The effect of salinity and photoperiod on thermal tolerance and growth of Atlantic and coho salmon reared from smolt to adult in recirculating aquaculture systems.

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The effect of salinity and photoperiod on thermal tolerance and growth of Atlantic and coho salmon reared from smolt to adult in recirculating aquaculture systems

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Abstract

Land-based, closed containment salmon aquaculture involves rearing salmon from smolt to adult in recirculating aquaculture systems (RAS). Unlike in open-net pen aquaculture, rearing conditions can be specified in RAS in order to decrease physiological stress. The environmental conditions that yield optimal growth and physiological stress tolerance in salmon are, however, unknown. To address this knowledge gap, we reared Atlantic (*Salmo salar*) and coho (*Oncorhynchus kisutch*) salmon in 7 separate RAS for 400 days post-smoltification under 2 photoperiods (12 or 24 hours of light) and 4 salinities (2.5, 5, 10 or 30 ppt) and assessed the effects of these conditions on growth and thermal tolerance. We found that salinity and photoperiod had significant effects on growth of Atlantic and coho salmon, but optimal conditions were not determined. Secondly, we found Atlantic salmon generally grew best under 24 hours of light until day 400 when the trend was lost and that coho salmon grew best under 12 hours of light in the freshwater (2.5 ppt) treatment. Finally, we found higher levels of maturation in Atlantic salmon reared under 24 hours of light, whereas the 12:12 photoperiod triggered greater levels of early maturation in coho. In order to evaluate the effects of photoperiod and salinity on the physiological stress tolerance we used critical thermal maxima or $CT_{\text{max}}$ tests as a performance proxy. We found that over the first 120 days post-smoltification, rearing coho under a 24 hour photoperiod resulted in a ~2°C lower $CT_{\text{max}}$ than in coho reared under a 12:12 photoperiod. This photoperiod effect did not persist at 200 and 400 days, which was coincident with an overall decrease in $CT_{\text{max}}$ in coho salmon relative to 120 days. Finally, Atlantic salmon had a higher $CT_{\text{max}}$ (~28°C) compared to coho (~26°C) at 400 days post-smoltification. Overall, these findings are important for the future implications of RAS and for the aquaculture industry to help identify physiologically sensitive time stages.
**Lay Summary**

I examined the effect of long-term exposure of salinities from almost freshwater to seawater and hours of light per day on the growth and ability for Atlantic and coho salmon. Furthermore, I investigated how coho salmon tolerated a high temperature challenge. I found that Atlantic salmon grew consistently across all salinities but showed the best growth under 24 hours of light per day, while coho salmon grew best in 2.5 ppt and 12 hours of light per day. It was also found that coho salmon had a higher thermal tolerance when exposed to 12 hours of light per day until day 200 of our trial when the trend disappeared.
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 Richards and Bob Shadwick. Experimental protocols involving use of animals in research were conducted according to The University of British Columbia’s Animal Care Committee protocols (#A13-0016 and #A17-0011).

Chapter 2 is based on a collaborative research project with Dr. Kevin T. Stiller, Yuanchang Fang and Victor Chan. 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 analysis.

Chapter 3 is a sub-project of the collaborative research project in Chapter 2. I was responsible for experimental design, implementation and data collection and received help from Dr. Christian Damsgaard on data analysis. This chapter has been published, with the full citation as follows: Hines, S.W., Fang, Y., Chan, V.K.S., Stiller, K.T., Brauner, C.J. and Richards, J.G. (2019). The effect of salinity and photoperiod on thermal tolerance of Atlantic and coho salmon reared from smolt to adult in recirculating aquaculture systems. Comparative Biochemistry and Physiology, Part A 230:1-6.
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$CT_{\text{max}}$ critical thermal maxima
FW freshwater
InSEAS Initiative for the Study of the Environment and its Aquatic Systems
Recirculating Aquaculture System
L:D hours of light: hours of dark
NKA $\text{Na}^+, \text{K}^+\text{-ATPase}$
RAS recirculating aquaculture system
SW seawater
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Finally, I want to thank my parents, John and Susan Hines who always made me mad by asking when I will be done my thesis and pushing me to be something better than I used to be. To my beautiful wife, Orysia Hines, you are my rock, without you none of this would have been possible.
Dedication

My thesis is dedicated to my grandparents Joseph Harold Chandler and Freddie Garris Chandler,
I know this would have made you proud and completely shocked that I turned myself around!
Chapter 1: Introduction

1.1 Salmon Aquaculture

In 1960, people consumed an average of 9.9 kilograms of fish annually. That number has now doubled to 20.1 kilograms (Cressey 2016). With the current global population estimated to be 7.5 billion people, that equates to 150,000,000 metric tonnes of fish consumed per year. The wild populations of fish are dwindling globally (Jachowski et al. 2016) and are unable to keep up with the global demand of seafood. To keep pace with the global demand for seafood, aquaculture is now the largest growing farming practice in the food production sector and can potentially alleviate the heavy fishing pressure experienced by wild populations of fish, although this point is often debated. Current estimates state that aquaculture accounts for half of the fish consumed in the world. (FAO 2019).

Salmon have been a focus of aquaculture since the 1970’s (Asche and Bjørndal 2011). Salmon fetch a high market price and are desired by consumers because of their firm flesh, subtle taste and their high levels of healthy omega fatty acids and protein. Currently the most common form of salmon aquaculture occurs in open sea net-pens.

1.2 Open Sea Net-Pen Culture

Fertilized salmon eggs are reared and hatched in an indoor freshwater (FW) facility. The parr (juvenile fish) then grow until they complete the parr-smolt transformation. This transformation is complex and well-studied that includes several morphological and physiological changes (Hoar 1988). The most notable change in this transformation is the development of seawater tolerance (McCormick et al. 2013). Atlantic salmon typically smolt
between 35-50 g. (Handeland and Stefansson 2001) and coho salmon smolt around 8-15 g. (Ebersole et al. 2006). The smolts are then transferred to open sea net-pens.

An open sea net-pen system is comprised of several submerged structures surrounded by mesh. This type of aquaculture is known as an open system aquaculture because of the lack of barrier between the environment and the rearing area. The site selection for a net-pen is crucial due to its intimate association with the environment. Sites with deep water and high current movement are selected to remove waste from the net pens as well as replenish the oxygen in the water (Cottee and Petersan 2009). Open sea aquaculture poses 3 major environmental risks, which are 1. wild/farmed fish interactions, 2. eutrophication of benthic communities, and 2. carbon footprint caused by large transportation costs.

One of the main negative interactions between wild and farmed fish is through putative disease transmission (Johansen et al. 2011). Due to the high densities of salmon in ocean net pens, disease outbreaks and sea lice congregation can be problematic for the industry. In Norway, which is the world’s largest producer of salmon grown in aquaculture, most of the disease outbreaks were caused by viruses. A list of the main diseases affecting salmon are; infectious pancreatic necrosis (IPN), infectious salmon anemia virus (ISAV), heart/skeletal muscle inflammation (HSMI) and cardiomyopathy syndrome (CS). Bacterial infections are also of concern, but not on the same scale of viral outbreaks (Johansen et al. 2011).

Parasites, particularly the salmon louse (sea lice; Lepeophtheirus salmonis), are also a concern for aquaculture. Very few salmon lice outbreaks in wild salmon have been reported before the introduction of open sea net-pen aquaculture (Johansen et al. 2011). Sea lice infestations have been estimated to cost farms approximately 9% of their revenues and the Norwegian farming industry $436 million dollars per year (Aolofia et al. 2017). With the farmed
fish being in such close proximity to each other, infection is inevitable. Sea lice become a problem for wild fish when their natural migratory routes take them through an active aquaculture site where the sea lice can be transferred from one host to the next quite easily (Johansen et al. 2011). When a sea louse is attached to a fish host, they feed on scales and mucus of the fish, which is the first line of defense for a fish. Infected fish undergo osmotic stress and become emaciated and not fit for sale to the market (Krkošek et al. 2005).

With open sea-net pens, there is the potential for the net to become compromised and farmed fish can escape. Genetically, farmed fish are different than wild salmon (Weir and Grant 2005). This is because fish farmers have been selectively breeding salmon for almost 50 years for increased growth, decreased maturation, improved flesh quality and many other traits. Escaped farmed fish have the potential to mate with wild fish, which would dilute the genetic diversity of wild fish. To minimize wild/farm fish interactions via breeding, the industry has turned to sterilizing farmed salmon by inducing triploidy (Le Page, 2018). The resulting extra set of chromosomes prevents the fish from producing viable gametes. While some farmed salmon will escape, if they are infertile, they will not be able to spawn with wild fish. Fertile farmed fish could spawn with wild fish and reduce population genetic diversity, overall fitness and over time, negatively affect wild salmon populations (Liu et al. 2013).

Net pens can also be associated with local environmental degradation. To try to mitigate the effect of eutrophication of surface and benthic communities caused by intensive feeding and waste settling at ocean floor, an effective net-pen site must be selected. An effective net-pen site is determined by adequate current flow to wash away excess feed and waste from the site. If there is not enough current around the site, the excess feed and waste settle below the site and decompose on the ocean floor. This decomposition process decreases the oxygen levels and can
potentially negatively affect benthic communities below the site. Furthermore, with intense commercial scale feeding, algal growth is promoted around the surface, which can be harmful (depending on the species) and may reduce water oxygen levels leading to environmental hypoxia.

The final concern with growing fish in remote net pens is the cost and environmental footprint of fish transport to market. Once the salmon reach market size, a harvest barge sails to the site for harvest. The freshly harvested fish are put on ice and immediately transported back to land. The process of harvesting and transport can be lengthy, so the market does not always receive the freshest fish. Currently, the majority of salmon sold in North America is flown in daily from Norway (FAO 2019). The cost and environmental footprint of transportation of open sea net-pen aquaculture can potentially make land-based recirculating aquaculture systems (RAS) a viable aquaculture alternative.

1.3 Recirculating Aquaculture Systems (RAS)

The negatives of open sea net-pen aquaculture have been mitigated by moving aquaculture to land-based, closed-containment systems, or RAS. The only major hinderance from RAS becoming more main stream in terms of fish culture is the large capital investment needed to start-up a RAS production facility and higher operating costs. However, there are several benefits of moving aquaculture away from open sea-net pens to land based closed containment recirculating systems or RAS. First, the water in a RAS system is recirculating and 99% of the water is recycled through the system (Tal et al. 2009). Furthermore, that 1% of water that is not recycled is treated before release to minimize eutrophication and impact on the environment (Lepine et al. 2016).
Since RAS does not require replenishment of water constantly, it is can be cost effective and environmentally friendly. The nitrogenous waste products created by the fish go through a series of steps to allow the water to be used continuously. There are three key pieces to removal of solids from the water; drum filter, swirl separator and a protein skimmer. All three components are crucial for removing solids before the water reaches the biofilter. A biofilter contains a colony of ammonia oxidizing bacterium and nitrite oxidizing bacterium that converts the toxic compounds ammonia and nitrite to the relatively harmless nitrate (Zhu et al. 2016).

The ability to grow fish in land-based facilities also decreases the environmental footprint of the operation due to decreased transportation costs. RAS facilities can be placed near major markets. This allows for fresh fish to be delivered short distances by truck to market which contrasts with the current model of a majority of salmon consumed in North America being flown in daily from Norway (FAO 2019).

Growing fish in a closed containment system allows complete control of environmental parameters (temperature, dissolved oxygen, pH, salinity, photoperiod and several more). For example, heaters and chillers are used to maintain or alter the water temperature, whereas fish in the ocean can experience potentially dangerous temperatures or temperature fluctuations. This control of the rearing environment can provide optimal conditions for growth. This has been seen in the gilthead seabream (Sparus aurata) aquaculture industry where they saw a 99% survival rate and feed conversion ratios 16% lower than the sea-pen grown counterparts (Tal et al. 2009). Currently, the optimal conditions for salmonid growth in RAS are not known.
1.4 Photoperiod

In aquaculture, it is common practice to rear fish under 24 hours of continuous light. This is done for three major reasons; to maximize the amount of time the fish can feed, induce smoltification earlier (Handeland and Stefansson 2001) and delay early maturation (Neil et al. 1993).

Salmon are classified as diurnal (during the day), visual feeders (Hoar 1942). The only exception to this rule is when temperature drops below 10°C and salmon become more nocturnal foragers (Björnsson et al. 1994). In our study and in commercial settings, the water temperature is above 10°C to accelerate growth. By rearing the fish under continuous light, they have continuous access to food. This decreases the time it takes for the fish to grow to market size and ultimately increases profitability of the operation.

As stated previously, pre-smolts (or parr) are reared in freshwater RAS until they undergo the parr-smolt transformation. It has been shown that rearing under continuous light accelerates the smoltification process (Handeland et al. 2003). This transformation affects the fish physiologically, behaviorally and morphologically (Morro et al. 2019). The main transformation that occurs during smoltification is the ability to tolerate seawater, which occurs on a physiological level. The transformation can be quantified by measuring increased gill Na+, K+-ATPase (NKA) activity (Imsland et al. 2014). The increase in NKA activity means the fish has a higher capacity for ion secretion in seawater (Morro et al. 2019). Behaviorally, the parr change from being food aggressive, benthic organisms to more passive and schooling after smoltification occurs (Morro et al. 2019). Morphologically, the dark, round parr change to a more chrome colour with a more stream-lined hydrodynamic shape (Riley et al. 2014).
Finally, maturation of salmon is triggered by changes in day light hours associated with season (Björnsson et al. 1994). Commercial salmon farmers use photoperiod to delay maturation and prevent precocious or early maturation of salmon (McClure et al. 2007). Early maturation results in decreased flesh quality due to metabolic energy reallocation from somatic tissue growth to gonad growth. Salmon, typically males, can mature as small as 70 g (Davidson et al. 2016). In the wild, males can become precocious because their size doesn’t greatly limit gamete storage and production. In females, body size is directly correlated with egg numbers, and thus bigger fish produce more gametes and increase that progeny’s chance of survival. Thus, there is less selection for early maturation in females relative to males. It is quite common to use of all female strains of salmon in aquaculture to reduce early maturation (Allyon et al. 2019), but hormone accumulation that potentially occurs in RAS can also accelerate that process (Good et al. 2017).

1.5 Salinity

The anadromous life cycle of Atlantic and coho salmon make them excellent osmoregulators and both species can tolerate a broad range of salinities (Maryoung et al. 2015). Salmon spawn and embryos hatch in FW where they feed until they are ready to undergo the parr-smolt transformation described in previous subchapter. The smolts then out-migrate to SW where they spend the next 1-4 years rapidly growing to maturity (McCormick and Regish 2018). The ability to tolerate 0 ppt to full-strength SW (~33 ppt) requires very different coping strategies.

In FW, ions are lost into the environment and water is gained in the fish from the environment via diffusion. Thus, the FW salmonids drink little water, actively take up ions
across the gills and produce copious amounts of dilute urine to conserve ions. In SW, water is lost to the environment and ions are gained in the fish via diffusion and must be excreted by the gills. Thus, the SW salmonid drinks copious amounts of water and produces small amounts of concentrated urine (McCormick and Regish 2018). The uptake of ions in FW and excretion of ions in SW requires active transport which is metabolically costly. There is no agreed upon value for the cost of osmoregulation, but it is estimated to be 5-50% of routine metabolic rate (Boeuf and Payan, 2000).

We hypothesize Atlantic and coho salmon will display the highest growth at 10 ppt. The salinity of the internal fluids of salmon is approximately 10 ppt. Therefore, the costs of osmoregulation could potentially be saved and redirected towards somatic growth. Previous studies on Atlantic salmon have shown that isosmotic (10 ppt) water improves growth (day 59, Emerman 2016, day 400, Fang 2018 and days 0-120, Chan 2018). Previous studies on coho salmon have shown increased growth rates in isosmotic conditions (days 59-156, Emerman 2016 and days 0-200, Fang 2018). There have been few experiments on the effect of salinity on growth of post-smolt salmonids, but an inverse relationship between salinity (between 0 and 32 ppt) and growth has been shown in adult rainbow trout (*Oncorhynchus mykiss*) where increased salinity results in a decrease in growth rate (McKay and Gjerde 1985).

1.6 Thermal Tolerance

Apart from measuring growth in response to different photoperiod and salinity regimes, I performed a physiological test to assess the overall performance of a fish and its ability to tolerate stress. As stated previously, the potentially high costs of osmoregulation can depress the
ability of a fish to tolerate stress, for example elevated water temperature if a water chiller failed overnight in a RAS facility.

To illustrate the potential of photoperiod and/or salinity being associated with the animal performance issues, this study utilized critical thermal maximum ($CT_{\text{max}}$) as a performance proxy. If a mechanical failure were to occur in a RAS facility, the water temperature could increase at a similar rate of a $CT_{\text{max}}$ ramp protocol, which would result in stress and potentially increase disease susceptibility or mortality.

$CT_{\text{max}}$ provides a relative measure of the maximum temperature at which biological processes (locomotion, escape response from conditions that will promptly lead to death, ventilation, etc.) will function properly, and above this, individual responses become disorganized (Wang et al. 2013, Zhang and Kieffer 2014); it remains the standard for determining the thermal tolerance of an organism because the behavioural response is universal throughout most organisms (Lutterschmidt and Hutchinson 1997).

A $CT_{\text{max}}$ test is performed by slowly increasing the water temperature at a constant rate until the fish loses its righting response or turns over in the water. There is no standard temperature ramp rate, some are as slow as 0.82°C/hour (Gallant et al. 2017) and go as high as 20°C/hour (Sardella et al. 2008). The ramp rate used in this experiment was 6°C/hour as the absolute values (which would be best achieved with a slow ramp rate) of the $CT_{\text{max}}$ trials were not as important as a high throughput and having comparable values between each treatment to determine the overall performance of the fish and if there is an osmorespiratory comprise occurring.
1.7 Objectives of Thesis

The objective of my thesis was to determine the optimal salinity and photoperiod for rearing Atlantic and coho salmon grown in RAS. True optimal conditions to maximize somatic growth and profitability/viability of RAS as an alternative to traditional fish culture is not known. In Chapter 2, I examined effects of salinity and photoperiod on the growth and maturation of Atlantic salmon (*Salmo salar*) and coho salmon (*Oncorhynchus kisutch*) by measuring change in mass, length, condition factor and occurrence of maturation. In Chapter 3, I looked at the thermal tolerance of these species by measuring the CT$_{max}$ of fish in each treatment as a performance proxy or stress resistance test.

The combination of the work done in both chapters has not been done before as this study differs from all previous literature because I am looking at the combined effect of salinity and photoperiod on growth and thermal tolerance on fish that have already undergone smoltification and carried out the project over a long time span (400 days). I predict that the isosmotic salinity (10 ppt) will yield the highest growth rates and highest thermal tolerance in both species due to decreased metabolic cost of osmoregulation.

The information obtained from this research aims to provide information to salmon producers how to optimize their growth rates and profit margins in RAS. This work also looks to further understand how salinity and photoperiod effect the amount of stress a fish can tolerate if a mechanical malfunction should occur.
Chapter 2: Effect of long-term photoperiod and salinity exposure on growth of Atlantic and coho salmon

2.1 Introduction

Aquaculture accounts for 35% (by value) of total fish and seafood production in Canada (Fisheries and Oceans Canada, 2011) of which 75% (by value) is composed of salmon farming, predominantly with Atlantic salmon (Salmo salar; Statistics Canada, 2012). Atlantic salmon have been cultured for decades, but there is increasing interest in developing coho salmon (Oncorhynchus kisutch) for aquaculture (Gaffney et al. 2016). To accommodate the increasing commercial demand for salmon, land-based recirculating aquaculture systems (RAS) are gaining in popularity as an additional means of producing adult salmon. Land-based RAS systems have a couple of issues that are currently limiting their success. RAS is expensive to build and maintain and the accumulation of hormones in the system which can lead to precocious maturation (Good et al. 2017). RAS does have some major advantages over open-net pens for the production of market sized fish, including the elimination of nutrient loading into natural waters and potential reduced transportation costs if RAS are built close to consumer markets (Martins et al. 2010). Another major advantage of RAS is the ability to control the environment in which fish are reared in order to target optimal conditions for fast growth; however, systematic studies to determine the optimal conditions for growth are generally lacking.

Salinity is an important abiotic factor in salmon aquaculture that can impact growth and physiology performance. Salmon are anadromous which means they are born in FW, migrate to SW and return to FW to spawn and as a result, they are considered to be excellent
osmoregulators. The trade-off is that there are metabolic costs of osmoregulation which have been estimated to account for 5 to 50% of routine metabolic rate (Boeuf and Payan, 2000). The salt concentration of a salmon’s blood is approximately 10 ppt. Therefore, these metabolic costs are likely to be highest in salmon reared in either freshwater (FW) or seawater (SW) due to the large osmotic gradients between the internal and external environment. Based upon these metabolic costs, we predict that salmon reared at isosmotic salinity (10 ppt) would result in the highest growth rates due to decreased osmoregulatory costs. The energy cost savings associated with rearing at isosmotic salinities could potentially be diverted to somatic growth yielding faster growth rates.

Salmon have the ability to detect photoperiod via their pineal organ and diurnal melatonin secretion. The pineal organ is a light-sensing organ complete with photoreceptors. Melatonin regulates the growth hormone release as well as pituitary gland activity (Fjelldal et al. 2005). This means, the more light sensed by the pineal organ, the more growth hormone released into the bloodstream of the fish. It is common in aquaculture, especially land-based RAS aquaculture, to grow salmon under 24 hours of continuous light to maximize the time the animals can feed and grow (Oppedal et al. 2007). The use of long daylengths also has the advantage of reducing the incidence of precocious maturation (Björnsson et al. 1994), which is associated with decreases in meat quality (McClure et al. 2007), feed conversion efficiency, and growth in fish (Good et al. 2017), which negatively affects profitability. We expect that fish exposed to 24 hours of continuous light will grow faster, which is in agreement with most previous literature.

The objective of this study is to investigate the combined effects of salinity (2.5, 5, 10 and 30 ppt) and photoperiod (12 and 24 hours of light) on growth, maturation and cataract formation of Atlantic and coho salmon during 200-400 days post-smoltification of continuous
rearing. To assess the effect of salinity and photoperiod on growth, we measured the mass and length at days 200 (at least 100 coho and entire population of Atlantics) and 300 and 400 (of the entire population within each tank). A subset of fish were implanted with PIT tags at the beginning of the study to assess individual growth rate throughout the experiment.

2.2 Methods

Animals used

Atlantic (Cermaq Canada, Campbell River, British Columbia, Canada) and coho salmon (Target Marine Hatcheries Ltd., Sechelt, British Columbia, Canada) were transported to the InSEAS RAS (Initiative for the Study of the Environment and its Aquatic Systems Recirculating Aquaculture System) facility at The University of British Columbia. The smolts (6,000 per species) were approximately one-year old and 100 g. Fish were initially held in flow-through, dechlorinated City of Vancouver tap water for one week at which point they were divided equally among their treatment tanks as described below. All experimental procedures were approved by UBC Animal Care Committee under protocol numbers A13-0016 and A17-0011.

RAS system set-up

The InSEAS RAS facility consists of 7 separate RAS which allows the independent control of salinity and photoperiod. Each RAS consists of two 5 m³ tanks, two 0.7 m³ tanks, and a radial-flow separator, microbead filter, biofilter and UV sterilization system. Within each of the 7 RASs, one 5 m³ tank housed approximately 850 coho salmon smolts and the other 5 m³ tank housed approximately 850 Atlantic salmon smolts. Thus, both species were exposed to identical
water within a given experimental treatment. Each 5 m$^3$ tank was wrapped in heavy black plastic and covered with a black tarpaulin to create an enclosure that eliminated external light sources. Inside each tarpaulin enclosure, a LED lamp (5000K/3000K - 20,000 Lumens - Natural White) was installed which was controlled by a timer (Stanley Timer Max Outdoor Pro mechanical timers; Seattle, Washington, USA) to provide either 12 or 24 hour photoperiods. Each 5 m$^3$ tank was equipped with an automatic drum feeder (Arvo-Tec drum 2000 feeder w/ Arvo-Tec WOLF control system Huutokoski, Finland) and fish from both photoperiod treatments were only fed during the 12 hour light period that corresponded with the 12 hour photoperiod.

Fish were allowed to acclimate to the RAS tanks in recirculating FW (City of Vancouver dechlorinated tap water) for 60 days before salinity and photoperiod were adjusted to the target salinities of 2.5, 5, 10 and 30 ppt (these salinities were used in similar previous experiments) and 12 or 24 hours of light. We chose to use 2.5 ppt as our FW treatment because lower salinities resulted in fungal outbreaks particularly in Atlantic salmon. To adjust salinity, Instant Ocean® Sea Salt, (Blacksburg, VA) was added to one of the 0.7 m$^3$ tanks in each RAS until the entire RAS reached the target salinity. InSEAS has 7 individual RAS, thus for three salinities (2.5, 5, and 10 ppt) and the two photoperiods (12 and 24 hours of light per day) there was a full factorial design where each salinity and photoperiod combination were represented in six RAS. In the remaining RAS, fish were exposed to a salinity of 30 ppt at a 24 hour photoperiod. Within each treatment group, coho salmon were reared in one 5 m$^3$ tank and Atlantic salmon were reared in the second 5 m$^3$ tank and thus were exposed to virtually identical water composition.

Target stocking density was 40 kg/m$^3$ throughout the growth trial. Stocking density was measured and then subsequently adjusted to 40 kg/m$^3$ every ~100 days by culling. Dissolved oxygen (DO), temperature and salinity were measured daily and DO was maintained at or above
75% air saturation and temperature maintained at 12°C. Salt was added to the system daily to maintain the target salinity. Ammonia, nitrite and nitrate were kept below 6, 4 and 160 ppm respectively. All water quality values were within the normal accepted range of salmon growth.

**Experimental Design**

Individual repeated measures of growth were made by implanting Biomark PIT tags (Boise, Idaho, USA) in approximately 120 smolts per tank after the FW RAS acclimation period. Smolts were lightly anesthetized in 0.1 g/L tricaine-methanesulfonate (MS-222) buffered with 0.2 g/L sodium bicarbonate. PIT tags were then implanted into the peritoneal cavity with Biomark implant guns.

At day 200, 300 and 400 post-smoltification, fork length and body mass was measured. Fork length was measured with a standard meter stick and body mass was recorded with a Kilotech benchtop scale (Lachine, Quebec, Canada). Both measurements were recorded in the Biotech Tag Manager Software.

Throughout the experiment, fish were checked several times a day. Any dead fish were removed and scanned for PIT tags. If the deceased fish was PIT tagged, a necropsy was conducted to remove tags. Fish were then weighed and added into the records to be used later for growth/biomass calculations.

**Growth measurements at day 200, 300 and 400 post-smoltification**

Due to high mortality observed during the FW acclimation period of Atlantic salmon, growth, maturation and cataracts of all Atlantic salmon and at least 100 coho salmon at day 200, including at least 30 PIT-tagged individuals, from each RAS treatment were measured. At 300 and 400 days post-smoltification, all Atlantic salmon and all coho salmon were sampled.
Prior to sampling, food was withheld for two days to ensure the salmon were in a post-absorptive state. Sampling 7 RAS (2 tanks per RAS) required 1 week to complete. The reported target day (day 200, 300, etc.) was the 3rd or 4th day of sampling, or the middle of the sampling week. Animals were anaesthetized following capture and transferred into 100 L buckets containing 0.1g/L tricaine-methane Sulfonate (MS-222) buffered with 0.2g/L of sodium bicarbonate mixed with water from the respective RAS system. Once the animals were lightly anaesthetized (as defined by the loss of righting response), individual animals were scanned for PIT tags using a PIT tag reader. The PIT tag reader would report any tagged fish into the Biomark Tag Manager Software along with their respective PIT tag HEX ID. The length, mass, presence and absence of maturation and cataracts for each individual fish sampled were recorded on a spreadsheet.

Fork length was measured with a standard meter stick and body mass was measured using a Kilotech benchtop scale (Lachine, Quebec, Canada). Both measurements were recorded in the Biotech Tag Manager Software. Cataracts were assessed under a spotlight by detecting the absence or presence of a semi-opaque colour on the animal’s optical lens. A fish with a positive determination for cataracts was then grouped as minor (not covering the entire lens) or major (covering the entire lens). 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. Measured fish were placed into a recovery bath at their respective salinity before being transferred back to their original tank.
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, 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, respectively that each animal was measured.

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:

\[ TGC = \frac{Mass_2 (g)^{1/3} - Mass_1 (g)^{1/3}}{Temperature \ (°C) \times (Time_2 - Time_1)} \times 1000 \]

where Temperature was the average temperature of the RAS between the first and second sampling time point, measured in 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.

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:

\[ CF = \left( \frac{Mass (g)}{Length (cm)^3} \right) \times 100 \]
Statistical analysis

The unbalanced experimental design caused by the 30 ppt/24 hour tank forced us to use a 3-way ANOVA using type 2 and 3 sum of squares to test the overall effects of photoperiod and salinity on growth and all of its interactions over the entire experiment. We realize the design of the InSEAS facility (7 separate RAS systems) creates pseudo replication, but true replication was not possible with the number of variables we investigated. Within each time point, a 2-way ANOVA was used to test for the effects of photoperiod and salinity on growth, and a 2-way ANOVA was used to compare condition factors within time periods and pairwise comparisons were calculated by Tukey’s Honest Significance test. Significance level was set 0.05.

Statistics were not conducted on PIT tagged fish due to low (less than 10) recapture rates throughout the experiment. Statistics were also not conducted on SGR, TCG, maturation or cataracts because they are tank population values and consequently there is no error associated with those values.

2.3 Results

Overall, for Atlantic salmon, the 3-way ANOVA revealed a significant effect of time, photoperiod and salinity (all p<<0.0001) on body mass with significant all-pairwise interaction terms (Table 2.2). Between each timepoint, the 2-way ANOVA revealed the significant effect (p < 0.05) of both salinity and photoperiod on growth can also be seen in Fig. 2.1 at every measurement interval. At day 200 and 300, fish exposed to the 24 hour photoperiod were significantly (p < 0.05) larger in 2.5, 10 and 30 ppt. At day 400, the magnitude of the response was reduced but there was still a statically significant effect of salinity and photoperiod (Table
2.2), however, this was largely due to a reduction in growth in the 5 ppt/24 hour treatment. Fig. 2.1 shows no significant effect of photoperiod or salinity on growth in all other treatments (2.5, 10 and 30 ppt) treatments at that time. The 5 ppt/24 hour, showed decreased growth for 200, 300 and 400 days post-smoltification (Fig. 2.1). Average length (cm), mass (g) and condition factor (K) can be seen in Table 2.3.

On days 200 and 300, Atlantic salmon exposed to 24 hour photoperiod had a higher standard growth rate (SGR) and thermal growth coefficient (TCG) than the fish exposed to 12 hour photoperiod. On day 200 however, the 5 ppt/24 hour fish had a negative SGR and TCG, and although the precise cause is unknown, a *Saprolegnia* infection was present in this treatment which resulted in high mortality and possibly confounded the results (Table 2.4).

The 24 hour photoperiod seemed to have a great effect on maturation of Atlantic salmon. At day 200, approximately 13% of fish in all salinities exposed to the 24 h photoperiod exhibited signs of sexual maturation, compared with only 1.4% in 12 hour photoperiod fish. The difference between photoperiods at day 300 was even more pronounced, where approximately 28% of the 24 hour photoperiod fish matured compared to less than 1% in the 12 hour photoperiod. However, by day 400, this affect disappeared and both photoperiods exhibited similar levels of maturation (43.5 and 37% on average for 24 and 12 hour photoperiod respectively, Table 2.5).

There were no large effects of salinity or photoperiod on visible cataract formation in Atlantic salmon, however, at day 400, 38.3% of the population in the 30 ppt/24 hour treatment had cataracts which was higher than the rest of the treatments (Table 2.6).

Overall, for coho salmon, the 3-way ANOVA showed significance (<<0.0001) for all factors and interactions (Table 2.8). Between each time point, the 2-way ANOVA revealed the significant effect (p < 0.05) of both salinity and photoperiod on growth can also be seen on Fig.
2.2 at every measurement interval. At day 300, the 2.5 ppt/12 hour treatment resulted in the largest body mass while the 30 ppt/24 hour treatment resulted in the lowest body mass. On day 400, the 0 ppt/12 hour group continued to show the greatest growth and the 5 ppt./12 hour treatment resulted in significantly greater body mass than the 5 ppt/24 treatment (Fig. 2.2). Average length (cm), mass (g) and condition factor (K) are reported in Table 2.9.

No visible trends for the effects of photoperiod or salinity were observed in the SGR or TCG of coho salmon (Table 2.10) or in early maturation at days 200 and 300. At day 400, there was an effect of salinity on maturation. The 24 hour photoperiod (salinities combined) resulted in about 3% early maturation compared to approximately 50% maturation in the 12 hour photoperiod (Table 2.11). No visible trends on the effect of photoperiod or salinity were observed for visible severe cataracts (Table 2.12). The PIT tag data analysis was excluded from this chapter due to low recapture and low statistical power, which was usually less than 4 fish per tank re-captured (Table 2.7 for Atlantic salmon and Table 2.13 for coho salmon).

2.4 Discussion

The objective of this study was to determine the combined effects of salinity (2.5, 5, 10 and 30 ppt) and photoperiod (12 and 24 hours) on growth, early maturation and cataracts in post-smolt Atlantic and coho salmon during long-term rearing from 200 to 400 days post-smoltification. The results of this study yielded three important and novel findings. First, we found salinity had a significant effect on growth of Atlantic and coho salmon. Secondly, we found Atlantic salmon grew best under 24 hours of light (except for the 5 ppt treatment that was infected with Saprolegnia) up until day 400. In contrast, coho salmon grew best under 2.5 ppt/
12 hours of light treatment. Finally, we found increased maturation in Atlantic salmon reared under 24 hours of light, whereas the 12 hour photoperiod triggered maturation in coho.

We predicted that both Atlantic and coho salmon would grow best in isosmotic conditions (~10 ppt, Ern and Esbaugh 2018), whereby they could potentially redirect energetic savings from the reduced costs of osmoregulation towards somatic growth. The potential energetic savings range from modest to large given that an estimated 5-50% of the standard metabolic rate is dedicated to osmoregulation (Boef and Payan 2001). The metabolic cost for osmoregulation did not prove to have a significant effect on growth even though there was a significant effect of salinity on growth of Atlantic salmon from days 200 to 400 (Fig. 2.1, Table 2.2). The predicted optimal salinity of 10 ppt was not correct as an optimal salinity was not determined. Our hypothesis that Atlantic salmon reared at 10 ppt would show the best growth due to decreased osmoregulatory costs was not supported by the data. Previous InSEAS experiments have shown improved growth in isosmotic conditions (day 59 Emerman 2016, day 400 Fang 2018). In the earlier time periods of this study not covered by this thesis, Chan showed that at 120 days post-smoltification, Atlantic salmon exposed to an isosmotic environment displayed the best growth (2018). It should be noted that the 5 ppt regardless of photoperiod showed slightly lower growth compared to the other salinities. The 5 ppt/24 hour treatment displayed slower growth rates which could be partially due to the confounding effect of the *Saprolegnia* infection, but no explanation for the 5 ppt/12 hour treatment is available at this time.

There was a significant effect of salinity on growth of coho salmon from days 200 to 400 (Fig. 2.2, Table 2.8). At day 300 to 400, coho salmon exhibited the best growth under 2.5 ppt (Fig. 2.2) which was in contrast with our original prediction. Emerman observed improved growth in 10 ppt at days 59 and 156 (2016), and Fang (2018) in the follow-up experiment also
saw improved growth at 10 ppt at day 257, but this effect was lost thereafter. In earlier time periods of this study, Chan (2018) saw no effect of salinity on growth of coho salmon aged 0-120 days. There have been few experiments on the effect of salinity on growth of post-smolt salmonids outside of the InSEAS projects. Tables 2.2 and 2.8 both indicate that salinity effects growth significantly, but the effect of photoperiod is greater.

Previous studies examining the effects of photoperiod on growth of Atlantic salmon suggest that a 24 hour photoperiod is best for somatic growth (Skilbrei et al. 1997, Handeland et al. 2013 and Døskeland et al. 2016). The findings of these short-term studies (with the longest occurring over 60 days) are consistent with our results for Atlantic salmon (in 2.5, 10 and 30 ppt water) where the 24 hour photoperiod elevated growth until day 400, beyond which the effect is no longer evident on Fig. 2.1. However, the statistical analysis in Table 2.2 shows a significant effect of photoperiod and salinity as well as their interaction that cannot be seen visibly on Fig 2.1. This may be caused by the confounding of the data by the 5 ppt/24 hour treatment. The opposite trend is seen in the 5 ppt treatments, where the fish exposed to 12 hours of light per day grew better than those exposed to 24 hours of light. This is most likely attributed to a Saprolegnia infection that occurred in the 24 hour, 5 ppt tank. This treatment experienced high mortality and did not receive food during the course of the infection and supplemental treatment. Therefore, the results from the 5 ppt treatment should be ignored as we do not believe the results to be a valid representation of the experiment.

Improved somatic growth under 24 hours of light can be explained by the pineal organ releasing higher amounts of growth hormone while suppressing pituitary gland activity (Johansen et al. 2011). It is common practice in the aquaculture industry to rear Atlantic salmon under 24 hours of light per day (Good and Davidson 2016) to maximize food availability,
decrease rearing time, avoid nightly oxygen drops by photosynthetic microorganisms consuming oxygen in the dark. By selectively breeding fish reared under these conditions, this may result in better performance under a 24 hour L:D photoperiod.

To further emphasize the effect of photoperiod on growth of Atlantic salmon, the condition factor (K) was calculated and is used as an indicator of general health of the fish that may be affected by different environmental conditions (Sutton et al. 2000). In this study, K is also highest in the 24 hour photoperiod consistent with increased somatic growth in that photoperiod. The mean K is 1.20, 1.31 and 1.39 (mean of all salinities in 24 hour photoperiod) compared to 1.17, 1.19 and 1.26 (mean of all salinities in 12 hour photoperiod) for days 200-400 respectively (Table 2.3).

The highest incidence of cataracts in Atlantic salmon occurred at 400 days in the 30 ppt tank at 14.2% (Table 2.6). A similar study has shown that fluctuation in salinity correlates to increased cataract development (Bjerkås and Sveier 2004). The 30 ppt treatment experienced the greatest amount of daily salinity fluctuation due to the high volume of salt that was added daily to maintain the target salinity. However, the increased incidence of cataracts was only seen at 400 days post-smoltification. Unlike Bjerkås and Sveier, we did not find a negative correlation between SGR and cataracts (2004).

Other salmonids such as masu salmon (Zhang et al. 2013) and rainbow trout (Taylor et al. 2006) have also been shown to exhibit enhanced growth under 24 hours light, so one would expect coho salmon to follow a similar trend. However, all previous literature on the effect of photoperiod on growth of salmonids disagrees with our findings that there was no effect of photoperiod until day 300 when the 0 ppt/12 hour treatment out-grew the others (Fig. 2.2). The same fish from my experiment showed no effects of photoperiod on growth from 0-120 days
post-smoltification (Chan 2018). As stated previously, the effect of photoperiod on the growth of
coho salmon was not seen until day 300 where the average mass (g) for coho in the 2.5 ppt/12
hour treatment was 1169.7 ± 22.0 compared to 951.1 ± 22.0 in the 24 hour photoperiod. At day
400, the 2.5 ppt/12 hour treatment again showed the most growth with an average mass of
2054.6 ± 52.2 compared to 1673.0 ± 39.2 in the 24 hour photoperiod. The 5 ppt/12 hour group
also showed a significantly higher average mass than the 24 hour treatment with 1727.9 ± 43 vs.
1520.0 ± 39.8 respectively (Table 2.8).

Interestingly, at day 300, the condition factor was lower in the 2.5 ppt/12 hour group
(1.28 ± 0.01) than the 24 hour group (1.31 ± 0.01), even though the 12 hour group showed
significantly higher growth. However, the SGR for the 12 hour treatment was higher (0.579) than
the 24 hour photoperiod (0.483, Table 2.9). At day 400, the same trend seen with Atlantics is
seen in coho salmon, where a higher condition factor correlates with a higher average mass. For
the 2.5 ppt treatment, the condition factors were 1.39 ± 0.01 and 1.31 ± 0.01 for the 12 and 24
hour photoperiod respectively. For the 5 ppt treatment, the condition factors were 1.43 ± 0.01
and 1.29 ± 0.02 for the 12 and 24 hour photoperiod respectively (Table 2.8). The SGR also
followed a similar trend, the 2.5 treatment for 12 hours was 0.563 whereas it was 0.565 for the
24 hour photoperiod and thus very similar. The SGR for the 5 ppt treatment at 400 days post-
smoltification was 0.603 for the 12 hour group and 0.478 for the 24 hour photoperiod (Table
2.9).

Much like Atlantic salmon, the coho salmon in the 30 ppt treatment at day 400 produced
the most visible severe cataracts with 7.0% of the population affected (Table 2.11). Again, this
may have been caused by the larger daily fluctuations in salinity due to challenges associated
with keeping such a high salt concentration. Consistent with the Atlantics salmon, we did not
find a correlation between SGR and cataract presence in coho salmon which has been found previously (Bjerkås and Sveier 2004).

To maximize the amount of time fish reared in RAS can eat and grow, they are commonly reared under 24 hours of light (Oppedal et al. 2007). The added benefit of rearing salmonids under 24 hours of light is that it is often shown to delay the maturation process (Unwin et al. 2005). Producing mature fish in an aquaculture setting reduces profitability (and cuts into the already thin margins of growing fish in RAS) because the meat quality is reduced. The Atlantic salmon strain we used for this experiment contained both males and females. In a culture environment, the problems of precocious maturation are greater in males because they divert energy towards gonadal development instead of somatic growth at a much earlier stage of development than females (Imsland et al. 2014). The result is a small fish (usually under 500 grams) that has changed from silver to gold/brown, possesses decreased meat quality and is full of gametes. All of those traits are clearly undesirable to the consumer of these fish.

While there was no significant effect of salinity on maturation (p>0.05), there was a significant effect of photoperiod. By day 200, approximately 13% of the 24 h photoperiod had sexually matured compared to 1.4% in the 12 hour photoperiod. At day 300, the differences were even larger with 28% of the 24 h photoperiod exhibiting sexual maturity compared to about 1% of the fish in the 12 hour photoperiod. The observed phenomena of the effect of photoperiod on maturation of Atlantic salmon is interesting in that it clearly disagrees with all of the previous literature on this topic (24 hours of light delays maturation). By day 400, however, both photoperiods exhibited similar levels of maturation (43.5 and 37% on average for 24 and 12 hour photoperiod respectively, Table 2.5).
For coho salmon, there was no maturation throughout any of the treatments for days 200 and 300 (except 1.7% of the population in the 10 ppt/24 hour treatment, Table 2.10). At day 400 however, the 24 hour photoperiod resulted in 3% maturation whereas the 12 hour photoperiod resulted in 50% of the population maturing. The coho used for the trial were an all female strain, which are expected to mature later than the males.

To further validate the findings in this study, a previous study by Emerman in the InSEAS systems showed no tank effects or differences in growth/performance between any of the RAS where coho salmon were reared at a consistent temperature and salinity (2016), thus, variation due to tank effects is likely minor. Hormone accumulation is an issue in RAS, where testosterone or estrogen can build up in RAS and promote early maturation (Good et al. 2017), but all systems were cleaned regularly, so that is unlikely as well. A study done on rainbow trout grown in RAS concluded that low pH (less than 7) could potentially have an effect on increased levels of cortisol and testosterone (Mota et al. 2017). This is also an unlikely explanation because all of the pH levels were relatively similar, and the only RAS to have a pH greater than 7 was the 30 ppt/24 hour system (Table 2.1). The trends seen could be a strain-specific trait or just an anomaly, but regardless, should be looked into in future research.

In summary, we found that salinity had a significant effect on growth of Atlantic and coho salmon. An optimal salinity for growth of Atlantic salmon could not be determined, but 2.5 ppt provided the optimal salinity for growth of coho salmon. Secondly, we found that Atlantic salmon grew the best under 24 hours of light, but the trend was lost past day 300. However, coho salmon grew better in the 12 hour photoperiod. Finally, at days 200 and 300, Atlantic salmon matured in the 24 hour photoperiod (contradictory to all literature) until day 400 where the trend was lost. Photoperiod had a significant effect on early maturation of coho salmon at day 400.
where the 12 hour photoperiod significantly increased early maturation relative to the 24 hour photoperiod, consistent with the literature (Björnsson et al. 1994, Oppedal et al. 2007).

Chapter 2 Figures and Tables

![Graph](image)

**Figure 2.1** Average body mass of approximately 30-123 Atlantic salmon (200, 300 and 400 days post-smoltification) reared at salinities of 2.5, 5, 10 and 30 ppt and exposed to 12 or 24 hour photoperiods. All points are means ± SEM. Letters indicate statistical differences in salinity and photoperiod within a time period. 2-way ANOVA Tukey’s post-hoc test (0.05).
Figure 2.2 Average body mass of approximately 110-459 coho salmon (200, 300 and 400 days post-smoltification) reared at salinities of 2.5, 5, 10 and 30 ppt and exposed to 12 or 24 hour photoperiods. All points are means ± SEM. Letters indicate statistical differences in salinity and photoperiod. 2-way ANOVA Tukey’s post-hoc test (0.05).
Table 2.1: Mean salinity, pH, oxygen, and temperature of RAS used in experiment from day 0 to 400 post-smoltification. Mean values ± SEM (n=400)

<table>
<thead>
<tr>
<th>Photoperiod</th>
<th>Salinity (ppt)</th>
<th>pH</th>
<th>Oxygen (ppm)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Target Actual</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hour light</td>
<td>2.5 2.17 ± 0.0</td>
<td>6.57 ± 0.0</td>
<td>11.2 ± 0.2</td>
<td>11.84 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>5   4.36 ± 0.1</td>
<td>6.23 ± 0.0</td>
<td>9.8 ± 0.1</td>
<td>12.29 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>10  9.02 ± 0.1</td>
<td>6.42 ± 0.0</td>
<td>9.9 ± 0.1</td>
<td>12.39 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>30  29.0 ± 0.0</td>
<td>7.15 ± 0.0</td>
<td>8.6 ± 0.1</td>
<td>12.35 ± 0.0</td>
</tr>
<tr>
<td>12 hour light</td>
<td>2.5 2.36 ± 0.0</td>
<td>6.47 ± 0.0</td>
<td>10.7 ± 0.1</td>
<td>12.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>5   4.52 ± 0.0</td>
<td>6.72 ± 0.0</td>
<td>11.1 ± 0.1</td>
<td>11.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>10  9.09 ± 0.1</td>
<td>6.51 ± 0.0</td>
<td>15 ± 4.0</td>
<td>12.01 ± 0.0</td>
</tr>
</tbody>
</table>

Table 2.2 Summary statistics of 30-123 Atlantic salmon (200, 300 and 400 days post-smoltification) reared at salinities of 2.5, 5, 10 and 30 ppt and exposed to 12 or 24 hours of light. A 2-way ANOVA was used within each time period (top) and a 3-way ANOVA was used for the overall statistics (bottom).

<table>
<thead>
<tr>
<th>Day</th>
<th>Salinity</th>
<th>Photoperiod</th>
<th>Photo x Salinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>1.2 x 10^-6</td>
<td>2.0 x 10^-16</td>
<td>2.0 x 10^-16</td>
</tr>
<tr>
<td>300</td>
<td>7.6 x 10^-16</td>
<td>2.0 x 10^-16</td>
<td>2.3 x 10^-8</td>
</tr>
<tr>
<td>400</td>
<td>1.2 x 10^-3</td>
<td>1.1 x 10^-9</td>
<td>1.4 x 10^-3</td>
</tr>
</tbody>
</table>

Overall analysis (3-way ANOVA)

<table>
<thead>
<tr>
<th>Day</th>
<th>Salinity x Photoperiod</th>
<th>Salinity x Day</th>
<th>Day x Photoperiod</th>
<th>Salinity x Day x Photoperiod</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;&lt;0.0001</td>
<td>&lt;&lt;0.0001</td>
<td>&lt;&lt;0.0001</td>
<td>&lt;&lt;0.0001</td>
<td>&lt;&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 2.3 Mean length (cm), mass (g) and condition factor (K) of 30-123 Atlantic salmon (200, 300 and 400 days post-smoltification) reared at salinities of 2.5, 5, 10 and 30 ppt and exposed to 12 or 24 hours of light.

<table>
<thead>
<tr>
<th>Photoperiod</th>
<th>Salinity</th>
<th>Length (cm)</th>
<th>Mass (g)</th>
<th>K</th>
<th>Length (cm)</th>
<th>Mass (g)</th>
<th>K</th>
<th>Length (cm)</th>
<th>Mass (g)</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>2.5</td>
<td>348.4 ± 4.9</td>
<td>529.4 ± 18.8</td>
<td>1.24 ± 0.02</td>
<td>403.4 ± 5.8</td>
<td>885.0 ± 34.4</td>
<td>1.30 ± 0.01</td>
<td>467.0 ± 11.8</td>
<td>1448.7 ± 93.3</td>
<td>1.41 ± 0.07</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>277.3 ± 2.3</td>
<td>213.8 ± 6.8</td>
<td>1.00 ± 0.02</td>
<td>336.2 ± 4.8</td>
<td>489.5 ± 23.1</td>
<td>1.26 ± 0.01</td>
<td>379.8 ± 7.0</td>
<td>805.5 ± 41.4</td>
<td>1.47 ± 0.05</td>
</tr>
<tr>
<td>10</td>
<td>2.5</td>
<td>353.3 ± 2.4</td>
<td>550.9 ± 10.7</td>
<td>1.23 ± 0.01</td>
<td>409.7 ± 4.0</td>
<td>933.0 ± 24.9</td>
<td>1.33 ± 0.01</td>
<td>466.1 ± 6.6</td>
<td>1373.8 ± 55.7</td>
<td>1.32 ± 0.02</td>
</tr>
<tr>
<td>30</td>
<td>2.5</td>
<td>337.8 ± 2.6</td>
<td>517.2 ± 11.6</td>
<td>1.32 ± 0.01</td>
<td>399.1 ± 3.8</td>
<td>885.6 ± 23.9</td>
<td>1.35 ± 0.01</td>
<td>473.4 ± 7.5</td>
<td>1444.1 ± 51.6</td>
<td>1.36 ± 0.04</td>
</tr>
<tr>
<td>12</td>
<td>2.5</td>
<td>330.5 ± 2.4</td>
<td>429.8 ± 9.6</td>
<td>1.18 ± 0.01</td>
<td>392.6 ± 3.6</td>
<td>739.8 ± 23.2</td>
<td>1.21 ± 0.03</td>
<td>460.0 ± 8.5</td>
<td>1293.3 ± 62.8</td>
<td>1.34 ± 0.07</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>319.2 ± 2.8</td>
<td>394.4 ± 11.4</td>
<td>1.19 ± 0.01</td>
<td>366.8 ± 4.3</td>
<td>594.1 ± 22.6</td>
<td>1.18 ± 0.02</td>
<td>450.9 ± 5.4</td>
<td>1086.5 ± 42.3</td>
<td>1.16 ± 0.04</td>
</tr>
<tr>
<td>10</td>
<td>2.5</td>
<td>335.3 ± 3.3</td>
<td>431.9 ± 12.8</td>
<td>1.13 ± 0.02</td>
<td>392.2 ± 10.5</td>
<td>686.7 ± 29.1</td>
<td>1.17 ± 0.01</td>
<td>455.4 ± 6.5</td>
<td>1215.6 ± 48.8</td>
<td>1.27 ± 0.02</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>2.5</td>
<td>0.918</td>
<td>1.854</td>
<td>0.514</td>
<td>1.276</td>
<td>0.493</td>
<td>1.448</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>0.060</td>
<td>-0.098</td>
<td>0.829</td>
<td>1.548</td>
<td>0.498</td>
<td>1.158</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.5</td>
<td>1.163</td>
<td>2.206</td>
<td>0.527</td>
<td>1.270</td>
<td>0.387</td>
<td>1.086</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>2.5</td>
<td>0.887</td>
<td>1.711</td>
<td>0.538</td>
<td>1.276</td>
<td>0.489</td>
<td>1.377</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>10</td>
<td>2.5</td>
<td>0.456</td>
<td>0.885</td>
<td>0.543</td>
<td>1.227</td>
<td>0.559</td>
<td>1.517</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>2.5</td>
<td>0.630</td>
<td>1.222</td>
<td>0.410</td>
<td>0.925</td>
<td>0.604</td>
<td>1.615</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>2.5</td>
<td>0.616</td>
<td>1.193</td>
<td>0.464</td>
<td>1.052</td>
<td>0.571</td>
<td>1.541</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.4 Standard growth rate (SGR) and thermal growth coefficient (TCG) of 30-123 Atlantic salmon (200, 300 and 400 days post-smoltification) reared at salinities of 2.5, 5, 10 and 30 ppt and exposed to 12 or 24 hours of light.
Table 2.5 Maturation (% of population) of 30-123 Atlantic salmon (200, 300 and 400 days post-smoltification) reared at salinities of 2.5, 5, 10 and 30 ppt and exposed to 12 or 24 hours of light.

<table>
<thead>
<tr>
<th>Photoperiod</th>
<th>Salinity</th>
<th>Day 200</th>
<th>Day 300</th>
<th>Day 400</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 2.5</td>
<td>18.2</td>
<td>28.6</td>
<td>31.7</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6.9</td>
<td>24.5</td>
<td>51.6</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>11.4</td>
<td>30.6</td>
<td>29.9</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>15.4</td>
<td>30.0</td>
<td>60.6</td>
<td></td>
</tr>
<tr>
<td>12 2.5</td>
<td>2.6</td>
<td>1.0</td>
<td>56.7</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.5</td>
<td>0.0</td>
<td>31.3</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.0</td>
<td>0.0</td>
<td>22.9</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.6 Visible severe cataracts (% of population) of 30-123 Atlantic salmon (200, 300 and 400 days post-smoltification) reared at salinities of 2.5, 5, 10 and 30 ppt and exposed to 12 or 24 hours of light.

<table>
<thead>
<tr>
<th>Photoperiod</th>
<th>Salinity</th>
<th>Day 200</th>
<th>Day 300</th>
<th>Day 400</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 2.5</td>
<td>10.1</td>
<td>4.9</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.2</td>
<td>3.6</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.6</td>
<td>4.2</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>12.7</td>
<td>4.0</td>
<td>14.8</td>
<td></td>
</tr>
<tr>
<td>12 2.5</td>
<td>9.8</td>
<td>9.7</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.6</td>
<td>10.4</td>
<td>12.1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>15.3</td>
<td>6.4</td>
<td>4.8</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.7 Standard growth rate (SGR in % wet mass per day) of 2-18 PIT-tagged Atlantic salmon between 200 and 400 days post-smoltification reared at salinities of 2.5, 5, 10 and 30 ppt and exposed to 12 hour or 24 hours of light.

<table>
<thead>
<tr>
<th>Day</th>
<th>Photoperiod</th>
<th>Salinity</th>
<th>Mean SGR ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>200-300</td>
<td>24</td>
<td>2.5</td>
<td>0.668 ± 0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0.687 ± 0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.519 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>0.540 ± 0.04</td>
</tr>
<tr>
<td>200-300</td>
<td>12</td>
<td>2.5</td>
<td>0.516 ± 0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0.381 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.444 ± 0.06</td>
</tr>
<tr>
<td>300-400</td>
<td>24</td>
<td>2.5</td>
<td>0.505 ± 0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0.554 ± 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.436 ± 0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>0.389 ± 0.09</td>
</tr>
<tr>
<td>300-400</td>
<td>12</td>
<td>2.5</td>
<td>0.489 ± 0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0.644 ± 0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.559 ± 0.11</td>
</tr>
</tbody>
</table>

Table 2.8 Summary statistics of of 110-459 coho salmon (200, 300 and 400 days post-smoltification) reared at salinities of 2.5, 5, 10 and 30 ppt and exposed to 12 or 24 hours of light. A 2-way ANOVA was used within each time period (top) and a 3-way ANOVA was used for the overall statistics (bottom).

<table>
<thead>
<tr>
<th>Day</th>
<th>Salinity</th>
<th>Photoperiod</th>
<th>Photo x Salinity</th>
<th>Salinity x Day</th>
<th>Salinity x Photoperiod</th>
<th>Day x Photoperiod</th>
<th>Salinity x Day x Photoperiod</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>1.2 x 10^{-6}</td>
<td>2.0 x 10^{-16}</td>
<td>2.0 x 10^{-16}</td>
<td>&lt;&lt;0.0001</td>
<td>&lt;&lt;0.0001</td>
<td>&lt;&lt;0.0001</td>
<td>&lt;&lt;0.0001</td>
</tr>
<tr>
<td>300</td>
<td>7.6 x 10^{-16}</td>
<td>2.0 x 10^{-16}</td>
<td>2.3 x 10^{-8}</td>
<td>&lt;&lt;0.0001</td>
<td>&lt;&lt;0.0001</td>
<td>&lt;&lt;0.0001</td>
<td>&lt;&lt;0.0001</td>
</tr>
<tr>
<td>400</td>
<td>1.2 x 10^{-3}</td>
<td>1.1 x 10^{-9}</td>
<td>1.4 x 10^{-3}</td>
<td>&lt;&lt;0.0001</td>
<td>&lt;&lt;0.0001</td>
<td>&lt;&lt;0.0001</td>
<td>&lt;&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 2.9 Mean length (cm), mass (g) and condition factor (K) of 110-459 coho salmon (200, 300 and 400 days post-smoltification) reared at salinities of 2.5, 5, 10 and 30 ppt and exposed to 12 or 24 hours of light.

<table>
<thead>
<tr>
<th>Photoperiod</th>
<th>Salinity</th>
<th>Length (cm)</th>
<th>Mass (g)</th>
<th>K</th>
<th>Length (cm)</th>
<th>Mass (g)</th>
<th>K</th>
<th>Length (cm)</th>
<th>Mass (g)</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>2.5</td>
<td>347.1 ± 586.5 ± 1.34 ± 5.2</td>
<td>22.2 ± 0.01</td>
<td>421.8 ± 951.1 ± 1.31 ± 1.6</td>
<td>21.0 ± 0.01</td>
<td>496.7 ± 1673.0 ± 1.31 ± 5.0</td>
<td>39.2 ± 0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>338.7 ± 531.1 ± 1.30 ± 7.1</td>
<td>25.3 ± 0.02</td>
<td>415.4 ± 942.2 ± 1.27 ± 2.0</td>
<td>19.5 ± 0.02</td>
<td>488.1 ± 1520.0 ± 1.29 ± 4.9</td>
<td>39.8 ± 0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>340.8 ± 537.3 ± 1.30 ± 6.2</td>
<td>20.1 ± 0.01</td>
<td>408.9 ± 978.8 ± 1.38 ± 2.5</td>
<td>18.2 ± 0.01</td>
<td>483.1 ± 1679.6 ± 1.55 ± 5.1</td>
<td>40.4 ± 0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>329.3 ± 495.8 ± 1.31 ± 7.8</td>
<td>24.3 ± 0.01</td>
<td>383.7 ± 778.2 ± 1.31 ± 3.3</td>
<td>21.3 ± 0.02</td>
<td>464.3 ± 1409.4 ± 1.36 ± 4.4</td>
<td>42.1 ± 0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2.5</td>
<td>365.2 ± 655.9 ± 1.29 ± 5.2</td>
<td>23.3 ± 0.02</td>
<td>446.8 ± 1169.7 ± 1.28 ± 2.8</td>
<td>22.0 ± 0.01</td>
<td>522.1 ± 2054.6 ± 1.39 ± 4.9</td>
<td>52.2 ± 0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>353.1 ± 562.8 ± 1.24 ± 4.3</td>
<td>19.3 ± 0.01</td>
<td>418.2 ± 940.9 ± 1.24 ± 2.7</td>
<td>19.3 ± 0.01</td>
<td>490.4 ± 1727.9 ± 1.43 ± 4.1</td>
<td>43.0 ± 0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>357.3 ± 597.8 ± 1.26 ± 6.0</td>
<td>20.9 ± 0.02</td>
<td>427.5 ± 1016.6 ± 1.28 ± 2.5</td>
<td>21.9 ± 0.01</td>
<td>485.0 ± 1643.6 ± 1.39 ± 4.8</td>
<td>44.4 ± 0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.10 Standard growth rate (SGR) and thermal growth coefficient (TCG) of 110-459 coho salmon (200, 300 and 400 days post-smoltification) reared at salinities of 2.5, 5, 10 and 30 ppt and exposed to 12 or 24 hours of light.

<table>
<thead>
<tr>
<th>Photoperiod</th>
<th>Salinity</th>
<th>Day 120-200</th>
<th>Day 200-300</th>
<th>Day 300-400</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SGR</td>
<td>TGC</td>
<td>SGR</td>
</tr>
<tr>
<td>24</td>
<td>2.5</td>
<td>0.816</td>
<td>1.728</td>
<td>0.483</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.683</td>
<td>1.372</td>
<td>0.573</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.760</td>
<td>1.505</td>
<td>0.600</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.534</td>
<td>1.064</td>
<td>0.451</td>
</tr>
<tr>
<td>12</td>
<td>2.5</td>
<td>0.970</td>
<td>2.029</td>
<td>0.579</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.710</td>
<td>1.536</td>
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</tr>
<tr>
<td></td>
<td>10</td>
<td>0.855</td>
<td>1.788</td>
<td>0.531</td>
</tr>
</tbody>
</table>
Table 2.11 Maturation (% of population) of 110-459 coho salmon (200, 300 and 400 days post-smoltification) reared at salinities of 2.5, 5, 10 and 30 ppt and exposed to 12 or 24 hours of light.

<table>
<thead>
<tr>
<th>Photoperiod</th>
<th>Salinity</th>
<th>Day (200)</th>
<th>Day (300)</th>
<th>Day (400)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>2.5</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.0</td>
<td>1.7</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.0</td>
<td>0.0</td>
<td>5.8</td>
</tr>
<tr>
<td>12</td>
<td>2.5</td>
<td>0.0</td>
<td>0.0</td>
<td>37.0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.0</td>
<td>0.0</td>
<td>65.7</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.0</td>
<td>0.0</td>
<td>42.5</td>
</tr>
</tbody>
</table>

Table 2.12 Visible severe cataracts (% of population) of 110-459 coho salmon (200, 300 and 400 days post-smoltification) reared at salinities of 2.5, 5, 10 and 30 ppt and exposed to 12 or 24 hours of light.

<table>
<thead>
<tr>
<th>Photoperiod</th>
<th>Salinity</th>
<th>Day (200)</th>
<th>Day (300)</th>
<th>Day (400)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>2.5</td>
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Table 2.13 Standard growth rate of 0-21 PIT-tagged coho salmon between 200 and 400 days post-smoltification reared at salinities of 2.5, 5, 10 and 30 ppt and exposed to 12 hour or 24 hours of light.

<table>
<thead>
<tr>
<th>Day</th>
<th>Photoperiod</th>
<th>Salinity</th>
<th>Mean SGR ± SE</th>
</tr>
</thead>
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<td>200-300</td>
<td>24</td>
<td>2.5</td>
<td>0.224 ± 0.01</td>
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<td>5</td>
<td>0.303 ± 0.04</td>
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<td>10</td>
<td>0.331 ± 0.05</td>
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<tr>
<td></td>
<td></td>
<td>30</td>
<td>0.456 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2.5</td>
<td>0.463 ± 0.06</td>
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<tr>
<td></td>
<td></td>
<td>5</td>
<td>0.450 ± 0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.421 ± 0.05</td>
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<tr>
<td>300-400</td>
<td>24</td>
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<td>0.302 ± 0.05</td>
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<td>0.527 ± 0.05</td>
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<td>0.404 ± 0.06</td>
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<td>0.548 ± 0.04</td>
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<td>0.537 ± 0.02</td>
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</tbody>
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Chapter 3: Effects of long-term salinity and photoperiod exposure on thermal tolerance of Atlantic and coho salmon

3.1 Introduction

As mentioned in Chapter 2, the optimal conditions for growth are still uncertain. Optimal conditions do not only apply to growth, but stress tolerance as well. Indeed, there are potential tradeoffs between conditions that enhance growth and the associated physiological stress tolerance, whereby conditions that yield faster growth have been reported to reduce stress resistance in fish (Solberg et al. 2016). In this study, we investigated how different RAS rearing conditions, in particular different salinities and photoperiods, affect thermal tolerance of both Atlantic and coho salmon during long-term rearing from smolt to adult.

Salinity is an important abiotic factor in salmon aquaculture that can impact growth and physiology performance. The metabolic costs of osmoregulation have been estimated to account for 5 to 50% of routine metabolic rate (Boeuf and Payan, 2000) and these metabolic costs are likely to be highest in salmon reared in either freshwater (FW) or seawater (SW) due to the large osmotic gradients between the internal and external environment. In theory, rearing salmon at a salinity that is isosmotic to blood (~10 ppt) may decrease the costs of osmoregulation and potentially increase growth rate and feed conversion, but may also affect tolerance to environmental stressors. The effect of salinities on thermal tolerance in salmonids is largely unstudied and what few studies exist suggest that the effects of salinity on thermal tolerance are minimal (Sardella et al. 2008; Chen and Chen 1999, Everatt et al. 2013, Denisse Re et al. 2006, 2012; Haney and Walsh, 2003). For example, Shaughnessy and McCormick (2018) measured
thermal tolerance in brook trout (*Salvelinus fontinalis*) during SW acclimation and while thermal tolerance decreased initially following exposure to SW compared with FW, it recovered after 16 days in SW. This study however only examined freshwater to seawater transfer and was of a relatively short duration, thus the effects of longer-term exposures to a variety of salinities remain unknown.

However, a 24 hour photoperiod is not a natural photoperiod for most salmonids and in Atlantic salmon may be stressful (Newman et al. 2015). While a 24 hour photoperiod appears to have positive effects on growth, the effect of this photoperiod regime on stress resistance, such as thermal tolerance, is not known. In addition, most of the available studies on the effects of photoperiod on salmon have been conducted on the parr-smolt stage (Oppedal et al. 2007) for example, with no studies conducted on salmon from smolt through to adult.

The objective of this study is to investigate the combined effects of salinity (2.5, 5, 10 and 30 ppt) and photoperiod (12 and 24 hours of light) on thermal tolerance of Atlantic and coho salmon during 400 days of continuous rearing from the smolt to adult life stages. To assess the effect of salinity and photoperiod on thermal tolerance, we measured the critical thermal maximum ($CT_{\text{max}}$) of coho salmon on days 60, 120, 200, 300 and 400 or rearing, while in Atlantic salmon, $CT_{\text{max}}$ was determined on day 400. $CT_{\text{max}}$ is a simple and repeatable measure of thermal tolerance (Lutterschmidt and Hutchison 1997) in fish and represents the temperature at which biological processes begin to fail (Wang et al. 2013, Zhang and Kieffer 2014).

### 3.2 Methods

*Animals used*

Atlantic (Cermaq Canada, Campbell River, British Columbia, Canada) and coho salmon (Target Marine Hatcheries Ltd., Sechelt, British Columbia, Canada) were transported to the
InSEAS RAS (Initiative for the Study of the Environment and its Aquatic Systems Recirculating Aquaculture System) facility at The University of British Columbia. The smolts (6,000 per species) were approximately one year-old and 100 grams. Fish were initially held in flow-through City of Vancouver tap water for one week until they were separated into their respective treatment tanks as described below. All experimental procedures were approved by UBC Animal Care Permits A13-0016 and A17-0011.

**RAS system set-up**

The InSEAS RAS facility consists of 7 separate RAS which allows the independent control of salinity and photoperiod. Each RAS is comprised of two 5 m³ tanks, two 0.7 m³ tanks, radial-flow separator, microbead filter, biofilter and UV sterilization system. Within each of the 7 RASs, one 5 m³ tank housed approximately 850 coho salmon smolts and the other 5 m³ tank housed approximately 850 Atlantic salmon smolts. Thus, both species were exposed to identical water within a given experimental treatment. Each 5 m³ tank was wrapped in black plastic and covered with a black tarpaulin to eliminate external light sources. Inside each 5 m³ tank, a LED lamp (5000K/3000K - 20,000 Lumens - Natural White) was installed which was controlled by a timer (Stanley Timer Max Outdoor Pro mechanical timers - Seattle, Washington, USA) to provide either 12 or 24 (hours of light) photoperiods. Each 5 m³ tank was equipped with an automatic drum feeder (Arvo-Tec drum 2000 feeder w/ Arvo-Tec WOLF control system Huutokoski, Finland) and fish from both photoperiod treatments were only fed during the 12 hour light period that corresponded with the 12 hour photoperiod.

After fish were acclimated to the RAS tanks in recirculating freshwater for 2 months, Instant Ocean was added to one of the 0.7 m³ tanks in each RAS to bring the salinity of that system up to the desired target salinities of 2.5, 5, 10 or 30 ppt. Because InSEAS has only 7
RAS, the treatment groups consisted of 2.5, 5 and 10 ppt at either 12 or 24 hour photoperiod, while the 30 ppt treatment was conducted only at a 24 hour photoperiod. Within each treatment group, coho salmon were reared in one 5 m³ tank and Atlantic salmon were reared in the second 5 m³ tank.

This study is part of a larger experiment that investigated the long-term effects of salinity and photoperiod on growth of Atlantic and coho salmon while being reared at 12°C. Fish were fed 1% biomass / day with the target stocking density of 40 kg/m³. Stocking density was measured and adjusted to the target 40 kg/m³ every 100 days by culling. Salinity was measured and salt added (Instant Ocean® Sea Salt, Blacksburg, VA) to the system once per day. The water quality over the experiment can be seen below in Table 1. Ammonia, nitrite and nitrate were kept below 6, 4 and 160 ppm respectively. All water quality values were within the normal accepted range of salmon growth.

**Thermal Tolerance**

$CT_{\text{max}}$ was determined for coho salmon at 60, 120, 200, 300 and 400 days following the start of experimental salinity and photoperiod treatments. For Atlantic salmon, $CT_{\text{max}}$ was determined only on day 400 because we had low animal numbers due to a *Saprolegnia* infection early in the experiment. Prior to the $CT_{\text{max}}$ test, 10 fish were randomly selected from each 5 m³ tank, weighed (to prevent use of fish that were considerably larger or smaller than the tank average) and transferred into one of the 0.7 m³ tanks within the respective RAS and held for 24 hours to ensure they were in a post-adsorptive state for the $CT_{\text{max}}$ trial. $CT_{\text{max}}$ was determined in a 170 L glass aquaria housed in an insulated fibreglass tank (0.75 x 1.2 x 1.0 m; outer chamber). The outer chamber had two pumps (Aquatop Powerhead 1000 LPH – 16 W – Brea, California, United States) to ensure that the water was well-mixed. A heater with a built-in circulator was
used in the outer chamber (IC-2 Heater 115V – 60 Hz – 1000 W, Brinkman Instruments, New York, United States) to heat the water as required. The inner chamber was filled with water from the respective RAS system to ensure constant salinity, and water was aerated to ensure > 80% air saturation throughout the $CT_{\text{max}}$ trial. Two fish were placed in the tank for each $CT_{\text{max}}$ trial, and each $CT_{\text{max}}$ trial was conducted 5 times for each treatment (ie. $n=5$, 2 fish per n, 10 fish per treatment). Three separate $CT_{\text{max}}$ experimental tanks were run simultaneously.

Fish were transferred from 0.7 m$^3$ tank into the 170 L test aquaria and allowed to habituate to the chamber for 120 minutes while temperature was maintained at 12°C. The $CT_{\text{max}}$ trials were initiated at 8:00 am and completed by 11:00 am in the three independent systems to control for time of day in our trials. The $CT_{\text{max}}$ was initiated by increasing the water temperature of the outer tank which through conduction increased the water temperature in the inner tank by 1°C every ten minutes, a rate consistent with that used by others (0.3°C min$^{-1}$ Sardella et al. 2008; 0.1°C min$^{-1}$ Ziegeweid et al. 2008, Wang et al. 2013). The water temperature increased until there was a loss of righting reflex of the first fish (experimental endpoint). At this point the temperature was recorded as $CT_{\text{max}}$ and the fish was removed from the chamber, euthanized and length and weight were recorded. The temperature was allowed to continue to increase in the inner chamber until the second fish reached the experimental endpoint and the fish euthanized as described above.

**Statistics and calculations**

A 3-way ANOVA was used to test for the effects of time, photoperiod and salinity on $CT_{\text{max}}$. Within each time point, a 2-way ANOVA was used to test for the effects of photoperiod and salinity on $CT_{\text{max}}$, and a one-way ANOVA was used to compare condition factors within
time periods and pairwise comparisons were calculated by Tukey’s Honest Significance test. Significance level was set 0.05.

Fulton’s condition factor (CF) was calculated by:

\[
CF = \left( \frac{\text{Mass}(g)}{\text{Length}(cm)^3} \right) \times 100
\]

3.3 Results

In coho salmon, there was a significant effect of photoperiod on CT\text{max} that changed over time, but no effect of salinity on CT\text{max} (3 way ANOVA; salinity p= 0.615, photoperiod= 2.53 \times 10^{-5}, day < 2 \times 10^{16}; Fig 3.1). On day 60 and 120, CT\text{max} was consistently lower in the 24 hour photoperiod than the 12 hour photoperiod (2-way ANOVA; day 60 salinity p= 0.27, photoperiod p = 2.3 \times 10^{-8}, interaction p= 0.19; day 120 salinity p= 0.2, photoperiod p = 5.2 \times 10^{-7}, interaction p= 0.36). At day 200, 300 and 400, however, CT\text{max} did not differ between the 12 and 24 hour photoperiod and there were no significant effects of salinity and no significant interaction terms (2-way ANOVA; day 200: salinity p= 1, photoperiod p = 0.26, interaction p= 0.99, day 300: salinity p= 0.96, photoperiod p = 0.25, interaction p= 0.99, day 400:salinity p= 0.94, photoperiod p = 0.84, interaction p= 0.95). Overall CT\text{max} decreased over time (p = 2.0 \times 10^{-5}).

The coho salmon used in the analysis of CT\text{max} grew over time as expected, but within each sampling time there were significant differences in length or body mass. There were however, a few differences of condition factor (CF) across treatments within sampling times (Table 3.1). On day 120, the CF was lower in the 2.5 ppt/24 hour treatment than all other
treatments and on day 400, CF of the 10 ppt/24 hour treatment was 0.08 higher than the other treatments.

In Atlantic salmon, there was no significant effect of salinity or photoperiod on \( CT_{\text{max}} \) (Fig 2; 2-way ANOVA, salinity \( p = 1 \), photoperiod \( p = 0.88 \), interaction \( p = 0.99 \)).

In Atlantic salmon, fish grew with time as expected, but there were few significant differences of condition factor (CF) within the different treatments (Table 3.2). The condition factors ranged from 1.26 to 1.42 due to early Saprolegnia issues which resulted in varying stocking densities.

3.4 Discussion

The objective of this study was to determine the combined effects of long-term salinity (2.5, 5, 10 and 30 ppt) and photoperiod (12 and 24 hour) exposure on \( CT_{\text{max}} \) in post-smolt Atlantic and coho salmon (from 0 to 400 days post-smoltification). The results of this study yield four important and novel findings. First, we found a clear and reproduceable effect of photoperiod on \( CT_{\text{max}} \) in coho salmon on days 60 and 120, where fish exposed to 12 hour photoperiod had a significantly higher \( CT_{\text{max}} \) than those exposed to 24 hours of light (Fig. 3.1). Second, \( CT_{\text{max}} \) decreased over time in coho salmon from approximately 29°C at 60 days to 26°C at 400 days post-smoltification (Fig. 3.1). Third, salinity had no effect on CTmax in coho or Atlantic salmon. Finally, at 400 days post smolt, Atlantic salmon had a higher \( CT_{\text{max}} \) (28°C) than coho salmon (26°C, Fig. 3.2).

Our findings, where a shorter photoperiod resulted in a 0.5 to 0.75°C higher \( CT_{\text{max}} \) in coho salmon over the first 120 days of the experiment (Fig 3.1) are in contrast with other studies on other teleost fish. For example, in killifish (\textit{Fundulus heteroclitus}), a longer photoperiod (14:10, L:D) resulted in a higher \( CT_{\text{max}} \) than a shorter 12 hour photoperiod (Healy and Schulte
Similarly, goldfish (*Carassius auratus*) exposed to a 16 hour photoperiod were more resistant to sudden, rapid increases in temperature compared to a group exposed to 8 hour photoperiod (Hoar and Robertson 1958). In blacknose dace (*Rhinichthys atratulus*) reared on a 16 hour photoperiod had a CT\text{max} that was 1°C higher than fish reared on the shorter 8 hour photoperiod (Terpin et al. 1976). The lack of consistency between our study and previous studies could be due to numerous factors including species differences life history differences, the photoperiods selected and rearing temperature.

The life cycle of coho is much more complex than *C. auratus* and *R. atratulus*. Coho emerge in late spring and grow in FW over summer and the following winter. The juveniles then undergo smoltification and migrate to estuaries and ultimately the ocean for ~ 18 months, but may return to FW earlier if they are precocious males (Wainwright and Weitkamp 2013). When our fish were transferred to their respective salinities, they were considered smolts, so the day 60 and 120 could represent a more sensitive stage when the smolts would naturally be in estuaries and close to shore compared to the older ocean-roaming stage. This “sensitive stage” also suggested that the unnatural 24 hour photoperiod may result in stress, which clearly reduced thermal tolerance, but could also decrease tolerance to other stressors, although Fang et al. (2018) did not reveal any effects on photoperiods on hypoxia tolerance or swim performance in this same group of fish.

In addition, the aforementioned studies in *C. auratus* and *R. atratulus* were conducted using natural photoperiods that aimed to simulate summer (eg. 18:6) and winter (e.g 8:16) conditions. In these temperate species, the longer summer photoperiod could serve as an intrinsic seasonal cue to increase thermal tolerance in preparation for higher summer temperatures. Similarly, coho salmon from British Columbia and Washington, USA, experience
a 12 hour photoperiod in roughly February and October, with the latter time corresponding to their outmigration from freshwater rivers to seawater. During this time, the river and estuarine temperatures are lower than those experienced in the summer, therefore, these winter temperatures may serve as a cure to lower thermal tolerance. Coho never experience 24 hours of light in their native environment, thus it is difficult to predict how it would affect thermal tolerance, but the decrease is thermal tolerance could have implications for aquaculture.

The lower CT$_{\text{max}}$ in coho reared for up to 120 day under 24 hours of light is of direct relevance to aquaculture, especially in RAS where there may be short periods where environmental parameters deviate from desired values due to equipment malfunction or power failure where temperature could change quite rapidly. The findings in this study indicate that fish may become more sensitive to temperature at different life stages and this is confounded by photoperiod. These findings have implications for fish farmers as there is a potential trade-off of using a 24 hour photoperiod to improve growth rates that negatively impacts thermal tolerance.

In our experiment, the CT$_{\text{max}}$ of coho decrease by ~3°C over the course of the 400 day trial (Fig. 1), with the greatest changes in CT$_{\text{max}}$ occurring between 120 – 200 days post-smoltification (~28.5 to 27°C). This time period could be an important transitional stage and may coincide with when coho would naturally migrate out to a much more thermally stable environment (ocean) compared to the less stable estuary (Wainwright and Weitkamp 2013). In addition, salmon size has been shown previously to affect CT$_{\text{max}}$, whereby larger salmonids generally have lower CT$_{\text{max}}$ compared with smaller salmonids. Specifically, Rio Grande cutthroat trout (Oncorhynchus clarkii) and Apache trout (Oncorhynchus apache) both showed a decrease in CT$_{\text{max}}$ (Recsetar et al. 2012) with increasing body size. The fish tested in their experiment however, ranged in size from ~ 50 to 200 g, which is smaller than the smallest fish
tested here (Table 2), but these studies suggest that differences in body size may be the causes of the lower CT\textsubscript{max}. Interestingly, rainbow trout (\textit{Oncorhynchus mykiss}) showed no decrease in CT\textsubscript{max} with an increase in size between 50 and 200 g (Recsetar et al. 2012).

Photoperiod is not the only factor that can affect thermal tolerance in fish; thermal history, time of day, and stage of development (Floyd 1985) have been documented as potential causes for altered thermal tolerance. The fish were all from the same generation of parents and reared at the same temperature (11.6-12.4°C), so thermal history should not have played a factor in these results. The CT\textsubscript{max} trials were conducted at 8:00 AM PST and only one trial per day was done to avoid a temporal effect on the results. The stage of development and its relation to CT\textsubscript{max} is interesting and unstudied.

Our study clearly demonstrates that long-term exposure to salinities ranging from FW to SW has no effect on thermal tolerance of coho or Atlantic salmon. These results are in general agreement with those of Shaughnessy and McCormick (2018) who demonstrated that FW to SW transfer in brook trout caused a transient decrease in CT\textsubscript{max}, which was recovered by 16 day post-transfer. Similarly, other studies suggest that salinity has no effect on CT\textsubscript{max} (Sardella et al. 2008; Chen and Chen 1999 and Everatt et al. 2013); however, there are other studies suggesting that salinity does impact thermal tolerance (Denisse Re et al. 2006, 2012; Haney and Walsh, 2003). Although, it is not possible to conclude how salinity affects thermal tolerance across diverse species, our study clearly demonstrates that salinity does not impact CT\textsubscript{max} in either coho or Atlantic salmon.

Another important finding of this study is that Atlantic salmon have an ~2°C higher CT\textsubscript{max} at day 400 than coho salmon. Atlantic salmon are a common aquaculture fish and their higher thermal tolerance may be a consideration when selecting aquaculture species. Our CT\textsubscript{max} values
for Atlantic salmon were ~28°C, which is at the higher end of values reported in the literature which suggests that CT_{max} lies between 24-26°C (Antilla et al. 2013) and 26-29°C (Bowden et al. 2018). The CT_{max} of coho salmon in our study decreased with time and at 400 days was ~26°C, which is in agreement with published reports suggesting they have a CT_{max} of 26.9°C (Chen et al. 2015). However, for all these previous studies, CT_{max} was determined on much smaller fish (<50 g) than we used in the present study. Although it is intriguing that Atlantic salmon appear to be more thermal tolerant than coho, this conclusions should be viewed as tentative since we observed early high mortality rates in the Atlantic salmon due to _Saprolignia_ infections, which could have removed the more sensitive animas from the population. Further investigations are required to confirm the specific-specific differences in CT_{max}.

In summary, the findings of the present study clearly indicate that thermal tolerance in coho salmon is affected by photoperiod during the first 120 day of rearing post-smolt, with the commonly used 24 hour photoperiod causing a reduction in CT_{max} compared with a 12 hour photoperiod. These photoperiod effects vanish between day 120 and 200, which is coincident with an overall decrease in CT_{max}. CT_{max} is also higher in Atlantic salmon than coho at 400 days of rearing. In both species, salinity has no effect on thermal tolerance. The findings of this study have several potential implications for salmon RAS aquaculture that are worth further exploration; decreasing photoperiod initially to increase thermal tolerance, decreasing temperature to provide a buffer should the facility experience equipment malfunctions and rearing the more thermally tolerant Atlantic salmon instead of coho salmon.
Chapter 3 Figures and Tables

Figure 3.1: Critical thermal maxima ($CT_{\text{max}}$) of coho salmon (Oncorhynchus kisutch) measured on Day 60, 120, 200, 300 and 400 days (from left to right) following exposure to a salinity of 2.5, 5, 10 or 30 ppt salinity and 12 or 24 hour photoperiod. 3-way ANOVA used to test effects of time, photoperiod and salinity on $CT_{\text{max}}$ and within each time point, a 2-way ANOVA was used to test for the effects of photoperiod and salinity on $CT_{\text{max}}$. Pairwise comparisons were calculated by Tukey’s Honest Significance test (0.05).
Figure 3.2: Critical thermal maxima ($CT_{\text{max}}$) of Atlantic salmon ($Salmo salar$) on Day 400 following exposure to a salinity of 2.5, 5, 10 or 30 ppt salinity and 12 or 24 hour photoperiod. 2-way ANOVA was used to test for the effects of photoperiod and salinity on $CT_{\text{max}}$. Pairwise comparisons were calculated by Tukey’s Honest Significance test (0.05). No significant differences found.
Table 3.1: Length, mass, and condition factor of the coho salmon used for the CT\textsubscript{max} trials from Day 60 to 400 days following exposure 2.5, 5, 10 or 30 ppt salinity and 12 or 24 hour photoperiods. All points are means ± SEM (n = 10). Values that share a letter within a measurement and timeframe, are not statistically different.

<table>
<thead>
<tr>
<th>Day</th>
<th>Photoperiod Salinity (ppt)</th>
<th>24 hours</th>
<th>12 hours</th>
</tr>
</thead>
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<td></td>
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<tr>
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<td>Length (mm)</td>
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<td>302 ± 3</td>
</tr>
<tr>
<td></td>
<td>Mass (g)</td>
<td>253 ± 12</td>
<td>266 ± 9</td>
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<td>0.99 ± 0.03</td>
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<tr>
<td>120</td>
<td>Length (mm)</td>
<td>329 ± 7</td>
<td>324 ± 2</td>
</tr>
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<td></td>
<td>Mass (g)</td>
<td>375 ± 10</td>
<td>392 ± 6</td>
</tr>
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<td>1.16 ± 0.01\textsuperscript{b}</td>
</tr>
<tr>
<td>200</td>
<td>Length (mm)</td>
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<td>369 ± 3</td>
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<td>Mass (g)</td>
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<td>657 ± 16</td>
</tr>
<tr>
<td></td>
<td>Condition Factor</td>
<td>1.30 ± 0.01</td>
<td>1.30 ± 0.01</td>
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<tr>
<td>300</td>
<td>Length (mm)</td>
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<td></td>
<td>Mass (g)</td>
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<td>Condition Factor</td>
<td>1.31 ± 0.02</td>
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<tr>
<td>400</td>
<td>Length (mm)</td>
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<td>503 ± 9</td>
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<tr>
<td></td>
<td>Mass (g)</td>
<td>1738 ± 62</td>
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</tr>
<tr>
<td></td>
<td>Condition Factor</td>
<td>1.39 ± 0.01\textsuperscript{a}</td>
<td>1.38 ± 0.02\textsuperscript{a}</td>
</tr>
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</table>
Table 3.2: Condition factor of 10 Atlantic salmon subject to CT\textsubscript{max} trials at 400 days post-smoltification. Fish were reared at salinities of 2.5, 5, 10 and 30 ppt and in 24 or 12 hours light. All points are means ± SEM. Letters indicate mean is statistically different within each treatment.

<table>
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<tr>
<th>Photoperiod</th>
<th>Salinity (ppt)</th>
<th>24 hours</th>
<th>12 hours</th>
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</thead>
<tbody>
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<td></td>
<td>2.5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>473 ± 9</td>
<td>403 ± 7</td>
<td>469 ± 472 ± 464 ± 463 ± 464 ±</td>
</tr>
<tr>
<td>Mass (g)</td>
<td>1491 ± 71</td>
<td>902 ± 64</td>
<td>1389 ± 1494 ± 1331 ± 1239 ± 1301</td>
</tr>
<tr>
<td>Condition Factor</td>
<td>1.41 ± 0.01</td>
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<td>1.35 ± 0.01</td>
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<td>0.01\textsubscript{ab}</td>
<td>0.01\textsubscript{a}</td>
<td>0.01\textsubscript{bde}</td>
</tr>
</tbody>
</table>

Table 3.3: Summary statistics for coho salmon

<table>
<thead>
<tr>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
</tr>
<tr>
<td>60</td>
</tr>
<tr>
<td>120</td>
</tr>
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<td>200</td>
</tr>
<tr>
<td>300</td>
</tr>
<tr>
<td>400</td>
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</table>

<table>
<thead>
<tr>
<th>Day</th>
<th>Salinity</th>
<th>Photoperiod</th>
<th>Salinity x Photoperiod</th>
<th>Salinity x Day</th>
<th>Day x Photoperiod</th>
<th>Salinity x Day x Photoperiod</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 x 10\textsuperscript{16}</td>
<td>0.63</td>
<td>3.6 x 10\textsuperscript{-5}</td>
<td>0.66</td>
<td>0.99</td>
<td>0.04</td>
<td>1</td>
</tr>
</tbody>
</table>
Chapter 4: Conclusion

4.1 Summary

The objective of my thesis was to further examine the optimal growth conditions and conditions that produced the lowest instance of early maturation of Atlantic and coho salmon grown in RAS between 200 and 400 days post-smoltification. I also wanted to quantify their stress resistance with thermal tolerance tests via $CT_{\text{max}}$ from 0 to 400 days post-smoltification. I predicted that fish reared in isosmotic (10 ppt) conditions would have a higher growth rate and thermal tolerance due to decreased costs of osmoregulation.

The results of the growth and maturation study yielded 3 novel findings; it was found that salinity had a significant effect on growth of Atlantic and coho salmon. Photoperiod also had a significant effect on growth of both species, but we found that Atlantic salmon showed highest growth rates under 24 hours of light per day (up to day 300) and coho salmon grew best under 2.5 ppt in the 12 hours of light treatment. Our findings of the long-term growth study differed with Emerman (2016), Fang (2018) or Chan (2018). They found a significantly improved growth rate at isosmotic conditions at day 59, 400 and day 120 respectively. This shows how complex the interaction between salinity, day and photoperiod is (all of which were found significant with the 3-way ANOVA).

For maturation, I found higher overall maturation in Atlantic salmon exposed to 24 hour photoperiod, whereas coho salmon in 12 hour photoperiods had higher maturation. My findings in Atlantic salmon were in contrast to several other studies that have shown that an increase in photoperiod correlates with a decrease in early maturation. The cause of this is unknown and early maturation in Atlantic salmon was seen in all 24 hour photoperiod treatments as early as
200 days post-smoltification. The instance of early maturation in Atlantic salmon is most likely attributed to the fact that we had a male/female strain vs. the all-female strain of coho. As discussed earlier, male salmonids are able to mature at a much smaller size (we had some fish mature around 50 g.) compared to their female counterparts. This can also be explained by year-to-year strain differences used in our trials and adding the complexity of a photoperiod trial on top of the previous work for this experiment. It wasn’t until day 400 when the 12 hour photoperiod treatment started to mature in over 2.5% of the population.

The results of the thermal tolerance study yielded 4 novel findings; we found a clear effect of photoperiod on CT\(_{\text{max}}\), where coho salmon reared under 12 hours of light had a significantly higher CT\(_{\text{max}}\) than those reared under 24 hours of light. This effect was seen on days 60 and 120 post-smoltification and disappeared after. It was also found that CT\(_{\text{max}}\) decreased by 3°C over time between 60 and 400 days post-smoltification. Contrary to our hypothesis, salinity had no effect on CT\(_{\text{max}}\) of Atlantic or coho salmon. Finally, Atlantic salmon were only exposed to thermal tolerance testing at day 400 due to high mortality caused by Saprolegnia infections, but they had a CT\(_{\text{max}}\) that was 2°C higher than coho at the same time-point.

4.2 Practical Applications for Industry

Due to contradicting findings in the studies conducted by Emerman (2016), Fang (2018), Chan (2018) and myself, I cannot recommend a salinity to optimize growth for Atlantic or coho salmon. This further highlights the points touched upon in section 4.1 such as; year-to-year variation in strains and this year’s addition of two photoperiods instead of a trial on salinity with only one photoperiod as conducted previously. As Chan’s (2018) experiment was the first 120 days of my experiment, it also suggests that the developmental stage of this fish is likely very
important. During the first 120 days of the growth trial, I found that photoperiod had a significant effect on thermal tolerance where the 12 hour photoperiod yielded a higher $CT_{\text{max}}$ than fish exposed to continuous light. This could potentially mean that the fish up to 4 months post-smoltification are more sensitive to potential stressors such as continuous light and/or non-isosmotic water.

I would recommend that Atlantic and coho salmon should be grown under 24 hours of light for the fastest growth rate. However, my work has demonstrated that growing Atlantic salmon under 24 hours of light may not always yield higher growth. Other factors such as developmental stage (first 4 months post-smoltification) should be further examined. Continuous light paired with continuous feed availability would likely provide the highest growth rates. In this experiment, fish in both photoperiods were fed for 12 hours as to not confound the data. The disadvantage of rearing in continuous light may be an increased incidence of maturation based upon the data in my study (for Atlantics), however most previous literature has shown that 24 hours of light delays maturation. Another trade-off to rearing coho under 24 hours of light is a decrease in thermal tolerance. This may be of significance in RAS if a pump failed, or some other mechanical failure occurred, as those fish may be more likely to be exposed to elevations in temperature. Assuming the reduction in $CT_{\text{max}}$ indicates increased susceptibility to stress in general, this may result in greater susceptibility to infections, diseases or mortality up until day 120.

From all of our InSEAS studies, it is not recommended to rear Atlantic or coho salmon above 10 ppt. No growth benefits are seen to warrant the higher costs of adding salt and having to replace pumps and other equipment of the RAS systems that may corrode due to high salt levels. Previous studies by Emerman (2016), Fang (2018) and Chan (2018) have shown that 10
ppt does increase growth rates over a broad time scale. These effects were not seen in my part of the experiment, but my experiment does agree with past studies that have concluded that there is no economic or physical benefit to growing salmonids above 10 ppt. This is interesting because Chan’s (2018) experiment covered the first-half (days 0-120) of the same growth trail where he found 10 ppt to be the optimal salinity for growth. Through my statistical analysis, I found no optimal salinity for growth and significant interaction terms between day, salinity and photoperiod. If fish are not reared at isosmotic conditions, it is recommended to have a salinity level greater than zero to prevent fungal infections such as *Saprolegnia*. Even though our Atlantic salmon in the 5 ppt/24 hour treatment experienced a massive *Saprolegnia* tank infection, it seems like an anomaly, possibly with a genetic basis as a different strain of fish was used from the previous experiment to this one.

Comparing the two species’ growth rates, I would recommend rearing coho salmon over Atlantic salmon. At the end of the similar duration growth trial, they averaged 400-500 grams larger than their Atlantic counterparts. Switching species from Atlantic to coho salmon may prove difficult as Norway and a few other countries have established Atlantic salmon as the species of choice in intensive aquaculture. The trade-off of rearing coho over Atlantic salmon may be a potentially decreased thermal/stress tolerance, but as Atlantic salmon were only tested at day 400, this cannot be confirmed. Another drawback of rearing coho salmon is that they are more aggressive during feeding, which can lead to increased eye and fin damage. This was told to me anecdotally by Dr. Don Furnell. In our experiment, however, we did not see a higher instance of eye damage in coho compared to Atlantic salmon, but with commercial-like densities, this may potentially pose an issue.
4.3 Strengths and Limitations

The major strength of the growth experiment is the ability to conduct research on a semi-industrial sized scale in the InSEAS facility. Having 7 separate RAS systems with two separate 5 m$^3$ tanks per system allows the knowledge gained to be accurately and appropriately applied to the industry. As far as I know, one of the best facilities in North America to test industry relevant densities coupled with as many other experimental factors as desired that can be entirely controlled via RAS technology. The one draw-back of the facility is the inability to replicate salinities due to lack of space to install more RAS. The current set-up allowed for comparison of 3 salinities with 2 corresponding photoperiods and an extra tank that was 30 ppt/24 hours of light. This did make running statistics a challenge, and the pseudo replication (and a true n = 1) is acknowledged, but Emerman (2016) validated the design by showing there was no significant tank effects. Another concern with the InSEAS facility was the placement of the drainage system. Typically, the drains are at the lowest point of the room, but in this experiment, it was the opposite. This caused major pooling (up to 10 cm.) of water. This pooling water can easily harbor fungal spores such as *Saprolegnia*. On the other hand, it is more likely that the fish came in already infected and the stress compromised their immune systems which presented the visible symptoms of infection.

The major strength of the thermal tolerance trials was it allowed us to use a physiological challenge as a proxy for performance and stress resilience to add to our comparative study. $\text{CT}_{\text{max}}$ testing is the gold standard for assessing the thermal tolerance of an organism (Lutterschmidt and Hutchinson 1997). I was able to do a relatively low temperature ramp protocol compared to most $\text{CT}_{\text{max}}$ testing done on salmonids in the literature. However, $\text{CT}_{\text{max}}$ has its limitations. Mainly that it is an acute temperature challenge and the temperature ramp
The protocol used does not necessarily mimic that of an industrial setting should a mechanical failure occur. Also I had to use 2 fish per thermal tolerance chamber due to time limitations for a true n=5. If I would have done 1 fish per experimental tank and 3 experimental tanks per day, it would have taken 24 days instead of 12, but provided me with more power. Since we were working with specific days post-smoltification, this margin would have been too wide to confidently report on days post-smoltification. For the purposes of our experiment, the protocol we used was perfect as a test to compare the relative values obtained from the CT_{max} tests between species and treatments (photoperiod and salinity).

### 4.4 Future Considerations

The future of growing fish in land-based RAS farms is an exciting possibility. Further research into the optimal growth parameters while minimizing early maturation (salinity, photoperiod, temperature, feed composition, water chemistry, and several more) should be conducted in the near future. With the increase in global demand for seafood, the wild fisheries will not be able to keep up. Furthermore, RAS has the potential to have a reduced environmental footprint by; recycling greater than 99% of the water, being located closer to markets reducing transportation costs and reducing some of the direct environmental impacts of open-sea net cages (such as eutrophication below net pens, wild/farmed interactions and escapes).

At the InSEAS facility, fewer treatments with two salinities (2.5 and 10 ppt which showed the best growth) and thus more replicates per salinity would increase the statistical power of the experiment. Genetic testing should also be conducted to ensure that the same strain is being used every year as I suspect genetics played a major role in the Atlantic salmon’s susceptibility to Saprolegnia.
For thermal tolerance, conducting a much slower temperature ramp on individuals that mimics possible temperature increases if a chiller went down overnight for example might prove more useful. It would be interesting to pair thermal tolerance tests with a protocol used by a PhD candidate in the Farrell lab Matt Gilbert who uses externally placed electrodes to measure when the heart is in arrythmia. His method allows for a more precise determination of thermal tolerance endpoint. When we compared our numbers, the endpoint of his fish was consistently around 2°C lower than mine. Fish appear to be able to tolerate higher temperatures with the ramping protocol, but the determined CTmax value is not very biologically relevant as it is the temperature at which they lose equilibrium and thus are unable to perform any normal tasks associated with maintaining life. The method used by Matt Gilbert is also less harmful to the fish as they are sedated and placed in a sling to minimize any thrashing or twitching.

The most important findings from this experiment that should be further examined are two-fold; the seemingly important developmental timeline from smoltification to approximately 120 days post-smoltification when intermediate environmental salinities appear to enhance growth and the interactions between time, photoperiod and salinity, which we proved to be extremely complex and intricate.
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