THE EFFECTS OF SALINITY AND PHOTOPERIOD ON GROWTH AND SWIMMING PERFORMANCE IN ATLANTIC AND COHO SALMON RAISED IN RECIRCULATING AQUACULTURE SYSTEMS FROM SMOLT TO ADULT.

by

Victor Chan

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis/dissertation entitled:

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Examining Committee:

Colin Brauner, Zoology

Co-supervisor

Jeffrey Richards, Zoology

Co-supervisor

Christopher Wood, Zoology

Supervisory Committee Member

Robert Shadwick, Zoology

Additional Examiner

Additional Supervisory Committee Members:

Supervisory Committee Member

Supervisory Committee Member

Abstract

Salmon are among the most popular seafood products and their culture continues to expand with improved aquaculture technology. Typical aquaculture production rears salmon from smolt to market size in net-pens in oceans, but this practice has been criticized due to potential environmental concerns such as eutrophication and interactions between escaped cultured fish and wild populations. Rearing fish in recirculating aquaculture systems (RAS) is a new technology to address some of the concerns over net-pen aquaculture, as well as enhance production of salmonids and other fish species. Salmon can inhabit a wide range of salinities with different osmoregulatory costs, but presumably these costs can be reduced if fish reside in water isosmotic to their blood plasma. Photoperiod has been shown to affect growth rates in salmon at different life stages but can also affect early maturation in salmon. To examine the effects of salinity and photoperiod on the growth of salmon, seven replicate RAS with salinities of 2.5, 5, 10 and 30ppt under 12:12 and 24:0 light:dark photoperiod were used to rear Atlantic and coho salmon from smolts onwards for 120 days. Salinity and photoperiod had an effect on Atlantic salmon growth, with those reared at 10ppt in 24:0 light showing the highest growth rates. However, neither photoperiod nor salinity affected coho salmon growth. To understand the effects of salinity and photoperiod on swimming performance and hematology, coho salmon from two separate studies underwent repeat maximum swimming speed (U_{max}) tests. In the first study, U_{max} was assessed in coho salmon that were reared for 350 days in 0 and 10ppt. In the second study, U_{max} was assessed in coho salmon that were reared for 60 and 150 days in 2.5, 10 and 30ppt under 12:12 and 24:0 light:dark photoperiod. In the first study, salinity had significant effects on resting plasma osmolality and chloride concentration. In the second year, salinity

affected first U_{max}, but neither salinity nor photoperiod affected repeated U_{max} and recovery ratio. There were also significant effects of salinity on the hematocrit, hemoglobin concentration, MCHC and plasma osmolality and chloride concentration of exhausted salmon after repeated swimming tests.

Lay Summary

In my research, I examined how salinity and photoperiod can improve growth and swimming performance of Atlantic and coho salmon reared in recirculating aquaculture systems at salinities ranging between freshwater and seawater under either continuous light or 12-hour light and dark cycles. I determined Atlantic salmon reared at salinities close to brackish water and under continuous light had the highest growth rate, while coho salmon seemed to grow equally well across all salinities and photoperiods tested. In coho salmon, I found salinity and photoperiod did not affect maximum swimming speeds but did influence blood chemistry changes at exhaustion. The results of my thesis show 24-hour light would provide improved growth for salmon in RAS production, with no compromises on swimming performance. Salinities close to brackish water also seemed to improve growth in Atlantic salmon, but not in coho salmon.

Preface

I conducted all research under the supervision of Drs. Colin J. Brauner and Jeffrey G. Richards. I wrote all 4 chapters of the thesis and received editorial feedback from Drs. Colin Brauner, Jeffrey G. Richards and Chris C.M. Wood. Experimental protocols involving use of animals in research were followed under the animal use protocol, in accordance with The University of British Columbia's Animal Care Committee, certificates #A13-0016 and #A17-0011.

Chapter 2 is based on a collaborative research project with Dr. Kevin T. Stiller, Yuanchang Fang and Chandler W. Hines under the same Animal Care Committee animal use protocols A13-0016 and A17-0011. I was equally responsible for the handling of the animals used in the research and the collection of data from these animals, which included daily animal care, experimental time point data collection and data analytic.

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12:12 light	12:12 light:dark photoperiod
24:0 light	24:0 light:dark photoperiod
BL	Body length
$[Cl^-]$	Plasma chloride ion concentration
FCR	Feed conversion ratio
[Hb]	Whole blood hemoglobin concentration
HiCN	Hemiglobincyanide
MCHC	Mean corpuscular hemoglobin concentration
NKA	Na^+/K^+ ATP-ase
ppt	Parts per thousand
RAS	Recirculating aquaculture system
RR	Recovery ratio
SGR	Specific growth rate
TGC	Thermal growth coefficient
U_{crit}	Critical swimming speed
U_{max}	Maximum swimming speed

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Dedication

To my grandmothers, who will hopefully be proud to have a grandchild that did something completely different and crazy.

Chapter 1: **Introduction**

1.1 Summary

Salmon is one of the most popular fish products on the seafood market and is in high demand worldwide. To meet increasing demands for fresh seafood, new aquaculture technology is continually developed to increase production and reduce costs. One such technology is Recirculating Aquaculture Systems (RAS). RAS provides greater flexibility and control of the environment than traditional open net-pen aquaculture, which can be used to maximize growth and performance. However, there are gaps in our understanding of how different environmental parameters affect the growth and performance of salmon reared in RAS, so it is important to determine optimal environmental conditions for enhancing salmon growth and physiological performance.

This chapter will focus on current practices in aquaculture, including the two key aquaculture systems used for rearing fish and the methods used for enhancing salmon growth. I will also describe the effects of salinity and photoperiod on salmon and how it potentially influences growth and performance. I will then outline my thesis objectives, which are to determine the effects of salinity and photoperiod on the growth of Atlantic salmon, *Salmo salar,* and the growth and swimming performance of coho salmon, *Oncorhynchus kisutch*.

1.2 Salmon aquaculture

Salmon is ranked highest in production value and revenue in the Canadian seafood industry. Between 2003 and 2013, Canadian aquaculture production grew by 63%, signifying both an increase in aquaculture products and an increase in international demand for Canadian aquaculture exports (Fisheries and Oceans Canada 2013). This increased production and value was made possible by the introduction of aquaculture technology to the industry in the 1970's and 1980's, which helped shift the supply base from wild-caught to aquaculture-based salmon (Asche & Bjørndal 2011). One of the most popular methods of aquaculture-based salmon production is open net-pen production.

1.3 Open net-pen salmon aquaculture

The biological process of salmon production starts with fertilizing and transporting eggs to a freshwater hatchery (Asche & Bjørndal 2011). After incubation for 2 months, larvae hatch and are reared in the hatchery until they become smolts, at which time they are then transferred to open net-pens in the sea. Generally, Pacific salmon fry smolt at 6-8g, 4-6 months post-hatch, while Atlantic salmon fry smolt at 40g, 16 months post-hatch. With the rapid growth of industry-selected Atlantic salmon smolts, which can instead reach 70-140g in 16 months, they now smolt and transfer to open net-pens at 8 months post-hatch (Asche & Bjørndal 2011). Typically, smolts are transferred to open net-pens during the warmer half of the year to make room for the next generation of salmon fry in freshwater hatchery tanks. The open net-pens that the smolts are transferred to are specialized for grow-out (ie. reaching market size), which requires 2 years to achieve a 2 to 8kg Atlantic salmon or 12-16 months to achieve a 2kg coho salmon.

Open net-pens depend on ocean currents to flush effluent out to sea and circulate water into the pens (Price 2004). The dependency on ocean water flow, along with intensive feeding and production regimes, can lead to eutrophication of the benthic communities beneath the open net-pens. Eutrophication is due to nutrient loading (particularly nitrogen and phosphorus from uneaten food and feces) in the environment that promotes algae growth, which can be toxic and result in oxygen depletion in the surrounding waters (Folke et al. 1994; Soto & Mena 1999). Salmon can also escape from net-pens due to torn nets, which can pose a significant ecological concern because of the potential for interbreeding with wild salmon, but aquaculture-reared salmon tend to be inferior in reproductive behavior, in some cases destroying wild salmon nests or males unsuccessfully fertilizing wild salmon eggs (Fleming et al. 2000; Naylor et al. 2005). In addition, escaped salmon can compete for the same resources as native salmon, decreasing overall abundance of habitat and food (Naylor et al. 2005).

To help mitigate some of the environmental concerns related to open net-pen farming, the aquaculture industry is exploring the use of land-based recirculating aquaculture systems (RAS) for growing salmon from smolt through to market-sized adults, which also minimizes transport costs and time to open net-pens (Martins et al. 2010; Tal et al. 2009).

1.4 Recirculating Aquaculture Systems

Generally, a RAS consists of two water loops: one for processing effluent water and one for providing water to the animal holding tanks. The effluent loop draws water from the tank containing fish and passes it through several stages of filtration, which generally includes particulate filtration for feces and uneaten food removal, and biological filtration for dissolved

ammonia and CO_2 removal. The second loop, which provides water to the animal holding tanks, is oxygenated and set to the appropriate temperature through a chiller or heater to provide optimal temperature for rearing. In a well-designed and properly maintained RAS, high stocking densities can be achieved with high recirculation efficiency, where almost no new water needs to be added and effluent produced from intensive RAS production can be potentially reduced by 96% through using waste as a carbon source for water denitrification and as biogas in a methanogenic bioreactor (Tal et al. 2009).

In addition to reducing water waste and effluent, the rearing conditions provided in RAS can improve fish growth rate when compared to traditional flow-through and open net-pen systems. A RAS design described by Tal et al. (2009) showed high survival rate and higher growth rates in gilthead seabream when compared to open net-pen production, providing strong support for RAS productions to manage and maintain optimal conditions for fish growth and survival. In rainbow trout, a similar increase in growth and feed conversion was also found following 77 days of continuous rearing in a RAS (70m^3 effective volume) relative to a flow-through system (18 m^3 effective volume) (Roque d'Orbcastel et al. 2009). This trial started with a similar starting density of 57kg/m^3 in both systems, but yielded a final density of 108kg/m^3 in RAS with an SGR of 0.85% after 77 days, while the final density was 98kg/m^3 in a flow-through system with an SGR of 0.68%; however, the differences in tank size may have also affected the outcome (Roque d'Orbcastel et al. 2009).

One of the greatest challenges facing the widespread adoption of RAS for commercial fish production is the significant cost associated with the initial RAS construction and ongoing

maintenance and operation. Badiola et al. (2017) attempted to rear 2,500 Atlantic cod in RAS in Northern Spain for 430 days and found employee and energy costs associated with operating RAS were the most significant contributions to overall production costs. Badiola et al. (2017) concludes that RAS production of less than 200 metric tons of Atlantic cod reared for 430 days is not economically viable, given the high salary and energy cost of the region. In British Columbia, financial analyses of open net-pen and RAS production for salmonids demonstrate that starting a RAS farm requires more than four times the initial investment compared with open net-pens (>$22 million vs. $5 million, respectively) (Boulet et al. 2010). Based on the initial investment costs and estimates of three-year income, return on equity for open net-pens (52%) is significantly higher than for RAS (4%). It is also worth noting that the profitability of RAS-reared salmon is much more sensitive to fluctuating market forces. With the high costs of construction and operation of RAS-based fish farms, it is paramount that any production efficiency for enhancing fish growth must be implemented to make RAS a viable solution for production. In current practices, there are methods already implemented in aquaculture to enhance growth and welfare for salmon, which can be translated to RAS production.

1.5 Current methods for improving salmon growth rate in aquaculture

The aquaculture industry has a long history of attempting to improve growth rates of economically important species and many of these strategies can also be implemented or utilized in RAS based fish farming. Selective breeding based on valued phenotypes is probably one of the most long-standing methods for domestication and maximizing production. By selecting and breeding favorable phenotypes, such as selecting fry with longer bodies for greater weight gain, this practice has yielded positive results in improving weight gain in Atlantic salmon (Friars et

al. 1990; Friars et al. 1995; O'Flynn et al. 1999). Genetic manipulation, such as sex reversal and induced polyploidy, has also been used for enhanced growth (Maclean & Penman 1990). Sex reversal is achieved by treating fry with methyltestosterone in food for 700^{o}C days (number of days × water temperature), which causes the fish to be phenotypically male while remaining genotypically female. This process produces sperm with only X chromosomes, allowing the next generation of fertilized eggs to be exclusively female. Induction of cellular polyploidy by thermal hydrostatic pressure shock after fertilization in salmon has long been implemented to induce sterility, which prevents net-pen escapees from breeding with wild populations or competing with wild salmon for breeding sites (Benfey 1998). However, other studies also show that triploids exhibit increased mortality and are more susceptible to stress than their diploid counterparts, making them less desirable in aquaculture, where production efficiency and survivability is critically important (Benfey 1998; O'Flynn et al. 1997).

As ectotherms, temperature variation can also play an important role in fish growth. In a study by Handeland et al. (1998), which examines the effects of salinity and temperature on growth in Atlantic salmon reared in 28% or 34% seawater at 4^{o}C or 8^{o}C for 60 days, temperature has a significant effect on growth, with mean weight being significantly higher in the 8^{o}C groups compared with the 4^{o}C groups. However, rearing salmon beyond their thermal optimum can result in poorer growth. For example, Atlantic salmon reared at 13^{o}C for 45 days relative to those reared at 19^{o}C for the same period had lower feed intake, lower specific growth rates, decreased mass and poorer condition factor (Hevrøy et al. 2013).

Genetic and environmental parameters have been shown to have strong effects on growth and are extensively used by the aquaculture industry to boost production. In recent years, however, the influences of behavior and welfare have also been considered for improving growth, as well as the marketability of salmon.

1.6 Density and welfare of salmon in aquaculture

The physical conditions, such as the animal stocking density and the interactions between animals, can play a major factor in determining the success and growth of fish in RAS. High stocking density in RAS can improve profitability, but there are also drawbacks of this practice. For example, Liu et al. (2017) found the incidences of fin damage increased, which is an indicator of greater aggression between individuals, as stocking density increased in Atlantic salmon post-smolts initially reared in low (9.8kg/m^3), medium (19.62kg/m^3) or high (28.79kg/m^3) stocking densities over 66 days. The high-density groups of this study also had higher serum cortisol levels, a common indicator of stress, and alkaline phosphatase, a lysosomal enzyme with a protective role in initial wound healing. This suggests that even though higher density may improve biomass yield, the visual quality and welfare of the animals can be compromised, which negatively impact their market value. Another study on rainbow trout reared in RAS at 57kg/m^3 also saw higher fin erosion, but in this case the issue was attributed to the high water velocity in RAS, which caused a higher incidence of fin erosion due to altered swimming behaviors (Roque d'Orbcastel et al. 2009). In contrast, Kolarevic et al. (2014) noted higher fin damage in flow-through systems compared to RAS and attributed it to the lower alkalinity in flow-through systems. These studies suggest that there are many factors that can be altered in RAS to reduce the incidence of fin erosion in salmon, including water flow rate, fish

density and water chemistry. Colson et al. (2015) showed no significant difference in shoaling behavior and swimming activity of rainbow trout before and after a condition stimulus (ie. inflow water turned off) in either RAS or flow-through systems, which indicated that grouping and swimming activity are not affected by the system design. This is further supported by low blood cortisol levels in fish reared in RAS, consistent with levels measured in fish reared in flow-through systems (Colson et al. 2015). Indeed, given the typical design of RAS, it is possible to manipulate many factors that can both improve the welfare of farmed animals and marketability of fish appearance.

Despite advances in understanding how the environment, biology and behavior of salmon can have varying effects on growth and health, there are still many other factors that are not well understood. With the shift to RAS, which provide greater possibilities for altering and maintaining different environmental conditions, new gaps to aquaculture practices are emerging and will need to be resolved to make RAS an efficient production system. Two environmental parameters, salinity and photoperiod, have been of interest because salinity is known to affect osmoregulatory demands that can in turn affect growth, and photoperiod is known to affect salmon maturation and growth.

1.7 Salinity

Salinity is a fascinating environmental condition to consider in aquaculture for improving growth because of a salmon's anadromous life cycle and ability to inhabit salinities ranging from freshwater to seawater. At different salinities, fish use different strategies to control plasma ion levels and osmolality (Marshall & Grosell 2006). For instance, in freshwater, fish will passively

lose ions and gain water, so to counteract the imbalance, the kidney becomes specialized to absorb electrolytes from urine while the mitochondrion rich cells in the gill epithelium will actively take up Na^+ and Cl^- into the blood (Evans 2008). While fish in freshwater compensate for ion loss via ionoregulatory pathways in the gills and the kidney, seawater fish use a different mechanism. In seawater acclimated fish, the gut plays a larger role in osmoregulation, where ions are actively absorbed from imbibed water, creating an osmotic gradient that favors water uptake (Sardella & Brauner 2007). The excess ions in the blood are then actively secreted at the gills, and a low volume of highly concentrated urine is released.

In freshwater or seawater, fish will need to expend energy, largely through activation of Na^+/K^+-ATPase (NKA) activity, to retain or excrete ions and water in the body to maintain homeostasis. However, there has been considerable attention towards intermediate salinities, where costs may be lower, relative to freshwater and seawater, due to its similar ionic composition to blood, thus allowing some of the saved costs in osmoregulation to shift to growth. A study by Morgan and Iwama (1991) on growth and metabolic rates in chinook salmon fry reared at 0, 5, 10, 20 and 28ppt over 11 weeks found growth is highest in freshwater, which decreases with increasing salinity to 28ppt. In addition, metabolic rates increased with salinity, showing significantly higher rates in hypertonic treatments relative to the freshwater treatments. Morgan and Iwama (1991) commented that the decreased growth and increased oxygen consumption in salmon fry reared in salinities above freshwater were from increased ion-osmoregulation energy costs. However, a later study by Morgan and Iwama (1998) on coho salmon smolts reared at 0, 10 and 28ppt for 6 weeks showed gill NKA activity, an indicator for osmoregulatory energetics, was higher in fish reared at 0 and 28ppt after 21 and 42 days of acclimation, relative to fish reared at

10ppt. Interestingly, plasma Na^+ and Cl^- concentrations of 28ppt fish returned to levels close to those of fish reared at 0ppt at 42 days, and oxygen consumption after 6 weeks of acclimation showed no significant differences between the three treatment groups. Based on these two studies, salinity seems to have strong effects on salmon growth and metabolic rates that are dependent on their natural life cycle: at the fry stage, there appears to be an increased metabolic rate and reduced growth at salinities above freshwater, which corresponds with the time prior to seaward migration when fish are not adapted to seawater. Likewise, at the smolt stage, physiological preparations for seawater migration enables osmoregulatory pathways that are not activated in fry, resulting in reduced NKA activity and energy demands at isosmotic salinities.

The osmoregulatory costs during different life stages and salinities presented by Morgan and Iwama (1991 and 1998) are further supported by other studies of salinity effects on growth in salmon. In Emerman's (2016) study, it was shown that coho salmon smolts reared at 10ppt under continuous light had enhanced growth rates during the first 59 days of acclimation, relative to other treatment groups at 0, 5, 20 and 30ppt. Similarly, Atlantic salmon parr acclimated to 0, 10, 20 and 31ppt exhibited stunted growth in the 20 and 31ppt treatments during the first two months of acclimation (Duston 1994). After the first two months, growth rates matched the 0 and 10ppt acclimated individuals, suggesting the effects of salinity on growth are determined by the time needed for the animals to re-establish efficient osmoregulation during smoltification. However, Emerman (2016) showed different results in Atlantic salmon, where there were no effects of salinity on growth rate in smolts reared at 0, 5, 10, 20 and 30ppt for 59 days. These results indicate salinity has strong interactions with salmon life stages, which can translate to enhanced growth that are only elicited at specific times.

Although many studies indicate salinity affects growth, other studies have shown salinity to have less of an effect when compared with the effects of temperature and photoperiod (Clarke et al. 1981; Handeland et al., 1998). Similarly, the effects of salinity are less important than feed ration in altering growth rate (McCormick et al. 1989). These results propose that other environmental parameters can have stronger effects on enhancing growth, relative to salinity, but these studies did not consider rearing salmon from smolt through to market size at different salinities, which requires a longer acclimation period that may elicit effects over time.

In salmon aquaculture, changes to salinity are also used for the treatment of infections and have impacts on cataract development. *Saprolegnia*, a species of oomycetes, is a salmon pathogen most commonly found in fish reared in freshwater. It is characterized by white or gray patches of wool-like filamentous mycelia that starts on the epidermal tissues, creating lesions that may be soft, necrotic and ulcerated (Robertson et al. 2009). Past veterinarian treatments for fish infected by *Saprolegnia* is to use malachite green, but the practice has been banned due to its toxic and carcinogenic effects on fish and humans. An alternative approach has been to increase salinity, where as little as 1.8ppt NaCl appears to protect against *Saprolegnia* infection (Robertson et al. 2009). In RAS, treatment of Atlantic salmon with 1-2ppt of aquarium salt has been observed to reduce *Saprolegnia* outbreaks, improving animal health and survival rates (Emerman 2016). Ocular cataract development is a concern for salmon farmers, as the issue is prevalent in intensive salmon aquaculture, which have been seen to have negative effects on growth and profitability because fish were not able to see their food as readily with cataracts (Bjerkås, Holst, & Bjerkås, 2004). Bjerkås and Sveier (2004) found optimal levels of dietary histidine prevented

cataract development in Atlantic salmon post-smolts but exposing them to varying salinities of 15 and 30ppm over 4 weeks and elevated temperatures still increased the risk of cataract development. The increased cataract development risk of Bjerkås and Sveier's (2004) study was attributed to the increased and varied ocular osmotic pressure imposed by the environment, relative to a stable salinity environment. The effects of seawater transfer on cataracts have also been observed: when yearling coho salmon of 25-75g were transferred to seawater in February and March, which was 1-2 months prior to their natural seawater migration time, the incidences of cataracts was found in all samples and persisted even after prolong seawater adaptation (Iwata et al. 1987). In contrast, when yearling coho salmon were transferred to seawater in April, cataract incidences decreased to 40% in the first week, none in the second and third week and 14% in the last week of the month. Iwata et al. (1987) concluded that cataract formation coincided with the animal's adaptation to seawater after smoltification and increased osmoregulatory ability. As such, the changes to salinity and the time at which salinity is introduced are important to reducing cataract development, which in turn will affect salmon growth in aquaculture.

1.8 Photoperiod

It is common practice in current aquaculture production to use continuous light in open net-pen grow-out facilities. Under continuous light conditions, salmon farmers can feed their animals continuously throughout the day to maximize growth to market weight. However, photoperiod has been attributed as the principal cue for entraining an endogenous rhythm for reproduction, which in turn affects the time of maturation and, inversely, growth (Bromage et al. 2001). This endogenous rhythm can heavily influence melatonin secretion from the pineal gland, which is

proportionately increased with the length of night and used as a strong indicator of seasonal cycle effects on growth and maturation in fish. Immature Atlantic salmon reared in sea cages under a natural photoperiod produce higher night-time melatonin levels when compared to those reared under continuous light (Porter et al. 1999). In addition, 61.5% of the Atlantic salmon were graded as mature when reared under a natural photoperiod, compared to 6.1% of fish graded as mature when reared with additional lighting at night. Another study by Hansen et al. (1992) also showed continuous light can increase growth rates after December and reduce second year sea-winter salmon maturation, relative to those reared under a natural photoperiod.

There are also different results from other studies, showing shorter photoperiod cycles to reduce maturation, especially in male salmon. RAS-reared immature Atlantic salmon of mixed sex, at approximately 850g, had reduced sex steroid and melatonin under 12:12 and 8:16 (light:dark) light cycles compared with continuous 24:0 light (Qiu et al. 2015). The shorter photoperiods were associated with lower incidence of gonadal development. In addition, the different photoperiods did not affect growth and survival rates, so Qiu et al. (2015) suggested that shorter photoperiods should be used for Atlantic salmon to prevent early maturation without compromising growth. Similarly, male Atlantic salmon parr reared for six weeks under 24:0 light showed 47% maturation incidences, while no maturation was found under 18:6 light (Fjelldal et al. 2011). In a follow-up experiment in the same study, under-yearling smolts reared under continuous light were subjected to either continuous 24:0 light or natural photoperiods in Norway for three months, with 8.3% of males in 24:0 light fully matured with female mimicry characteristics (absence of hooked lower jaw and presence of dark yellow-greenish skin coloration) at the end of the experiment, while no males matured under natural conditions.

1.9 Exercise and swimming performance

Fish swimming performance, particularly maximum swimming speeds, have often been considered as an indicator of aerobic capacity and fish health, which can be affected by environmental and physiological challenges. Hammer (1995) and Jain et al.'s (1998) studies established that environmental pollutants, reduced dissolved oxygen, changes in temperature and disease can significantly reduce swimming performance. In an ecological perspective, critical swimming speeds (U_{crit}) can also indicate the ability of salmon to swim through strong currents during upstream migration, which ultimately affects their reproductive capabilities (Jain et al. 1998). Physiologically, salmon swimming near U_{crit} reach their maximum cardiac output, which provides additional value when comparing maximum aerobic capacity between and within salmon species (Jain et al. 1997; Thorarensen et al. 1996). As such, analysis of fish swimming performance has important implications for aquaculture because it can provide insight into overall animal health and physiological performance.

Exercise increases oxygen demand, which requires increased O_2 and CO_2 flux across the gills through increased ventilation volume, functional gill surface area and increased blood perfusion (Sardella & Brauner 2007). However, the improved gas exchange that supports greater oxygen consumption comes at the price of increased osmoregulatory disturbances because of the increased diffusion of ions between the animal and the environment. Postlethwaite and Mcdonald (1995) found there were significant increases in whole animal Na^+ and Cl^- efflux in freshwater rainbow trout exercised at 1.8BL/s over 96 hours. Upon 24-hour recovery from exercise, these fish then increased Na^+ and Cl^- ion influx, presumably to compensate for the exercise-induced loss. As salinity can be changed in RAS to enhance growth or health, there are

potentially higher energy costs when faster swimming speeds are used (chasing by farmers using nets) because of increased osmoregulatory disturbance imposed by oxygen demand.

There is limited literature looking at whether enhanced growth due to salinity and photoperiod can compromise or compliment maximum swimming performance, as it can be predicted that higher muscle mass equates to animals achieving higher maximum speeds. On the other hand, higher mass may also require higher metabolic costs to move the animal, which limits the animal's ability to achieve higher speeds because of substantial energy costs. As such, the trade-off between growth and swimming performance should be studied to recognize any compromises that are imposed by changes to the RAS environment.

1.10 The Initiative for the Study of the Environment and its Aquatic Systems

Despite the potential benefits of using RAS and its ability to control and maintain specific environmental parameters, one of the major hurtles preventing the widespread adoption of RAS for salmon aquaculture is the fact that we don't know what environmental conditions yield optimal growth, welfare and physiological performance of any salmon species. To address this knowledge gap, the Initiative for the Study of the Environment and its Aquatic Systems (*InSEAS*) at The University of British Columbia was built with the aim to identify the optimal conditions for rearing salmon in RAS, in the hope of improving salmon production in Canada. *InSEAS* consists of seven identical, 15,000L RAS, each with two $5m^3$ and two $0.7m^3$ blue fibreglass tanks, which allow various fish species to be reared in identical conditions at different densities (shown in Figure 1.1) (Emerman 2016).

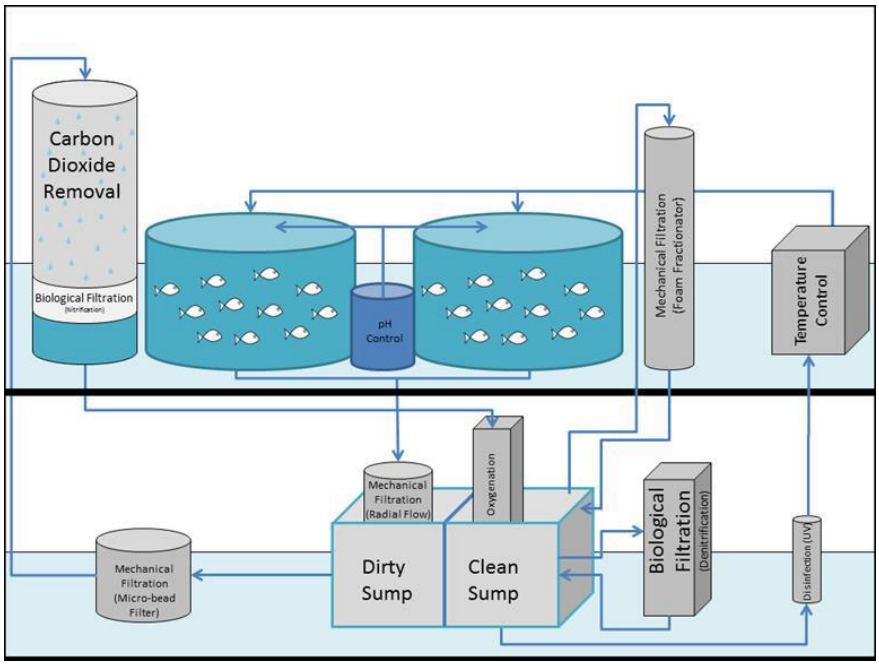

Figure 1.1 Diagram of an *InSEAS* recirculation aquaculture system, consisting of two 5m^3 tanks and associated mechanical and biological components. Arrows represent direction of water flow through the system (Emerman 2016).

1.11 Objectives of this thesis

The goal of this thesis was to examine the effects of salinity and photoperiod on the growth of Atlantic salmon (*Salmo salar*) and growth and swimming performance of coho salmon (*Oncorhynchus kisutch*). These species were chosen because of the extensive aquaculture production of Atlantic salmon around the world, and the interest in developing a British Columbia native salmon, coho salmon, for production.

There has been extensive research into understanding the effects of salinity, photoperiod and exercise on salmon growth and performance; however, most studies to date are limited to short acclimation time from 24 hours to several weeks. Very few of these studies have been conducted

from smolt to adult. In addition, there is little to no research that considers the interactions of salinity and photoperiod and their effects on growth and performance of salmon.

This work aims to provide information and recommendations to salmon producers for better management of their RAS facilities, in hopes to meet the demands for fish products by the global market and reduce cost and price of such commodity. This work also looks to further understand how a range of salinity and photoperiod can affect the physiology of Atlantic and coho salmon.

Chapter 2: **Effects of Salinity and Photoperiod on Salmonid Growth**

2.1 Summary

For RAS to be a viable and profitable technology for producing fish, environmental conditions must be managed appropriately to minimize costs and maximize production. However, as RAS is still a relatively new technology, there are knowledge gaps about which rearing conditions can be manipulated to maximize growth. The effects of salinity and photoperiod on salmonid physiology have been extensively studied and manipulation of these variable can affect growth and performance. This data chapter investigates the optimal conditions of, and the interactions between, salinity and photoperiod that can maximize growth in Atlantic and coho salmon. Animals were reared in seven identical RAS, each at one of four salinities (2.5, 5, 10 and 30ppt) and one of two photoperiods (12:12 and 24:0 light:dark cycles) over 120 days; a 30ppt treatment under 12:12 light was not conducted due to limited equipment. Growth, maturation and cataracts were measured at 60 and 120 days of acclimation. Atlantic salmon reared at 10ppt had the highest mass and growth rate by the end of the experiment, relative to other salinities. In addition, Atlantic salmon reared in 24:0 light across all salinities had higher growth rates relative to their 12:12 light counterparts. However, there were no differences in coho salmon growth across salinities and photoperiods.

2.2 Introduction

Traditionally, salmon aquaculture has been conducted using open-net aquaculture, which involves incubating and rearing salmon larvae in a freshwater hatchery until they reach sizes that elicit smoltification for open net-pen seawater transfer (Asche & Bjørndal 2011). Open net-pens depend on ocean currents to provide clean water to the animals and carry effluent generated in the pens out to sea (Price 2004). However, the use of seawater currents to remove waste has been shown to have environmental impacts, such as eutrophication, which promotes algae growth and oxygen depletion in the surrounding ecosystem (Folke et al 1994; Soto & Mena 1999). As some open net-pens are situated near wild populations of salmon, farmed salmon that escape from the pens can also compete with the wild population for the habitat, food and breeding sites (Naylor et al. 2005).

The development of new aquaculture technology has been underway for numerous years to address some of the issues related to open net-pen farming, with land-based recirculating aquaculture systems (RAS) showing great potential to reduce waste, transport cost and time for rearing salmon, relative to open net-pen aquaculture (Martins et al. 2010; Tal et al. 2009). Past studies show higher growth rates in gilthead seabream and rainbow trout reared in RAS compared with fish reared in open net-pen and flow-through systems (Roque d'Orbcastel et al. 2009; Tal et al. 2009). However, the higher costs for energy and labor with reduced profitability during the initial years of starting up a RAS facility are some of the limiting factors for moving the industry forward with this technology (Badiola et al. 2017; Boulet et al. 2010). In addition,

the optimal environmental parameters for rearing fish in RAS are still not known, which can potentially impact production time and investment returns.

Salinity has been shown to have significant effects on growth and metabolic rates in salmon at different life stages; chinook salmon fry reared at 0, 5, 10, 20 and 28ppt for 11 weeks show growth rates decreasing and metabolic rates increasing with increasing salinity (Morgan & Iwama 1991). Similar results were also seen in Atlantic salmon parr reared at 0, 10, 20 and 31ppt for two months, with stunted growth at higher salinities (Duston 1994). Coho salmon smolts reared at 0, 5, 10, 20 and 30ppt in RAS for 59 days exhibited enhanced growth at 10ppt, while Atlantic salmon smolts reared at the same salinities did not differ in growth over the same period (Emerman 2016). Salinities as low as 1.8ppt have acted as an effective, preventative measure for *Saprolegnia* infection, an oomycetes species that creates soft, necrotic and ulcerated lesions in salmonids that can lead to mortality (Robertson et al. 2009).

Continuous light is commonly used in aquaculture production to allow farmers to feed fish continuously throughout the day, but such a photoperiod regime has been attributed for entraining an endogenous rhythm that affects maturation time and, inversely, growth (Bromage et al. 2001). Atlantic salmon reared under natural photoperiods and continuous light have shown mixed outcomes in terms of maturation, which has important implications for farmers, as early maturation will result in smaller fish that are less marketable and profitable (Fjelldal et al. 2011; Hansen et al. 1992; Porter et al. 1999; Qiu et al. 2015).

Based on past research, manipulation of both salinity and photoperiod can affect growth and early maturation of salmon. Manipulation of salinity, particularly the use of isosmotic salinities relative to salmon blood osmolality, show promise for enhancing growth by reducing osmoregulatory costs. The impacts of continuous light on maturation and growth is not well understood because most studies focus on juvenile fish or near-maturing fish. In addition, both salinity and photoperiod showed varying effects in previous literature on their effectiveness in improving growth rates and efficiency in salmon farming.

The aim of this research was to identify the effects of salinity and photoperiod, and their interactions, on growth and physiological changes in Atlantic salmon and coho salmon. Based on past studies, I hypothesized that both salinity and photoperiod would influence growth rates and maturation of both salmonid species, with isosmotic salinity and continuous daylight showing the greatest growth improvements and lowest incidences of maturation.

2.3 Methods

2.3.1 Overview

The experiments were conducted at the Initiative for the Study of the Environment and its Aquatics Systems (*InSEAS* at The University of British Columbia; Vancouver, BC, Canada), which consisted of seven identical RAS. To process and filter water, the system was equipped with a radial-flow separator for large waste particulates, a microbead filter for finer particulates and a biological filter with CO_2 stripping capabilities for nitrogen cycling. The water then passed through a low head oxygenator to directly inject oxygen for the desired dissolved oxygen levels

in the system. The low head oxygenator also injected ozone, which was used to oxidize tannins in fish feed; tannins causes water turbidity and off-flavors in fish fillet. Finally, the water was treated through a protein skimmer to remove waste protein, and then passed through a UV light to sterilize the water of harmful bacteria and viruses. The water was conditioned in a heat exchanger to reach the desired temperature for rearing fish. Within each tank, automatic drum feeders connected to a feed control system delivered a pre-programmed amount of feed per tank over the course of the day to provide consistent and labor-free feeding. In past research, it has been determined that the different RAS can produce consistent growth under similar conditions, allowing researchers to look at salmonid growth under different environmental conditions with minimal tank effects (Emerman 2016). Experiments were conducted under UBC Animal Care Permit #A13-0016 and #A17-0011.

2.3.2 Animals used

Atlantic and coho salmon were obtained from Cermaq Canada (Campbell River, BC, Canada) and Target Marine Hatcheries (Sechelt, BC, Canada), respectively. Both species were brought in as smolts at approximately 1 year of age and 100g. 6,000 coho salmon were received on September 15, 2016 and held in two RAS across four $5m^3$ tanks in dechlorinated City of Vancouver tap water prior to starting salinity and photoperiod treatments. 6,000 Atlantic salmon were received on October 22, 2016 and held in another two RAS across four $5m^3$ tanks in City of Vancouver tap water. All RAS were initially kept on flow-through water and within the first week of arrival, Atlantic salmon were prophylactically treated with Parasite-S (37% formaldehyde solution) for one hour, then Vidalife (<5% tetrasodium EDTA) for one hour to remove parasites and fungus. Coho salmon were not treated because they have not been shown to

have the same disease susceptibility as Atlantic salmon. Both species were held in these tanks until January 12, 2017, during which they were fed a ration ~0.5% of biomass per day. A low feed ration was used during the holding period to avoid nitrogenous waste accumulation in the RAS while the nitrogen-consuming bacteria colonized the biological filters. Mortality was checked daily in each tank and dead fish were removed and weighed. Due to an outbreak of *Saprolegnia* in Atlantic salmon during this holding period, there was mass mortality, so feeding rate was reduced to <0.5% of biomass per day, as per the advice of the university veterinarian, until the start of the salinity and photoperiod treatments.

2.3.3 Equipment used

All tanks were wrapped with black opaque plastic sheets along the elevated mesh that lines the circumference of the RAS tank, and covered with tarpaulin over the tank to isolate photoperiod in each treatment. LED lamps (5000K/3000K - 20,000 Lumens - Natural White) were installed into each $5m^3$ tank. Three RAS with LED lamps providing 12:12 light:dark photoperiod (12:12 light) were fitted with STANLEY TimerMax Outdoor Pro mechanical timers (Seattle, Washington, USA) to turn on at 8:00 and off at 20:00. Four RAS with LED lamps providing 24:0 light:dark photoperiod (24:0 light) were directly connected to electrical outlets. Laboratory room lights were set to turn on at 7:00 and off at 21:00 to reduce stray light from entering the tanks. Each tank was fitted with an Arvo-Tec drum 2000 feeder with a central Arvo-Tec WOLF control system (Huutokoski, Finland) to automatically feed the animals. The feeders delivered feed according to a pre-set "salmon" formula, which included fish number, average body mass and environmental parameters, such as temperature and dissolved oxygen (DO). Daily mortalities were accounted in the control system to ensure feeding rate was proportionate to tank

biomass. Feeders operated from 8:00 to 20:00 so that all fish would eat at similar times and were able to see the feed dropped into the tanks. Water ammonia, nitrite and nitrate in each RAS was measured at least twice a week and was maintained below 6ppm, 4ppm and 160ppm, respectively. Nitrate levels of 160ppm was the upper limit of the nitrogen test kits used, but concentrations close to this value have been shown to have little toxicity effects on juvenile tilapia, lake trout and lake whitefish survival and growth (McGurk et al. 2006; Monsees et al. 2017). Instant Ocean® aquarium salt (Blacksburg, Virginia, USA) was used to maintain salinity in each system. Salinities were measured and corrected for daily by adding the salt into the RAS's 0.7m^3 tanks, which did not house fish regularly. Prior to the growth experiment, salinities for all RAS, except for the ones used for holding animals, were increased to their respective treatment levels of 2.5, 5, 10 and 30ppt. The growth experiment used the following salinity and photoperiod combinations for each RAS: one of four salinities (2.5, 5, 10 and 30ppt) and one of two photoperiods (12:12 light and 24:0 light) with Atlantic salmon held in one of the 5m^3 tanks of a given system and coho salmon in the other to ensure that both species were exposed to identical salinities and photoperiods. A treatment for fish reared at 30ppt in 12:12 light was not used because *InSEAS* had limited equipment to replicate an eighth system.

2.3.4 Experimental design

To track individual growth rate, a subset of fish (110 to 120 individuals from each tank) were implanted with Biomark PIT tags (Boise, Idaho, USA). To implant the PIT tags, fish were removed from their tank and lightly anesthetized in 100L of 0.1g/L Tricaine-methanesulfonate (MS-222) buffered with 0.2g/L of sodium bicarbonate. PIT tags were then implanted into the peritoneal cavity of each fish using implant guns. Fork length and body mass were measured,

using a standard ruler and a Kilotech benchtop scale, respectively, and recorded in the Biomark Tag Manager Software. Fish were recovered in an aerated 100L freshwater bucket. When the barrel was visually full, animals were transported to one of the seven RAS systems. The remaining Atlantic and coho salmon were sorted by bulk biomass measurements on an Ohaus Defender bench platform scale in a 100L freshwater bucket, which were then distributed across the seven RAS to result in similar tank density. This was done by calculating total biomass of PIT-tagged fish from the collected mass data and matching bulk measurements between systems. Once all animals were evenly distributed, Instant Ocean® aquarium salt was added to each RAS that initially held the fish during the holding period to reach their target salinity.

As mentioned previously, because of the *Saprolegnia* outbreak in Atlantic salmon, the starting Atlantic salmon biomass was significantly lower than coho salmon. The initial stocking density for Atlantic and coho salmon ranged between 6.6-7.2kg/m^3 and 27.0-27.8kg/m^3, respectively.

Throughout the experiment, any dead fish was removed immediately and scanned for PIT tags. If PIT tags were found, necropsy was conducted to remove tags. Mass of the dead fish was recorded in the animal care records, which were later used for growth calculations. Carcasses were placed in plastic bags and stored in freezers for later incineration.

During the first 60 days of growth, *Saprolegnia* infections in Atlantic salmon were often observed. Any animals found with infections and mortality were removed immediately to reduce spreading the infection to healthy animals. Feeding rate was kept low (~0.5% of biomass per day) for both species during the first 60 days under the advice of the university veterinarian and

to keep water ammonia levels below 6ppm to reduce further stress. At the 60th day of acclimation, feed amount per day was increased to 1% of biomass and maintained throughout the remaining growth experiment. At the 120th day of acclimation, I discovered the feeder for Atlantic salmon reared at 5ppt in 24:0 light had not been dosing the correct amount of feed because of a faulty wire, which resulted in a reduced, unknown amount of feed given to these animals, relative to Atlantic salmon in the other six RAS. As such, the results of Atlantic salmon reared at 5ppt in 24:0 light may not accurately represent the effects of salinity and photoperiod.

2.3.5 Growth measurements at Day 60 and 120

Growth, maturation and cataracts of all Atlantic salmon and at least 100 coho salmon, including at least 30 PIT-tagged individuals, from each RAS treatment were measured at the 60th and 120th day post-acclimation. Sampling 14 RAS required 7-8 days to complete, so the 60th and 120th day was approximately the 3rd or 4th day of sampling. Two days prior to sampling a treatment group, animals were fasted to ensure a post-absorptive state. Animals were netted from the tanks and placed into 100L buckets containing 0.1g/L Tricaine-S (MS-222) buffered with 0.2g/L of sodium bicarbonate and water from the same RAS for anesthesia. Once the animals were lightly anaesthetized, as defined by the inability of the fish to stay upright, individuals were removed from the anesthetic bath and scanned for PIT tags using a PIT tag reader connected to a laptop computer with the Biomark Tag Manager Software. The length, mass, presence and absence of maturation and cataracts were recorded on a spreadsheet. If a PIT tag was detected, the same data collected on the fish were recorded into the Biomark Tag Manager Software with their respective PIT tag HEX ID. Body mass was measured using a Kilotech benchtop scale. Following body mass measurement, fork length was measured using a standard ruler. Cataracts were assessed as

the absence or presence of a semi-opaque, white color on the animal's optical lens that can be clearly seen in ambient room light. Maturation was evaluated using external features and was defined as having two of the following characteristics: (1) a soft, rounded belly due to developed gonads, (2) presence of milk for milt due to developed testes, (3) a considerably smaller body size, considered as a jack, or (4) a distinct skin coloration change that is indicative of spawning adults.

After measurements were taken, fish were placed into a well-aerated 100L bucket with water from the same RAS, along with 66ppm of Vidalife to preserve the fish's natural mucous layer during handling. Recovering fish were checked every time a fish was processed to ensure animals were recovering from the anesthesia and handling by the researchers. Recovered fish, observed as individuals swimming upright, were then transferred to their respective RAS's $0.7m^3$ tanks to free up space in the buckets for the remaining fish to be sampled. Once all data were collected, fish in both the recovery buckets and the $0.7m^3$ tanks were then transferred back into their respective $5m^3$ holding tanks.

2.3.6 Calculations

Specific growth rate (SGR) was calculated for both PIT-tagged individuals and the whole tank (ie. average growth rate of all animals in a tank). A higher SGR equated to faster growth rates per day and was calculated according to:

$$SGR = \frac{\ln Mass_2(g) - \ln Mass_1(g)}{Time_2 - Time_1} \times 100$$

where $Mass_1$ and $Time_1$ referred to the first sampling time point, respectively, and $Mass_2$ and $Time_2$ referred to the second sampling time point. For PIT-tagged individuals, $Mass_2$ and $Mass_1$ was the mass at the second and first time point of each individual, respectively.

Effective feed conversation ratio (FCR) was analyzed for the whole tank. A higher FCR equated to lower efficiency in converting feed to body mass and was calculated according to:

$$FCR = \frac{Total\ feed\ fed\ within\ time\ period\ (kg)}{\left(Biomass_{final}(kg) + Culled\ biomass(kg)\right) - Biomass_{initial}(kg)}$$

where $Biomass_{final}$ and $Biomass_{initial}$ referred to the total biomass of the tank at the second and first sampling time point, respectively. Culled biomass was the total biomass of mortality and culled fish used for other experiments in the project.

Thermal growth coefficient (TGC) was calculated for the whole tank. A TGC was used to standardize salmonid growth rates at different temperatures. A higher TGC equated to greater mass gain per degree-day and is calculated according to:

$$TGC = \frac{Mass_2(g)^{1/3} - Mass_1(g)^{1/3}}{Temperature\ (℃) \times (Time_2 - Time_1)} \times 1000$$

where Temperature was the average temperature of the RAS between the first and second sampling time point, given as degrees Celsius. $Mass_1$ and $Time_1$ referred to the first sampling time point, respectively, and $Mass_2$ and $Time_2$ referred to the second sampling time point. A plot of cube root of weight against time gives a straight line when fish are reared under constant temperature; hence, the equation uses cube root when calculating for TGC (Jobling, 2003).

Fulton's condition factor (CF) was calculated for individual fish measured at each time point. A higher CF equated to a larger, healthier fish and was calculated according to:

$$CF = (\frac{Mass(g)}{Length(cm)^3}) \times 100$$

2.3.7 Statistical analysis

All data are presented as mean ± standard error of the mean unless otherwise indicated. A 3-way ANOVA with treatment factors of salinity, photoperiod and time was used to test statistical differences of mass and condition factor. The 30ppt treatment was also included in the 3-way ANOVA using Type II and III sum of squares due to the unbalanced design. Due to the scale of each RAS with limited time and space to conduct the experiment, individual animals sampled were used as the unit of analysis (n=100+). It is recognized that this represents pseudo replication, but due to the scale of the project true replication was not possible at this time. Data were considered as significant if $P \leq 0.05$, and a post-hoc Tukey test using pairwise comparisons were completed if significant results were found. Chi-square tests were used to test if maturation and cataracts incidences were related to salinity and photoperiod. Any sampling periods that had 0% maturation or cataracts across all treatments were not tested. If any sampling periods had more than 20% of the expected values less than 5, a randomization test was used with 10,000 Monte-Carlo simulations. The post-hoc test was used to look for specific differences between treatment groups within each sampling period. No statistical analyses were performed for SGR and FCR because the calculations were based on tank averages and therefore there is no error.

2.4 Results

2.4.1 Atlantic salmon

Biomass and culled biomass at each sampling period are listed in Appendix A. Atlantic salmon were placed into their respective treatments at ~100g (Figure 2.1A and B) and fed 0.5% of their biomass per day for the first 60 days and then 1% of their biomass per day between the 60th and 120th days post-acclimation. 3-way ANOVA revealed that there were significant effects of salinity ($P<0.01$), photoperiod ($P<0.01$) and time ($P<0.01$) on average mass. There were significant interactions between salinity and time ($P<0.01$), photoperiod and time ($P<0.01$) and between all three factors ($P=0.01$). Using Tukey pairwise comparison, there were no significant effects of salinity and photoperiod on the first day of acclimation. On the 60th day of acclimation, fish reared at 10ppt in 24:0 light had highest average mass, relative to fish reared at all salinities in 12:12 light. On the 120th day of acclimation, fish reared at 10ppt in 24:0 light again had the highest average mass compared to all other treatments.

Condition factor is shown in Table 2.1. There were significant effects of salinity ($P<0.01$) and time ($P<0.01$), but there were no effects of photoperiod ($P=0.14$). There were significant interactions between salinity and time ($P<0.01$) and photoperiod and time ($P<0.01$). A Tukey pairwise comparison test revealed that at the 60th day, fish reared in 5ppt and 10ppt in 12:12 light had the highest CF. At the 120th day, fish reared in 5ppt and 10ppt in 12:12 light continued to have the highest CF.

Only PIT-tagged individuals that were re-captured at both the 60th and 120th day were reported; re-captured individuals that were caught on the 60th day but not on the 120th day, and vice versa,

were not reported. I successfully recaptured 7 to 22 PIT-tagged animals from each treatment, which were used to assess individual changes in body mass over time (Figure 2.2). There were no significant effects of salinity or photoperiod on body mass of the PIT-tagged Atlantic salmon ($P=0.53$ and 0.17, respectively), but there was a significant effect of time ($P<0.01$), whereby all fish grew larger with time, as expected. There were also significant interactions between salinity and time ($P=0.02$) and photoperiod and time ($P<0.01$). Using Tukey pairwise comparisons, there were no differences between all treatments at each time point. PIT-tagged animal SGR calculated from body weight data are shown in Figure 2.3. There were significant effects of salinity ($P=0.02$), photoperiod ($P=0.05$) and time ($P<0.01$) on SGR with no interactions between any of the three treatment factors. Using Tukey pairwise comparison, there was a difference between fish reared at 10ppt in 24:0 light and 2.5ppt in 12:12 light.

SGR and TGC of the whole tank is shown in Table 2.2. Over the 120 days of acclimation, animals reared at 10ppt in 24:0 light showed the greatest growth rate. TGC was also the highest in this group. During the first 60 days, SGR was almost half the rate compared to SGR between the 60^{th} and 120^{th} day across all treatments. This was most likely due to the low feeding rate given during the first 60 days to reduce ammonia build-up before biological filtration was successful, as well as following veterinarian advice for reducing *Saprolegnia* infections. TGC followed the same trend as SGR, with the highest growth coefficient, standardized for temperature, at 10ppt in 24:0 light.

FCR is shown in Table 2.3, and presence of maturation and cataracts are shown in Table 2.4. The amount of feed given within each sampling period for FCR calculation can be found in Appendix

B. Between the first and 60th day of acclimation, all treatments except for fish reared at 10ppt and 30ppt in 24:0 light showed negative FCR. Feeding rate was reduced to below 0.5% of biomass per day as previously described, hence the results are not reported. Between the 60th and 120th day of acclimation, fish reared at 5ppt in 12:12 light had the best feed conversion, and fish at 5ppt in 24:0 light had the poorest feed conversion. In the first 60 days of acclimation, incidences of maturation were independent of salinity ($P=0.51$) and photoperiod ($P=1.0$), and no incidences of cataracts were found. Between the 60th and 120th day of acclimation, the incidences of maturation were dependent on salinity ($P=0.02$) but not photoperiod ($P=1.0$). Incidences of cataracts were also dependent on salinity ($P<0.01$) but not photoperiod ($P=0.09$).

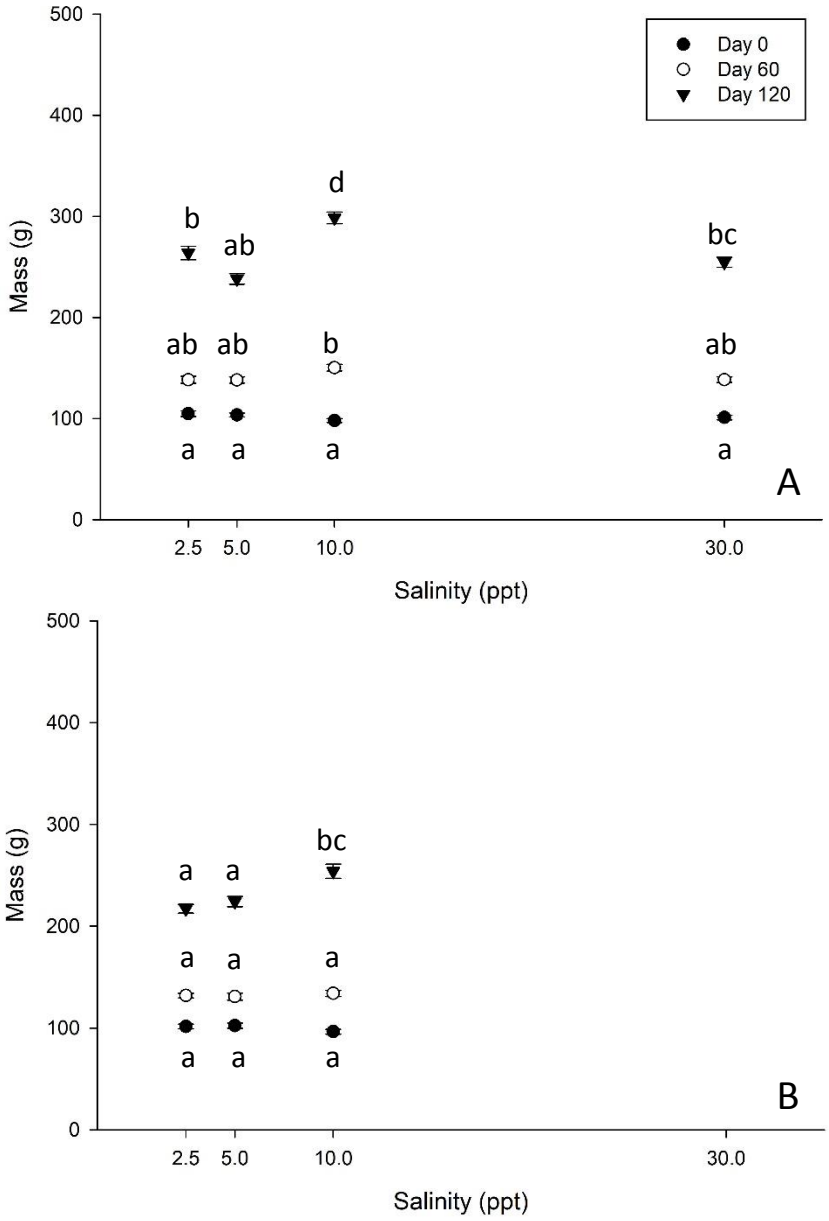

Figure 2.1 Average body mass of approximately 50-140 Atlantic salmon reared at salinities of 2.5, 5, 10 and 30ppt in 24:0 light (A) and 12:12 light (B). Filled circles represent the first day of the growth trial, open circles represent the 60th day of acclimation, and filled triangles represent the 120th day of acclimation. All points are means ± SEM. Letters that differ within each sampling period indicate statistical differences in salinity and photoperiod.

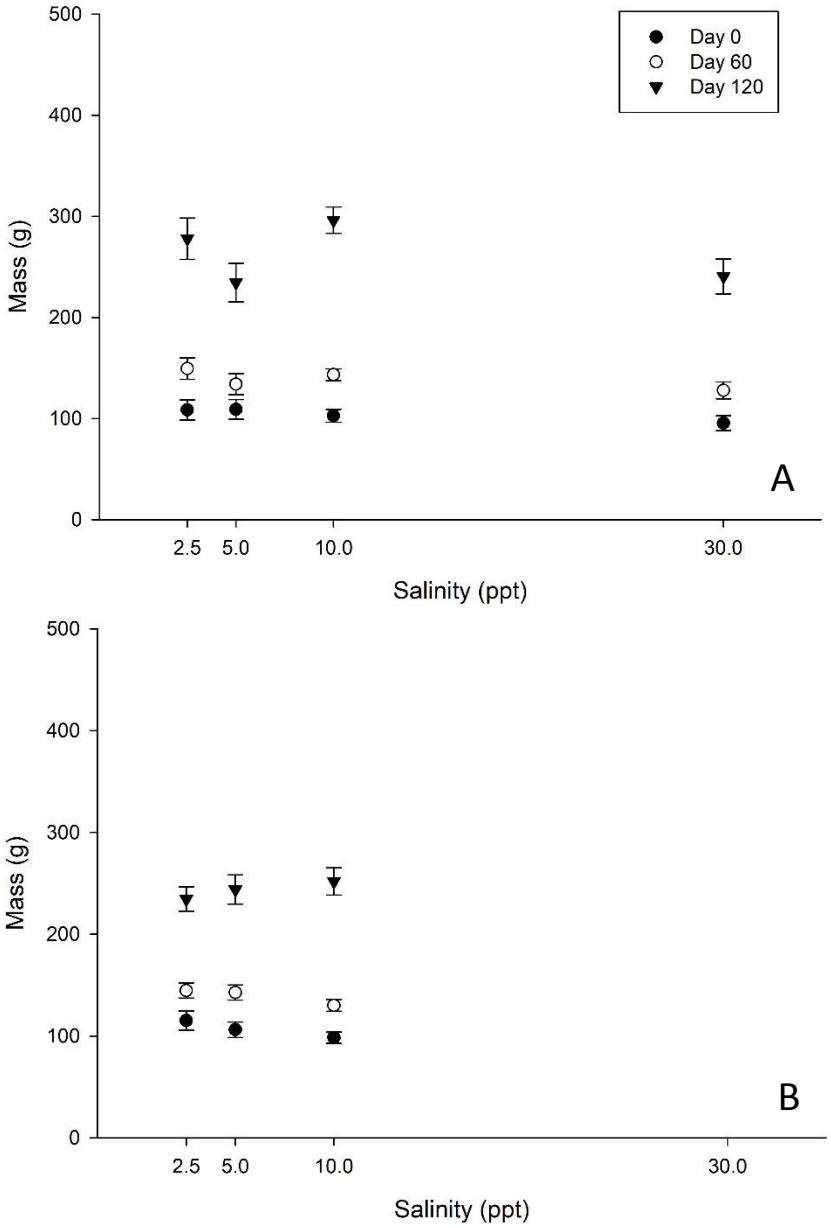

Figure 2.2 Average body mass of PIT-tagged Atlantic salmon (n=7-22) reared at salinities of 2.5, 5, 10 and 30ppt in 24:0 light (A) and 12:12 light (B). Filled circles represent the first day of growth trial, open circles represent the 60th day of acclimation, and filled triangles represent the 120th day of acclimation. All points are means ± SEM. No statistical differences were observed within a given sampling time.

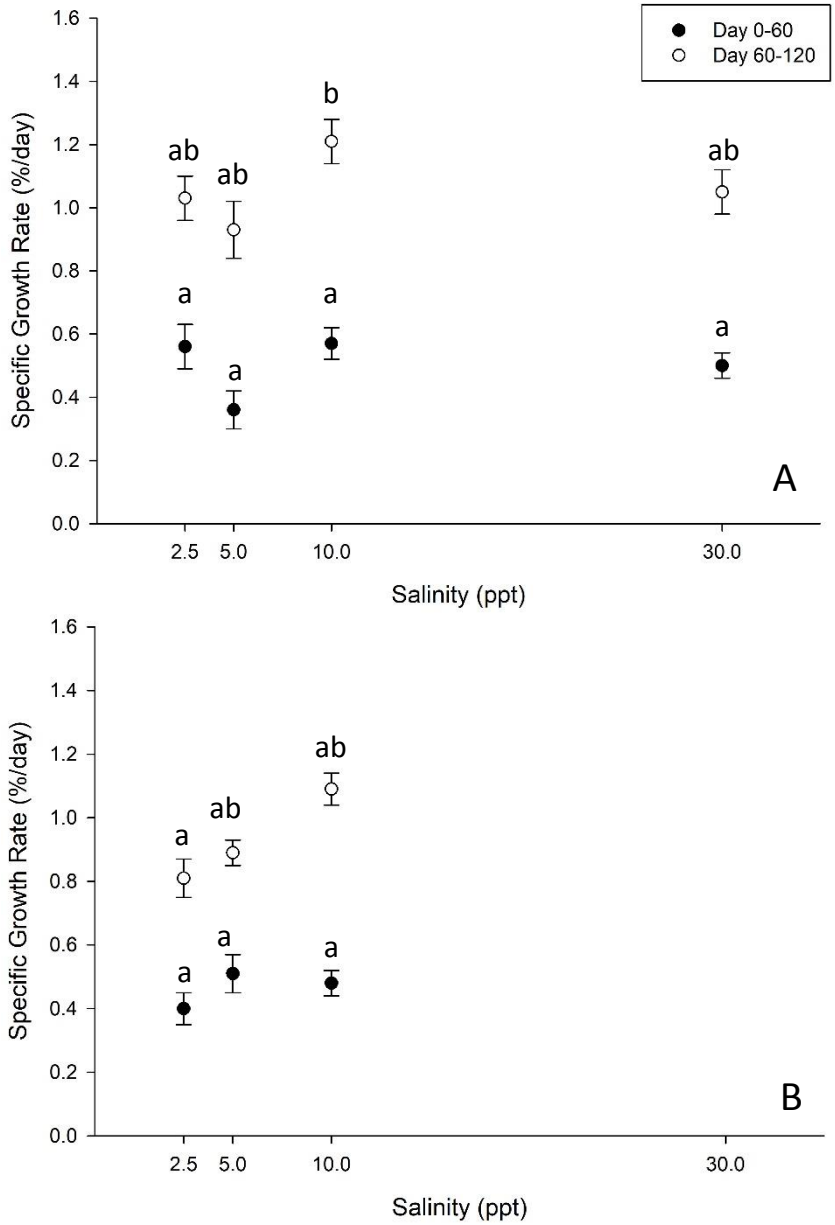

Figure 2.3 Specific growth rate of PIT-tagged Atlantic salmon (n=7-22) reared at salinities of 2.5, 5, 10 and 30ppt in 24:0 light (A) and 12:12 light (B). Filled circles represent SGR between the beginning of the trial and the 60th day of acclimation, and open circles represent SGR between the 60th day and the 120th day of acclimation All points are means ± SEM. Letters that differ within each sampling period indicate statistical differences in salinity and photoperiod.

Table 2.1 Condition factor of approximately 50-140 Atlantic salmon reared at salinities of 2.5, 5, 10 and 30ppt and in 24:0 light and 12:12 light. All points are means ± SEM. Letters that differ within each sampling period indicate statistical differences in salinity and photoperiod.

Photoperiod	Salinity (ppt)	Day 0	Day 60	Day 120
24-hr light	2.5	$1.01\pm0.01_{ab}$	$1.06\pm0.02_{ab}$	$1.16\pm0.01_{ab}$
	5	$1.02\pm0.01_{b}$	$1.03\pm0.01_{a}$	$1.09\pm0.01_{a}$
	10	$0.99\pm0.01_{ab}$	$1.09\pm0.01_{b}$	$1.16\pm0.01_{b}$
	30	$1.02\pm0.01_{b}$	$1.07\pm0.01_{ab}$	$1.24\pm0.01_{c}$
12-hr light	2.5	$1.03\pm0.01_{b}$	$1.07\pm0.01_{ab}$	$1.23\pm0.02_{bc}$
	5	$1.03\pm0.01_{b}$	$1.10\pm0.01_{b}$	$1.25\pm0.01_{c}$
	10	$0.97\pm0.01_{a}$	$1.10\pm0.01_{b}$	$1.30\pm0.04_{c}$

Table 2.2 SGR and TGC of approximately 50-140 Atlantic salmon reared at salinities of 2.5, 5, 10 and 30ppt and in 24:0 light and 12:12 light.

Photoperiod	Salinity (ppt)	Day 0-60 SGR (%/day)	Day 0-60 TGC	Day 60-120 SGR (%/day)	Day 60-120 TGC
24-hr light	2.5	0.46	0.61	1.08	1.68
	5	0.48	0.66	0.91	1.45
	10	0.71	0.97	1.13	1.73
	30	0.52	0.69	1.02	1.57
12-hr light	2.5	0.44	0.58	0.83	1.26
	5	0.41	0.58	0.90	1.42
	10	0.55	0.74	1.07	1.68

Table 2.3 Feed conversion ratio of approximately 50-140 Atlantic salmon reared at salinities of 2.5, 5, 10 and 30ppt and in 24:0 light and 12:12 light.

Photoperiod	Salinity (ppt)	Day 60-120 FCR (kg feed/kg mass gain)
24-hr light	2.5	1.04
	5	1.33
	10	1.06
	30	1.18
12-hr light	2.5	0.96
	5	0.86
	10	1.13

Table 2.4 Presence of maturation and cataracts of approximately 50-140 Atlantic salmon reared at salinities of 2.5, 5, 10 and 30ppt and in 24:0 light and 12:12 light.

		Day 0		Day 60		Day 120	
Photoperiod	Salinity (ppt)	Mature (%)	Cataract (%)	Mature (%)	Cataract (%)	Mature (%)	Cataract (%)
24-hr light	2.5	3	0	0	0	6	3
	5	0	0	0	0	0	1
	10	0	1	0	0	0	1
	30	0	0	1	0	0	7
12-hr light	2.5	0	0	0	0	0	0
	5	0	0	0	0	1	1
	10	0	0	1	0	3	0

2.4.2 Coho salmon

Biomass and culled biomass at each sampling period are listed in Appendix A. On the first day of sorting and acclimating salmon to their respective salinity and photoperiod, average mass was ~160g (Fig 2.4A and B). Time showed a significant effect on average mass ($P<0.01$), but no effects of salinity ($P=0.07$) and photoperiod ($P=0.35$) were seen, and there was no interaction between any of the three treatment factors. Using Tukey pairwise comparisons, there were no differences between all treatments at each sampling period.

Condition factor is shown in Table 2.5. There was a significant effect of time ($P<0.01$), but no effects of salinity ($P=0.20$) and photoperiod ($P=0.22$) on condition factor. There were significant interactions between salinity and photoperiod ($P=0.03$) and between photoperiod and time ($P=0.01$). Using Tukey pairwise comparison, there were differences at the 60^{th} day of acclimation, showing that fish reared at 5ppt and 10ppt in 12:12 light were significantly larger than those reared at 2.5ppt in 24:0 light.

SGR and TGC is shown in Table 2.6. During the first 60 days of acclimation, fish reared at 10ppt in 24:0 light had the highest growth rate, and between the 60th and 120th day, fish reared at 2.5ppt and 30ppt in 24:0 light had the highest growth rate. Like Atlantic salmon, SGR for coho salmon during the first 60 days was approximately half the rate compared with SGR between the 60th and 120th day, largely due to the lower feeding rate used during the first 60 days. TGC was the highest for fish reared at 10ppt in 24:0 light during the first 60 days and at 5ppt in 24:0 light between the 60th and 120th day.

Only PIT-tagged individuals that were re-captured at the 60th and 120th day were reported; re-captured individuals that were only caught on the 60th day but not the 120th day, and vice versa, were not reported. I successfully recaptured 8 to 12 PIT-tagged animals from each treatment, which were used to assess individual changes in body mass over time (Figure 2.5). There were no significant effects of salinity nor photoperiod on body mass, but there was a significant effect of time ($P<0.01$). However, there were interactions between salinity and photoperiod ($P<0.01$). However, using Tukey pairwise comparisons did not reveal any differences in body mass between treatments within a sampling time. PIT-tagged coho salmon SGR data are shown in Figure 2.6. There were no significant effects of salinity ($P=0.44$) nor photoperiod ($P=0.64$), and there were no significant interactions between any of the three treatment factors.

FCR is shown in Table 2.7, and presence of maturation and cataracts are shown in Table 2.8. The amount of feed fed within each sampling period for FCR calculation can be found in Appendix B. During the first 60 days, fish reared at 10ppt in 24:0 light had the best feed conversion, while fish reared at 2.5ppt in 24:0 light at the poorest. Between the 60th and 120th day, fish at 2.5ppt

and 5ppt in 24:0 light had the highest feed conversion, while fish at 10ppt in 24:0 light had the poorest. In the first 60 days of acclimation, incidences of cataracts were dependent on salinity (P=0.01) but not photoperiod (P=0.42), and no incidences of maturation were found. Between the 60^{th} and 120^{th} day of acclimation, the incidences of cataracts were independent of salinity (P=0.87) and photoperiod (P=0.67), and no incidences of maturation were found.

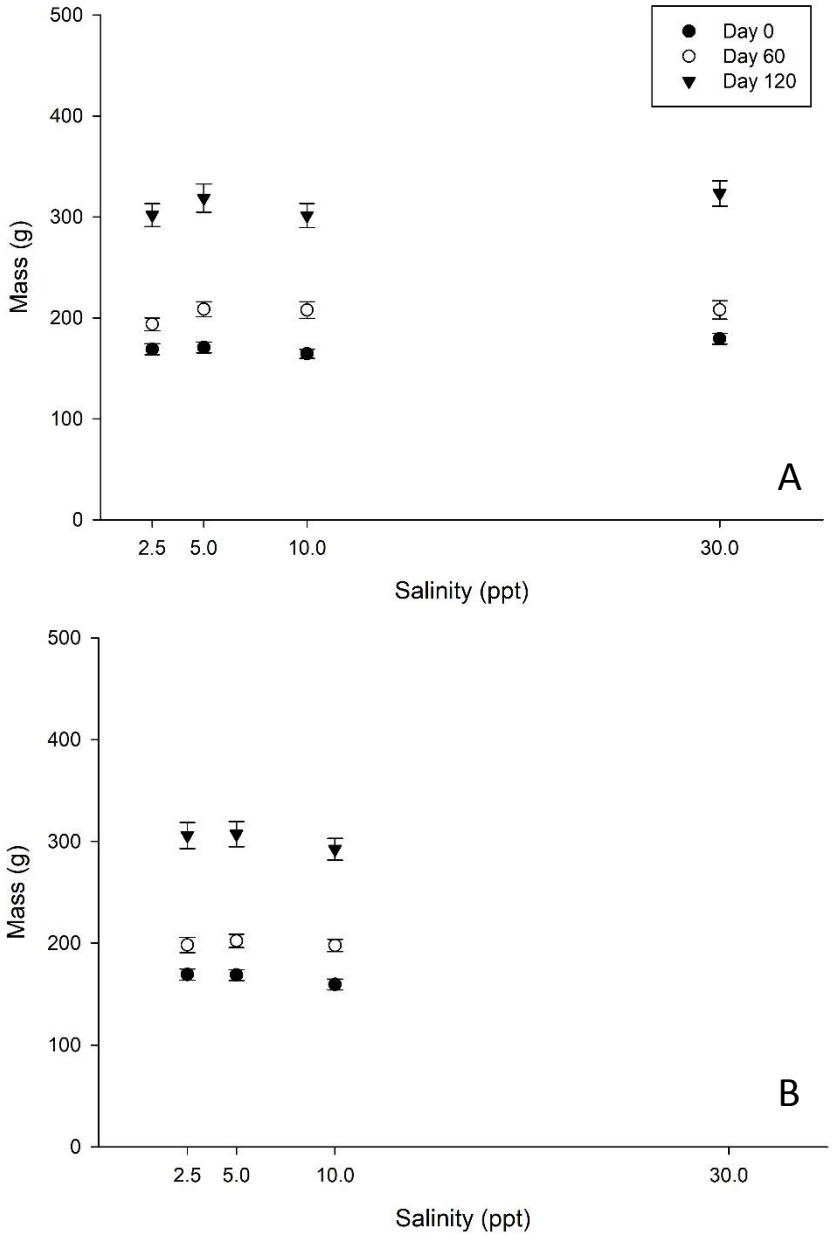

Figure 2.4 Average body mass of at least 100 coho salmon (randomly selected in a cohort of 500-600 fish per tank) reared at salinities of 2.5, 5, 10 and 30ppt in 24:0 light (A) and 12:12 light (B). Filled circles represent the first day of growth trial, open circles represent the 60th day of acclimation, and filled triangles represent the 120th day of acclimation. All points are means ± SEM. No statistical differences were observed within sampling times.

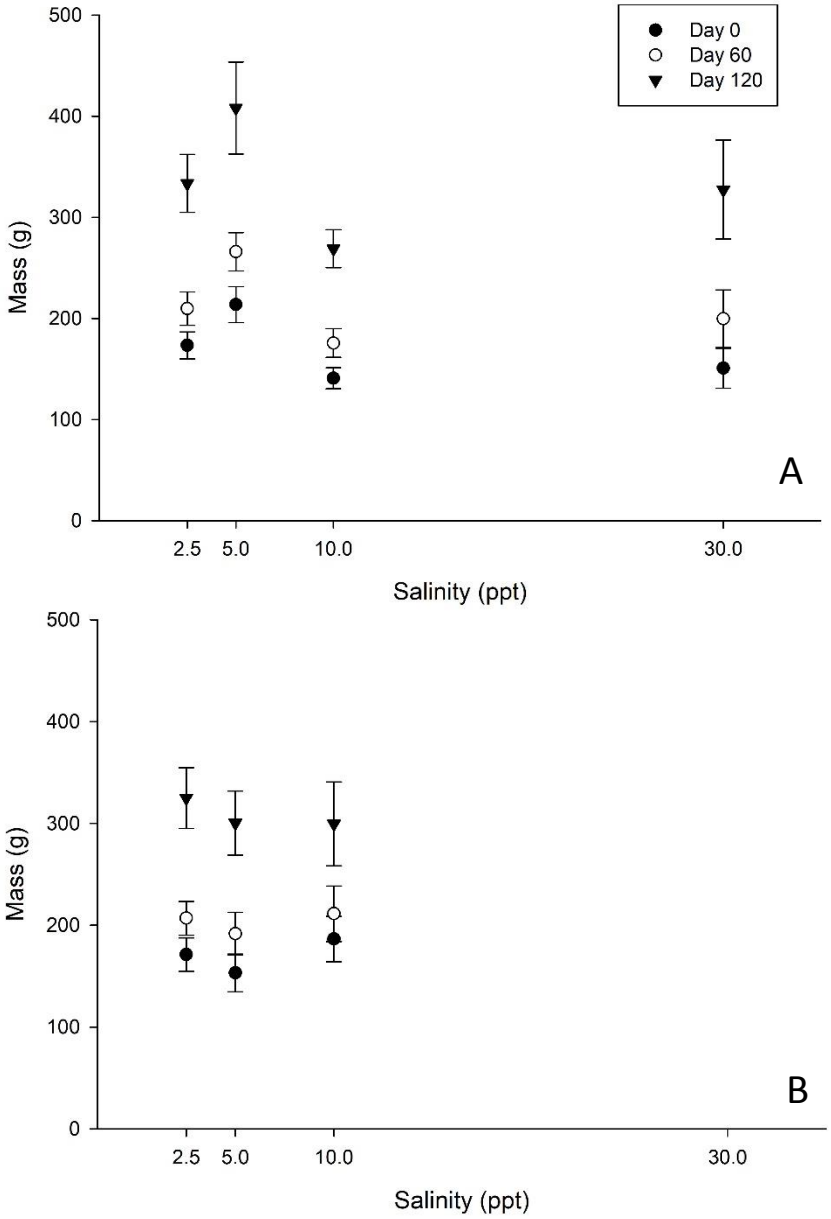

Figure 2.5 Average body mass of PIT-tagged coho salmon (n=8-12) reared at salinities of 2.5, 5, 10 and 30ppt and in 24:0 light (A) and 12:12 light (B). Filled circles represent the first day of growth trial, open circles represent the 60th day of acclimation, and filled triangles represent the 120th day of acclimation. All points are means ± SEM. No statistical differences were observed within sampling times.

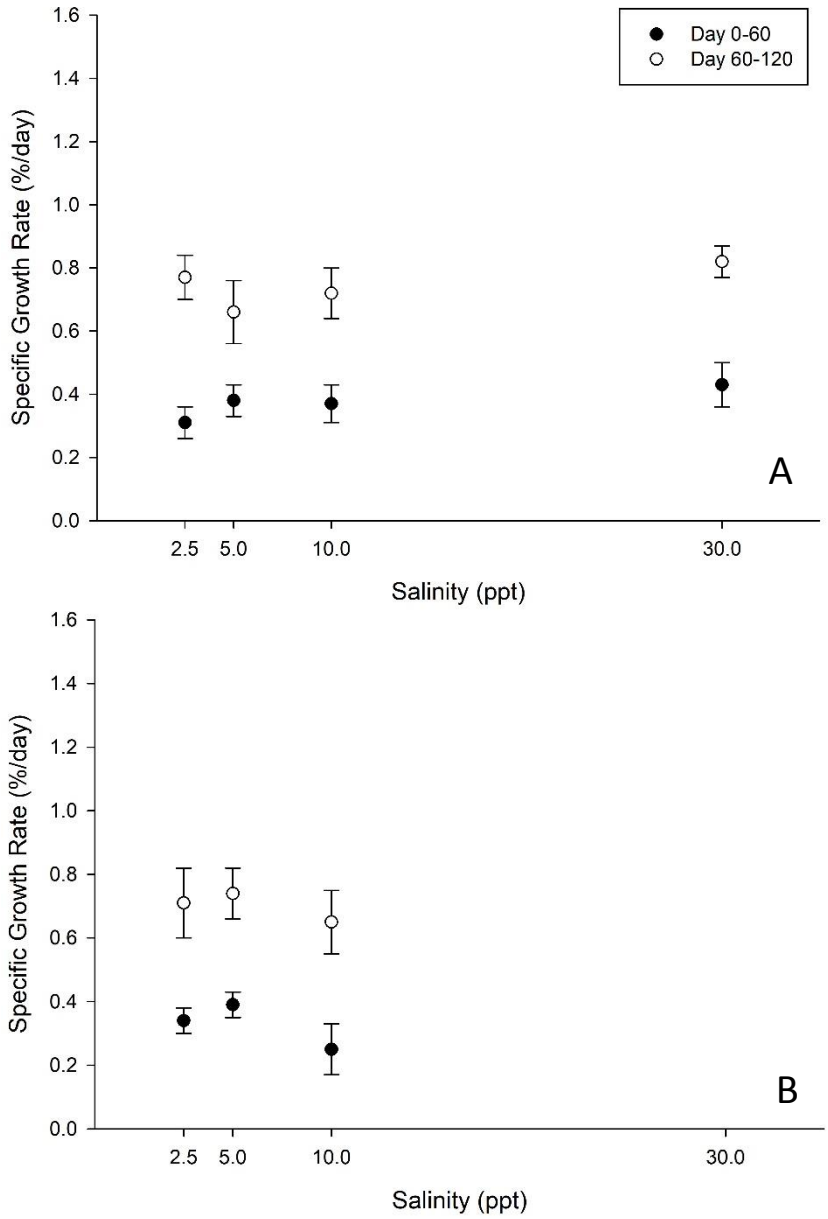

Figure 2.6 Specific growth rate of PIT-tagged coho salmon (n=8-12) reared at salinities of 2.5, 5, 10 and 30 ppt in 24:0 light (A) and 12:12 light (B). Filled circles represent SGR between the beginning of the trial and the 60th day of acclimation, and open circles represent SGR between the 60th day and the 120th day of acclimation All points are means ± SEM. All points are means ± SEM. No statistical differences were observed within sampling times.

Table 2.5 Condition factor of at least 100 coho salmon (randomly selected in a cohort of 500-600 fish per tank) reared at salinities of 2.5, 5, 10 and 30ppt and in 24:0 light and 12:12 light. Letters that differ within each sampling period indicate statistical differences in salinity and photoperiod.

Photoperiod	Salinity (ppt)	Day 0	Day 60	Day 120
24-light	2.5	$1.03\pm0.01_{a}$	$1.03\pm0.01_{a}$	$1.13\pm0.01_{a}$
	5	$1.06\pm0.01_{ab}$	$1.05\pm0.01_{ab}$	$1.13\pm0.03_{a}$
	10	$1.04\pm0.01_{a}$	$1.07\pm0.01_{ab}$	$1.17\pm0.03_{a}$
	30	$1.05\pm0.01_{ab}$	$1.07\pm0.01_{ab}$	$1.17\pm0.01_{a}$
12-light	2.5	$1.07\pm0.01_{b}$	$1.08\pm0.01_{ab}$	$1.12\pm0.02_{a}$
	5	$1.06\pm0.01_{ab}$	$1.09\pm0.01_{b}$	$1.14\pm0.02_{a}$
	10	$1.05\pm0.01_{ab}$	$1.08\pm0.02_{b}$	$1.11\pm0.01_{a}$

Table 2.6 SGR and TGC of at least 100 coho salmon (randomly selected in a cohort of 500-600 fish per tank) reared at salinities of 2.5, 5, 10 and 30ppt and in 24:0 light and 12:12 light.

Photoperiod	Salinity (ppt)	Day 0-60 SGR (%/day)	Day 0-60 TGC	Day 60-120 SGR (%/day)	Day 60-120 TGC
24-hr light	2.5	0.23	0.35	0.74	1.25
	5	0.33	0.54	0.71	1.27
	10	0.39	0.60	0.62	1.00
	30	0.25	0.38	0.74	1.26
12-hr light	2.5	0.26	0.41	0.72	1.24
	5	0.30	0.50	0.70	1.25
	10	0.36	0.56	0.65	1.12

Table 2.7 Feed conversion ratio of at least 100 coho salmon (randomly selected in a cohort of 500-600 fish per tank) reared at salinities of 2.5, 5, 10 and 30ppt and in 24:0 light and 12:12 light.

Photoperiod	Salinity (ppt)	Day 0-60 FCR (kg feed/kg mass gain)	Day 60-120 FCR (kg feed/kg mass gain)
24-hr light	2.5	2.35	1.33
	5	1.06	1.33
	10	0.90	1.77
	30	1.75	1.50
12-hr light	2.5	1.43	1.45
	5	1.25	1.42
	10	1.22	1.72

Table 2.8 Presence of maturation and cataracts of at least 100 coho salmon (randomly selected in a cohort of 500-600 fish per tank) reared at salinities of 2.5, 5, 10 and 30ppt and in 24:0 light and 12:12 light.

		Day 0		Day 60		Day 120	
Photoperiod	Salinity (ppt)	Mature (%)	Cataract (%)	Mature (%)	Cataract (%)	Mature (%)	Cataract (%)
24-hr light	2.5	0	0	0	0	0	2
	5	0	0	0	1	0	2
	10	0	0	0	3	0	2
	30	0	0	0	1	0	2
12-hr light	2.5	0	0	0	0	0	2
	5	0	1	0	2	0	0
	10	0	2	0	5	0	2

2.5 Discussion

The current study found both salinity and photoperiod influenced Atlantic salmon growth up to 120 days, with those reared at 10ppt and 24:0 light showing the greatest growth change over time. However, there were no effects of salinity nor photoperiod on coho salmon growth. Both species showed no effects of salinity and photoperiod on cataract and maturation incidences.

2.5.1 Atlantic salmon

2.5.1.1 Growth

Salinity had significant effects on growth in Atlantic salmon, as indicated by changes in body mass over time. Animals reared at 10ppt in both photoperiods consistently had higher average mass by day 120, relative to other salinity treatments of their respective photoperiods. In 1kg rainbow trout, 62% of gross intake energy was distributed between energy for new tissue (40%) and maintenance energy for life (22%) (Cho & Bureau 1995). Over time, the maintenance energy saved from reduced osmoregulation may be transferred to energy for synthesizing new tissue for growth. Enhanced growth at 10ppt in my study could be associated with a lower energy demand

for osmoregulation and maintenance relative to animals reared in a freshwater or seawater environment, and instead a divergence of energy towards new tissue production. Sea bream reared at 7, 15 and 35ppt in a RAS also show enhanced growth at 15ppt (Woo & Kelly 1995). This was evident by the 15ppt group's higher growth rates and protein efficiency ratio (gram of mass gain per gram of protein consumed) relative to the other salinity treatments. In addition, rates of oxygen consumption and ammonia excretion was lowest at 15ppt, which derived from a lower metabolic demand that is potentially higher in freshwater and seawater due to higher osmoregulatory costs. Woo and Kelly (1995) concluded in an isosmotic environment relative to blood osmolality, sea bream may re-organize metabolic demand to utilize carbohydrates and spare protein, which in turn enhanced growth performance in these animals.

Condition factor of my study was consistent with the increased mass gain of the 10ppt treatment groups, which showed Atlantic salmon are gaining mass proportionally quicker than their body length gain. However, this changed after 120 days of acclimation, with fish reared at 10ppt in 24:0 with lower condition factors relative to fish reared at 30ppt in the same photoperiod. The basis for this phenomenon is unclear, and future sampling periods will inform on whether condition factors change with salinity as salmon grow even larger.

Photoperiod seemed to have a strong effect on Atlantic salmon mass gain: fish reared at 2.5, 5 and 10ppt in 24:0 light exhibited higher average mass relative to their 12:12 light counterpart by 120 days of acclimation at a feeding rate of approximately 1% of biomass per day. One possible explanation could be continuous photoperiod simulated longer daylight period like summer months, which is known to increase growth in smolts preparing for seawater migration.

Handeland et al. (2013) reared Atlantic pre-smolts (starting at 15.9g) under continuous light and natural daylight periods over 11 months, showing fish reared under continuous light have higher growth rates, increased gill NKA activity and elevated plasma growth hormone levels. The enhanced rates and activities of these three parameters elicited smoltification approximately 4-5 months earlier than the natural season for parr-smolt transformation. Based on Handeland et al.'s (2013) results, the earlier smolt development due to continuous light can potentially kick-start and enhance growth at a younger age for Atlantic salmon, which aligns with the results found in my study.

Other studies examining the effects of photoperiod on Atlantic salmon juveniles and pre-smolts also demonstrated that rearing fish under continuous light or extended daylight serves to increase salinity tolerance and gill NKA activity, as well as trigger earlier production of growth hormones (McCormick et al. 1995; McCormick et al. 1987). As such, photoperiod is thought to be a major regulator and trigger for salmon to prepare for seawater entry and improved growth. In my study, 24:0 light could have promoted higher growth hormone production because of its closer proximity to summer light cycles, relative to 12:12 light, during initial acclimation by promoting smoltification at an earlier time. In turn, this enhanced growth over the first 120 days of the study, while salmon reared under 12:12 light exhibited growth enhancements later in the treatment.

An interesting result was the statistically significant effects of salinity and photoperiod that differed between PIT-tagged individuals and the whole tank data of all fish from each RAS. The averaged data of 50 to 140 Atlantic salmon showed significant effects of salinity and photoperiod

on growth, but this was not observed in the PIT-tagged individuals, even both data showed similar trends of higher average mass at 10ppt. This was potentially due to the small sample size achieved with PIT-tagged animals, which may not have provided enough statistical power to accurately determine if salinity and photoperiod had effects on individual growth over time.

2.5.1.2 SGR, TGC, FCR

The specific growth rate observed in my study was similar to records of 1-year old Atlantic salmon smolts starting at approximately 90g fed in excess in freshwater (Imsland et al. 2011). As mentioned previously, feed amount in my study was restricted to approximately 0.5% of biomass per day for the first 60 days, but the results showed growth rate exceeded this, which could be due to a significant amount of water incorporated into the tissues when dry feed was converted to somatic growth. Work by Imsland et al. (2011) also demonstrated acute starvation can significantly reduce growth rates in both freshwater and seawater acclimated salmon. However, the animals can then increase their growth rates once food is provided again and can even exceed growth rates of fully fed groups, a phenomenon that Imsland et al. (2011) noted as compensatory growth. In my study, the restricted feed regime in the first 60 days could have been compensated once feed ration was increased to 1% of biomass per day, which significantly improved SGR between the 60^{th} and 120^{th} day.

The SGR of PIT-tagged fish showed significant effects of salinity and photoperiod, though the differences were not observed in the Tukey pairwise comparison tests. This datum was like the SGR results found from the averaged data of all fish in the RAS, which suggested that salinity

and photoperiod had an overall effect on growth rates over time but was not apparently observed within each time point.

The TGC results showed a similar trend as SGR: Atlantic salmon reared at 10ppt had the highest TGC relative to all other salinity at both sampling periods. In addition, TGC of 24:0 light acclimated salmon was consistently higher than their counterparts in 12:12 light at both sampling periods. It has been shown that smolted Atlantic salmon TGC is affected by salinity, with higher TGC and growth rates in seawater relative to freshwater when observed for four weeks (Krogdahl et al. 2004). However, my data only showed such a phenomenon during the first 60 days. By the 120th day, TGC of fish at 2.5ppt in 24:0 light was greater than those at 30ppt in 24:0 light. As TGC can be used as a predictor of growth rates at different temperatures, I speculate that rearing Atlantic salmon at 10ppt and under continuous light could improve growth at all temperature ranges; however, this needs to be experimentally validated.

The feed conversion ratio during the first 60 days were not reported because of high mortality and low feed ration, which produced negative FCR results. FCR between the 60th and 120th day showed fish reared at 2.5 and 5ppt in 12:12 light were most efficient at converting feed to body mass. The higher feed conversion ratio at 2.5 and 5ppt in 12:12 light, relative to 10ppt in 12:12 light, seemed to contrast the SGR and TGC results, which showed higher somatic growth in fish reared at 10ppt in 12:12 light instead. It was unknown why there was such a contrast between growth rates and feed conversion ratio, so later sampling periods, when feeding rates and mortality returned to acceptable levels, can provide more accurate data on growth.

2.5.1.3 Maturation and Cataracts

Atlantic salmon used in my study were a mixed sex cohort with both males and females, which commonly develop jacks (mature male with significantly smaller body size and large gonads), signaled by longer daylight cycles or constant daylight (Fjelldal et al. 2011). My results did not show any trends that pinpointed a specific salinity or photoperiod that increased maturation rates. Melo et al. (2014) reared post-smolt Atlantic salmon in freshwater or seawater and under 24:0 light or 12:12 light at 16^{o}C, which resulted in 93% of males reaching maturation when reared in seawater in 24:0 light, relative to 70-80% mature males in the 12:12 light treatment. As the Atlantic salmon in my study were reared at a lower temperature and were smaller than those used in Melo et al.'s (2014) study (376 ±115g, which is greater than the largest average mass observed in my study), the onset of maturation may not have been triggered within the period of the present study.

Cataracts are a major concern for salmonid growth, as cataract development is negatively correlated with growth rates (Bjerkås & Sveier 2004). Cataracts were only observed during the first day of the experiment and on the 120th day of acclimation. Atlantic salmon reared at 30ppt in 24:0 light had the highest incidence of cataracts at 7%, while other treatment groups ranged between 0-3%. Photoperiod seemed to have a consistent effect on developing cataracts, with all salinity treatments in 24:0 light showing 1-7% of cataract incidences in the sampled individuals compared with 0-1% of incidences in 12:12 light. Fluctuating salinity between 15 and 30ppm is known to increase the likelihood of cataract development because of intraocular pressure stress from osmotic disturbances between the lens and the environment (Bjerkås & Sveier 2004). As our RAS had continuous filtration and maintenance that disposed some recirculating water, the

regular upkeep of adding new saline water could have imposed small salinity fluctuations that could affect cataract development. However, the effects of fluctuating salinity were not considered at the time of the experiment, so it would be hard to tell if the RAS maintenance duties could influence cataract development.

2.5.2 Coho salmon

2.5.2.1 Growth

Salinity had no effect on growth at any sampling period up to 120 days. In a past study by Emerman (2016), coho salmon reared in RAS at approximately the same size as my study grow fastest at 10ppt relative to those reared in freshwater and seawater for 59 and 156 days. Fish in that study was fed approximately 1% of biomass per day at the start of the experiment, while my study limited feeding to 0.5% of biomass per day for the first 60 days, which suggested there was an enhanced growth effect during the first 60 days of salinity acclimation that was dependent on feed ration. Rainbow trout of 10 months and 1.5 years reared at 0, 10, 20, 24, 28 and 32ppt for 12 weeks exhibited reduced growth rates and appetite with increasing salinity (McKay & Gjerde 1985). Higher growth rates at lower salinities were also seen in rainbow trout, steelhead trout and chinook salmon fry reared at different salinities for 77-84 days (0, 9 and 18ppt for rainbow trout; 0, 4, 8, 12 and 16ppt for steelhead trout; 0, 5, 10, 20 and 28ppt for chinook salmon) (Morgan & Iwama 1991). As the effects of salinity on growth have been observed in several *Oncorhynchus* species at different life stages and acclimation periods, growth enhancements are induced only if specific requirements, such as fish age and feed availability, are met in a small period of time.

Photoperiod did not result in any significant effects on growth in coho salmon. I speculate that photoperiod may have a greater influence on growth if it were to be introduced during the pre-smolt and smolting life stages, similar to what has been observed in fish reared at different salinities. Coho salmon fry reared under a weekly photoperiod change of 12-hour to 15-hour to 12-hour light for 12 weeks have higher growth rates, when compared to being reared under constant 12-hour light or a weekly photoperiod change of 12-hour to 9-hour to 12-hour light (Clarke et al. 1981). This is likely attributed to a photoperiod regime that stimulates growth hormone production for smoltification and preparation for seawater transfer. Increasing daylight hours and temperature, similar to a spring season light and temperature cycle, can elevate growth hormone levels in salmonids, which promote somatic growth and stimulate branchial NKA activity (Björnsson 1997). However, two-year-old rainbow trout starting at approximately 280g have greater mass gain and increased SGR when they were reared under 24:0 light and 18-hour light for five months (Noori et al. 2015). As such, there could be potential growth enhancements in coho salmon under 24:0 light after 120 days of acclimation in my study. Based on the results of my study and past results of photoperiod effects, photoperiod could have an effect on growth at different life stages for different salmon species.

PIT-tagged data of coho salmon also showed no significant effect of salinity or photoperiod. This data only accounted for 8-12 recaptured individuals, which, like Atlantic salmon, may not have provided enough statistical power to accurately determine if salinity and photoperiod had effects on individuals over time.

2.5.2.2 SGR, TGC, FCR

Specific growth rates of coho salmon did not show any trends across salinity and photoperiod. These results may support the above speculation that this study missed an enhanced growth period found closer to when smoltification would occur: SGR seemed to be slightly enhanced, but because of the low food ration at 0.5% of biomass per day, growth rate was not increased significantly to show differences in average mass across the treatments. TGC showed a similar trend as SGR, indicating temperature may have little interaction with salinity and photoperiod to enhance growth. TGC in rainbow trout also showed little variation across freshwater and seawater when they were reared in these conditions for four weeks (Krogdahl et al. 2004). Compared to the Atlantic salmon in this study, coho salmon had poor FCR: between the 60th and 120th day, FCR for all treatments groups were 1.33-1.77kg feed/kg mass gain, representing inefficiency in coho salmon to convert digested energy to somatic growth. As coho salmon did not experience the same *Saprolegnia* infection and mass mortality as Atlantic salmon, FCR could be low because coho salmon allocated more gross intake energy towards maintenance energy, which could improve immune responses to infections; however, this remains to be tested specifically.

2.5.2.3 Maturation and cataracts

In this study, I found no maturation in any of the treatment groups. Similar to Atlantic salmon, coho salmon may not have reached the size needed to induce maturation. In addition, the coho salmon used in my study were an all-female cohort, so no male jacks would have been observed. A study by Noori et al. (2015) concluded that two-year-old rainbow trout gonad development was highly influenced by short photoperiods of 6-hour light and natural photoperiod, while

individuals reared under 24:0 light and 18-hour light exhibited delayed gonad development. Gonad size and oocyte diameter increased at the third month of photoperiod acclimation in Noori et al.'s (2015) study and continued until the fifth month of the experimental endpoint, at which time, the rainbow trout mass was above 450g. The coho salmon in my study had not reached this mass yet, so it may have been too early to detect precocious maturation in coho salmon.

Salinity had a statistically significant effect on cataract incidence in coho salmon during the first 60 days of acclimation, although it was not clear which salinity had a greater impact. Furthermore, although there was a statistically significant effect, the biological significance of salinity on cataract incidence is questionable as the number of observed cataracts was quite low in all salinities. There was no statistically significant effect of photoperiod on cataract development in coho salmon over the first 120 days of rearing. This growth trial continued beyond the sampling reported here and so it will be interesting to see how salinity and photoperiod affect the development of cataracts throughout the remainder of the growth trial.

2.6 Conclusion

In this study, I found differences in how salinity and photoperiod affected growth and physiological changes, which were different between the two species. In Atlantic salmon, salinity and photoperiod influenced growth by day 120, with animals reared at 10ppt showing the highest growth in both light regimes. Likewise, animals reared in 24:0 light were observed to also have higher growth rates, relative to their 12:12 light counterparts at the same salinity. These growth effects were also generally observed in the calculation of SGR, TGC and FCR, with Atlantic salmon reared at 10ppt having the highest SGR and TGC in both photoperiods, as well as the lowest FCR, relative to other treatment groups. However, PIT-tagged fish average mass data showed salinity and photoperiod did not have an as apparent effect as observed in the whole-tank average mass results. Coho salmon growth was not affected by salinity or photoperiod over the 120 days of acclimation, a conclusion supported by the PIT-tagged fish data, SGR, TGC and FCR calculations. This is exciting information for producers to consider when making decisions for optimizing environmental conditions in RAS for their specific fish species. It is important then to continue this study to rear both salmon species to market weight to determine how salinity and photoperiod affects their full life cycle in RAS. This study is currently underway.

Chapter 3: **Effects of Salinity and Photoperiod on Swimming Performance**

3.1 Summary

Exercise and swimming performance is an important factor of salmon behavior and may be greatly influenced by the environment they are reared in, especially in RAS. However, there are gaps in our understanding of how swimming performance and growth interact physiologically, as both are energetically expensive processes. This data chapter looks at the effects of, and the interactions between, salinity and photoperiod on swimming performance of coho salmon conducted in two separated studies over two years. In the first year, coho salmon was reared at 0 and 10ppt for 350 days. In the second year, coho salmon was reared in either 0, 2.5, 10 and 30ppt in 12:12 light or 24:0 light for 60 and 150 days. Repeated maximum swimming speeds, U_{max}, and hematology were measured at each of these time points. In the first year, salinity had significant effects on resting osmolality and chloride ion concentration. In the second year, there were significant effects of salinity and time on first U_{max} and hematology, such as hematocrit, mean corpuscular hemoglobin concentration, and plasma osmolality and chloride ion concentration. However, there were no effects of photoperiod on swimming performance and hematology. Based on the results, there did not seem to be a trade-off between growth and swimming performance at different salinity and photoperiod.

3.2 Introduction

Salmon are active fish that swim throughout their life and often orient into currents, so the metabolic costs associated with swimming are an important component of their daily energy budget. As such, there could be potential trade-offs between swimming and other energetically demanding processes, such as growth. The considerations for constant or intermittent swimming conditions in fish aquaculture are becoming a more important factor to balance captive reared fish growth and health (Palstra & Planas 2013). For instance, maximum swimming speeds for farmed fish have been considered as a way to select for appropriate water velocities in fishways and culverts (Peake 2008). RAS permits high levels of control on water velocity and exercise for salmon, but without understanding the maximum swimming performance of the animals reared, salmon producers run the risk of exhausting their fish due to mismanaged water velocities, and even reducing productivity and growth.

Providing exercise for salmon through sustained swimming has been documented to improve growth rates due to skeletal muscle changes (Palstra & Planas 2011). For instance, yearling brook charr reared at 0.85 BL/s exhibited higher growth rates and lipid storage, relative to other individuals reared in still water or higher water velocities, possibly due to greater feed conversion efficiency as a result of sustained exercise (East & Magnan 1987). Similarly, adult Atlantic salmon reared in raceways with a water flow rate of 28.0 ± 11.8cm/s showed significantly higher weight gain, better feed conversion and higher condition factors, relative to salmon reared in standard cages where fish were not able to swim (Totland et al. 1987).

Exercising fish at different salinities, particularly freshwater and seawater, have been shown to impose osmotic and ionic disturbances due to the increase in oxygen demand at higher swimming speeds. At higher swimming speeds, there is an increased demand for O_2 and CO_2 transport across the gill that is accomplished through an increased functional gill surface area and blood perfusion at the cost of greater plasma sodium and chloride ion flux (Sardella & Brauner 2007). A study by Postlethwaite and Mcdonald (1995) show significant efflux of plasma sodium and chloride ions when freshwater rainbow trout were exercised over 96 hours, with subsequent sodium and chloride ion influx after 24 hours of recovery to compensate for ions lost during exercise. While fish in freshwater compensate for ion loss via ionoregulatory pathways in the gills, seawater fish use their gut more prominently for osmoregulation as the gut actively absorbs ions from imbibed water, which passively increases water absorption (Sardella & Brauner 2007). The excess ions absorbed by this process is then excreted back into the environment via the gills.

When coho salmon parr were acutely introduced from freshwater to seawater for 24 hours, their critical swimming speed (U_{crit}) significantly decreased, along with increases to plasma sodium and chloride ions (Brauner et al. 1992). Interestingly, parr that continued to acclimate in seawater for five to seven days showed less changes to their U_{crit}, despite significant increase in plasma sodium and chloride ions. It can be hypothesized that after an extended acclimation period to seawater, even as short as five to seven days, compensatory mechanisms are activated to return swimming performance and osmoregulatory disturbance to levels observed before acclimation. Repeated swimming performance, which involves swimming fish a second time after a first exhaustive swim with brief recovery, has been used as an indicator of health of salmonids under stress. It has been shown that juvenile coho salmon's ability to recover from exercise stress after

exposure to freshwater or seawater for 24 hours is affected by salinity, with those exposed to seawater exhibiting a significantly reduced second U_{crit} (Brauner et al. 1994). Plasma sodium ions at rest and exhaustion were similar to those found in Brauner et al. (1992) study, suggesting recovery from exercise and stress can potentially be affected by salinity.

It is unknown whether enhanced growth under a range of conditions is directly associated with better or poorer swimming performance, as it would be predicted that higher muscle mass can equate to animals achieving higher maximum speeds. On the other hand, higher mass may also require higher metabolic costs to move the animal, which can limit the animal's ability to achieve higher speeds because of substantial energy costs. At *InSEAS*, we identified both salinity and photoperiod to have significant effects on salmon growth, which presented a unique opportunity to observe how growth enhancements due to salinity and photoperiod may be traded off with swimming performance. If there are direct relationships between growth and swimming performance, this can potentially be an important indicator that salmon farmers can use to better manage RAS water flow rates and further improve growth and welfare of their animals.

The aim of this research is to understand the effects of salinity and photoperiod, and their interactions, on swimming performance and hematology in coho salmon. I hypothesized that salinity, but not photoperiod, would influence maximum swimming speeds and hematology, with salmon reared at a salinity of 10ppt showing the highest swimming speeds.

3.3 Methods

3.3.1 Overview

The experiments were conducted at the Initiative for the Study of the Environment and its Aquatics Systems (*InSEAS* at The University of British Columbia*;* Vancouver, BC, Canada). This consisted of seven identical RAS and these RAS have been shown to yield similar growth rates in coho salmon when all five of the seven RAS are held under identical environmental conditions (Emerman, 2016). The design of the RAS is described in detail by Emerman (2016). Two separate studies were conducted over two years to look at swimming performance at different life stages. In the first year, the animals used were acclimated to their respective salinity for 350 days, while in the second year, the animals used were acclimated to their respective salinity and photoperiod for 60 and 150 days. Experiments were conducted under UBC Animal Care Permits #A13-0016 and #A17-0011.

3.3.2 Animals used

Coho salmon smolts in the first and second year study were purchased from Target Marine Hatcheries (Sechelt, BC, Canada). Once received at *InSEAS*, they were housed in $5m^3$ tanks at a biomass of approximately $40kg/m^3$. In the first year, salinity trials began on April 27, 2015, and coho salmon smolts were reared at 0, 10 and 30ppt on a 24:0 light for approximately 350 days. Prior to swimming tests, 10 individuals of similar size were randomly selected from each salinity treatment and held in $0.7m^3$ tanks in the same RAS. These individuals were fasted for a minimum of 72 hours to ensure they were in a post-absorptive state.

In the second year, salinity trials began on January 11, 2017, and coho salmon smolts were reared at 2.5, 10 or 30ppt on a 24:0 light and 2.5ppt and 10ppt on a 12:12 light for 60 and 150

days, resulting in a total of five salinity and photoperiod permutations (See chapter 2). Prior to swimming tests, nine individuals of similar size were randomly selected from each salinity treatment and held in 0.7m^3 tanks in the same RAS. These individuals were fasted for a minimum of 24 hours to ensure they were in a post-absorptive state.

3.3.3 Equipment used

I used a Loligo 185L swim tunnel (Viborg, Denmark) to conduct repeated swimming performance tests to increase and maintain water velocity throughout the experiment. The swim chamber, where the animals were held for swimming, was constantly flushed with aerated and temperature-controlled water (11.7^{o}C and 12.8^{o}C). Between salinity changes, the swim tunnel was thoroughly rinsed with freshwater to remove waste build-up. Before each repeated U_{max} test, chamber water DO and ammonia were measured to ensure consistent water conditions between tests.

The front half of the swim chamber was covered by black plastic sheets to encourage the individuals to swim into a hiding area. Swim chamber water velocity was controlled by a variable frequency driver (VFD). The water velocity was measured as frequency (Hz) and converted to water velocity based upon a frequency water velocity calibration conducted using a Hontzsch hand-held anemometer (HFA series with a connected Vane Wheel FA fan) (Waiblingen, Germany). Water velocity was directly measured at the floor and mid-depth of the test chamber. The velocity at each frequency was calculated by averaging flow speeds measured on the left and right side and middle of the chamber at each depth. A velocity slope from multiple frequency tests for flow speeds was generated from this result and used to calculate

velocity at any specific frequency (found in the Appendix C). For this study, I used water velocity collected at the mid-depth of the test chamber because that was closer to where most fish would swim for a majority of the test. The following equation was used to calculate velocity:

$$V\ (cm/s) = 5.4382 \times Hz - 3.1446$$

The swim chamber was filled with dechlorinated water for 0ppt tests or with saline water of 10 and 30ppt prepared in a separate water aging barrel using Instant Ocean. Water from the animal's RAS holding tanks (also prepared using Instant Ocean) were not used due to high turbidity. Salinity was kept within ±1ppt of its target salinity.

3.3.4 Repeated swimming performance

The swim chamber was set to ~23cm/s prior to placing fish into the unit. Individuals were removed from the 0.7m^3 RAS tanks and placed into the swim chamber using a net with as little air exposure as possible to reduce handling stress. The swim chamber was then sealed, and the fish was left to orient itself to the flowing water. Once the fish was oriented against the water flow, a 10-minute practice swim was performed. This was based on a concept by Jain et al. (1997) to effectively habituate fish to a swim chamber prior to a ramped U_{crit} test, which significantly reduces testing time without compromising U_{crit} results, when compared to traditional step-test U_{crit} protocols. In the first year, fish were swum individually, and water velocity was increased by ~6cm/s every minute, until the velocity reached ~75cm/s (average 1.4BL/s), or until the fish became exhausted. At the end of the practice swim, water velocity was reduced to ~23cm/s and the fish was given 30 minutes to rest prior to beginning the test. In the second year, fish were swum in groups of three at the same time to increase sample size and reduce experiment time. Water velocity was increased by ~3cm/s every minute, until the velocity

reached ~53cm/s (average 2.0BL/s at the 60th day; average 1.5 BL/s at the 150th day). The slower acceleration was because of the smaller size of the animals used, relative to the first year. At the end of the practice swim, water velocity was reduced to ~23cm/s and the fish was given up to 60 minutes to rest prior to beginning the test. If a fish either continued to become disoriented in the chamber (constantly facing the incorrect direction) or unwilling to swim (remaining stationary in the corners of the chamber and unresponsive to disturbances), they were removed and euthanized.

A repeat maximum swimming speed (U_{max}) protocol was used to quantify an individual's swimming performance and ability to recover from exhaustion. U_{max} was defined as the water velocity at which the animal reaches exhaustion under a constant acceleration profile. Exhaustion was defined as either the time when the fish was unable to remove itself from the rear screen after five seconds, despite prodding. The protocol used was derived from and outlined by Nendick et al. (2009) and Farrell (2008), which involved stepwise increase of water velocity by ~3cm/s every minute to exhaustion to determine the first U_{max}. Fish were then allowed to recover for 60 minutes at ~23cm/s, followed by a repeated U_{max}. Fish were encouraged to swim into the hiding area of the swim chamber throughout the test. Any fish that fell back into the exposed area of the swim chamber were gently prodded by the researcher to ensure continuous swimming until exhaustion was observed. U_{max} is different from U_{crit}: U_{crit} usually refers to methods using incremental velocity tests that relies on aerobic performance but requires a longer time to complete one test, relative to U_{max} (Farrell, 2008). However, U_{max} may not allow for a steady state of aerobic performance, making it difficult to differentiate aerobic and anaerobic capacity.

At the end of the repeated U_{max} test, the fish was removed within 5 minutes and euthanized. In the first year, individuals were euthanized with a percussive blow to the brain, and in the second year, they were euthanized with MS-222 (0.5g/L) buffered in sodium bicarbonate. The animals were euthanized in the same salinity as their treatment group to ensure there was no alterations to osmolality and ions in blood during lethal sampling. Length and weight were measured with a standard ruler and Kilotech benchtop scale, respectively. 3mL of blood was sampled from the caudal vein using a heparinized syringe and stored in 1.5mL centrifuge tubes on ice for later measurement of plasma chloride ions ($[Cl^-]$) and osmolality. A minimum of 3 heparinized capillary tubes were filled, sealed and put on ice to measure hematocrit (Hct). To measure hemoglobin concentration ([Hb]), 10µL of blood was pipetted into triplicate 1mL Drabkin's solution, which were shaken thoroughly and held on ice.

3.3.5 Hematology analysis

Capillary tubes were centrifuged for a minimum of 1-2 minutes in a hematocrit capillary centrifuge. Three capillary tubes per individual was measured for Hct, and results are reported as the average of the three. Hct was calculated as the proportion of red blood cells to the full length of blood in the capillary tube using a standard ruler and was reported as a percentage. Centrifuge tubes were centrifuged for 5 minutes at approximately 10,000RPM in a benchtop centrifuge. After centrifugation, equal portions of blood plasma were pipetted into two separate bullet tubes and stored in a -80^{o}C freezer for later analysis, as described later in this section. The remaining red blood cells were discarded. Whole blood [Hb] was measured spectrophotometrically by measuring absorbance at 540nm and calculated using the equation in the Calculations section.

Blood plasma samples from exhausted fish were frozen at -80^{o}C until analysis. Blood plasma samples was also collected from resting fish of the same cohort during the growth experiment, as described in the previous chapter, to look at the hematology of resting animals. Blood samples from resting fish were matched time wise to the exhausted fish to reduce variations that may exist at different life stages. Hematology samples were not collected for resting fish during the growth experiment. Osmolality was measured using a Wescor VAPRO® vapor pressure osmometer with 10µL of plasma. The vapor pressure osmometer measures osmolality by using vapor pressure depression, achieved by thermocouple hygrometry (Wescor Inc. 2002). Measurements were recorded as mmol/kg. [Cl^-] was measured using a Labconco digital chloridometer as mEq/L with 10µL of plasma. Triplicate measurements of osmolality and [Cl^-] for each animal was completed, and results reported are the average of the three.

3.3.6 Calculations

Recovery ratio (RR) was calculated by dividing the repeated- U_{max} by the first U_{max} as an indicator of how well an animal recovers from exhaustion.

[Hb] was calculated using Beer's Law: A = εcl (A = absorbance, ε = millimolar activity (L/mmol·cm), c = concentration, l = lightpath length). To determine [Hb] (mmol/L), the following equation was used:

$$[Hb] = \left(\frac{A}{\varepsilon} \times \frac{1}{4}\right) \times 100$$

Where millimolar activity of hemiglobincyanide (HiCN) at 540nm, a compound of hemoglobin converted by Drabkin's solution, is ε = 11 L/mmol·cm, and light path length used in this

experiment is l = 1 cm (Zwart 1993). As red blood cells contain a heme group of 4 hemoglobin molecules, the absorbance value is divided by 4 to determine individual hemoglobin molecule concentration.

Mean cell hemoglobin concentration (MCHC) was calculated as:

$$MCHC = \frac{[Hb]}{(\frac{Hct}{100})}$$

3.3.7 Statistical analysis

All data were presented as means ± standard error of the means when standard error could be calculated. Outliers that were not within normal distribution were removed in statistical analyses, but still reported in the results. Outliers are defined as values lying outside the limits set by:

$$Outlier = \bar{x} \pm (2 \times SD)$$

where $\bar{x}$ was the sample mean and SD was the standard deviation of the mean. Outliers were removed in this data because it created statistical issues in normal distribution. For the first year, a 1-way ANOVA with salinity as a treatment factor was used to test statistical differences of swimming performance and hematology due to salinity. In the second year, 3-way ANOVA's with treatment factors of salinity, photoperiod and time were used to test statistical differences of swimming performance and hematology in relation to these factors and their interactions. The 30ppt treatment was also included in the 3-way ANOVA. Due to limited time and swim tunnels to conduct the experiment in the second year, individual animals sampled were used as the unit of analysis. It was recognized that this represented only a pseudo replication of the results, instead of considering each swim test, consisting of three animals, as the unit of analysis. Data was considered as significant if $P \leq 0.05$, and a post-hoc Tukey test using pairwise comparisons

were completed if significant differences were found. The post-hoc test was used to look for specific differences between treatment groups within each sampling period. Paired t-tests were used to determine if there were significant differencs in osmolality and plasma [Cl^-] between at rest and at exhaustion with each treatment group at each sampling period.

3.4 Results

3.4.1 Swimming performance

Recovery time between the two U_{max} tests varied between 30 to 60 minutes because the time was used for processing blood samples from previous samples, which sometimes took longer than expected. Past studies have shown recovery time between two U_{crit} tests of approximately 45 minutes allows for sufficient recovery for adult salmonids, so there should be little effects of the varied recovery time in my study (Farrell et al. 2003; Jain et al. 1998).

First U_{max} is shown in Figure 3.1. At the 150th day post-acclimation, one individual reared at 2.5ppt in 24:0 light and at 10ppt in 24:0 light was removed from the statistical analyses because of higher-than-normal speed. In the first year, there were no significant effects of salinity ($P=0.13$) on the first U_{max}. In the second year, there were significant effects of salinity ($P=0.04$) and time ($P<0.01$) on the first U_{max}, but there were no effects of photoperiod ($P=0.69$) and interactions between the three factors. Using Tukey pairwise comparison, there were no differences between any treatments at each time point.

Repeated U_{max} is shown in Figure 3.2. In the first year, there were no significant effect of salinity (P=0.40). In the second year, there were no significant effects of salinity (P=0.92) or photoperiod (P=0.95) on repeated U_{max}, but there was a significant effect of time (P<0.01). There were no interactions between any of the three factors.

Recovery ratio is shown in Figure 3.3. At the 60th day post-acclimation, two individuals reared at 10ppt in 24:0 light were removed from the statistical analyses because of their higher-than-normal RR. In the first year, there was no significant effect of salinity (P=0.45). In the second year, there were no significant effects of salinity (P=0.52), photoperiod (P=0.66) or time (P=0.28). There were no interactions between any of the three factors.

3.4.2 Hematology

Hematocrit, [Hb] and MCHC of blood plasma collected from individuals after the repeated U_{max} are shown in Table 3.1. In the first year, there were no effects of salinity on hematocrit (P=0.45). In the second year, there were significant effects of salinity (P<0.01) and time (P=0.04) on hematocrit. There was also a significant interaction between photoperiod and time (P<0.01), but there was no significant effect of photoperiod (P=0.16). Using Tukey pairwise comparison, exhausted fish reared at 2.5ppt in both photoperiods following 60 days of acclimation had the highest hematocrit in their respective photoperiod. Following 150 days of acclimation, hematocrit was significantly higher in exhausted fish reared at 2.5ppt in 24:0 light than those at 30ppt in 24:0 light.

In the first year, there were no significant effects of salinity on [Hb] (P=0.53). In the second year, there were significant effects of salinity (P<0.01) and photoperiod (P=0.05) on [Hb], but there were no effects of time (P=0.44). There were significant interactions between salinity and photoperiod (P<0.01), and photoperiod and time (P=0.01). Using Tukey pairwise comparison, [Hb] was significantly higher following 60 days of acclimation in exhausted fish reared at 2.5ppt in both photoperiods relative those at 10ppt in 12:12 light. Following 150 days of acclimation, [Hb] was significantly higher in exhausted fish reared at 2.5ppt in 12:12 light than all fish reared in 24:0 light.

In the first year, there was no significant effect of salinity on MCHC (P=0.86). In the second year, there were significant effects of salinity (P=0.04), but there were no effects by photoperiod (P=0.66) and time (P=0.10). There were interaction effects between salinity and photoperiod (P<0.01). Using Tukey pairwise comparison, MCHC was significantly higher following 60 day of acclimation in exhausted fish reared at 10ppt in 24:0 light, 2.5ppt in 12:12 light and 10ppt in 12:12 light, relative to those at 2.5ppt in 24:0 light at the. Following 150 day of acclimation, MCHC was significantly higher in exhausted fish reared 30ppt in 24:0 light than those at 2.5ppt in 24:0 light at the.

Plasma osmolality and [Cl^-] of coho salmon after a repeated U_{max} test and at rest are shown in Table 3.2. In the first year, there were no significant effects of salinity on osmolality at exhaustion (P=0.24). In the second year, there were significant effects of salinity (P<0.01), photoperiod (P=0.02) and time (P<0.01) on osmolality at exhaustion. There were no interactions between any of the three factors. Using Tukey pairwise comparison, osmolality at exhaustion

was significantly higher following 60 and 150 days of acclimation in fish reared at 30ppt in 24:0 light compared to those at 2.5ppt in 24:0 light and 2.5 and 5ppt in 12:12 light. In the first year, there was significant effect of salinity on osmolality at rest ($P<0.01$). Using Tukey pairwise comparison, fish reared at 10ppt was significant higher compared to those at 0ppt. In the second year, there were also significant effects of salinity ($P<0.01$), photoperiod ($P=0.02$) and time ($P<0.01$) on osmolality at rest. There was significant interaction between photoperiod and time ($P<0.01$). Using Tukey pairwise comparison, osmolality at rest was significantly higher following 60 days of acclimation in fish reared at 30ppt in 24:0 light compared to those at 2.5 and 10ppt in 24:0 light and 2.5ppt in 12:12 light. Following 150 days of acclimation, osmolality at rest was significantly higher in fish reared at 30ppt in 24:0 light compared to all other treatment groups.

In the first year, there was no significant effect of salinity on $[Cl^-]$ at exhaustion ($P=0.07$). In the second year, there were significant effects of salinity ($P<0.01$) and time ($P<0.01$) on $[Cl^-]$ at exhaustion, but there was no significant effect of photoperiod ($P=0.48$). There was also significant interaction between salinity and time ($P<0.01$). Using Tukey pairwise comparison, $[Cl^-]$ at exhaustion was significantly higher following 60 and 150 days of acclimation in fish reared at 30ppt in 24:0 light relative to all other treatments. In the first year, there was a significant effect of salinity on $[Cl^-]$ at rest ($P<0.01$). Using Tukey pairwise comparison, fish reared at 10ppt was significant higher compared to those at 0ppt. In the second year, there was also a significant effect of salinity ($P<0.01$) on $[Cl^-]$ at rest. However, there was no significant effect of photoperiod ($P=0.34$) and time ($P=0.97$). There was significant interaction between all three treatment factors ($P=0.03$). Using Tukey pairwise comparison, $[Cl^-]$ at rest was

significantly higher following 60 days of acclimation in fish reared at 30ppt in 24:0 light compared to those at 2.5ppt in both photoperiod treatments. Following 150 days of acclimation, $[Cl^-]$ at rest was significantly higher in fish reared at 30ppt in 24:0 light compared to those at 2.5ppt in both photoperiod treatments and 10ppt in 12:12 light.

In the first year, the change in osmolality from rest to exhaustion was significant at 0ppt ($P<0.01$), but not at 10ppt ($P=0.63$). The change in $[Cl^-]$ was significant at 10ppt ($P=0.01$), but not at 0ppt ($P=0.20$). In the second year, at 60 days of acclimation, the change in osmolality from rest to exhaustion was significant in all treatment groups ($P=0.01$ at 2.5ppt in 12:12 light; $P<0.01$ for all other treatment groups). The change in $[Cl^-]$ from rest to exhaustion was significant in fish reared at 2.5ppt in 24:0 light ($P<0.01$), 2.5ppt in 12:12 light ($P=0.02$) and 30ppt in 24:0 light ($P<0.01$) but was not significant in fish reared at 10ppt in 24:0 light ($P=0.06$) and 10ppt in 12:12 light ($P=0.11$). Following 150 days of acclimation, the change in osmolality from rest to exhaustion was significant in all treatment groups ($P<0.01$). The change in $[Cl^-]$ from rest to exhaustion was also significant in all treatment groups ($P=0.02$ at 30ppt in 24:0 light; $P<0.01$ for all other treatment groups).

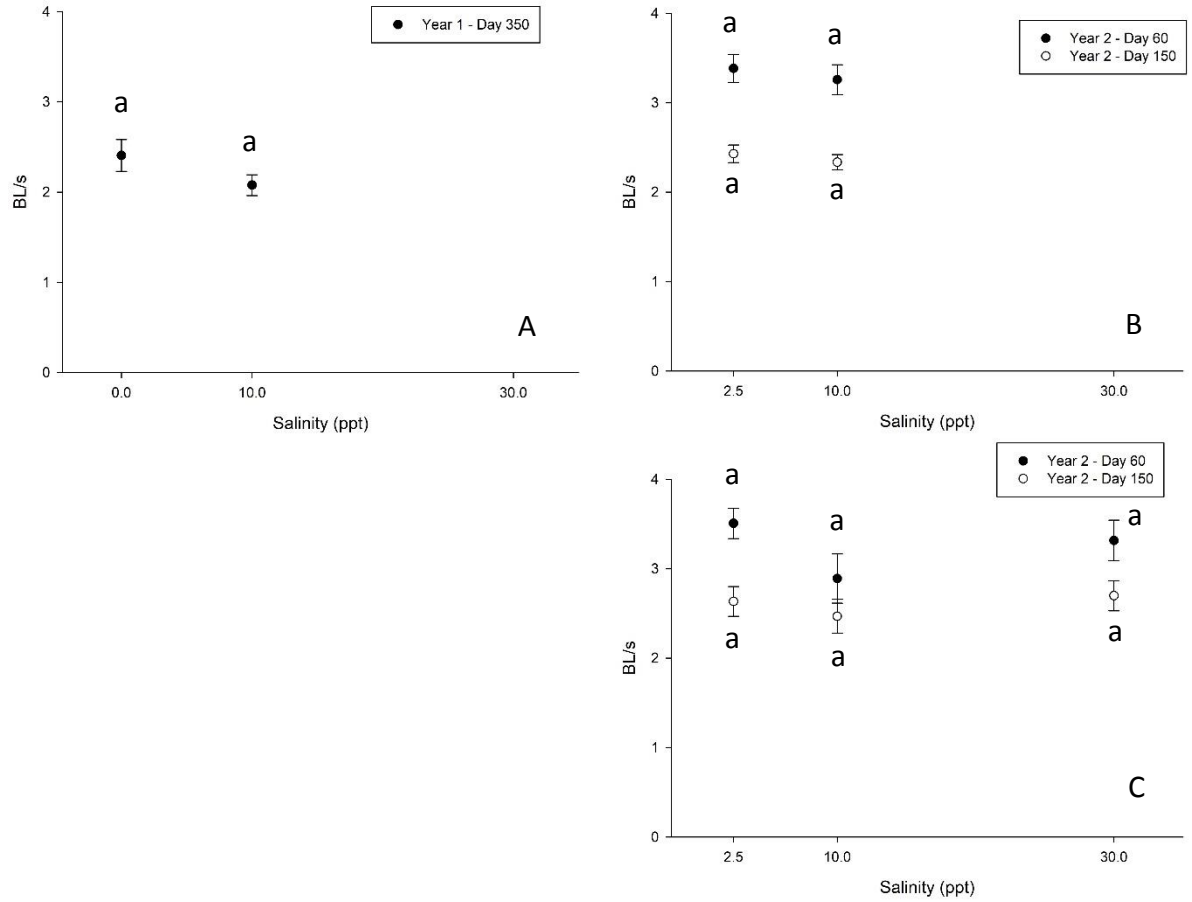

Figure 3.1 First U_{max} of 10 coho salmon (randomly selected in a cohort of 500-600 fish per tank) in Year 1 reared at salinities of 0ppt and 10ppt in 24:0 light (A). First U_{max} of 9 coho salmon (randomly selected in a cohort of 500-600 fish per tank) in Year 2 reared at salinities of 2.5, 10 and 30ppt in 24:0 light (B) and 12:12 light (C). All points are means ± SEM. Letters that differ within each sampling period indicate statistical differences in salinity and photoperiod.

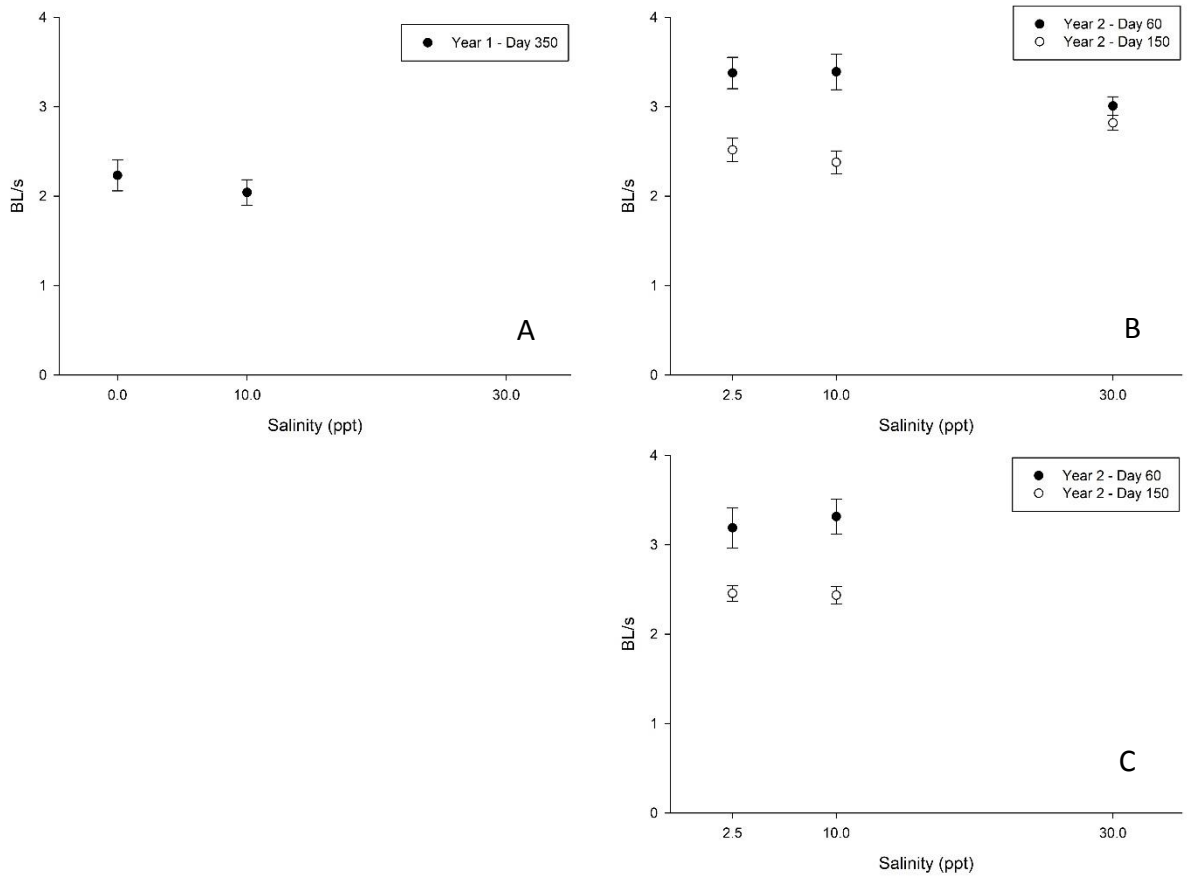

Figure 3.2 Repeated U_{max} of 10 coho salmon (randomly selected in a cohort of 500-600 fish per tank) in Year 1 reared at salinities of 0ppt and 10ppt in 24:0 light (A). Repeated U_{max} of 9 coho salmon (randomly selected in a cohort of 500-600 fish per tank) in Year 2 reared at salinities of 2.5, 10 and 30ppt in 24:0 light (B) and 12:12 light (C). All points are means ± SEM. No statistical differences were observed within sampling times.

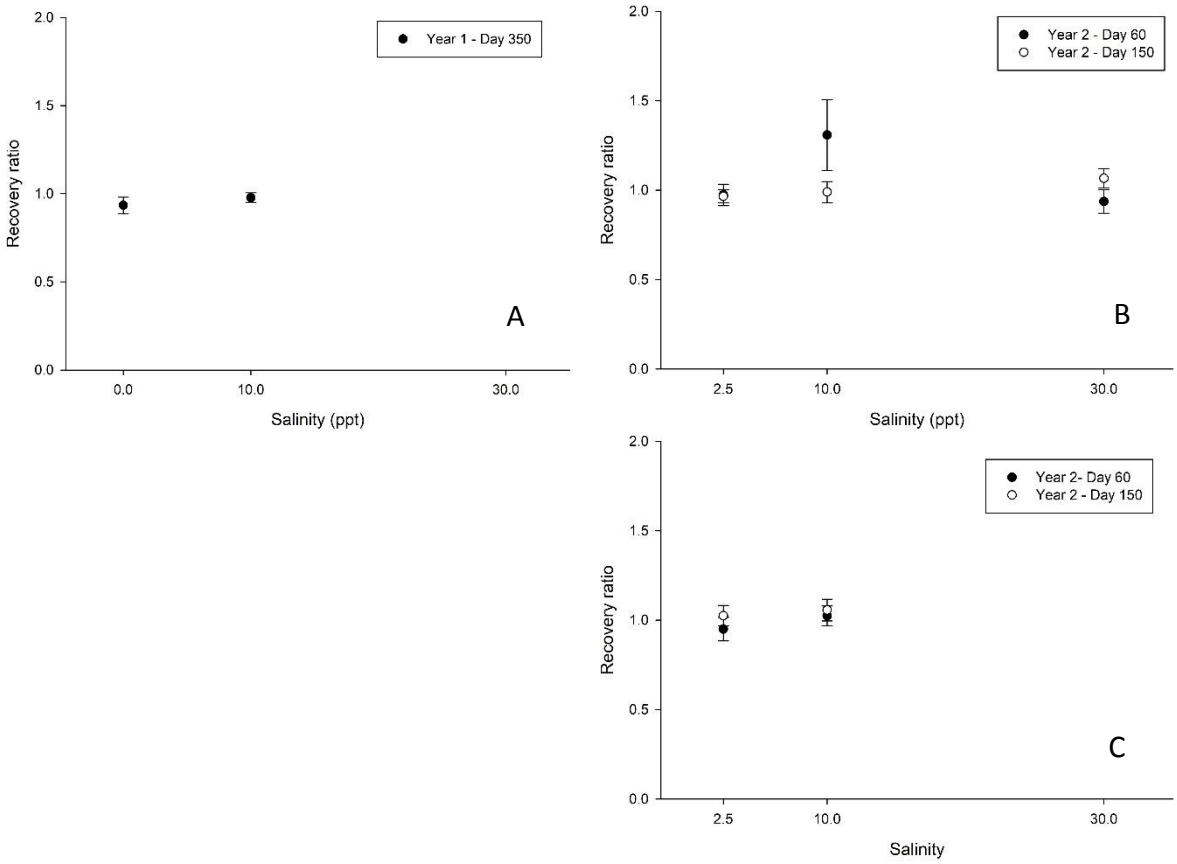

Figure 3.3 Recovery ratio of 10 coho salmon (randomly selected in a cohort of 500-600 fish per tank) in Year 1 reared at salinities of 0ppt and 10ppt in 24:0 light (A). Recovery ratio of 9 coho salmon (randomly selected in a cohort of 500-600 fish per tank) in Year 2 reared at salinities of 2.5, 10 and 30ppt in 24:0 light (B) and 12:12 light (C). All points are means ± SEM. No statistical differences were observed within sampling times.

Table 3.1 Hct, [Hb] and MCHC of coho salmon after repeated U_{max} test. Coho salmon in Year 1 reared at 0ppt and 10ppt in 24:0 light. Coho salmon in Year 2 reared at 2.5ppt, 10ppt and 30ppt in 24:0 light and 12:12 light. All values are mean ± SEM. Letters that differ within each sampling period indicate statistical differences in salinity and photoperiod.

Year 1 - Day 350				
Photoperiod	Salinity (ppt)	Hematocrit (%)	[Hb] (mmol/L)	MCHC ($mmol/L_{RBC}$)
24:0 light	0	$42\pm2_{a}$	$1.59\pm0.08_{a}$	$3.75\pm0.14_{a}$
	10	$40\pm2_{a}$	$1.52\pm0.08_{a}$	$3.78\pm0.15_{a}$

Year 2 - Day 60				
Photoperiod	Salinity (ppt)	Hematocrit (%)	[Hb] (mmol/L)	MCHC ($mmol/L_{RBC}$)
24-hr light	2.5	$46\pm1_{a}$	$1.48\pm0.05_{a}$	$3.25\pm0.13_{a}$
	10	$38\pm1_{b}$	$1.42\pm0.05_{ab}$	$3.73\pm0.12_{b}$
	30	$36\pm1_{bc}$	$1.32\pm0.05_{ab}$	$3.66\pm0.12_{ab}$
12-hr light	2.5	$38\pm1_{b}$	$1.43\pm0.05_{a}$	$3.79\pm0.06_{b}$
	10	$30\pm3_{c}$	$1.12\pm0.11_{b}$	$3.72\pm0.09_{b}$
Year 2 - Day 150				
24-hr light	2.5	$43\pm2_{ab}$	$1.36\pm0.04_{a}$	$3.21\pm0.10_{a}$
	10	$39\pm1_{bc}$	$1.35\pm0.04_{a}$	$3.45\pm0.06_{ab}$
	30	$35\pm1_{c}$	$1.26\pm0.02_{a}$	$3.59\pm0.07_{b}$
12-hr light	2.5	$46\pm2_{a}$	$1.54\pm0.05_{b}$	$3.34\pm0.11_{ab}$
	10	$41\pm1_{abc}$	$1.38\pm0.04_{ab}$	$3.38\pm0.08_{ab}$

Table 3.2 Plasma osmolality and [Cl^-] of coho salmon after repeated U_{max} test and at rest. Coho salmon in Year 1 reared at 0ppt and 10ppt in 24:0 light. Coho salmon in Year 2 reared at 2.5ppt, 10ppt and 30ppt in 24:0 light and 12:12 light. All values are mean ± SEM. Letters that differ within each sampling period indicate statistical differences in salinity and photoperiod. Asterisks indicate statistical differences between measurements at rest and at exhaustion within the same treatment group.

Year 1 - Day 350							
Photoperiod	Salinity (ppt)	Osmolality at rest (mmol/kg)	Osmolality at exhaustion (mmol/kg)	ΔOsmolality from rest (mmol/kg)	[Cl^-] at rest (mEq/L)	[Cl^-] at exhaustion (mEq/L)	Δ[Cl^-] from rest (mEq/L)
24-hr light	0	$291.4\pm2.5_a$	$324.4\pm4.6_a$	33.0*	$122.0\pm1.2_a$	$129.2\pm3.1_a$	7.2
	10	$313.8\pm3.7_b$	$316.6\pm4.5_a$	2.8	$131.4\pm1.3_b$	$135.6\pm1.4_a$	4.3*

Year 2 - Day 60							
Photoperiod	Salinity (ppt)	Osmolality at rest (mmol/kg)	Osmolality at exhaustion (mmol/kg)	ΔOsmolality from rest (mmol/kg)	[Cl^-] at rest (mEq/L)	[Cl^-] at exhaustion (mEq/L)	Δ[Cl^-] from rest (mEq/L)
24-hr light	2.5	$293.9\pm2.3_a$	$331.1\pm4.8_a$	37.3*	$133.7\pm1.0_a$	$118.1\pm2.5_a$	-15.6*
	10	$309.6\pm1.2_b$	$335.9\pm3.8_{ab}$	26.2*	$135.5\pm1.3_{ab}$	$139.6\pm2.0_b$	4.0
	30	$321.3\pm3.8_c$	$358.1\pm7.5_b$	36.8*	$141.2\pm2.1_b$	$163.9\pm2.1_c$	22.7*
12-hr light	2.5	$299.6\pm1.2_a$	$315.7\pm5.4_a$	16.1*	$131.9\pm1.7_a$	$124.3\pm1.8_a$	-7.6*
	10	$313.2\pm1.0_{bc}$	$333.4\pm5.0_a$	20.2*	$134.9\pm1.3_{ab}$	$138.6\pm1.5_b$	3.6
Year 2 - Day 150							
24-hr light	2.5	$291.4\pm2.6_a$	$344.4\pm2.2_a$	53.0*	$132.1\pm0.9_a$	$120.5\pm1.9_{ab}$	-11.6*
	10	$304.6\pm1.4_b$	$358.3\pm7.1_{ab}$	53.7*	$138.3\pm0.8_{bc}$	$128.8\pm1.6_b$	-9.5*
	30	$329.1\pm2.2_c$	$380.5\pm8.2_b$	51.4*	$142.1\pm1.1_c$	$152.9\pm3.9_c$	10.7*
12-hr light	2.5	$281.5\pm2.3_a$	$335.6\pm5.1_a$	54.0*	$137.2\pm1.3_b$	$118.7\pm2.1_a$	-18.5*
	10	$289.2\pm3.3_a$	$346.3\pm5.7_a$	57.0*	$136.4\pm1.3_{ab}$	$126.7\pm1.1_{ab}$	-9.7*

3.5 Discussion

The current study found swimming performance of coho salmon was not significantly affected by salinity or photoperiod in both years of the study. After a repeated swimming performance test, salinity only affected resting plasma osmolality and chloride concentration in the first year, while salinity, but not photoperiod, showed effects on all hematological analyses at rest and at exhaustion. Based on these results, there did not seem to be a compromise between growth and swimming performance due to salinity and photoperiod.

3.5.1 Swimming performance

3.5.1.1 U_{max}

My results demonstrated that there were no significant effects of salinity or photoperiod on the first U_{max} of coho salmon within each sampling time, despite showing significant effects across the 60^{th} and 150^{th} days post-acclimation. There were also no effects of salinity and photoperiod on the repeated U_{max}. Other studies demonstrate acute exposure of juvenile coho salmon to seawater or freshwater can affect swimming performance (Brauner et al. 1994, 1992). Juvenile coho salmon that were acutely exposed to seawater, after transfer from freshwater rearing, for 24 hours have lower U_{crit} when compared to individuals that were retained in freshwater (Brauner et al. 1992). However, when the individuals from Brauner et al.'s (1992) study were acclimated to seawater for five to seven days in the same study, their U_{crit} values are no different from those that are acclimated to freshwater. It could be that the extended acclimation period of this study, which was 60 days and 150 days, provided sufficient time for compensatory mechanisms for osmoregulatory disturbances were fully activated and allowed the animal to return to a similar

level of swimming performance prior to salinity challenges, similar to what Brauner et al. (1992) observed in their experiment.

3.5.1.2 Recovery ratio

There were also no significant effects of salinity and photoperiod in coho salmon recovery after a repeated U_{max} test. As most salmonids are active fish, constantly swimming for a majority of their life, they should be adept at maintaining and recovering from exhaustive swimming that would be commonly encountered in the wild. For instance, rainbow trout can fully recover from U_{crit} tests in as little as 70 minutes (Jain et al. 1998). In addition, the animals in my study were reared under RAS conditions that provided a constant swimming environment, which have been shown to enhance recovery in salmonids after exhaustive exercise. Rainbow trout adults trained with sustained swimming of 0.9BL/s have significantly lower blood lactate and cortisol concentration when chased to exhaustion, compared to those that are reared in still water of 0BL/s (Milligan et al. 2000). Arterial pH, muscle glycogen and lactate of these rainbow trout adults also return to resting levels after approximately two hours of recovery, while still water acclimated trout do not recover for up to six hours. Similar results are seen in coho salmon parr acutely acclimated to one day in seawater, five days in seawater, one day in seawater before returning to freshwater, and staying in freshwater: muscle lactate concentration returns to resting levels for all treatments after two hours of recovery from exhaustion (Brauner et al. 1992). It is most likely biochemical processes linked to compensation for metabolic acidosis and anaerobic swimming are optimized differently for different environmental conditions to efficiently return the body to normal conditions. Therefore, it is likely coho salmon reared at all salinities in this

study were able to recover equally well because they had the appropriate compensatory processes in place for their specific environment.

The lack of difference in recovery ratio across all treatment groups also indicated coho salmon had equally good physical status and health under the salinities and photoperiods used in this study. When mature sockeye salmon were exposed to dehydroabietic acid, a toxic component that wild salmon may encounter, or were suffering from *Vibrio* and *Sporocytophagosis* infections, recovery ratio was significantly reduced, with infected salmon exhibiting a recovery ratio as low as 0.61 (Jain et al. 1998). In this study, the equally high recovery ratio across all treatment groups could be directly linked to the consistent SGR, condition factor and average mass observed, which clearly showed the coho salmon are healthy under all environmental conditions to which they were acclimated.

3.5.2 Hematology

3.5.2.1 Hematocrit, [Hb] and MCHC

Based on the 3-way ANOVA and Tukey pairwise comparison tests, the results were difficult to determine if there were relationships between hematology, salinity and photoperiod. However, the statistical significance of salinity and photoperiod in different treatment groups still provided evidence that environmental conditions could still affect hematology at exhaustion, which warrants for future studies with a larger sample size and at different sampling periods.

Similar results of lower [Hb] at higher salinities are found in European flounders acutely acclimated from isosmotic salinity to seawater and decreased hematocrit at higher salinities in

juvenile coho salmon when acutely acclimated to seawater (Brauner et al. 1994, 1992; Jensen et al. 2002). In studies of both European flounders and juvenile coho salmon, changes in osmolality and salinity did not affect MCHC. The low U_{crit} values of juvenile coho salmon acutely acclimated to seawater are attributed to limited oxygen delivery due to increased plasma ion levels from the environment, which in turn caused red blood cell shrinkage (ie. decreased hematocrit), increased plasma volume and reduced muscle water content (Brauner et al. 1994; Randall & Brauner 1991). However, Randall and Brauner (1991) demonstrated that fish transferred to saline waters will eventually restore their aerobic swimming capacity via recruitment of gill chloride cells, which increase osmoregulatory capacity. As the coho salmon used in this study were acclimated for 60 days and beyond, gill chloride cells could have been effectively recruited to reduce osmotic and ionic disturbances during exercise, thus maintaining equal performance across all salinities. In the future, it will be worthwhile to look at hematocrit and [Hb] changes over time after exhaustion to determine if the rate of red blood cell recovery from shrinkage is significantly different in fish reared at 30ppt, relative to those at lower salinities.

3.5.2.2 Plasma Osmolality and [Cl^-]

At rest, this study found plasma osmolality to be consistent with observations in Atlantic salmon parr and Chinook salmon reared at 0, 10, 20 and 31/34ppt, which showed plasma osmolality increases with salinity (Duston 1994; Stewart et al. 2016). At exhaustion, osmolality differences between 0 and 30ppt were also observed, consistent with the results in resting fish, where plasma osmolality from fish reared at 0ppt were significantly lower than those reared at 30ppt. Based on the Tukey pairwise comparisons of this study, there was an increase in plasma osmolality at

exhaustion and rest at 30ppt when compared to all other treatments, so osmotic differences were still clearly present, despite acclimation to the salinity treatment for 60 days and more. Changes in osmolality from rest to exhaustion varied over time. In the first year, the greatest change to osmolality were in fish reared at 0ppt in 24:0 light and the least at 10ppt in 24:0 light. In the second year at 60 days of acclimation, the greatest change to osmolality were in fish reared at 2.5ppt in 24:0 light and the least change at 2.5ppt in 12:12 light. At 150 days, the change in plasma osmolality at exhaustion was similar between all treatments.

This trend for an increase in plasma osmolality during exercise in seawater has previously been observed in chinook salmon (Gallaugher et al. 2001). Fish that were swum up to 80% U_{crit} and then U_{crit} exhibited an increase in plasma osmolality from rest, most likely due to dehydration. The authors hypothesized this was associated with a reduction in gut blood flow for improved blood flow to locomotory muscles, which decreases gut water reabsorption (Gallaugher et al. 2001). However, Gallaugher et al. (2001) mentioned that freshwater adapted fish should hydrate after exercise, resulting in a reduction in plasma osmolality, while seawater adapted fish should dehydrate. Interestingly, plasma osmolality increased after exhaustion in all salinity treatments tested, which contrasted with what Gallaugher et al (2001) noted. One possible explanation is that after smoltification and extended acclimation to a specific salinity, coho salmon can reduce osmoregulatory and metabolic costs such that plasma osmolality and swimming performance remain consistent across a wide range of salinities. European seabass have been seen to maintain exercise performance, plasma osmotic homeostasis and tissue water balance at U_{crit} when they were transferred from seawater to seawater, 10% seawater, 5% seawater or freshwater for 18 hours (Chatelier et al. 2005). Adriatic sturgeon reared that were transferred from freshwater or

brackish water to seawater for 24 hours resulted in a reduction in U_{crit} and an increase in plasma osmolality at both rest and at U_{crit} relative to sturgeon that were held in freshwater or brackish water McKenzie et al. (2001). Interestingly, McKenzie et al. (2001) found a negative linear relationship between plasma osmolality, Na^+ or Cl^- concentration, and U_{crit}, which suggests that plasma osmotic and ionic imbalances have a direct effect on swimming performance. In this thesis, no significant differences in U_{max} or plasma osmolality at rest or following exhaustion were observed across the different salinities tested, indicating that coho salmon acclimated efficiently by the 60^{th} and 150^{th} day, which in turn decreased osmoregulatory and metabolic costs that impede swimming performance.

Changes in plasma $[Cl^-]$ also changed with salinity. In the first year, plasma $[Cl^-]$ at exhaustion was not significantly different between the two treatments, though $[Cl^-]$ at rest was significantly higher in fish reared at 10ppt relative to 0ppt. In both treatments, plasma $[Cl^-]$ increased from rest to exhaustion. However, the results of the second year differed, showing a reduction in plasma $[Cl^-]$ at exhaustion as salinity decreased. At 60 days and 150 days post-acclimation, fish reared at 2.5ppt under both photoperiods had reduced plasma Cl^- ions when the individual reached exhaustion, while those reared at 30ppt in 24:0 light had increase plasma Cl^- ions. Blood plasma $[Cl^-]$ is correlated with swimming performance in coho salmon parr acutely acclimated to seawater and freshwater, where plasma $[Cl^-]$ is reduced in fish reared in freshwater and increased in seawater at exhaustion (Brauner et al. 1992). The net flux of Cl^- ions in coho salmon reared at different salinities in this study was most likely associated with the osmorespiratory compromise, where oxygen demand increased near exhaustion and this negatively affected ionoregulatory homeostasis. Based on the similar U_{max} and recovery ratios that coho salmon achieved across all

salinities of this study, the changes to plasma osmolality and [Cl^-] at exhaustion was an indication of sublethal differences influenced only by salinity but not by swimming performance. Hence, the effects of salinity, when fish were acclimated for 60 days and beyond, may not influence osmotic and ionic disturbances enough at exhaustion to stunt maximum swimming speeds and recovery.

3.6 Conclusion

Although swimming performance was largely unaffected by salinity and photoperiod, these two factors affected hematology and plasma ions. The results of the two U_{max} trials found showed no significant effect of salinity and photoperiod, which suggest the effects of salinity on osmoregulatory compromises during the initial transfer are minimized after 60 days of acclimation. Hematocrit at exhaustion was affected by both salinity and photoperiod, while [Hb] and MCHC was only influenced by salinity. Osmolality was consistently increased at exhaustion across all treatment groups, while there was a decrease of plasma [Cl^-] in exhausted fish reared at 0ppt and increase of plasma [Cl^-] in exhausted fish reared at 30ppt. It is interesting to note that there are little to no studies on the effects of salinity and photoperiod on fish acclimated in these conditions for up to 300 days, while most other studies only looked at acute acclimation between 24 hours and 7 days. When I compared the results of growth to swimming performance, there did not seem to be any trade-off between these two factors. As such, the results of this study were not consistent with the hypothesis that salinity and photoperiod influence maximum swimming speeds but did support the hypothesis that these conditions will affect the hematology of coho salmon.

Chapter 4: **General Conclusion**

4.1 Summary

The overall goal of this thesis was to examine the effects of salinity and photoperiod on the growth and swimming performance of Atlantic and coho salmon reared in RAS. I tested salinity and photoperiod effects on salmonid growth in replicate systems at The University of British Columbia *InSEAS* research facility, where fish were continuously reared for 120 days. I found salinity and photoperiod had species-specific effects, with contrasting results from previous research on salinity in the same systems by Emerman (2016). In this study, Atlantic salmon growth was significantly affected by salinity and photoperiod, but coho salmon growth was not. I also conducted repeated U_{max} tests on coho salmon reared at different photoperiods and salinities to determine how these environmental conditions affect swimming performance. I found there were effects of salinity only on the first U_{max} of the repeated swimming performance test, but there were no other significant effects of salinity and photoperiod on the repeated U_{max} and recovery ratio. In the first year, resting plasma osmolality and $[Cl^-]$ was significantly affected by salinity. In the second year, all parameters of hematology were significantly affected by salinity, both at rest and at exhaustion. [Hb] and resting and exhausted plasma osmolality was also significantly affected by photoperiod.

Salinity showed a greater effect on Atlantic salmon growth, with fish reared at 10ppt having the highest growth rate. Photoperiod resulted in some effects on growth rates, which may require longer acclimation time to enhance the effects for clear significance. SGR and TGC also seemed to be significantly enhanced by salinity, which consistently showed higher SGR and TGC in

10ppt treatment groups at both sampling periods, when compared to all other salinity treatments. This could potentially be due to the species' extensive breeding for optimized growth, so any metabolic cost savings could be immediately directed to somatic growth. It was unfortunate that no swimming performance could be collected from Atlantic salmon, as it would have been interesting to see if the enhanced growth observed at 10ppt influenced swimming performance; however, because we were limited in fish numbers, we focused on just growth in Atlantic salmon.

There were no significant effects of salinity and photoperiod that influenced growth and swimming performance in coho salmon. However, in the second year of swimming performance tests, there were significant effects of salinity and photoperiod on hematology, including hematocrit, [Hb], MCHC, plasma osmolality and [Cl^-]. This suggested that coho salmon acclimated to different salinities and photoperiods at and beyond 60 days were still osmotically challenged by their environment when they reached maximum swimming speeds. However, this environmental challenge on the animal's physiology was not significant enough to compromise their growth and performance.

The results presented in this thesis can be applied to salmon farming and RAS management practices. At *InSEAS*, I was given the opportunity to look at two different salmon species, which provided strong evidence that different species behaved differently to their environment and continued to support salinity as an effective environmental condition for enhancing salmon growth. However, as there were contrasting results of salinity effects on growth between this study and Emerman's (2016), who suggested that salinity has an effect in coho salmon, but not in

Atlantic salmon, in the same RAS facility. When the study was continued by Fang (in prep), who reared the same Atlantic and coho salmon as Emerman (2016) up to 460 days, salinity started to show significant effects in Atlantic salmon growth starting at 295 days post-acclimation, while salinity continued to show significant effect on coho salmon growth starting at 59 days post-acclimation. Therefore, I cannot provide a solid support of any one salinity that optimizes growth in these two species until more replicate studies are performed to enhance statistical power. Based on my research, I recommend 24:0 light to enhance growth and provide continuous feeding. As the growth study only looked at the first 120 days of growth, which did not allow for salmon to reach market weights, it is important to continue this study to look at how salinity and photoperiod affect growth in both Atlantic and coho salmon at later stages in life, particularly in maturation and cataract development.

4.2 Applications of this Research to Salmon Farming

The results of this research can provide several recommendations to salmon producers to improve rearing conditions for optimized growth and animal health. Based on the data, it is inadvisable to maintain salinity above 10ppt, as there were no added growth benefits for either species beyond 10ppt. In addition, maintaining a high salinity RAS is costly and difficult to manage. For example, to maintain 30ppt in one 15,000L RAS at *InSEAS*, approximately 757L of seawater mixed from Instant Ocean® (or 27.2kg of salt) at approximately $30CAD per box was needed each day to maintain salinity. Even if a facility is built with a seawater pump to decrease salt costs, Emerman (2016), Fang (in prep) and this study still show no benefits of using salinity as high as 30ppt for enhanced growth. Emerman (2016) and Fang (in prep) note salinity has an effect coho salmon, with those reared in 10ppt with enhanced growth relative to 0, 5, 20 and

30ppt, while there are no effects found in Atlantic salmon. In contrast, my study found there were effects of salinity on Atlantic salmon, with enhanced growth at 10ppt relative to 2.5, 5 and 30ppt, but no effects were found in coho salmon. As both studies used the same RAS, it suggests there were other factors that influence growth, but were not measured. However, some level of salinity, rather than using complete freshwater, may still provide health benefits, most notably for reducing *Saprolegnia* infections and maintaining pH that can support better salmonid health. Furthermore, salinity did not seem to significantly affect swimming performance and recovery of coho salmon after exercise or stress, so any salinity used for rearing salmon would not reduce health and performance.

Based on this study, continuous photoperiod was best for promoting growth, and if paired with continuous feeding, as was the case in past research at *InSEAS*, it could further enhance mass gain. More data points over time are needed to provide a clearer picture of photoperiod's effect on growth, especially on early maturation, which was not observed up to this time point because the animals had not reached mature sizes. Just like salinity, photoperiod did not appear to affect swimming performance and health of the fish, so continuous light could be used to enhance growth without compromising health of the animals.

4.3 Strengths and Limitations

In this study, we were able to provide further data to extend those collected previously at the *InSEAS* facility, showing salinity affects growth in salmonids. Even though this study showed that salinity only influenced Atlantic salmon growth, it still supported salinity as a key environmental factor that could potentially influence growth rates in other species. This study

also delved into greater detail of the osmoregulatory mechanisms that are known to be affected by salinity in multiple species. The results of the ionic and osmotic disturbances in coho salmon at exhaustion provided more solid support of salinity as an important rearing condition that salmon producers must consider and carefully manage to optimize growth and health of their animals.

The *InSEAS* facility offered a unique opportunity to rear two different species of salmon in two identical tanks running with the same water condition as each other. In this study, it was shown that two species behave differently when acclimated to the same conditions, which provided strong evidence that not only was growth dependent on environmental conditions, but genetic and physiological variances could affect how a species fares in RAS under different environmental conditions. In this study, we found no differences in maturation between salinity and photoperiod. It was most likely because we were measuring these animals early in their life stage, so maturation may not have set in, as well as using a non-invasive method of identifying maturation.

One of the greatest limitations of this study was the number of tank replicates that could be achieved to improve statistical confidence on the effects of salinity and photoperiod on growth. As the experiment's scope was to provide producers and stakeholders with timely and immediately usable recommendations in a near-commercial scale, it was logistically and economically not possible to replicate tanks in a reasonable time frame. Another major limitation of the growth study was the maintenance and troubleshooting of the RAS design. As it was a relatively new design with a multitude of life support equipment (ie. filtration and oxygenation),

which required extensive maintenance and knowledge to operate, there were potential biological and technical factors that we did not consider but could have significant effects on the growth and health of the animals. However, the results of the growth study still emerged with interesting data that indicated salinity and photoperiod could influence enhanced growth in some salmonid species, which argues for continued research in environmental effects on cultured fish species for maximized production.

Swimming performance results were greatly improved by using a constant acceleration profile that achieved exhaustion in fish in a short period of time, allowing for more individuals to be tested at each sampling period. However, a constant acceleration profile was limited to only understand burst performance and how coho salmon managed their anaerobic capacity, which did not represent how they handled aerobic exercise or sustained swimming. The hematological analyses in this study could also benefit from cannulating and drawing blood from fish over different speeds, providing better results of how osmoregulatory compromises were affected as exercise and oxygen demand increased over time.

Another limitation of the swimming performance study, like the growth study, was the limited tank replicates that were available to reduce potential, random effects of tanks. In the future, it would be advisable to increase the sample size of each salinity and photoperiod. That said, the swimming performance study provided new results and insights into how fish perform under stress and exercise when exposed to different environmental conditions for multiple months, while previous research has limited environmental challenges to between several hours and days.

As such, this experiment should continue to be replicated over different acclimation periods to further understand how fish acclimate to environmental challenges and conditions.

4.4 Future Directions

This study gave a better understanding and new evidences of the influence of salinity and photoperiod on rearing salmon in RAS. As the growth results of this study were contrary to the past study by Emerman (2016) and Fang (in prep), it is clear that further experiments are needed to parse out the relationship of environmental conditions, like salinity, to growth and performance. In addition, further studies on the same 7 RAS should investigate fewer treatments with greater tank replicates to increase statistical power. As there were species-specific differences in growth due to salinity and photoperiod, it will also be interesting to test other farmed salmon species and their responses to different salinity and photoperiod.

Swimming performance of salmonids and other fish species have been extensively tested under acute acclimation to different or extreme conditions, but very few look at how the animals will perform if they were given more than 24 hours to acclimate to a new environment that is within their tolerable range. In this study, I demonstrated Atlantic and coho salmon could acclimate effectively to a new environment within their tolerable salinity range of 0 to 30ppt by showing no differences in achieving similar U_{max} and recovery ratio across salinities and photoperiods, even though there were physiological differences in their hematology. For future studies at *InSEAS,* it will be worthwhile to look at how hematology changes as different swimming speeds are imposed on the animal, as well as plasma ions and osmolality fluctuations over time after exhaustion. In addition, fillet quality of the animal should be assessed, as this is important to

salmon producers to raise a visually and gastronomically pleasant product. Muscle composition at rest and at exhaustion will give clearer insights to how individuals reared in their respective conditions utilize their energy storage and for what purpose.

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Appendices

Appendix A : Biomass and culled biomass for FCR calculations

Tank biomass and culled biomass of Atlantic and coho salmon reared at salinities of 2.5, 5, 10 and 30ppt and in 24:0 light and in 12:12 light, reported for the beginning of the trial, the 60th day and the 120th day post acclimation.

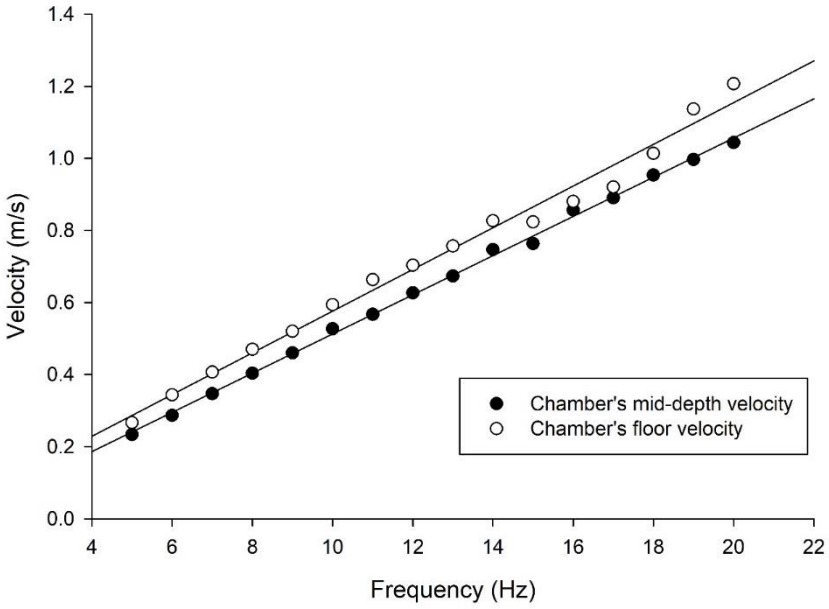
			Biomass (kg/m^3)			Culled Biomass (kg/m^3)	
Species	Photoperiod	Salinity	Day 0	Day 60	Day 120	Day 0-60	Day 60-120
S. salar	24:0 light	2.5ppt	36.00	12.02	20.57	12.17	1.24
		5ppt	34.00	20.16	25.73	12.21	4.22
		10ppt	32.00	24.34	35.53	11.28	3.83
		30ppt	34.00	30.17	35.40	5.68	12.78
	12:12 light	2.5ppt	33.00	12.93	11.30	13.52	7.47
		5ppt	34.00	10.08	16.37	16.66	1.59
		10ppt	33.00	14.48	18.55	13.81	3.42
O. kisutch	24:0 light	2.5ppt	135.00	130.49	171.78	15.71	16.20
		5ppt	136.00	139.28	193.19	21.52	9.45
		10ppt	139.00	159.71	201.40	12.19	10.63
		30ppt	135.00	140.74	178.19	10.13	16.20
	12:12 light	2.5ppt	139.00	139.56	178.35	18.57	17.19
		5ppt	136.00	140.79	194.89	16.87	7.53
		10ppt	136.00	148.52	186.91	10.62	12.11

Appendix B : Average feed amount per day for FCR calculations

Total feed amount (Kg) delivered to each tank per month. First day of acclimation was January 25, 2017; 60th day post-acclimation was March 9, 2017; 120th day post-acclimation was May 19, 2017.

			Average feed amount (kg/day)					
Species	Photoperiod	Salinity	Jan. 24, 2017	Feb. 2017	Mar. 9, 2017	Mar. 31, 2017	Apr. 2017	May 19, 2017
S. salar	24:0 light	2.5ppt	0.49	1.77	0.38	2.56	6.46	1.17
		5ppt	0.79	3.21	0.68	4.17	7.17	1.70
		10ppt	0.83	3.43	0.71	5.02	8.81	2.11
		30ppt	1.22	4.77	0.94	6.34	11.90	2.94
	12:12 light	2.5ppt	0.77	2.55	0.47	2.08	2.86	0.66
		5ppt	0.72	2.87	0.51	2.04	3.78	0.94
		10ppt	0.79	3.14	0.55	2.67	4.64	1.13
O. kisutch	24:0 light	2.5ppt	3.96	18.41	4.02	24.76	43.75	10.16
		5ppt	3.94	18.29	4.03	25.97	46.88	11.38
		10ppt	4.27	20.78	4.55	30.11	53.82	12.53
		30ppt	3.73	19.76	4.32	26.30	46.65	10.67
	12:12 light	2.5ppt	4.00	19.10	4.25	26.88	46.95	10.81
		5ppt	3.96	18.90	4.22	27.00	48.58	11.71
		10ppt	4.07	19.70	4.40	28.16	50.23	11.83

Appendix C : Water velocity of Loligo 185L swim tunnel



Relationship between motor frequency (Hz) and water velocity in a Loligo 185L swim tunnel. Filled circles represent the velocity measured at the mid-point of the chamber and open circles represent the velocity measured at the floor of the swim chamber.