

**Thursday September 6th – Langeve / Cartier
Husbandry / Physiology
Moderator – Hamish Rodger (Fish Vet Group)**

3:15 PM	Husbandry / Physiology	<u>Timmerhaus</u> - Effects of Low to Very High Water Velocities on Atlantic Salmon Post-Smolts: Part I: Growth, Muscle Development and Schooling
3:30 PM		<u>Lazado</u> - Effects of Low to Very High Water Velocities on Atlantic Salmon Post-Smolts: Part II: Welfare, Mucosal Health and Stress Responses
3:45 PM		<u>Misk</u> - Assessing the Effects of High Oxygen Freshwater Saturation on Atlantic Salmon (<i>Salmo salar</i>) Growth, Food Conversion Ratio and Overall Health Within a Simulated Commercial Hatchery Setting
4:00 PM		<u>Stockwell</u> - Determining the Effects of Oxygen Supplementation on Cultured Salmon Behavior Using Acoustic Telemetry
4:15 PM		<u>Barker</u> - Saprotect™ – A Plant Derived Product for the Maintenance of Optimal Health of Fish and Fish Eggs.



8th International Symposium on Aquatic Animal Health

September 2-6, 2018 - Charlottetown, Prince Edward Island, Canada



Effects of Low to Very High Water Velocities on Atlantic Salmon Post-Smolts: Part I: Growth, Muscle Development and Schooling

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Beneficial effects of induced water velocities on Atlantic salmon smolts have been described in several studies. These effects include elevated growth rates, feed conversion rates and disease resistance. However, the optimum water velocity for farmed salmon smolts remains unknown. Thus, in this study, we addressed the effects of different water velocities on growth and muscle development (histology and gene markers) to estimate optimum conditions for rearing of post smolts in a recirculating aquaculture system. In addition to individual parameters, we addressed the behavioral response in regards to schooling. We divided 2400 salmon smolts (average start weight 80g) into twelve tanks (200 fish per tank) and set the water velocities for four triplicate tanks to *low* – 0.5 body length per second (BL/s); *medium* – 1.0 BL/s; *high* – 1.8 BL/s; and *very high* – 2.5 (BL/s). The velocity for the *very high* group was the highest tested for salmon smolts to date. The trial lasted three month and organ samples were collected at three time points. Time-laps cameras were used to observe the schooling behavior in increasing water velocities and showed that fish in the *low* and *medium* group distributed mostly evenly in the tanks. In contrast, fish in *high* and *very high* displayed strong schooling behavior at specific spots in the tanks. We observed a close to linear relationship between water velocity and growth rate, which resulted in 5.4% higher average body weight in the *very high* group than the *low* group at the end of the trial. The condition factors of fish from the *low* group was lower than in the other groups and an analysis of the contour of the fish bodies showed that fish in higher velocities grew wider (distance between back and belly outlines). Histological analyses of the muscle fibers revealed increased somatic growth in *high* and *very high* groups, while the expression of some genes of myosomatic growth pathway s were increased in the same fish. In conclusion, the increased body weight of fish reared in high water velocities was likely due to enhanced somatic growth of muscle fibers. Thus, these findings provide further evidence that elevated water velocities have positive effects on the growth rate of smolts even at the highest levels tested to date.

Participants are highly advised to consult the talk entitled: Effects of low to very high water velocities on Atlantic salmon post-smolts: Part II: Welfare, mucosal health and stress responses. by Carlo C. Lazado et al., for additional results.

Conference Session Designation: (Aquatic Animal Health Management)
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Effects of Low to Very High Water Velocities on Atlantic Salmon Post-Smolts: Part II: Welfare, Mucosal Health and Stress Responses

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There is ample evidence showing the beneficial effects of induced swimming, or exercise training in farmed fish. In Atlantic salmon, it has been shown that training through elevated water velocities has positive impacts on growth, feed conversion efficiency and robustness. However, there is little evidence demonstrating the effects on mucosal and stress responses, as well as on the external welfare of fish. More so, there is a question whether limits exist on the beneficial functions of elevated water velocities in salmon. In this study, we subjected salmon post-smolts (initial body weight circa 80 g) to four different training intensities by manipulating the water velocity in the tank: low – 0.5 body length per second (BL/s); medium – 1.0 BL/s; high – 1.8 BL/s; and very high – 2.5 (BL/s), for three months. The water velocity in the very high group has not been tested before in salmon. The external welfare status of fish was assessed following the FISHWELL handbook. Increased incidence of skin damage (i.e., scale loss, hemorrhaging) and pelvic fin damage (i.e., splitting) in the high and very high groups was documented. Nonetheless, the overall external welfare scores remained favourable in all groups. The skin and gills were subjected to quantitative histomorphometry and qPCR analysis of genes relevant to the mucosal defence. The expression of immune defence genes (e.g., *cd8α*, *tcrα*, *mhc1*, *mhc2*, *mblc2*) in the skin was negatively affected in the very high group, where significantly lower transcript levels compared with the other groups were observed. Interestingly, no significant differences between treatments were observed in the expression of selected marker genes in the gills. Histomorphological analyses of skin and gills are on-going. Plasma samples were collected and analysed for stress indicators. Plasma cortisol, glucose and lactate varied remarkably between groups at the beginning of the trial but such differences were not observed at the termination of the experiment. In conclusion, the welfare scores and the gene expression results in the skin revealed that the very high velocity may have some unfavourable consequences. Nonetheless, results from other response variables are suggesting that salmon subjected to a water velocity higher than the level previously thought to be the upper limit does not pose substantial negative consequences to health and welfare. The results of the study offer new frontiers in producing robust salmon through the benefits of induced swimming at higher water velocities.

Participants are highly advised to consult the talk entitled: **Effects of low to very high water velocities on Atlantic salmon post-smolts: Part I: Growth, muscle development and schooling.** by Gerrit Timmerhaus et al., for additional results.

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Assessing the Effects of High Oxygen Freshwater Saturation on Atlantic Salmon (*Salmo Salar*) Growth, Food Conversion Ratio and Overall Health Within a Simulated Commercial Hatchery Setting

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Atlantic salmon is a diadromous fish species and spends about 50% of its entire life cycle in culture conditions in freshwater land-based hatcheries. A critical aspect in commercial hatcheries is the provision of appropriate levels of oxygen within the freshwater environment to provide optimal growing conditions. Oxygen can represent a significant operational cost to the hatchery cash flow depending on its source, which can be further exasperated depending on the efficiency of the delivery system. Conversely, improper oxygen delivery and maintenance of elevated levels may be detrimental if this leads to physiological issues, such as gas bubble disease in the absence of appropriate stripping of other gases to control the total gas pressure. This project provide benchmarking between triplicate tanks treated with ambient freshwater dissolved oxygen concentration ($90\% \pm 10\%$) with triplicate tanks receiving added dissolved oxygen concentrations of $150\% \pm 10\%$ and $200\% \pm 10\%$, respectively. Study water was made up on demand using a proprietary gas infusion system to infuse oxygen to raise the measured dissolved oxygen concentrations while removing nitrogen from the water to maintain water total gas pressure at near 100%. This study design was maintained from the time when the tested Atlantic salmon were about 3g throughout the entire freshwater stage to provide a simulated freshwater hatchery environment. Specific growth rate and Fulton's condition factor were calculated using data collected during non-lethal sampling ($n=10 \times 3$ /group) either monthly or during planned cutbacks that match the simulation production plan based on stocking density. Fish survival from each of the treatment replicate tanks was recorded through documentation of removed mortalities. Overall fish health was assessed by a lethal sub-sampling of each tank population ($n=10 \times 3$ /group) during cutbacks, including hematocrits and general necropsy. After the first density-split, fish held in 150% and 200% dissolved oxygen saturation freshwater had higher survival compared with controls held within ambient conditions. The overall mean growth rate in 150% and 200% oxygen saturation was also higher than from ambient conditions by 47% and 44%, respectively. Interestingly, while fry held in 150% and 200% oxygen saturation had similar overall performance, only fish exposed to 200% oxygen saturation had significantly lower hematocrit values. This highlights the ability of exposed fry to perform better in increased oxygen saturation but yet limit their intake of dissolved oxygen as the increase in dissolved oxygen saturation was not directly correlated with increased performance.

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Determining the Effects of Oxygen Supplementation on Cultured Salmon Behavior Using Acoustic Telemetry

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The health and welfare of farmed fish is highly dependent on water quality, being dissolved oxygen (DO) one of the most critical factors. Some farms suffer from episodic low DO, which can be exacerbated with predicted rising sea level temperatures causing solubility of oxygen to decrease. The negative impacts of low DO have caused the farm managers to seek alternative solutions for sustaining the health of farmed fish by supplementing sea cages with oxygen. Low oxygen levels negatively affect fish behavior, which is a key component in determining fish welfare, and can therefore could be used as an early warning indicator of stress from low DO. In this study, the behavior of Atlantic salmon (*Salmo salar*), located in Southern Nova Scotia, was studied in response to the introduction of supplemental oxygen for 3 months (mid July-mid Oct) to test the suitability of using fish behavior as an early indicator of fish health with relation to changes in oxygen levels. Swimming depth and biomass density were recorded, before, during, and after oxygen supplementation trials, using CageEye, a sonar system used for tracking total biomass movement within aquaculture cages in real time. Additionally, health factors, such as mortality rate, swimming activity, and feed intake, were recorded to help understand the behavior during changing dissolved oxygen levels. Future work will combine this technology with VEMCO acoustic tags to test the applicability of using fish behavior as an indicator of other stress drivers such as storms, temperature changes, and diseases. Preliminary results suggest that real time data collection of fish behavior allows for an early warning indicator of fish health and can help to improve farm management.

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SAPROTECT™ - A Plant Derived Product for The Maintenance of Optimal Health of Fish and Fish Eggs

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Infections by Oomycete “water mould fungi” (e.g., *Saprolegnia* spp.) are problematic at most freshwater fish hatcheries, including Canadian salmon hatcheries, with egg losses between 10-50%. The most commonly used approved therapeutant is formalin (Parasite-S™); however, there are concerns about its safety for the fish and the user. Other treatments (e.g., iodophores, salt, H₂O₂, etc.) exist but each has its limitations. Consequently, there is a need to develop an alternative product that can be safely applied to all stages of eggs and fish. RPS Biologiques, a PEI-based Canadian Biotechnology Company working in the field of aquatic, human and animal health, has developed a plant derived product called SAPROTECT™ to maintain health of fish and fish eggs. SAPROTECT™ is cost-competitive with existing anti-fungal therapeutants and the raw materials can be used for human consumption. As part of the ongoing product development and pre-regulatory testing process, a third party test facility (Huntsman) was contracted to evaluate target animal safety (TAS) studies. In pilot studies using an *in-vitro* infection model, low concentrations (LC₅₀ = 5.28-26.18%, mean (± SD) = 17.47 ± 8.88%) of SAPROTECT™ had similar efficacy as standard formalin concentrations. Using these baseline concentrations, an exploratory safety evaluation on Atlantic salmon, *Salmo salar*, embryos (E), alevins (A) and fry (F) was conducted, based on the standardized EAF-test methods of Environment Canada. For toxicity testing exposures, 50% SAPROTECT™ represented the highest dose, with subsequent dilutions 25, 12.5, 6.25, 3.13 and 1.56%. One reference control used hatchery water and a second reference control was formalin applied at typical treatment doses used in salmon culture settings (250 ppm for eggs, 167 ppm for fry). The study began with 160 fertilized, ‘eyed’ eggs per treatment (4 replicates of 40 eggs per treatment). For the E-stage, a series of one-hour, static bath treatments occurred every Monday, Wednesday and Friday (n=13 treatments). During the A-stage, there were daily observations but no treatments to facilitate yolk sac absorbance by the alevins without disturbance. For the F-stage, a series of one-hour, static bath treatments occurred every Monday, Wednesday and Friday (n=12 treatments). During all stages, there were no patterns of mortality associated with treatment. Because of low overall mortality and low percentage of any measured effects, values of LC₅₀, EC₅₀ and EC₂₅ could not be reliably predicted (by definition of the algorithm). Using mortality as an endpoint, the NOEC (no observable effect concentration) for fry was 50% SAPROTECT™ and the LOEC (lowest observed effect concentration) was > 50% SAPROTECT™. The results from the exploratory TAS study did not report any quantifiable toxicological effect associated with using SAPROTECT™ (1.56, 3.13, 6.25, 12.5, 25 and 50%) in repeated 1h static bath exposures on live embryos (within eggs) and fry of Atlantic salmon. RPS is now engaged in the regulatory approval process and has applied for product registration. It is anticipated that pilot scale production will soon begin.

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