $CaCO_3$) to the spring water, which provides increased buffering

719    capacity to toxicants and other general advantages for fish production. Mean alkalinity was

720    maintained at $> 200$ mg/L as $CaCO_3$ for each grow-out trial. During periods of maximum feed

721    loading the biofilter consumed alkalinity at a faster rate than supplied by the make-up water.

722    Periodic addition of sodium bicarbonate was used to maintain alkalinity at > 100 mg/L to ensure

723    optimal biofilter nitrification.

724

725    *4.3.4.   Carbon dioxide*

726         Post-smolt Atlantic salmon were cultured at average $CO_2$ concentrations $\leq$ 14 mg/L;

727    however, maximum $CO_2$ for the CS1 and CS2 trials reached 20 and 24 mg/L, respectively (Table

728    7). Despite exposure to slightly elevated $CO_2$ levels, the CS1 and CS2 cohorts grew at a faster

729    rate and demonstrated greater survival compared to SJR salmon which were cultured at $CO_2$

730    concentrations ranging from 9 to 16 mg/L. Thus, the Cascade salmon were apparently not

731    impacted by occasional $CO_2$ concentrations of 20-24 mg/L. These findings are consistent with

732    those of another on-site study which reported no difference in post-smolt Atlantic salmon health,

733    performance, or welfare in replicated RAS with mean $CO_2$ levels of 10 versus 20 mg/L when the

734    systems were maintained at 12-13 $^{\circ}$C and dissolved oxygen kept near saturation (Good et al.,

735    2012). It is important to note that the alkaline culture water available during the present trials

736    sharply contrasts the water quality in Norway, which is typically very soft with low alkalinity

737    and pH (Bergheim et al., 2009). Factors, such as alkalinity, pH, water temperature, and dissolved

738    oxygen interact to influence the $CO_2$ tolerance threshold of Atlantic salmon and other fish

739    (Fivelstad et al., 1999; Wedemeyer, 1996; Good et al., 2010); therefore, $CO_2$ concentrations

740    measured during this study appear to be acceptable under the tested conditions but might not be

741    appropriate for commercial salmon grow-out in locations with differing water quality. For

742    example, Fivelstad et al (2015) found that post-smolt salmon growth was suppressed when $CO_2$

743    exceeded 19 mg/L in flow through systems using full strength seawater, and nephrocalcinosis

744    occurred at 16 mg/L. The recommended $CO_2$ concentration for salmon smolt farms in Norway is

745 &lt;15 mg/L (FOR, 2004; Bergheim, 2009). More research evaluating $CO_2$ thresholds for salmon

746 reared in RAS environments is needed.

747

748 *4.3.5.  Total suspended solids*

749 The RAS grow-out system was maintained with relatively low suspended solids by

750 optimizing tank hydraulics, fractionating solids using a dual-drain tank design, and polishing the

751 recycled flow with a microscreen drum filter. In addition, clarity of the culture water was

752 maintained by nearly continuous low-dose ozone injection within the LHO. A culture tank with

753 clearer water could enhance the ability of the fish to see, feed optimally, and grow (Sigler et al.,

754 1984) and allows the farmer to observe fish health, behavior, and feeding activity (Christensen et

755 al., 2000); thus clear water with low suspended solids is likely advantageous for Atlantic salmon

756 production in RAS.

757

758 *4.4. Waste discharge*

759 Treatment, capture, or removal of phosphorus, nitrogen, and organic matter is seldom

760 achieved in typical culture systems used to produce market-size Atlantic salmon. In contrast, the

761 RAS grow-out system created two discrete effluents, which were treated to remove wastes prior

762 to discharge. The larger of the two effluents was the system overflow, which was nearly equal in

763 volume to the makeup water added to the pump sump. Water quality of the system overflow was

764 similar to the mean tank water quality reported in Table 5; thus, this effluent contained mean

765 concentrations of 0.7-0.9 mg/L total phosphorus, 20-25 mg/L of total inorganic nitrogen (nearly

766 all $NO_3$-N), and 1-3 mg/L of TSS. These nutrients were concentrated in a relatively small flow

767 that could be treated further, if required by a specific discharge permit. For example, woodchip

768    bioreactors could likely be used to remove the majority of inorganic nitrogen remaining in the

769    effluent (Lepine et al., 2015). A much smaller volume discharge was created by the drum filter

770    backwash and sediment trap flow flushed from the base of the radial flow settler (Fig. 1).

771    Reduced scale (1/12) research using replicated systems with the same technology (Davidson et

772    al., 2013) has shown that combined drum filter and settler flushing flows generally average 0.5

773    % of the total recycle flow; thus, the estimated grow-out system backwash volume was

774    approximately 23 L/min. In addition, approximately 22 % of the feed is converted to fecal

775    matter, resulting in suspended solids waste that is flushed from the system in these two discrete

776    discharges (Davidson and Summerfelt, 2005). The backwash and flushing flow was treated and

777    dewatered on-site using gravity thickening settlers described by Sharrer et al. (2010) to produce a

778    slurry of approximately 9 % dry weight, which was removed by a contract hauler during the

779    present study. Nitrogen and phosphorus contained in these biosolids could potentially be

780    reclaimed as a soil amendment when applied at agronomic rates to row crops or hay fields.

781    However, in a RAS using brackish water or full-strength seawater, the captured biosolids would

782    have to be further pressed or centrifuged to remove saltwater, producing a relatively dry cake

783    before its use as a soil amendment. A mass balance indicates that approximately 90 % of TSS

784    was captured in the gravity thickening settlers, while approximately 10 % of the TSS was

785    contained in the supernatant overflowing the gravity thickening settlers and the grow-out system

786    overflow. The combined gravity thickening settler and grow-out system overflows were treated

787    along with other fish production system flows by a central microscreen drum filter, which is

788    followed by a pair of fish exclusion barriers. The treated water leaving the filtration systems

789    discharges to a tributary of the Chesapeake Bay watershed, and was therefore  monitored

790    monthly (and more recently weekly) under a pollution discharge permit.

791

*4.5. Fish exclusion*

792

Due to the closed containment design of the RAS grow-out system and the associated waste

793

794 treatment systems, no salmon escaped the facility. Inherently, it is very difficult for fish to escape

795 the tank and bypass the drain structures; however, in the rare occurrence that a fish passes

796 through the piping, built-in fish exclusion barriers are in place to trap even the smallest fry. The

797 ability of land-based RAS to effectively contain fish and prevent fish escapement to the wild is

798 an added advantage of this technology (Summerfelt and Vinci, 2008).

799

*4.6. Harvesting logistics*

800

801 Important insight regarding harvesting logistics was gained during each trial. For example,

802 when market-size salmon were harvested at a slower rate, as was the case for the SJR and CS1

803 groups, the mean weight of fish removed during subsequent harvests continued to increase

804 incrementally. For example, harvesting of SJR and CS1 began when the salmon were just over 4

805 kg, but mean harvest weights of 4.7 and 5.7 kg were recorded at the conclusion of each

806 respective trial. In contrast, when larger salmon biomasses were removed each week through

807 selective top-grading (as was the case for CS2), the harvesting window was shortened; smaller

808 fish representative of the lower size distribution did not have ample time to grow; and the

809 average harvest weight decreased with time. These experiences demonstrate the importance of

810 harvesting rate when designing a bioplan for market-size salmon production in land-based RAS.

811 A minimum depuration period of 6 days was used to effectively purge off-flavor compounds

812 (MIB and geosmin) from the salmon flesh. Clean, biofilm-free partial reuse systems operated

813 with rapid water exchange rates (2-3 hr HRT) were used to depurate the salmon. Required time

814    for depuration may vary among cohorts and production sites; therefore, it is important for each

815    production facility to establish their own standard operating procedure (Davidson et al., 2014).

816

817    *4.7. Product quality*

818        In general, product quality measurements from salmon cultured to market-size during these

819    trials were comparable to data reported for commercial net cage operations. Fillet fat content of

820    4-5 kg salmon harvested from RAS during the present trials ranged from approximately 14 to 20

821    %. Mørkøre et al. (2001) reported average fillet fat content ranging from 14.5-21.8 % for market-

822    size Atlantic salmon harvested from four Norwegian cage farms where fish were fed standard

823    diets containing 33 % lipid and average starvation time prior to harvest was 17 days. Jensen et al.

824    (2012) reported fillet fat content of approximately 12 % from Norwegian farmed salmon

825    weighing approximately 3.5 kg; and Acharya (2011) measured an average fillet fat content of 15-

826    16 % from Norwegian Atlantic salmon > 5 kg. Mørkøre and Rørvik (2001) found that fillet lipid

827    content of net cage-reared salmon varied seasonally and was dependent on ocean water

828    temperature. Many additional factors can influence the fillet lipid content of cultured salmonids,

829    particularly dietary lipid (Einen and Skrede, 1998; Chaiyapechara et al., 2003). Based on this

830    abbreviated literature review, salmon cultured during the present trials had fillet fat levels that

831    were comparable to Atlantic salmon from the net cage industry.

832        During the present study, an omega-6:3 ratio of 0.48-0.63 was measured. Jensen et al (2012)

833    assessed the fatty acid content of commercially farmed salmon from Norway and reported an

834    omega 6:3 ratio of 0.44. A standard North American commercial salmon diet was used during

835    the present trials; therefore, the fillet fatty acid content was likely similar to that of commercially

836    available salmon. Most importantly, the omega-3 fatty acids, EPA and DHA, which are known

837    for consumer health benefits were maintained in Atlantic salmon fillets during these trials.

838        Average head-on-gutted yield (slaughter yield) of market-size salmon harvested during the

839    present trials ranged from 87.8-91.1 %. Similar results were described by Acharya (2011), which

840    reported a slaughter yield of 90.7-90.8 % for market-size salmon (> 5 kg) sampled from the

841    Norwegian net-cage industry. Another study reported a slaughter yield for 4-5 kg Norwegian

842    salmon of 90-93 % depending on starvation period (Einen et al., 1998). The average butterfly

843    fillet yield or boneless, untrimmed fillet yield measured during the present study was consistent

844    between grow-out trails, ranging from 74.7-74.8 %. Acharya (2011) reported an average

845    untrimmed fillet yield from farmed Norwegian Atlantic salmon (> 5 kg mean weight) of 72.2 %.

846        Fillet color gradually increased with time, as carotenoids contained in the feed were

847    deposited in the fillet. When each cohort reached market-size, the red/orange color of fillets still

848    tended to be increasing, indicating some potential for improvement. Optimal salmon fillet

849    coloration is dependent on uptake and storage of astaxanthin and cantaxanthin in the flesh, which

850    is largely affected by composition of these carotenoids in the feed and the feeding regimens

851    employed (Nickell and Springate, 2001). During the present trials, 30 ppm astaxanthin and 30

852    ppm cantaxanthin were included in the diets. Inclusion of astaxanthin and cantaxanthin in salmon

853    diets is allowable up to a total combined concentration of 80 ppm in the U.S.; therefore,

854    subsequent on-site studies plan to evaluate the effect of maximum carotenoid inclusion.

855        Generalized comparisons of product quality between RAS-produced salmon and commercial

856    salmon should be considered with perspective, as many variables such as genetics, feed

857    composition, and depuration period, to name a few, can impact these metrics. The provided

858    product quality information is meant to serve as a baseline that can be referenced by prospective

859  fish farmers planning to culture salmon in RAS under similar conditions. Other product quality

860  measurements, including fillet thickness, belly flap thickness, and fillet texture are also included

861  as reference for industry.

862

863  **5.  Conclusions**

864  This study suggests that land-based closed-containment systems can be used to produce

865  market size Atlantic salmon in the face of water resource limitations, pollution restrictions, and

866  mounting disease challenges common in open environments. As a proof of concept, the study

867  also suggests that producing salmon in RAS is biologically and technically viable. Data from

868  three successive cohorts of Atlantic salmon highlighted that the fish grow to market size in

869  freshwater in approximately 2 years from hatch with high survival, efficient FCR, acceptable

870  health and welfare, and almost no therapeutic treatment cost. Use of seawater or brackish water

871  was not necessary.

872  This study did not evaluate production economics; however, Liu et al. (2016) recently

873  provided a detailed assessment of the fixed and variable costs, market development, and

874  potential sale price for market-size Atlantic salmon production in land-based RAS compared to

875  traditional net pen production. The prevalence of early maturing male salmon encountered in the

876  three trials would pose a serious constraint to the economics of production in land-based closed-

877  containment systems. Fortunately, previous yet unpublished research at The Conservation Fund

878  Freshwater Institute indicates that an all-female germplasm can be cultured to market-size to

879  eliminate early maturing male salmon. An all-female source of Atlantic salmon was not available

880  when these three trials were conducted, but at least one source is commercially available, as of

881  2016.

882    These findings significantly advance our understanding of Atlantic salmon performance in

883    freshwater recirculation systems. They also inform the existing salmon farming industry,

884    government officials, funders, and conservation advocates on the potential of land-based,

885    freshwater, closed-containment systems for grow-out of Atlantic salmon to market size. If land-

886    based, freshwater, closed-containment systems for producing market-size Atlantic salmon

887    ultimately prove to be cost competitive, this could enable the salmon farming industry to expand

888    production to inland areas adjacent to large markets, where fish escapes, disease, and/or genetic

889    interactions between farmed and wild fish stocks would be less likely.

890

903

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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.

| | Days Posthatch | | | Mean Weight (kg) | | |
|---|---|---|---|---|---|---|
| Pre-Smolt Salmon Production Milestones | St. John River | Cascade I | Cascade II | St. John River | Cascade I | Cascade II |
| Stocked in nursery system, first feeding | 42 | 34 | 32 | ~ 0.0002 | ~ 0.0002 | ~ 0.0002 |
| $S_0$ winter photoperiod begins | 269 | 202 | 227 | ~ 0.054 | ~ 0.039 | ~ 0.063 |
| $S_0$ winter photoperiod ends, 24-h light resumed | 312 | 239 | 268 | ~ 0.075 | ~ 0.060 | ~ 0.114 |
| Precocious males removed | 439 | 249 | 270 | ~ 0.305 | 0.070 | 0.107 |
| Stocked in intermediate partial reuse system | - | 250 | 270 | - | 0.070 | 0.107 |

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.

| | Days Posthatch/ Days in Grow-out | | | Mean Weight (kg) | | |
|---|---|---|---|---|---|---|
| Post-Smolt Salmon Production Milestones | St. John River | Cascade I | Cascade II | St. John River | Cascade I | Cascade II |
| Stocked in commercial scale WRAS | 465/ 1 | 417/ 1 | 428/ 1 | 0.34 | 0.75 | 0.51 |
| First male (grilse) salmon harvest | 616/ 152 | 564/ 148 | 617/ 190 | 2.7 | 2.6 | 2.1 |
| Last male (grilse) salmon harvest | 728/ 264 | 582/ 166 | 634/ 207 | 3.7 | 2.6 | 2.0 |
| First premium ($\geq$ 4 kg) salmon harvest | 759/ 294 | 679/ 262 | 746/ 318 | 4.2 | 4.1 | 4.9 |
| Last premium ($\geq$ 4 kg) salmon harvest | 831/ 367 | 812/ 396 | 788/ 361 | 4.7 | 5.7 | 3.5 |
| Number of weekly premium harvests | 5 | 16 | 7 | - | - | - |

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.

| | Number of Fish | | | % of Population | | |
|---|---|---|---|---|---|---|
| | St. John | Cascade I | Cascade II | St. John | Cascade I | Cascade II |
| Mortalities | 82 | 155 | 182 | 3.9 | 2.7 | 2.6 |
| Culls (Fungus, Unthrifty fish) | 114 | 221 | 343 * | 5.6 | 3.9 | 4.9 * |
| Jumpers | 41 | 21 | 47 | 1.9 | 0.4 | 0.7 |
| Premium Salmon harvested for market | 435 | 2,752 | 2,952 | 21.2 | 48.7 | 43.0 |
| Early Maturing Males harvested | 751 | 2,178 | 1,174 | 36.6 | 38.5 | 17.1 |
| Bottom cull to reduce biomass density | 0 | 0 | 1,778 | 0.0 | 0.0 | 25.7 |
| Salmon harvested for other research | 629 | 324 | 430 | 30.7 | 5.8 | 6.2 |
| Total | 2,052 | 5,651 | 6,906 | 100 | 100 | 100 |

* 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.

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

* 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.

| Water Quality | Mean | | | Min/Max Range | | |
|---|---|---|---|---|---|---|
| | St. John | Cascade I | Cascade II | St. John | Cascade I | Cascade II |
| Alkalinity (as $CaCO_3$) | $212 \pm 7$ | $226 \pm 3$ | $209 \pm 9$ | 114 - 281 | 175 - 272 | 89 - 270 |
| Carbon dioxide | $9 \pm 0$ | $14 \pm 1$ | $13 \pm 1$ | 4 - 16 | 2 - 24 | 2 - 20 |
| Dissolved oxygen | $10.9 \pm 0.0$ | $11.3 \pm 0.1$ | $11.9 \pm 0.1$ | 9.5 - 12.5 | 9.5 - 13.9 | 9.6 - 14.4 |
| Hardness | ~ 300 | ~ 300 | ~ 300 | ~ 300 | ~ 300 | ~ 300 |
| Nitrite nitrogen | $0.01 \pm 0.00$ | $0.01 \pm 0.00$ | $0.02 \pm 0.01$ | 0.00 - 0.13 | 0.00 - 0.02 | 0.00 - 0.21 |
| Nitrate nitrogen | $19 \pm 2$ | $19 \pm 1$ | $24 \pm 3$ | 3 - 60 | 5 - 32 | 4 - 65 |
| Temperature ($^o$C) | $15.6 \pm 0.0$ | $15.6 \pm 0.1$ | $15.2 \pm 0.0$ | 14.3 - 17.7 | 12.8 - 17.9 | 13.0 - 16.7 |
| Total ammonia nitrogen | $0.11 \pm 0.01$ | $0.22 \pm 0.01$ | $0.30 \pm 0.03$ | 0.01 - 0.56 | 0.04 – 0.36 | 0.03 - 0.54 |
| Total phosphorous | $0.9 \pm 0.1$ | $0.7 \pm 0.0$ | $0.9 \pm 0.1$ | 0.6 - 1.3 | 0.1 - 1.4 | 0.1 - 2.2 |
| Total suspended solids | $1.2 \pm 0.1$ | $2.3 \pm 0.2$ | $2.5 \pm 0.1$ | 0.3 - 4.5 | 0.5 - 5.0 | 1.2 - 4.8 |

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

| | St. John River | Cascade I (4 kg) | Cascade I ($\geq$5 kg) | Cascade II |
|---|---|---|---|---|
| Number of Fish | 21 | 6 | 6 | 5 |
| Days of Purging | 10 | 6 | 6 | 6 |
| Initial Whole Body Weight (kg) | $3.81 \pm 0.13$ | $4.21 \pm 0.18$ | $5.30 \pm 0.14$ | $4.75 \pm 0.30$ |
| Head-On-Gutted Yield (%) | - | $91.1 \pm 0.4$ | $90.5 \pm 0.6$ | $87.8 \pm 1.3$ |
| Butterfly Fillet Yield (%) | $74.8 \pm 0.5$ | $74.8 \pm 0.4$ | $74.7 \pm 0.9$ | - |
| Skin-On Fillet Yield (%) | - | $61.2 \pm 0.6$ | $61.9 \pm 0.9$ | - |
| Skin-Off Fillet Yield (%) | $57.8 \pm 0.5$ | $57.0 \pm 0.6$ | $57.3 \pm 0.9$ | - |
| Belly Flap Thickness (mm) | - | - | $15.8 \pm 0.4$ | - |
| Fillet Thickness (mm) | $30.3 \pm 0.6$ | $36.3 \pm 0.8$ | $34.3 \pm 1.2$ | - |

- Indicates data was not collected for specified parameter

Table 7. Summary of fillet quality attributes for premium market-size Atlantic salmon from each cohort.

| | St. John River (< 4 kg) | Cascade I (4 kg) | Cascade I (>5 kg) | Cascade II |
|---|---|---|---|---|
| Fillet Moisture (%) | 63.8 ± 0.6 | 63.1 ± 0.6 | 62.0 ± 0.3 | - |
| Fillet Protein (%) | 15.9 ± 0.7 | 20.0 ± 0.2 | 19.8 ± 0.3 | - |
| Fillet Fat (%) | 20.4 ± 0.2 | 15.2 ± 0.7 | 17.0 ± 0.3 | 14.4 ± 2.7 |
| Fillet Ash (%) | 1.5 ± 0.1 | 1.2 ± 0.0 | 1.1 ± 0.0 | - |
| Total Omega-3 Fatty Acids (mg/g) | 17.6 ± 0.7 | 21.6 ± 2.8 | 23.6 ± 0.5 | - |
| Total Omega-6 Fatty Acids (mg/g) | 11.3 ± 0.4 | 10.4 ± 1.3 | 14.8 ± 0.7 | - |
| Fillet Color (L) | 35.3 ± 0.5 | 39.1 ± 1.1 | 38.5 ± 0.8 | - |
| Fillet Color (A) | 8.9 ± 0.4 | 12.9 ± 0.6 | 9.2 ± 0.6 | - |
| Fillet Color (B) | 9.5 ± 0.3 | 15.5 ± 0.5 | 11.9 ± 1.0 | - |
| Rouche Color Fan Score | 28-29 | 26-27 | 27-28 | 26-28 |
| Fillet Texture (g/g wt) | 414 ± 13 | 387 ± 33 | 394 ± 21 | - |

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.

| Fatty acids (mg/g) | St. John River | | Cascade I (4 kg) | | Cascade I (≥ 5 kg) | |
|---|---|---|---|---|---|---|
| | % of Total FA | mg/g | % of Total FA | mg/g | % of Total FA | mg/g |
| 12:0 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.07 ± 0.01 | 0.06 ± 0.01 | 0.04 ± 0.02 | 0.05 ± 0.03 |
| 14:0 | 5.06 ± 0.07 | 4.79 ± 0.26 | 5.63 ± 0.13 | 4.82 ± 0.54 | 4.54 ± 1.24 | 5.36 ± 1.81 |
| 14:1 | 0.06 ± 0.00 | 0.06 ± 0.00 | 0.13 ± 0.06 | 0.12 ± 0.06 | 0.05 ± 0.03 | 0.05 ± 0.04 |
| 15:0 | 0.39 ± 0.00 | 0.36 ± 0.02 | 0.28 ± 0.06 | 0.24 ± 0.06 | 0.38 ± 0.03 | 0.44 ± 0.05 |
| 16:0 | 17.6 ± 0.16 | 16.6 ± 0.86 | 16.1 ± 0.66 | 14.0 ± 1.96 | 18.3 ± 0.82 | 21.4 ± 2.40 |
| 16:1 | 8.17 ± 0.11 | 7.66 ± 0.35 | 8.14 ± 0.49 | 7.06 ± 0.94 | 8.97 ± 0.56 | 10.5 ± 1.42 |
| 17:0 | 0.34 ± 0.00 | 0.32 ± 0.02 | 0.29 ± 0.04 | 0.24 ± 0.05 | 0.31 ± 0.01 | 0.36 ± 0.03 |
| 18:0 | 4.08 ± 0.06 | 3.86 ± 0.22 | 5.74 ± 0.66 | 4.95 ± 0.90 | 3.97 ± 0.20 | 4.63 ± 0.26 |
| 18:1, n-9t | 0.52 ± 0.12 | 0.53 ± 0.15 | 0.11 ± 0.05 | 0.09 ± 0.05 | 0.37 ± 0.23 | 0.43 ± 0.25 |
| 18:1, n-9c | 29.3 ± 0.15 | 27.6 ± 1.38 | 23.7 ± 0.96 | 20.1 ± 2.16 | 28.4 ± 0.72 | 33.2 ± 3.10 |
| 18:2, n-6c (LA) | 10.5 ± 0.11 | 9.73 ± 0.38 | 9.48 ± 0.19 | 8.18 ± 1.01 | 10.4 ± 0.51 | 12.1 ± 1.24 |
| 18:3, n-6 | 0.29 ± 0.01 | 0.27 ± 0.01 | 0.43 ± 0.05 | 0.36 ± 0.06 | 0.25 ± 0.02 | 0.29 ± 0.04 |
| 18:3, n-3 (ALA) | 3.87 ± 0.04 | 3.62 ± 0.16 | 2.56 ± 0.13 | 2.23 ± 0.31 | 2.83 ± 0.12 | 3.31 ± 0.25 |
| 20:0 | 0.15 ± 0.00 | 0.15 ± 0.01 | 0.14 ± 0.01 | 0.12 ± 0.02 | 0.13 ± 0.03 | 0.14 ± 0.03 |
| 20:1 | 2.69 ± 0.04 | 2.51 ± 0.12 | 1.98 ± 0.13 | 1.71 ± 0.23 | 2.08 ± 0.22 | 2.42 ± 0.24 |
| 20:2 | 0.52 ±0.01 | 0.49 ± 0.04 | 0.79 ± 0.17 | 0.69 ± 0.18 | 0.57 ± 0.11 | 0.67 ± 0.13 |
| 20:3, n-6 | 0.33 ± 0.01 | 0.31 ± 0.02 | 0.37 ± 0.02 | 0.31 ± 0.04 | 0.29 ± 0.04 | 0.34 ± 0.05 |
| 20:3, n-3 | 0.31 ± 0.01 | 0.30 ± 0.02 | 0.23 ± 0.01 | 0.20 ± 0.03 | 0.25 ± 0.06 | 0.29 ± 0.07 |
| 20:4, n-6 | 0.71 ± 0.01 | 0.66 ± 0.02 | 1.04 ± 0.03 | 0.89 ± 0.11 | 0.80 ± 0.05 | 0.93 ± 0.05 |
| 20:5, n-3 (EPA) | 5.17 ± 0.08 | 4.81 ± 0.18 | 7.24 ± 0.25 | 6.19 ± 0.73 | 5.48 ± 0.44 | 6.38 ± 0.40 |
| 22:0 | 0.11 ± 0.01 | 0.11 ± 0.02 | 0.02 ± 0.00 | 0.01 ± 0.00 | 0.01 ± 0.01 | 0.01 ± 0.01 |
| 22:1, n-9 | 0.35 ± 0.01 | 0.33 ± 0.02 | 0.26 ± 0.02 | 0.23 ± 0.03 | 0.23 ± 0.08 | 0.27 ± 0.09 |
| 22:6, n-3 (DHA) | 9.26 ± 0.19 | 8.56 ± 0.30 | 15.2 ± 1.20 | 13.0 ± 1.84 | 11.2 ± 1.09 | 13.0 ± 0.97 |
| 24:0 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.00 ± 0.00 | < det | 0.00 ± 0.00 | < det |
| 24.1 | 0.18 ± 0.01 | 0.16 ± 0.01 | 0.14 ± 0.04 | 0.11 ± 0.03 | 0.16 ± 0.06 | 0.18 ± 0.06 |
| n-6 | 12.2 ± 0.13 | 10.96 ± 0.42 | 11.3 ± 0.20 | 9.75 ± 1.19 | 11.7 ± 0.49 | 13.7 ± 1.29 |
| n-3 | 19.3 ± 0.26 | 17.29 ± 0.64 | 25.2 ± 1.28 | 21.6 ± 2.76 | 19.8 ± 1.54 | 23.0 ± 1.36 |
| n-6: n-3 | - | 0.63 ± 0.01 | - | 0.45 ± 0.03 | - | 0.60 ± 0.06 |

< 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).
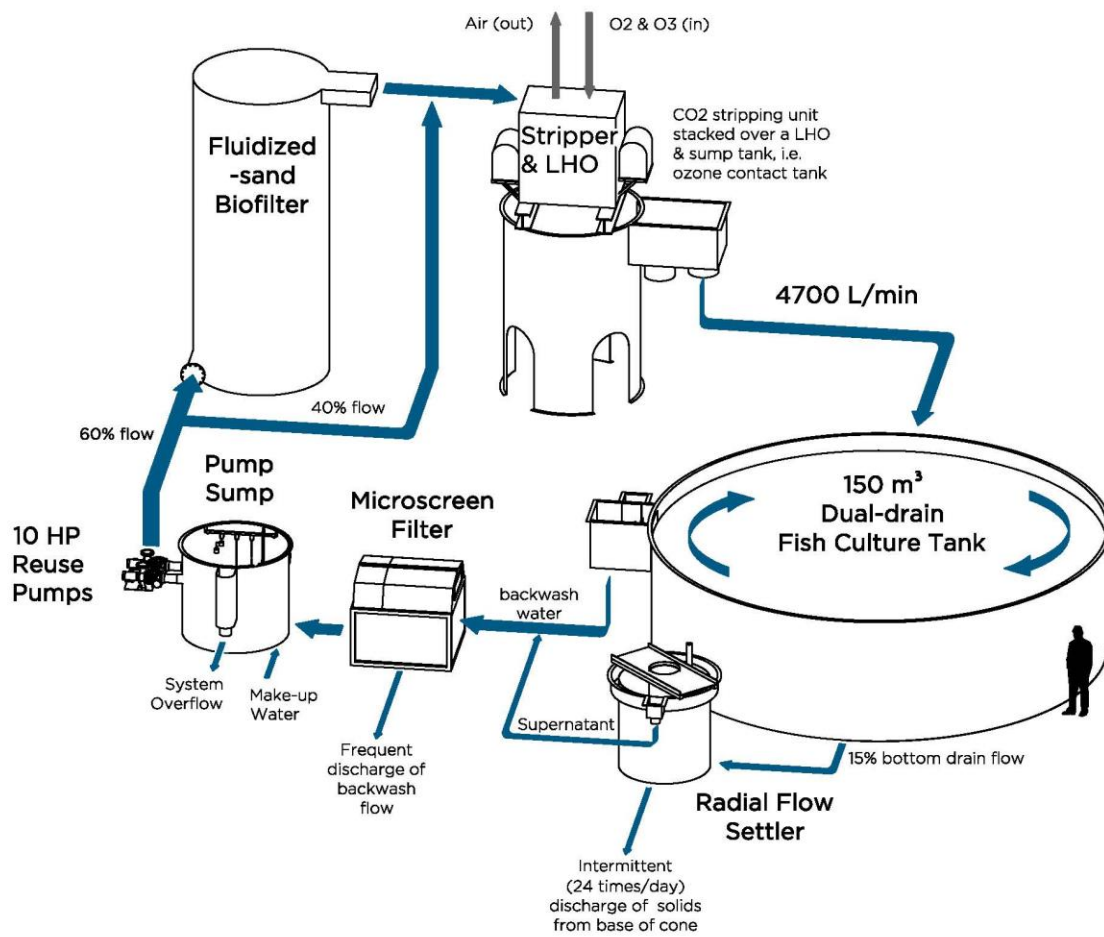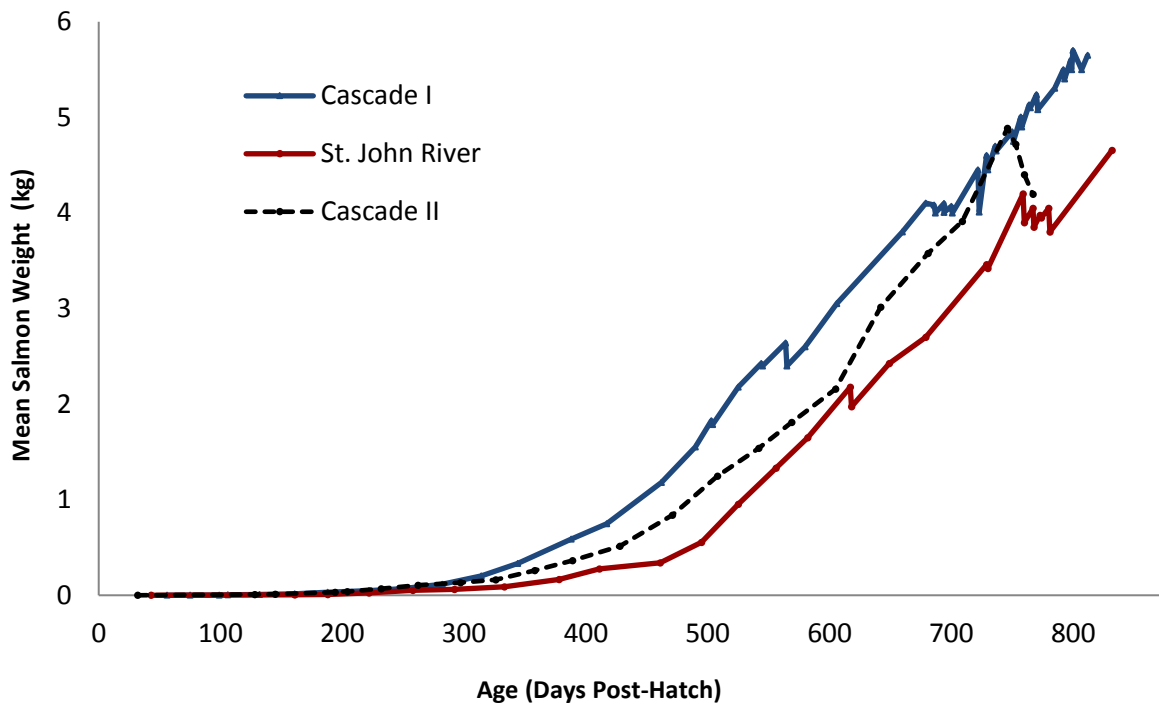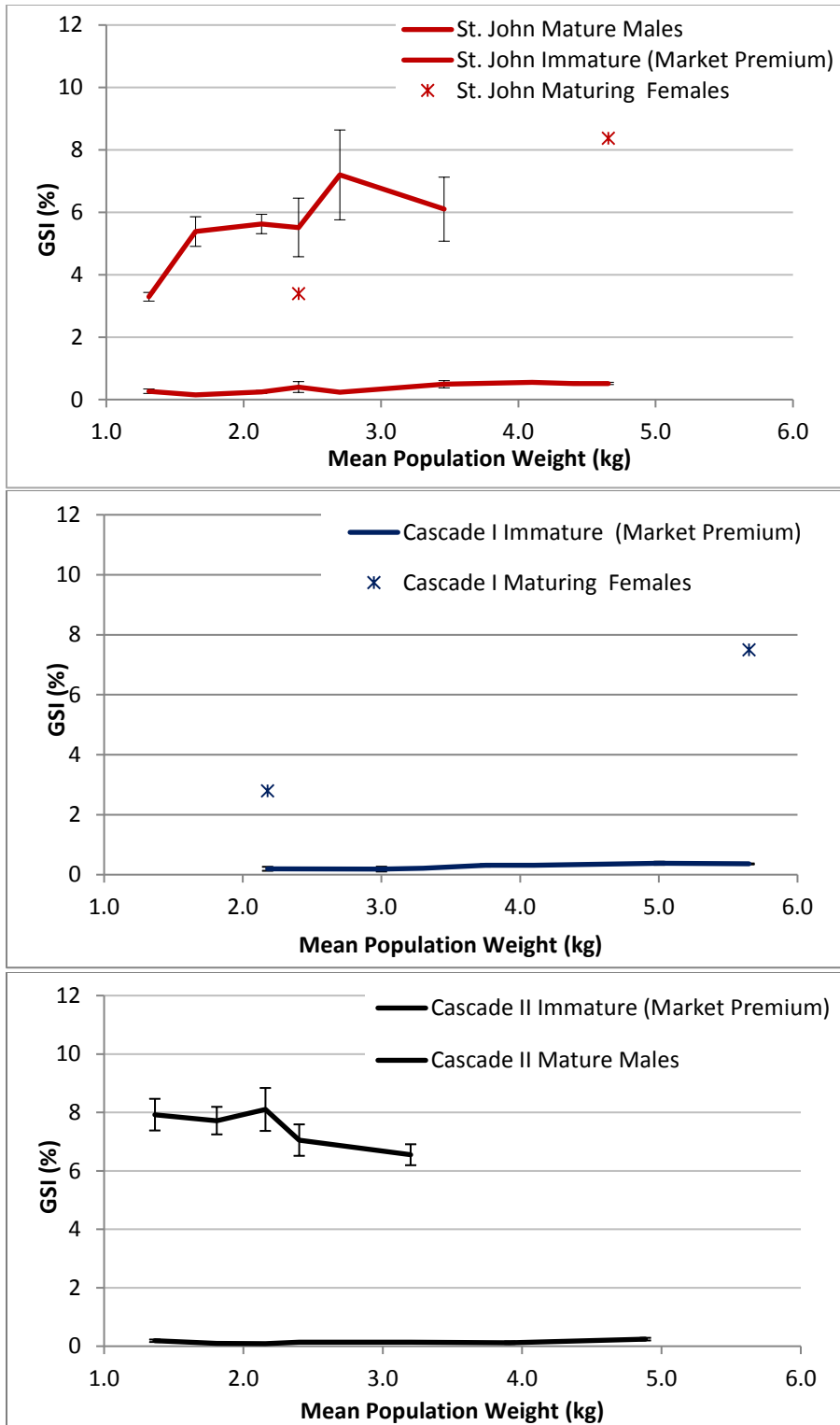
**Figure 1**

**Figure 2**

**Figure 3**

**\*** 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.
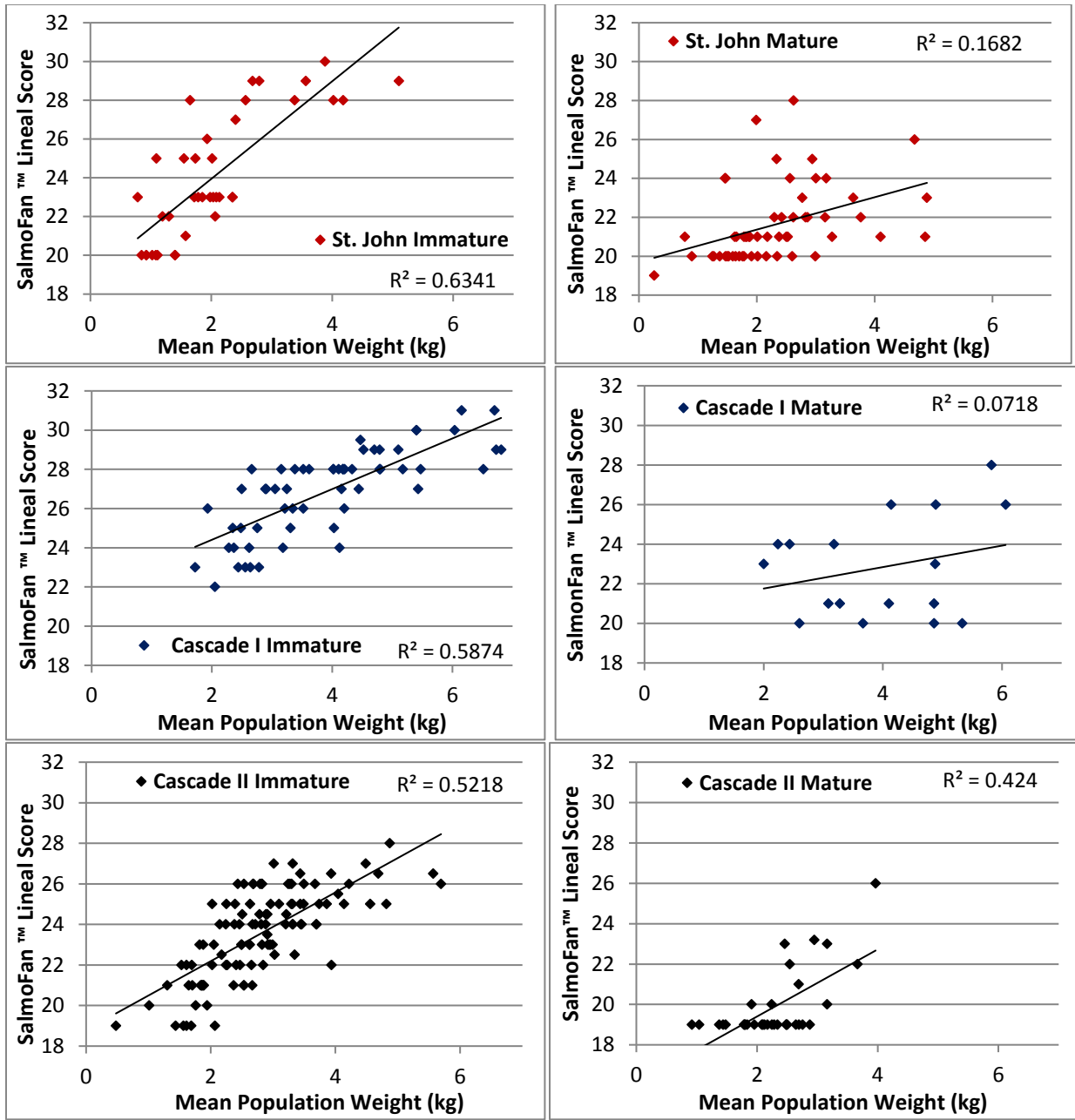
**Figure 4**