EFFECTS OF SALINITY AND PHOTOPERIOD ON GROWTH, AEROBIC SCOPE AND HYPOXIA TOLERANCE OF ATLANTIC AND COHO SALMON IN RECIRCULATING AQUACULTURE SYSTEMS

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Abstract

Recirculating aquaculture systems (RAS) are an emerging technique in aquaculture to rear salmon in land-based facilities, but the systems are also associated with high costs, so rearing fish under optimal conditions for maximum growth is required for profitability. However, few systematic studies have been conducted to determine optimal conditions for growth of salmon from smolt through to market size in RAS and the related effects on physiological performance. To address this knowledge gap, the first part of my thesis investigated the effect of salinity on growth, metabolism and hypoxia tolerance of Atlantic and coho salmon. Smolts were reared at salinities of 0, 5, 10, 20 and 30 ppt under 24 h of light in RAS for up to 460 days. Between Days 200 and 400, respirometry was conducted to measure routine, maximum metabolic rate and aerobic scope, while time to loss of equilibrium at 10% air saturation was measured to determine hypoxia tolerance. The initial effect of salinity on growth was found in Atlantic and coho salmon at Day 295 and 59, respectively, after which growth was generally enhanced at intermediate salinities of 5 and 10 ppt. No clear relationship was found between salinity and metabolic measurements in either species. Hypoxia tolerance of Atlantic salmon was enhanced at 5 and 10 ppt, but salinity had no effect on hypoxia tolerance of coho salmon. The second part of this thesis aimed to (1) determine metabolism and hypoxia tolerance of coho salmon during their early growth stages where the effect of salinity on growth was the most profound and (2) explore the interactive effect of photoperiod. A new cohort of coho salmon smolts were reared in RAS at 2.5, 5, 10 and 30 ppt under 12:12 and 24:0 (light:dark) photoperiods, while respirometry and hypoxia trials (15% air saturation) were conducted at Day 60 and 120. No effect of salinity and photoperiod was found on metabolic measurements and hypoxia tolerance in the younger coho salmon during these periods.

Overall, my data suggest that there is some potential to enhance growth of salmon by manipulating environmental conditions in RAS without compromising other physiological performance.

Lay Summary

Salmon are increasingly reared in land-based recirculating aquaculture systems (RAS) which have the potential to minimize negative impacts on the environment. Growing salmon under optimal environmental conditions to enhance growth is required to compensate for the high costs associated with RAS. However, little information is available about how salinity (dissolved salt level) and photoperiod can affect growth, metabolic rate and hypoxia (low-oxygen) tolerance of salmon in RAS. I found that growth of Atlantic salmon was enhanced at intermediate salinities, which was also associated with an increase in hypoxia tolerance, but there was no clear relationship between metabolic rates and salinity. Coho salmon also showed enhanced growth at intermediate salinities, but no relationship was found between salinity/photoperiod and metabolic rates/hypoxia tolerance. The current thesis demonstrates that rearing conditions can be manipulated to achieve fast growth of salmon in RAS without any significant compromise on other physiological performance measures.

Preface

For the 2015-2016 growth trial, I collaborated with Joshua D. Emerman, Victor Chan and Kevin T. Stiller under the supervision of Drs. Colin J. Brauner and Jeffrey G. Richards. I conducted all the respirometry, hypoxia challenge trials and data analysis under the supervision of Drs. Colin J. Brauner and Jeffrey G. Richards. I wrote all 4 chapters of this thesis and received editorial feedback from Drs. Colin J. Brauner, Jeffrey G. Richards and Patricia M. Schulte. All the experimental protocols involved with animals were approved by Animal Care Committee at the University of British Columbia (certificate number: A17-0011).

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ANCOVA	analysis of covariance
ANOVA	analysis of variance
AS	aerobic scope
ATP	adenosine triphosphate
ATPase	adenosinetriphosphatase
°C	degree Celsius
Ca^{2+}	calcium
Cl^{-}	chloride
eFCR	economic feed conversion ratio
GR	growth rate
GSI	gonadosomatic index
K^{+}	potassium
LOE	loss of equilibrium
Mg^{2+}	magnesium
MMR	maximum metabolic rate
MRC	mitochondria-rich cell
MS222	tricaine methanesulfonate

Na^+	sodium
O_2	oxygen
OCLTT	oxygen and capacity-limited thermal tolerance
P_{crit}	critical oxygen tension
ppt	parts per thousand
RAS	recirculating aquaculture system
RMR	routine metabolic rate
SEM	standard error of mean
SGR	specific growth rate
SMR	standard metabolic rate
TGC	thermal growth coefficient
T_{opt}	optimal temperature

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Chapter 1: General Introduction

The aquaculture industry has proposed recirculating aquaculture systems (RAS) to supplement net pen salmon farming in order to fulfil the large market demand for salmon and to mitigate some of the negative environmental impacts of traditional net-pen systems. The construction and use of RAS for rearing salmon, however, is extremely costly. Therefore, it is necessary to rear fish under optimal environmental conditions (e.g., temperature, salinity, pH and photoperiod) to maximize production and profitability. Little is known, however, about the effect of salinity on the growth of salmon in RAS, and how photoperiod can influence the growth of salmon in conjunction with salinity. Measuring metabolic rate of fish enables us to understand energetic allocation under different environmental conditions. However, it is also unclear how the metabolic rate of salmon changes across different rearing salinities and photoperiods in RAS. Hypoxia (low oxygen level in the water) is commonly seen in fish culture due to the high stocking density for maximized production output. Hence, hypoxia tolerance of fish is of great importance in aquaculture, but little information is available about hypoxia tolerance of salmon reared under different conditions of salinities and photoperiods in RAS. In the current thesis, I present the results of experiments in which I cultured salmon under different salinities and photoperiods in RAS to define the optimal salinity and photoperiod for growth of salmon in RAS. Respirometry was conducted to measure the metabolic rate of the fish to provide insight into the energetic costs and aerobic capacity associated with continuous rearing at different salinities and photoperiods. In addition, hypoxia challenge trials were conducted to examine how salinity and photoperiod can affect hypoxia tolerance of the fish.

This chapter will first describe the benefits and drawbacks of RAS in aquaculture. Background information about osmoregulation will then be provided, followed by a discussion of how salinity might alter fish growth. The chapter will then discuss how salinity can potentially affect metabolic rate and hypoxia tolerance. Photoperiod will be introduced as a second environmental parameter that can be manipulated in RAS, after which its effect on growth and potential relationship with metabolic rate and hypoxia tolerance will be further discussed.

1.1 Salmon Farming and Recirculating Aquaculture Systems

Aquaculture has played an increasingly important role in supplying protein for human consumption, with about 44% of global seafood produced by aquaculture in 2014 (FAO 2016a). The reliance on aquaculture to supply protein is likely to increase in the coming decades as the world population continues to grow and salmon are expected to be an important global aquaculture species. In 2015, the production of farmed salmon comprised about 65% of the total aquaculture production in Canada, with approximately 76% of the farmed salmon produced in British Columbia (Statistics Canada 2015). Atlantic salmon (*Salmo salar*) are the most widely cultured fish among all the salmon species. In 2014, there were about 23.3 million tonnes of Atlantic salmon produced globally in aquaculture, and this number comprised 93% of the total world farmed salmon production (FAO 2016b). Coho salmon (*Oncorhynchus kisutch*) are native to the coastal streams in British Columbia (DFO, Salmon Facts 2017), and are also being developed into an aquaculture species, with 170,000 tonnes of coho produced globally in aquaculture in 2014 (FAO, FishStat).

In traditional salmon aquaculture, post-smolts are reared in open net pens which generally consist of floating net cages anchored to the seabed in coastal areas. However, there is no control over the environmental parameters in open net pens, so locations suitable for salmon farming are very restricted. Moreover, net pen aquaculture has raised a series of environmental concerns including potential issues with interbreeding between escapees and wild strains of salmon, transmission of pathogens, and eutrophication of benthic areas caused by uneaten feed and fish faeces (Naylor et al. 2005; Folke et al. 1994; Kutti et al. 2008). A proposed alternative to address some of the problems associated with net pen aquaculture is to rear salmon post-smolts in recirculating aquaculture systems (RAS). Compared with net pen systems, RAS have improved biosecurity while reducing some environmental impacts and permit more complete control over water quality parameters (e.g., temperature, salinity, photoperiod and pH). However, the profit margins associated with growing salmon in RAS are low compared with net pens due to high costs associated with construction of the facility and its operation. Given that the rearing conditions of RAS can be adjusted to a much greater extent than in net pens, this raises the possibility of rearing fish under water conditions that can maximize growth and feed conversion, which further reduces production cycle and feed cost (~50% of the total cost), thus improving profitability. Among all the environmental parameters that can be manipulated in RAS, temperature has been widely studied regarding how it affects growth of salmon post-smolts (Edsall et al. 1999; Handeland et al. 2008; Hevrøy et al. 2013). For example, Handeland et al. (2008) reared Atlantic salmon post-smolts at 6, 10, 14 and 18 ℃, and they found that the optimal temperature for growth rate was 14 ℃ and the enhanced growth rate at 14 ℃ was about 100% higher than 6 ℃. There are also many other water parameters that can be manipulated to influence growth rate in fish. Osmoregulation

is an energy-consuming physiological process, so in theory salinity could be manipulated to lower osmoregulatory costs, which might improve growth rates of fish. However, there are few studies exploring the effect of salinity on growth of salmon post-smolts and only a small number of salinity treatments were examined (0, 10 and 30 ppt, McCormick et al. 1989; 0 and 30 ppt, Krogdahl et al. 2004). Furthermore, of the studies that have examined the effect of salinity on growth of salmon, they have been of limited durations (6 weeks, McCormick et al. 1989; 4 weeks, Krogdahl et al. 2004) so no study has so far investigated how a wide range of salinities may affect growth of salmon post-smolts through to market size in RAS. Photoperiod is commonly manipulated in salmon aquaculture to stimulate growth and suppress sexual maturation, but its effect on growth has only been studied in conjunction with temperature in Atlantic salmon up to ~ 600 g (Handeland et al. 2013; Imsland et al. 2014; Døskeland et al. 2016). Therefore, the interactive effects of salinity and photoperiod on fish growth from smolts to market size still remains unexplored.

1.2 Salinity

Salmon hatch and spend their early life stage in freshwater (roughly 0 parts per thousand (ppt) salinity) for 2 to 4 years, until they smolt and migrate downstream to seawater (35 ppt) (Noble et al. 2009). After adult salmon become sexually mature after 1 or more years in the ocean, they will migrate back to freshwater to spawn (Noble et al. 2009). Therefore, salmon experience a large variation in salinity during their life cycle. Despite changes in the environmental salinity, salmon actively osmoregulate and maintain the blood osmolality at a relatively constant level (~350 mOsm

kg^{-1}, which is equivalent to 11-14 ppt) (Gordon 1977), which is accomplished by a number of physiological mechanisms.

When salmon are in the water that is hyposmotic relative to their blood plasma (e.g., freshwater), they passively gain water by osmosis and lose ions to the environment across the gills and skin by diffusion. To counteract the movement of water into the fish, the kidney of freshwater salmon produces large quantities of dilute urine (Duffy et al. 2011). The fish kidney also plays a role in compensating for ion loss in hyposmotic water by reabsorbing ions in the distal nephron (Nishimura and Fan 2003) and potentially also in the proximal nephron (Marshall and Grosell 2006). Additionally, pavement cells and mitochondria-rich cells (MRC) in gills actively absorb ions, including Na^+, Cl^- and Ca^{2+} (Laurent et al. 1985). MRCs express a high activity of Na^+, K^+-ATPase (NKA) which uses ATP to assist in the uptake of ions from freshwater (McCormick et al. 2009). Perry (1998) found freshwater fish had an increased number of MRCs in the gills, and believed that the proliferation of MRCs can not only promote ion uptake at gills, but also thicken the gill lamellae, which further reduces the passive ion loss to the environment. Freshwater fish also compensate for ion loss by obtaining ions from the diet across the intestine (Flik and Verbost 1993; Bucking and Wood 2007).

When salmon are in water that is hyperosmotic relative to their blood plasma (e.g., seawater), they passively lose water by osmosis and gain ions from the environment across the gills and skin by diffusion. To compensate for water loss, seawater fish drink (Nobata et al. 2013). The kidney of marine fish plays a role in osmoregulation: water is reabsorbed in the proximal region of the

nephron, while divalent cations (i.e., Ca^{2+} and Mg^{2+}) are excreted into the renal tubule and eventually into the surroundings via concentrated urine (Duffy et al. 2011). As described before, MRCs in gills are capable of absorbing ions when fish are in freshwater, whereas they are also the primary site of ion excretion for fish in seawater (McCormick et al. 2009). Besides NKA, there are also other ion transport pathways in MRCs (e.g., Na^+-K^+-$2Cl^-$ cotransporter and cystic fibrosis transmembrane conductance regulator), and all of these transporters form a sophisticated but complete system for ion excretion (Hwang et al. 2011). The intestine of marine teleosts also contributes to osmoregulation. In seawater fish, the intestinal epithelia secrete bicarbonate to form precipitates with Ca^{2+} and Mg^{2+} in the intestine (Grosell et al. 2001), which further reduces osmolality in the intestinal fluids, promoting water absorption and preventing absorption of these divalent cations across the gut (Brauner et al. 2013).

It is well understood that the numerous physiological mechanisms required for osmoregulation in freshwater and seawater are energy-consuming, but the precise costs associated with osmoregulation are debated. Bœuf and Payan (2001) reviewed all the data available at the time and suggested that most studies estimate the costs of osmoregulation to comprise between 20 and 50% of the total resting metabolic rate, with a few theoretical estimates at a lower osmoregulatory cost of about 10% of total metabolic rate (Kirschner 1993). In theory, when ambient salinity is iso-osmotic (~10 ppt) to blood plasma, the costs of osmoregulation should be reduced. Therefore, energy might be saved from costly osmoregulation in fish acclimated to iso-osmotic salinity, and the spared energy could be reallocated to other physiological processes, such as growth.

The effects of salinity on growth have been studied in a variety of fish species but the findings are species-dependent. Studies on rainbow trout (*Oncorhynchus mykiss*) (McKay and Gjerde 1985), rainbow and steelhead trout (Morgan and Iwama 1991) and Abant trout (*Salmo trutta abanticus*) (Kocabas et al. 2011) revealed that these fish species generally had the highest growth rate in freshwater and the growth rate decreased with increasing salinity up to seawater. Juvenile turbot (*Scophthalmus maximus*) were found to have an higher growth rate at intermediate salinities between 10 and 19 ppt, compared with seawater (Gaumet et al. 1995). Lambert et al. (1994) also reported that Atlantic cod (*Gadus morhua*) reared at intermediate salinity (~ 14 ppt) had an enhanced specific growth rate which was up to 63% higher than the ones reared at 28 ppt. However, Arnesen et al. (1993) found no effect of salinity (10 to 35 ppt) on the growth of Arctic charr (*Salvelinus alpinus*) with a mean body weight of 150 g. In terms of the salinity effect on growth of salmon, McCormick et al. (1989) investigated growth of Atlantic salmon post-smolts (23–70 g) over 6 weeks at different salinities (0, 10 and 30 ppt) and feeding rations, and they found that salinity did not affect growth when the feeding ration was under 3.1% dry weight per day, while fish reared at 10 ppt had the lowest growth at the highest feeding ration of 5.1% dry weight per day. Another 4-week growth trial (0 and 30 ppt) on Atlantic salmon (199 g) showed that seawater fish had a faster growth rate than freshwater fish with either a low or high carbohydrate diet (Krogdahl et al. 2004). In contrast, an 8-month growth trial found coho salmon fry reared at 5 to 10 ppt had the highest growth rate over the pre-smoltification period (Otto 1971). From these data, it appears as if there is no consistent effect of salinity on the growth of salmon, which may be due to these studies also differing in many other aspects (e.g., feeding ration, species, life stage). A previous study, Emerman (2016), grew a cohort of Atlantic and coho salmon smolts at different salinities (0, 5, 10, 20 and 30 ppt) under 24 h photoperiod up to 5 months in RAS to determine the

effect of salinity on the early growth stages of both species (Figure 1.1). Emerman (2016) found that salinity did not affect the growth of Atlantic salmon during the first 96 days of salinity exposure (Figure 1.1, A). In contrast, in coho salmon, salinity did have an effect on growth over the first 156 days of salinity exposure, with those animals reared at iso-osmotic salinity (10 ppt) showing the highest body mass gain (Figure 1.1, B). The current thesis continued the growth trial started by Emerman 2016 in order to investigate the effect of salinity on the later growth stages of both species in RAS. Despite the importance of salmon in aquaculture, information about the effects of salinity on salmon growth is still very limited, and nothing is known about how salinity affects growth and performance of salmon from smolts through to market size in RAS.

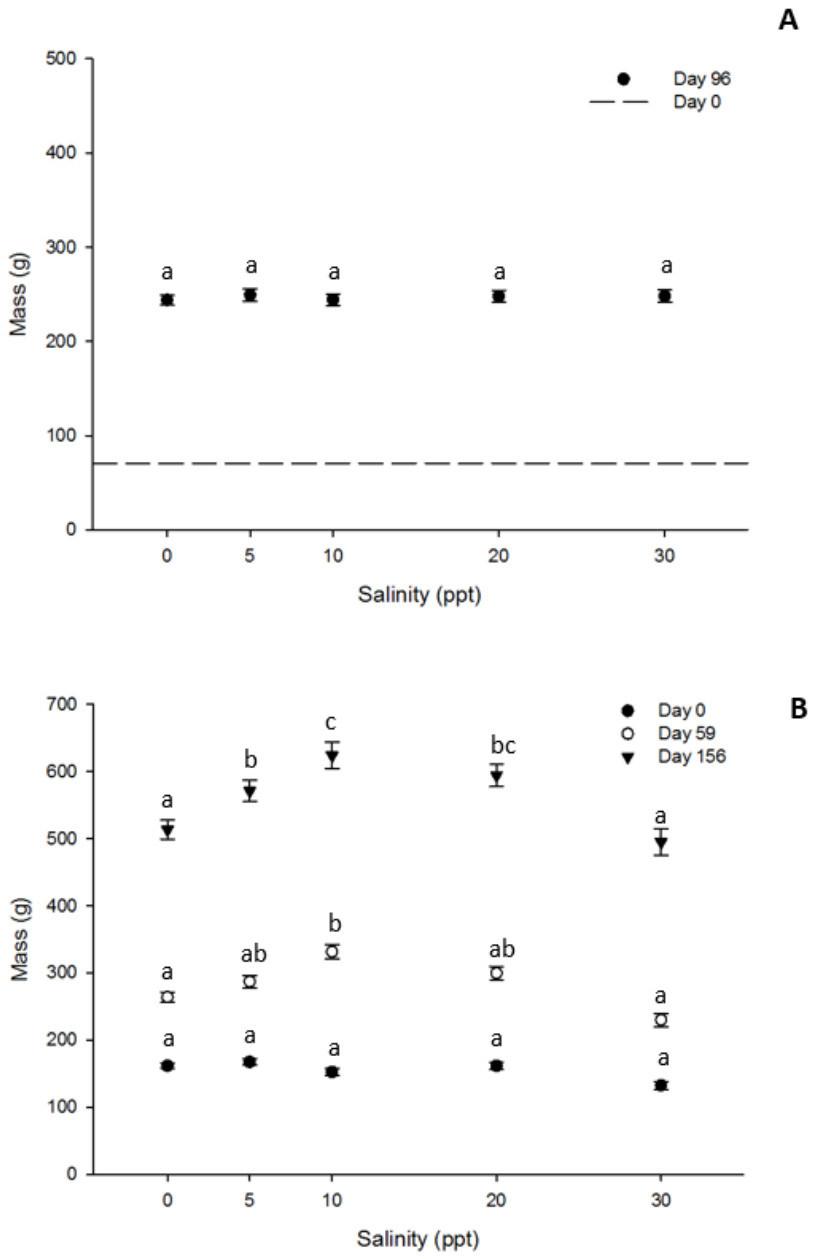

Figure 1.1 Body mass (n = 100) of Atlantic and coho salmon reared at salinities of 0, 5, 10, 20 and 30 ppt during the early growth trial (Emerman 2016). Atlantic salmon (A, top) were sampled for body mass at Day 0 and 96. The Dashed line represents mean body mass (70.58 g ± 1.11) of Atlantic salmon at the time that they were distributed into different salinities (i.e., Day 0). Coho salmon (B, bottom) were sampled for body mass at Day 0, 59 and 156. All data are mean ± SEM. Letters that differ indicate statistically significant differences within a given sampling time period ($P < 0.05$).

1.3 Metabolic Rates and Respirometry

Measurements of metabolic rate reflect the total energy turnover associated with ongoing physiological processes, which is influenced by the state of the animal. Standard metabolic rate (SMR) describes the energy turnover associated with the maintenance of a fish's basic needs without digestion, movement or sexual activities (Chabot et al. 2016b). However, SMR is nearly impossible to measure in active fish species like salmon which need to move their fins to maintain their vertical position in the water. In contrast, routine metabolic rate (RMR) has been widely used and is defined as the oxygen consumption rate of the fish that have minimal necessary movement (Brett 1962). Maximum metabolic rate (MMR) is defined as the maximal oxygen consumption rate of fish at exhaustion during or immediately after intensive exercise. Swim-tunnel respirometry and manual exhaustive exercise are the two major ways to induce and measure MMR in fish (Norin and Clark 2016). In swim-tunnel respirometry, flow rate in the swim-tunnel is gradually increased to a point where the fish can no longer swim against the current and fall back to the grid of the swim-tunnel. Metabolic rate of the fish is measured either as the highest attainable oxygen consumption rate observed during swimming or the oxygen consumption rate measured upon exhaustion. By contrast, in the exhaustive exercise protocol, the fish is placed in a circular container and chased by hand or fish net until the fish is unable to burst swim. At exhaustion, the fish is transferred into a respirometer for a measurement of the post-exercise oxygen consumption rate, which is assumed to be equivalent to MMR. The difference between MMR and SMR (or RMR) is defined as aerobic scope (AS) (Eliason and Farrell 2016). AS indicates the metabolic capacity in an animal to support its additional energy-consuming processes (e.g., growth and reproduction) beyond its basic needs (Rosewarne et al. 2016). The implications of differences in

aerobic scope was first described by Fry (1947), and it has been a useful tool to explore how environmental changes such as increasing temperature influence the metabolic capacity of an organism. The aerobic scope curve formed over a wide range of temperatures was named the Fry aerobic scope curve to honor its importance (Farrell 2009). The Fry curve is bell-shaped as a function of temperature, and the temperature where the peak of the curve (maximum AS) occurs is termed optimal temperature (T_{opt}) (Farrell 2016). The theory of oxygen and capacity-limited thermal tolerance (OCLTT), which is based on the idea of the Fry curve, further describes the loss of aquatic animals' capacity to deliver oxygen to their tissues when temperatures move towards what is considered extreme for that species (Pörtner, 2001; Pörtner and Farrell 2008; Pörtner 2010; Farrell 2016).

Although the analysis of AS has proven useful in many studies for assessing the impacts of environmental temperatures on fish's metabolism (e.g., Farrell 2009; Eliason and Farrell 2016), far fewer studies have assessed the impacts of other environmental conditions on AS. For example, even though salinity is an important environmental factor for many fish species, only a few studies have investigated the impact of salinity acclimation on AS and in only a few species: European sea bass (*Dicentrarchus labrax*) (Claireaux and Lagardère 1999), barramundi (*Lates calcarifer*) (Norin et al. 2016), killifish (*Fundulus heteroclitus*) (Brennan et al. 2016) and perch (*Perca fluviatilis*) (Christensen et al. 2017). Nothing is known about the effects of salinity on AS of salmon. Despite the lack of information on the effect of salinity of AS, numerous studies have examined the effects of salinity on RMR and/or SMR measurements in several species including: Rainbow trout (*Oncorhynchus mykiss*) (Rao 1968; Rao 1971; Morgan and Iwama 1991), Mozambique tilapia (*Oreochromis mossambicus*) (Fiess et al. 2007; Zikos et al. 2014), California Mozambique

tilapia (*Oreochromis mossambicus* x *O. urolepis hornorum*) (Sardella and Brauner 2008) and several species in the family Cyprinodontidae (Haney and Nordlie 1997; Plaut 2000; Nordlie 2014). In terms of salmon, Morgan and Iwama (1991) found salinity (0 - 28 ppt) had no effect on routine metabolic rate of Chinook salmon (*Oncorhynchus tshawytscha*) fry (~ 6 g). Similarly, another study reported that there was no difference in routine metabolic rate of juvenile coho salmon (~ 20 g) acclimated in freshwater, brackish water and seawater (Morgan and Iwama 1998). The few studies related to salmon only focused on the juvenile stage, so no information is available regarding the effect of salinity on metabolism of salmon at various stages from smolt through to market size adults and it is still largely unclear that how metabolic energy allocation is influenced by environmental salinity in salmon.

Metabolic rate can be measured directly by the traditional calorimetry which measures the heat produced by all chemical reactions occurring in animals, but calorimetry is especially difficult to perform on aquatic organisms (Nelson 2016). The indirect methodology, or respirometry, measures the oxygen consumption of fish to estimate their metabolic rate. Oxygen consumption rate measured by respirometry only accounts for aerobic metabolism in animals and therefore excludes anaerobic metabolism which does not depend on oxygen to produce ATP. However, under normal conditions, aerobic metabolism plays a much more essential role than anaerobic metabolism in organismal function as it produces ATP much more efficiently (Chabot et al. 2016a), so respirometry can generally well reflect the metabolic rate of fish .

Closed, flow-through and intermittent respirometry are three major types of technologies that have been used in respiratory measurements of fish. In a closed respirometry protocol, a fish is placed in a sealed respirometer and oxygen decline is measured over time. Closed respirometry has advantages of low cost, simple design and easy operation, but if the measurement lasts for a long period of time, the fish will experience issues including hypoxia, elevated carbon dioxide (hypercapnia) and accumulated nitrogenous products (ammonia and nitrite) in the water, which potentially compromises experimental results (Svendsen et al. 2016). Flow-through respirometry measures the difference between inflowing and outflowing oxygen tensions of the respirometer. If the flow rate is known, the oxygen consumption rate of the fish can be calculated. Flow-through respirometry has continuous clean water flowing through the chamber, so it does not have the issues discussed previously in the closed respirometry (oxygen depletion and accumulated metabolic waste). However, maintaining a proper ratio of flow rate to respirometer volume is very important to minimize the error caused by disturbances (e.g., fish transference into respirometer and changes in metabolic rate of the fish) (Steffensen, 1989; Eriksen 2002). A high ratio of flow rate to volume enables the system to quickly response to changes in oxygen tensions, but the respirometer volume is constrained to the fish size and the flow rate should be within a range where the difference between oxygen tension readings is still detectable (Eriksen 2002). Intermittent respirometry is the combination of closed and flow-through respirometry. In intermittent respirometry, the measurement of oxygen reduction is carried out when the respirometer is closed, followed by a period to flush the respirometer with new water, which also removes the metabolic waste produced by fish. During the measurement of oxygen reduction, even though the respirometer is closed, water inside is still recirculated by a water pump to ensure the water is well mixed. Respirometry without a mixing device was found to yield highly variable measurements of

oxygen consumption rate and dramatically underestimate the results compared to the ones with a mixing device (Rodgers et al. 2016). Oxygen tension is usually measured with one oxygen probe in intermittent respirometry, which makes it easier to set up compared to flow-through respirometry with two probes. The design, setup and related calculations for intermittent respirometry were discussed in details by Svendsen et al. (2016). Steffensen (1989) compared the benefits and drawbacks of the three types of respirometry and highly recommended using intermittent respirometry to measure oxygen consumption of aquatic animals.

1.4 Hypoxia

Using RAS to rear salmon is costly, so a high stocking density is often maintained to maximize production and profitability. When a high biomass is maintained in RAS, there is an increasing risk for water quality deterioration. Under high stocking densities, hypoxia can be commonly seen in RAS and other aquaculture systems. Exposure to a hypoxic environment can compromise fish health or cause mortalities. Even mild or periodic hypoxia has been shown to have impact on fish growth and performance. For example, Atlantic salmon post-smolts reared under periodic hypoxia were found to have reduced feeding, growth (Remen et al. 2014) and compromised leucocyte function (Burt et al. 2013). Therefore, it is important to understand how hypoxia tolerance of fish can be altered by rearing conditions in RAS.

Fish have multiple behavioural, morphological, physiological and biochemical strategies to survive hypoxia (Richards et al. 2007). When the water first becomes hypoxic, many fish species

will attempt to behaviourally acquire more oxygen by ventilating the more oxygenated water at the water-air interface, which is defined as aquatic surface respiration (ASR) (He et al. 2015). In response to hypoxia, fish can also raise the frequency and amplitude of the buccal-opercular pump to increase ventilation. At the physiological and biochemical level, fish will downregulate energy-consuming pathways to save energy in response to reduced oxidation of carbohydrates, lipids and amino acids for energy production in hypoxia (Mandic et al. 2013). When oxygen is limited, aerobic metabolism becomes ineffective at generating metabolic energy to meet demands, so fish will switch to anaerobic metabolism for energy production. Therefore, in hypoxia, ATP production is maintained primarily by increased enhanced fermentation which produces lactate and maintains glycolytic flux (Richards et al. 2007).

There are several ways to measure hypoxia tolerance in fish. Fish can be categorized into oxygen conformers and regulators based on their responses of metabolic rate to the decreasing oxygen level in the water. Specifically, when the oxygen level decreases, oxygen conformers will reduce the metabolic rate, while oxygen regulators will maintain a relatively stable metabolic rate until the critical oxygen tension (P_{crit}) is reached. When the oxygen level decreases below P_{crit}, oxygen regulators reduce their metabolic rate with the decreasing oxygen level (Barnes et al. 2011). In general, hypoxia tolerance is negatively correlated to P_{crit}, whereby hypoxia tolerant fish usually have lower P_{crit}. As water oxygen levels decrease, fish eventually display an inability to maintain its vertical position in the water, called loss of equilibrium (LOE) (He et al. 2015). Other measurements of hypoxia tolerance are based on the incidence of LOE: the oxygen tension where LOE occurs (LOE_{crit}) and time to LOE at a constant level of hypoxia (He et al. 2015; Borowiec et al. 2016).

Hypoxia tolerance of fish could be influenced by the oxygen demand for routine physiological activities (RMR, the load). If a fish has very low routine metabolic costs, less oxygen is required to maintain routine physiological processes, so fish can remain functional longer when water oxygen is limited. On the other hand, the capacity to extract oxygen (MMR, the limit) might also affect hypoxia tolerance of fish. If a fish has a very efficient oxygen extraction, more oxygen will be available for physiological processes when needed. Capacity of oxygen extraction could be influenced by the thickness of gill lamella, which can be explained by the osmorespiratory compromise describing the trade-off between the ionoregulatory functions and gas exchange of the gills (Wood and Grosell 2015). When there is an osmoregulatory challenge in ion-poor water, proliferation of MRCs at fish gills can occur for two purposes: (1) to promote active ion uptake and (2) to restrict passive ion loss by thickening gill lamella (Perry 1998). Thickening gill lamella in freshwater-acclimated fish increases the water-to-blood diffusion barrier, which restricts passive movement of ions and water thereby potentially reducing osmoregulatory costs, but impairs oxygen uptake at gills, as the gas exchange efficiency at gills is negatively correlated to the water-to-blood barrier (Henriksson et al. 2008). Hence, fish with thicker gill lamella (reduced oxygen extraction and hence MMR) might have reduced hypoxia tolerance. However, most of the studies on the osmorespiratory compromise have been conducted on freshwater fish (Greco et al. 1995; Greco et al. 1996; Perry 1998; Wood et al. 2009), with only a few studies that have used fish acclimated to brackish or seawater (Henriksson et al. 2008). Eventually, considering RMR and MMR together, hypoxia tolerance of fish could be associated with AS, where fish with higher AS (low RMR and/or high MMR) have more capacity for stressors and thus may have enhanced hypoxia tolerance. Despite the fact that hypoxia is a commonly-seen stressor in fish culture,

hypoxia tolerance of salmon associated with different rearing salinities still remains unknown. If this aspect is better understood, it could help with managing the risk posed by hypoxia in the salmon farming.

1.5 Photoperiod

Photoperiod is regarded to be the most important environmental cue for modulating circadian rhythms in animals, including fish (Castanheira et al. 2011). Interestingly, circadian rhythms of fish can show a great plasticity in response to environmental change (Reebs 2002). In fish, the patterns of activities change based on the day/night cycle, and fish can be categorized into diurnal, nocturnal and crepuscular dependent on when they are active (Reebs 2002). Metabolic rate reflects the number of ongoing behavioral, physiological and biochemical activities in fish body. Since fish can alter their activities based on the circadian clock, photoperiod would have a potential impact on the metabolic rate of fish. For example, routine metabolic rate was to be highest during the dark in the nocturnal Senegalese sole (*solea senegalensis*) (Castanheira et al. 2011). Another study found that Nile tilapia (*Oreochromis niloticus*) also showed a circadian pattern in their metabolic rate which was higher during the day and lower during the night (Ross and McKinney 1988).

Photoperiod is widely manipulated in salmon aquaculture to supress early sexual maturation. Sexually mature salmon are extremely undesirable in the aquaculture industry because early maturation slows growth, reduces the feed conversion ratio and results in deterioration of the fish

muscle (Good et al. 2016). Seasonal changes in day length are believed to be the main environmental cue of sexual maturation in most of fish species (Bromage et al. 2001). Therefore, as opposed to natural photoperiod, a variety of artificial photoperiod regimes are used in salmon farming to prevent early sexual maturation. Many studies have found that extended photoperiod relative to natural photoperiod is most efficient in suppressing early maturation of salmonids (Guerrero-Tortolero and Bromage 2008; Good et al. 2016; Unwin et al. 2005; Björnsson et al. 1994). Some studies, however, showed that extended photoperiod can actually accelerate maturation (Duncan et al. 1999; Imsland et al. 2014). Photoperiod is believed to regulate sexual maturation of fish by affecting the excretion of gonadotropin-releasing hormone (GnRH). The hypothalamic-pituitary-gonadal axis plays a pivotal role in reproduction of vertebrates. GnRH is secreted from the hypothalamus of the brain, and GnRH further stimulates the pituitary gland to release two types of gonadotropins (GTHs), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Ando and Urano 2005). FSH stimulates ovarian follicles to secrete estradiol-17 β (E2) which promotes the liver to produce vitellogenin for the initiation of maturation, while LH is more responsible for inducing the final stage of maturation (Choi et al. 2010). Reduced photoperiod has been found to elevate the mRNA level of GnRH in masu salmon (*Oncorhynchus masou*) (Amano et al. 1995), as well as in rainbow trout (*Oncorhynchus mykiss*) (Choi et al. 2010). In contrast, another study showed that the mRNA expression of GnRH and other reproductive hormones increased under an extended photoperiod regime in goldfish (*Carassius auratus*) (Shin et al. 2014). In addition to preventing early sexual maturation before harvesting, photoperiod is also often manipulated to stimulate growth in fish farming. Freshwater Atlantic salmon parr-smolts were found to have about 15% more growth over 5 months under continuous photoperiod, compared with fish reared at the natural photoperiod (Stefansson et al. 1991). A similar growth

enhancing effect of continuous photoperiod was also seen in seawater-acclimated Atlantic salmon smolts which had an increase of 19% to 178% (varied in time and strains) in specific growth rate, compared with the natural photoperiod (Handeland et al. 2003). The growth enhancing effect of continuous photoperiod was documented in other species as well, including rainbow trout (*Oncorhynchus mykiss*) (Noori et al. 2015), Atlantic halibut (*Hippoglossus hippoglossus* L.) (Simensen et al. 2000; Gústavsson et al. 2010), and Atlantic cod (*Gadus morhua*) (Imsland et al. 2007). Noori et al. (2015) suggested this positive effect of extended photoperiod might be associated with an improved appetite or a better food assimilation in fish.

Photoperiod manipulation for maturation suppression and growth stimulation in salmon farming plays an important role and has been extensively studied. However, nothing is known about whether the photoperiod-induced changes in maturation and growth can be associated with a metabolic shift in salmon, and furthermore how that will affect welfare of fish including hypoxia tolerance. Since salmon are widely cultured, it is important to know the metabolic and welfare status of salmon reared under different photoperiod regimes. Also, nothing is known so far about how photoperiod can interact with rearing salinity to affect the growth of salmon, which would contribute to a better understanding of salmon aquaculture from a physiological perspective of view.

1.6 Thesis Objectives and Predictions

The general goal of this thesis was to determine the effects of salinity and photoperiod on growth, aerobic scope and hypoxia tolerance of Atlantic and coho salmon reared in RAS from smolt to market size. The current study aimed to define the optimal salinity and photoperiod for growing salmon in RAS, which largely helps the industry to reduce the duration of the production cycle or improve feed conversion efficiency. In addition, the respirometry and hypoxia tolerance trials contribute to a better understanding of the physiological and welfare status of salmon reared under different environmental conditions in RAS.

As described above, in the early 2015-2016 growth trial, Emerman (2016) found no effect of salinity on growth of Atlantic salmon, but an enhanced growth on coho salmon at iso-osmotic salinity (Figure 1.1). However, Emerman (2016) only followed these fish for 96 to 156 days of exposure to the various salinities. Therefore, the current thesis continued the 2015-2016 growth trial and aimed to see if the growth-enhancing effect of iso-osmotic salinity would present in Atlantic salmon during their late growth stages, and if the continuation of this effect could still be seen in the larger coho salmon. In addition, respirometry was carried out on both species, the aim of which was to determine whether salinity had an effect on metabolic rate and aerobic scope. Hypoxia tolerance was also determined by measuring time to loss of equilibrium (LOE) of both species in hypoxic water with corresponding rearing salinities.

In the 2016-2017 growth trial, the interactive effects of salinity and photoperiod (12:12 and 24:0 light : dark) were assessed in a new cohort of Atlantic and coho salmon (the 2015-2016 growth trial was only under 24:0 photoperiod). This was done for several reasons: (1) to replicate the

previous growth trial and validate the effect of salinity that was previously observed; (2) to test if salinity has an effect on aerobic scope and hypoxia tolerance of coho salmon during the early growth stages where salinity had the greatest impact on growth; (3) to see how photoperiod can interact with salinity to influence growth, aerobic scope and hypoxia tolerance of fish. All data on the effects of salinity and photoperiod on growth of Atlantic and coho salmon were reported in Chan (2018). The current thesis, however, aimed to explore the effects of salinity on aerobic scope and hypoxia tolerance of coho salmon during their early growth. Therefore, respirometry and hypoxia tolerance trials were again conducted with similar but improved setups and protocols on the second cohort of coho salmon. Atlantic salmon were not involved in the 2016-2017 physiological experiments due to insufficient fish number for those experiments.

Figure 1.2 shows a schematic for how I believe growth, aerobic scope and hypoxia tolerance are related in fish reared across different salinities and photoperiods and that serves as predictions for the thesis. In theory, little energy is required for osmoregulation in fish acclimated to iso-osmotic salinity due to reduced osmotic pressure between the aquatic environment and blood plasma of the fish. Therefore, the curve of osmoregulatory cost is predicted to be U-shaped as a function of salinity, with the lowest cost at iso-osmotic salinity. Even though Emerman (2016) found no difference in body mass of Atlantic salmon during the early stages, salinity might have an effect later in the growth trial, while salinity clearly had an effect on coho salmon from the beginning. Therefore, the overall growth of salmon is predicted to be enhanced at iso-osmotic salinity (10 ppt) and reduced as salinity increases or decreases. If there are metabolic saving at iso-osmotic salinity, energy may be re-allocated to growth at a constant routine metabolic rate (RMR). Due to the osmorespitory compromise, the ability to extract oxygen from the water at the gills (MMR) is

predicted to be optimal at iso-osmotic salinity and reduced in freshwater and seawater. If RMR is subtracted from MMR, the curve of AS will also be bell-shaped as a function of salinity, with the peak at iso-osmotic salinity. As AS indicates the metabolic capacity for handing additional environmental stressors, fish with higher AS might have an enhanced hypoxia tolerance, so the curve of hypoxia tolerance will be bell-shaped as well, enhanced at iso-osmotic salinity and reduced in freshwater and seawater. If two photoperiod regimes (12 and 24 h light) are introduced into the experiments, salmon reared under 24 h photoperiod would be predicted to grow faster than those under 12 h, since studies have shown enhanced growth of salmonids under an extended photoperiod (Stefansson et al. 1991; Handeland et al. 2003; Noori et al. 2015). Therefore, as the cost of growth increases under 24 h photoperiod, and osmoregulatory cost and MMR are assumed to be independent of photoperiod, RMR would correspondingly increase, which further leads to a decrease in AS and hypoxia tolerance (Figure 1.2).

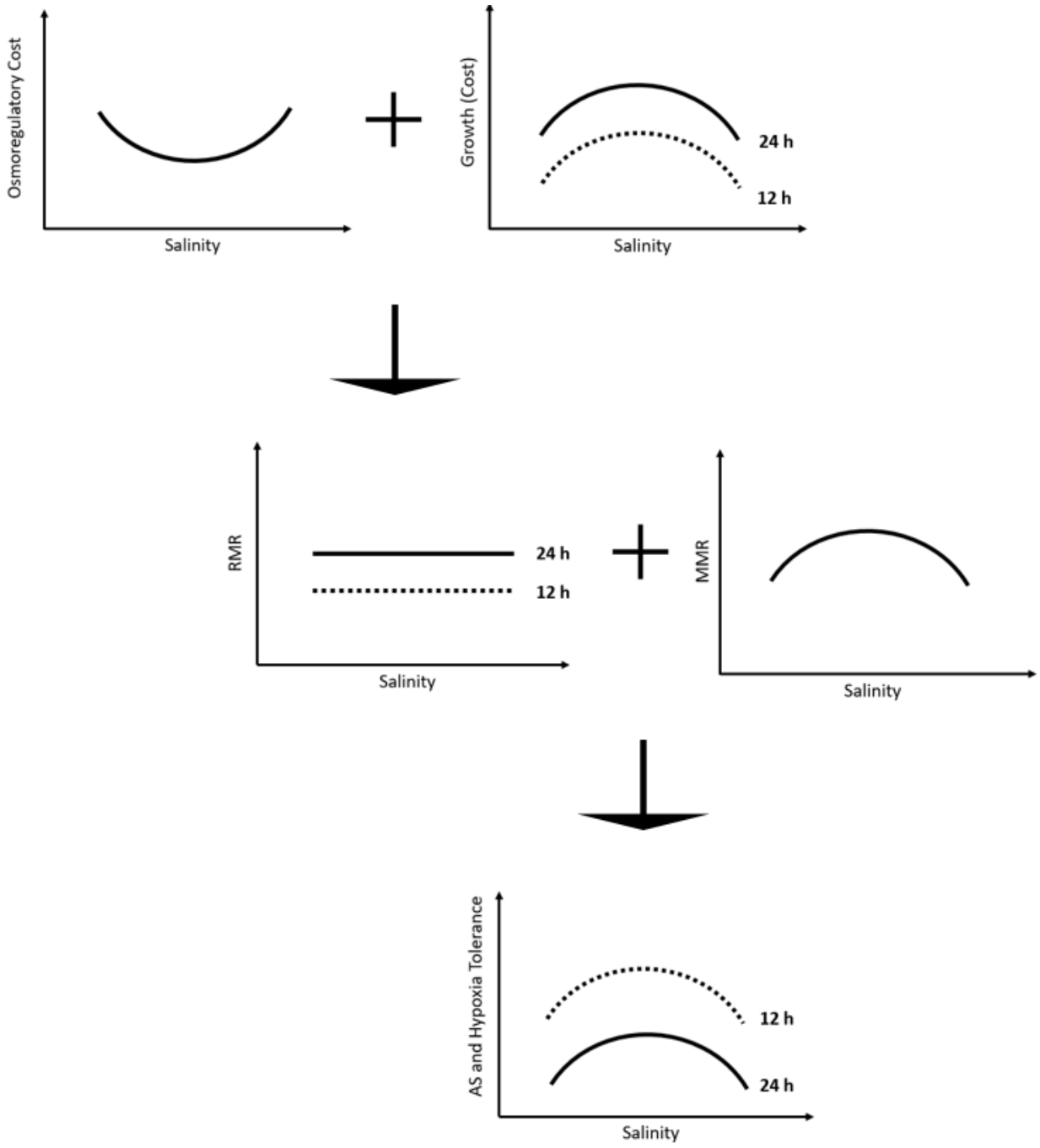

Figure 1.2 Predictions of growth, osmoregulatory cost, routine metabolic rate (RMR), maximum metabolic rate (MMR), aerobic scope (AS) and hypoxia tolerance of Atlantic and coho salmon reared at a wide range of salinities under 12 and 24 h photoperiods. Dashed and solid lines represent 12 and 24 h photoperiods, respectively. Predictions are based on two assumptions: (1) growth and osmoregulation are the two major energy-consuming activities; (2) photoperiod has no impact on osmoregulatory cost and MMR.

Chapter 2: The Effects of Salinity on Growth, Aerobic Scope and Hypoxia Tolerance in Atlantic and Coho Salmon

2.1 Introduction

The production of seafood from capture fisheries has remained largely unchanged since the late 1980s, while aquaculture has been steadily developing to supply seafood for human consumption (FAO, 2016a). Salmon are an important aquaculture species with a global farmed salmon production of 23 million tonnes in 2014 (FAO, 2016b). In Canada, British Columbia produces the most salmon with 92,926 tonnes produced in 2015 which comprised about 76% of Canadian farmed salmon (Statistics Canada, 2015). Traditional salmon aquaculture employs open net pens which have been criticized for a number of ecological issues including pollution and escapees (Naylor et al. 2005; Folke et al. 1994; Kutti et al. 2008). Recirculating aquaculture systems (RAS) are an emerging technology to grow fish on land in aquaculture. RAS permits better control of outflow waste and no risk of escapees from the fish farms, while the capability to manually control water parameters (e.g., temperature, salinity, photoperiod and pH) in RAS provides the potential to rear fish under ideal conditions. RAS, however, is expensive, thus fish production and feed conversion need to be maximized for economic viability. Growing fish under optimal conditions for fast growth is one of the ways to maximize the production.

Salinity has a great potential to be manipulated in aquaculture to affect growth of salmon and achieve production efficiency because salmon can tolerate a wide range of salinities from 0 to 35 ppt and osmoregulation is energy-consuming, which affects energy distribution and hence growth of fish. When there is an osmotic difference between the external aquatic environment and internal blood plasma, passive movement of ions and water will occur across the fish's integument, which must be compensated. There are many mechanisms and organs (e.g., gills, kidney and intestine) involved with osmoregulation in fish; however, all require active transport of ions and thus osmoregulation may be costly. There are many estimates of the metabolic cost of osmoregulation in fish, but they vary quite dramatically. Some estimates are as low as 0.5% of resting metabolic rate (Eddy 1982), while others are as high as 70% (Bushnell and Brill 1992). A theoretical model that incorporated whole animal ionic flux rates and presumed ATP requirements in a freshwater fish yield an estimated cost of osmoregulation around 10% of standard metabolic rate (Kirschner 1993), a value that is likely closer to the real value (Bœuf and Payan 2001). In theory, if fish are reared at iso-osmotic salinities (~ 10 ppt) where osmotic gradients are minimized, osmoregulatory costs should be reduced and energy could be allocated to other physiological processes including growth. A growth enhancing effect of intermediate salinities on fish growth has been reported in many studies (Otto 1971; Lambert et al. 1994; Gaumet et al. 1995; Emerman 2016). However, the current knowledge about how salinity can affect growth of salmon from smolt through to market size in RAS still remains unknown and this is a central focus of my thesis.

Respirometry is a useful tool to understand how energetic cost and capacity are associated with changes in environmental conditions. Routine metabolic rate (RMR) estimates the metabolic cost of minimal but necessary activities in fish, while maximum metabolic rate (MMR) measures the

maximum oxygen consumption rate of a fish at exhaustion post-exercise. The difference between maximum and routine metabolic rates is aerobic scope (AS) which reflects the metabolic capacity available for additional activities above routine. Respirometry has been widely conducted to study the effect of temperature on metabolism in fish associated with global warming, but little is known about how salinity can affect metabolic rates of salmon (Morgan and Iwama 1991; Morgan and Iwama 1998), which I investigate here. Economic viability in RAS requires a high stocking density, and this can result in low oxygen levels if water flow is not high enough or supplemental oxygenation systems malfunction. Therefore, understanding hypoxia tolerance of fish under different conditions is of great importance for animal welfare, as hypoxia has been documented to compromise feeding, growth and immunity of fish (Remen et al. 2014; Burt et al. 2013); however, the related information on how salinity can affect hypoxia tolerance of salmon is currently unavailable.

The objective of this chapter was to continue the growth trial that had been conducted in Emerman (2016) to investigate the effect of salinity on late growth stages (~ 500 to 2,500 g) of Atlantic and coho salmon reared in RAS (wild salmon at these stages usually live in the ocean). Atlantic and coho salmon were reared in separate tanks in RAS at one of five salinities (0, 5, 10, 20 or 30 ppt) under 24 h light. Every 3 months, fish were sampled for body length and mass to calculate growth (growth rate, specific growth rate and thermal growth coefficient) and feed conversion, while sexual maturation and cataracts were also assessed during the samplings. In addition, respirometry was conducted to measure the routine metabolic rate, maximum metabolic rate and aerobic scope of fish reared at different salinities. Fish were also exposed to progressive hypoxia down to 10% air saturation at all salinities, after which point time to loss of equilibrium (LOE) was measured as

an indicator of hypoxia tolerance to determine how it was affected by salinity. The results of this chapter will help the aquaculture industry to define the optimal salinity for growth of salmon reared in RAS, as well as to understand the effect of salinity on metabolic performance and hypoxia tolerance of salmon.

2.2 Methods

2.2.1 Fish Rearing System Overview

The InSEAS (Initiative for the Study of the Environment and its Aquatic Systems) is an aquatic facility with seven independent 12,000 L recirculating aquaculture systems at the University of British Columbia. Each recirculating system consists of two 5 m^3 and two 0.7 m^3 fiberglass fish tanks. The 5 m^3 fish tanks were used to continuously rear fish and the 0.7 m^3 fish tanks were used to temporarily hold fish for respirometry and hypoxia challenge trials. Each system contained a water sump where oxygen, pH and salinity probes were located and used to remotely and continuously monitor water quality.

Outflow from fish tanks was mechanically filtered by a radial flow separator (coarse filtration), a micro-bead filter (fine filtration) for any solid waste, and a foam fractionator for organic compounds. The systems also contained a degasser for removing carbon dioxide, two bio-filters for nitrification and denitrification, an oxygenator, a pH controller for stabilizing pH by dosing sodium bicarbonate ($NaHCO_3$) into water, an ultraviolet (UV) sterilizer, and a heat exchanger for regulating temperature. An automatic feeder (T Drum 2000 feeder, Arvo-Tec, Finland) was suspended above each 5 m^3 fish tank, and a feeding control system (Arvo-Tec Professional Feeding Control System, Arvo-Tec, Finland) was used to deliver a pre-determined amount of feed regularly throughout the day (see below). Illumination was provided by fluorescent lighting on the ceiling of the fish rooms.

2.2.2 Growth Trial

In December 2014, coho salmon smolts with a mean body mass of 40 g were obtained from Target Marine Hatcheries (Sechelt, British Columbia, Canada) and transported to the aquatic facility at the University of British Columbia. Fish then were randomly and evenly distributed into 5 independent RAS. Each RAS has two 5 m^3 fish tanks and coho salmon were held in one of the two tanks in each RAS with about 600 fish per tank (the other tank in each system was used to hold upcoming Atlantic salmon). Salinities in the five recirculating systems were maintained at one of 0, 5, 10, 20 and 30 ppt by adding sea salt (Instant Ocean® Sea Salt, Blacksburg, VA) into the systems. In each system, pH and temperature were maintained at around 7 and 12 ℃ respectively, while photoperiod was kept at 24 h by fluorescent lighting in the rooms. It took time for beneficial bacteria in the biofilters to establish their population and remove ammonia efficiently, so to keep ammonia levels low, fish were only given feed of 0.2% of body mass per day by the automatic feeders for the first 6 months.

Once the biofilters were established and began to efficiently remove nitrogenous wastes, fish were fed with a daily dose of 1% of body mass. At this time point, 100 coho from each tank were randomly selected and placed in light anesthesia (0.1 g L^{-1} tricaine methanesulfonate (MS-222) buffered with 0. 2 g L^{-1} sodium bicarbonate). Once fish were anesthetized, fork length and body mass were measured for calculations of growth rates and feed conversion. Fish were then transferred to a barrel of well-oxygenated water (of their respective rearing salinities) for recovery, after which they were returned to their rearing tanks. This first measurement is referred to as Day 0 for the growth trial. Beyond this time point, length and mass were repeatedly measured every

two or three months at Day 59, 156, 257, 355 and 460. Data for Day 0, 59 and 156 were previously reported in Emerman 2016 while the current study conducted the measurements at Day 257, 355 and 460. During the entire growth trial, stocking density was calculated after each sampling. If the stocking density was over 40 kg m^{-3}, any extra number of fish were randomly selected and euthanized with an overdosed MS222 solution of 0.2 g L^{-1} tricaine methanesulfonate buffered with 0.4 g L^{-1} sodium bicarbonate, in order to keep the stocking density consistent among all the systems.

In June 2015, Atlantic salmon smolts were obtained and transported from Omega Pacific Hatchery (Great Central Lake, Port Alberni, British Columbia, Canada) to UBC. Fish were held in two large flow-through dechlorinated freshwater tanks and treated with formalin periodically to prevent growth of fungus. A month later, 200 fish from each of the two flow-through tanks were randomly selected, anesthetized and measured for body length and mass with the same protocol as discussed above and are referred to as Day 0 values for Atlantic salmon. The 200 sampled fish along with the rest of the population were then randomly distributed into the other one of the two 5 m^3 tanks in each of the five systems of different salinities with about 600 fish per tank (coho salmon were held in the other tank in each system). Thus, within each system, coho and Atlantic salmon were held in two different tanks, but shared the same system water. Atlantic salmon were also fed by automatic feeders with a daily dose of 1% of body mass. Body length and mass were measured at Day 96, 197, 295 and 405. The measurements at Day 0 and 96 were previously reported in Emerman 2016 while the current study conducted the measurements at Day 197, 295 and 405. Similar to coho salmon, the stock density of Atlantic salmon was maintained at around 40 kg m^{-3} through culling.

During the sampling in October 2015, Day 156 for coho salmon and Day 96 for Atlantic salmon, the 100 randomly selected fish from each treatment were implanted with pit tags (Biomark HPT12, ID, USA). The pit tag was inserted into the body cavity posterior to the tips of the pectoral fins. Fish tag IDs were scanned by a reader (Biomark 601 Handheld Reader, ID, USA) and corresponding body length and mass of tagged fish were recorded in a computer program (Tag Manager Software, Biomark, ID, USA). During the later general samplings, all fish that were sampled for length and mass were scanned for the presence of tags. When tags were found, the length and mass of the tagged fish were recorded in the program and used to calculate individual growth rates.

In addition to body length and mass in the general samplings, sexual maturation and cataracts were also identified and quantified for Atlantic salmon at Day 295 and 405, and coho salmon at Day 355 and 460. Sexual maturation was defined as the presence of dark brown skin color, hook on the jaw and soft belly, while cataracts were defined as the presence of white precipitate in the eyes. Also, in the terminal sampling, up to 50 fish (or all fish in the tank if there were less than 50) in each salinity treatment were sampled for gonad mass which was then corrected for body mass as gonadosomatic index (GSI).

2.2.3 Calculations of Growth Related Parameters

2.2.3.1 Growth Rates

Growth rate (GR) was calculated as:

$$GR\ (g\ day^{-1}) = \frac{Mass_2\ (g) - Mass_1\ (g)}{Time_2\ (day) - Time_1\ (day)}$$

where $Mass_1$ and $Mass_2$ are the mean body mass (or body mass of individual tagged fish) from sampling $Time_1$ and $Time_2$, respectively.

To standardize for body size of the fish, specific growth rate (SGR) was then calculated:

$$SGR\ (\%\ day^{-1}) = \frac{\ln Mass_2\ (g) - \ln Mass_1\ (g)}{Time_2\ (day) - Time_1\ (day)} \times 100$$

where $Mass_1$ and $Mass_2$ are the mean body mass (or body mass of individual tagged fish) from sampling $Time_1$ and $Time_2$, respectively.

Furthermore, to standardize for fish body size and rearing temperature, thermal growth coefficient (TGC) was calculated:

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

where $Mass_1$ and $Mass_2$ are the mean body mass (or body mass of individual tagged fish) from sampling $Time_1$ and $Time_2$, respectively, while temperature is the mean rearing temperature (°C) between sampling $Time_1$ and $Time_2$.

2.2.3.2 Condition Factor

In order to know the growth pattern and health of the fish, condition factor was calculated for each fish sampled:

$$Condition\ Factor = \frac{Mass\ (g)}{Fork\ Length\ (mm)^3} \times 10^5$$

2.2.3.3 Economic Feed Conversion Ratio

Economic feed conversion ratio (eFCR) was calculated to estimate the efficiency of fish in converting feed into biomass:

$$eFCR = \frac{Total\ Feed\ Fed\ (kg)}{Total\ Live\ Biomass\ Gained\ (kg) + Total\ Mortality\ Biomass\ Gained\ (kg)}$$

Feeding amount and mortalities were recorded daily. eFCR is an estimation that helps the industry with predicting the feed amount that is required for fish to achieve a certain body mass.

2.2.4 Respirometry

Respirometry was conducted from June to August 2016 which was close to Day 400 for Atlantic and Day 460 for coho salmon in the growth trial. The respirometer chamber (see Figure 2.1) was made of an opaque polyvinyl chloride (PVC) pipe, with a diameter of 15 cm, a length of 82 cm, and a total volume of about 15 L. The design was an intermittent respirometer that consisted of a

single loop with a water pump (Cobalt EXT Inline Pump, 800 lph, Cobalt Aquatics, SC, USA). The Foxy System (Ocean Optics, FL, USA), with an oxygen probe (Foxy-OR125), a cable (BIFBORO-1000-2-Series Bifurcated Fiber Assemblies) and a footprint phase fluorimeter (NeoFox-GT), was used to measure air saturation (% air sat) in the water, while a computer program (NeoFox Viewer, v2.3, Windows 7) was used to display and record the data. The oxygen probe was sealed by a rubber stopper and inserted into a PVC tee next to the water pump. The whole respirometer was submerged in one of the 0.7 m^3 fish tanks associated with the respective RAS system. By doing so, the same rearing tank water was used for respirometry measurements. When the respirometer was in a flushing mode (Figure 2.1, A), the PVC screw connectors were disconnected. Well-oxygenated ambient tank water was sucked into the respirometer via the inflow by the pump displacing deoxygenated water within the respirometer. To convert the respirometer to a recirculating mode (Figure 2.1, B), the PVC screw connectors were connected, and the pump recirculated water inside the respirometer. As a fish was consuming oxygen in the respirometer, the decline in water oxygen level was recorded and used to calculate metabolic rate of the fish.

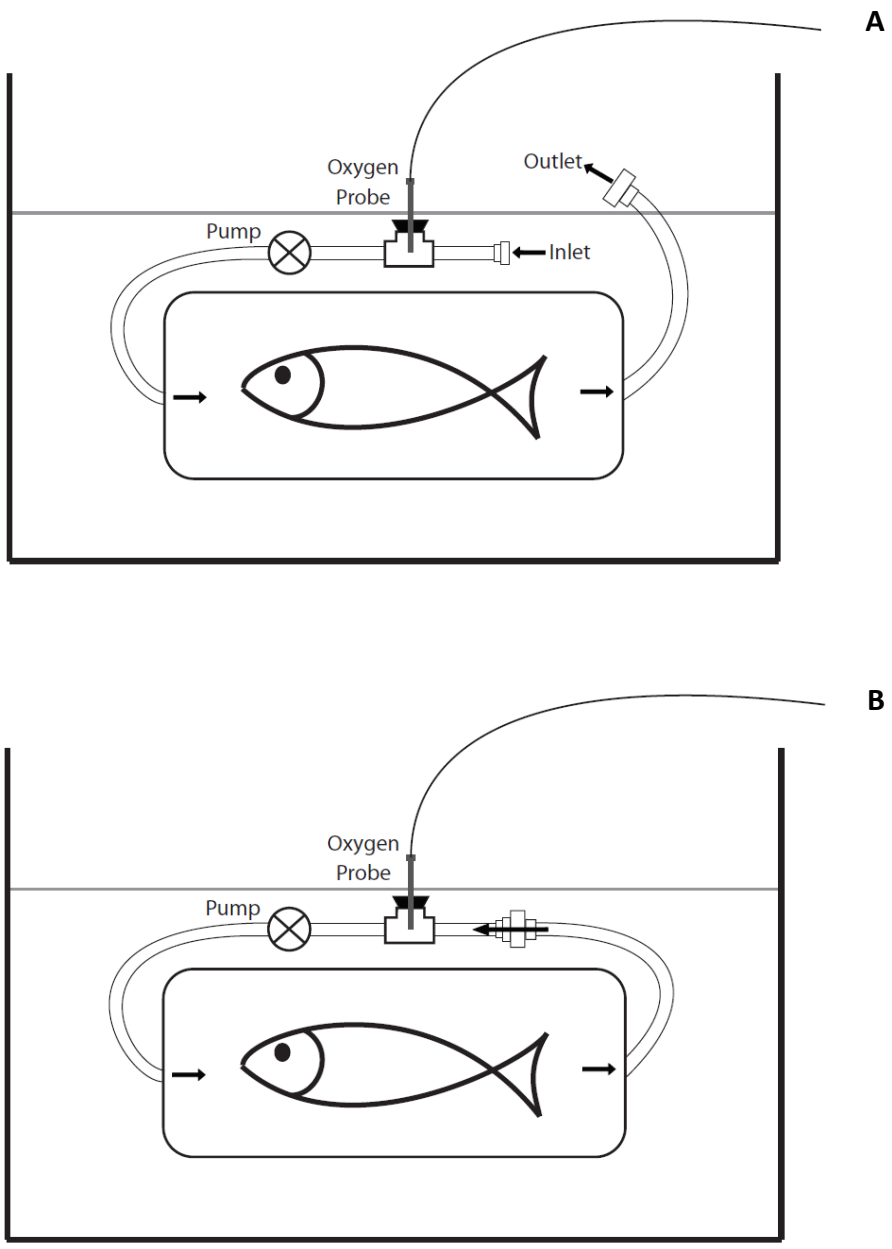

Figure 2.1 The design of intermittent respirometers that were used in the current study. Figure A represents the flushing mode while Figure B represents the recirculating mode.

Fish were randomly captured from the large rearing tanks, placed into the respirometers, and acclimated overnight in the flushing mode until the next experiment day. Coho salmon reared at 0, 10 and 20 ppt, and Atlantic salmon reared at 0, 10, 20, and 30 ppt were used for the experiments (8 fish of each species per salinity treatment). In all cases, respirometry was conducted at the salinity where fish were reared. Prior to each measurement, the oxygen probe was calibrated at 12 ℃, the system temperature. After the respirometer was switched to the recirculating mode, the Foxy System then continuously measured and recorded (every 2 s) the real-time value of air saturation inside the respirometer. Each measurement continued for 10 minutes, after which the respirometer was switched to the flushing mode. Once deoxygenated water inside was fully flushed out, the respirometer was closed again for the next repeated measurement of oxygen consumption rate. As the measurements above were conducted when the fish was under a resting state, those resting oxygen consumption rates would be defined as routine metabolic rate (RMR), and in total 3 measurements of RMR were conducted for each fish, after which an average value was obtained from the 3 measurements.

Once the measurements of RMR were finished, the fish was then removed from the respirometer and placed into a 19 L bucket filled with water from the respective rearing tank. The fish was chased by hand to exhaustion defined as the inability to escape from the chasing hand. After that, the fish was immediately transferred back to the respirometer, and the decline in water oxygen levels in the subsequent 5 minutes was used to calculate maximum metabolic rate (MMR). During the measurements of oxygen consumption rates for both RMR and MMR, water air saturation levels were not permitted to drop below 80% to avoid hypoxia. Once the measurements were completed, the fish was removed from the respirometer and euthanized with an over-dosed MS222

solution of 0.2 g L^{-1} tricaine methanesulfonate buffered with 0. 4 g L^{-1} sodium bicarbonate, after which the body mass was measured and recorded.

2.2.5 Calculations of Metabolic Rates and Aerobic Scope

The routine and maximum metabolic rates of fish were calculated as the following:

$$\dot{M}o_2 = \frac{[\frac{d(\%\ air\ sat)}{dt} \times \frac{3{,}600s}{h}] \times \sigma_{O_2} \times V}{M_f}$$

where $\dot{M}o_2$ is the metabolic rate with a unit of μ mol g^{-1} h^{-1}, $\frac{d(\%\ air\ sat)}{dt}$ is the slope of decreasing air saturation over the time with a unit of % air sat per s, σ_{O_2} is the oxygen solubility with a unit of μ mol L^{-1} per % air saturation, V is the effective respirometer volume (where fish mass was subtracted from the respirometer volume, assuming 1 kg body mass equals 1 L) with a unit of L, and M_f is the body mass of the fish with a unit of g.

Aerobic scope was determined as follows:

$$Aerobic\ Scope = Maximum\ Metabolic\ Rate - Routine\ Metabolic\ Rate$$

2.2.6 Hypoxia Tolerance

The hypoxia challenge trials were conducted from January to June in 2016 which covered Day 197 and 295 for Atlantic salmon, and Day 257 and 355 for coho salmon in the growth trial. A 100 L experimental aquarium was set up above a 0.7 m^3 tank of a given system, while the tank water was pumped into the aquarium and was allowed to overflow into the 0.7 m^3 tank. In each hypoxia tolerance trial, 3 fish were randomly selected and transferred from the rearing tanks to the experimental aquarium and 5 to 6 trials were conducted per salinity treatment per species. Atlantic salmon reared at 0, 5, 10, 20 and 30 ppt were used for the hypoxia trials and coho salmon reared at 0, 10, 20 and 30 ppt were selected due to the shortage of fish in the treatment of 5 ppt due to a mortality event. Fish were acclimated in the aquarium to allow recovery from handling stress for about 2 days prior to the experiment. The pump was turned off and hypoxia was induced by bubbling nitrogen from a gas cylinder into the aquarium. A pump was placed inside the aquarium to ensure the water was well mixed and air saturation was consistent throughout. A piece of bubble wrap was place on the surface of the water to minimize gas exchange with the air and to prevent fish from performing aquatic surface breathing. A pre-calibrated oxygen probe (YSI Pro2030, OH, USA) was placed in the aquarium to continuously monitor air saturation which was recorded every 2 minutes. The air saturation was reduced from 100% to the target tension of 10% within about 37 minutes, after which nitrogen bubbling was stopped. Occasionally, air was bubbled to maintain air saturation at 10% over the hypoxia exposure. Once 10% air saturation was reached, time to loss of equilibrium of the fish (LOE, inability to maintain the vertical position in the water) was recorded. Fish were then immediately removed from the aquarium, euthanized with an overdose of MS222 (0.2 g L^{-1} tricaine methanesulfonate buffered with 0. 4 g L^{-1} sodium bicarbonate), and their body mass was measured.

2.2.7 Statistical Analysis

Anderson Darling test was used to test normality of the data, using an α of 0.05. If the data were not normally distributed, transformation of the data was attempted to satisfy the assumption of normality. After each analysis of variance (ANOVA) or covariance (ANCOVA), if significant differences were detected, Tukey post hoc multiple comparison was conducted to reveal where the significant differences occurred, using an α of 0.05.

For the analysis of growth, I considered each fish as a single replicate even though they were all sampled from the same tank. Two-way analysis of variance (ANOVA) with salinity and exposure time as main effects was performed on body mass and condition factor of the fish randomly sampled from the tanks. If the main effects were significant, Tukey post hoc multiple comparison analysis was conducted to reveal where the significant differences occurred, using an α of 0.05 (same for all other statistical analysis). No statistical analysis could be conducted for whole-tank GR, SGR, TGC and eFCR because their calculations were based on the difference between two means (i.e., n = 1). Two-way ANOVA with salinity and exposure time as main effects was conducted for tagged fish GR, SGR and TGC. Final GSI was not normally distributed, so the Kruskal-Wallis test was performed on the data to test for significant differences.

Measurements of RMR, MMR and AS might be affected by body mass, so one-way analysis of covariance (ANCOVA) was performed on these measurements with salinity as a factor and body mass as a covariate. RMR of coho salmon was not normally distributed so the data were

transformed into $\frac{1}{RMR}$ to satisfy normality and then one-way ANCOVA was performed. Time to LOE was transformed into $\sqrt{time\ to\ LOE}$ and $\lg time\ to\ LOE$ in Atlantic and coho salmon, respectively, to satisfy normality of variance. Hypoxia tolerance might also be affected by body mass, so body mass was included as a covariate for the analysis of hypoxia tolerance. Since multiple fish were involved in one trial, a mixed effects model was used with time to LOE as a dependent variable, salinity as a fixed factor, trial ID as a random factor and body mass as a covariate.

2.3 Results

2.3.1 Growth, Maturation and Cataract

2.3.1.1 Atlantic Salmon

The growth data for Atlantic salmon at Day 0 and 96 were previously reported in Emerman 2016. The current study continued the growth trial and measured growth of the Atlantic salmon at Day 197, 295 and 400 (Figure 2.2). There was a significant effect of salinity ($P < 0.001$) and exposure time ($P < 0.001$) on body mass of Atlantic salmon, while the interaction between salinity and exposure time was also significant ($P < 0.001$). At Day 197, no significant difference was found in body mass of the fish from the different salinity treatments. At Day 295, Atlantic salmon reared at 10 ppt had the largest average body mass which was significantly higher than those reared at 0 ppt. At Day 400, Atlantic salmon reared at 5 ppt were significantly larger than any other salinity treatments. Body mass of fish at 10 and 20 ppt appeared to be intermediate while fish reared at two salinity extremes, 0 and 30 ppt, had the lowest body mass (Figure 2.2).

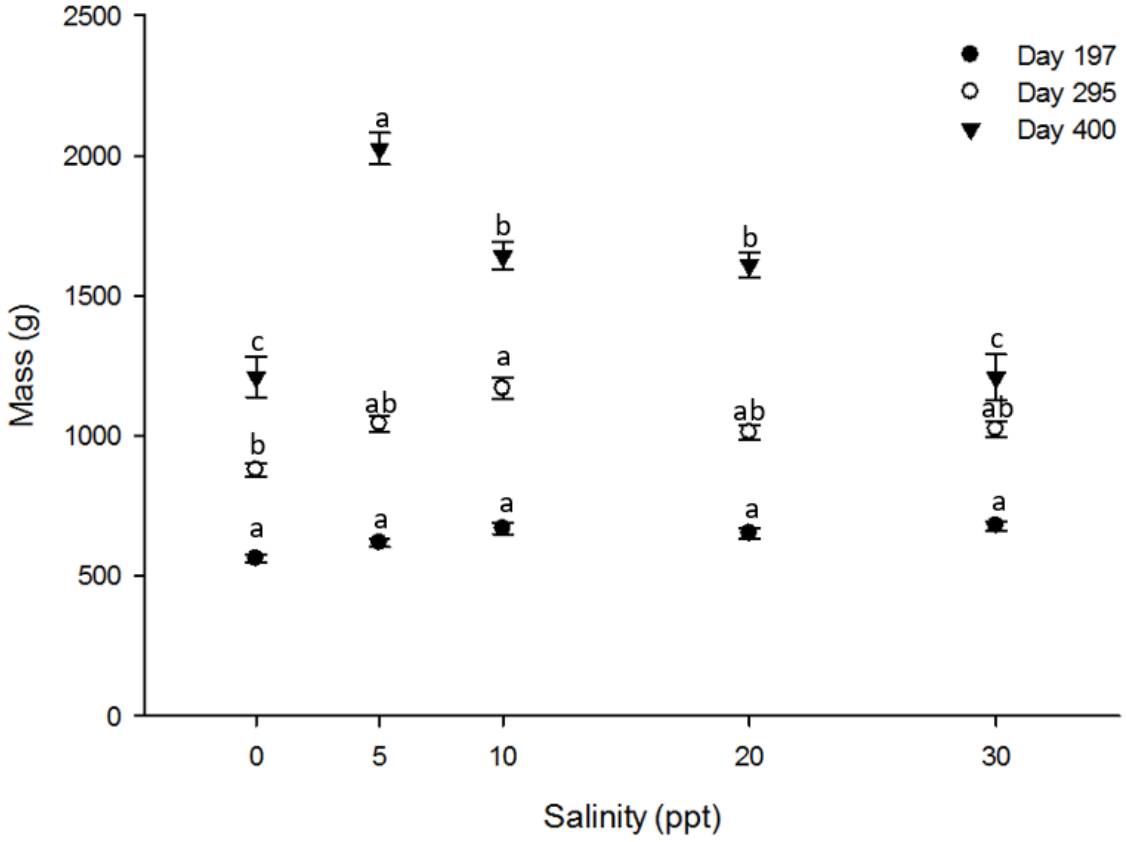

Figure 2.2 Mean body mass of Atlantic salmon reared at salinities of 0, 5, 10, 20 and 30 ppt sampled at Day 197, 295 and 400. Means represent a sample size larger than 100 fish with the exception at Day 400 where there were only 21 fish at 0 ppt and 26 fish at 30 ppt. Filled circles, open circles and filled triangles represent Day 197, 295 and 400, respectively. All data are mean ± SEM. Letters that differ indicate statistically significant differences within a given sampling time period ($P < 0.05$).

Whole-tank Growth rate (GR), specific growth rate (SGR) and thermal growth coefficient (TGC) of Atlantic salmon were highest at 10 ppt from Day 197 to 295 (Table 2.1). However, from Day 295 to 400, those parameters were greatest at 5 ppt. Whole-tank GR, SGR and TGC were generally lower in freshwater and seawater, relative to intermediate salinities. Economic feed conversion ratio (eFCR) of Atlantic salmon was highest at 0 ppt and lowest at 10 ppt from Day 197 to 295, while from Day 295 to 400, eFCR was again highest at 0 ppt but lowest at 5 ppt instead. There was a significant effect of salinity ($P < 0.001$) and exposure time ($P < 0.001$) on condition factor of Atlantic salmon, while the interaction between salinity and exposure time was also significant ($P < 0.001$), but there was no clear general pattern in condition factor of fish reared at different salinities over time (Table 2.1).

At Day 295, Atlantic salmon reared at 0 and 30 ppt had the highest sexual maturation rate of 22% and 23%, respectively, about twice observed in 5 and 10 ppt treatments. At Day 400, around 50% of the fish reared at 0, 10 and 20 ppt were sexually mature, while that at 30 ppt reduced from previous 23% (Day 295) to 15%, which is most likely due to a high mortality event that may have differentially selected mature fish. Salinity significantly influenced gonadosomatic index (GSI) of the fish at Day 400 ($P < 0.001$) when a reduction (up to 60%) was seen in GSI at 5 and 30 ppt, compared with the rest of salinities. Cataract prevalence of Atlantic salmon at Day 295 was highest (66%) at 20 ppt, while only 3% and 8% of fish reared at 0 and 30 ppt respectively exhibited the presence of cataracts. However, at Day 400, fish reared at 0, 10 and 20 ppt showed a reduction in the cataract presence rate. The reasons for the abnormal reduction in the cataract prevalence could be: (1) researchers' variations in defining cataracts and (2) potential mortalities of stress-sensitive fish with cataracts at 30 ppt due to the system malfunction as mentioned above. However, the

observations from Day 295 to 400 generally showed a trend that cataract prevalence was higher from 5 to 20 ppt and lower in freshwater and seawater (Table 2.1).

In pit tagged Atlantic salmon, there was a significant effect of salinity ($P < 0.001$) but no effect of exposure time ($P = 0.292$) on GR, while the interaction between salinity and exposure time was significant ($P = 0.008$). There was a significant effect of salinity ($P < 0.001$) and exposure time ($P = 0.031$) on SGR, while the interaction between salinity and exposure time was also significant ($P = 0.001$). There was a significant effect of salinity ($P < 0.001$) but no effect of exposure time ($P = 0.454$) on TGC, while the interaction between salinity and exposure time was significant ($P = 0.002$). In general, from Day 197 to 294, GR, SGR and TGC of tagged Atlantic salmon were not statistically different across salinities. However, from Day 295 to 400, those parameters at 5 ppt were significantly higher than other salinities, and those parameters were overall lowest at 0 and 30 ppt (Table 2.1).

Table 2.1 Growth rate (GR), specific growth rate (SGR), thermal growth coefficient (TGC), economic feed conversion ratio (eFCR), condition factor (n = 21 - 132), presence of sexual maturation, gonadosomatic index (GSI) (n = 21 - 57) and cataracts of Atlantic salmon reared at 0, 5, 10, 20 and 30 ppt over the growth period from Day 197 to 400. GR, SGR and TGC were calculated on both the whole-tank mean values (n = 1) and individual tagged fish (n = 2 - 19). Data are mean ± SEM. Letters that differ indicate statistically significant differences in measurements among salinities within each respective sampling time period ($P < 0.05$).

		Salinity (ppt)				
		0	5	10	20	30
Growth Rate (g day^{-1})	Day 197-295	3.23	4.32	5.1	3.68	3.51
	Day 295-400	3.17	9.37	4.51	5.77	1.77
Tagged Fish Growth Rate (g day^{-1})	Day 197-295	NA	4.06±0.81a	4.51±2.00a	3.45±0.28a	2.74±0.68a
	Day 295-400	3.04±1.01b	10.52±0.83a	6.31±0.84b	5.41±0.50b	0.07±1.02b
Specific Growth Rate (% day^{-1})	Day 197-295	0.46	0.53	0.57	0.45	0.42
	Day 295-400	0.31	0.63	0.32	0.45	0.16
Tagged Fish Specific Growth Rate (% day^{-1})	Day 197-295	NA	0.49±0.04a	0.38±0.15a	0.67±0.07a	0.36±0.09a
	Day 295-400	0.25±0.09bc	0.66±0.02a	0.41±0.03b	0.43±0.03b	-0.02±0.12c
Thermal Growth Coefficient	Day 197-295	1.07	1.32	1.48	1.14	1.06
	Day 295-400	0.85	1.92	0.98	1.27	0.45
Tagged Fish Thermal Growth Coefficient	Day 197-295	NA	1.21±0.12a	1.07±0.43a	1.45±0.12a	0.89±0.22a
	Day 295-400	0.74±0.25bc	2.04±0.09a	1.26±0.11b	1.20±0.08bc	-0.03±0.30c
Economic Feed Conversion Ratio	Day 197-295	3.19	1.54	1.19	2.43	1.95
	Day 295-400	20.81	0.38	2.8	2.61	1.8
Condition Factor	Day 197	1.15±0.01b	1.15±0.01b	1.20±0.03ab	1.14±0.01b	1.26±0.03a
	Day 295	1.17±0.01a	1.16±0.01a	1.13±0.01a	1.14±0.01a	1.13±0.01a
	Day 400	1.15±0.03bc	1.31±0.01a	1.24±0.01b	1.21±0.01b	1.05±0.02c
Sexual Maturation (%)	Day 295	22	11	11	18	23
	Day 400	52	29	48	46	15
Gonadosomatic Index (%)	Day 400	4.38±1.07a	2.18±0.42bc	3.75±0.5ab	3.16±0.48ab	1.63±0.49c
Cataract (%)	Day 295	3	39	49	66	8
	Day 400	0	39	37	34	15

2.3.1.2 Coho Salmon

The growth data of coho salmon at Day 0, 59 and 156 were previously reported in Emerman (2016), and those at Day 257, 355 and 460 reported here represent a continuation of that study (Figure 2.3). There was a significant effect of salinity ($P < 0.001$) and exposure time ($P < 0.001$) on body mass of coho salmon. At Day 257, coho salmon reared at 10 ppt had the largest mean body mass which was significantly higher than fish at 0 ppt ($P = 0.028$), while fish reared at 5, 20 and 30 ppt had an intermediate body mass. At Day 355, no statistically significant difference was detected on the body mass of fish among all the groups, but the fish reared at 10 ppt had the highest value of mean body mass. At Day 460, fish reared at 10 ppt had the greatest mean body mass which was significantly higher than that of fish reared at 20 ppt ($P = 0.041$), while fish reared at lower salinities (0 and 5 ppt) had an intermediate body mass. No data are presented for Day 460 at 30 ppt because that treatment had to be terminated prior to that sampling time due to a system failure (Figure 2.3).

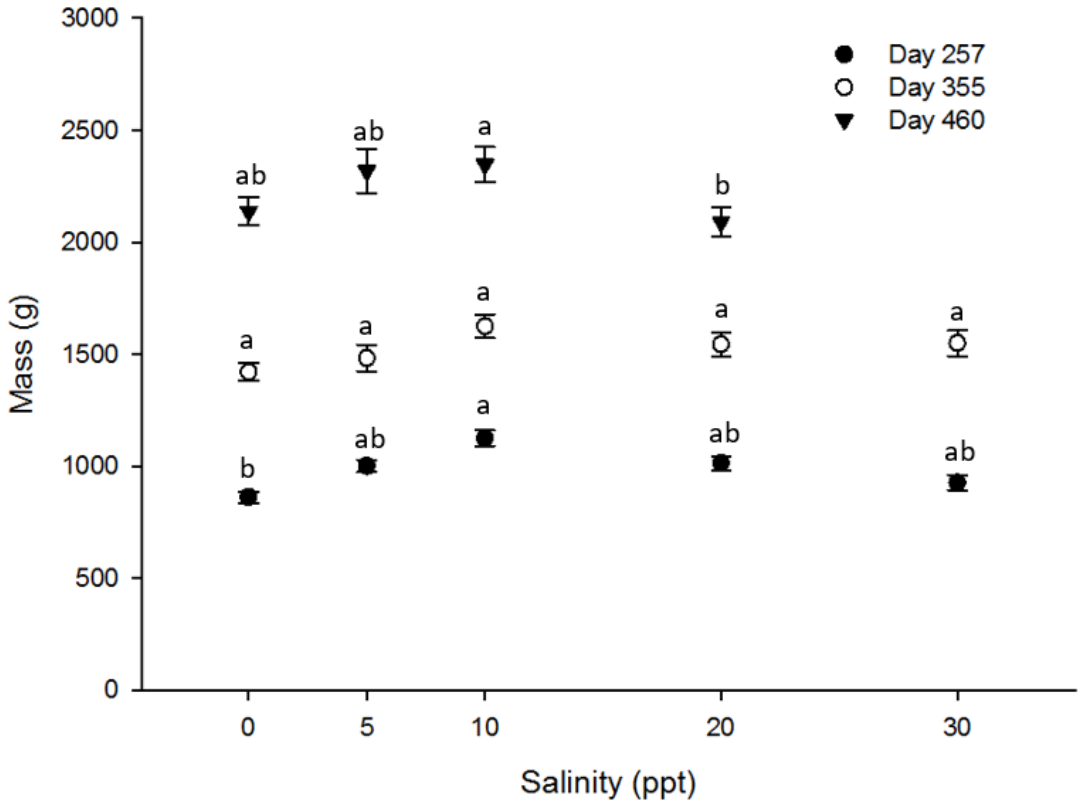

Figure 2.3 Mean body mass of coho salmon reared at salinities of 0, 5, 10, 20 and 30 ppt sampled at Day 257, 355 and 460. Filled circles represent Day 257 (n = 100 - 104), open circles represent Day 355 (n = 66 - 102) and filled triangles represent Day 460 (n = 57 - 131). All data are mean ± SEM. Letters that differ indicate statistically significant differences in body mass among salinities within the respective sampling time period ($P < 0.05$).

Whole-tank GR, SGR and TGC of coho salmon were highest at 30 ppt from Day 257 to 355, and at 5 ppt from Day 355 to 460, respectively (Table 2.2). From Day 257 to 460, despite that coho salmon reared at 10 ppt always had the highest body mass, whole-tank GR, SGR and TGC appeared to be intermediate among all salinities, while eFCR of this group remained the highest and it increased dramatically over the growth period. There was a significant effect of salinity and exposure time on condition factor of coho salmon, while the interaction between salinity ($P < 0.001$) and exposure time ($P < 0.001$) was also significant ($P < 0.001$), but there was no clear general pattern in this interaction as the effect of salinity on condition factor changed over time (Table 2.2).

No sexual maturation was seen in coho salmon till the end of the growth trial. Also, coho salmon showed no statistically significant difference in GSI across salinities at Day 460 ($P = 0.083$). Cataracts were only seen in few coho salmon reared at 0 and 20 ppt at Day 257, and again the reduction of the presence of cataracts in these two group at Day 460 can probably be explained by researchers' variations in defining cataracts, but overall coho salmon showed nearly no cataract (Table 2.2).

In pit tagged coho salmon, there was a significant effect of salinity ($P < 0.001$) and exposure time ($P = 0.004$) on GR. There was a significant effect of salinity ($P = 0.004$) and exposure time ($P = 0.003$) on SGR. There was a significant effect of salinity ($P < 0.001$) but no effect of exposure time ($P = 0.541$) on TGC. In general, from Day 257 to 355, there was no statistical difference in GR, SGR and TGC of tagged coho salmon reared at different salinities. However, from Day 355 to 460,

GR, SGR and TGC of coho salmon reared at 5 and 10 ppt were overall higher than the others (Table 2.2).

Table 2.2 Growth rate (GR), specific growth rate (SGR), thermal growth coefficient (TGC), economic feed conversion ratio (eFCR), condition factor (n = 57 - 131), presence of sexual maturation, gonadosomatic index (GSI) (n = 50 - 59) and cataracts of coho salmon reared at 0, 5, 10, 20 and 30 ppt over the growth period from Day 257 to 460. GR, SGR and TGC were calculated on both the whole-tank mean values (n = 1) and individual tagged fish (n = 8 - 40). Data are mean ± SEM. Letters that differ indicate statistically significant differences in measurements among salinities within each respective sampling time period ($P < 0.05$).

		Salinity (ppt)				
		0	5	10	20	30
Growth Rate (g day-1)	Day 257-355	5.71	4.92	5.1	5.42	6.37
	Day 355-460	6.82	7.96	6.88	5.21	NA
Tagged Fish Growth Rate (g day-1)	Day 257-355	4.69±0.59a	5.02±0.40a	4.96±0.55a	4.42±0.42a	5.70±0.70a
	Day 355-460	6.85±0.88ab	7.62±0.55a	7.35±0.61a	4.34±0.55b	NA
Specific Growth Rate (% day-1)	Day 257-355	0.51	0.4	0.38	0.43	0.53
	Day 355-460	0.39	0.43	0.35	0.29	NA
Tagged Fish Specific Growth Rate (% day-1)	Day 257-355	0.45±0.04a	0.44±0.04a	0.39±0.0a	0.40±0.03a	0.48±0.04a
	Day 355-460	0.37±0.04ab	0.42±0.02a	0.36±0.02ab	0.26±0.03b	NA
Thermal Growth Coefficient	Day 257-355	1.39	1.15	1.12	1.26	1.51
	Day 355-460	1.29	1.4	1.19	0.92	NA
Tagged Fish Thermal Growth Coefficient	Day 257-355	1.18±0.10a	1.21±0.08a	1.12±0.07a	1.11±0.09a	1.35±0.12a
	Day 355-460	1.24±0.13a	1.35±0.08a	1.22±0.07a	0.80±0.10b	NA
Economic Feed Conversion Ratio	Day 257-355	0.84	1.48	1.95	1.27	1.62
	Day 355-460	0.85	0.48	3.06	2.35	NA
Condition Factor	Day 257	1.38±0.01b	1.39±0.02ab	1.41±0.01ab	1.41±0.02ab	1.45±0.01a
	Day 355	1.40±0.01ab	1.34±0.02bc	1.36±0.01bc	1.33±0.01c	1.47±0.01a
	Day 460	1.50±0.02a	1.44±0.02ab	1.51±0.02a	1.42±0.02b	NA
Sexual Maturation (%)	Day 355	0	0	0	0	0
	Day 460	0	0	0	0	NA
Gonadosomatic Index (%)	Day 460	0.37±0.03a	0.35±0.03a	0.36±0.02a	0.28±0.02a	NA
Cataract (%)	Day 355	8	0	0	7	0
	Day 460	0	0	0	4	NA

2.3.2 Respirometry

2.3.2.1 Atlantic Salmon

Body mass had no effect on RMR of Atlantic salmon (P = 0.580). There was a statistically significant effect of salinity on RMR of Atlantic salmon (P = 0.022), but Tukey post hoc analysis found no significant difference in RMR across salinities. (Figure 2.4, A). Body mass negatively affected MMR of fish (P = 0.003). After MMR was adjusted for body mass (covariate), it was found that salinity had a significant effect on MMR of Atlantic salmon (P = 0.008), and the multiple comparison showed that MMR of fish reared at 30 ppt was significantly higher than the fish reared at 0 ppt, while MMR of the fish reared at 10 and 20 ppt appeared to be intermediate. (Figure 2.4, B). Body mass negatively affected AS of Atlantic salmon (P = 0.003). After AS was adjusted for body mass (covariate), salinity showed a significant effect on AS of Atlantic salmon (P = 0.037), and AS of the fish reared at 30 ppt was significantly higher than the fish reared at 20 ppt, while fish reared at 0 and 10 ppt had an intermediate AS. (Figure 2.4, C).

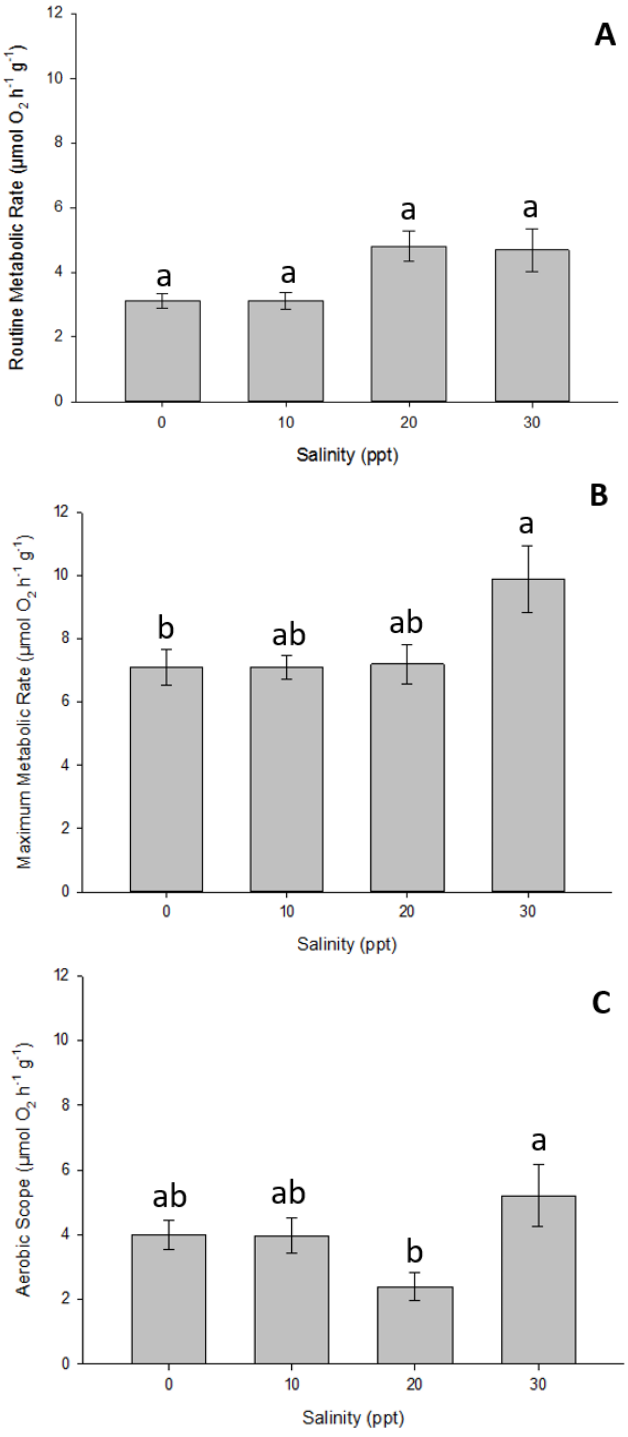

Figure 2.4 Routine metabolic rate (A), maximum metabolic rate (B) and aerobic scope (C) of Atlantic salmon reared at 0, 10, 20 and 30 ppt close to Day 400 (n = 6 - 9, mean body mass = 1531.6 g). All data are mean ± SEM. Letters that differ indicate statistically significant differences ($P < 0.05$).

2.3.2.2 Coho Salmon

Body mass had no effect on RMR of coho salmon (P = 0.674), and no effect of salinity was found on RMR of fish, either (P = 0.064) (Figure 2.5, A). Body mass negatively affected MMR of the fish (P = 0.001). After MMR was adjusted for body mass, there was a significant effect of salinity on MMR of coho salmon (P = 0.031). MMR at 20 ppt was significantly higher than that at 10 ppt, while MMR of the fish reared at 0 ppt was intermediate (Figure 2.5, B). Body mass negatively affected AS of coho salmon (P = 0.008). After AS was adjusted for body mass, no effect of salinity on AS was detected (P = 0.709) (Figure 2.5, C).

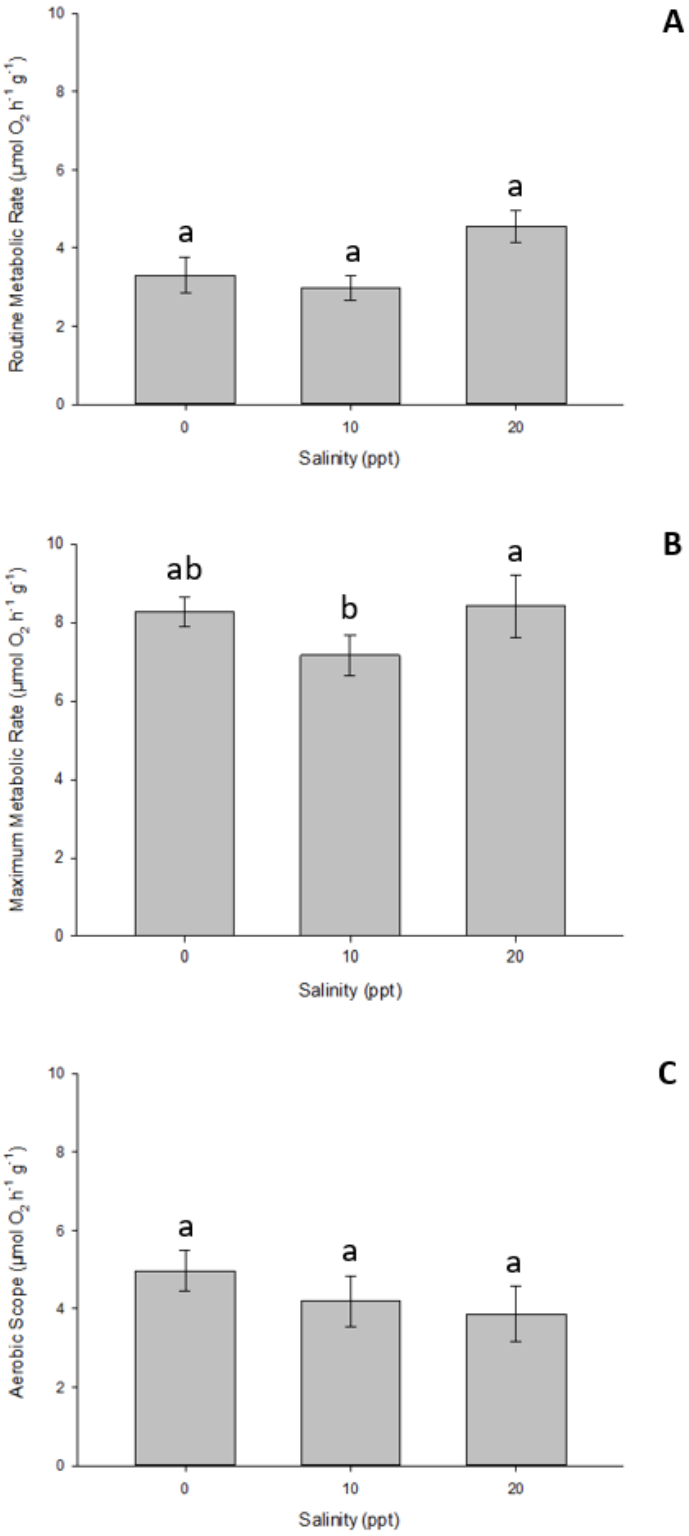

Figure 2.5 Routine metabolic rate (A), maximum metabolic rate (B) and aerobic scope (C) of coho salmon reared at 0, 10 and 20 ppt close to Day 460 (n = 5 - 8, mean body mass = 1957.1 g). All data are mean ± SEM. Letters that differ indicate statistically significant differences ($P < 0.05$).

2.3.3 Hypoxia Tolerance

In Atlantic salmon, body mass negatively affected time to LOE of fish held at 10% air saturation ($P = 0.031$). After time to LOE was adjusted for body mass, there was a statistically significant effect of salinity on time to LOE ($P = 0.005$). Atlantic salmon reared at 5 and 10 ppt had significantly higher time to LOE than the ones reared at 0 ppt, while time to LOE of the fish reared at 20 and 30 ppt was intermediate (Figure 2.6). In coho salmon, there was no statistically significant effect of body mass ($P = 0.553$) or salinity ($P = 0.134$) on time to LOE (Figure 2.7).

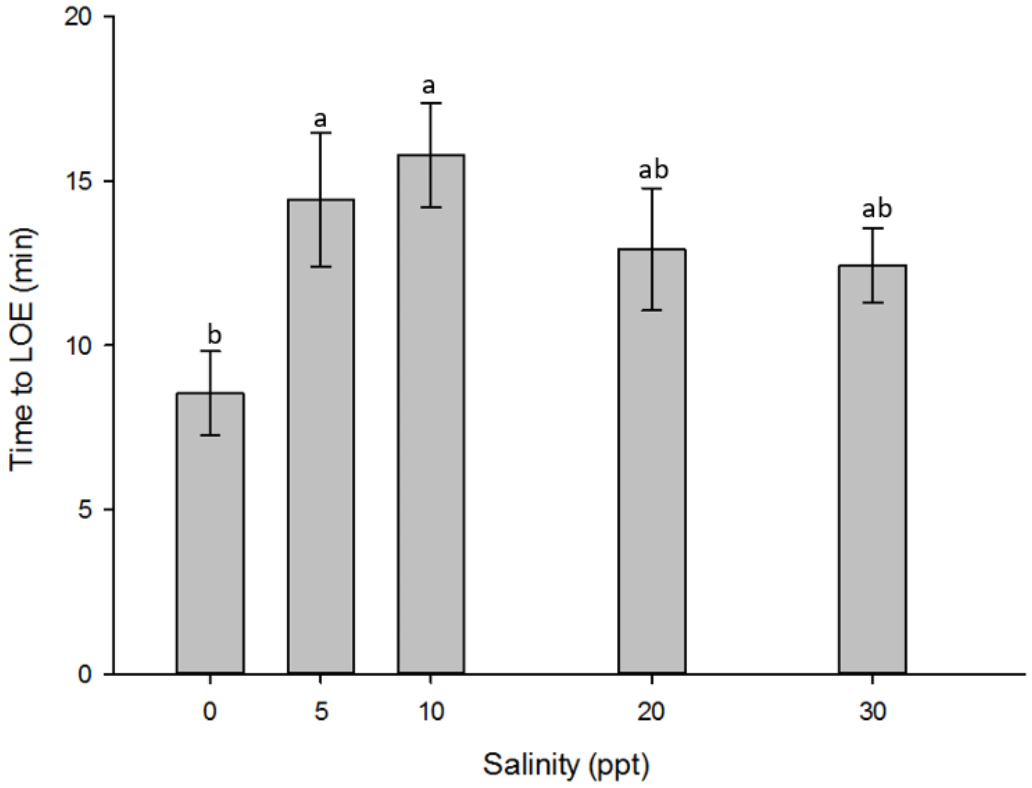

Figure 2.6 Time to loss of equilibrium (LOE) of Atlantic salmon at 10% air saturation (12℃) reared at 0, 5, 10, 20 and 30 ppt (n = 10 - 14, mean body mass = 902.3 g). All data are mean ± SEM. Data with different letters indicate significant differences ($P < 0.05$).

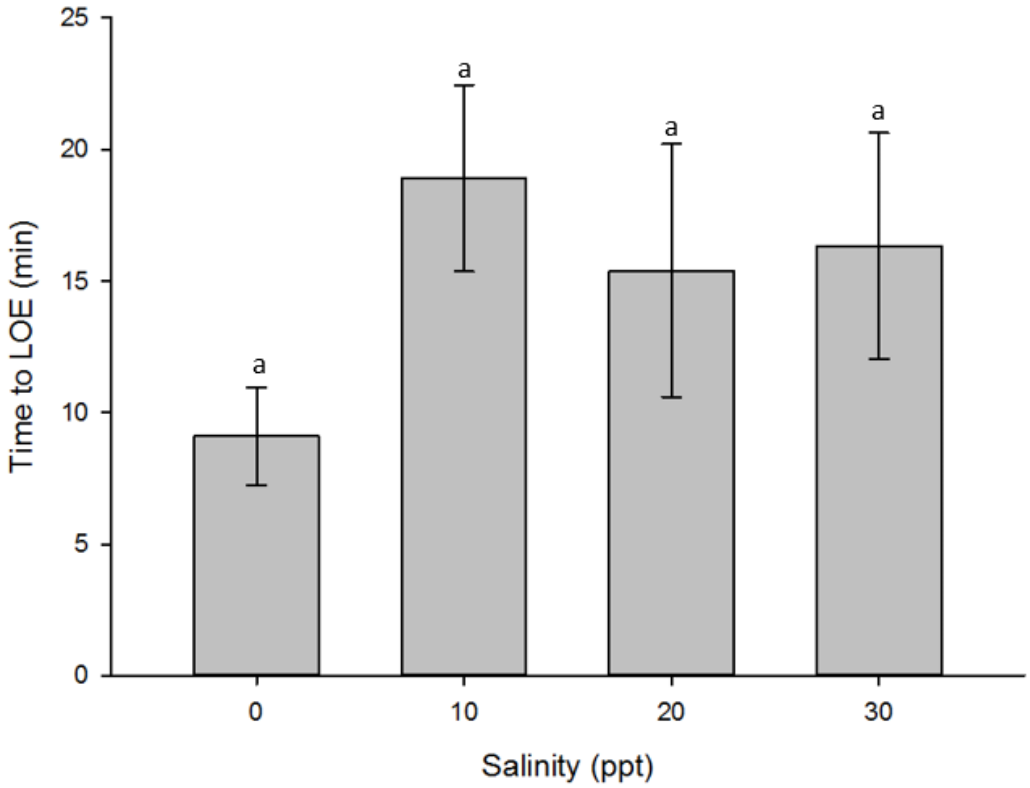

Figure 2.7 Time to loss of equilibrium (LOE) of coho salmon at 10% air saturation (12℃) reared at 0, 10, 20 and 30 ppt (n = 9 - 11, mean body mass = 1484.0 g). All data are mean ± SEM. Data with different letters indicate significant differences ($P < 0.05$).

2.4 Discussion

The current study found that growth of Atlantic and coho salmon was enhanced at intermediate salinities (5 and 10 ppt), which is in agreement with my prediction (Figure 1.2). The enhanced growth of Atlantic salmon was associated with relatively lower rates of sexual maturation, but higher incidence of cataracts. In contrast, coho salmon showed no sexual maturation and cataracts. No clear relationship between salinity and metabolic traits (RMR, MMR and AS) was found in either species. Hypoxia tolerance of Atlantic salmon was enhanced at intermediate salinities and reduced in freshwater and seawater (consistent with the prediction, Figure 1.2). However, salinity had no impact on hypoxia tolerance of coho salmon (not consistent with the prediction, Figure 1.2).

2.4.1 Growth, Maturation and Cataract

The first part of this growth trial reported in Emerman (2016) found no effect of salinity (0, 5, 10, 20 and 30 ppt) on growth of Atlantic salmon up to ~250 g at Day 96 (Figure 1.1). Body mass of Atlantic salmon was still not significantly different across salinities around 600 g at Day 197 (Figure 2.2). This result is consistent with McCormick et al. (1989) who conducted a 6-week growth trial and found no effect of salinity (0,10 and 30 ppt) on growth of Atlantic salmon post-smolts (initially 23 – 70 g) at a feeding ration of less than 0.8% wet weight (1% wet weight was used in the current study). At Day 295, Atlantic salmon started to show significant differences in body mass which was highest at 10 ppt and lowest at 0 ppt (Figure 2.2). From Day 197 to 295,

even though there was no statistical difference in tagged fish GR, SGR or TGC across salinities, I still observed lowest eFCR and highest whole-tank GR, SGR and TGC at 10 ppt (Table 2.1).

From Day 295 to 400, Atlantic salmon reared at 5 ppt achieved the largest body mass (Figure 2.2) with lowest eFCR and highest GR, SGR and TGC among all the treatments in both whole-tank and tagged fish measurements (Table 2.1). At Day 400, the larger body mass at 5 ppt was also associated with these fish having one of the lowest incidence of sexual maturation and GSI (Table 2.1). Sexual maturation in fish is often associated with a reduction in growth rate due to diversion of energy from somatic growth to gonadal development. As such, there may be a link between the lower incidence of sexual maturation and higher growth rate at 5 ppt. Interestingly, the previously-seen growth-enhancing effect of iso-osmotic salinity (10 ppt) at Day 295 was not maintained through to Day 400 but was associated with a high final maturation rate and GSI (Table 2.1). Unfortunately, between Day 295 and 400, there was a malfunction in the biofilter resulting in high ammonia in the 30 ppt treatment, and about 60% of the population in that treatment of Atlantic salmon were lost. Upon the detection of high ammonia, an immediate water change was conducted, but a large number of stress-sensitive mature Atlantic salmon might have died, which could explain the abnormal reduction in maturation rate at 30 ppt from 23% at Day 295 to 15% at Day 400 (Table 2.1).

Another distinct observation at Day 400 is that Atlantic salmon at 0 and 30 ppt had the lowest body mass and the smallest mass gain since Day 295 (Figure 2.2). Furthermore, condition factor obtained at Day 400 was relatively lower at 0 and 30 ppt (Table 2.1), which may indicate poor

health. The poor growth in freshwater Atlantic salmon was largely due to the presence of fungal infection which compromised fish health and appetite, as well as the corresponding formalin treatment which was also physiologically stressful. The high ammonia levels at 30 ppt mentioned above might largely compromise fish health, resulting in poor growth in that treatment.

A very high cataract prevalence from 34% to 66% was observed in Atlantic salmon reared at 5, 10 and 20 ppt, while 0 and 30 ppt resulted in lower incidences of cataracts from 0% to 15% (Table 2.1). Cataracts have been shown to negatively impact growth of Atlantic salmon due to reduced food consumption caused by visual disturbance and blindness (Ersdal et al. 2001; Breck and Sveier 2001). However, in the present study, Atlantic salmon reared at 5, 10 and 20 ppt had relatively higher incidences of cataract compared with freshwater and seawater, but this does not appear to have negatively impacted growth. Cataracts can be caused by many factors, including temperature fluctuation (Bjerkås et al. 2001), nutritional deficiencies (Ersdal et al. 2001), UV radiation exposure (Cullen et al. 1994), high CO_2 levels (Neves and Brown 2015). However, all of these variables were controlled in the current study, so it is possible that salinity might have a direct effect on the incidence of cataracts, clearly an area worthy of further investigation.

Coho salmon reared at 10 ppt were found to have enhanced growth rate over the first 59 days in Emerman (2016) (Figure 1.1), which then led to the fish reared at 10 ppt being continuously the largest through to Day 460 seen in the current study (Figure 2.3). Interestingly, there was likely a compensatory growth of coho salmon reared at 5 ppt near the terminal sampling because fish in this treatment had lowest eFCR, as well as the highest whole-tank and tagged fish GR, SGR and

TGC from Day 355 to 460 (Table 2.2). In an 8-month growth trial, Otto (1971) found a salinity of 5 to 10 ppt to be optimal for growth in coho salmon fry through to smolt. The current study in conjunction with Otto (1971) show that intermediate salinities (5 – 10 ppt) are optimal for growth of coho salmon from juvenile to adult stages.

Over the entire growth trail, there was no incidence of early sexual maturation observed in coho salmon. However, they were an all-female strain, and early maturation is typically seen in male salmonids (Silverstein and Hershberger 1992). Indeed, Atlantic salmon used in the current study were from a mix sexed population (male:female = 1:1) and most of mature Atlantic salmon were male. In addition, despite the high incidence of cataracts in Atlantic salmon, the coho salmon showed nearly no cataract except for few fish at 0 and 20 ppt (Table 2.2). This result in coho salmon is contrary to that reported in Iwata et al. (1987) who observed the presence of cataracts earlier in coho salmon smolts, but the cataract incidences seen in those coho salmon smolts were due to low osmoregulatory ability which led to osmotic disturbance in aqueous humor and hence caused cataracts in the eyes.

Despite different timing, intermediate salinities from 5 to 10 ppt generally resulted in a growth-enhancing effect on both Atlantic and coho salmon in RAS. The finding is in agreement with my predication (Figure 1.2), as well as many other studies that proposed and demonstrated the growth-enhancing effect of the iso-osmotic salinity and salinities close to it (Otto 1971; Suresh and Lin 1992; Lambert et al. 1994; Gaumet et al. 1995; Woo and Kelly 1995). As discussed above, enhanced growth of Atlantic salmon at 5 ppt might be partially due to a low sexual maturation rate,

which is not the case for coho salmon as no maturity was found in all salinity treatments, so there could be other factors related to salinity influencing growth of the fish.

In the current study, the enhanced growth at intermediate salinities was also found to associate with relatively high feed conversion efficiency (low eFCR), which indicates that intermediate salinities may offer growth advantages by potentially maximizing feed utilization in fish. Gracia-López et al. (2006) found juvenile common snook (*Centropomus undecimalis*) reared at iso-osmotic salinity had lower apparent heat increment, meaning lower cost of digestion, compared with freshwater and seawater. Thus, with the same amount of energy, fish at iso-osmotic salinity are able to process more food, which could eventually result in a faster growth. Tsuzuki et al. (2007) reared juvenile fat snook (*Centropomus parallelus*) at 5, 15 and 35 ppt, and found fish reared at 15 ppt had the lowest FCR which was associated with the highest gut alkaline proteinase and amylase activities, indicating that the intermediate salinity might improve feed digestion by increasing digestive enzyme activities. In the current study, gut digestive enzyme activities were not measured, but this would clearly be interesting in further studies to provide insight into the basis for low FCR and high growth in Atlantic and coho salmon reared at intermediate salinities.

2.4.2 Routine, Maximum Metabolic Rates and Aerobic Scope

Rearing fish under iso-osmotic conditions has long been associated with a reduction in SMR due to a reduced osmoregulatory cost at that salinity, and it has been proposed that this could yield higher growth rates (Bœuf and Payan 2001). In the current study, fish were only acclimated

overnight prior to respirometry, and thus were not in a post-absorptive state when the respirometry was conducted. Hence, the metabolic rate measurements should be defined as RMR rather than SMR and account for metabolic cost of digestion for growth. After 300 to 400 days of rearing, salinity had no effect on RMR in either Atlantic (Figure 2.4, A) or coho salmon (Figure 2.5, A), which is consistent with my predictions (Figure 1.2). These findings may indicate that the metabolic energy saved from osmoregulation at intermediate salinities was re-allocated to growth and the enhanced growth did not elevate total metabolic rate (RMR). Unfortunately, the current study was not able to conduct respirometry on fish at 5 ppt, which would have been useful in understanding the effect of 5 ppt on growth in both species near the end of the growth trial. The findings in the current study are also consistent with Morgan and Iwama (1998) who found no significant difference in RMR of coho salmon smolts reared at 0, 10 and 28 ppt. Morgan and Iwama (1998) also found gill Na^+, K^+-ATPase activities were lower at 10 ppt, and higher at 0 and 28 ppt, indicating a reduced osmoregulatory cost at iso-osmotic salinity. Gaumet et al. (1995) found juvenile turbot reared at iso-osmotic salinity grew significantly faster than those in seawater, but the enhanced growth was associated with a reduction (up to 40%) in RMR. Unlike coho salmon in the current study, for the fish in Gaumet et al. (1995), it seems the metabolic energy saved from osmoregulation at iso-osmotic salinity was not fully contributed to growth. Another study on salmonids found RMR of rainbow trout, steelhead trout and chinook salmon fry actually increased with salinity while the growth rate reduced with salinity (Morgan and Iwama 1991). Instead of iso-osmotic salinity, freshwater provided the metabolic and growth optimum to three species in that study. Morgan and Iwama (1991) indicated that freshwater is the natural habitat for these species during their juvenile stage, which might explain why those fish fry had the best performance in freshwater rather than iso-osmotic salinity. Finally, Morgan and Iwama (1991) came to a

conclusion that it is hard to establish a relationship between salinity and metabolic rate, because this relationship is also affected by species, lifestyle, developmental stages and other physiological processes.

MMR of Atlantic salmon overall increased with salinity (Figure 2.4, B), indicating that seawater Atlantic salmon had the greatest capacity to extract oxygen from the water. AS of Atlantic salmon was highest at 30 ppt (Figure 2.4, C), meaning seawater fish had the greatest metabolic capacity to support extra activities. MMR of coho salmon was U-shaped as a function of salinity, with the lowest values at 10 ppt (Figure 2.5, B), suggesting fish reared at 10 ppt had the poorest capacity to extract and deliver oxygen. Despite the effect of salinity on MMR of coho salmon, there was no effect of salinity on AS (Figure 2.5, C). These results with MMR and AS of Atlantic and coho salmon are not consistent with my predictions where I predicted MMR and AS could be enhanced at iso-osmotic salinity (Figure 1.2). In either species of salmon, the iso-osmotic salinity did not seem to provide higher oxygen extraction, indirectly measured as MMR, despite the minimal osmotic pressure and hence reduced osmorespiratory compromise at this salinity. In fact, MMR of coho salmon was even reduced at iso-osmotic salinity.

Rainbow trout exposed to soft-water were found to have a 50% increase in both chloride cell coverage area and water-to-blood diffusion barrier at gills, which could promote ion uptake and prevent passive ion loss but compromise oxygen extraction (Greco et al. 1996), potentially resulting in a lower MMR. By contrast, fish reared at iso-osmotic salinity likely have reduced passive ion movement, so it is not necessary for the fish to thicken gills to prevent the passive ion

transport, which implies fish at iso-osmotic salinity might have the most efficient oxygen extraction and hence higher MMR. Christensen et al. (2017) acclimated perch (*Perca fluviatilis*) to different salinities (0, 10 and 15 ppt) and temperatures (5, 10 and 20 ℃), and found about 20% reductions in MMR of fish reared in (0 ppt and 20 ℃) and (15 ppt and 5 ℃) treatments, compared with 10 ppt at the corresponding temperatures. Christensen et al. (2017) also indicated that the large reductions of MMR might be explained by reduced oxygen extraction which was the result of decreased gill permeability to maintain the internal osmolality in the hypo- and hyper-saline environments. Additionally, the reduced MMR of perch was seen to be associated with a decrease (~ 25%) in AS (Christensen et al. 2017). Similarly, another study found that acclimation to iso-osmotic salinity in killifish (*Fundulus heteroclitus*) provided metabolic advantages (lower RMR, higher MMR and higher AS), compared with freshwater (Brennan et al. 2016). However, the findings in these studies are not consistent with my results where there was no general relationship between MMR (and/or AS) and salinity in either Atlantic or coho salmon.

2.4.3 Hypoxia Tolerance

Continuous rearing of Atlantic salmon at 5 and 10 ppt resulted in an improvement in hypoxia tolerance relative to those reared in freshwater and higher salinities (Figure 2.6), which is consistent with my predication of a bell-shaped curve of hypoxia tolerance as a function of salinity (Figure 1.2). However, the variations in hypoxia tolerance of Atlantic salmon were not related to differences in RMR, MMR or AS (Figure 2.4). By contrast, salinity had no effect on hypoxia tolerance of coho salmon (Figure 2.7), which is not consistent with my prediction (Figure 1.2). Similarly, in coho salmon, there was no effect of salinity on RMR or AS and only a small effect

of salinity on MMR which was lower at 10 ppt compared with 0 and 20 ppt (Figure 2.5). As a result, there appears to be a species-specific effect of salinity on hypoxia tolerance, and within and between species there was no strong relationship between hypoxia tolerance and metabolic measurements.

This lack of a clear relationship between hypoxia tolerance and my measurements of metabolic rate could be due to the measurements being done on different ages of the fish with different body mass. Fish used for the hypoxia challenge trials (Atlantic salmon = 902 g; coho salmon = 1484 g) were younger and smaller than those in respirometry (Atlantic salmon = 1532g; coho salmon = 1957 g). In fact, the statistical analysis found significant effects of body mass on MMR, AS and hypoxia tolerance in Atlantic salmon, and MMR and AS in coho salmon. Therefore, fish age and body mass could potentially influence the results gained from hypoxia challenge trials and respirometry. Future work should attempt to use fish of the similar size for the two physiological measurements so that the data of metabolic rate and hypoxia tolerance can be more comparable. However, the relationship between salinity and hypoxia tolerance could be species-dependent. Henriksson et al. (2008) acclimated freshwater prickly sculpin (*Cottus asper*) and seawater Pacific staghorn sculpin (*Leptocottus armatus*) to 0, 15 and 30 ppt, and they found prickly sculpin at 0 ppt had the highest P_{crit} (potentially the poorest hypoxia tolerance) while salinity acclimation did not affect P_{crit} of Pacific staghorn sculpin. Hence, in the current study, it might not be uncommon to see different responses between Atlantic and coho salmon to hypoxia across salinities.

Hypoxia is a commonly challenge in salmon farming due to high stocking densities, frequent feeding and periodic changes in oxygen levels. The current study found intermediate salinities can enhance growth of Atlantic and coho salmon, but any re-allocation of energy saved from osmoregulation towards growth did not appear to compromise hypoxia tolerance of coho salmon, and even actually improved hypoxia tolerance of Atlantic salmon, which indicates a potential physiological benefit of using intermediate salinities to rear salmon in RAS.

2.4.4 Conclusions

The data presented here demonstrate that salinity had no effect on growth of Atlantic salmon up to 600 g at Day 197, after which increases in growth were observed at intermediate salinities (5 and 10 ppt), compared with 0, 20 and 30 ppt. The higher growth and larger body mass of Atlantic salmon reared at 5 ppt for 400 days could partially result from a low incidence of sexual maturation (29%) as more energy was used for somatic growth rather than gonadal development. Atlantic salmon reared at intermediate salinities had relatively higher incidence of cataracts relative to 0 and 30 ppt, but the high incidence of cataracts did not impact fish growth. For coho salmon, the previously reported increase in growth rate over the first 59 days at 10 ppt (Emerman, 2016) set these fish on a trajectory where they were always larger than the ones at 0, 5, 20 and 30 ppt through to a body mass of ~2,300 g. Coho salmon did not show signs of early maturation and few cataracts were found. In either Atlantic or coho salmon, salinity did not affect RMR, and there is no clear relationship between MMR, AS and salinity. Hypoxia tolerance of Atlantic salmon was enhanced at 5 and 10 ppt, compared with lower and higher salinities, but salinity had no effect on hypoxia

tolerance of coho salmon. There seems to be no connection between metabolic traits (RMR, MMR and AS) and hypoxia tolerance in these species.

Chapter 3: The Effects of Salinity and Photoperiod on Aerobic Scope and Hypoxia Tolerance of Coho Salmon in RAS

3.1 Introduction

Emerman (2016) and the data in Chapter 2 of my thesis demonstrate that the growth of coho salmon was enhanced at iso-osmotic salinity, relative to either lower or higher salinities, as early as the first two months of the growth trial, and this growth-enhancing effect was carried forward to Day 460 such that the coho salmon reared at 10 ppt were consistently bigger than fish reared in other salinity treatments. The respirometry and hypoxia challenge trials in Chapter 2 were conducted on larger coho salmon near the middle to the end of the growth trial when calculated growth rates at 10 ppt were not statistically higher than the others. As a result, it is perhaps not surprising that my analysis of metabolic rates and hypoxia tolerance of coho salmon did not yield large differences. Therefore, it would be interesting to see whether salinity had an effect on metabolic rates and hypoxia tolerance of coho salmon during their early growth stages when the growth-enhancing effect of iso-osmotic salinity was the greatest. Hence, the first objective of this chapter was to perform respirometry and hypoxia challenge trials at this earlier critical window to explore the effect of salinity on aerobic scope and hypoxia tolerance of coho salmon within the first 120 days of growth.

Photoperiod plays an important role in regulating circadian rhythms and activities of fish during the day and night cycle (Castanheira et al. 2011). While activity rhythms of salmonids are variable,

in general, most salmonids show diurnalism where they are more active during the day than at night (Reebs 2002). In salmon aquaculture, photoperiod is often manipulated to suppress sexual maturation and stimulate growth. Many studies have shown that longer periods of daylight have a positive effect on the growth of salmonids (Stefansson et al. 1991; Handeland et al. 2003; Noori et al. 2015), which might be associated with increased feed conversion in fish (Noori et al. 2015). Another purpose of utilizing extended photoperiod in salmon farming is to enable fish to feed continuously over a 24 h cycle, which can avoid fluctuations in dissolved oxygen levels in the water due to sudden feeding. However, this unnatural continuous feeding schedule might lead to an elevation in routine metabolic rate with fish being more active, which might further result in a reduced aerobic scope to handle environmental stressors, including hypoxia. Hence, the second objective of this chapter was to explore how photoperiod affects aerobic scope and hypoxia tolerance of coho salmon reared in RAS.

To address these objectives, a second cohort of Atlantic and coho salmon smolts was obtained and reared in the same RAS (as that of Chapter 2) under 12:12 h photoperiod (salinities of 2.5, 5 and 10 ppt) and 24:0 h photoperiod (salinities of 2.5, 5, 10 and 30 ppt). The new growth trial was designed to partly replicate the 2015-2016 salinity growth trial described in Chapter 2 and assess the interactive effects of photoperiod on growth in both species of salmon, which was a collaborative research presented in Chan (2018). For the purpose of this study, I focused on the effects of salinity and photoperiod on aerobic scope and hypoxia tolerance in coho salmon during the early growth stages (Day 60 and 120) when the grow-enhancing effect of 10 ppt was the greatest in the previous growth trial. For the hypoxia challenge trials, an increased air saturation of 15% was used in order to allow more time for fish to respond to hypoxia (10% air saturation in

Chapter 2). The findings from this chapter can not only enable us to see if there is some consistency in aerobic scope and hypoxia tolerance between smaller and larger coho salmon reared at different salinities, but also explore how photoperiod can interact with salinity to affect the physiology of coho salmon in RAS.

3.2 Methods

3.2.1 Fish Culture

After the 2015-2016 growth trial (Chapter 2) was finished, fish in all systems were removed and euthanized with an over-dose of MS222 (0.2 g L^{-1} tricaine methanesulfonate buffered with 0. 4 g L^{-1} sodium bicarbonate). All the systems were cleaned, disinfected with hydrogen peroxide (H_2O_2) and flushed for 30 days. LED lights (LED Area Light - 160 W - Natural White 5000K - 20,000 lumens, Super Bright LEDs, Inc., MO, USA) were installed above each 5 m^3 fish tank, and each tank was covered with a black plastic sheet to prevent ambient light contamination. Each LED light was connected to a timer which was used to control the photoperiod. All other aspects of the RAS system setups were identical to that described Chapter 2.

Approximately 6,000 Coho (Target Marine Hatcheries, Sechelt, British Columbia) and 6,000 Atlantic (Cermaq Canada, Campbell River, British Columbia) salmon were transported to UBC, held in flow-through systems, and maintained on a low feed ration (~0.3% body mass per day). In January 2017, 700 coho and 700 Atlantic salmon were randomly selected from the stock tanks for measurement of fork length and body mass using the same protocol described in Chapter 2. These values served as the starting values for the new growth trial. All the measured fish were then transferred to each of the 7 recirculating systems so that within a RAS, one tank contained 100 coho and the other tank contained 100 Atlantic salmon. The remaining ~5,000 fish per species were randomly distributed among the 7 systems so that each tank contained ~800 fish in total. The

photoperiod and salinity of the 7 recirculating systems were set up as: 12:12 light and dark from 8 am to 8 pm (2.5, 5 and 10 ppt) and 24:0 light and dark (2.5, 5, 10 and 30 ppt). Due to the fact that health of the Atlantic salmon reared in freshwater was compromised by fungus in the first year and the holding period in the second year, the 0 ppt salinity treatment was replaced with a slightly saline treatment of 2.5 ppt to help control fungal outbreaks. Within each system, a pH of 7 was maintained through the addition of $NaHCO_3$, and temperature was kept at 12 ℃. Fish were fed by automatic feeders with a relatively lower feeding rate at 0.5% body mass per day for the first 60 days to allow nitrifying bacteria to populate the biological filters. After the first 60 days, the feeding rate was adjusted to 1% body mass per day. Fish were only fed from 8am to 8pm in both 12:12 and 24:0 photoperiod treatments. Beyond Day 0, fork length and body mass of 100 randomly-selected fish per species were measured at Day 60 and 120 with the same protocol described above. Stocking density was adjusted at the end of each sampling period to maintain at ~40 kg m^{-3} throughout the growth trial. The measurements of body mass and growth rates over the first 120 days of Atlantic and coho salmon were presented in Chan (2018).

3.2.2 Respirometry

Routine and maximum metabolic rates were determined using the same protocol as described in Chapter 2, except the size of the respirometer was decreased to accommodate the smaller fish (a diameter of 15 cm, a length of 41 cm and a total volume of about 8 L). Analysis of 8 fish from each treatment took about 15 days respectively in each sampling time at Day 60 and 120.

3.2.3 Hypoxia Tolerance

The hypoxia challenge trials were also conducted over a 15-day period in each sampling period in March 2017 for Day 60 and July 2017 for Day 120. The setup and protocol for determining hypoxia tolerance was slightly different from that described in Chapter 2. Eight 19 L white opaque containers were placed in a large water bath that was maintained at 12℃ with an electric chiller. Eight fish from each treatment were randomly selected and each fish was transferred to one container filled with water from the respective RAS. Fish were allowed to acclimate to the container in darkness overnight under fully aerated conditions. Two cylinders of nitrogen and compressed air were connected to 2 mass flow controllers (SmartTrak C100L, Sierra, CA, USA). The flow rates of mass flow controllers were set up to achieve a ratio of nitrogen:air = 17:3, resulting in gas outflow of 15% air saturation. A manifold was used to distribute gas outflow into the 8 containers by bubbling via diffusers. Similar to the previous hypoxia trials, bubble wrap was placed on the water surface of containers to prevent gas exchange and aquatic surface breathing of fish. An YSI dissolved oxygen probe was moved among 8 containers to monitor oxygen levels. The oxygen levels were lowered from 100% to 15% air saturation within about 43 minutes during which bubbling rates were kept relatively consistent among 8 containers by adjusting the needle valves. After the oxygen levels reached 15% air saturation, a timer was started, the bubbling was maintained to displace CO_2 and ensure an O_2 level at 15% air saturation. The activity of fish was observed through the translucent bubble wrap. Similar to the 2015-2016 hypoxia trials, the procedure continued until fish displayed loss of equilibrium as defined by the inability to maintained their vertical position in the water. After fish lost equilibrium, the time was noted, the fish were removed from the containers and euthanized with over-dosed MS222 (0.2 g L^{-1} tricaine

methanesulfonate buffered with 0.4 g L^{-1} sodium bicarbonate), followed by a measurement of their body mass.

3.2.4 Statistical Analysis

Anderson Darling test was used to test normality of the data with an α of 0.05, and if the data were not normally distributed, transformation of the data was attempted to satisfy the assumption of normality. Data of RMR and time to LOE were not normally distributed so they were transformed into $\frac{1}{RMR}$ and $\lg(time\ to\ LOE + 1)$, respectively. Three-way ANCOVA was performed on $\frac{1}{RMR}$, MMR, AS and $\lg(time\ to\ LOE + 1)$, with exposure time (i.e., Day 60 and 120), salinity and photoperiod as factors, and body mass as a covariate due to the potential effect of variation in body mass on metabolic measurements and hypoxia tolerance. If significant differences were detected, Tukey post hoc multiple comparison was conducted to reveal where the significant differences occurred, with an α of 0.05. Pearson correlation coefficient was conducted to test the strength of linear relationship between time to LOE and body mass separately at Day 60 and 120 in order to explore how body mass affected time to LOE over time.

3.3 Results

3.3.1 Respirometry

There was no effect of body mass (P = 0.682), exposure time (P = 0.437), salinity (P = 0.090) and photoperiod (P = 0.196) on RMR of coho salmon (Figure 3.1, A and B). There was no effect of body mass (P = 0.402), exposure time (P = 0.310) and photoperiod (P = 0.130) on MMR of coho salmon. Salinity significantly affected MMR of fish (P = 0.005) and the interaction between salinity and photoperiod was also significant (P = 0.01), but Tukey post hoc analysis within a single time frame (either Day 60 or 120) did not reveal any significant difference between salinities and photoperiod (Figure 3.1, C and D). There was no effect of body mass (P = 0.382) and exposure time (P = 0.438) on AS of coho salmon. Salinity (P = 0.016) and photoperiod (0.018) significantly affected AS of coho salmon, and the interaction between salinity and photoperiod was also significant (P =0.003), but Tukey post hoc analysis did not show any significant difference in AS of fish across different salinities and photoperiods over time (Figure 3.1, E and F)..

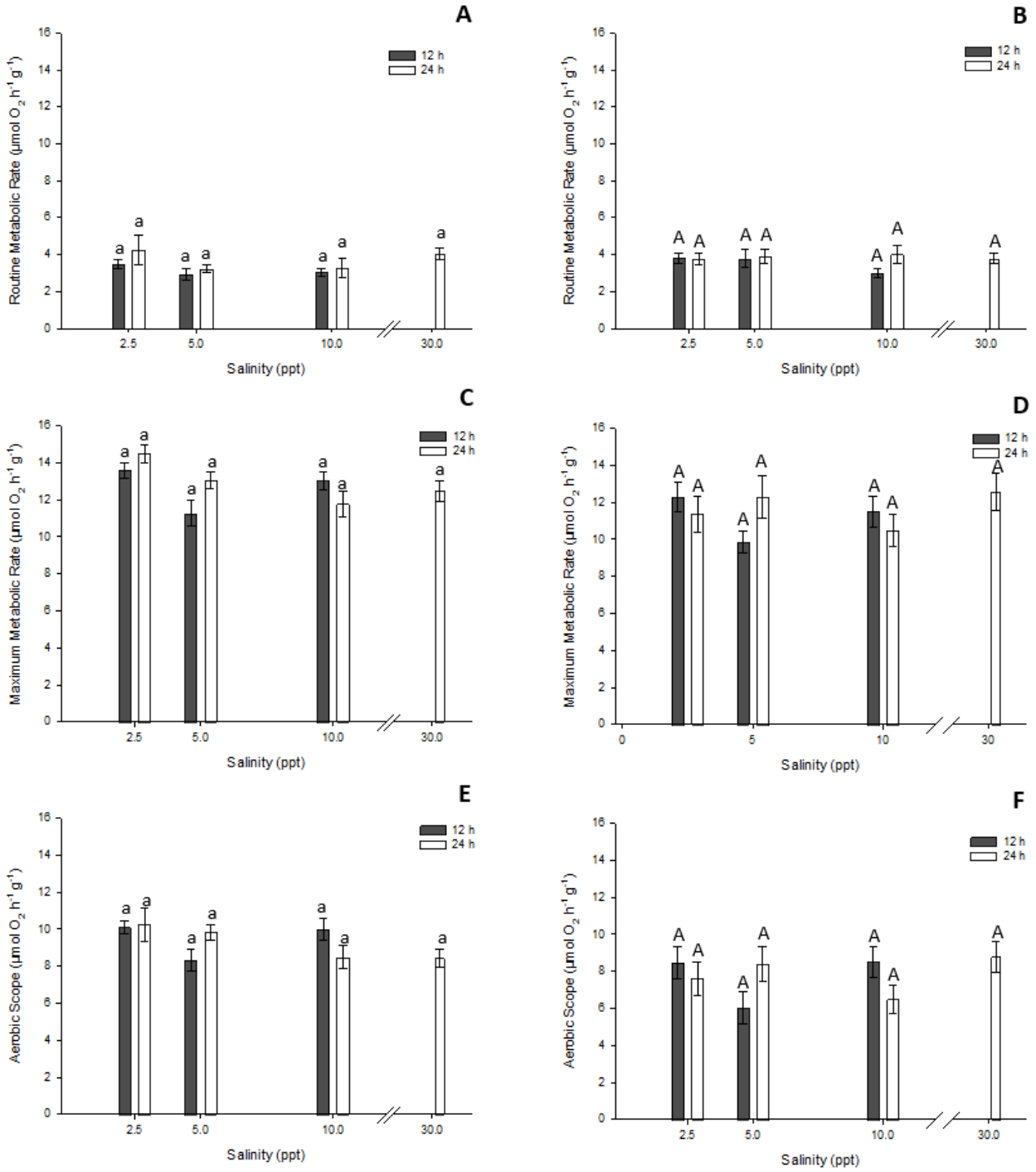

Figure 3.1 Routine metabolic rate (RMR), maximum metabolic rate (MMR) and aerobic scope (AS) at Day 60 and 120 of coho salmon reared at salinities of 2.5, 5, 10 and 30 ppt under 12 and 24 h photoperiods. Figures A, C and E represent Day 60 (mean body mass = 256.3 g), while figures B, D and F represent Day 120 (mean body mass = 439.7 g). Grey and white bars represent 12 and 24 h photoperiods, respectively. All data are mean ± SEM. Letters that differ indicate statistically significant differences among salinity and photoperiod treatments within either Day 60 or 120 ($P < 0.05$).

3.3.2 Hypoxia Tolerance

Body mass was a significant covariate in this analysis of hypoxia tolerance as assessed by time to LOE ($P < 0.001$). To investigate the effect of body mass in more detail, Pearson correlations were performed between time to LOE and body mass, and the correlation coefficient increased from -0.537 at Day 60 ($P < 0.01$) to -0.264 at Day 120 ($P = 0.05$), which suggests that the correlation between time to LOE and body mass weakened over time. At Day 60, larger fish generally had a poorer hypoxia tolerance than smaller fish, but at Day 120, the effect of body mass on hypoxia tolerance was reduced (Figure 3.2). After time to LOE was corrected to body mass, ANCOVA revealed that there was no effect of exposure time ($P = 0.057$), salinity ($P = 0.301$) and photoperiod ($P = 0.688$) on hypoxia tolerance of coho salmon (Figure 3.3).

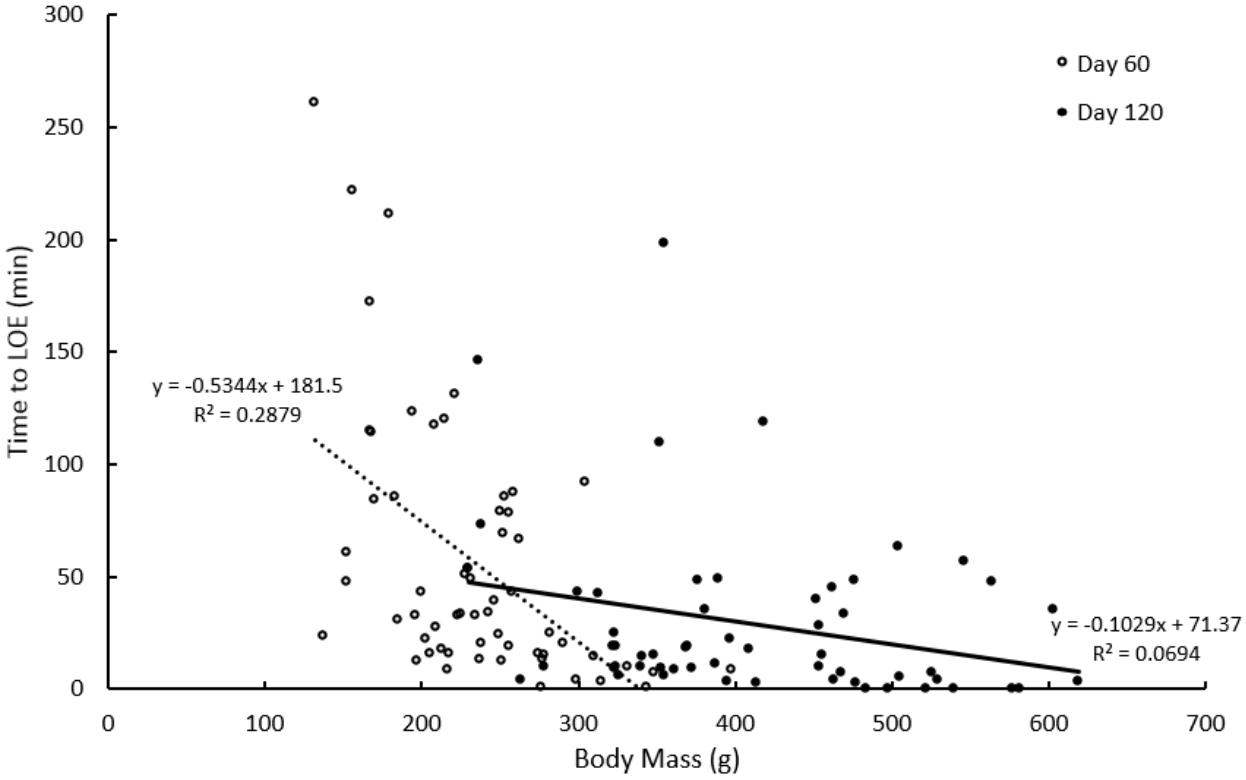

Figure 3.2 Relationship between time to loss of equilibrium (LOE) and body mass of coho salmon sampled at Day 60 and 120, with salinity and photoperiod combined. Open and closed circles represent Day 60 and 120, respectively. Dashed line is the regression line for Day 60: $y = -0.5344x + 181.5$, $R^2 = 0.2879$, mean body mass = 237.4 g; Solid line is the regression line for Day 120: $y = -0.1029x + 71.37$, $R^2 = 0.0694$, mean body mass = 413.5 g.

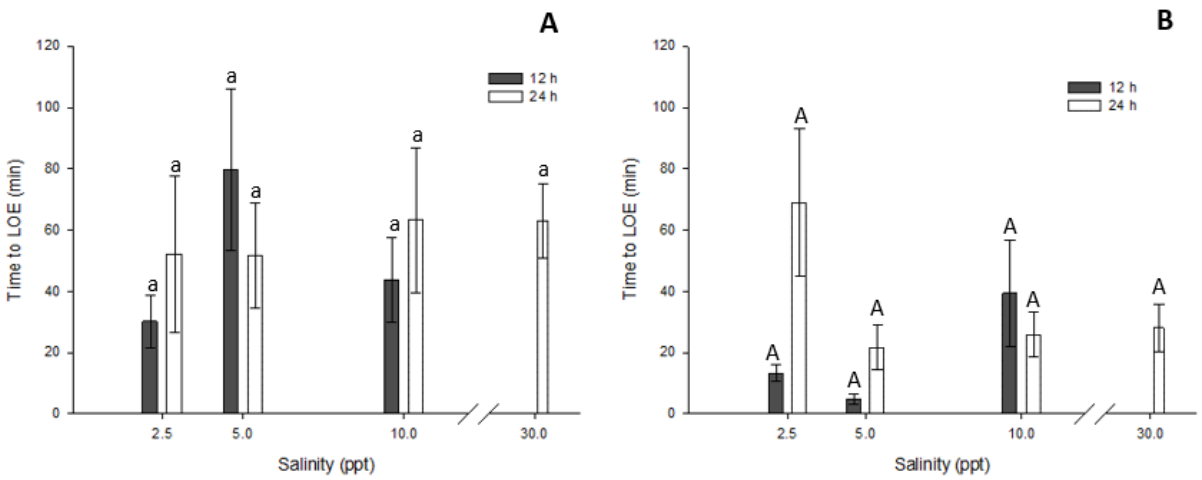

Figure 3.3 Time to loss of equilibrium (LOE) at 15% air saturation of coho salmon reared at 2.5, 5, 10 and 30 ppt under 12 and 24 h photoperiods, sampled at Day 60 (A; mean body mass = 237.4 g) and 120 (B; mean body mass = 413.5 g). Grey and white bars represent 12 and 24 h photoperiods, respectively. All data are mean ± SEM. Letters that differ indicate statistically significant differences among salinity and photoperiod treatments within either Day 60 or 120 ($P < 0.05$).

3.4 Discussion

In this chapter, respirometry and hypoxia challenge trials were conducted on coho salmon during their early growth stages. It was found that at either Day 60 or 120 of rearing, there was no effect of salinity and photoperiod on RMR, MMR, AS and hypoxia tolerance of these coho salmon.

3.4.1 Routine, Maximum Metabolic Rates and Aerobic Scope

In this chapter, I performed respirometry on a new cohort of smaller coho salmon at Day 60 and 120 under the assumption that we would see the highest growth rate at 10 ppt during this early growth window, which was seen in Emerman (2016). However, in contrast with Emerman (2016), Chan (2018) demonstrated in the present 2016-2017 growth trial that salinity had no effect on growth of coho salmon up to Day 120, so we were unable to replicate the previous year's increase in growth rate at 10 ppt. Hence, not surprisingly, there was no effect of salinity on RMR, MMR and AS of coho salmon at either Day 60 or 120 (Figure 3.1), which together imply that osmoregulation did not comprise a significant portion of the total metabolic energy for those fish.

Photoperiod is well-known to affect behavioural patterns in fish, and indeed, salmonids tend to be more active during the daylight (Godin 1981; Brännäs and Alanärä 1997; Bolliet et al. 2001). In salmon farming, fish are often reared under extended photoperiods relatively to the natural photoperiod so that they can be fed continuously in order to avoid fluctuations in water oxygen levels that are associated with feeding events. However, these unnatural photoperiod regimes

might cause metabolic rates of fish to be elevated, which would result in lower AS and hence less capacity to support additional physiological activities. Interestingly, the results of the current chapter showed photoperiod had no effect on RMR, MMR and AS of coho salmon measured during the daytime within either Day 60 or 120 (Figure 3.1), which disagrees with my predictions that fish reared under continuous light should have higher RMR and hence lower AS (Figure 1.2). Similarly, Ross and McKinney (1988) measured the metabolic rate of Nile tilapia (*Oreochromis niloticus*) under 12:0 photoperiod in respirometers for 9 days, and they found a circadian rhythm occurred in metabolic rate which was higher during the daytime and lower during the night time. Ross and McKinney (1988) then adjusted the photoperiod to 24 h light, and they found the circadian rhythm remained while the metabolic rate still peaked and bottomed out at the same levels as observed in the 12:0 photoperiod regime. Fraser et al. (1993, 1995) reported that Atlantic salmon showed more nocturnalism at lower temperatures. The authors also observed a supressed activity of fish at low temperatures even under a summer photoperiod. Eventually, Fraser et al. (1993, 1995) concluded that such behavioural changes in activities of fish are dependent on temperature and independent of photoperiod. This may explain my findings that no difference was found in metabolic rates of coho salmon between 12:12 and 24:0 photoperiods given that temperature was consistent among all RAS. Nevertheless, the findings in this chapter also indicate that it is feasible to rear and feed coho salmon under 24 h light in RAS without elevating metabolic rate and reducing aerobic scope.

In the 2016-2017 respirometry trials, the body mass of the coho salmon used ranged from 150 to 400 g at Day 60 and from 250 to 770 g at Day 120, but it was found that body mass had no effect on RMR, MMR and AS of coho salmon over the period. These results are however, not entirely

consistent with the previous 2015-2016 respirometry described in Chapter 2: body mass had no effect on RMR but a negative effect on MMR and AS in the larger and older coho salmon (1200 – 3200 g). Clark et al. (2012) found RMR and MMR increased with body mass in coho salmon (200 - 6000 g), but AS was consistent among the different sizes of fish examined. Similarly, Cutts et al. (2002) found SMR and MMR increased with body mass in juvenile Atlantic salmon (1 – 9 g), but AS decreased with body mass. Therefore, the effect of body mass on metabolic rates can be influenced by species and developmental stages.

3.4.2 Hypoxia Tolerance

In the 2016-2017 hypoxia challenge trials, no statistical difference was found in hypoxia tolerance of coho salmon reared under different conditions of salinities and photoperiods at either Day 60 and 120 (Figure 3.3). This result is consistent with the previous chapter where no effect of salinity was seen in hypoxia tolerance of the larger coho salmon (Figure 2.7). The result was also in agreement with Henriksson et al. (2008) who found that salinity (0, 15 and 30 ppt) had no effect on P_{crit} of staghorn sculpin (*Leptocottus armatus*), which further indicates that salinity might not have a large effect on hypoxia tolerance of the fish. As described in the previous chapter, hypoxia tolerance of fish might be influenced by both the oxygen demand required to support routine activities, and the capacity to extract oxygen from water, which can be indirectly assessed through measurements of RMR and MMR, respectively. When a fish has a low RMR, that means the fish does not require that much oxygen and ATP to support its fundamental physiological processes, so the fish might be able to sustain itself in hypoxia for a longer period of time. For a fish to attain a high MMR, it must have the means to extract oxygen from the environment at a high rate, thus

MMR can be used as an indirect assessment of the capacity for oxygen extraction. Therefore, one might predict that fish with larger AS (low RMR and high MMR) would have an enhanced hypoxia tolerance. As the current chapter has demonstrated, salinity and photoperiod had no effect on AS of the coho salmon, which might contribute to explaining the lack of effects of salinity and photoperiod on hypoxia tolerance of these fish. These findings also suggested that hypoxia tolerance is not compromised in coho salmon reared under different salinity and photoperiod regimes, which provides salmon farmers with more options of rearing salinities and photoperiods without any concerns in altering fish's hypoxia tolerance.

In the coho salmon examined at Day 60, large fish tended to have lower hypoxia tolerance than small fish, but this negative relationship between hypoxia tolerance and body mass was weakened at Day 120 (Figure 3.2). The previous hypoxia challenge trials from 2015-2016 showed no effect of body mass on hypoxia tolerance of coho salmon (600 – 2200 g) at 10% air saturation. Therefore, combined, my data suggests that body mass negatively affects hypoxia tolerance of coho salmon during the early growth stage, but this effect is reduced as fish grow older. Burleson et al. (2001) exposed a group of largemouth bass (*Micropterus salmoides*) (23 - 3000 g) to an oxygen gradient ranging from 10 to 95% air saturation and found that small fish had a tendency to reside in less oxygenated water within the chamber. Burleson et al. (2001) suggested that this behaviour of the small fish was to avoid competition and predation, and it implied greater hypoxia tolerance relative to the larger fish, which is consistent with my findings in the current chapter. However, Roze et al. (2013) examined the effect of body mass on lethal oxygen tension of rainbow trout and found larger fish had better hypoxia tolerance. Similarly, Almeida-Val et al. (2000) measured survival time of Oscar cichlid (*Astronotus ocellatus*) (13 to 260 g) exposed to severe hypoxia, and found

larger fish had better hypoxia tolerance. A review on the effect of body mass on hypoxia tolerance in fish (Nilsson and Östlund-Nilsson 2008) suggested bigger fish do have a larger gill respiratory surface, but this will be compensated by a higher whole-body oxygen consumption, so body mass essentially has little impact on hypoxia tolerance of fish. Ultimately, Nilsson and Östlund-Nilsson (2008) concluded that natural adaptations to hypoxic environments can outweigh the impact of body mass, which might explain some of the variation and inconsistency in the effect of body mass on hypoxia tolerance in current and other studies.

3.4.3 Conclusions

This chapter found salinity, photoperiod as well as body mass had no effect on RMR, MMR and AS of coho salmon at either Day 60 or 120. Similarly, there was no effect of salinity or photoperiod on hypoxia tolerance of the coho salmon at either Day 60 or 120, but there was a negative relationship between hypoxia tolerance and body mass in coho salmon, with the relationship fading as fish grew larger.

Chapter 4: General Discussion and Conclusions

4.1 Summary

To determine the optimal salinity to grow salmon in RAS and how metabolism and hypoxia tolerance were accordingly affected, Atlantic and coho salmon smolts were reared at salinities of 0, 5, 10, 20 and 30 ppt over 400 days in RAS, while their growth, metabolic rates and hypoxia tolerance were measured.

In Atlantic salmon, Emerman (2016) found salinity had no effect on growth up to Day 96 (~250 g), but I found salinity started to affect growth at Day 295 (~1000 g) through to Day 400 (~1700 g), with overall greater growth rates and body mass seen at intermediate salinities (5 and 10 ppt). The increase in growth rate at 5 ppt may partially be due to a relatively lower incidence of sexual maturation, whereby early sexual maturation is associated with a reduction in somatic growth. Higher incidences of cataracts were observed in fish reared at 5, 10 and 20 ppt compared with 0 and 30 ppt, but the high incidences of cataracts did not seem to compromise growth of the fish. There was no general pattern in RMR, MMR and AS of Atlantic salmon as a function of salinity. Hypoxia tolerance of Atlantic salmon at 5 and 10 ppt was significantly higher than 0 ppt. However, there seems to be no relationship between metabolic measurements and hypoxia tolerance in Atlantic salmon.

In coho salmon, Emerman (2016) found that iso-osmotic salinity enhanced growth as early as Day 59 (~180 g). I found in the later growth trial that the impact of this early increase in growth rate was observed through to Day 460 (~2300 g) with coho salmon reared at 10 ppt always being larger than the ones at 0, 5, 20 and 30 ppt. Coho salmon showed no sexual maturation and few cataracts over the entire growth trial. The experiments conducted on both larger coho salmon in Chapter 2 and smaller coho salmon in Chapter 3 indicate that there was no general pattern in RMR, MMR and AS as a function of salinity, while salinity also had no effect on hypoxia tolerance of coho salmon. Similarly, there seems to be no connection between metabolic measurements and hypoxia tolerance in coho salmon. Continuous photoperiod is often used in salmon farming so that fish can be fed throughout the day to avoid fluctuations in water oxygen levels due to feeding activities, but there was no information available about how this unnatural photoperiod may impact metabolism and hypoxia tolerance. Results from my Chapter 3 show that coho salmon reared at different photoperiod regimes (12:12 and 24:0, light:dark) did not show variations in RMR, MMR, AS or hypoxia tolerance at either Day 60 or 120.

4.2 Industrial Applications

The current study found that growth of Atlantic and coho salmon might be enhanced at intermediate salinities (5 to 10 ppt) in RAS. However, from an industrial perspective, a salinity of 5 ppt might be a better option than the iso-osmotic salinity (10 ppt) to rear Atlantic salmon in RAS, since using saline water to rear fish may be costly depending on the location of the facility. The same applies to coho salmon. Coho salmon reared at 10 ppt had the highest mean final body mass,

but the value was quite similar to that at 5 ppt. Therefore, profit gained from the minor enhancement in the final body mass of coho salmon reared at 10 ppt, compared with the ones at 5 ppt, might not cover the increasing cost of using more salt at 10 ppt, which suggests using 5 ppt to rear coho salmon might be a better option. Additionally, the rearing of coho salmon in RAS has some benefits over Atlantic salmon, as coho salmon exhibited very little precocious sexual maturation (as they were an all-female strain) and few cataracts.

Despite the fact that we found enhancement in growth of Atlantic and coho salmon at intermediate salinities, there was no significant difference in RMR of both species reared at different salinities, which indicates the variations in growth did not change the overall metabolic expenditure. The current thesis also found hypoxia tolerance was independent of environmental salinity in coho salmon, and it was even enhanced in Atlantic salmon reared at intermediate salinities. These findings indicate that the enhanced growth of both species at intermediate salinities did not occur at a cost of compromising their hypoxia tolerance. Therefore, in the salmon farming, it is feasible to rear these two salmon species in RAS at intermediate salinities with a high stocking density to achieve optimal growth and production without a negative effect on oxygen consumption rate or hypoxia tolerance.

In salmon farming, fish are often reared under 24 h light so that they can be fed continuously throughout the day to avoid fluctuations in water oxygen levels due to feeding activities. The current study found no difference in routine oxygen consumption rates of coho salmon reared at 12:0 and 24:0 photoperiods. This finding demonstrates a feasibility of rearing and feeding coho

salmon under continuous light, without elevating their routine metabolic rate or further reducing aerobic scope.

4.3 Limitations and Future Directions

The current study only had one system for each salinity treatment in the 2015-2016 growth trial, or two systems with two photoperiod regimes for each salinity in the 2016-2017 growth trial due to the limitation in laboratory space. Even though about 100 fish were sampled for body size for each species and each treatment, statistically they can only be considered as a sample size of one, so it was difficult to distinguish between effects of tanks and salinity on the results obtained from the growth trial. Prior to the first-year growth trial, Emerman (2016) grew Atlantic salmon in freshwater with other rearing conditions controlled in the 7 RAS for 5 months, and he found that there was no difference in growth of the fish, suggesting that there is no tank effect on growth of fish in this experimental design. Nevertheless, repeated growth trials might be necessary to ensure no tank effect and permit a more detailed statistical analysis. Alternatively, we could narrow down the treatment range and focus on the most interesting and representative treatments (e.g., 0, 10 and 30 ppt for freshwater, iso-osmotic salinity and seawater, respectively).

As the calculations of the whole-tank growth rates were based on the difference in whole-tank average body mass obtained from two sampling times, no statistical analysis could be conducted on the whole-tank growth rates. To solve this issue, we implanted pit tags in 100 fish in each treatment in order to obtain repeated measures of growth data from individual fish so that statistical

analysis could be conducted on those data. In my thesis, both whole-tank and tagged fish growth rates showed similar trends across salinities, but there were some variations in numeral values between those two different ways of measurements, which is probably due to the low sample size of tagged fish (n = 2 - 40). Hence, for any future studies associated with growth trials, it would be very beneficial to capture all the tagged fish for a greater statistical power and a more accurate calculation of growth rates.

My thesis demonstrates the potential growth-enhancing effect of intermediate salinities (5 and 10 ppt) on growth of salmon in RAS. The observed elevation of growth was associated with enhanced feed conversion. Hence, it would be interesting to measure the intestinal digestive enzyme activities of fish reared at different salinities to shed further insight into the physiological basis for improved growth at intermediate salinities.

The InSEAS recirculating systems at The University of British Columbia provide complete system setups with a semi-industrial scale and are able to simulate practical RAS operation in aquaculture. With this capacity, more different types of experiments could be conducted in the future to explore the effects of other environmental conditions, and a variety of physiological responses could be examined. Rearing fish in RAS has many potential advantages, such as the ability to manually control rearing conditions, growing fish with high stocking densities, enhanced biosecurity and less impact on the environment, so the usage of RAS in aquaculture is of increasing interest. Despite the high construction and operation costs of RAS, if more studies are conducted to improve

the biological and technical efficiency of this technique, it will ultimately increase profitability of and thus the potential for RAS in aquaculture.

References

Almeida-Val, V. M. F., Val, A. L., Duncan, W. P., Souza, F. C. A., Paula-Silva, M. N. and Land, S., 2000. Scaling effects on hypoxia tolerance in the Amazon fish *Astronotus ocellatus* (Perciformes: Cichlidae): contribution of tissue enzyme levels. Comparative Biochemistry and Physiology, Part B, 125(2), 219-226.

Amano, M., Hyodo, S., Kitamura, S., Ikuta, K., Suzuki, Y., Urano, A. and Aida, K., 1995. Short photoperiod accelerates preoptic and ventral telencephalic salmon GnRH synthesis and precocious maturation in underyearling male masu salmon. General and Comparative Endocrinology, 99(1), 22-27.

Ando, H. and Urano, A., 2005. Molecular regulation of gonadotropin secretion by gonadotropin releasing hormone in salmonid fishes. Zoological Science, 22(4), 379-389.

Arnesen, A. M., Jørgensen, E. H. and Jobling, M., 1993. Feed intake, growth and osmoregulation in Arctic charr, *Salvelinus alpinus* (L.), following abrupt transfer from freshwater to more saline water. Aquaculture, 114(3), 327-338.

Barnes, R., King, H. and Carter, C. G., 2011. Hypoxia tolerance and oxygen regulation in Atlantic salmon, *Salmo salar* from a Tasmanian population. Aquaculture, 318(3), 397-401.

Bjerkås, E., Bjørnestad, E., Breck, O. and Waagbø, R., 2001. Water temperature regimes affect cataract development in smolting Atlantic salmon, *Salmo salar* L. Journal of Fish Diseases, 24(5), 281-291.

Björnsson, B. T., Taranger, G. L., Hansen, T., Stefansson, S. O. and Haux, C., 1994. The interrelation between photoperiod, growth hormone, and sexual maturation of adult Atlantic salmon (*Salmo salar*). General and Comparative Endocrinology, 93(1), 70-81.

Bœuf, G. and Payan, P., 2001. Review: How should salinity influence fish growth? Comparative Biochemistry and Physiology Part C 130 : 411-423

Bolliet, V., Aranda, A. and Boujard, T., 2001. Demand-feeding rhythm in rainbow trout and European catfish. Synchronisation by photoperiod and food availability. Physiology & Behavior, 73(4), 625.

Borowiec, B. G., Crans, K. D., Khajali, F., Pranckevicius, N. A., Young, A. and Scott, G. R., 2016. Interspecific and environment-induced variation in hypoxia tolerance in sunfish. Comparative Biochemistry and Physiology, Part A, 198, 59-71.

Brännäs, E. and Alanärä, A., 1997. Is diel dualism in feeding activity influenced by competition between individuals? Canadian Journal of Zoology, 75(5), 661-669.

Brauner, C. J., Gonzalez, R. J. and Wilson, J. M., 2013. Extreme environments: hypersaline, alkaline, and ion-Poor Waters. Pages 435-476 in S. D. McCormick, A. P. Farrell, and C. J. Brauner (Eds.), Euryhaline Fishes: Fish Physiology, Vol 32. Academic Press.

Breck, O. and Sveier, H., 2001. Growth and cataract development in two groups of Atlantic salmon (*Salmo salar* L) post smolt transferred to sea with a 4 week interval. Bulletin of the European Association of Fish Pathologists 21, 91–103.

Brennan, R. S., Hwang, R., Tse, M., Fangue, N. A. and Whitehead, A., 2016. Local adaptation to osmotic environment in killifish, *Fundulus heteroclitus*, is supported by divergence in swimming performance but not by differences in excess post-exercise oxygen consumption or aerobic scope. Comparative Biochemistry and Physiology, Part A, 196, 11-19.

Brett, J. R., 1962. Some considerations in the study of respiratory metabolism in fish, particularly salmon. Journal of the Fisheries Research Board of Canada, 19(6), 1025-1038.

Bromage, N., Porter, M. and Randall, C., 2001. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. Aquaculture, 197(1), 63-98.

Bucking, C. and Wood, C. M., 2007. Gastrointestinal transport of Ca^{2+} and Mg^{2+} during the digestion of a single meal in the freshwater rainbow trout. Journal of Comparative Physiology B, 177(3), 349-360.

Burleson, M. L., Wilhelm, D. R. and Smatresk, N. J., 2001. The influence of fish size on the avoidance of hypoxia and oxygen selection by largemouth bass. Journal of Fish Biology, 59(5), 1336-1349.

Burt, K., Hamoutene, D., Perez-Casanova, J., Kurt Gamperl, A. and Volkoff, H., 2013. The effect of intermittent hypoxia on growth, appetite and some aspects of the immune response of Atlantic salmon (*Salmo salar*). Aquaculture Research, 45(1), 124-137.

Bushnell, P. G. and Brill, R. W., 1992. Oxygen transport and cardiovascular responses in skipjack tuna (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) exposed to acute hypoxia. Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology, 162(2), 131-143.

Castanheira, M. F., Martins, C. I. M., Engrola, S. and Conceição, L. E. C., 2011. Daily oxygen consumption rhythms of Senegalese sole solea senegalensis (Kaup, 1858) juveniles. Journal of Experimental Marine Biology and Ecology, 407(1), 1-5.

Chabot, D., McKenzie, D. J. and Craig, J. F., 2016a. Metabolic rate in fishes: Definitions, methods and significance for conservation physiology. Journal of Fish Biology, 88(1), 1-9.

Chabot, D., Steffensen, J. F. and Farrell, A. P., 2016b. The determination of standard metabolic rate in fishes. Journal of Fish Biology, 88(1), 81-121.

Chan, V., 2018. Determining the optimal salinity and photoperiod for Atlantic and coho salmon growth and swimming performance raised in recirculating aquaculture systems from smolt to adult. Master thesis. The University of British Columbia.

Choi, S., Lee, C. H., Park, W., Kim, D. and Sohn, Y. C., 2010. Effects of shortened photoperiod on gonadotropin-releasing hormone, gonadotropin, and vitellogenin gene expression associated with ovarian maturation in rainbow trout. Zoological Science, 27(1), 24-32.

Christensen, E. A. F., Svendsen, M. B. S. and Steffensen, J. F., 2017. Plasma osmolality and oxygen consumption of perch *Perca fluviatilis* in response to different salinities and temperatures. Journal of Fish Biology, 90(3), 819-833.

Claireaux, G. and Lagardère, J. -P., 1999. Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. Journal of Sea Research, 42(2), 157-168.

Clark, T. D., Donaldson, M. R., Pieperhoff, S., Drenner, S. M., Lotto, A., Cooke, S. J., Hinch, S. G., Patterson, D. A. & Farrell, A. P., 2012. Physiological benefits of being small in a changing world: Responses of coho salmon (*Oncorhynchus kisutch*) to an acute thermal challenge and a simulated capture event. PloS One, 7(6) : e39079.

Cullen, A. P. Monteith-McMaster, C. A. and Sivak, J. G., 1994. Lenticular changes in rainbow trout following chronic exposure to UV radiation, Current Eye Research, 13:10, 731-737

Cutts, C. J., Metcalfe, N. B. and Taylor, A. C., 2002. Juvenile Atlantic salmon (*Salmo salar*) with relatively high standard metabolic rates have small metabolic scopes. Functional Ecology, 16(1), 73-78.

DFO, Salmon Facts, 2017. Salmon facts – Pacific salmon – coho salmon. [accessed 2017 December 29]. http://www.pac.dfo-mpo.gc.ca/fm-gp/species-especes/salmon-saumon/facts-infos-eng.html#coho

Døskeland, I., Imsland, A. K. D., Fjelldal, P. G., Stefansson, S. O., Roth, B., Eriksen, K. E., Mikalsen, B. and Handeland, S., 2016. The effect of low temperatures and photoperiods on growth and vertebra morphometry in Atlantic salmon. Aquaculture International, 24(5), 1421-1434.

Duffy, N. M., Bui, P., Bagherie-Lachidan, M. and Kelly, S. P., 2011. Epithelial remodeling and claudin mRNA abundance in the gill and kidney of puffer fish (*Tetraodon biocellatus*) acclimated to altered environmental ion levels. Journal of Comparative Physiology B, 181(2), 219-238.

Duncan, N., Mitchell, D. and Bromage, N., 1999. Post-smolt growth and maturation of out-of-season 0+ Atlantic salmon (*Salmo salar*) reared under different photoperiods. Aquaculture, 177(1), 61-71.

Eddy, F. B., 1982. Osmotic and ionic regulation in captive fish with particular reference to salmonds. Comparative Biochemistry and Physiology -- Part B: Biochemistry and Molecular Biology, 73(1), 125-141.

Edsall, T. A., Frank, A. M., Rottiers, D. V. and Adams, J.V., 1999. The effect of temperature and ration size on the growth, body composition, and energy content of juvenile coho salmon. Journal of Great Lakes Research, 25(2), 355-362.

Eliason, E. J. and Farrell, A. P., 2016. Oxygen uptake in pacific salmon *Oncorhynchus* spp.: When ecology and physiology meet. Journal of Fish Biology, 88(1), 359-388.

Emerman, J. D., 2016. Establishing the optimal salinity for rearing salmon in recirculating aquaculture systems. Master thesis. The University of British Columbia.

Eriksen, N. T., 2002. Accuracy and precision of aquatic respirometers with emphasis on monophase oxystats. Fish Physiology and Biochemistry, 26(2), 139-147.

Ersdal, C., Midtlyng, P. J. and Jarp, J., 2001. An epidemiological study of cataracts in seawater farmed Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms, 45(3), 229-236.

FAO. 2016a. The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome. 200 pp.

FAO, 2016b. Commodity Statistics Update, Salmon, by GLOBEFISH.

FAO, FishStat. Global Aquaculture Production for Species, *Oncorhynchus kisutch*. [accessed 2017 August 31]. http://www.fao.org/fishery/culturedspecies/Oncorhynchus_kisutch/en

Farrell, A. P., 2009. Environment, antecedents and climate change: Lessons from the study of temperature physiology and river migration of salmonids. The Journal of Experimental Biology, 212(Pt 23), 3771-3780.

Farrell, A. P., 2016. Pragmatic perspective on aerobic scope: Peaking, plummeting, pejus and apportioning. Journal of Fish Biology, 88(1), 322-343.

Fiess, J. C., Kunkel-Patterson, A., Mathias, L., Riley, L. G., Yancey, P. H., Hirano, T. and Grau, E. G., 2007. Effects of environmental salinity and temperature on osmoregulatory ability, organic osmolytes, and plasma hormone profiles in the Mozambique tilapia (*Oreochromis mossambicus*). Comparative Biochemistry and Physiology, Part A, 146(2), 252-264.

Flik, G. and Verbost, P. M., 1993. Calcium transport in fish gills and intestine. Journal of Experimental Biology, 184, 17.

Folke, C., Kautsky, N. and Troell, M., 1994. The costs of eutrophication from salmon farming: Implications for policy. Journal of Environmental Management, 40(2), 173-182.

Fraser, N. H. C., Heggenes, J., Metcalfe, N. B. and Thorpe, J. E., 1995. Low summer temperatures cause juvenile Atlantic salmon to become nocturnal. Canadian Journal of Zoology, 73(3), 446-451.

Fraser, N. H. C., Metcalfe, N. B. and Thorpe, J. E., 1993. Temperature-dependent switch between diurnal and nocturnal foraging in salmon. Proceedings of the Royal Society of London. Series B: Biological Sciences, 252(1334), 135-139.

Fry, F. E. J., 1947. Effects of the environment on animal activity. Publications of the Ontario Fisheries Research Laboratory 68, 1–62.

Gaumet, F., Bœuf, G., Severe, A., Le Roux, A. and Mayer-Gostan, N., 1995. Effects of salinity on the ionic balance and growth of juvenile turbot. Journal of Fish Biology, 47(5), 865-876.

Godin, J. -G. J., 1981. Circadian rhythm of swimming activity in juvenile pink salmon (*Oncorhynchus gorbuscha*). Mar. Biol. 64, 341–349.

Good, C., Weber, G. M., May, T., Davidson, J. and Summerfelt, S., 2016. Reduced photoperiod (18 h light vs. 24 h light) during first-year rearing associated with increased early male maturation in Atlantic salmon *Salmo salar* cultured in a freshwater recirculation aquaculture system. Aquaculture Research, 47(9), 3023-3027.

Gordon, M. S., 1977. Water and solute metabolism. Pages 295–363 in M.S. Gordon (ed.), Animal Physiology-Principles and Adaptations (3rd edn). Collier Macmillan, London.

Gracia-López, V., Rosas-Vázquez, C. and Brito-Pérez, R., 2006. Effects of salinity on physiological conditions in juvenile common snook *Centropomus undecimalis*. Comparative Biochemistry and Physiology, Part A, 145(3), 340-345.

Greco, A. M., Fenwick, J. C. and Perry, S. F., 1996. The effects of soft-water acclimation on gill structure in the rainbow trout *Oncorhynchus mykiss*. Cell and Tissue Research, 285(1), 75-82.

Greco, A. M., Gilmour, K., Fenwick, J. C. and Perry, S. F., 1995. The effects of soft-water acclimation on respiratory gas transfer in the rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 198, 2557–2567.

Grosell, M., Laliberte, C. N., Wood, S., Jensen, F. B. and Wood, C. M., 2001. Intestinal HCO_3^- secretion in marine teleost fish: evidence for an apical rather than a basolateral Cl^-/HCO_3^- exchanger, Fish Physiol. Biochem. 24: 81.

Guerrero-Tortolero, D. A. and Bromage, N., 2008. Growth and maturation of Atlantic salmon (*Salmo salar*) populations with different grilse proportions under natural photoperiod and superimposed nighttime light. Aquaculture, 285(1), 63-66.

Gústavsson, A., Imsland, A. K., Gunnarsson, S., Árnason, J., Arnarson, I., Jónsson, A. F., Smáradóttir, H. and Thorarensen, H., 2010. Growth and blood chemistry of Atlantic halibut

(*Hippoglossus hippoglossus* L.) in relation to salinity and continuous light. Aquaculture International, 18(3), 433-445.

Handeland, S. O., Imsland, A. K. and Stefansson, S. O., 2008. The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. Aquaculture, 283(1), 36-42.

Handeland, S. O., Imsland, A. K., Björnsson, B. T. and Stefansson, S. O., 2013. Long-term effects of photoperiod, temperature and their interaction on growth, gill Na^+, K^+-ATPase activity, seawater tolerance and plasma growth-hormone levels in Atlantic salmon *Salmo salar*: Manipulation of smoltification in *Salmo salar*. Journal of Fish Biology, 83(5), 1197-1209.

Handeland, S. O., Porter, M., Björnsson, B. T. and Stefansson, S. O., 2003. Osmoregulation and growth in a wild and a selected strain of Atlantic salmon smolts on two photoperiod regimes. Aquaculture, 222(1), 29-43.

Haney, D. C. and Nordlie, F. G., 1997. Influence of environmental salinity on routine metabolic rate and critical oxygen tension of *Cyprinodon variegatus*. Physiological Zoology, 70(5), 511-518.

He, W., Cao, Z. and Fu, S., 2015. Effect of temperature on hypoxia tolerance and its underlying biochemical mechanism in two juvenile cyprinids exhibiting distinct hypoxia sensitivities. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology, 187, 232-241.

Henriksson, P., Mandic, M., Richards, J. G., 2008. The osmorespiratory compromise in sculpins: Impaired gas exchange is associated with freshwater tolerance. Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches, 81(3), 310-319.

Hevrøy, E. M., Hunskår, C., de Gelder, S., Shimizu, M., Waagbø, R., Breck, O., Takle, H., Sussort, S. and Hansen, T., 2013. GH–IGF system regulation of attenuated muscle growth and lipolysis in Atlantic salmon reared at elevated sea temperatures. Journal of Comparative Physiology B, 183(2), 243-259.

Hwang, P., Lee, T. and Lin, L., 2011. Ion regulation in fish gills: Recent progress in the cellular and molecular mechanisms. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology, 301(1), R28-R47.

Imsland, A. K., Foss, A., Koedijk, R., Folkvord, A., Stefansson, S. O. and Jonassen, T. M., 2007. Persistent growth effects of temperature and photoperiod in Atlantic cod *Gadus morhua*. Journal of Fish Biology, 71(5), 1371-1382.

Imsland, A. K., Handeland, S. O. and Stefansson, S. O., 2014. Photoperiod and temperature effects on growth and maturation of pre- and post-smolt Atlantic salmon. Aquaculture International, 22(4), 1331-1345.

Iwata, M., Komatsu, S., Collie, N. L., Nishioka, R. S. and Bern, H. A., 1987. Ocular cataract and seawater adaptation in salmonids. Aquaculture, 66(3), 315-327.

Kirschner, L. B., 1993. The energetics of osmotic regulation in ureotelic and hypoosmotic fishes. The Journal of Experimental Zoology, 267:19-26.

Kocabas, M., Basçinar, N., Sahin, S. A. and Okumus, I., 2011. Effect of salinity on growth of abant trout *Salmo trutta abanticus*. Journal of Fish Biology, 79(7), 1961-1967.

Krogdahl, Å., Sundby, A. and Olli, J. J., 2004. Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) digest and metabolize nutrients differently. effects of water salinity and dietary starch level. Aquaculture, 229(1), 335-360.

Kutti, T., Ervik, A. and Høisæter, T., 2008. Effects of organic effluents from a salmon farm on a fjord system. III. linking deposition rates of organic matter and benthic productivity. Aquaculture, 282(1), 47-53.

Lambert, Y., Dutil, J.-D. and Munro, J., 1994. Effects of intermediate and low salinity conditions on growth rate and food conversion of Atlantic cod (*Gadus rnorhua*). Can. I. Fish. Aquat. Sci. 51 : 1569-1576.

Laurent, P., Hõbe, H. and Dunel-Erb, S., 1985. The role of environmental sodium chloride relative to calcium in gill morphology of freshwater salmonid fish. Cell and Tissue Research, 240(3), 675-692.

Mandic, M., Speers-Roesch, B. and Richards, J. G., 2013. Hypoxia tolerance in sculpins is associated with high anaerobic enzyme activity in brain but not in liver or muscle. Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches, 86(1), 92-105.

Marshall, W. S. and Grosell, M., 2006. Ion transport, osmoregulation, and acid-base balance. Pages 177-210 in D. H. Evans and J. B. Claiborne (eds.), The Physiology of Fishes, 3rd edn. Taylor and Francis Group, Boca Raton.

McCormick, S. D., Regish, A. M. and Christensen, A. K., 2009. Distinct freshwater and seawater isoforms of Na^+/K^+-ATPase in gill chloride cells of Atlantic salmon. The Journal of Experimental Biology, 212(Pt 24), 3994-4001.

McCormick, S. D., Saunders, R. L. and MacIntyre, A. D., 1989. The effect of salinity and ration level on growth rate and conversion efficiency of Atlantic salmon (*salmo salar*) smolts. Aquaculture, 82(1), 173-180.

McKay, L. R. and Gjerde, B., 1985. The effect of salinity on growth of rainbow trout. Aquaculture, 49(3), 325-331.

Morgan, J. D. and Iwama, G. K., 1991. Effects of salinity on growth, metabolism, and ion regulation in juvenile rainbow and steelhead trout (*Oncorhynchus mykiss*) and fall chinook salmon (*Oncorhynchus tshawytscha*). Can. I. Fish. Aquat. Sci. 48: 2083-2094.

Morgan, J. D. and Iwama, G. K., 1998. Salinity effects on oxygen consumption, gill Na^+, K^+-ATPase and ion regulation in juvenile coho salmon. Journal of Fish Biology, 53(5), 1110-1119.

Naylor, R., Hindar, K., Fleming, I. A., Goldburg, R., Williams, S., Volpe, J., Whoriskey, F., Eagle, J., Kelso, D. and Mangel, M., 2005. Assessing the risks of escaped fish from net-pen aquaculture. BioScience, 55(5), 427-437.

Nelson, J. A., 2016. Oxygen consumption rate v. rate of energy utilization of fishes: A comparison and brief history of the two measurements. Journal of Fish Biology, 88(1), 10-25.

Neves, K. J. and Brown, N. P., 2015. Effects of dissolved carbon dioxide on cataract formation and progression in juvenile Atlantic cod, *Gadus morhua* L. Journal of the World Aquaculture Society, 46(1), 33-44.

Nilsson, G. E. and Östlund-Nilsson, S., 2008. Does size matter for hypoxia tolerance in fish? Biological Reviews, 83(2), 173-189.

Nishimura, H. and Fan, Z., 2003. Regulation of water movement across vertebrate renal tubules. Comp Biochem Physiol 136A:479–498

Nobata, S., Ando, M. and Takei, Y., 2013. Hormonal control of drinking behavior in teleost fishes; insights from studies using eels. General and Comparative Endocrinology, 192, 214-221.

Noble, R. A. A., Bredeweg, B., Linnebank, F., Salles, P. and Cowx, I. G., 2009. A qualitative model of limiting factors for a salmon life cycle in the context of river rehabilitation. Ecological Informatics, 4(5), 299-319.

Noori, A., Mojazi Amiri, B., Mirvaghefi, A., Rafiee, G. and Kalvani Neitali, B., 2015. Enhanced growth and retarded gonadal development of farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum) following a long-day photoperiod. Aquaculture Research, 46(10), 2398-2406.

Nordlie, F. G., 2014. Influences of body mass, temperature, oxygen tension, and salinity on respiratory oxygen consumption of cyprinodontoid fishes of three families. Reviews in Fish Biology and Fisheries, 24(1), 269-315.

Norin, T. and Clark, T. D., 2016. Measurement and relevance of maximum metabolic rate in fishes. Journal of Fish Biology, 88(1), 122-151.

Norin, T., Malte, H., Clark, T. D. and Konarzewski, M., 2016. Differential plasticity of metabolic rate phenotypes in a tropical fish facing environmental change. Functional Ecology, 30(3), 369-378.

Otto, R. G., 1971. Effects of salinity on the survival and growth of pre-smolt coho salmon (*Oncorhynchus kisutch*). Journal of the Fisheries Research Board of Canada, 28(3), 343-349.

Perry, S. F., 1998. Relationships between branchial chloride cells and gas transfer in freshwater fish. Comp. Biochem. Physiol. Vol. 119A, No. 1, pp. 9–16.

Plaut, I., 2000. Resting metabolic rate, critical swimming speed, and routine activity of the euryhaline *Cyprinodontid, Aphanius dispar*, acclimated to a wide range of salinities. Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches, 73(5), 590-596.

Pörtner, H. O. and Farrell, A. P., 2008. Physiology and climate change. Science 322, 690–692.

Pörtner, H. O., 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88, 137–146.

Pörtner, H. O., 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. Journal of Experimental Biology 213, 881–893.

Rao, G. M. M., 1968. oxygen consumption of rainbow trout (*Salmo gairdneri*) in relation to activity and salinity. Canadian Journal of Zoology, 46, 781.

Rao, G. M. M., 1971. Influence of activity and salinity on the weight-dependent oxygen consumption of the rainbow trout *Salmo gairdneri*. Marine Biology, 8(3), 205-212.

Reebs, S. G., 2002. Plasticity of diel and circadian activity rhythms in fishes. Reviews in Fish Biology and Fisheries, 12(4), 349-371.

Remen, M., Aas, T. S., Vågseth, T., Torgersen, T., Olsen, R. E., Imsland, A. and Oppedal, F., 2014. Production performance of Atlantic salmon (*Salmo salar L.*) post smolts in cyclic hypoxia, and following compensatory growth. Aquaculture Research, 45(8), 1355-1366.

Richards, J. G., Wang, Y. S., Brauner, C. J., Gonzalez, R. J., Patrick, M. L., Schulte, P. M., Choppari-Gomes, A. R., Almeida-Val, V. M. and Val, A.L., 2007. Metabolic and ionoregulatory responses of the Amazonian cichlid, *Astronotus ocellatus*, to severe hypoxia. J Comp Physiol B. 177:361–374

Rodgers, G. G., Tenzing, P. and Clark, T. D., 2016. Experimental methods in aquatic respirometry: The importance of mixing devices and accounting for background respiration: Methods in aquatic respirometry. Journal of Fish Biology, 88(1), 65-80.

Rosewarne, P. J., Wilson, J. M. and Svendsen, J. C., 2016. Measuring maximum and standard metabolic rates using intermittent-flow respirometry: A student laboratory investigation of aerobic metabolic scope and environmental hypoxia in aquatic breathers. Journal of Fish Biology, 88(1), 265-283.

Ross, L. G. and McKinney, R. W., 1988. Respiratory cycles in *Oreochromis niloticus* (L.), measured using a six-channel microcomputer-operated respirometer. Comparative Biochemistry and Physiology -- Part A: Physiology, 89(4), 637-643.

Roze, T., Christen, F., Amerand, A. and Claireaux, G., 2013. Trade-off between thermal sensitivity, hypoxia tolerance and growth in fish. Journal of Thermal Biology, 38(2), 98-106.

Sardella, B. A. and Brauner, C. J., 2008. The effect of elevated salinity on 'California' Mozambique tilapia (*Oreochromis mossambicus* x *O. urolepis hornorum*) metabolism. Comparative Biochemistry and Physiology, Part C, 148(4), 430-436.

Shin, H. S., Song, J. A., Choi, J. Y., Kim, N. N., Choi, Y. J., Sung, S. N., Park, M. S., Min, B. H. and Choi, C. Y., 2014. Effects of various photoperiods on Kisspeptin and reproductive hormones in the goldfish, *Carassius auratus*. Animal Cells and Systems, 18(2), 109-118.

Silverstein, J. T. and Hershberger, W. K., 1992. Precocious maturation in coho salmon (*Oncorhynchus kisutch*): Estimation of additive genetic variance. The Journal of Heredity, 83(4), 282.

Simensen, L. M., Jonassen, T. M., Imsland, A. K. and Stefansson, S. O., 2000. Photoperiod regulation of growth of juvenile Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquaculture, 190(1), 119-128.

Statistics Canada, 2015. Aquaculture Statistics. Catalogue no. 23-222-X.

Stefansson, S. O., Björnsson, B. Th., Hansen, T., Haux, C., Taranger, G. L., and Saunders, R. L., 1991. Growth, parr-smolt transformation, and changes in growth hormone of Atlantic salmon (*Salmo salar*) reared under different photoperiods. Canadian Journal of Fisheries and Aquatic Sciences, 48(11), 2100-2108.

Steffensen, J. F., 1989. Some errors in respirometry of aquatic breathers: How to avoid and correct for them. Fish Physiology and Biochemistry, 6(1), 49-59.

Suresh, A. V. and Lin, C. K., 1992. Tilapia culture in saline waters: A review. Aquaculture, 106 : 201-226.

Svendsen, M. B. S., Bushnell, P. G. and Steffensen, J. F., 2016. Design and setup of intermittent-flow respirometry system for aquatic organisms. Journal of Fish Biology, 88(1), 26-50.

Tsuzuki, M. Y., Sugai, J. K., Maciel, J. C., Francisco, C. J. and Cerqueira, V. R., 2007. Survival, growth and digestive enzyme activity of juveniles of the fat snook (*Centropomus parallelus*) reared at different salinities. Aquaculture, 271(1), 319-325.

Unwin, M. J., Rowe, D. K., Poortenaar, C. W. and Boustead, N. C., 2005. Suppression of maturation in 2-year-old Chinook salmon (*Oncorhynchus tshawytscha*) reared under continuous photoperiod. Aquaculture, 246(1), 239-250.

Woo, N. Y. S. and Kelly, S. P., 1995. Effects of salinity and nutritional status on growth and metabolism of *Sparus sarba* in a closed seawater system. Aquaculture, 135(1-3), 229.

Wood, C. M. and Grosell, M., 2015. Electrical aspects of the osmorespiratory compromise: TEP responses to hypoxia in the euryhaline killifish (*Fundulus heteroclitus*) in freshwater and seawater. The Journal of Experimental Biology, 218(Pt 14), 2152-2155.

Wood, C. M., Iftikar, F. I., Scott, G. R., De Boeck, G., Sloman, K. A., Matey, V., Domingos, F. X. V., Duarte, R. M., Almeida-Val, V. M. F. and Val, A. L., 2009. Regulation of gill transcellular permeability and renal function during acute hypoxia in the Amazonian oscar (*Astronotus ocellatus*): New angles to the osmorespiratory compromise. The Journal of Experimental Biology, 212(Pt 12), 1949-1964.

Zikos, A., Seale, A. P., Lerner, D. T., Grau, E. G. and Korsmeyer, K. E., 2014. Effects of salinity on metabolic rate and branchial expression of genes involved in ion transport and metabolism in Mozambique tilapia (*Oreochromis mossambicus*). Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology, 178, 121.