FINAL REPORT

FRESHWATER GROWOUT TRIAL OF ST JOHN RIVER STRAIN ATLANTIC SALMON IN A COMMERCIAL-SCALE, LAND-BASED, CLOSED-CONTAINMENT SYSTEM

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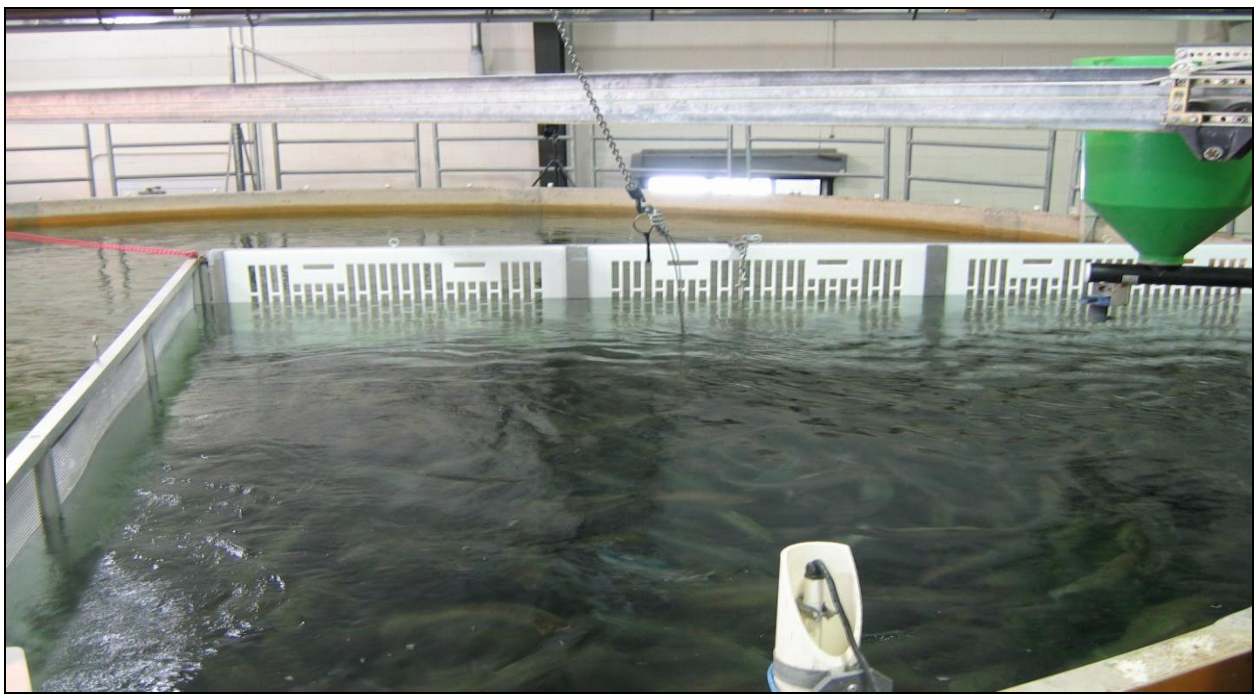

EXECUTIVE SUMMARY

This growout trial employed a pre-existing salmonid growout closed containment facility and technical expertise at The Conservation Fund's Freshwater Institute in Shepherdstown, WV. The project demonstrated as proof of concept that Atlantic salmon can be reared from post-smolt (~340 g) to harvest size (4-4.6 kg) within approximately 12 months in a land-based, freshwater, closed-containment system.

This project determined the following key parameters for Atlantic salmon grown to harvest size in our growout system:

- Growout cycle from post-smolt to 4.6 kg was just over 12 months.
- Total loss to mortality (3.9%), culls (5.6%), and jumpers (1.9%) accounted for 11.4% of the population.
- Fish densities were modest and only reached 35 kg/m3 just before harvest events were initiated.
- Feed conversion average 1.09 over the entire study.
- Male salmon grilse made up approximately 37% of the population and were harvested early and sent to processing for hot smoking.
- No obligate pathogens, sea lice, or Kudoa were detected in the fish or the system.
- No fish were vaccinated.
- No antibiotic, pesticide, or harsh organic chemotherapeutants were used.
- No salmon escaped from the production facility.
- Biosolids backwashed from the system's drum filter and settler were dewatered on site using gravity thickening settlers to produce a slurry of approximately 9% dry weight. Biosolids capture efficiency averages about 92%; captured biosolids were land-applied by a contract hauler.
- The technical and economic feasibility of a commercial-scale, land-based closed containment farm for Atlantic salmon was evaluated through a concept-level design and associated costing for a 3,300 metric ton (mt) facility. The capital cost developed for the 3,300 mt facility was $31 million (this is dependent upon many variables such as feed, electricity, processing, etc. where costs will vary per region).
- The operating cost developed was similar to that published for the net pen industry at approximately $3.90–$4.00 per kg of head-on gutted salmon.

This production trial provided the Atlantic Salmon Federation, other ENGO's, salmon farming companies, aquacultural engineering and supply companies, and government agencies (such as Canada's Department of Fisheries and Oceans) with actual performance data and key production costs during a salmon production trial within a commercial-scale, freshwater, closed-containment system. For example, on March 8, 2012, A Canadian Government Parliamentary Committee studying land-based closed-containment systems for salmon farming visited TCFFI to learn more about the technology.

These results will provide vital information to the North American salmon farming industry, government officials, funders, and conservation advocates, in order to inform decision-making regarding land-based, freshwater, closed-containment systems for Atlantic salmon growout.

In addition, this growout trial provided stakeholders with one of the first cohorts (approximately 7 tons) of Atlantic salmon raised in land-based, closed-containment systems for test marketing opportunities. Results of this growout trial suggest that full-scale closed-containment systems have the potential to meet all five tests of sustainability put forth by the Conservation Council of New Brunswick:

• It does not degrade the environment on which it is dependent.
• It is in harmony with other economic, cultural, and social activities that use the same natural resources.
• It does not diminish the ability of future generations to use the same resources.
• It invests in local communities, and decision-making is local.

• It produces a reasonable and relatively stable net income to both producers and society by using natural resources on a long-term, renewable basis.

The successful adoption of land-based, freshwater, closed-containment systems for Atlantic salmon growout could ultimately enable the salmon farming industry to shift production to inland areas, which would contain these fish and any potential diseases and prevent interactions with wild species.

INTRODUCTION

The Atlantic Salmon Federation (ASF) and TCFFI believe that land-based closed-containment systems are a cost-effective and environmentally friendly farming method, with the potential to eliminate the use of vaccines, antibiotics, pesticides, and other harsh chemicals in salmon farming. Our goal with this technology is to provide fish farmers and entrepreneurs an opportunity to choose an innovative, alternative method to grow fish – a method which we believe is not only better for the environment, but for business as well. We recognize, however, that many challenges exist in promoting positive change in the aquaculture industry, and that overcoming the status quo and its inherent resistance to paradigm shifts may take considerable time. Nonetheless, we hope that the myriad benefits of closed containment, water recirculation technologies will speak for themselves, and that industry will embrace these innovations following successful, commercial-scale demonstration of their feasibility.

This report summarizes the results of a commercial-scale Atlantic salmon growout trial in a freshwater closed-containment system. This initiative provided important, novel data on growth rate, survival, fish densities, feed conversion, primary variable costs, waste loads, fish health, pesticide/antibiotic use, and other key parameters for Atlantic salmon production to food-size over 12 months within our freshwater commercial-scale closed containment system. The findings of this study will assist future decision-making by the North American salmon farming industry, government regulators, funders, and conservation advocates, resulting in better-informed decisions as the industry continues to grow.

BACKGROUND INFORMATION

Prior to the production trial commencement, a number of project milestones were developed to guide the study's progress; these milestones are summarized in Table 1.

The growout trial officially began with the stocking of 2,052 St. John River strain Atlantic salmon in TCFFI water recirculation growout system on May 2, 2011. All fish used in the growout trial were the same cohort and age and originated from the same batch of specific pathogen-free eyed salmon eggs procured from Cooke Aquaculture (Maine, USA). The fish were stocked at a mean weight of 340 grams +/- 6.27 grams. Prior to the stocking event, approximately 6 % of the salmon were culled as precocious males. These fish were removed based on observable morphometric and coloration characteristics. Precocious males are easily differentiated from immature salmon, as they typically are smaller, display parr marks, colorful spots, and an olive to brown coloration. In contrast, the immature salmon (i.e. non-precocious males) are larger and silver in coloration. Precocity was confirmed post-mortem via visual gonadal assessments.

INCUBATION AND HATCHING SYSTEM

Salmon used in this trial arrived as eyed eggs on January 14th, 2010. Eggs were iodine disinfected upon arrival, following facility standard operating procedures. The eggs were incubated in vertical heath style trays at an average incubation water temperature of 7.5^{0}C. The eggs hatched at approximately 414 ATU's (accumulated thermal units) on January 22, 2010. The date of 50 % hatching is a critical milestone, as it represents Day 0 in age for any given cohort. Accumulated thermal units were tracked to follow egg and fry development and to predict important milestones such as hatching and ponding. After hatch, Atlantic salmon have large yolk sacs and do not require any extraneous feed until 35-45 days of age, depending on several factors such as water temperature, rearing conditions and fry activity, and species strain. Survival percentages at hatching and ponding for the cohort used in the production trial were 91% and 84%. Unfortunately, the small increase in mortality between these two events was due to a fungal outbreak that occurred while the alevins resided in the incubation trays. Fungal outbreaks were controlled by addition of hydrogen peroxide to the incubation trays as a short duration static bath. Mortalities and eggs shells were removed on a daily basis to promote good tank hygiene.

Water quality in the incubation system was excellent. Oxygen was maintained at atmospheric saturation, and carbon dioxide levels were close to zero due to the combined effects of the water cascading between incubation trays and the low level of respiration due to lack of feeding (i.e., alevins are not fed while held in the incubation trays). It is important to note that the low CO_2 levels and subsequent higher pH in hard water can precipitate calcium carbonate crystals from the water, which deposit on eggs and equipment. In our experience, however, this has not impacted hatching success at TCFFI. Water temperatures were maintained close to 7.5˚C during the incubation phase, and slowly increased to 12˚C over a 3-day period to acclimate fish for ponding into the nursery system.

FIRST FEEDING, PARR, AND SMOLT SYSTEM

The alevins[1] (sac fry) were stocked into 560 L (148 gallon) circular nursery tanks on March 5, 2010 at approximately 704 ATU's, i.e., when all or most (90%) of the yolk sac had been consumed. These sac fry were stocked a few days early before first feeding began, as they had some remaining yolk reserves. The time for first feeding can vary based on facility, species, and water temperature; at TCFFI, fish are usually stocked and fed around 35-45 days of age using an incubation temperature of 8˚C, but timing ultimately depends on observation of yolk sac reserves

First feeding is a critical time for alevins. If first feeding is started too early, fish will not feed and water quality can deteriorate leading to fish health issues. If feeding begins too late, there is a risk of fish not adapting to feed and eventually dying from malnutrition. Atlantic salmon were fed commercially available diets from Bio-Oregon (Westbrook, ME) and Zeigler Brothers (Gardner's, PA) specifically formulated for salmonid fry and parr.

The salmon were reared in the 12-tank nursery system and other similar tanks through fry, parr, and smolt stages, until May 2, 2011, when they were stocked into the growout production tank. The salmon fry and parr were reared with 24-hour continuous light and feeding events at least once per hour, 24 hours daily. After reaching a mean size of 40 g, while still in the nursery system the salmon parr were treated with a 6-week artificial winter photoperiod (consisting of days with 12 hours light and 12 hours dark) in order to produce what industry refers to as an S_0 smolt. An S_0 smolt is a salmon that has undergone smoltification in a period within approximately six months from hatching, rather than the usual 12 or 24 months. After the end of the 6-week winter photoperiod, fish were returned to a 24-hour continuous lighting regime. The fish started to smoltify within 1-3 weeks after resuming the continuous 24-hour photoperiod.

Previous research indicates that Atlantic salmon grow faster to 4-5 kg if smoltification has been induced by a 6-week artificial winter (compared to fish that were simply exposed to continuous 24-hour lighting), despite being raised to market size in freshwater only. Thus, providing a 6-week artificial winter photoperiod to produce smoltification is now standard practice when rearing Atlantic salmon to market-size in the freshwater closed-containment system.

Water quality was excellent while salmon were cultured in the nursery system. Temperature and dissolved oxygen were recorded daily, whereas carbon dioxide, hardness, alkalinity, total ammonia nitrogen, nitrite nitrogen, pH, and total suspended solids were measured once weekly.

[1] Alevins are a life cycle stage that refers to the time between hatching and full utilization of their endogenous yolk sac.

Table 1. Date and fish age when major milestones were achieved in the Atlantic salmon growout trial.

Date	Days Post-Hatch	Milestone
January 14th, 2010	n/a	Eyed eggs received
January 22, 2010	1	50% hatch date
March 5, 2010	42	Ponding and first feeding
October 18, 2010	269	S_0 winter period begins (12 hours dark, 12 hours light per day)
November 30, 2010	312	S_0 winter period ends, fish resume 24- hour photoperiod
April 6, 2011	439	Precocious males removed from population
May 2, 2011	465	**ASF trial begins;** Salmon stocked into TCFFI growout system
September 30, 2011	616	1st male harvest
January 20, 2012	728	2nd male harvest
February 20, 2012	759	Harvest of premium salmon
February 28, 2012	767	Harvest of premium salmon
March 5, 2012 and other dates	773	Harvest of premium salmon for various additional research studies
March 12, 2012	780	Harvest of premium salmon
May 2, 2012	831	Final harvest of premium salmon; completion of ASF trial

Figure 1 depicts mean weight growth from first stocking in the nursery tanks until just prior to startup of the growout trial, when the fish averaged 340 grams. The data suggest that the Atlantic salmon growth rate decreased during, and just after, the 6-week S_0 winter, as parr approached and then underwent smoltification. Salmon growth rapidly accelerated after approximately 333 days post-hatch.

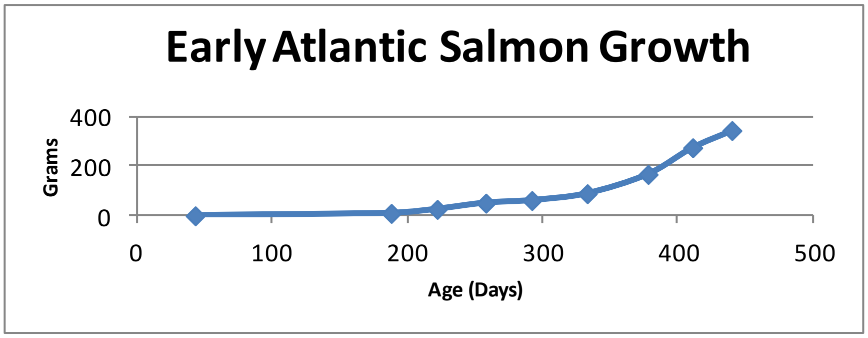

Figure 1. Early salmon growth for the Atlantic salmon cohort used in the production trial. Growth during the first year post-hatch is typically slow in this species, followed by rapid growth in the second year.

GROWOUT TRIAL IN THE FRESHWATER CLOSED-CONTAINMENT SYSTEM

Approximately 2,052 Atlantic salmon post-smolt were stocked into a 150 m^3 culture tank within a freshwater recirculation system on May 2, 2011, when the fish were 460 days post-hatch. The trial ended on May 2, 2012 after a 372 day period. The final mean weight of the salmon was 4.655 kg.

Production System Description - The water recirculation system (Figure 2) uses two 5-HP centrifugal pumps to move 4,640 L/min of water from the system's lowest hydraulic grade line elevation, i.e., the pump sump, to the system's highest elevation, i.e., at the top of the cyclonic fluidized-sand biofilter. Water exiting the top of the biofilter flows by gravity through a forced-ventilated cascade aeration column, a low head oxygenation (LHO) unit, a LHO sump, and a channel UV irradiation unit (Figure 2) before entering the 150 m^3 fish culture tank. Water exits the fish culture tank by gravity and flows through a microscreen drum filter (installed with 90-µm sieve panels) and into the sump pump, where the water is pumped again. The water flow rate was selected to exchange the water volume in the fish culture tank approximately once every 30 minutes. Ozone was generated in the 99.5% pure oxygen feed gas. The ozonated-oxygen feed gas was subsequently injected into the recirculating system at the LHO, where the pure oxygen feed gas was used to supplement dissolved oxygen levels to increase the carrying capacity of the system and maintain dissolved oxygen concentrations at saturation throughout the study (Summerfelt et al., 2009).

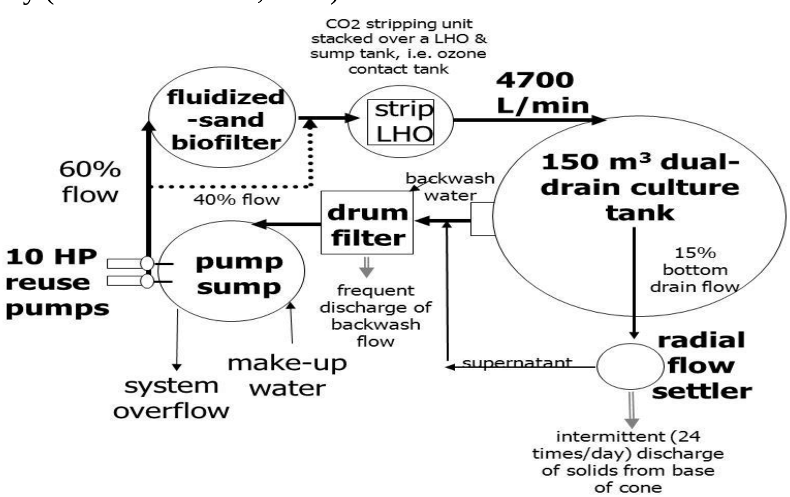

Figure 2. Process flow drawing of the water recirculation salmonid growout system (Summerfelt et al., 2009).

A constant 24-hour photoperiod was provided. In addition, to produce a nearly constant biological respiration and waste production rate, timer-controlled mechanical feeders were used to feed fish equivalent portions during eight feeding events daily, i.e., approximately one feeding every 3 hours.

Growth - Atlantic salmon were stocked into the growout trial tank at an average weight of 340 grams, which equated to a starting biomass of 694 kg. The first harvest of premium salmon began 294 days (~10 months) later at a mean size of approximately 4.2 kg. At the end of the 372-day (~12-month) growout trial, the salmon had reached an average weight of 4.655 kg and all harvests were complete. Figure 5 provides the entire growth curve for this St. John River strain Atlantic salmon, comparing these data with St. John River strain salmon commercially farmed in a net pen located near Jonesport, Maine (Wolters, 2010). These data suggest that it can take considerably longer, 626 days total during growout, to culture the same strain of Atlantic salmon in an ocean net pen from a mean stocking size of 120 g to a mean size of 4.729 kg (Wolters, 2010).

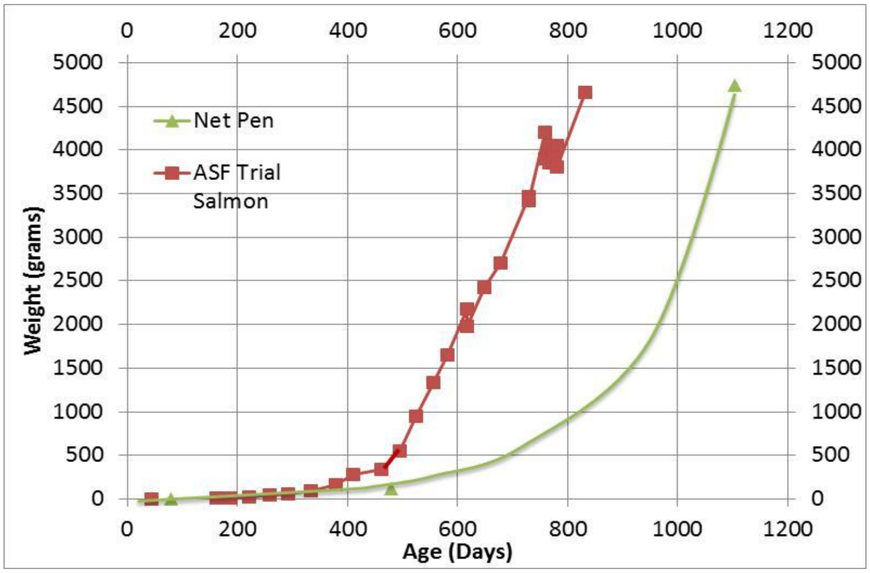

Figure 3. Overall growth curve for Atlantic salmon in the present growout trial compared with salmon grown in net pens off the coast of Maine (Wolters, 2010).

Feeding - The salmon were fed commercially available diets during the production trial. The vast majority of the diet used and fed was produced by Ewos (Surrey, BC) and is sold under the Dynamic brand. This salmon diet contained 42% protein and 30% fat as well as 30 ppm of astaxanthin and 30 ppm of canthaxantin to promote a red fillet color. The Ewos Dynamic formulation is available in a Summer Red and Winter Blue formulations; these salmon were fed the Summer Red variant, based on the relatively constant 15-17^{o}C water temperatures in TCFFI's growout production tank. It should be noted that pre-trial salmon in the nursery system were fed a commercially available diet from Bio-Oregon (Westbrook, ME) that contained 47% Protein, 24% fat, and 40 ppm astaxanthin. Both diets produced good growth and feed conversion ratio (FCR).

FCRs were calculated as follows: FCR = Cumulative Feed Delivered / Fish Biomass Gain

The overall feed conversion ratio was estimated to be 1.09 (1.09 Lbs. feed fed produced 1 Lb. of weight gain) during the production trial. Feed conversion through March 12, 2012, was calculated based on 7317 kg (16,097 lbs.) feed and 6702 kg (14,744 lbs.) gain (i.e., 1.09 FCR). We had to stock a second cohort of Atlantic salmon into the growout tank on March 12, 2012, which was about 1.5 months before our final harvest. However, this next cohort was 11 months younger and much smaller than the St. John River strain salmon. We were able to selectively harvest the largest salmon from the culture tank on May 2, 2012. When we include the total feed fed to the mixed cohort after March 12, the FCR is 1.08 with 7673kg (16,916 lbs.) feed fed to 7115kg (15,686 lbs.) gain.

Mortality, Culling, and Survival - Table 2 summarizes the inventory of salmon in the growout production tank over the 12-month trial. The grow-out trial began with an initial stocking of 2,052 salmon. Overall mortality accounted for 3.9% of the fish during the growout production phase. There were a total of 82 mortalities over the 372 day period; however, 59 of these mortalities occurred within the first 10 days after the initial stocking event. The majority of these stocking mortalities were most likely fish that succumbed to stress from transportation, handling, and netting. In addition, Atlantic salmon can be susceptible to fungal infections, especially after moderate handling and stress. After the initial mortalities, a total of 23 mortalities were removed from the tank over a 357 day period, which averaged one mortality every two weeks.

In any aquaculture facility, culling and removing unthrifty fish is part of fish health and biosecurity best management practices. Unthrifty fish can be sick, malnourished, and/or exhibit very slow growth. Following standard operating procedures, these fish are removed from the population to reduce the risk of clinical disease and consequent amplification of fish pathogens. During the 372-day time period described above, a total of 114 fish were removed due to fish health concerns (i.e., external fungal infections), deformities, or slow growth. The vast majority of these fish were simply slower-growing, unthrifty fish. In total, 5.6% of the salmon population was removed as culls.

Atlantic salmon is a naturally athletic species, and on a few occasions fish accidently jumped from the culture tank, over an approximately 3-ft tall jump screen, to the floor. A total of 41 salmon (1.9%) were lost in this manner over the entire growout period, with the majority of jumpers occurring early on in the trial.

Total loss to mortality (3.9%), culls (5.6%), and jumpers (1.9%) accounted for 11.4% of the population (Table 2).

Another 30.7% of the fish that could have gone to harvest were used for research objectives that included:

- Approximately 130 fish removed monthly to quantify fish length and weight, condition factor, sex, fillet color, and gonad index.
- A total of 629 fish used in studies on depuration kinetics and fillet attributes before and after 10 days' purging in flow-through tanks.
- Sixty fish sampled for pathogen screening.

The remaining fish were harvested and sent to processing.

Table 2. Final numbers for all fish outcomes during the growout trial

Fish outcomes	# fish	%
Mortalities	82	3.9
Intentional culls (unthrifty fish)	114	5.6
Jumpers	41	1.9
Premium salmon harvested for fillet processing	435	21.2
Precocious males harvested	751	36.6
Used for other research purposes (e.g. fillet attributes)	629	30.7
TOTAL salmon in growout trial	2,052	100.0

Harvests – Grilse were hand culled from the population twice: on September 30, 2011, at a mean size of about 2 kg, and January 20, 2012, at a mean size of 4 kg. Almost all of the grilse were male salmon that were either sexually maturing or already sexually mature. Male salmon that are sexually maturing have a distinctive broad nose, an extended jaw, and enlarged kype (hook) at the end of the lower jaw. In addition, the silvery sides changes to a light or dark brown as the male matures. Only a few sexually mature females were encountered. These fish are darker in coloration, have ovipositors and are beginning to develop soft bellies. Fish that are not mature have hard bellies and silvery sides. The best indicator of a maturing female is a protruding ovipositor. Every salmon with this characteristic had enlarged ovaries and reduced fillet color. Fish with these ovipositors tend to have a swollen vent and are easily spotted.

Belly firmness is not always a consistent indicator of maturity. It is difficult to feel the eggs in the abdomen due to the thick underbellies and high fat content in the cavity. Females with fully developed ovaries had pale and nearly white flesh.

Premium salmon were harvested February 20 and 28, March 5 and 12, and May 2. In addition, premium salmon were removed several other occasions for use during research on purging kinetics and slaughter technologies, plus fish health testing.

Biomass Density – The biomass density was maintained at less than 40 kg/m3 to ensure optimal growth throughout the growout trial (Figure 5). The consequence of the low biomass density was a significantly reduced production capacity within the system, because it was operated at about one-third of its carrying capacity.

Condition Factor, Fillet Color, Sexual Maturity, Proximate Analysis and Fillet Yields - During the growout trial, fish were periodically sampled and assessed for sexual maturity and fillet color; sexual maturity and full fillet attribute assessment (i.e., color, proximate analysis, fatty acid content, and fillet yield) was carried out during the final study sampling. Figure 6 illustrates the color and fillet attributes of a large premium salmon harvested at the trial's completion. Final salmon mean weight was 4,655 ± 131 grams.

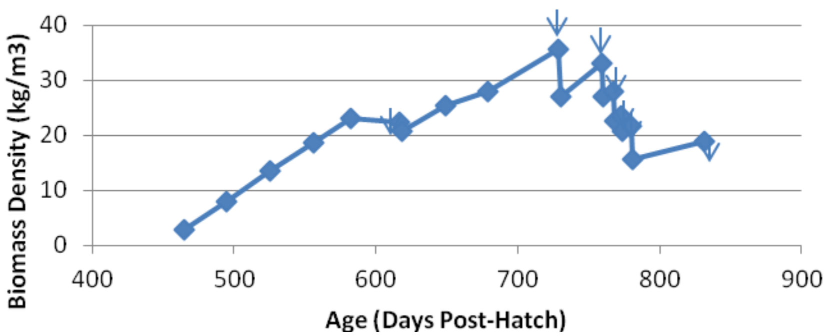

Figure 4. Biomass density within the 150 m^3 culture tank during the growout trial; vertical arrows indicate harvest events.

Figure 5. Thick portion of female salmon fillet showing color and fat levels.

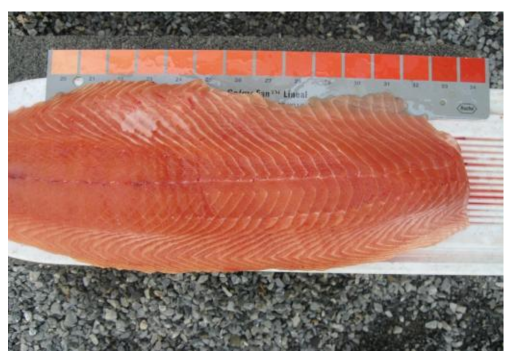

Figure 6. Fillet of female salmon at final harvest.

A total of 129 fish were sampled periodically during the growout trial. These fish were sampled for total length (mm), total weight (g), fillet color, gonad weight (to determine GSI index), sex, and condition factor. Of the 129 fish sampled, 86 fish were females, the remaining fish were males. Table 5 summarizes the overall mean condition factor for the entire sample as well as broken down into males and females. Fillet color was influenced by carotenoid pigments (30 ppm astaxanthin and 30 ppm canthaxantin) incorporated into the feed. Figure 6 shows the fillet color at the final harvest.

Overall, fillet color scores increased based on assessments of small samples of males and females taken over the course of the trial (Figure 7). The slow but steady increase in fillet color score was more evident in samples from female fish, and it is likely that similar pigment accumulation in males was hindered by peripheral lipid utilization during precocious sexual maturation. The final pigmentation was similar to that found in Atlantic salmon cultured in net pens.

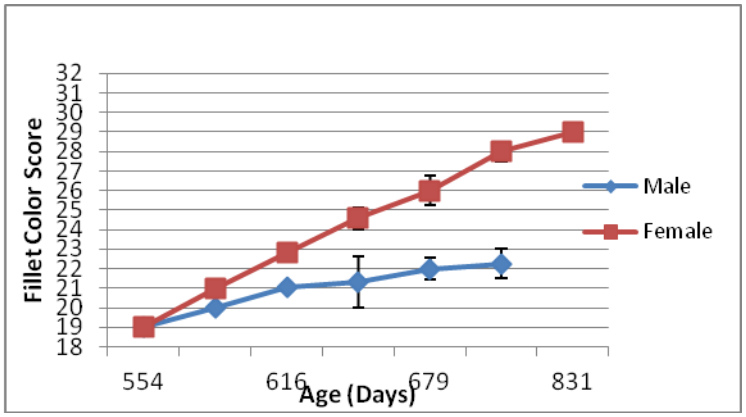

Figure 7. Mean fillet color scores of sampled male and female salmon.

It is critical that salmon are harvested before they reach full maturity. Sexually mature fish can lose fillet color and develop softer fillet characteristics; these characteristics can make the product much less desirable to the consumer.

Water Quality and Quantity - Water quality monitoring was carried out daily or weekly, depending on which parameters were being tested. On a daily basis, temperature, oxidation-reduction potential (ORP), pH, make up flow rate, and dissolved oxygen concentration were recorded and monitored to ensure that these parameters stayed within safe and optimal levels for fish culture. Total ammonia nitrogen, total nitrite nitrogen, total nitrate nitrogen, carbon dioxide, alkalinity, total suspended solids, and total phosphorous concentrations were monitored weekly.

The water treatment processes in the recirculating system were sufficient to maintain excellent water quality. Throughout the growout trial, dissolved oxygen and carbon dioxide were maintained at an average of 10.8 mg/L (approximately at 100% saturation) and 9.2 mg/L, which provide the opportunity for maximum fish growth. Total ammonia nitrogen and nitrite nitrogen were also controlled at extremely low levels for a water recirculation system, averaging 0.11 mg/L and 0.013 mg/L, respectively, over the duration of the study. Nitrate nitrogen averaged approximately 20 mg/L, which represents the amount of ammonia and nitrite nitrogen that were produced and converted to nitrate nitrogen at the mean loading rate on the system. The combination of ozonation and microscreen filtration maintained relatively low total suspended solids concentrations, i.e., they averaged 1.2 mg/L, which is just above the concentration in our spring water source. Thus, this study suggests that larger commercial facilities using properly designed water recirculation systems can also maintain exceptional water quality at similar feed loading levels.

Fish Health – The only fish health issue observed during the production trial was the occurrence of external fungal infections. Atlantic salmon, in general, are more susceptible to infections by ubiquitous water molds such as *Saprolegnia* spp., compared to other salmonids (e.g. rainbow trout and Arctic charr). There was one moderate outbreak of external fungus that occurred after the initial stocking event in the growout system. A total of 59 fish were lost in a six-day period following the initial stocking. These mortalities were likely related to harvesting stress, fungal infection, or through a combination of both factors. After this initial mortality event, only 23 mortalities occurred for the remainder of the growout trial. All subsequent occurrences of external fungus were easily controlled by the addition of salt to the production tank. Dosages of salt were added during treatments to bring the salinity in the production tank to 2.5 ppt; however, the salt would stay in the production tank longer due to the reduced makeup and longer system hydraulic retention time. A total of 6532 kg (14,400 lbs.) of salt was used to prevent and control any spread of fungus over the course of the study.

The practice of culling fish was also utilized to remove any unthrifty, sick, or lethargic fish. Culling fish may help to prevent potentially sick fish from infecting other fish, or to curtail the transition to overt clinical disease and the consequent amplification of fish pathogens in the recirculation system water. The use of extended salt treatments at low levels (2.5 ppt) coupled with culling was a major tool in helping to remove and prevent fungal infection outbreaks and mortalities.

On January 25, 2012, 60 salmon from the production lot were sampled and tested, according to American Fisheries Society-Fish Health Section's "Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens" (2010) protocols, by the certified fish health laboratory Kennebec River Biosciences (Maine, USA). This testing was completed to provide a 95% confidence (assuming 5% pathogen apparent prevalence) that the salmon population was not infected with the following pathogens: infectious salmon anemia virus (ISAV), infectious pancreatic necrosis virus (IPNV), infectious hematopoietic virus (IHNV), viral hemorrhagic septicemia virus (VHSV), *Oncorhynchus masou* virus, Spring Viremia of Carp virus, *Aeromonas salmonicida* (causative agent of furunculosis), *Yersinia ruckeri* (enteric redmouth disease), *Renibacterium salmoninarum* (bacterial kidney disease), *Ceratomyxa shasta* (ceratomyxosis), or *Myxobolus cerebralis* (whirling disease). In addition, 30 salmon fillets were tested on May 17, 2012 for the myxosporean parasite, *Kudoa thrysites*, using real time PCR. No pathogens were detected at any point, and furthermore no clinical signs resembling diseases associated with important viral or bacterial pathogens were noted during the entire study period, and no external parasites, such as *Lepeophtheirus salmonis* (sea lice), were observed at any point.

Aquaculture Drugs and Chemicals - No chemicals or drugs besides sodium chloride were used to treat the salmon during the 12-month production growout trial. Sodium chloride was used to relieve stress and control fungus during the production trial. Hydrogen peroxide was used occasionally in the incubation and fry culture stages to control fungus before the trial began. No vaccines were administered, and no formalin or other harsh chemotherapeutic agents were used during any phase of the growout trial. No antibiotic- or pesticide-containing feed was administered to any of the salmon during the growout trial.

Fish Exclusion - Fish containment is desired to protect the captive fish populations, but also to prevent fish escape and potential interbreeding with wild populations, or the exchange of pathogens between wild and farmed populations. Land-based closed-containment systems confine eggs and fish within solid-wall vessels to create optimized conditions for fish husbandry. However, these solid walled vessels must also include inlet ports, drains, and overflows to allow for water exchange. Thus, if a barrier across a drain/overflow fails, it is always possible for eggs or fish to escape out of their primary containment vessel with the water flow. Three physical barriers, each with openings sized small enough to exclude the life-stage of fish under culture, were located across the path that water must flow from the fish/egg culture vessel to the facility discharge. This approach is usually accepted as adequate redundancy to eliminate fish escapes. At TCFFI, these barriers were designed and maintained using standard operating practices to prevent the eggs or fish from exiting with the water discharge, or failing that, to recognize and remove escaped fish if observed in front of the second or third barriers. Screens and physical filters were used for fish exclusion, because they allow for water passage if properly maintained. Problems occur if the screens become fouled or are hydraulically overwhelmed, allowing water flow to overtop or bypass the barrier. Thus, standard operating practices are used to observe for escapees and to maintain the barriers.

No Atlantic salmon from the production trial was observed to escape from the growout tank. Any fish that escaped the production tank would be trapped in the downstream fish exclusion area. No salmon were observed in the exclusion filter area.

Waste Loads – The water recirculating systems had two discrete discharges, the largest discharge volume is the overflow from the grow-out system, which is equal to the makeup water added to the sump pump and averaged 66 L/min (17.5 gpm) over the entire production cycle, i.e., approximately 1.5% of the total flow recirculating through the system. The smaller volume discharge is the drum filter backwash flow and sediment trap flushing flow from the base of the radial flow settler, which are combined under slab in a single pipe. Research on 1/12-scale replicates of the same system (Davidson et al., In Press) indicate that the combined drum filter and settler flushing flows average 0.5% of the total recirculating flow, i.e., 23 L/min (6 gpm). Approximately 22% of the feed ends up as suspended solids that are flushed from the system in the two discrete discharges (Davidson and Summerfelt, 2005), i.e., the relatively clean overtopping flow (which averages about 1.2 mg/L TSS and 0.1 kg TSS/day) and the combined backwash flow. The combined drum filter and settler flushing flows contains a mass that averaged 4.7 kg TSS/day with a concentration of 142 mg/L TSS (based on a mass balance estimate); this 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. The biosolids capture efficiency averages 92% across the gravity thickening settlers (Sharrer et al., 2010). Thus, approximately 4.3 kg TSS/day (dry weight) – or 90% of the solids – were captured in the gravity thickening settlers, while approximately 0.5 kg/day – 10% of the solids – were contained in the supernatant overflowing the gravity thickening settlers and the overflow from the growout system. This combined flow was treated with other fish production system flows across a microscreen drum filter and a pair of fish exclusion barriers. The drum filter and fish exclusion barriers treat all water exiting the production facilities before discharge to the receiving water. After drum filtration, total biosolids waste capture was estimated to have exceeded 90% over the duration of the study. The final outfall then discharges into the Rockymarsh Run watershed in Jefferson County, WV, which is a tributary to the Potomac River and flows into the Chesapeake Bay.

The biosolids captured were removed as a slurry (9% dry weight) by a contract hauler. The nitrogen and phosphorus contained in these biosolids can thus be reclaimed as a soil amendment when applied at agronomic rates to row crops or hay fields.

Economic Feasibility - The technical and economic feasibility of a commercial-scale, land-based closed containment farm for Atlantic salmon was evaluated through a concept-level design and associated costing for a 3,300 metric ton (mt) facility. Technical feasibility is based on the data developed by The Freshwater Institute's growout trials of Atlantic salmon over the last three years. Thermal growth coefficient values were based on data currently being collected in a growout trial with Cascade strain Atlantic salmon (purchased as eyed-eggs from Icicle Seafoods), because the Cascade strain fish grew significantly faster in freshwater than the St John River strain fish. Growout trial data, specifically feed conversion, mortality, head-on gutted yield, and other performance indicators, were used to develop a biological plan for a 3,300 mt facility. Bioplanning includes predicting fish growth at each lifestage and determining all locations and movements of fish throughout the lifecycle. Completion of the bioplan allowed the progression to a concept-level facility design including water recirculation system designs for each fish grouping developed in the bioplan. Concept designs for fry, smolt, pre-growout, and growout rearing areas, as well as a final purging system, were completed using water quality criteria required for optimal growth and successfully demonstrated in The Freshwater Institute growout trials.

Concept Design – The concept-level design developed for the 3,300 mt facility included independent designs for egg incubation, fry rearing, smolt rearing, pre-growout rearing, growout rearing and final purging. Each design included multiple recirculation modules to allow for staging and movement of fish throughout the facility. The following table (Table 3) summarizes the concept-level design characteristics for each rearing area:

Table 3. Concept-level design characteristics for each rearing area in a 3,300 mt Atlantic salmon farm.

Rearing Area	Recirculation Modules	Tanks per Module	Tank Diameter by Depth	Total Rearing Volume	Module Recirc Flowrate
Fry Rearing	2	18	2 m by 1 m	114 m^3	1.5 m^3/min
Smolt Rearing	2	4	9 m by 2 m	1,018 m^3	11.4 m^3/min
Pre-Growout Rearing	3	4	10 m by 3	2,827 m^3	22 m^3/min
Growout Rearing	8	5	16 m by 4.25 m	34,180 m^3	94.6 m^3/min
Final Purging	1	2	16 m by 4.25 m	1,709 m^3	37.9 m^3/min

Water supply required for the entire 3,300 mt facility was based on maintaining no more than 75 mg/L nitrate-nitrogen at maximum loading in each recirculation system, assuming no passive denitrification. The amount of water supply needed to maintain this nitrate-nitrogen level in the recirculation systems was calculated to be 7.6 m^3/min, including 1.1 m^3/min for purging. This water supply requirement could be reduced by reusing the 1.1 m^3/min from final purging as water supply to the growout systems and by including the effect of passive denitrifcation found to occur in fluidized sand biofilters; the overall effect would be to reduce the water supply required by 50% to 3.8 m^3/min.

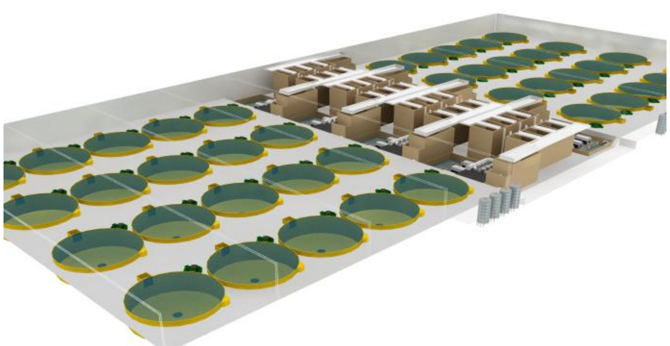

Figure 8. Concept-level design for the growout rearing area in a 3,300 mt Atlantic salmon farm. There are eight water recirculation modules for the growout rearing area. The total building area required for this portion of the facility is 21,400 square meters.

Capital and operating costs – Completion of the design for each system allowed the progression of capital and operating costs for comparison to other similar designs or enterprises. The concept-level capital cost estimate developed for the 3,300 mt facility was $31 million (Table 4). Cost data used in the development of the concept-level estimate provided here was a combination of industry standard published cost data (RS Means) and project specific vendor quotations. The operating cost developed was similar to that published for the net pen industry at approximately $3.90–$4.00 per kg of head-on gutted salmon. See Table 5 for the operating expense breakdowns. Note that these operating costs are subject to change with site selection due to differences in power costs, feed shipping costs, and other factors. For example operating costs presented here do not include the cost of heating or cooling that may or may not be required based on the geographic location of the facility.

Table 4. Capital expense breakdown for a 3,300 mt Atlantic salmon farm.

Facility Component	Cost
Water Recirculation Systems	**$21,126,000**
• Fry Rearing	$224,000
• Smolt Rearing	$997,000
• Pre-Growout Rearing	$2,147,000
• Growout Rearing	$16,944,000
• Final Purging	$814,000
Processing	**$1,878,000**
Buildings	**$8,379,000**
Influent/Effluent Treatment	**$0**
Total (+/- 30%)	**$31,383,000**

Critical assumptions used in the cost analysis were based upon data generated during the salmon growout trials at the Freshwater Institute. We also assumed that that all female eyed eggs were available four times annually, which a salmon egg supplier in Norway claims will be available in the near future (OddGeir Oddsen, SalmoBreed, Bergen, Norway, personal communication); all female eggs are assumed so as to eliminate the problem created by the early maturing males. Assumptions are presented in Table 6. The assumption for feed conversion ratio during growout is one of the most critical values in the estimation because it drives the largest component of the cost of production – feed cost during growout. Performance data from the Freshwater Institute trials indicate a feed conversion ratio of 1.09; utilizing this value during growout instead of 1.20 reduces the cost of production to $3.72 per kg head-on gutted product. The effect of feed conversion ratio during growout is shown in Figure 9.

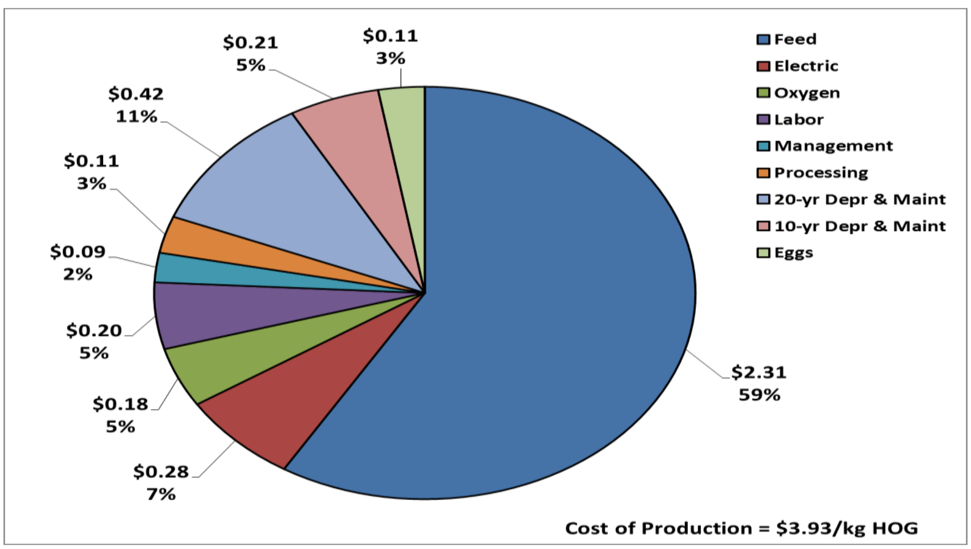

Figure 9. Cost of production as a function of feed conversion ratio during growout phase of a 3,300 mt Atlantic salmon farm.

Table 5. Operating expense breakdown for a 3,300 mt Atlantic salmon farm.

	Unit Price Assumption	Quantity per Year	$/kg HOG	% of Total
Feed	$1.50/kg	5,072,047 kg	$2.31	59%
Electric	$0.05/kWh	18,720,120 kWh	$0.28	7%
Oxygen	$0.20/kg	3,043,228 kg	$0.18	5%
Farm Labor	$45,000/person/year	15 full time employees	$0.20	5%
Management	$300,000/year	allowance	$0.09	2%
Processing Labor	$37,500/person/year	10 full time employees	$0.11	3%
Depreciation & Maintenance	Straight-Line		$0.63	16%
Eyed Eggs	$0.30 per egg	1,193,929	$0.11	3%
			$3.93	100%

Table 6. Critical assumptions used in the analysis for a 3,300 mt Atlantic salmon farm.

Feed Conversion Ratio – Fry	**0.75**
Feed Conversion Ratio – Smolt	**0.90**
Feed Conversion Ratio- Pre-Growout	**1.10**
Feed Conversation Ratio – Growout	**1.20**
Thermal Growth Coefficient – Fry	**1.25**
Thermal Growth Coefficient – Smolt	**1.40**
Thermal Growth Coefficient – Pre-Growout	**2.00**
Thermal Growth Coefficient - Growout	**2.30**
Purge Weight Loss	**5%**
Head-On Gutted Yield	**88%**

CONCLUSIONS

This growout trial served as a proof of concept, suggesting that it is biologically and technically feasible to culture Atlantic salmon from post-smolt (approximately 340 g) to harvest size over twelve months in a land-based, freshwater, closed-containment system, at least when at a suitable commercial scale. These fish that achieved a 4.6 kg mean size by almost exactly 2 yrs post-hatch. Survival during the growout phase was approximately 90% and feed conversion was 1.09:1. No obligate fish pathogens or sea lice were detected and no antibiotics, pesticides, or harsh chemotherapeutics were used. The fish were also never vaccinated.

Unfortunately, the majority (approximately 80%) of male salmon were observed to reach sexual maturity within the 24-month growout trial. The maturing males were readily identifiable based on observable morphometric and coloration characteristics and were harvested early and sent to processing as a hot smoked product. However, the fillet color and yield of maturing males are lower than for non-mature fish. This is a serious constraint to production in land-based closed-containment systems. Fortunately, previous research at The Conservation Fund Freshwater Institute indicates that an all-female strain (the Gaspe strain) can be produced to eliminate early maturing male salmon. Thus, an all-female source of Atlantic salmon will have to be developed to supply eyed eggs to land-based closed-containment systems that intend to produce food-size fish. Developing an all-female egg supply is not considered a huge barrier, as several other species are currently commercially available as all-female eyed eggs, including rainbow trout and Arctic char. In addition, the Atlantic salmon egg source should provide eyed eggs at least once every 6 months to maximize the production capacity within land-based closed-containment systems.

Growout trial data, specifically thermal growth coefficient, feed conversion, mortality, head-on gutted yield, and other performance indicators, were used to develop a biological plan, system design, and economic assessment for a 3,300 mt facility. Assuming that all female eyed eggs were available four times annually, the capital cost for this egg to plate facility was estimated to be approximately $31 million and its production cost was estimated at $3.90-$4.00 per kg of head on gutted salmon.

Acknowledgements

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