Environmental and maternal effects on hatch characteristics and early growth of fishes in Newfoundland and Labrador

by

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ABSTRACT

Early life history can set individuals on phenotypic trajectories that subsequently affect their ability to survive. Additionally, early life stages are the most vulnerable to sub-optimal conditions and predation so early success can be fundamentally important for overall fitness and health of an individual. This thesis focuses on two important adaptations, phenotypic plasticity and growth compensation. In Chapter 2 I looked at how temperature and conductivity impacted embryonic development and found that most of the variation in hatch success was explained by temperature and not conductivity levels. In Chapter 3 I showed that hatch synchrony was affected by both temperature variability and water pH. However, the main focus was on the relative contributions of maternal and environmental factors (temperature variability and pH) on embryonic development and how maternal effects influenced the degree of phenotypic plasticity. Overall maternal factors were more important than environmental factors in explaining early life history characteristics and the degree of phenotypic plasticity that embryos expressed.

Both Chapters 4 and 5 were field-based research chapters where I examined the relationship between growth rate and hatch timing. Through daily aging of otoliths I found no relationship between age and fork length in young of the year salmonids, suggesting that older fish were not necessarily larger and that later hatchers were likely growing faster than early hatchers. This was supported across four species from six different locations in Newfoundland and Labrador and may be a within-population compensatory growth adaptation for a shorter growing season that late hatchers experience. The populations I examined were from northern latitudes (Labrador-Chapter 4 and Newfoundland-Chapter

5) where the relationship cannot be explained by changes in environmental conditions or age alone, which may point to a within-population adaptation to a short growing season.

Overall, this thesis supported previous work that abiotic factors affect early development. I found that environmental and maternal factors can impact hatch success and size, and that the timing of hatch can affect early growth rates. This is significant because small changes in growth and survival resulting from environmental changes can have far reaching implications.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER 1: GENERAL INTRODUCTION

1.1 BACKGROUND

Life history is the specific patterns and timing in an individual's life including reproductive phenology, number and size of offspring, and age and size at maturity (Stearns, 1976). Life history theory focuses on trade-offs (costs) and 'decisions' made by an individual that results in a positive change in one trait at the detriment of another (Stearns, 1989). Examples include anadromy versus residency (Hendry *et al.*, 2004), age versus size at maturity (Folkvord *et al.*, 2014) or size versus number of offspring in a brood for a female (Svärdson, 1949). Females have finite resources available for reproduction; therefore optimal egg size depends on environmental conditions and the quantity of the mother's internal resources. Theoretically, this is part of the parent-offspring conflict (Trivers, 1974) where it is advantageous to the individual offspring to be bigger, but the mother's fitness is greater when creating a higher number of offspring even if each individual offspring is smaller (Smith and Fretwell, 1974; Godfray and Parker, 1991). While larger offspring size is generally associated with a higher chance of survival (Pepin, 1991; Pess *et al.*, 2011; Rollinson and Hutchings, 2013; Pick *et al.*, 2016), all else being equal the mother's strategy should be to have the largest number of offspring survive, so there will be an optimal offspring size for each female which is not necessarily the optimal size for the individual offspring (Smith and Fretwell, 1974).

From the offspring's perspective the results of life history trade-offs can have large ramifications and consequences because early life sets the stage for the future (Stearns, 1989). Early experience feeds directly into alternative life history strategies which then

impact size and age of maturity, which affects fitness (Moore *et al.*, 2015) and overall population structure (Begg *et al.*, 1999; Braun and Reynolds, 2014). An optimal start, such as a large body size at hatch or birth, increases the likelihood of survival (Pess *et al.*, 2011) and future reproductive success (Sinervo and Doughty, 1996; Dickerson *et al.*, 2005). Whereas a poor start means the individual has to try to catch up, but may never get ahead. Additionally, compared to adults and sub-adults, early life stages are more vulnerable to predation (Sogard, 1997) and are more susceptible to changes in the environment (Pankhurst and Munday, 2011; DePasquale *et al.*, 2015). They have a lower ability to deal with increased metabolic stress due to lower energy stores and a higher surface area-volume ratio that which increases the effect of pollutants or chemistry changes per volume (Pankhurst and Munday, 2011; DePasquale *et al.*, 2015). Therefore, the impacts of environmental factors, such as climate change, will not be equally distributed among species or life stages, whereby early life history stages such as larvae and juveniles will be affected greater than sub-adults and adults. Knowledge of early life history can provide insight into a species' ecology and evolution, and how anthropogenic impacts might affect recruitment and survival of populations.

There are intrinsic and extrinsic factors that act in concert to affect early life history and create an integrated phenotype which is a series of correlated and functionally-related traits. Intrinsic factors include genetics and body size (Pepin, 1991), while extrinsic factors include abiotic variables such as temperature (Crisp, 1981; Benjamin *et al.*, 2013) and water chemistry (Hawkins *et al.*, 2003); and biotic variables such as competition (Cutts *et al.*, 1999; Berg *et al.*, 2014), prey availability (Hutchings, 1991; Segers and Taborsky, 2011)

and predator abundance (Belk, 1998; Biro *et al.*, 2004). While fundamentally important for health and fitness, the relative contributions of intrinsic and extrinsic factors are not always well understood. For instance, if the environmental conditions (extrinsic) are within an optimal range, does maternal influence (intrinsic) matter more than when environmental conditions are sub-optimal? When conditions are sub-optimal there are several adaptations that individuals may use to cope and increase survival. In my thesis I focus on phenotypic plasticity and growth compensation and how these factors impact early life history characteristics.

1.2 PHENOTYPIC PLASTICITY

Phenotypic plasticity is the ability of a genotype to produce different phenotypes when exposed to different environmental conditions and is generally measured as a reaction norm. A reaction norm is the slope of the line that describes the phenotypic expression pattern (Woltereck, 1909; Gupta and Lewontin, 1982; Schlichting and Pigliucci, 1998; West-Eberhard, 2003). There is a difference between active and passive phenotypic plasticity, where active plasticity is an anticipatory and adaptive, often highly integrated response to the environment, often involving changes in developmental pathways. Passive plasticity stems from direct environmental influences on biological processes, where the plasticity is a consequence of the environment rather than anticipatory and adaptive (Forsman, 2015). Active phenotypic plasticity can be adaptive by allowing a degree of flexibility depending on specific factors experienced by an individual. When one genotype has the ability to express a range of phenotypes it allows for contingencies based upon what the individual is experiencing. The ability to be plastic allows for relatively immediate

adaptation within the individual to the environment, rather than requiring selection on a genotype which affects subsequent generations. For example, when facing periods of climate change, plasticity has been shown to help individuals cope with sub-optimal conditions (Richter *et al.*, 2012).

However, there are instances where phenotypic plasticity is not adaptive. Dewitt and colleagues provided an excellent overview of costs and limitations of phenotypic plasticity including costs of genetically and physiologically maintaining or producing multiple phenotypic possibilities, and negative impacts of developmental instability. Maintenance costs can occur if plasticity possibilities require maintaining multiple sensory or regulatory mechanisms. Developmental instability is maladaptive, where phenotypic variance occurs within a single environment or through fluctuating asymmetry (DeWitt *et al.*, 1998). The benefits of plasticity can also be limited, through information liability, lag-time limitations and developmental range limits (DeWitt *et al.*, 1998).. Additionally, when populations display a high degree of phenotypic plasticity it can negatively impact ecological processes due to decreased predictability (Miner *et al.*, 2005).

Phenotypic plasticity has been studied for decades; however, researchers do not yet fully understand plasticity to multiple environmental variables (context-dependent plasticity), or the nuances of how it works across generations (transgenerational plasticity or non-genetic parental effects). Context-dependent phenotypic plasticity occurs when the plasticity to one environment interacts with the plasticity to another (Pigliucci, 2001, 2005). Transgenerational plasticity is the effect of a parent's phenotype on the reaction norm or plasticity of their offspring. While parental effects are similar, they are defined as the

impact of the parents phenotype (non-genetic resources or benefits) on the offspring's phenotype independent of environmental impacts, for example bigger eggs produce larger offspring due to a large investment in each egg (Mousseau and Fox, 1998; Ezard *et al.*, 2014). In this thesis I focus on how context-dependent and transgenerational plasticity affect development, in an effort to better understand the relative importance of environmental and maternal influences on the integrated phenotype.

1.3 GROWTH COMPENSATION

One response to less than ideal conditions is growth compensation, where after a period of slow growth, compensatory behaviours such as bolder foraging (Nicieza and Metcalfe, 1997; Damsgård and Dill, 1998; Biro *et al.*, 2004) alter growth trajectories which results in faster growth (Metcalfe and Monaghan, 2001). Growth compensation may be considered an intrinsic plastic response to changes in the environment (Zhu *et al.*, 2003; Carlson, *et al.*, 2004) that is triggered by a depletion of stored lipid resources (Ali *et al.*, 2003). To date, most experiments examining growth compensation have focused on comparing growth rates between a normally fed group (control group, non-stunted) and a food limited group (stunted; see Figure 1.8.1 for a representation). Once food availability increases, the stunted group grows faster than the control group despite being offered the same amount of food. The result is often that the low fed group grows so fast that they end up compensating for the time period of low food, so much so that they are no longer stunted (Won and Borski, 2013). However, there are costs to compensatory growth. For example, in order to accomplish a faster growth rate the individual tends to be bolder in order to forage more which increases predation risk (Ali *et al.*, 2003). Additionally, there are

metabolic consequences that can negatively affect lifespan (Lee *et al.*, 2013; Metcalfe and Monaghan, 2001).

In this thesis, I examine whether hatch timing produces a similar phenomenon to growth compensation via nutritional deficit, whereby a fish may compensate for hatching late, as they are disadvantaged by a shorter growing season, so they grow faster than individuals that hatched earlier (see Figure 1.8.2 for graphical representation). Essentially, I predict that later hatchers will grow faster to compensate for the late start because their growing season will be shorter than early hatchers. However to date, there has been no empirical evidence for growth compensation based upon hatch time.

1.4 STUDY SPECIES

I chose to use fish as models because they are relatively easy to rear in laboratory settings, have many offspring, and are external developers, which makes them ideal vertebrate candidates for the types of questions I wanted to ask. Questions about early life history and phenotypic plasticity can be best answered in species that clonally reproduce where large numbers of the same genotype can be tested; however, there are few vertebrate examples (e.g., through parthenogenesis in Squamates; Avise, 2015). Additionally, most are inaccessible or impractical, for example twinning or polyembrony produces too small of a sample size to work with in the lab, therefore the next best option is to work with species that have many offspring for comparison. The fact that they are external developers means we can easily manipulate and reproduce developmental conditions experienced by the embryos. Therefore, my thesis focuses on early life history in several species of Salmonidae (Order: Salmoniformes) and one species of Fundulidae (Order:

Cyprinodontiformes).

In Chapter 2 I focused on banded killifish (*Fundulus diaphanus*) which are a warm fresh water species, and their optimal thermal reproductive range is likely over 20°C (Brown *et al.*, 2011). But at the edge of their range, in Newfoundland, Canada, populations experience a much cooler climate than mainland populations. Therefore in Newfoundland they can be considered to be residing in sub-optimal thermal habitat for the species. However, the question of how sub-optimal conditions impact reproduction, embryonic and juvenile development remains unknown. This makes them an ideal species to study for questions relating to species distribution edges and reproduction.

In subsequent research questions I worked with several salmonid species including: brook trout (*Salvelinus fontinalis*; Chapters 3, 4, and 5); Arctic charr (*Salvelinus alpinus*; Chapter 4); Atlantic salmon and brown trout (*Salmo salar* and *S. trutta*; Chapter 5). Salmonids have gametes that are easy to collect manually, have large eggs, and they spawn in areas that are relatively accessible. As a group, salmonid species are variable in terms of freshwater habitat, propensity of migrating to sea (Hendry *et al.*, 2004), spawning season (spring or fall), spawning time within a season (Heggberget, 1988; Webb and McLay, 1996), and how many times an individual spawns in a life time (Ducharme, 1969; Berg *et al.*, 1998). For instance, within the *Salvelinus* genus there are differences in migration patterns where lake trout (*S. namaycush*) populations tend to stay in lakes, most brook trout populations are freshwater residents but some do go to sea, while Arctic charr populations are often anadromous (Curry *et al.*, 2010; Swanson *et al.*, 2010). In the genus *Oncorhynchus* (Pacific salmonids) species tend to only spawn once then die (semelparity)

while the *Salmo* genus (containing Atlantic salmon and brown trout) has the capability to spawn more than once (iteroparity; for e.g., Atlantic salmon <10% are repeat spawners, Mills, 1989; ~60% of brown trout are repeat spawners; Klemetsen *et al.*, 2003).

Perhaps more important than the high degree of inter-specific variation, is that the amount of life history variation within a single species can be incredibly high. In 2013, Klemetsen stated that Arctic charr are the most variable vertebrate on earth in terms of range, life history, size, and phenotypic plasticity (Klemetsen, 2013). The large intra-specific differences within salmonids makes them ideal subjects for questions related to variation in early life history and phenotypic plasticity. What is common across all salmonids is the way they breed, although there is considerable variation in spatial and temporal factors. However, all salmonids have to lay their eggs in fresh water (usually rivers or streams, but sometimes lakes). There can be a great deal of clutch size variation within and among populations (Beacham and Murray, 1993). There is also variation among clutches across years for a single mother in terms of size (Reid and Chaput, 2012) and nutrient composition of the yolk (Palm, Penney, Stein and Purchase, *in prep*).

Salmonids are ecologically important. Trophically, salmonids can fill multiple niches, for example the polymorphic Arctic charr can be found to be benthivorous, planktivorous, and piscivorous in the same lake (Snorrason *et al.*, 1994). Many salmonid species are anadromous, which means they impact both freshwater and marine ecosystems. They are often trophically important making up large parts of other animal's diet, for example, some populations of killer whales (*Orcinus orca*) feed primarily on Chinook salmon (*Oncorhynchus tshawytscha*) off the coast of British Columbia, Canada (Ford *et*

al., 1998). Pacific salmonids cycle vast quantities of nutrients into the river systems of British Columbia, becoming sources of marine nutrients for the streams and rivers (Cederholm *et al.*, 1999), and they have had a large effect by helping fertilization of the Great Bear rainforest, which has impacted plant diversity (Hocking and Reynolds, 2011).

Lastly, salmonids have a high socio-economic value. For example in 2012, Canada-wide Arctic charr landings were around 57 tonnes, worth over 185,000 CAD (Fisheries and Oceans Canada, 2014), and the commercial fishery for northern Labrador Arctic charr has harvested over 2600 total tonnes since 1975 (Fisheries and Oceans Canada, 2001). Additionally, there is a recreational and subsistence fishery for charr in the area (Fisheries and Oceans Canada, 2001). In fact, Arctic charr and Atlantic salmon are the two most important fish for many aboriginal communities with up to 90% of northern Labrador households fishing or trading for fish (Felt *et al.*, 2011). In Newfoundland and Labrador, the direct total economic value for the Atlantic salmon recreational fishery was estimated to be over $37 million dollars for 2010 (Gardner-Pinfold, 2011).

1.5 WHY EARLY LIFE HISTORY CHARACTERISTICS MATTER IN FISHES

There is incredible variation in early life history both within and across species. In fishes, common traits of importance include hatch size, hatch timing, and growth rate. Selection acts on all of these factors to produce an optimal hatch size, time, and growth rate which interact to affect survival and future reproductive success. For example, there is a strong relationship between hatch size and competition which subsequently affects survival, particularly in salmonids. Larger hatchlings are better able to compete for food

and refugia space, and therefore are more likely to grow faster and survive (Johnsson *et al.*, 1999; Pess *et al.*, 2011).

Within a season, the timing of hatch dictates the environment that the offspring experiences (Sternecker *et al.*, 2014). Most organisms have an ideal birth or hatch timing that is selected upon (McNamara *et al.*, 2011). In salmonids, the ideal hatch time is created by a combination of food availability, predator abundance, temperature, and water flow. In turn, growth rate is impacted by metabolic and environmental components, body size, temperature, food quantity and quality.

1.6 RESEARCH QUESTIONS

The research questions for my thesis fell under two main themes: phenotypic plasticity and age-based growth compensation.

1. **How do extrinsic (environmental) factors affect early development?**

Nature is more complicated than looking at how one factor affects a biological trait; however, many laboratory studies focus on how the manipulation of one variable impacts development. While these studies are important for understanding the fundamental impacts of those factors, it is more realistic to manipulate multiple variables to determine how the interaction results in the expression of phenotypic traits. Therefore, in Chapter 2 and Chapter 3 I examine how multiple variables impacted embryonic development (hatch size and hatch time) to try to illuminate how multiple variables affect early development.

2. **How do intrinsic (maternal effects) and extrinsic factors (environmental conditions) interact during development?**

Studies often focus on maternal and/or genetic effects or environmental conditions. While fundamental understanding of these factors is important it is often less understood

how they interact or cumulatively impact phenotypes. Therefore, in Chapter 3 I examined the relative contributions of maternal and environmental impacts on embryonic development (hatch time and size).

3. **Are delays in hatch phenology enough to induce growth compensation in late hatching individuals?**

Hatch time has been shown to be an important predictor of survival; however, to date no study has shown whether hatch time affects growth rate, other than from an abiotic perspective i.e., individuals with different hatch times experience different abiotic conditions such as temperature or flow rate. However, when environmental factors between early and late hatchers are similar, can we detect differences in growth rates among the hatch times? Both Chapters 4 and 5 examined the relationship between growth rate and hatch timing. We tested this question with two species in Labrador (Chapter 4) and three salmonid species in Newfoundland (Chapter 5).

Overall this thesis will examine abiotic and transgenerational factors that affect early development. Understanding early life history can be key to predicting future trends; however, in practise it becomes quite difficult to tease apart all the contributing factors. Here, I focus on phenotypic plasticity and growth compensation as two potential adaptations and how they impact early life characteristics.

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1.8 FIGURES

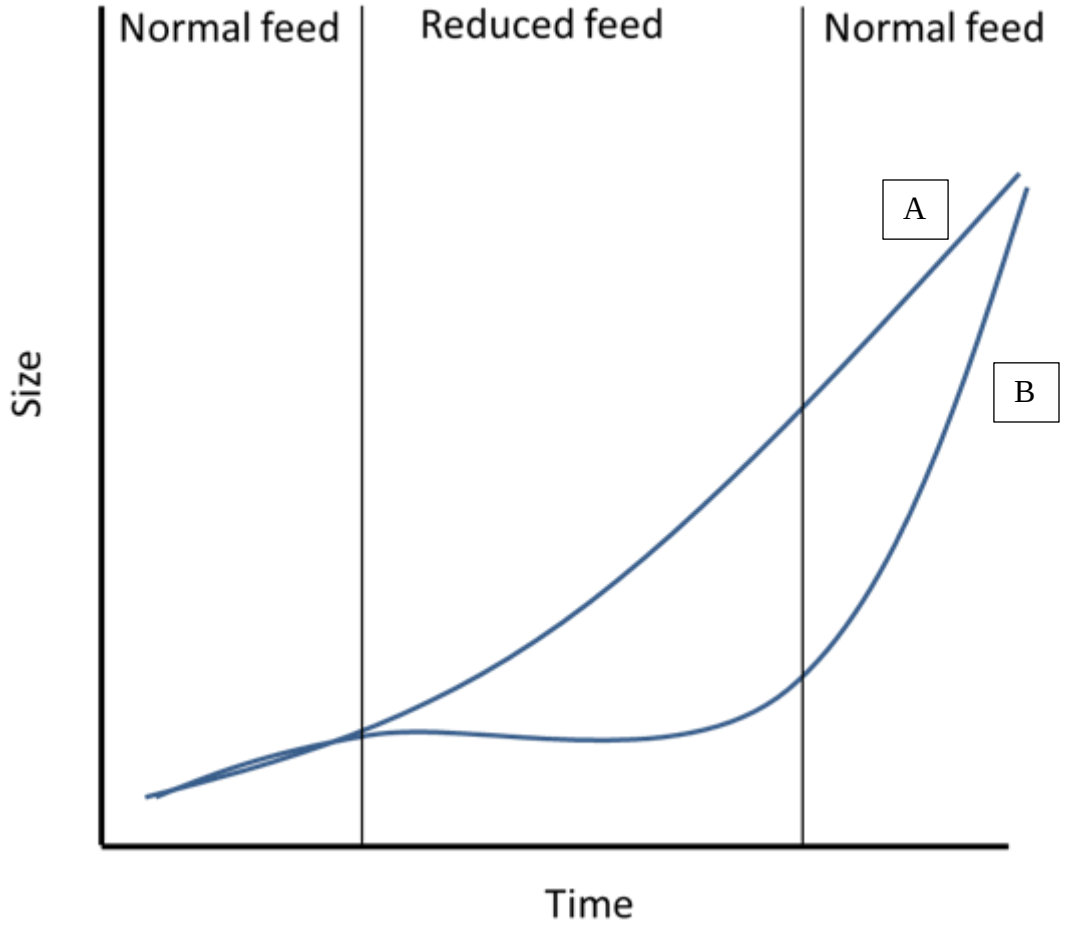

Figure 1.8.1. A representation of a hypothetical experiment showing growth compensation. Each line represents a level of food pattern, A) is the control group that received the same amount of food through the whole time period, B) has a period of reduced food which stunts their growth.

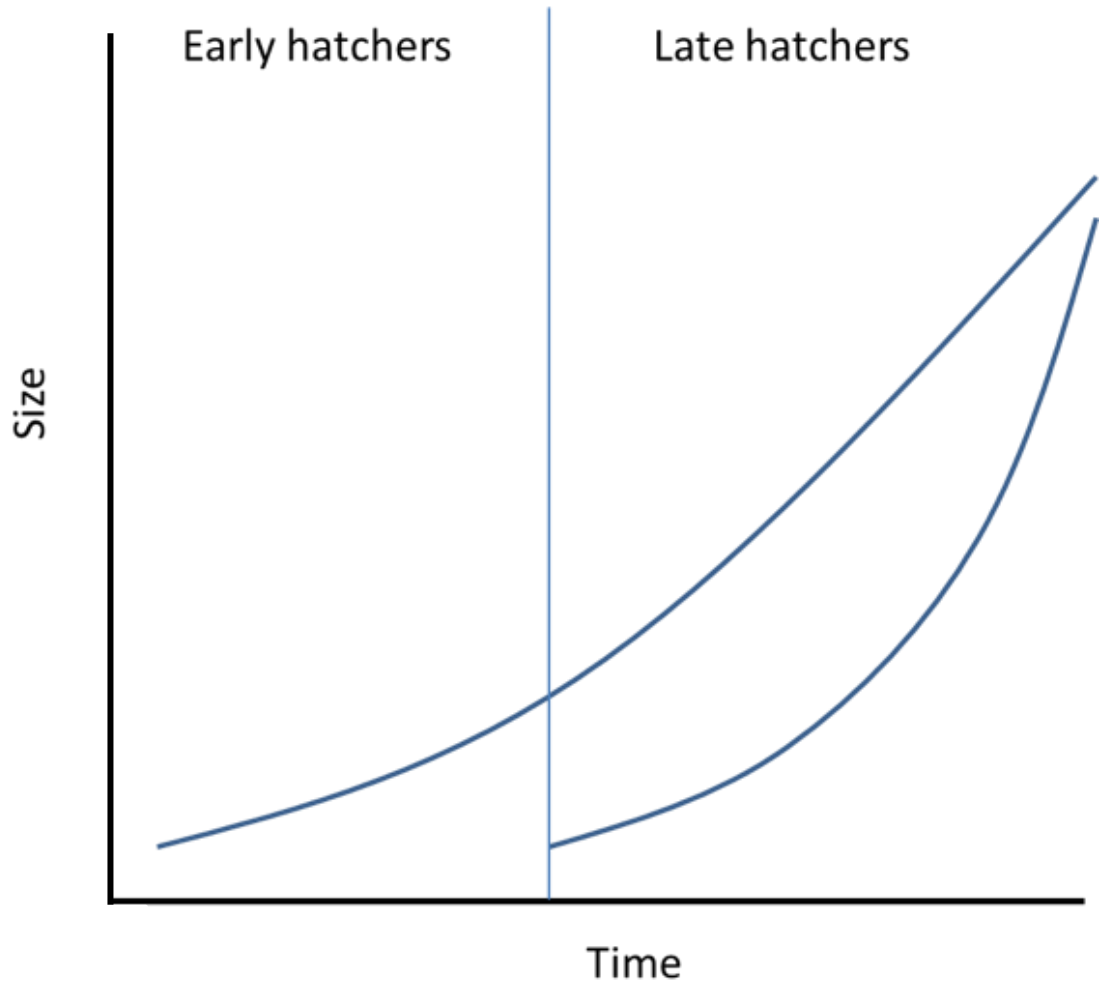

Figure 1.8.2. A representation of the hypothetical growth rates of early and late hatchers over time. The hatchers start and end at the same size; however, late hatchers have to grow much faster to compensate for the shorter amount of time.

CO-AUTHORSHIP STATEMENT

The work described in the present thesis was conducted by Heather Penney with guidance from her supervisory committee Craig Purchase, Ian Fleming and Travis Van Leeuwen. This thesis is presented in manuscript format, and as such each chapter has 2 or 3 coauthors. Heather Penney was primarily responsible for field and laboratory data collection and analysis (with assistance by Craig Purchase) for Chapters 2, 3, and 4. Chapter 2 field collection was conducted by Aline Litt, and Violaine Shikon. Laboratory data collection was conducted by Aline Litt and Heather Penney. Chapter 3 field and laboratory data were collected by Heather Penney and Jose Beirao, and David Schneider helped with data analyses. Chapter 4 had field data collection assistance from Rob Perry and Don Keefe, and laboratory assistance from Linda Lait and Aline Litt. Brian Dempson helped plan field sites and made suggestions on the manuscript. Chapter 5 laboratory otolith data collection preparation was conducted by Aline Litt and otolith aging was conducted by Heather Penney. However, field data collection, specifically fish collection and length measurements, and the otolith microchemistry work were conducted by Lucas Warner. The microchemistry section of this chapter is an excerpt from his thesis (See Appendix A for discussion of this). All chapters were analyzed and written by Heather Penney with intellectual and editorial input by Craig Purchase.

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CHAPTER 2: ABIOTIC FACTORS AT A RANGE EDGE CONSTRAINS REPRODUCTION IN AN ECTOTHERM

Coauthors: H.D. Penney, M.A. Litt, and C.F. Purchase

ABSTRACT

Reproduction at the edges of a species' range presents challenges because conditions are usually sub-optimal. In general, embryos and juveniles are vulnerable to sub-optimal environmental conditions, which makes early growth and survival particularly challenging at range margins. Banded killifish (*Fundulus diaphanus*) are a small, freshwater fish that have a wide but patchy distribution in Newfoundland, Canada, which contributes to Newfoundland populations being listed as 'of concern' by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). Members of the *Fundulus* genus, including the banded killifish are often found and breed in estuarine conditions; however, Newfoundland's freshwater has relatively low conductivity. Additionally, we posit that because Newfoundland is the northern and eastern edge of the species range and the summer climate is much cooler than other locations at similar latitudes this may contribute additional issues particularly for young, more vulnerable life stages. The objective of this chapter was to determine how low temperatures and conductivities affect embryonic development, which we measured as developmental stage reached, hatch success, hatch time and hatch size. A 4x2 experiment was conducted, where individual embryos were sorted into four temperature (10, 16, 22, 28°C) and two conductivity (0.6, 1.2 mS/cm) treatments. Results suggest that temperature was an important factor, where warmer temperatures lead to more developed embryos, a higher hatch success and faster hatch time. Conductivity and temperature interacted to affect hatch size. Therefore, banded killifish are likely challenged by the lower than optimal temperature and conductivity

conditions in Newfoundland which may result in reproductive declines, and perhaps complete cohort failures in cooler years.

2.1 INTRODUCTION

Species' distributions are limited by physical or environmental barriers (Goldberg and Lande, 2007; Hardie and Hutchings, 2010). Physical barriers such as mountain ranges create a border that prevents individuals from dispersing. While, environmental barriers include biotic or abiotic factors (Hardie and Hutchings, 2010) they are often considered partial barriers because they generally slow rather than fully stop dispersal (Goldberg and Lande, 2007). Biotic interactions can be interspecific, such as predator-prey dynamics (Baur 1993; Brooks and Dodson 1965), or intraspecific such as competition for space or resources (Payo-Payo *et al.*, 2017). Abiotic barriers include factors such as moisture and temperature that can limit species through inadequate environmental conditions for reproduction and survival (Dansereau, 1957; Harsch and Hille Ris Lambers, 2016).

Water is a determinant factor for most species' distribution through availability (e.g.,Western 1975, Casanova 2011) and its particular chemistry (e.g., Tessier and Horwitz 1990; see review by Hawkins et al. 2003). However, in many cases the relative impacts of water are not the same across an entire life cycle. For example, toads and land crabs live on land as adults, but have to lay their eggs in water, whereas most turtles have the opposite constraint. For animals that have an aquatic part of their life cycle, water chemistry such as salinity is also important. Most aquatic species can live in either fresh water or salt water, which limits their distribution (e.g., Fritz and Garside 1974); however, there are exceptions, such as diadromous or estuarine species. Different life history stages may have more

nuanced water chemistry requirements, partially because older/larger individuals have lower surface area to volume ratios and a greater ability to osmoregulate (Bœuf and Payan, 2001; Varsamos *et al.*, 2005). For example, anadromous salmonids can live in fresh or salt water as adults, but are constrained to spawn in fresh water because their sperm will not activate in salt water (Vladiĉ and Järvi, 1997) and their embryos cannot tolerate high salinities (Otto, 1971; Crisp, 1993; Gibson, 1993; Thorpe, 1994). Research has shown that even minor changes in water chemistry can greatly impact fertilization success and embryonic development (Daye and Glebe, 1984; Purchase, 2018), which can determine the effectiveness of reproduction and dictates the likelihood of success for species that expand into new areas.

In addition to hydrological factors, temperature limits species' distributions particularly for ectotherms, because individuals cannot regulate their internal body temperature and climatically it changes over latitude and altitude. However, seasonality, weather, and microclimate also make specific temperature effects much harder to predict. Species have thermal maxima and minima thresholds, which are affected by acclimation, life history stage, and local adaptation. Constraints such as the thermal tolerance range (temperatures organisms can tolerate without adverse effects) and thermal optimum range (temperature where growth and reproduction is optimized) affect distribution, growth, reproduction, and survival (Johnson and Kelsch, 1998). Within species, work has shown evidence of local adaptation in that the thermal optima can change with latitude (i.e., decreases heading away from the equator), and a broader temperature tolerance range for populations farther away from the equator (Schaefer, 2012). Often within an individual's

life, young individuals, particularly embryos and juveniles, have a narrower thermal tolerance and optima than adults, which means that reproduction presents extra challenges at the edges of species ranges. For example, brown trout adults can tolerate up to 28ºC (Carline and Machung, 2001), but their thermal tolerance is lower in earlier life stages: parr and smolt can survive up to 26ºC, alevins up to 24ºC, but embryos can tolerate up to 13ºC (Elliott and Elliott, 2010). As a result, many species have temperature-phenology breeding plasticity (flexible reproductive timing) as an adaptation to coincide reproduction with more optimal temperatures (Bowler and Terblanche, 2008). Breeding phenology can also be an adaptation in areas where there is a strong seasonal time constraint that is often seen at northern or southern range edges (Rowe and Ludwig, 1991; Edge *et al.*, 2017). However, even with such adaptations, temperature can be a limiting factor of species' distributions.

Studying species at their distributional extremes can be informative, particularly for species that may be exposed to multiple sub-optimal conditions that may require acclimation (Buckley *et al.*, 2010; Alofs and Jackson, 2015). Species are more likely to experience sub-optimal conditions at or near their range edges, which can have some negative, but non-lethal impacts on individuals, such as a slow growth rate resulting in small size. However, multiple sub-optimal factors may result in additive or amplifying effects, where one sub-optimal condition affects an individual's ability to tolerate another sub-optimal condition that otherwise would only have a slightly negative impact (Rogell *et al.*, 2009). For instance, there is an inverse relationship between water temperature and dissolved oxygen, therefore the physiological stress of approaching both the thermal maxima and low oxygen thresholds may pose additional problems for some fish species (Kleypas, 2015). Similarly, low temperatures affect a fish's ability to transition from salt

water to fresh water or vice versa, for example salmon smolts going to sea in the spring (Otto 1971, Glova and McInerney 1977).

For this study, we chose to work on the banded killifish (*Fundulus diaphanus*), which are small, freshwater fish that have a wide but patchy distribution across eastern North America. Fundulidae generally spawn in estuaries because their embryos perform better in salinities around 20‰; however, banded killifish spawn in warm, fresh water (Fritz and Garside, 1974) despite adults being able to survive salinities higher than sea water (Griffith, 1974). Their distributional range includes much of the eastern United States and Canada, as far south as South Carolina to the northeastern edge of Atlantic Canada, including Newfoundland, which is an isolated island (April and Turgeon, 2006). Newfoundland's climate is not well predicted by latitude because the conditions are much cooler later in the spring and summer than in other parts of the banded killifish's range at similar latitudes, such as in the Maritime provinces of New Brunswick and Nova Scotia. Additionally, banded killifish have a relatively high conductivity (salinity) tolerance compared to other freshwater fish (April and Turgeon, 2006), but conductivity is relatively low in Newfoundland watersheds (Department of Environment and Conservation, 2015, n.d.).

Despite the cited concerns over the survival of Newfoundland's peripheral populations (Osborne and Brazil 2006; COSEWIC, 2014), there is a dearth of information regarding their early life history. It has already been established that there have been shifts in spawning phenology for banded killifish, where mainland populations begin spawning in April and May but Newfoundland populations do not begin spawning until June or July

(Mitchell and Purchase, 2014); however, it is unknown whether the combined effects of the cool temperatures and low conductivities will have an additive impact on embryonic development.

In fishes, both water chemistry and temperature affect many parts of life history, including size at maturity, growth rate (Schultz *et al.*, 1996), and reproductive factors, such as hatch time (Wilson and Hubbs 1972, Pepin 1991, DiMichele and Westerman 1997, Gillooly et al. 2002, Penney et al. 2018) and hatch size (Brown et al. 2011, Penney et al. 2018). Therefore, the objective of this chapter was to determine how embryonic development may be affected by multiple conditions (temperature and conductivity; manipulated in the lab) that banded killifish experience at a range edge. We had two predictions: 1) that temperature would have a strong effect, with fish having higher hatch success, faster time to hatch (Schaefer, 2012), and larger size at hatch in warmer temperatures (Brown *et al.*, 2011); and 2) that the embryos would have a higher hatch success and be larger at the higher conductivity (Brown *et al.*, 2012).

2.2 MATERIALS AND METHODS

2.2.1 Temperature data

Air temperature, water source, water body size, flow rate, and solar radiation impact the rate that fresh water warms up in spring. While the most ideal way to examine differences in water temperature between Newfoundland and the Maritimes would have been to examine similar sized water bodies in several places, the only relevant historical water temperature data we could retrieve were based on rivers and were not very detailed. Our study site is a relatively small, shallow pond that would likely warm up quickly.

Therefore, to determine temperature differences between Newfoundland and the Maritimes we collected air temperature data from multiple temperature stations from historical Environment Canada records (http://climate.weather.gc.ca) from 2010 to 2017 from 7 sites in Newfoundland and 8 sites in the Maritimes (New Brunswick and Nova Scotia). We averaged data from 8 years, and focused on the banded killifish's potential growing season (April 1st to October 31st; Chippett, 2004; Figure 2.7.1A). We acknowledge issues with using air temperature as a proxy for water temperature; however, we did not have viable alternatives. We also calculated the thermal summed units when the temperature was over 15°C (approaching the thermal minima, as we began to have hatch success at 16) for the daily average for each area (Figure 2.7.1B, see Appendix Table 2.8.A1).

2.2.2 Population and collection information

We collected banded killifish embryos (n=107) (*Fundulus diaphanus*) from the Burton's Pond population in St. John's, NL, Canada (47.574°N, 52.728°W) between June 24 and July 11, 2014. This population of fish was introduced in 1999 (Mitchell & Purchase, 2014) from Indian Bay, NL (COSEWIC, 2014). Embryos were collected on artificial (yarn, ~10 to 15 cm long threads) spawning mops (~20), which were used to mimic the plant substrate that banded killifish eggs typically adhere to. Each spawning mop consisted of a float and an anchor so that they hung vertically in the middle of the water column (depth ranged between ~30 and 60 cm of water). The mops were checked twice daily. Embryos stripped from the mops during the first collection were not included because fertilization time (within 20 hours) was unknown. The second collection took place four hours later and therefore embryos were known to be within the first four hours of development. Each individual embryo was checked under a dissecting microscope for cell division to ensure

fertilization, and embryos from each collection day were distributed relatively evenly into the different treatments (Figure 2.7.2), and the experiment ran from June 24 to July 23, 2014. The majority of the embryos were collected between June 27th and July 1st (n=84). Because of low hatch success at the lower temperatures, we collected additional embryos (n=23; between July 9th and 11th) and added them evenly into the 10°C and 16°C treatments to increase the sample size.

2.2.3 Experimental set up and design

A 4x2 experimental design was conducted (Figure 2.7.2), where embryos were sorted into four temperature treatments (10, 16, 22, 28°C) and two conductivity treatments (0.6, 1.2 mS/cm) that allowed us to examine the possibility of an interaction between temperature and conductivity on embryonic development. We chose a wide range of temperature treatments, and two realistic conductivities. Temperature treatments were chosen based on the documented thermal range for spawning 19-24°C (Chippett, 2004) but realistic of worst-case to best-case temperatures typical of Newfoundland summers (10, 16°C) and elsewhere in the banded killifish's range (22 and 28°C). The two conductivities were chosen to be representative of the conductivity typical of Newfoundland's fresh water systems. The two water conductivities were prepared using sea salt (Instant Ocean Spectrum Brands, Blacksburg, VA, USA) and deionized water. Individual embryos were fully immersed in water in Petri dishes placed in incubators at their respective temperatures, and the water was topped up daily.

2.2.4 Development and size metrics

Every 12 hours from collection to hatch or death, each individual embryo was photographed in its Petri dish using a Leica M80 stage microscope. Petri dishes with

embryos were not out of their incubators for more than 10 minutes during the photographic process. Because to our knowledge no published documentation on embryonic developmental stages of the banded killifish exists, we compared development of our embryos to a congeneric species, the common mummichog (*Fundulus heteroclitus*), as outlined by Armstrong and Child (1965). Using this comparison it was possible to distinguish the same 6 stages in banded killifish that were found in common mummichogs: cleavage (2-64 cell stage), blastula, gastrula, neuralae, segmentation (further embryo growth and organ development), and hatch (Figure 2.7.3). Any embryo that did not develop for seven consecutive days was considered to have ceased development.

All size measurements were collected from digital photos in ImageJ (ImageJ, 2011; Schneider *et al.*, 2012). Embryo size was determined from the first photo on collection day and was measured as the average of two axes of the yolk (the longest and its perpendicular; see Figure 2.7.3A). Standard length of the larvae on hatch day was used for hatch size.

2.2.5 Data analyses

General approach:

All of the figures (using package 'ggplot2'), data processing and statistics (using packages car, ggpmisc, lme4, multcomp, plyr, and survival) were created and conducted in R version 3.3.3 (R Development Core Team, 2015). For all statistical tests an alpha of 0.05 was used. Assumptions of normality and heteroscedascity were tested using the residuals and no deviations were observed using a normal error structure (hatch size) and binomial structure (hatch success, survival stage).

$$DV \sim S + T + C + SxT + SxC + TxC + error$$

[model 1]

The same model parameters (model 1) were run for all of the dependent variables (DVs): last developmental stage reached before development ceased (herein 'survival stage'), hatch success, and hatch size. Independent variables included: embryo size (S, covariate), temperature (T), conductivity (C) and all possible 2-way interactions among the variables. We did explore the 3-way interactions (SxTxC) for each DV; however it was only significant for hatch success ($p=0.02$). The hatch success pattern was the same for both conductivities (i.e., 22°C treatment had the highest and the 10°C treatment had the lowest hatch success). So, we elected to split the data to analyze each conductivity treatment separately, in both models the only significant factor was temperature. Therefore, we did not include the 3-way interaction in the final analyses because it did not change any of our conclusions.

Survival stage:

For survival stage the dependent variable was the final developmental stage reached (6 stages: cleavage, blastulae, gastrulae, neurulae, segmentation, and hatch), therefore the data were considered ordinal. To determine whether the temperature and conductivity treatments affected survival to different developmental stages, an ordinal regression was conducted as a Cumulative Link Model (CLM, see Guisan and Harrell, 2000 for a similar analysis) on model 1 with an 'equidistant' threshold, using the 'ordinal' package in R.

Hatch success, time and size:

To determine impacts of the main effects (model 1) on: 1) hatch success- we conducted a generalized linear model (GzLM) with a binomial error structure, then an analysis of deviance was performed to determine significance; and 2) hatch time and hatch size- we conducted a GzLM with normal error structure, then an analysis of deviance was performed to determine significance. The 10°C treatment had no embryos hatch, and the 16°C treatment only had 2 embryos hatch, therefore, for hatch time and size the tests were only on the temperature treatments with successful hatching. For hatch size, there was a significant interaction between temperature and conductivity, therefore a Tukey post hoc test was conducted.

2.3 RESULTS

2.3.1 Temperature differences

There are some differences in temperature between Newfoundland and the Maritimes, in that Newfoundland warms up later in the season and does not reach the same highs as the Maritimes (Figure 2.7.1A). There was a difference in accumulated thermal units (Figure 2.7.1B) with a 15°C cut off, which means there was a difference in annual growth potential between the two areas. By the end of October, the Maritimes reaches over 1700°C thermal units, while Newfoundland reaches around 1000°C (ATU, see Appendix Table 2.8.A1).

2.3.2 Hatch success

We determined that hatch success was significantly affected by temperature; however, embryo size, conductivity and the interaction between conductivity and temperature were not significant (Table 2.6.1). Neither of the 10°C treatments had embryos

hatch, while the 22 and 28°C treatments had reasonable hatch success (>74%), and the two 16°C treatments had very low hatch success (0 and 10%) (Figure 2.7.4).

2.3.3 Survival stage

We used a cumulative link model (CLM), and determined that temperature significantly affected the stage reached by developing embryos, but the effects of embryo size, conductivity and the interactions between embryo size, conductivity, and temperature were not significant (Table 2.6.1). The 22 and 28°C treatments had relatively high hatch success, and those that did not hatch died during all five stages of development (Figure 2.7.5). No fish hatched in the 10°C treatment, and the embryos died over all 5 stages. However, in the 16°C treatment most embryos developed until organ growth and differentiation but did not hatch.

2.3.4 Hatch time

For the larvae that did hatch (n=34), there was a significant interaction ($p<0.001$) between size and temperature on time to hatch in accumulated thermal units (Table 2.6.1). Embryo size, conductivity, and the interaction between conductivity and temperature were not significant (Figure 2.7.6). The 28°C temperature treatment hatched in the fewest accumulated thermal units and the shortest amount of time (accumulated thermal unit mean: 233.1 ATU, sd: 10.8; days mean: 8.3 sd: 0.4), followed by the 22°C (mean: 274.4 ATU, sd: 12.9; days mean: 12.5 sd:0.6) and 16°C (mean: 335.7ATU, sd: 56.6; days mean: 21.0 sd: 3.5) treatments. Note: mean and standard deviation were calculated using all individuals in that temperature treatment regardless of conductivity treatment.

2.3.4 Hatch size

There was a significant interaction between temperature and conductivity on hatch size (Table 2.6.1). Embryo size was not significantly related to hatch size. Results of the Tukey post hoc test showed that the 0.6 mS/cm conductivity and 28°C temperature treatment produced significantly smaller larvae (mean: 6.4 mm, sd: 0.2, $p<0.001$) at hatch than the other 4 treatment combinations (mean 7.1 mm, sd:0.2) that resulted in hatchlings (Figure 2.7.7).

2.4 DISCUSSION

The objective of this study was to determine how embryonic development may be affected by multiple conditions (temperature and conductivity) that banded killifish experience at a range edge. We found partial support for our first prediction in that temperature had a very important effect; higher temperatures resulted in higher hatch success and less time to hatch. There was a significant interaction between temperature and conductivity on hatch size, where there were much shorter hatchlings in the lower conductivity at 28 degrees. Additionally, we found no support for our second prediction that embryos would have a higher hatch success and be larger at the higher conductivity.

Low temperatures negatively affected embryonic development in banded killifish. We tested a cold (10°C), cool (16°C), warm (22°C) and warmer (28°C) temperature that would capture the range of Newfoundland's summer climate to investigate impacts on embryonic development. It is not uncommon for Newfoundland's freshwater to remain far below the developmental thermal optimum range (between 22 and 28°C according to this study, and between 19 and 24 according to Chippett, 2004) well into the summer (Figure

2.7.1A). In fact, in the period examined here, the average daily air temperature (8 years from 7 locations) never reaches 22°C in Newfoundland (Appendix Table 2.8.A1). Results showed that temperature significantly affected the stage that the embryos reached before development ceased, where lower temperatures resulted in lower hatch success and the embryos ceasing development at earlier stages. The low temperatures had little (10% at 16°C) to no hatch success (10°C). In fact, at 10°C only 10% of embryos reached the neurulae stage, but at 16°C, ~75% of embryos reached the neurulae stage. Therefore, it seems that if an embryo can develop past the neurulae stage to the segmentation and growth phase of development they were much more likely to hatch. At 22 and 28°C there was high hatch success (at least 74% of embryos hatched), which is unsurprising given that previous work has shown that the optimal thermal range for killifish development is between 19 and 24°C (Chippett 2004). Additionally, the highest temperature (28°C) had high hatch success despite being 4 degrees over the predicted optimal temperature range for development. These results indicate that there may be a temperature threshold between 16 and 22°C that needs to be reached in order for the embryos to develop properly. More research should be conducted on development between 16 and 22°C, as well above the optimal spawning temperature, for example between 28 and 32°C.

Unsurprisingly, time to hatch was faster at warmer temperatures. However, perhaps the most interesting finding of this study was that while hatching in fewer days is expected at warmer temperatures, generally in fishes when growing at a warmer temperature it takes more accumulated thermal units to hatch than at cooler ones (e.g., salmonids; Crisp 1988; Atlantic cod *Gadus morhua* Geffen *et al.*, 2006; and European plaice *Pleuronectes platessa*

Ryland and Nichols, 1975). Our study found the opposite, not only did it take fewer days to hatch but it took fewer accumulated thermal units as temperature increased. However, a similar result has also been shown in other Cyprinodontiformes species (striped killifish *F. majalis,* Abraham 1985, desert pupfish *Cyrpinodon macularius* Kinne and Kinne, 1962). While we are not sure why this occurred, it is possible that the warmer temperatures in our study were closer to optimal growth due to banded killifish preferring relatively warmer water, compared to a salmonid that has a much lower thermal maxima, therefore they may be more efficient and require fewer thermal units at the warmer temperatures.

Our results showed that conductivity did not affect developmental stage reached, hatch success, or timing. The only factor that was affected by conductivity was hatch size, as an interaction with temperature. It is clear that the treatment with lower conductivity (0.6 mS/cm) at 28°C had a much smaller size than the other treatments, which was likely driving the significant interaction. This lack of effect of conductivity on development and survival is somewhat surprising given that previous work has shown that banded killifish had optimal hatch success and growth rates at conductivities 3 to 4 times higher than what we tested (Griffith, 1974). However, we only examined two conductivities that were both relatively low. There are three possible explanations for this result: 1) perhaps there is local adaptation to low conductivity in Newfoundland; 2) alternatively, conductivity did not have an effect in treatments where temperature was not a stressor (see Penney *et al.*, 2018). In other words, the embryos could cope with the low conductivity in the treatments that approached optimal temperature (22°C treatment), whereas at 28 degrees, the embryos were experiencing stress from both high temperature and low conductivity which has

additive energetic costs resulting in smaller embryos; 3) we were only testing two conductivities, and both of them were low (0.6 and 1.2mS/cm). Perhaps if we had tested a wider spectrum of conductivities, we would have obtained a clearer result of conductivity's effect on development. Previous work has shown that adult banded killifish can survive salinities more than twice the salinity of sea water (Griffith, 1974) so testing embryonic development at conductivities between 1 and 32 mS/cm would be a logical next step.

Officially there are over 40 populations of banded killifish on the island of Newfoundland, and COSEWIC considers them as their own designatable unit (DU) due to small population sizes and limited dispersal. Newfoundland's populations of banded killifish are at risk and currently listed by the province of Newfoundland and Labrador as a vulnerable species (Osborne and Brazil, 2006), the Species at Risk Act (SARA) as a species of special concern, and the International Union for Conservation of Nature (IUCN) as having a threat impact level of "high" (COSEWIC, 2014). Given the results in this study we conclude that their reproductive potential of banded killifish is probably negatively affected in Newfoundland because the temperature is much lower than has been shown to be the ideal or optimal for the species. Some years in Newfoundland, the water never reaches 20°C, and in years that it does, it can take until July to do so, which puts pressure on killifish reproduction. One documented adaptation of the banded killifish is a shift in spawning period from April to May, as it occurs on the mainland, to late June to July in Newfoundland (Mitchell and Purchase 2014). One limitation to our study is that we only worked with one source population, and we are unsure if they have specialized adaptations for Newfoundland climate. Therefore, more work needs to be conducted investigating

spawning times and other reproductive differences in other populations on the island of Newfoundland and compare those to populations in eastern North America because the selective pressures may differ throughout their range. We are unsure about the effects of this shift in spawning time on size at the end of the first growing season, and consequently how this affects juvenile overwinter survival.

Another avenue to explore is the banded killifish's ability to perform behavioural thermoregulation. The killifish spawn in shallow waters on freshwater plants. These areas are warmer than other areas of the ponds and lakes they inhabit. It is likely that they are giving their embryos an advantage with more degree days. However, it is unknown if this increases the risk of desiccation, or affects predation rate on eggs. Future work could examine whether egg laying location changes with temperature or among populations.

The banded killifish are affected by edge conditions in 3 ways: 1) because of the cool spring temperatures they spawn late which means the growing season is short; 2) the growing season is cold, so there are fewer accumulated thermal units available to use for development; and 3) because they have great difficulty developing when it is 16 degrees Celcius or colder, they will be challenged in years that Newfoundland has cool summers. These factors may result in additive negative impacts, which may mean reproductive declines and perhaps complete cohort failures in moderately cool years. It is possible that Newfoundland banded killifish are locally adapted to reproduce in colder water than elsewhere, but this remains untested. For example, in a congeneric species (mummichog, *Fundulus heteroclitus*) it has been shown that populations from different latitudes have differences in behavioural thermoregulation (Fangue *et al.*, 2009) and temperature

tolerance ranges (Fangue *et al.*, 2006, 2008). As such, special care should be taken to understand whether the Newfoundland populations of banded killifish are adapting to less than optimal conditions in order to help protect these populations.

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2.6 TABLES

Table 2.6.1. A summary of analyses of deviance on the fixed effects- embryo size (size), temperature (temp), conductivity (cond), and a temperature x conductivity interaction, from general linear models (GLM) for hatch success (proportion), hatch time (accumulated thermal units, °C), and hatch size (length in mm). χ^2 values, degrees of freedom, and the corresponding p-value, are given for each effect.

	Hatch success			**Stage reached**			**Hatch time**			**Hatch size**		
Factor	**df**	**χ^2**	**p**	**df**	**χ^2**	**p**	**df**	**χ^2**	**p**	**df**	**χ^2**	**p**
Size	1	2.68	0.10	1	4.59	**0.03**	1	2.63	0.10	1	0.53	0.47
Temp	3	72.12	**<0.00001**	3	46.66	**<0.0001**	2	226.56	**<0.0001**	2	23.20	**<0.00001**
Cond	1	0.003	0.95	1	0.78	0.38	1	3.14	0.08	1	2.82	0.09
Size x Cond	1	0.02	0.88	1	0.002	0.96	1	2.21	0.14	1	1.11	0.29
Size x Temp	3	0.20	0.98	3	5.44	0.14	2	22.40	**<0.0001**	2	1.23	0.54
Temp x Cond	3	3.02	0.39	3	3.84	0.28	1	2.32	0.14	1	13.64	**0.0002**

2.7 FIGURES

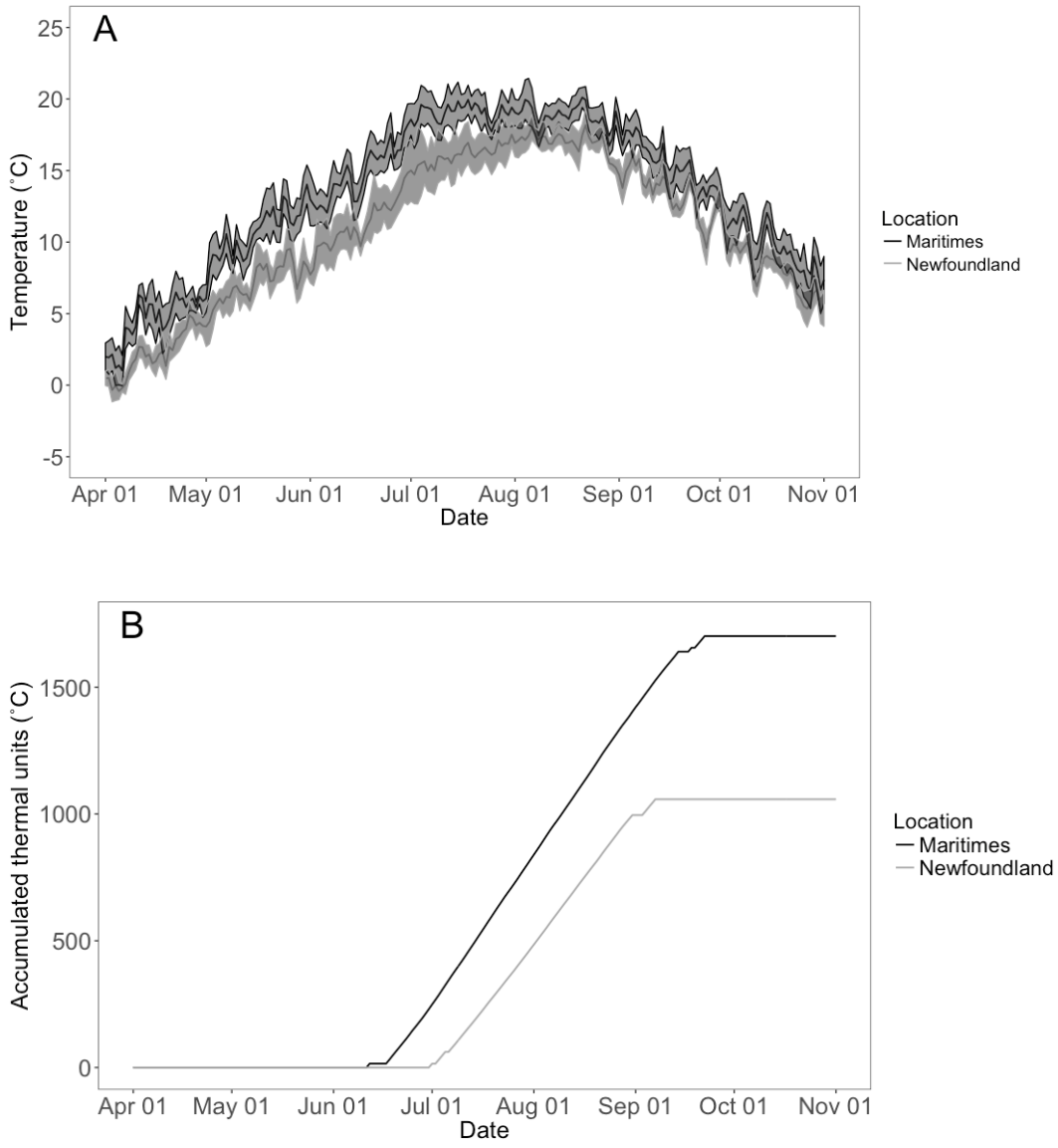

Figure 2.7.1. A) Temperature comparisons and B) thermal summed units (when over 15°C) from weather stations in Newfoundland (grey, n=7) and the Maritimes (black, n=8) from April to the end of October (average temperature from all stations for 2010 to 2017). Newfoundland has approximately 2/3 the accumulated thermal units as the Maritimes. (Note: grey shows standard deviation among stations in A, the deviation in B is negligible at this scale, so does not appear on this graph).

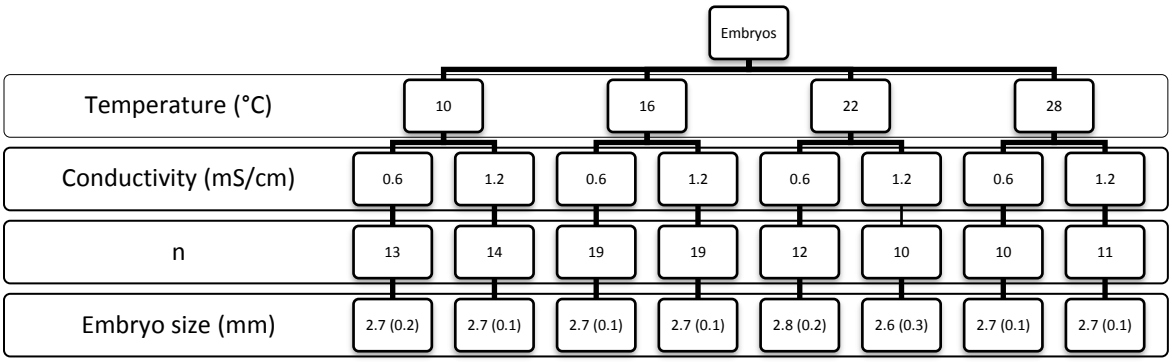

Figure 2.7.2. The number of embryos and the mean embryo size (standard deviation) for the temperature and conductivity treatments.

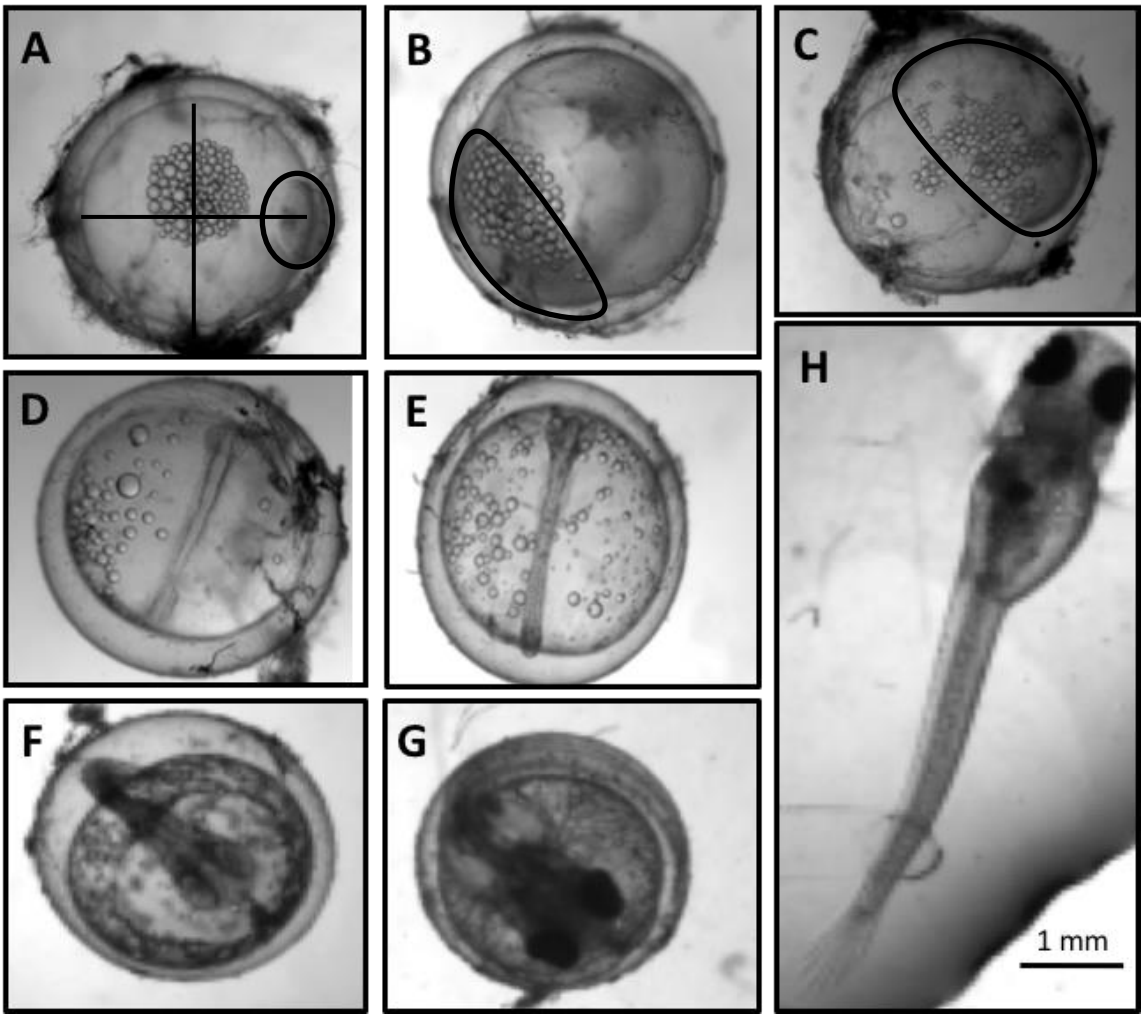

Figure 2.7.3. Photo examples for different stages of development. A) cell division (4 cell stage); B) blastulae; C) gastrulae; D) neurulae; E to G) segmentation (growth, differentiation, and development); H) newly hatched larva. Stages were based on drawings in Armstrong and Child (1965). The perpendicular lines in panel A indicate how embryo size was determined. Black circles were added in panels A through C to indicate where the cells were. Note: the large round drops are oil globules from the yolk not embryonic cells.

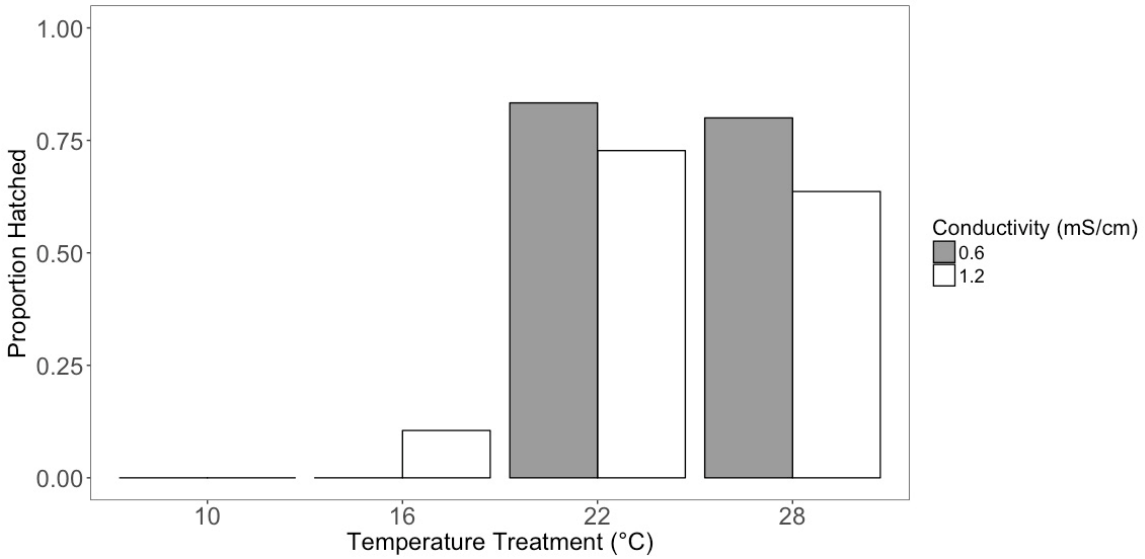

Figure 2.7.4. Hatch proportion for embryos reared at each conductivity and temperature. There are replicate embryos reared individually, not replicate groups, therefore there are no error bars.

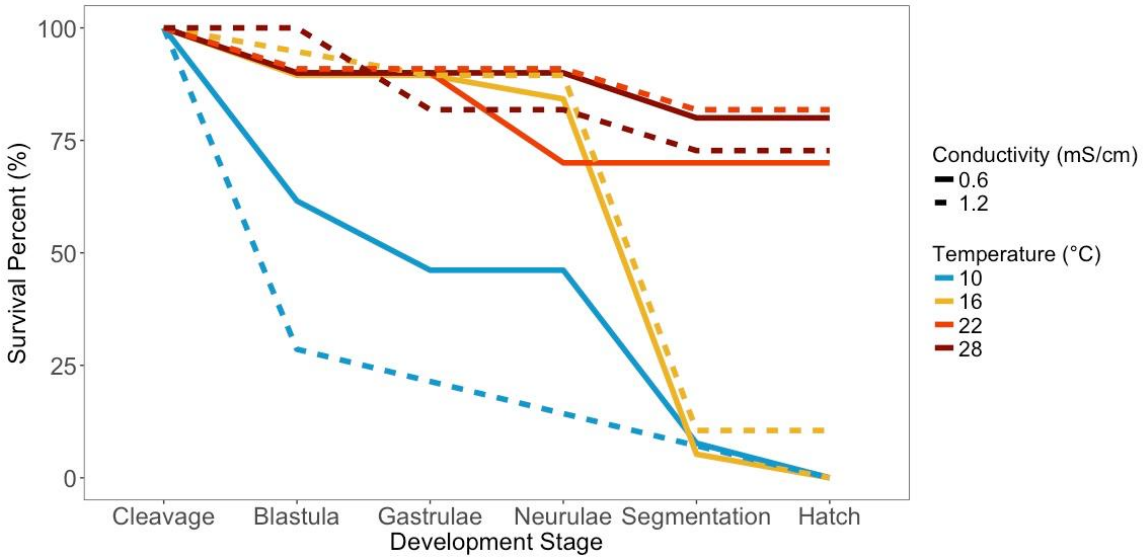

Figure 2.7.5. Survival curves through the different stages of embryonic development for each temperature (colour) and conductivity (solid or dashed line) treatments.

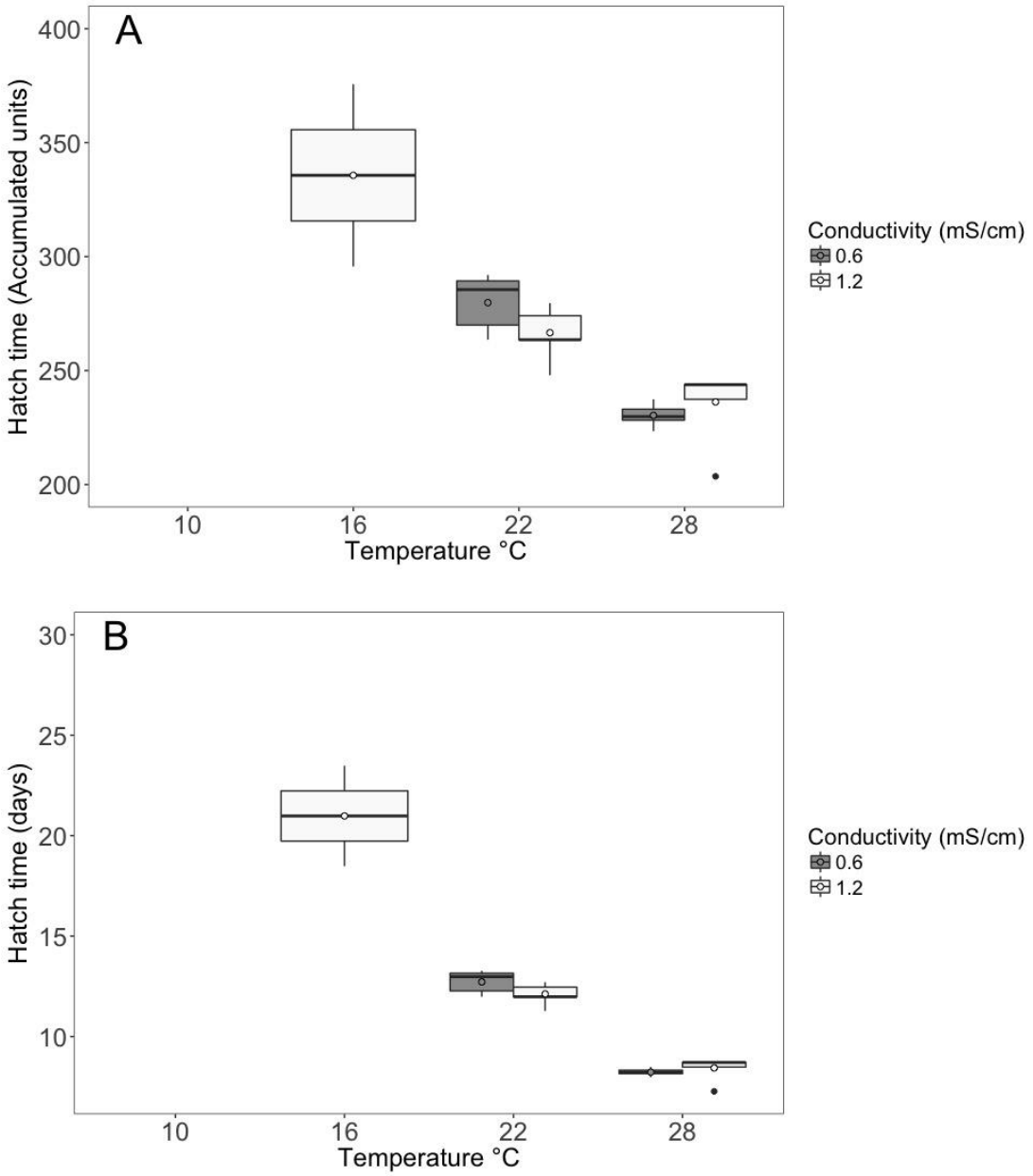

Figure 2.7.6. Hatch time in accumulated thermal units (A) and days (B) for each conductivity and temperature. The box plot shows the interquartile range (IQR, 25 and 75%), and the line shows the median value for hatch time among individuals. Whiskers represent the next quartile (1.5 x IQR), outliers are represented by the black dots. The open circles represent the mean. Note: no fish hatched at 10°C in either conductivity, or in the 16°C-0.6mS/cm treatment.

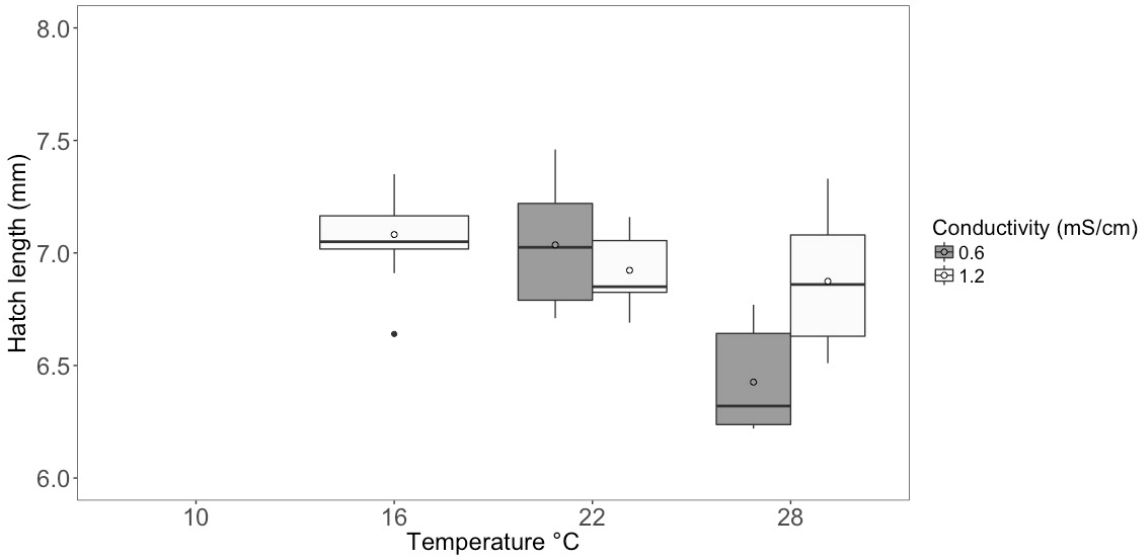

Figure 2.7.7. Hatch length for each conductivity and temperature. Where the box plot shows the interquartile range (IQR, 25 and 75%), and the line is the median value for length. Whiskers represent the next quartile (1.5 x IQR), outliers are represented by the black dots. The open circles represent the mean. Note: no fish hatched at 10°C in either conductivity, or in the 16°C-0.6mS/cm treatment.

2.8 Appendix Chapter 2

Table 2.8.A1: Differences in accumulated thermal units (°C, when over 15°C) between Newfoundland[1] (average from 2010-2016 from 7 locations) and the Maritimes[2] (average from 2010-2016 from 8 locations in Nova Scotia and New Brunswick). This table shows the Accumulated thermal units (ATUs) reached on different dates, and at which date it reached specific ATUs. The maximum thermal units and the last day accumulating thermal units are also shown. Data acquired from Environment Canada's archive.

	Cumulative ATUs			**Date to # of ATUS**	
Date	**Newfoundland**	**Maritimes**	**ATUs**	**Newfoundland**	**Maritimes**
May 31	0	0	50	July 5	June 20
June 30	0	230	100	July 9	June 23
July 31	468	820	500	Aug 2	July 15
Aug 31	996	1403	750	Aug 17	July 28
Sept 30	1058	1701	1000	Sept 4	Aug 10
			1250	NA	Aug 23
			1500	NA	Sept 6
			First day accumulating thermal units		
				July 1	June 12
Maximum thermal units			*Last day accumulating thermal units*		
	1058	1701		Sept 7	Sept 22

[1]Newfoundland sites included: Burgeo, Deer Lake, Gander, Harbour Breton, Port aux basques, Stephenville, and St. John's

[2]Maritimes sites included: Fredericton, NB; Moncton, NB; Saint John, NB; Halifax, NS; Kentville, NS; Pockwock Lake, NS; Sydney, NS; and Yarmouth, NS

CHAPTER 3: PHENOTYPIC PLASTICITY DURING EXTERNAL EMBRYONIC DEVELOPMENT IS AFFECTED MORE BY MATERNAL EFFECTS THAN MULTIPLE ABIOTIC FACTORS IN BROOK TROUT

*Note: A version of this chapter has been published in Evolutionary Ecology Research and should be cited as follows: Penney, H.D., Beirão, J., and Purchase, C.F. 2018. Phenotypic plasticity during external embryonic development is affected more by maternal effects than multiple abiotic factors in brook trout. *Evolutionary Ecology Research*, 19: 171-194.

ABSTRACT

Phenotypic plasticity is the response of a genotype to an environmental gradient. However, plasticity can occur in multiple dimensions (context dependent plasticity) and can span generations (transgenerational plasticity). In this chapter we examined the contribution of transgenerational and context dependent phenotypic plasticity on developing brook trout (*Salvelinus fontinalis*) embryos. We tested 3 hypotheses: 1) that hatch time is affected more by environmental than maternal effects; 2) that maternal effects have a larger impact on hatch size (dry weight, yolk volume and hatchling length) than environmental effects due to the large investment (i.e., yolk) salmonid mothers make in their offspring; and 3) egg size affects the degree of plasticity of hatch time and hatchling size. Brook trout embryos were individually incubated in four treatments consisting of two possible temperatures: stable (5^{o}C), and fluctuating (ranging from 2 to 8^{o}C, mean 5^{o}C), and two possible pH levels: benign (6.5) and stressful (5.25). As predicted, hatch time (synchrony) was affected by the environmental variables in that the fluctuating temperature/benign pH treatment combination had a significantly longer hatch range (decreased hatch synchrony) than other treatment combinations. However, maternal effects (egg size) overshadowed any potential

environmental effects on hatch size, where larger eggs produced longer hatchlings. We found that maternal effects influenced the degree of plasticity where larger eggs were more plastic. Our results suggest researchers need to pay special attention to transgenerational effects when attempting to examine early phenotypic plasticity phenomena.

3.1 INTRODUCTION

Phenotypic plasticity is the ability of a genotype to produce different phenotypes when exposed to different environmental conditions (Schlichting and Pigliucci, 1998; West-Eberhard, 2003). Plasticity is an important aspect of evolutionary ecology because while it can be neutral or maladaptive, it often offers a protective, adaptive mechanism in times of environmental change (Hutchings, 1996; Crispo and Chapman, 2010; Chevin and Lande, 2015). Reaction norms are a measure or function of the potential phenotypic responses of a genotype to different environments (Woltereck, 1909; Gupta and Lewontin, 1982; Pigliucci, 2001). While plasticity studies usually examine two or three levels of an environmental variable, reality is much more complicated and oversimplification may result in misunderstanding important aspects of phenotypic plasticity.

When considering multiple environmental variables' impacts on a phenotypic trait, it should be addressed that phenotypic plasticity to one environmental variable can interact with other environmental variables. This type of plasticity can be considered context-dependent or multi-dimensional phenotypic plasticity, where the complexity of multiple reaction norms can be combined to create an n-dimensional reaction surface (Pigliucci, 2001, 2005). This can complicate our interpretation of environmental effects, but is more realistic than single, isolated environmental gradients. For example, temperature and moisture gradients both vary, and their combination can affect the phenotype of lizard

embryos (Flatt *et al.*, 2001). Many studies have examined phenotypic plasticity to one environmental variable (Stearns, 1989; DeWitt *et al.*, 1998; Pigliucci, 2005); however, it is likely due to logistic complications and difficulty with interpretation that there are few studies on context-dependent phenotypic plasticity (Pigliucci *et al.*, 1995; West-Eberhard, 2003; Brooks *et al.*, 2010).

Complicating matters further, the environment can also include biotic interactions. Transgenerational plasticity, more commonly known as non-genetic parental effects, are the impact of the parent's phenotype on their offspring's phenotype (Mousseau and Fox, 1998; Ezard *et al.*, 2014). They can include biomolecules (nutrients and hormones transferred to offspring), environmental (natal environment, temperature, or timing of reproduction) and behavioural (parental care) factors (Crean and Bonduriansky, 2014). Due to similar environmental factors (potential spatial and temporal autocorrelation) between parents and offspring, it has been shown that transgenerational plasticity can be adaptive through adjusting offspring phenotype to better match the likely environmental conditions (Uller, 2008; Ezard *et al.*, 2014).

Both context-dependent and transgenerational phenotypic plasticity have been examined the most thoroughly in plants (Pigliucci *et al.*, 1995; Sultan, 2000, 2003, 2004; Valladares *et al.*, 2007; Brooks *et al.*, 2010; Herman and Sultan, 2011), and vertebrates (Wimberger, 1992; Bashey, 2006; Westneat *et al.*, 2009; Xu *et al.*, 2010; Scordato *et al.*, 2012; Salinas and Munch, 2012; Burton *et al.*, 2013; Berejikian *et al.*, 2014; AbGhani and Merilä, 2014; Donelson *et al.*, 2016; Jonsson and Jonsson, 2016). Most of the work has been conducted on transgenerational plasticity to one environmental variable (Burgess and Marshall, 2011; Ezard *et al.*, 2014); however, no study has examined whether these

parental signatures can influence the next generation's plastic response to multiple abiotic factors.

While we acknowledge that paternal effects can be important, maternal effects tend to have a larger impact than paternal contributions especially in very early life (Crean and Bonduriansky, 2014), therefore maternal effects are the focus of this chapter. When maternal investment is low (small eggs) environmental factors will likely have a large effect on development. However, when mothers make a large investment into each offspring (large eggs), maternal effects may overshadow effects of abiotic variation. Plasticity in developing animal embryos is usually best studied using species with external development, because the embryonic microenvironment can be manipulated and recreated relatively easily. For internal developers, the environment is relatively stable, and there is little opportunity to observe mechanisms that impact development. Working with externally spawning fish for example, allows repeatable studies, and precise control and manipulation of the abiotic environment. Hatch timing (Witzel and MacCrimmon, 1983) and size (Xu *et al.*, 2010; Régnier *et al.*, 2013; Leblanc *et al.*, 2014) are traits that can predict growth and survival and can be influenced by several variables including abiotic factors (such as temperature; Jensen *et al.*, 1989, and water chemistry; Leduc *et al.*, 2009; Purchase, 2018), non-genetic parental effects (Bagenal, 1971; Einum and Fleming, 1999), and parental behaviour (e.g., nest site selection and spawning time; (Sternecker *et al.*, 2014; Beer and Steel, 2017).

Using four populations of brook trout (*Salvelinus fontinalis)* from Cape Race, Newfoundland, Canada, that have been studied since the late 1980's (see Hutchings, 1991) we focused on two potentially maternally influenced early life history characteristics (hatch

time and size) of brook trout that produce very large eggs that develop externally over an extended time period. While previous research on the Cape Race populations has worked with early phenotypic plasticity, it focused on post-hatch development (Hutchings, 1991). The objective of our study; however, was to determine the relative contributions of maternal and environmental effects on embryonic development, and whether degree of plasticity is influenced by maternal effects. We examined how maternal effects (variation in egg size) influenced the plastic response of embryos to environmental conditions. To test this we used a split-brood approach that tracked half sibling families and then manipulated two environmental factors: 1) temperature (stable or fluctuating), as fluctuating temperatures may affect hatch synchronization and is rarely examined in plasticity studies (Post *et al.*, 2001; Dammerman *et al.*, 2016); and 2) acidity (stressful or benign), as pH level can affect resource conversion efficiency due to metabolic stress (Jordahl and Benson, 1987; Kamler, 2008). We tested three hypotheses: 1) that hatch time is affected more by environmental than maternal effects because previous work in salmonids has shown that there is a weak relationship between egg size and hatch time but there is a strong positive relationship between egg size and hatch size (Beacham *et al.*, 1985; Hutchings, 1991); 2) that maternal effects have a larger impact on hatch size (dry weight, yolk volume and hatchling length) than environmental effects due to the large investment (i.e., yolk) salmonid mothers make in their offspring (Einum and Fleming, 1999; Berejikian *et al.*, 2014); and 3) egg size affects the degree of plasticity of hatch time and hatchling size.

3.2 MATERIALS AND METHODS

3.2.1 Gamete collection and embryo fertilization:

Sexually mature brook trout (n: ♀=24, ♂=48) were captured by electrofishing four populations on Cape Race, Newfoundland, Canada: Freshwater River (FW), Watern Cove River (WN), Cripple Cove River (CC), and Ouananiche Beck River (OB) (see Table 3.6.1 for stream details) in October 2012. Fish were held overnight (~12 hours) in flow-through cages in their home streams until gamete collection took place the following morning, and were then promptly released unharmed.

Eggs (FW and WN) and sperm (FW, WN, CC and OB) were collected into 50 mL plastic containers and 1.5 mL Eppendorf tubes, respectively. They were held <4^{o}C and transported back to Memorial University, where fertilization took place 8 to 11 hours after gamete collection. We ensured that sperm from each male used were viable by microscopic examination. Previous work on the same maternal populations showed that Cape Race females have low fecundity (FW: 47 eggs, sd±25, and WN: 55 eggs, sd±28; Hutchings, 1991). In our study we had few eggs per female (FW 29-77; WN 22-80) therefore each cross was small (11-40 embryos). Egg size was used to quantify maternal effects (see below), and the relationship between mother's fork length and egg volume can be seen in Figure 3.7.1.

Each female's brood was split in two and fertilized with sperm from two sources: one random male from her native stream and one random male from a foreign stream. Although these trout spawn in consistent locations (Purchase and Hutchings, 2008), they have natural differences in spawning phenology in the different rivers, therefore conducting a full reciprocal factorial cross design was not possible because when we were sampling females from FW and WN the females from OB and CC were not in spawning condition. We crossed each female with males from two sources for initial plans to evaluate

population hybridization on long term development (Figure 3.7.2). However, due to logistical problems with adequate samples, this second planned experiment was not conducted, therefore we ignored native/foreign status. Of the 48 crosses, 45 produced embryos that hatched (FW♂× FW♀=12; CC♂× FW♀ =11; WN♂×WN♀ =12; and OB♂×WN♀=10). The three unsuccessful crosses were due to issues with the sperm, as the mother had eggs hatch from the cross with the other male (Appendix Table 3.8.A1 for family details). Each brood was then split evenly into the four environmental treatments, see "*Incubation set up*" below.

3.2.2 Yolk size measurements

We refer to eggs and egg size as unfertilized maternal components (oocytes) that may influence developmental timing and size of embryos. Each individual was photographed twice, immediately after fertilization (as an embryo) to measure egg size and within 24 hours post-hatch (as a hatchling), with a camera (Leica DFC420) mounted on a dissecting microscope (Leica M80). The yolk in each photograph was measured (two perpendicular width measurements) to a known scale using ImageJ (ImageJ, 2011; Schneider *et al.*, 2012). Yolk volumes for each individual egg and hatchling (not an average per brood) were then calculated using: length x height^2 x (π/6) (Koskinen *et al.*, 2002).

3.2.3 Incubation set up:

We examined phenotypic plasticity during embryonic development using a split-brood experiment among siblings, that controls for genetic effects of family (Figure 3.7.2). Each embryo (n=1162) was placed in an individual 50 mL tube, that contained a gravel bottom (embryo sat on top of the gravel to be visible). Embryos were incubated in the dark in incubators with a programmable temperature cycle (ThermoScientific Precision Model

818 Incubator) in their assigned temperature treatment. Incubator and position were randomized every two days to avoid a potential incubator or position effect. Water changes took place 1-2 times per week until hatch. Once hatched, the hatchlings (n=299) were euthanized using an overdose of clove oil.

3.2.4 Influences of two environments on phenotype:

The plasticity of two early life history traits were investigated (hatch time and size, where size was measured by three metrics: dry weight, yolk volume and hatchling length). Each individually incubating embryo was examined every 24 hours to establish hatch time, which was converted to thermal summed units (sum of daily mean degrees Celcius). Hatchlings were photographed within 24h after hatching (see above), and hatch length was measured from the tip of the snout to the end of the notochord (in millimeters). Hatchlings (including unabsorbed yolk) were then placed in a drying oven for a minimum of 48 hours at 60°C to obtain a dry weight (milligrams).

To investigate the plasticity of these traits we manipulated two environmental variables (Figure 3.7.2): temperature and pH (stable-benign (SB), stable-acidic (SA), fluctuating-benign (FB) and fluctuating-acidic (FA) treatments). We used a stable (S; 5.0^{o}C) or fluctuating (F; mean: 5.0^{o}C, range: 2.0 and 8.0^{o}C) temperature. Temperature changed every 18 hours, in a cyclical pattern: 2 to 5 to 8 to 5 to 2 to 5 to 8 and so on. This cycle was chosen so that both treatments had the same number of thermal units each week. Thermal units are important in ectothermic animals because it factors in time and temperature, which combine to affect development.

We produced water with a conductivity of 0.8 mS/cm by adding aquarium salt to deionized water and then added sulphuric acid (H_2SO_4), which has been shown to be a source of surface water acidification in freshwater streams (Rodhe *et al.*, 1995; Leduc *et al.*, 2004) to achieve the required acidity. We created stressful (acidic (A); 5.25 pH) and non-stressful (benign (B); 6.5 pH) conditions based on the pH of the mothers' home rivers. A pH of 5.25 was chosen as an acidic pH, because it would be stressful but not cause deformities or mortalities (Fiss and Carline, 1993; Leduc *et al.*, 2004). Data collected in 2010 and 2011 by Wood *et al*. (2014) indicated the average pH for Freshwater River (6.59 pH) and Watern Cove (6.51 pH) was 6.55 (see Table 3.6.1).

3.2.5 Statistical analyses and model selection:

General approach:

For all analyses, α was set at 0.05. Residuals were examined to test for normality and heteroscedascity, and no deviations were observed. All graphs were created in base plot or ggplot2 in R version 3.2.2. We conducted general linear models, and general linear mixed effects models on our data using factors described in Table 3.6.2.

Mother's population (Mpop) and father's population (Ppop) were considered random factors for our main model (see below). However, they were treated as fixed effects in models 1 and 2 because we wanted to test for differences among populations.

Testing for survivorship differences:

To ensure that there were no survivorship differences among the four treatments (hatch percent: SB= 18.6%, SA= 23.8%, FB= 16.4%, and FS= 21.4%), populations (FW= 21.9%, WN= 17.9%) or egg sizes that could potentially bias further analyses we conducted

an analysis of deviance on a general linear mixed effect model with a binomial error structure:

$$HS\sim pH + T + pH \times T + ES + Mpop + (1|Mother) + error$$

[model 1]

where hatch success (HS, binomial yes/no) is explained by fixed effects: egg size (ES), mother's population (Mpop), the pH and temperature (T) treatments with a 2-way interaction; and the random effect of mother's ID (1|Mother). We determined that the pattern of hatch success differed between the two pH treatments ($\chi^2_{(1)}$=6.52,p=0.01) where the benign pH treatments (17.4%) had a slightly lower hatch success than the acidic treatments (22.6%). However, hatch success did not differ among temperature treatments ($\chi^2_{(1)}$=1.47,p=0.22), or by the pH temperature interaction ($\chi^2_{(1)}$=0.06, p=0.91), maternal population ($\chi^2_{(1)}$=3.12, p=0.41), or egg size ($\chi^2_{(1)}$=0.09, p=0.77).

Testing for differences in maternal and paternal populations:

As our key interest was related to maternal effects, to determine if we should treat the two maternal populations separately in further analyses we conducted an Analysis of Deviance on model 2:

$$AES\sim Mpop + ML + Mpop \times ML + error$$

[model 2]

Where AES is average egg size per mother, Mpop (fixed factor) is mother's population and ML (covariate) is mother's length. The interaction between mother's length and mother's population was not significant ($F_{(1,28)}$=3.77, p=0.06).

To determine if there were differences among father's populations we evaluated if the slopes and intercepts differed significantly between male populations by performing ANCOVAs on each linear model (Figures 3.7.3A, 3.7.4, 3.7.5A, 3.7.6A, 3.7.7A, and 3.7.8) with the following format:

$$DV\sim ES + Ppop + ES \times Ppop + error$$

[model 3]

Where the DV is each dependent variable, ES (covariate) is the individual egg size, and Ppop (fixed factor) is the paternal population. If the main effects or interactions were significant we plotted separate lines and presented separate equations for each paternal source (see Appendix Table 3.8.A2). Note: in all of the remaining analyses we treated mother's population and father's population as random factors. We could not treat the parental populations as random factors in models 1, 2 and 3 because we needed to include it in the interaction term to determine how to plot the equations.

Main model:

For hatch timing and size we conducted analyses using the following model:

$$DV\sim pH + T + pH \times T + ES + (1|Mpop) + (1|Ppop) + (1|Mother) + (1|Father) + error$$

[model 4]

Where the dependent variable (DV) is explained by fixed effects: egg size (ES), the pH and temperature (T) treatments with a 2-way interaction; and included random effects: mother's population (1|Mpop), father's population (1|Ppop), mother (1|Mother) and father (1|Father) IDs (Table 3.6.3) where using parental IDs as random effects allows us to control

for additive genetic effects, in the repeated sibling groups across environmental conditions. A model including the interaction terms with egg size did not change conclusions therefore a simplified version is presented here.

Analyzing hatch characteristics:

To test our first hypothesis that hatch time is affected more by environmental than maternal effects we conducted a Cox proportional hazard mixed effects model (Coxme; using the "coxme" R package) on model 4, which is a common method to analyze time-to-event data (Rich *et al.*, 2010) but can also include random effects. Our second hypothesis was that maternal impacts have a larger impact than environmental effects on hatch size characteristics. To determine whether there were differences in our main effects, we conducted an analysis of deviance on the Linear Mixed Effect model (LME, model 4) (lme4 package in R) for each dependent variable: dry weight, yolk volume, and hatchling length. There was more inter- than intra- brood variability in egg size (see variance estimates in Table 3.6.3), therefore the variance from the random effect of 'mother' encompassed most of the variation in egg size.

Analyzing magnitude of phenotypic plasticity:

We analyzed plasticity at the half-sibling level because we did not have enough hatchlings to run the analysis at the full-sibling level (i.e., collapsed father's population). We included families that had embryos hatch in at least two treatments (n=23 families, Freshwater n=12; Watern n=11). While many studies assess reaction norms through analyzing line slopes (e.g., Purchase and Moreau, 2012; Fuhrman *et al.*, 2017), it has to be examined differently when the environments are categorical or do not have a natural

gradient (i.e., are plotted in an arbitrary order on the x-axis), therefore plasticity is measured in terms of character states (de Jong, 1995; Van Leeuwen *et al.,* 2015) or comparing the mean for each environment to the overall mean (Via *et al.*, 1995). Therefore, we determined plasticity for each mother's brood for each DV as follows: 1) we calculated a grand mean of all

$$\Delta x_{(SA,SB,FA,FB)} \sim ES + (1|Mpop) + error \quad \text{[model 5]}$$

5) For each dependent variable we ran an Analysis of Deviance on a linear mixed model (model5) with the averages $\Delta x_{(SA,SB,FA,FB)}$ for each DV (hatch time, dry weight, yolk volume and hatch length), with the egg size (ES) as the fixed factor and mother's population (1|Mpop) as a random factor.

3.3 RESULTS

3.3.1 Egg size:

We found that the majority of variance in egg size came from inter-brood not intra-brood variation. We examined the variation (standard deviation, sd) in egg yolk size measurements as a whole (mean= 58.5 mm^3, sd=15.9), and among mothers within each population (FW: mean=51.3 mm^3, sd=14.3; WN: mean=66.6 mm^3, sd=13.5). We obtained an average of the intra-brood standard deviations to determine the difference in standard deviation within (average sd=6.1 mm^3) and among broods (average sd=15.9mm^3). The

average egg volume per mother's length (mm^3 $_{egg\ volume}$ / mm $_{mother\ length}$) was very similar to previous work conducted by Hutchings (1991) for both Freshwater (current study: 0.53, Hutchings: 0.53) and Watern mothers (current study: 0.48, Hutchings: 0.43).

3.3.2 Hatch time:

Across all parental populations, and all four treatments, on average it took 425.6 TSUs to hatch. The Cox proportional hazards mixed effects model (Coxme) showed that hatch time was not affected by egg size ($z=0.76$, $p=0.45$). However, there was a significant interaction between temperature and pH ($z=-3.40$, $p<0.0001$; Table 3.6.3). When examining Figure 3.7.3A it is clear that the fluctuating-benign (FB) treatment was less synchronous, in that they started hatching earlier but finished hatching around the same time compared to the other 3 treatments. This means that the FB treatment had a wider hatch range (more variability), which may be driving the significant relationship for the Coxme rather than a directional difference in mean hatch time.

3.3.3 Hatch size:

For hatch size, we measured hatchling yolk size (mm^3, mean: 47.25), dry weight (mg, mean: 14.16), and length (mm, mean: 13.64); we compared all three metrics to each other (Figure 3.7.4A,B,C). Salmonids hatch with a large amount of yolk left, so unsurprisingly, we found that yolk size and dry weight were very strongly correlated (Pearson's $r=0.91$, $p<0.0001$). Dry weight and hatchling length were mildly correlated (Pearson's $r=0.35$, $p<0.0001$), as were hatchling length and yolk volume (Pearson's $r=0.48$, $p<0.0001$).

The analysis of deviance on the LME showed that for both dry weight (Figure 3.7.5) and yolk volume (Figure 3.7.6) a large amount variance was explained by the random

effects of mother's population and Mother ID but no main effects were significant (Table 3.6.3). This indicates that the maternal variables encompass much of the variance for dry weight and yolk volume (more variation in egg size among mothers than within mothers).

According to the analysis of deviance on the LME, hatch length was affected by egg size ($\chi^2_{(1)}$=9.1**,** p=0.003), but no other factors were significant, including the pH and temperature treatments which had no effect on length at hatch. There was also a large amount of variance explained by the mother's population and Mother ID. Overall, the pattern showed that bigger eggs tended to produce longer hatchlings (Figure 3.7.7).

3.3.4 Phenotypic Plasticity:

The degree of phenotypic plasticity of hatch time and all three size metrics was affected by egg size indicating that there was a strong maternal effect impacting plasticity where larger eggs were more plastic. Linear models were run on the plasticity scores (mean Δx_i) for hatch time, dry weight, yolk volume and length (Figure 3.7.8). An ANCOVA was conducted on each model(Table 3.6.4). For the Δx_i among treatment there was a significant effect of egg size on the plasticity of hatch time ($\chi^2_{(1)}$=4.52, p=0.03), dry weight at hatch ($\chi^2_{(1)}$=17.97, p<0.0001), and hatch length ($\chi^2_{(1)}$=8.51, p=0.004); but not yolk volume at hatch ($\chi^2_{(1)}$=3.22, p=0.07). We found small to medium effect sizes of egg size on plasticity of hatch size (R^2 values include 0.13, 0.29 and 0.46).

3.4 DISCUSSION

Maternal (transgenerational) (Burton *et al.*, 2013) and environmental effects (Wood and Budy, 2009) have been shown to be important in early development. However, this is the first study that compares the contributions of maternal effects and context-dependent

phenotypic plasticity (both pH and fluctuating temperatures) during embryonic development. We found evidence for at least partial support for all three of our hypotheses: 1) that environmental effects have a larger impact than maternal effects on hatch time; 2) maternal effects have a larger impact than environmental effects on hatch size, we found that one out of three of our size metrics (length) was significantly affected by egg size; and 3) egg size affects the degree of plasticity of hatch time and two of the measures of hatchling size.

Hatch time was not affected by egg size, which supports previous work that has shown no relationship between egg size and hatch time in the same two maternal populations examined in our study (Hutchings, 1991). The environmental effects of pH and temperature significantly affected hatch time. Our results suggest that there is a difference in hatch synchrony (variability), rather than differences in mean hatch time, among the different groups. This result is largely driven by the fluctuating-temperature benign-pH treatment group, in that they began hatching earlier so had a wider hatch range. Fluctuating temperatures seem to be warranting more research as of late (Richter-Boix *et al.*, 2015; Jeuthe *et al.*, 2016; Beer and Steel, 2017) likely because of impacts of climate change (Paaijmans *et al.*, 2013), but has not been examined in terms of context-dependent phenotypic plasticity. Even moderate fluctuations (here we have 6°C of variation) in temperature can affect hatch time, and high degree of asynchrony may have negative consequences for competition in establishing feeding territories if the asynchrony in hatch time translates into differences in emergence timing (Einum and Fleming, 2000) and avoiding predators (Mirza *et al.*, 2001).

On the other hand, the variance in hatch size (dry weight, yolk volume, and hatch length) was mostly encompassed by maternal impacts (random effects of maternal population and Mother ID) and there were no effects of the pH and temperature treatments (context-dependent abiotic influences). Our effect sizes ranged from small to medium, which implies not only statistical, but at least some biological significance. As with previous work (Solberg *et al.*, 2014), larger eggs produced longer hatchlings. Other work has shown that maternal effects are very strong in early life and decrease over time, when environmental and potentially paternal effects also play a much larger role (Heath *et al.*, 1999). Previous work has shown effects of pH (Fiss and Carline, 1993) and temperature (Dammerman *et al.*, 2016; Beer and Steel, 2017; Fuhrman *et al.*, 2018) on developing fish embryos and juveniles. However, our manipulation of pH and temperature were well within natural ranges, so the environmental variables may have simply had no effect, or the magnitude of the maternal effects (transgenerational signature) may have masked the environmental effects. When we ran our models the random effects of 'mother' and 'mother population' explained the majority of the variance, so much so that it overshadowed the effect of individual egg size for dry weight and hatchling's yolk volume. The term 'mother' encompasses several different variables within a sibling brood including genetic, epigenetic, maternal size, egg size, and yolk composition effects. Although the relative components are beyond the scope of this study, most of the egg size variation was across broods, not within a brood. Variation in eggs size likely exists as a part of the parent-offspring conflict, where the optimal egg size and number for a mother is at odds with the optimal egg size for the offspring (Trivers, 1974).

The most interesting finding in our study was the evidence for an interaction between maternal (transgenerational) and environmental effects (context-dependent) on the degree of plasticity of hatch length. In that, mothers with larger eggs (maternal effect) had families whose siblings showed more plasticity to multiple abiotic conditions than smaller eggs. However, it is unclear if the result is due to plasticity or constraint. In salmonids, a portion of the yolk is retained in a yolk sac and absorbed while the hatchling is in the gravel nest. In general, if a hatchling is heavier during the absorption stage it is likely that it is at an earlier developmental stage, where conversely being longer is indicative of being more developed. Each egg has a fixed amount of nutritional material that the embryo and hatchling can convert into growth (length and weight), meaning that at theoretical 100% conversion efficiency there is a fixed maximum dry weight prior to exogenous feeding. Smaller eggs are constrained by a lower maximum size (some combination of length and weight depending on developmental stage at hatch) which affects the size range that a hatchling can be, while larger eggs have more flexibility depending on environmental conditions through differences in nutrients available and conversion efficiency. Thus, maternal investment affects how susceptible embryos are to environmental conditions, where a large investment allows for more flexibility. This novel finding could show that if embryos from larger eggs are more plastic, they may have potential for greater range of expression which may provide advantages to fit their environment. If the plasticity for size continues through from hatchling and juvenile stages to adults, it may confer benefits and be one explanation for increased survival in larger juveniles (Einum and Fleming, 2000; Xu *et al.*, 2010; Pess *et al.*, 2011).

We attempted to tease apart maternal effects from multiple environmental effects on embryonic development and we found that maternal effects observed in this study were so strong, particularly for hatch size, that environmental effects could not be detected. We have known for decades that maternal effects may override potential environmental effects during embryonic development, which has implications for future studies on early life phenotypic plasticity. While a great deal of research has been conducted on maternal effects or environmental plasticity the results of this study suggest that future work should consider the various types of plasticity due to the interactive effects between transgenerational and environmental impacts. Therefore, carefully designed experiments are required to control for maternal effects when testing questions on phenotypic plasticity.

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3.6 TABLES

Table 3.6.1. River locations (GPS), pH values (average of 2010 and 2011), temperature (°C) from our loggers placed in spawning areas (ground water seeps) during incubation (November 7, 2012 to May 2, 2013), population size estimate (95% CI for 2011), mean length, and mean egg size (volume in mm^3) of spawning fish (standard deviation is shown in brackets).

	GPS[1]	pH[2]	Temp[1] (SD)	Population size[2]	Average size in mm (SD)						
					Male[1]		*Female*			*Egg size (mm^3)*	
					Our study	*n*	Previous work[3]	Our study[1]	*n*[1]	Previous work[3]	Our study[1]
FW	46°38.760N, 53°13.304W	6.59	7.4 (0.6)	5076-5743	99 (16.1)	31	109 (15)	96 (14.0)	19	57.9 (0.01)	51.3 (14.3)
OB	46°38.944N, 53°11.137W	5.90	NS	3355-6269	131 (18.9)	18		NS	NS		NS
WN	46°37.942N, 53°09.546W	6.51	5.3 (1.5)	7225-10255	129 (20.0)	18	129 (11)	138 (20.0)	13	55.1(0.01)	66.6 (13.5)
CC	46°38.750N, 53°06.164W	6.05	NS	2231-2632	154 (34.8)	24		NS	NS		NS

NS = not sampled. FW: Freshwater River, OB: Ouananiche Beck, WN: Watern Cove River, CC: Cripple Cove River

[1] Our study

[2] Data extracted from Wood *et al*. (2014)

[3] Data extracted from Hutchings (1991), Note: egg diameters have been converted to egg volume.

Table 3.6.2. List of the factors, description, levels and type of factor used for our models.

Factor	Description	Levels	Type of factor
pH	pH	stressful, benign	Fixed
Temp	Temperature	stable, fluctuating	Fixed
Mpop	Maternal population	FW, WN	Fixed in models 1,2 Random in models 4,5
Ppop	Paternal population	FW, WN, CC, OB	Fixed in model 3 Random in model 4
ES	Egg size	Continuous variable	Covariate
ML	Mother length	Continuous variable	Covariate
MotherID	Mother's ID code	FW1-12, WN 1-12	Random
FatherID	Father's ID code	FW1-12, WN 1-12, CC 1-11, OB 1-10	Random

Table 3.6.3. A summary of the Cox proportional mixed effects model for hatch time and the analyses of deviance on the fixed effects from linear mixed effects (LME) models for dry weight, yolk volume, and hatch length. χ^2 or z values, degrees of freedom, and the corresponding p-value, are given for each effect.

DV	Effect	Variance	SE			**z**	df	p
Hatch Time	Random							
	Father	0.14						
	Mother	0.0004						
	Mpop	0.34						
	Ppop	0.02						
	Fixed							
	Intercept							
	pH					5.03	1	**<0.00001**
	Temp					8.40	1	**<0.00001**
	Egg size					0.76	1	0.45
	pHxTemp					-3.40	1	**<0.0001**
	Effect	Variance	SE	χ^2	Estimate	t	df	p
Dry Weight	Random							
	Father	0.14						
	Mother	9.53						
	Mpop	8.89						
	Ppop	0.004						
	Residuals	1.19						
	Fixed							
	Intercept		2.27		13.31	5.85		
	pH		0.18	0.80	-0.06	-0.34	1	0.37
	Temp		0.17	0.50	0.15	0.86	1	0.48
	Egg size		0.01	1.84	0.01	1.36	1	0.17
	pHxTemp		0.26	0.24	-0.13	-0.49	1	0.62
Yolk Volume	Random							
	Father	0.00						
	Mother	168.96						
	Mpop	177.82						
	Ppop	0.00						
	Residuals	46.87						
	Fixed							
	Intercept		10.42		44.91	4.31		
	pH		1.17	0.51	0.66	0.56	1	0.53
	Temp		1.12	2.94	1.51	1.36	1	0.09
	Egg size		0.06	0.11	0.02	0.30	1	0.77
	pHxTemp		1.68	0.09	-0.23	-0.14	1	0.89
Hatch Length	Random							
	Father	0.36						
	Mother	0.05						
	Mpop	0.53						
	Ppop	0.02						

Residuals	2.44					
Fixed						
Intercept	0.78		11.66	15.50		
pH	0.26	0.07	0.08	0.26	1	0.78
Temp	0.25	0.73	-0.04	-0.19	1	0.39
Egg size	0.01	9.07	0.03	3.01	1	**0.003**
pHxTemp	0.38	0.48	-0.27	-0.69	1	0.49

Table 3.6.4. A summary of the analysis of deviance on the linear mixed effects models on plasticity of hatch time (thermal summed units), dry weight (mg), yolk volume (mm^3) and hatch length (mm) among environmental treatments (average Δx_i). The standard error, χ^2-values, degrees of freedom and the corresponding p-value are given for the fixed effects.

DV	Effect	Variance	SE	χ^2	df	p
Δx_i **Hatch Time**	Random					
	Mpop	21.53				
	Residuals	42.48				
	Fixed					
	Intercept		7.08			
	Egg size		0.11	4.52	1	**0.03**
Δx_i **Dry weight**	Random					
	Mpop	0.008				
	Residuals	0.07				
	Fixed					
	Intercept		0.25			
	Egg size		0.004	17.97	1	**<0.0001**
Δx_i **Yolk volume**	Random					
	Mpop	0.00				
	Residuals	4.39				
	Fixed					
	Intercept		1.66			
	Egg size		0.03	3.22	1	**0.07**
Δx_i **Hatch length**	Random					
	Mpop	0.00				
	Residuals	0.07				
	Fixed					
	Intercept		0.20			
	Egg size		0.003	8.51	1	**0.004**

3.7 FIGURES

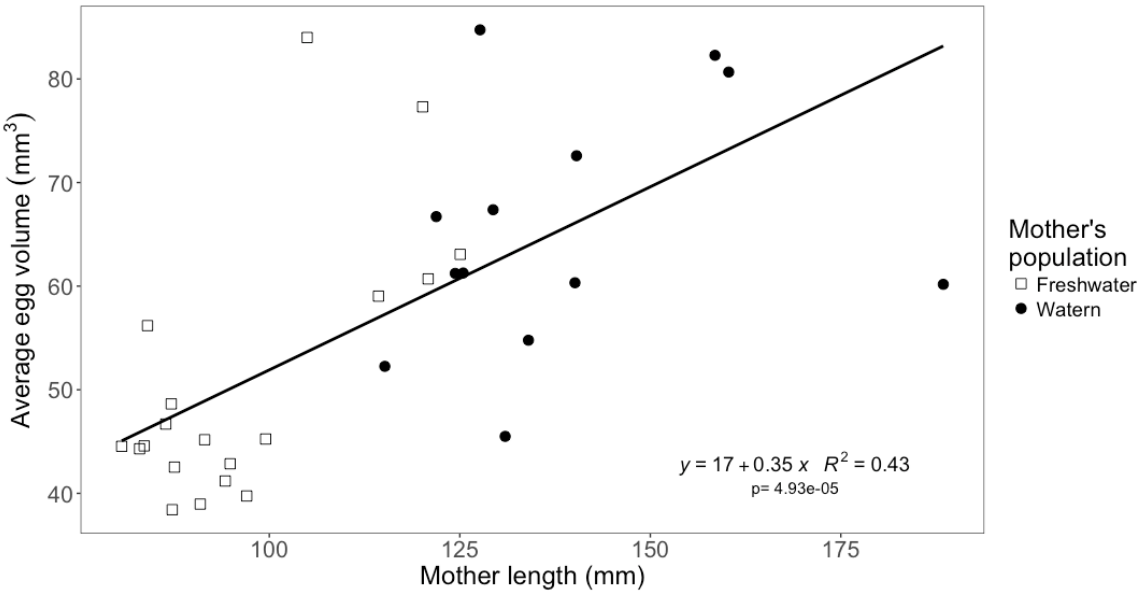

Figure 3.7.1. The relationship between mother fork length (mm) and her average egg volume (mm^3) for Freshwater (FW) and Watern (WN) populations.

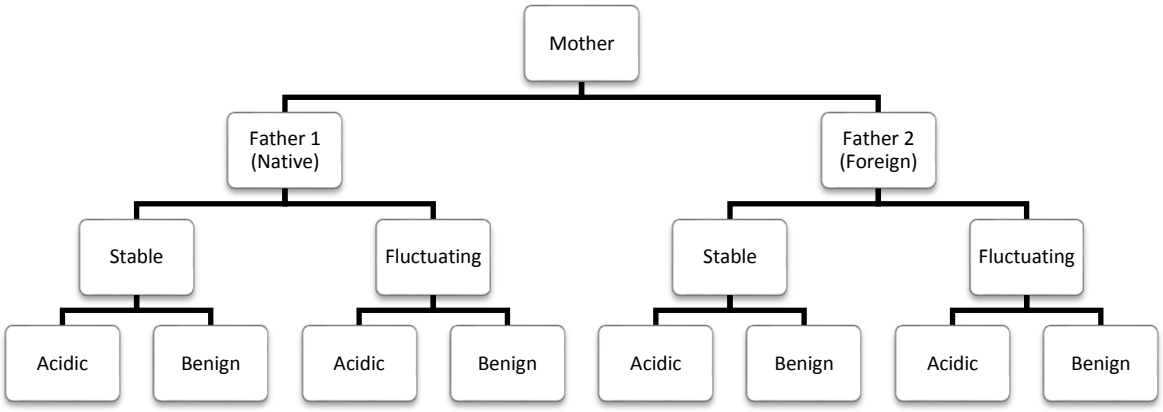

Figure 3.7.2. Cross design and the split-brood, common garden experiment set up. Half of each female's brood (FW and WN) was crossed with a male from her native stream (FWxFW; WNxWN) and half with a male from a foreign stream (FWxCC; WNxOB). Each cross was then split into two temperature treatments and two acidity treatments.

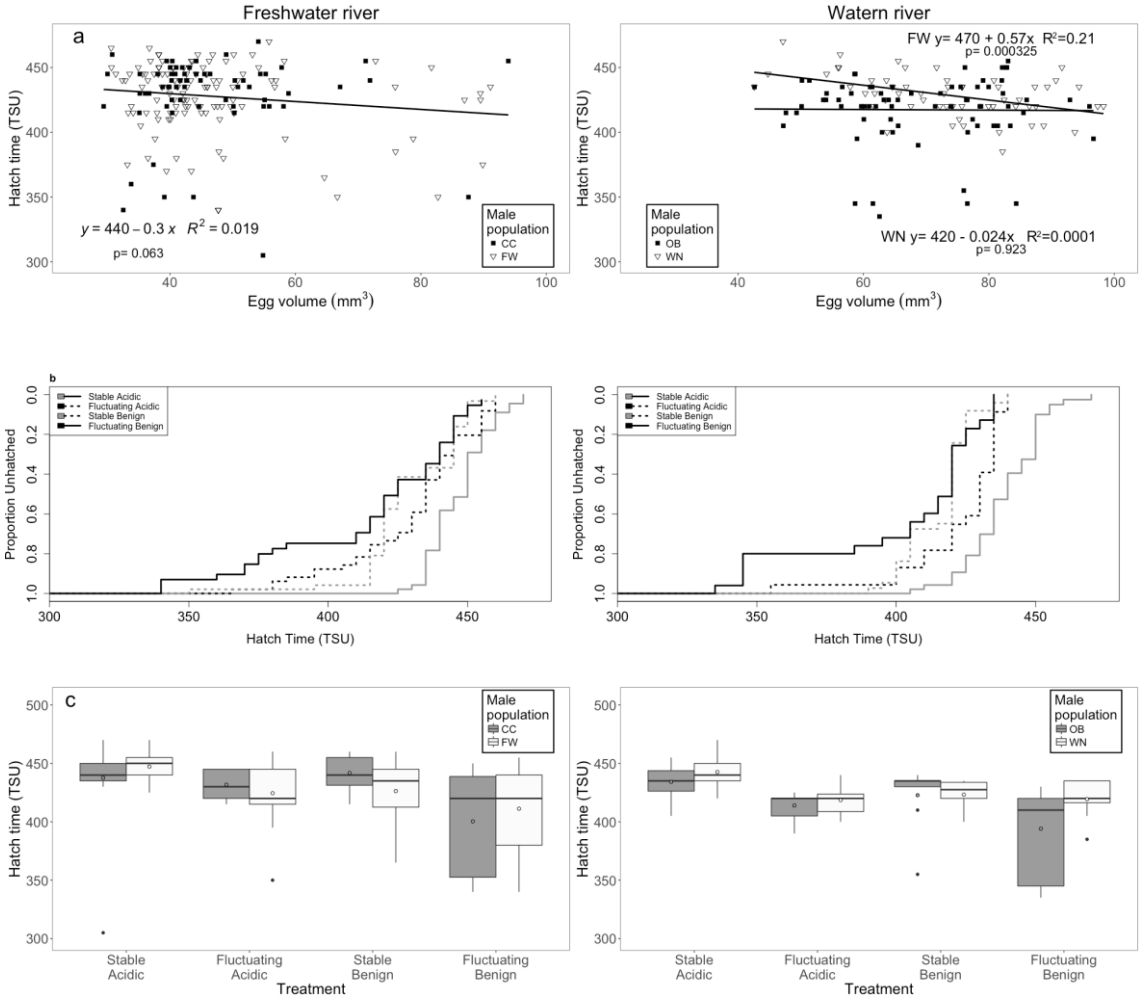

Figure 3.7.3. Hatch time in thermal summed units (TSU) for each full-sibling family. (a) Relationship between egg volume (individual egg) and hatch time for native and foreign male (father) sourced embryos from (i) Freshwater and (ii) Watern mothers. (b) Hatch time for each pH and temperature treatment in the Kaplan Meier analysis, different lines represent treatment combinations. (c) Hatch time for each pH and temperature treatment from (i) Freshwater and (ii) Watern. The boxplot shows the median (line) the interquartile range (IQR, 25 and 75%), whiskers represent the next quartile of the data (1.5 *IQR), and outliers are represented by dots. Open circles represent the mean for each group.

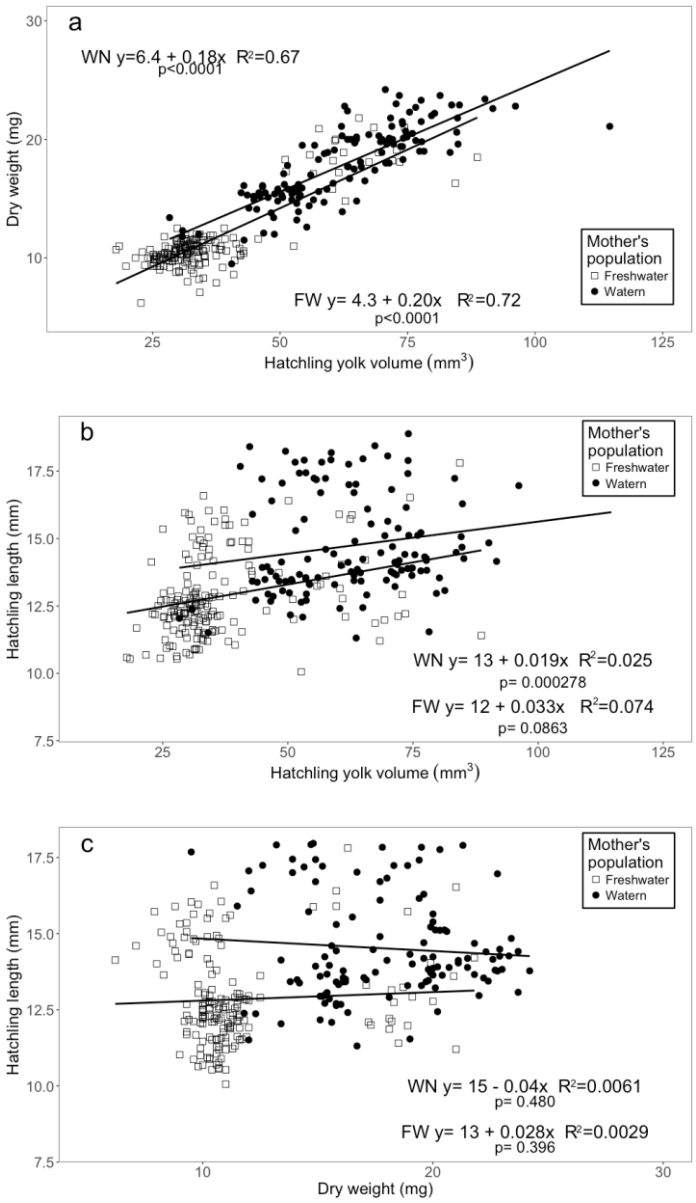

Figure 3.7.4. Relationship between (a) hatchling yolk volume (mm3) and dry weight (mg); (b) hatchling yolk volume (mm3) and length (mm); (c) and hatchling dry weight (mg) and length (mm) for each population.

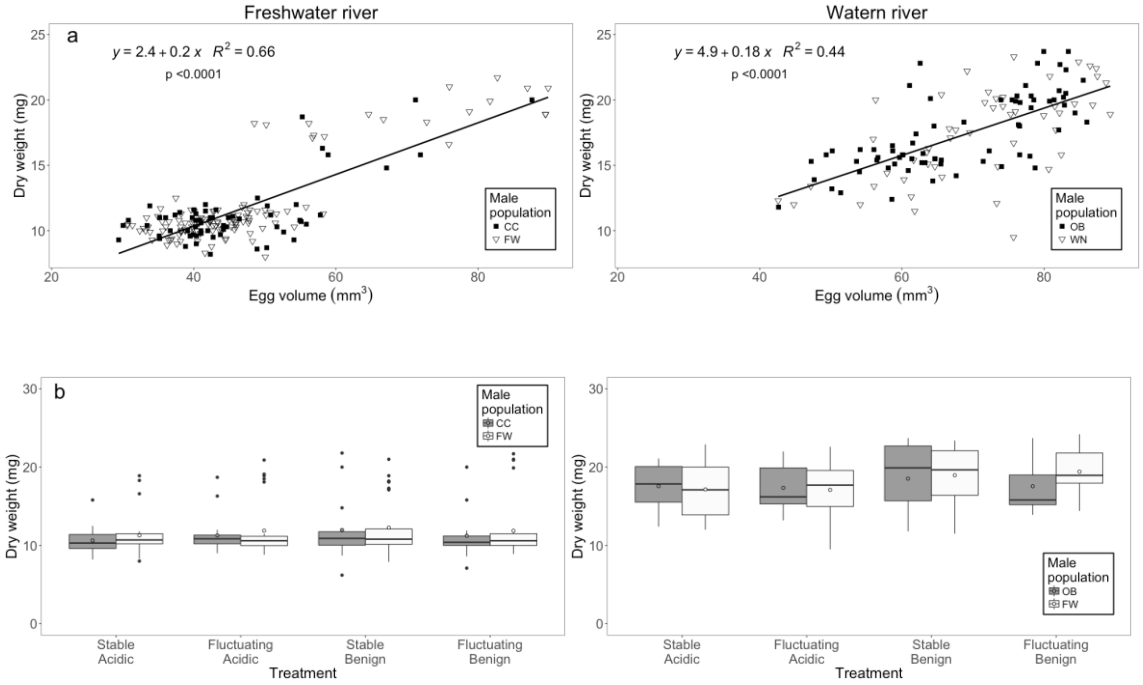

Figure 3.7.5. Relationship between egg volume (individual egg) and hatchling dry weight (mg) (a), for native and foreign male (father) sourced embryos from (i) Freshwater and (ii) Watern mothers. (b) Hatchling dry weights for each pH and temperature treatment from (i) Freshwater and (ii) Watern. The boxplot shows the median (line) the interquartile range (IQR, 25 and 75%), whiskers represent the next quartile of the data (1.5 *IQR), and outliers are represented by dots. Open circles represent the mean for each group.

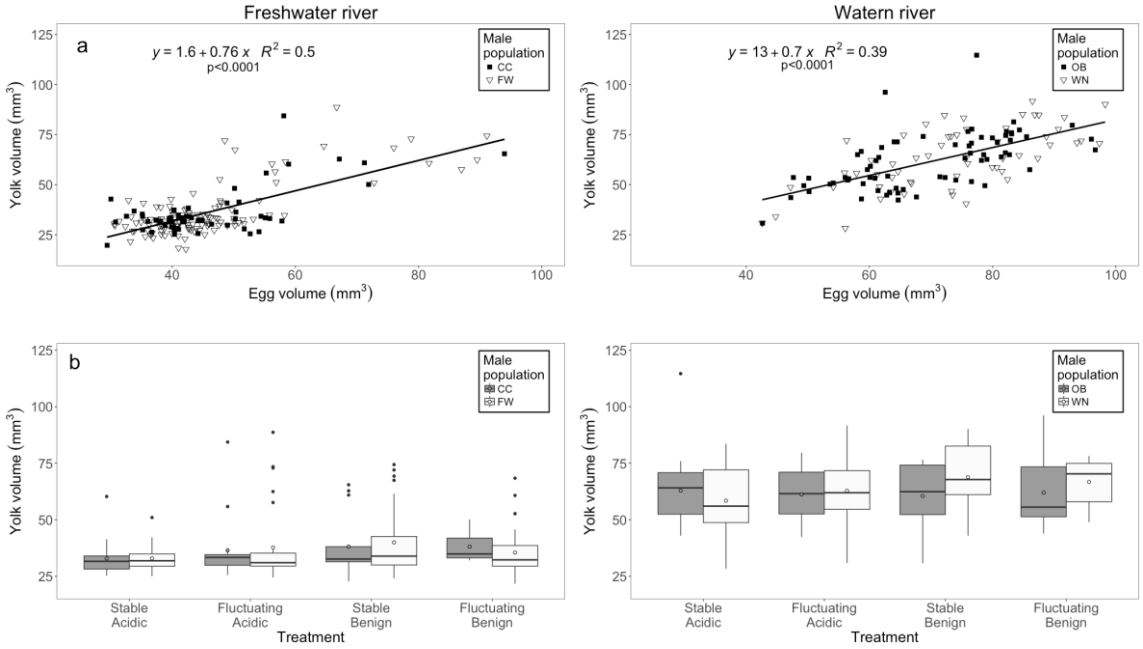

Figure 3.7.6. Relationship between egg volume (individual egg) and hatchling yolk volume (mg) (a), for native and foreign male (fathers) sourced embryos from (i) Freshwater and (ii) Watern mothers. (b) Hatchling yolk volume for each pH and temperature treatment from (i) Freshwater and (ii) Watern. The boxplot shows the median (line) the interquartile range (IQR, 25 and 75%), whiskers represent the next quartile of the data (1.5 *IQR), and outliers are represented by dots. Open circles represent the mean for each group.

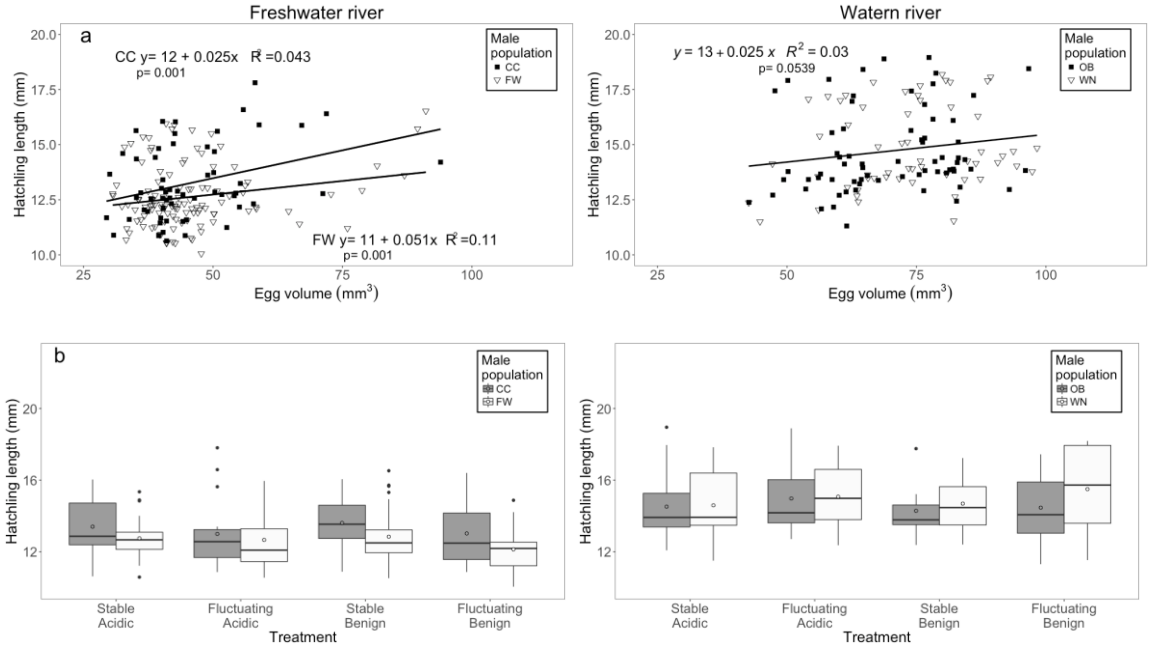

Figure 3.7.7. Relationship between egg volume (individual egg) and hatchling length (a), for native and foreign male (father) sourced embryos from (i) Freshwater and (ii) Watern mothers. (b) Hatchling length for each pH and temperature treatment from (i) Freshwater and (ii) Watern. The boxplot shows the median (line) the interquartile range (IQR, 25 and 75%), whiskers represent the next quartile of the data (1.5 *IQR), and outliers are represented by dots. Open circles represent the mean for each group.

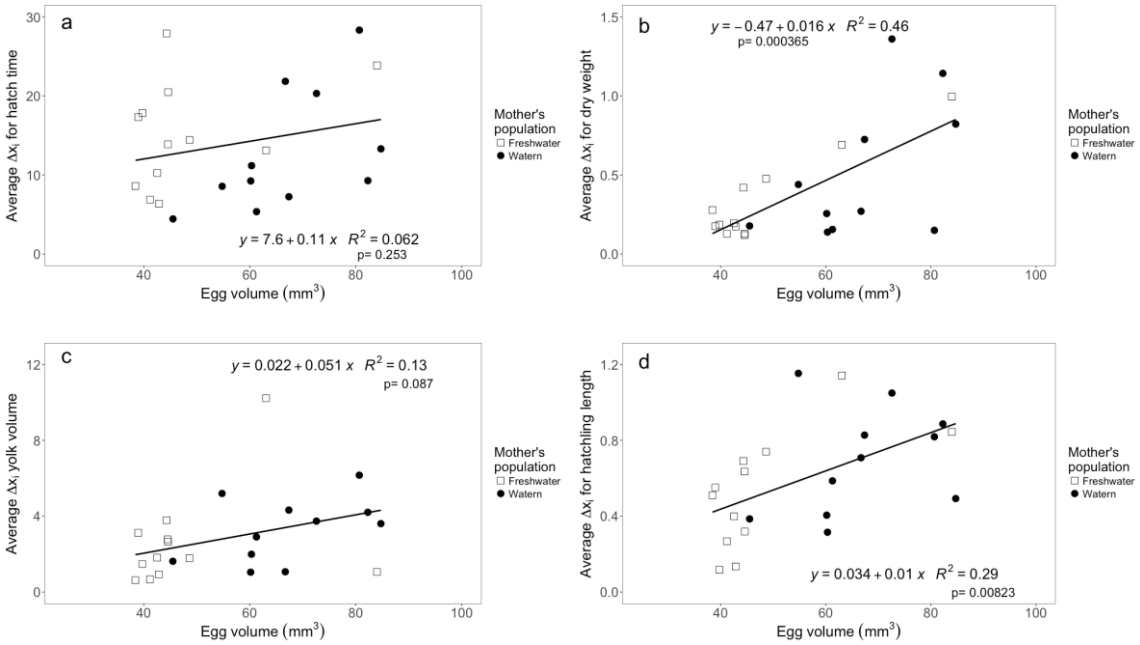

Figure 3.7.8. The relationship between egg size (average egg volume for a mother) and the absolute average Δx_i for each family for (a) hatch time; (b) dry weight; (c) yolk volume; (d) length; for mothers from Freshwater (FW) and Watern (WN).

3.8 Appendix Chapter 3

Table 3.8.A1: Summary of number of embryos, number hatched, hatch percent and average egg size for each cross. Mother and Father IDs indicate which river they came from (letters) and a unique identifier (number).

Mother	Father	Number of embryos	Number hatched	Hatch percent (%)	Mean egg size (mm)
FW1	FW1	24	5	20.8	5.45
	CC1	10	2	20.0	5.43
FW5	FW5	23	8	34.8	4.33
	CC5	24	2	8.3	4.21
FW7	FW7	21	13	61.9	4.47
	C11	21	13	61.9	4.28
FW8	FW19	16	8	50.0	4.53
	CC12	16	6	37.5	4.58
FW9	FW20	18	5	27.8	4.38
	CC13	18	6	33.3	4.56
FW11	FW22	16	11	68.8	4.29
	CC15	15	4	26.7	4.36
FW12	FW23	22	10	45.5	4.32
	CC16	22	10	45.5	4.22
FW13	FW24	15	10	66.7	4.45
	CC17	14	6	42.9	4.41
FW14	FW25	40	10	25.0	4.92
	CC18	37	5	13.5	5.03
FW15	FW26	29	11	37.9	4.14
	CC19	28	2	7.1	4.26
FW16	FW28	15	6	40.0	4.32
	CC20	15	3	20.0	4.51
FW18	FW30	21	17	81.0	4.34
	CC23	20	0	0.0	4.41
TOTAL		500	173	34.6	
MEAN		**20.8**	**7.2**	**4.5**	**4.61**
WN1	WN1	40	2	5.0	4.95
	OB1	40	10	25.0	4.88
WN2	W2	40	12	30.0	5.52
	OB18	40	9	22.5	5.52
WN3	WN3	10	1	10.0	5.46
	OB3	12	3	25.0	5.40
WN4	WN4	40	8	20.0	5.52
	OB4	40	10	25.0	5.43
WN5	WN5	29	1	3.4	4.89
	OB5	28	7	25.0	4.95
WN6	WN6	22	12	54.5	5.23

	OB6	23	12	52.2	5.22
WN7	WN7	28	4	14.3	5.03
	OB7	28	10	35.7	4.86
WN8	WN8	26	7	26.9	5.13
	OB17	26	2	7.7	5.01
WN9	WB9	28	2	7.1	5.08
	OB9	28	5	17.9	5.03
WN10	WN10	28	1	3.6	4.91
	OB10	28	0	0.0	4.92
WN11	WN11	19	2	10.5	4.48
	OB16	20	1	5.0	4.44
WN12	WN12	20	5	25.0	4.77
	OB12	20	0	0.0	4.74
TOTAL		663	126	19.0	
MEAN		27.6	5.25	18.8	5.06

Table 3.8.A2: Results from ANCOVAs on interactions of the linear models for each of the figures. If the p value is significant it means the slopes are significantly different and would need to include two lines and equations on the graphs. Subsequently, the equations and p-values shown on the graphs are for each of these models without the interaction because none were significant.

			Intercept			Slope		
Figure	Model	Population	F	df	p	F	df	p
1	ES ~ ML+ MPop + ML x MPop		0.001	1,28	0.98	3.77	1,28	0.06
3a	DD ~ ES + Ppop + ES x Ppop	Freshwater	0.04	1,182	0.84	1.44	1,182	0.23
		Watern	**10.2**	**1,127**	**0.002**	3.30	1,127	0.07
4a	DW ~YV+ MPop + YV x MPop		**25.2**	**1,297**	**0.0001**	0.86	1,297	0.35
4b	HL ~ YV + MPop + YV x MPop		**18.2**	**1,298**	**0.0001**	0.40	1,298	0.53
4c	HL ~ DW + MPop + DW x MPop		**44.1**	**1,297**	**0.0001**	0.83	1,297	0.36
5a	DW ~ ES + Ppop + ES x Ppop	Freshwater	1.34	1,181	0.19	1.71	1,181	0.19
		Watern	0.24	1,127	0.24	0.24	1,127	0.63
6a	YV ~ ES + Ppop + ES x Ppop	Freshwater	0.01	1,169	0.92	1.47	1,169	0.23
		Watern	0.40	1,122	0.53	0.40	1,122	0.52
7a	HL ~ ES + Ppop + ES x Ppop	Freshwater	**7.12**	**1,169**	**0.008**	1.58	1,169	0.21
		Watern	0.14	1,122	0.71	0.01	1,122	0.94
8a			4.45	1,19	0.05	0.41	1,19	0.52
8b			1.70	1,19	0.21	0.002	1,19	0.97
8c			0.16	1,19	0.69	0.03	1,19	0.87
8d			0.12	1,19	0.74	0.93	1,19	0.35

DD: hatch time (accumulated thermal units ^{o}C), DW: hatchling dry weigh (mg), ES: egg size (mm^3), HL: hatch length (mm), ML: mother's length (mm), MPop: maternal population, Ppop: paternal population, Δx_i plasticity of the trait. YV: hatchling yolk volume (mm^3)

CHAPTER 4: EVIDENCE OF HATCH TIME-BASED GROWTH COMPENSATION IN THE EARLY LIFE HISTORY OF TWO NORTHERN SALMONID SPECIES

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ABSTRACT

Initial body size can indicate quality in many species, with large size increasing the likelihood of survival. However, some populations or individuals may have body size disadvantages due to latitudinal differences in temperature, photoperiod, or food availability. Animals often compensate for a slow start either by locally adapting at the population level or behavioural adjustments at the individual level by increasing food intake after periods of deprivation (growth compensation). In this study, we posit a theoretical extension of growth compensation to include within-population differences related to short growing seasons due to delayed hatch time. The main objective of this chapter was to test the hypothesis that individual fish that hatch later grow faster than individuals that hatched earlier. The relative magnitude of such a response was compared to growth variation among populations (rivers) and between related species. We sampled young of the year Arctic charr and brook trout from five rivers in northern Labrador. Daily increments from otoliths were used to back-calculate size to a common age and calculate growth rates. Older individuals were not larger at capture than younger fish. This occurred because animals that hatched later grew faster than those that hatched earlier, which may indicate age-based growth compensation.

4.1 INTRODUCTION

Early phenotype can establish individuals on trajectories towards alternate life history strategies, and influence fitness related traits such as growth and survival (Taborsky, 2006; Varpe *et al.*, 2007; Jonsson and Jonsson, 2014; Rohde *et al.*, 2015; Walsh *et al.*, 2015; Clarke *et al.*, 2016; Karjalainen *et al.*, 2016). In turn, phenology (timing) of important events such as germination, hatch, or birth can affect early phenotypes (Beer & Anderson, 2001; Brännäs, 1995; Einum & Fleming, 2000; Sternecker, Denic, & Geist, 2014). Therefore, there is often strong selective pressure on organisms to undertake reproductive events at an optimal time (McNamara, Barta, Klaassen, & Bauer, 2011; Morgan & Christy, 1994; Morin, Lawler, & Johnson, 1990). For example, reproductive phenology has been shown to affect reproductive success in plants (Satake *et al.*, 2001), corals (Guest *et al.*, 2008; Mercier *et al.*, 2011), insects (Maino *et al.*, 2017), amphibians (Morin *et al.*, 1990), fishes (Morbey and Ydenberg, 2003), birds (Reed *et al.*, 2009; Shoji *et al.*, 2015), and mammals (Rotella *et al.*, 2016). However, there can be considerable variation in hatch or birth time within a population, which may or may not be adaptive

Within a population hatch or birth timing is affected by environmental conditions (McNamara *et al.*, 2011), breeding timing (Sternecker *et al.*, 2014), maternal condition (Berejikian *et al.*, 2014), and female investment in offspring (Beacham *et al.*, 1985; Maino *et al.*, 2017). In salmonids, the phenology of several important reproductive events have been associated with fitness, including spawning (Beer and Anderson, 2001; Sternecker *et al.*, 2014), hatch timing (Solberg *et al.*, 2014), and emergence timing (Einum and Fleming, 2000). When salmonids hatch, they remain under the gravel (the nest) for several weeks

until they are ready to emerge and feed. Evidence suggests that salmonids have an optimal spawning time which results in an emergence phenology that allows offspring to take advantage of the best possible environmental conditions in an average year. For example, a study by Jensen, Johnsen and Heggberget (1991) found that spawning Atlantic salmon (*Salmo salar*) in Norway timed egg deposition and therefore emergence and first feeding to occur after the peak spring freshets and after temperatures reached 8°C. By doing so, their offspring are less likely to be displaced by floods and could take advantage of temperature dependant food sources (Jensen *et al.*, 1991).

Conversely, sub-optimal emergence phenology can result in a mismatch in trophic dynamics with predators or prey (Brännäs, 1995), whereby food is unavailable to newly emerging offspring. Optimal emergence timing is stochastic year to year, but may be relatively stable across generations. Emerge too early and there may be no food and/or floods may displace fry. However, late emergers are at a competitive disadvantage for feeding territories compared to early emerging fry due to dominance hierarchies. Known as a prior residence advantage, it has been shown that individuals holding a territory are more likely to hold it than be ousted from it (Metcalfe and Thorpe, 1992; Cutts *et al.*, 1999), which ultimately could result in slower growth due to lower food availability for these individuals. Prior residence advantage has also been shown for interspecific competition between Atlantic salmon and brown trout (*S. trutta*; Skoglund *et al.*, 2012). Thus, sub-optimal hatch timing can result in slower growth rates and lower chances of survival (Snucins *et al.*, 1992; Einum and Fleming, 2000; Borcherding *et al.*, 2010; Skoglund *et al.*, 2012).

Intraspecific variation in growth rates is ubiquitous. Among-population differences often exist due to latitudinal and altitudinal gradients in temperature and photoperiod, with individuals in more northern latitudes or at higher elevations experiencing shorter growing seasons (Campos *et al.*, 2009; Sinnatamby *et al.*, 2014). Populations often adapt to such conditions in a pattern deemed counter-gradient variation in growth rates. Populations experiencing shorter growing seasons evolve greater capacity for growth than those experiencing longer growing seasons, and this can mitigate some negative environmental effects on size (Arendt and Wilson, 1999; Purchase and Brown, 2000). Within-populations, individuals may experience a period of depressed feeding opportunities that result in diminished growth rates; however compensatory behaviours can allow them to catch up by the end of the growing season (Metcalfe and Monaghan, 2001). This growth compensation may be considered an intrinsic plastic response to changes in the environment (Zhu *et al.*, 2003; Carlson, *et al.*, 2004) that is triggered by a depletion of stored resources in particular lipids (Ali *et al.*, 2003). Compensating growth can have positive effects on individuals, through an increased likelihood of survival (associated with larger body size); however, growth compensation has also been shown to have increased risks associated with bolder foraging behaviours which put these individuals at a greater risk of predation (Nicieza and Metcalfe, 1997; Damsgård and Dill, 1998; Biro *et al.*, 2004). While the immediate effect of accelerated growth rate can be quite beneficial, negative consequences have been shown to occur later in life. For example, Atlantic salmon that compensated for slow growth rate in early life later exhibited delayed maturity and reduced fat deposition as adults (Morgan & Metcalfe, 2001).

Previous work has established that hatch time is related to growth rate across-populations (e.g., Lapolla, 2001) and periods of faster growth will occur to compensate for periods of slow growth due to limited food (Metcalfe and Monaghan, 2001). In this study, we posit a theoretical extension of growth compensation to include within-population differences related to short growing seasons due to delayed hatch time. An individual may compensate for hatching late, where they are disadvantaged by a short growing season, by growing faster than other individuals within their population that hatched earlier, thereby making the best of a bad situation. We tested this hypothesis in two sympatric salmonid species (*Salvelinus spp.*) where the relative magnitude of such a response was compared to growth variation between the species and across populations (five rivers) in northern Labrador.

4.2 MATERIALS AND METHODS

4.2.1 Environmental information

Sampling occurred on secondary and tertiary streams of five river systems in northern Labrador, Canada: Hebron River, Kamanatsuk Brook, Fraser River, Anaktalik Brook, and Igluvigaluk Brook (Figure 4.7.1; Appendix Table 4.8.A1).

Temperature loggers (HOBO TidbiT v2, UTBI-001) were installed during the spawning season in October 2012, and removed during the June 2013 sampling period at two sampling sites, Fraser and Anaktalik rivers; and one that we did not sample, Ikadlivik Brook (Appendix Table 4.8.A1). Loggers were fastened to rebar and firmly placed in river beds. We were able to retrieve 4 loggers to use for our analyses (Figure 4.7.2). Salmonids often spawn in groundwater seeps having steady flows of water at stable temperatures. The

temperature loggers placed in Fraser River were in a spawning aggregation where redds were observed, while the loggers Anaktalik River and Ikadlivik Brook were placed in the main flow of the river. The temperature estimates from our loggers are likely underestimates (through winter) compared to those experienced in the redds because the loggers were in the water column and not in the gravel (where salmonids lay their eggs, and when in the presence of groundwater seeps, tend to have more stable temperatures).

4.2.3 Fish collection

We collected young of the year Arctic charr and brook trout (*Salvelinus alpinus* and *S. fontinalis*) using a Smith-Root LR24 backpack electrofisher between June 24th and June 29th, 2013. Potential sites to collect young of the year were viewed from a helicopter with each tributary selectively sampled by electrofishing upstream. Areas where we were unlikely to find young of the year, such as sandy substrate, and turbulent or deep water, were not surveyed. The minimum stretch of water sampled was ~100 meters per stream, and care was taken to not oversample clusters of fish to avoid collecting multiple siblings from a family. After capture, the fish were euthanized, measured (fork length), and a tissue sample taken and placed in 95% ethanol for genetic species identification. The fish were then frozen (at -20°C) for later otolith extraction.

4.2.3 Genetic identification to species

The small physical size of the newly emerged alevins made morphological species identification difficult. Therefore, we used genetic barcoding to determine the species identity of each fish (brook trout or Arctic charr; n=436). We extracted DNA from tissue samples using a Qiagen DNeasy Blood and Tissue Kit according to the manufacturer's

protocol (Qiagen, Hilden, Germany). A 520 base pair fragment of the cytochrome oxidase 1 (CO1) gene was amplified by PCR using standard COI barcoding primers (Cox1-1F AACGTAATTGTCACCGCCCATG and Cox1-1R CACCTCAGGGTGTCCGAAG-AAT). We purified the PCR products with an Exo-SAP clean-up method and sent them to Genome Quebec (McGill University, QC) for sequencing using standard dideoxy methods. We aligned the sequences in MEGA v6.0 (Tamura *et al.*, 2013) and species identification was determined.

4.2.4 Otolith work and hatch date

Sagittal otoliths were extracted from young of the year fish using established methodology (Radtke 1996). Each otolith was fixed to a glass slide and polished using 3 and 30 μm lapping film. In salmonids a layer of calcium carbonate is deposited every day; this forms an increment that can be used to interpret fish age (see Radtke 1989, 1996; and Adams et al. 1992 for methods). When a band appears darker and thicker than the others, it is considered a check. Checks can occur for a variety of reasons including: stress due to hatch, emergence, or environmental change such as a storm; lack of food; or handling stress in aquaculture settings (Adams et al., 1992; Campana & Neilson, 1985). The result of this stress is slower growth, and therefore two daily rings merge into one thicker ring (Adams *et al.*, 1992). In this study, we were interested in both hatch and emergence checks. The hatch check is a thick ring that encircles all of the primordia (nuclei upon which the otolith is built) near the core of the otolith. The emergence check occurs when fry leave their gravel nest and begin exogenous feeding (Figure 4.7.3). After the establishment of the emergence

check, growth often accelerates and therefore subsequent rings are further apart and more translucent (Campana, 2001; Campana & Neilson, 1985).

Of the original 436 individuals, only rivers with a sample size n $\geq$10 fish of a species, and for which we could obtain daily age readings were included in further analyses (324 fish: 206 Arctic charr; 118 brook trout) (see Appendix Table 4.8.A2 for details). We were unable to age 112 fish due to otolith loss or breakage during processing. Based on the number of daily increments present and the date of capture, each fish's hatch date was back-calculated (Table 4.6.1). Photographs of otoliths were taken using a compound microscope under 100x magnification. The photographs were cropped, grey-scaled, and the colour range of greys was reduced to make ring visualization easier using Photoshop.

Each otolith was assigned a blind code and read without knowledge of species or river origin. We reinterpreted age on a random subset of 50 fish to determine precision. Precision estimates were based on coefficient of variation (CV) values (Chang, 1982). A review found that a CV of less than 7.6% is generally acceptable for aging studies (Campana, 2001). Our precision estimate was 7.1% for days post-hatch and 8.0% for days post-emergence. In addition, of the 50 subsampled otoliths we also identified emergence checks in 40. When we compared these checks against their original interpretations 7 of the 40 (17.5%) did not agree. Therefore, determining emergence checks was deemed unreliable for these otoliths, and we did not examine emergence in further analyses. All otoliths were aged by the same reader (HDP).

4.2.5 Growth rate and back calculated lengths

We used the ObjectJ plugin for ImageJ (Schneider *et al.*, 2012) to calculate the fish's daily growth rate (mm/day) based on the width of the daily otolith rings. Daily growth was then calculated based upon total growth of the fish (length at capture minus estimated hatch size; defined below) compared to the width of each daily otolith ring [model 1]. The total radius of the otolith was measured from the hatch line to the last visible increment. Detailed information on the ImageJ plugin ObjectJ can be found at https://sils.fnwi.uva.nl/bcb/objectj.

Previous studies have shown that the relationship between otolith size and fish size is metabolically driven and therefore can be influenced by temperature, with higher temperatures uncoupling the relationship between the otolith radii and fish length (>13°C for Arctic charr; Mosegaard et al. 1988). However, in this instance, the otolith size-fish length relationship was satisfactory given the temperatures experienced by our fish remained at a level well below concern, because temperatures would not have been high enough to uncouple the relationship that early in the growing season (Figure 4.7.2).

We back-calculated fry length to 25 and 50 days post-hatch (Table 4.6.1) using the biological intercept model [model 1](Campana, 1990), which has been shown to be one of the better models for back-calculation due to its relative accuracy and simplicity (Vigliola and Meekan, 2009).

$$L_a = L_c + \frac{(O_a - O_c)(L_c - L_o)}{(O_c - O_o)}$$

[model 1]

Where L was fish length (size), O was otolith radius, L_c and O_c were size at capture, L_a and O_a were size at age, and L_o and O_o were size at hatch. While the model had limitations and required some assumptions, we felt that this was the best-fit model for our data. One of the weaknesses of this model was that hatch length needs to be estimated in order to estimate post-hatch lengths. We used a hatch length (L_o) of 18 mm based on previous work in brook trout (Penney, Beirao, and Purchase, 2018; Chapter 3). Additionally, we conducted a sensitivity analysis with an assumed hatch size of 16mm and 20mm and it made no difference on the overall conclusions.

4.2.6 Data analyses and statistics

For descriptive purposes and to determine if we could examine our hypotheses at a species level, we tested whether river (population) had an affect on any of our dependent variables (hatch date, growth rate, fork length) (Table 4.6.2). To determine if hatch date (HD, age on June 24th) or post-hatch growth rate (GR, mm/day) differed between species (Sp) or among rivers (R) we conducted two analyses of variance (ANOVA) using the structure of model 2. There was no difference in growth rates in the first 25 days and the entire post-hatch life, so we used lifetime growth rates in the analysis.

$$HD \text{ or } GR \sim Sp + R + error$$

[model 2]

In addition, we ran an analysis of deviance on a linear mixed effect model (LME) to examine differences in fork length (mm) between species and among rivers at two time points (25 and 50 days post-hatch) [model 3]. Where FL was fork length, A was age, Sp was species, and R was river. ID was a unique identifier that was a random factor which

allowed for paired results between the two ages within an individual. We did not test for the interaction between species and river for model 2 or 3 because we did not have representatives from both species in each river.

$$FL \sim A + Sp + R + (1|ID) + error$$

[model 3]

There was no effect of river on hatch date (above), fork length at capture, or growth rate (Table 4.6.2), therefore the populations were pooled for further analyses. Therefore, we conducted linear regressions for each species (regardless of river) to determine: 1) if there was an association between fork length at capture and age, and 2) if there was an association between hatch date and growth rate. For all analyses, α was set at 0.05. Residuals were examined to test for normality of data and heteroscedascity, and no deviations were observed. The map was created in ArcGIS. All graphs (ggplot2), data processing and statistics(using packages lubridate, reshape, were done in R version 3.3.3 (R Development Core Team, 2015; using packages car, ggpmisc, Hmisc, lme4, and lubridate).

4.3 RESULTS

The hatch dates (Table 4.6.1) for brook trout (mean: April 26; range: March 30 to May 17) and Arctic charr (mean: April 24; range: March 21 to May 17) did not differ between species, or among rivers (Table 4.6.2 and Figure 4.7.4). Individual fish were longer when they were older (50 vs 25 days), and brook trout (26.5 ± 3.7 SD) were slightly shorter than Arctic charr (27.7 ± 1.8 SD), (Table 4.6.2 and Figure 4.7.5). There was a significant

difference in individual growth rates by species, with Arctic char growing faster than brook trout (Table 4.6.2).

Subsequent to pooling populations, we conducted linear regressions and found no relationship between age and fork length at capture for Arctic charr or brook trout (Figure 4.7.6). Older fish were not bigger than younger fish. To better understand the absence of a relationship between age and body size, we conducted additional tests correlating hatch date to daily growth rate at three time points. We found a positive relationship at all three time points (first 25 days, June 1st to 21st, and entire post-hatch life) for both Arctic charr and brook trout (Figure 4.7.7), whereby, fish with earlier hatch dates had slower growth rates than fish that had later hatch dates.

4.4 DISCUSSION

The objective of this chapter was to test the hypothesis that hatch time affects growth rate, where individuals that hatch later in the season are disadvantaged by a shorter growing season than those that hatch earlier and therefore would grow faster to compensate, potentially making the best of a bad situation. We tested our hypothesis and found support for our prediction in two sympatric species of salmonid (*Salvelinus spp.*). Overall, there was more variation in growth rate among individuals than across populations and between species. There was no relationship between age and size of young of the year charrs, which means that older hatchlings were not larger than younger hatchlings. We found that this occurred because fish that hatched later grew faster, potentially as a form of growth compensation. Growth compensation, correcting for stunted growth, has been found in other studies and has been linked to initially poor environmental conditions such as drought

stress on plants (Oesterheld & McNaughton, 1991), predation (McNaughton, 1983), density dependence (Sundström *et al.*, 2013), or resource availability (Metcalfe and Monaghan, 2001; Walling *et al.*, 2007). Thus, differences in growth rates due to hatch timing may be a theoretical extension of growth compensation as an adaptive response to a short growing season experienced by late hatchers.

Growth rate can be affected by a variety of factors depending on the conditions that the individual experiences including food availability and abiotic factors (e.g., Nicieza & Metcalfe, 1997). Changes in growth rates can be adaptive on a population level (counter-gradient variation; e.g., Carlson, et al., 2004; McCairns, 2004; Yamahira & Conover, 2002) and on an individual level (growth compensation; e.g., Mortensen & Damsgård, 1993; Nicieza & Metcalfe, 1997). For example, there are predictable latitudinal and altitudinal variations in temperature and photoperiod that contribute to counter-gradient variation in growth potential among populations, where northern populations demonstrate faster rates of growth when exposed to equivalent temperatures to that of populations living in more southerly latitudes (Lapolla, 2001; Campos *et al.*, 2009; Sinnatamby *et al.*, 2014). On an individual basis, a period of faster compensatory growth often occurs after a depletion of resources causes a period of slow growth (Metcalfe and Monaghan, 2001; Ali *et al.*, 2003).

Fish may deplete their endogenous resource stores if they have few opportunities to gather resources. This situation can arise through a food shortage brought on by a competitive disadvantage from not establishing feeding territories before others in their cohort (Metcalfe and Thorpe, 1992; Cutts *et al.*, 1999), or shorter growing seasons (Arendt and Wilson, 1999; Campos *et al.*, 2009). In fishes, this compensatory mechanism seems to

be triggered after a period of stunted growth (resource depletion), where growth rate increases until the resource stores are back to normal (Ali *et al.*, 2003). Individuals counteract or compensate for this disadvantage by growing faster, which allows them to reach a similar size to individuals who were not stunted at a later time point. We tested this at three time points, during initial growth, for the entire life post-hatch, and for the first three weeks of June. We found that during all three time periods individuals that hatched later grew faster than early hatchers. This phenomenon could be observed in daily otolith growth increments, where incremental growth of late hatching fry tended to be larger than that of early hatching fry. The June growth comparisons indicate that late hatchers grow faster even under the same abiotic conditions and food availability. To our knowledge this is the first time individual growth compensation has been shown due to hatch timing.

Previous work in other salmonid species has shown that larger eggs tend to produce bigger offspring, and larger offspring may emerge from the nest (i.e., ready to begin exogenous feeding) earlier than smaller offspring (e.g., Solberg *et al.*, 2014; Cogliati *et al.*, 2018). The probable difference in resource availability (both diminished fat stores and yolk resources) in early life may be enough to trigger growth compensation response in the late hatching fish. Additionally, individuals that are larger have a higher absolute growth rate but a lower relative or proportional growth rate (Van Buskirk *et al.*, 2017). Cogliati et al. (2018) found that when comparing early and late hatchers there was no difference in growth rate, but did find that fish from small eggs had a significantly larger increase in size over time. In our case, the older fish grew slower, therefore the potential bias is in a conservative direction because the small fish had a higher absolute growth rate and a higher proportional

growth rate. We do not know whether intrinsic effects such as hatch time or extrinsic environmental conditions experienced at different hatch times were the main causes for differences in growth rate. There are likely notable differences in food supply (e.g., zooplankton and insect abundance increases through the spring) and photoperiod in the experiences of the early and late hatchers, which might explain the differences in growth rates. One could also assume that temperature would be an extrinsic explanation for this pattern. However, when comparing the first 25 days of life early hatchers (early April, ~1°C) and late hatchers (mid-May, ~2°C), the temperature profiles were likely not different enough to explain the difference in growth rates. Most importantly, the comparison during the first 3 weeks of June controls for differences in both abiotic and biotic effects, and showed that the pattern was the same.

Capture sizes and hatch estimates for Arctic charr (size: 27.7±1.8 mm; age: 60.4±10.8 days) and brook trout (size: 26.5±3.7mm; age: 58.6±10.5 days) (see Table 1 for an overall break down) were similar to previous work conducted on Arctic charr from Labrador (size: 28.8 ± 2.8 to 46.2±5.1 mm; growing days: 59 to 116; Sinnatamby et al., 2014). Additionally, our estimated hatch timing fits with estimated temperatures (~1 to 2°C), because we know that the Labrador populations spawn in mid to late October (CFP pers. obs) and we would expect a hatch range of late March to mid-May (~160-190 days at 1-2°C). Previous work has shown that Arctic char hatch between 331 to 416 degree days (at 8.5°C, 39 to 49 days; Yanik, Hisar, & Bölükbasi, 2002) and brook trout between 477 to 483 degree days (at ~10-11°C, 43 to 48 days; Witzel and MacCrimmon 1983, Penney et al. 2018). We found relatively similar results in lab experiments conducted on Arctic charr

embryos from the Fraser River, Labrador (400±34.9 degree days; Penney and Purchase unpublished data).

Growth rate and size are two important early life history traits in fishes and understanding the nuances of factors that affect early growth can help explain how different early phenotypes project into different adult life history strategies (Clarke *et al.*, 2016). Future work designed to empirically test how hatch phenology affects growth rate and survival through the first year of life and how that translates into differences in fitness for salmonids is recommended. For example, a study by Lee et. al. (2013) found a direct growth rate-lifespan trade off in three-spined sticklebacks. Sticklebacks that grew faster to compensate for early slow growth had a shorter life span than those that did not (Lee *et al.*, 2013). Furthermore, future work should be conducted to determine if there are similar results in salmonids. Whatever the case, the result of this study has shown that the timing of hatch affected subsequent growth rate, providing more evidence that hatch phenology can play an important role in early life history. Future predicted changes in climate are likely to affect hatch phenology, and therefore, more research should be done to understand the consequences of changes in complex northern ecosystems.

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4.6 TABLES

Table 4.6.1. Information for species in each site collected June 24 to June 29th, 2013 in Labrador, Canada. Site number refers to locations in Figure 4.7.1. Descriptive statistics for: mean age (in days, on June 24th – first day of electrofishing), hatch date, mean otolith radius at capture (mm), fork length at capture, and estimated fork length at 25 and 50 days old (mm). Only samples from species in rivers that had a sample size of at least 10 individuals were included (N = sample size; SD = standard deviation).

Species	Site	River	N	Mean ± SD* age (days)	Mean hatch date	Hatch Range	Otolith radius (mm) at capture ± SD*	Otolith radius (mm) at hatch ± SD*	Mean ± SD* capture fork length (mm)	Mean ± SD* estimated fork length at 50 days (mm)	Mean ± SD* estimated fork length at 25 days (mm)
S. alpinus	1	Hebron	83	60±10	Apr 28	Mar 21-May 13	0.17±0.03	0.13±0.03	27.8±2.2	25.8±1.7	22.0±1.0
	2	Fraser	19	60±9	Apr 27	Apr 11-May 15	0.16±0.03	0.14±0.05	26.8±1.2	25.2±1.4	21.8±0.9
	4	Anaktalik	92	61±12	Apr 24	Mar 27-May 17	0.17±0.03	0.13±0.04	27.9±1.6	26.1±1.7	22.2±1.0
	5	Igluvigaluk	12	60 ±8	Apr 20	Apr 10-May 8	0.17±0.02	0.12±0.02	27.6±3.0	25.9±2.4	21.9±1.2
S. fontinalis	3	Kamanatsuk	100	58±8	Apr 23	Mar 30-May 17	0.17±0.03	0.13±0.04	26.8±2.7	25.2±3.2	21.7±1.8
	5	Igluvigaluk	18	59±11	Apr 25	Apr 9-May 8	0.17±0.02	0.13±0.03	25.0±3.8	23.8±2.3	21.0±1.1

*SD= standard deviation

Table 4.6.2. Results of analysis of variance (ANOVA) for hatch date and average growth rate (mm/day) between fish species (*Salvelinus alpinus* and *S. fontinalis*) and analysis of deviance (χ^2) for fork length (mm) for samples collected among five rivers June 24 to June 29th, 2013 in Labrador, Canada (df = degrees of freedom; F = calculated F statistic; p – probability; χ^2 = analysis of deviance).

	Hatch date			Fork length (mm)			Growth rate (mm/day)		
Factor	**F**	**df**	**p**	χ^2	**df**	**p**	**F**	**df**	**p**
Species	0.21	4,318	0.65	4.98	1,318	0.03	4.21	1,318	**0.04**
River	0.16	1,318	0.96	7.38	4,318	0.12	1.99	4,318	0.10
Age	NA	NA	NA	3271.76	1,318	**0.0001**	NA	NA	NA

4.7 FIGURES

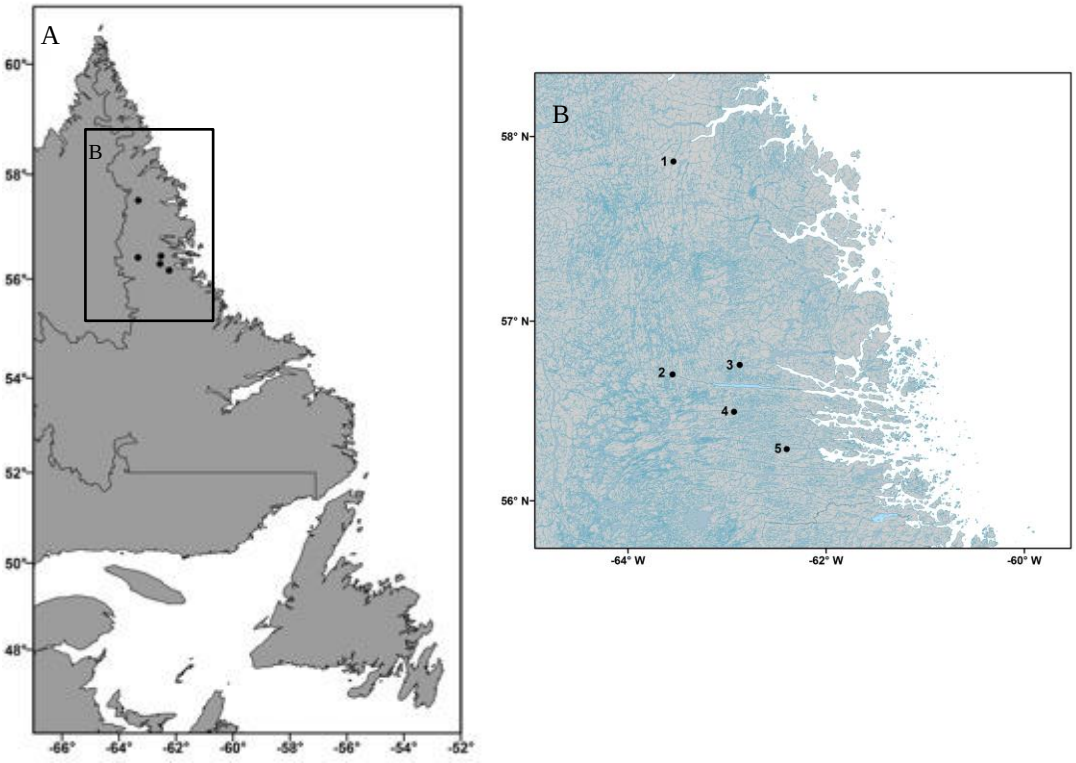

Figure 4.7.1. A) Map of Newfoundland and Labrador, Canada B) Inset of study area. For both maps, points indicate sampling sites, see Table A1 for site details and GPS locations.

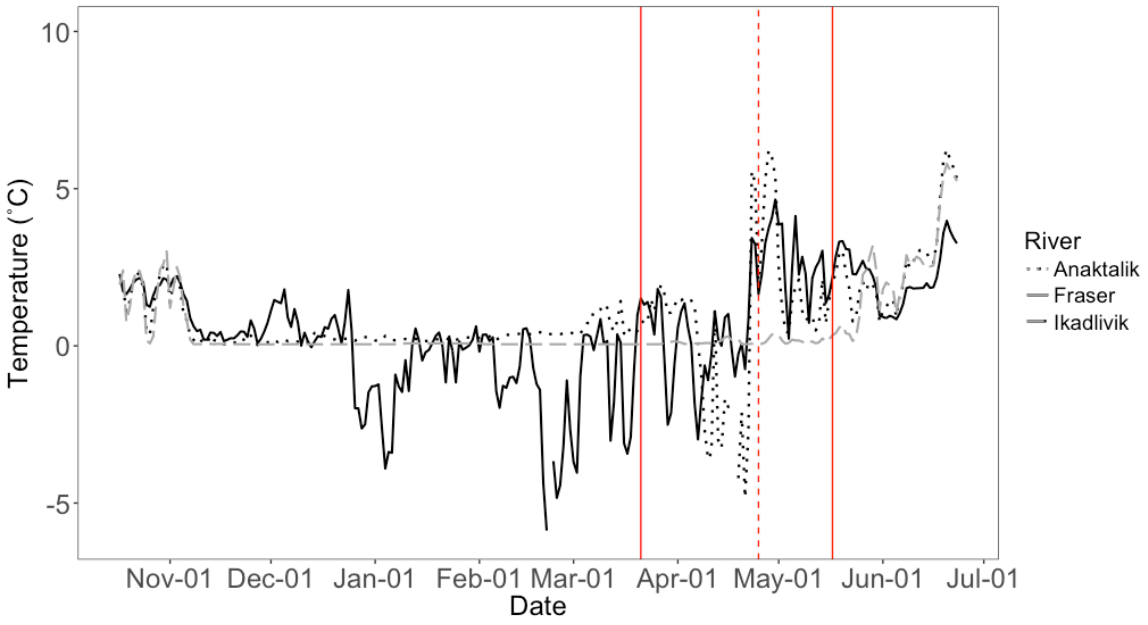

Figure 4.7.2. Temperature profiles from Fraser (black line) (average of 2 loggers), Ikadlivik (grey, dashed line) and Anaktalik (black, dotted line) rivers in Labrador, Canada from loggers in place from October 2012 to June 2013. Vertical lines indicate hatching dates for both species and all rivers (solid=range, dashed= mean). Note: negative values likely indicate being frozen in ice.

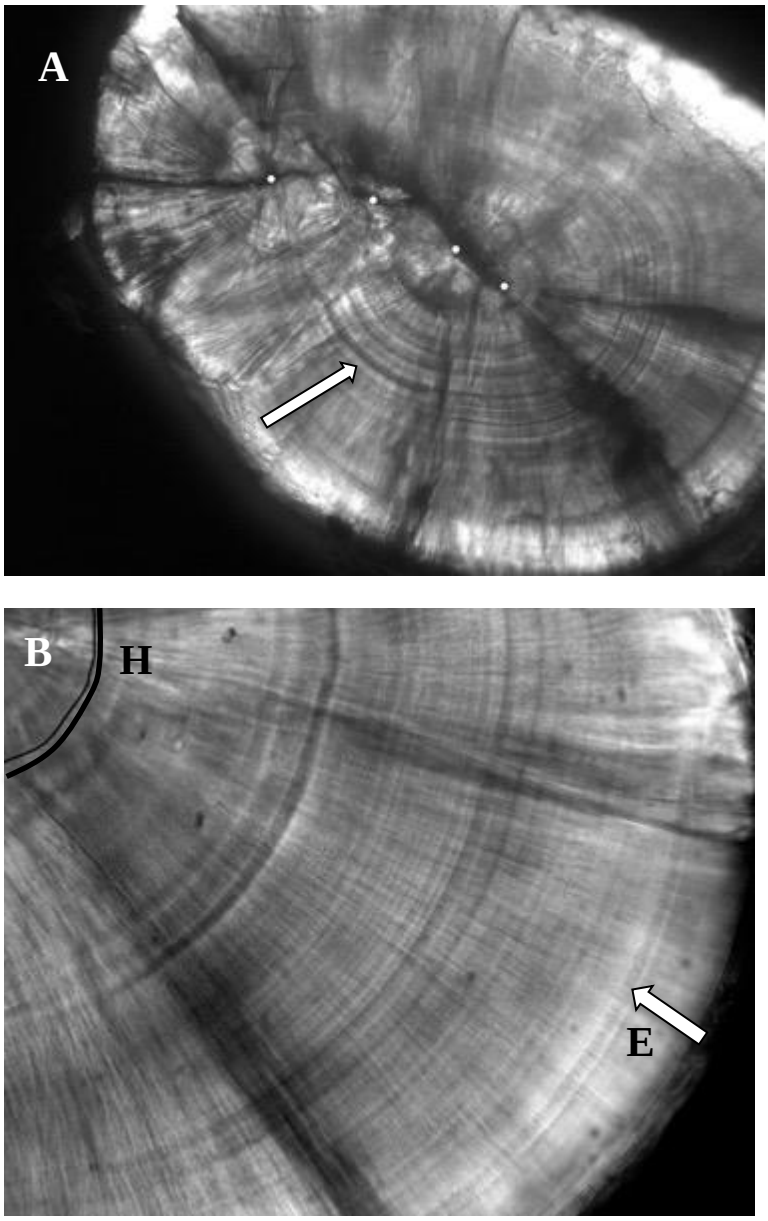

Figure 4.7.3. Photographs of otoliths from Arctic charr sampled from Anaktalik river, Labrador taken under a compound microscope and then manipulated in Photoshop. A) A whole salmonid otolith (40x), and B) a close-up photo (100x, under oil immersion), with hatch (H) and emergence (E) checks indicated. White dots indicate primordia.

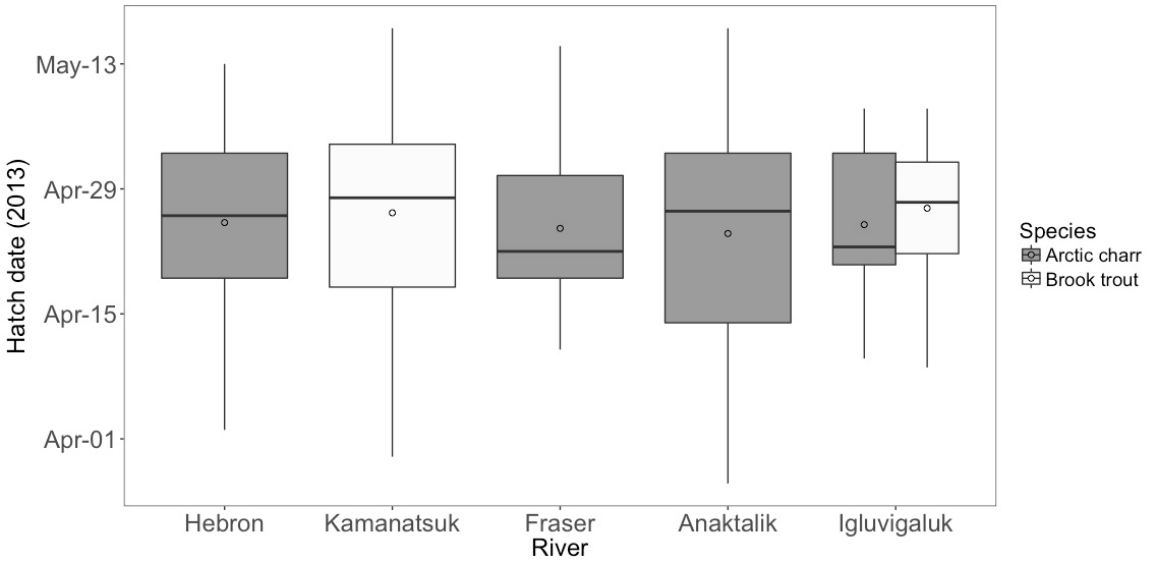

Figure 4.7.4: Box plot comparisons of hatch dates for Arctic charr and brook trout (*Salvelinus alpinus* and *S. fontinalis*) by river (only rivers with a sample size greater than 10 were included) sampled June 24 to June 29th, 2013 in northern Labrador, Canada. The boxplot shows the median (line) the interquartile range (IQR, 25 and 75%), whiskers represent the next quartile of the data (1.5 *IQR), and outliers are represented by dots. Open circles represent the mean for each group.

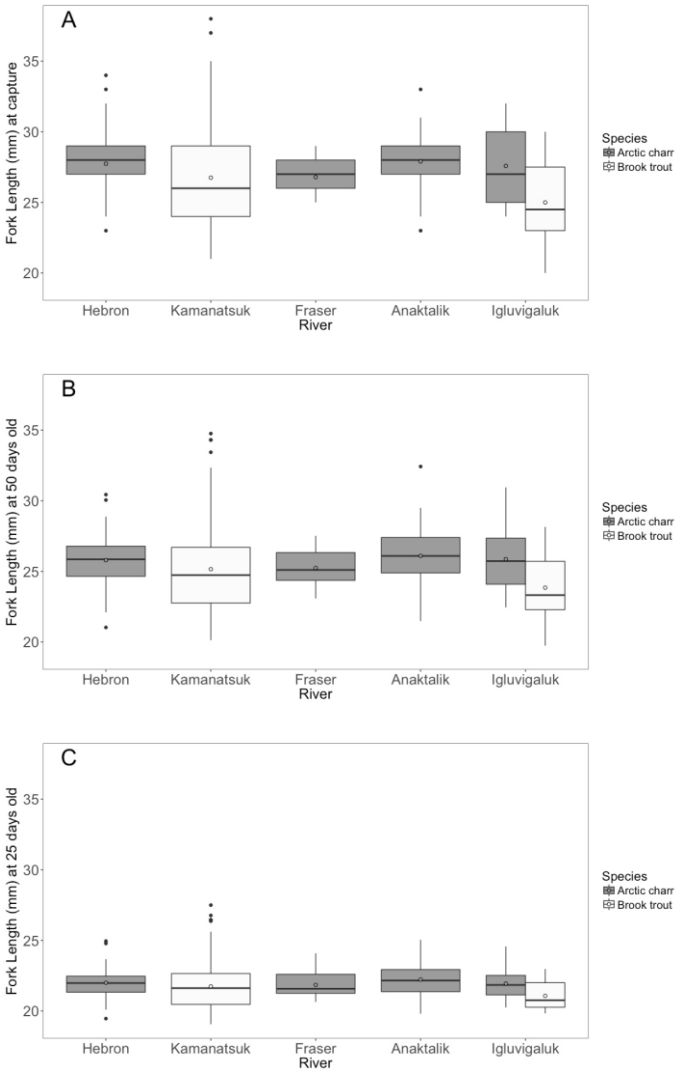

Figure 4.7.5. Box plot comparisons of fork length at (A) capture, and back-calculated fork lengths for (B) 50 and (C) 25 days old for Arctic charr and brook trout (*Salvelinus alpinus* and *S. fontinalis*) and river (only rivers with a sample size greater than 10 were included) sampled June 24 to June 29th, 2013 in northern Labrador, Canada. The boxplot shows the median (line) the interquartile range (IQR, 25 and 75%), whiskers represent the next quartile of the data (1.5 *IQR), and outliers are represented by dots. Open circles represent the mean for each group.

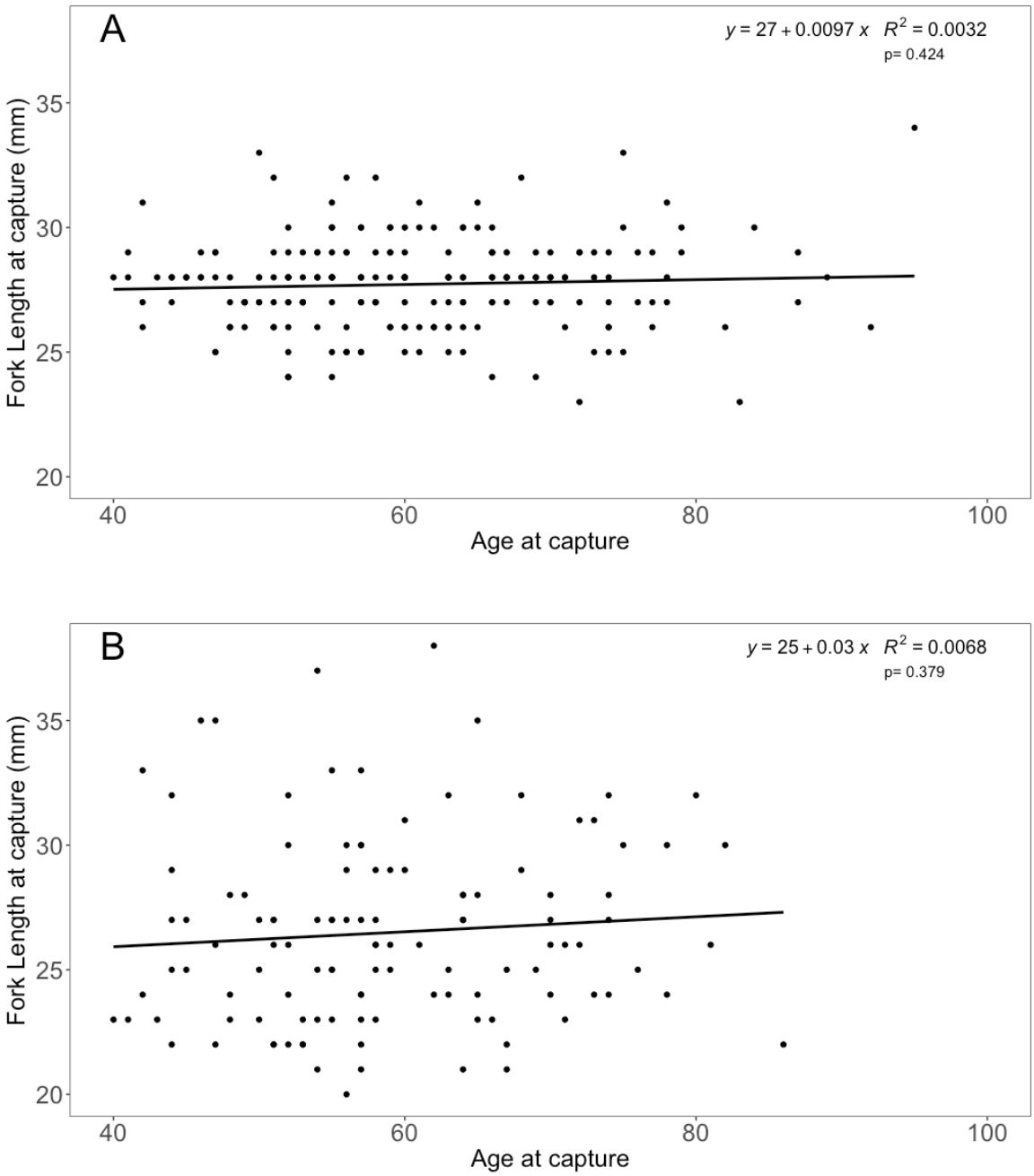

Figure 4.7.6. Plots showing lack of a relationship between age (days) and fork length (mm) at capture for (A) Arctic charr and (B) brook trout (*Salvelinus alpinus* and *S. fontinalis*) among five rivers in northern Labrador, Canada.

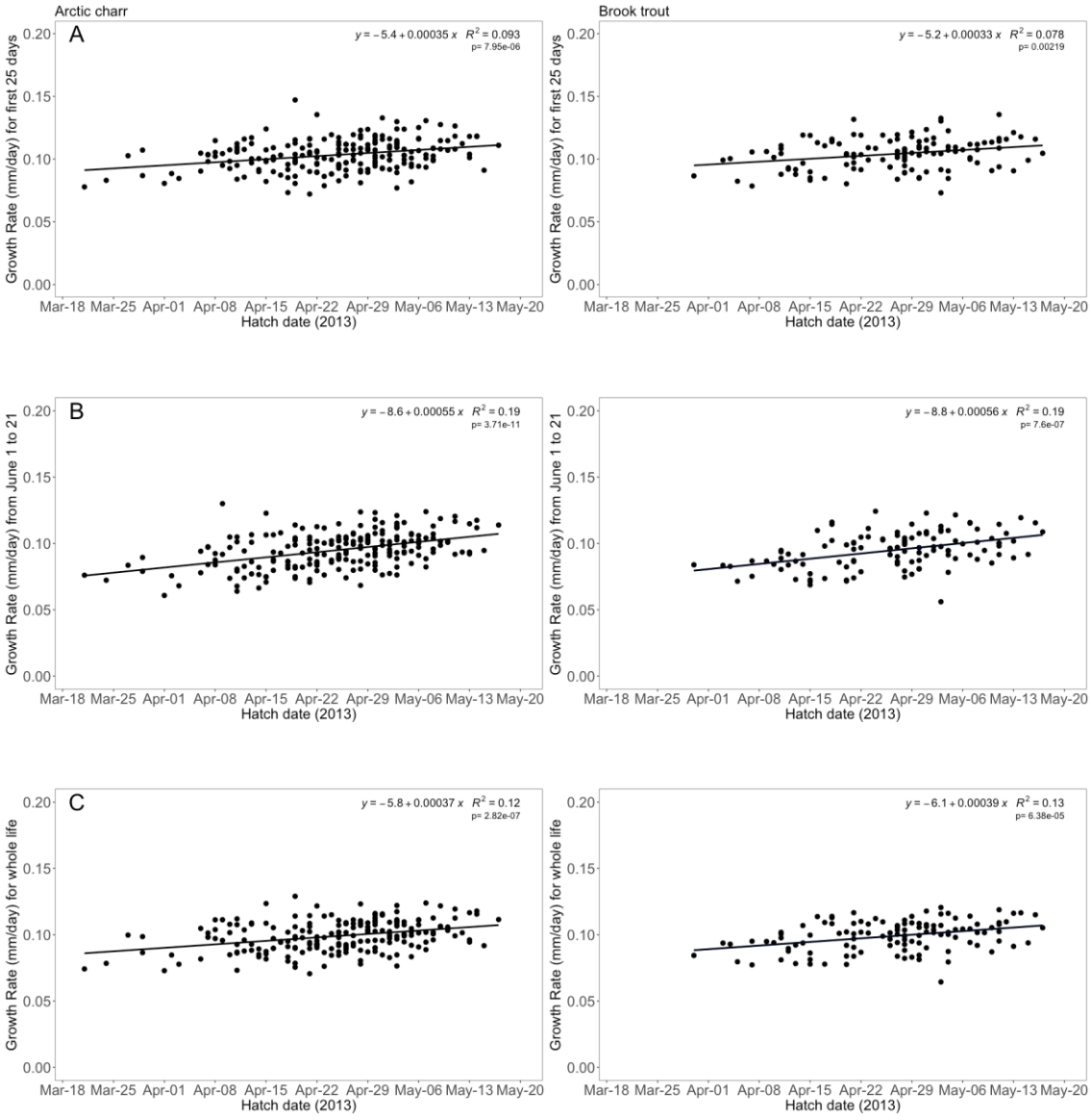

Figure 4.7.7. Plots showing simple linear regression models for the relationship between hatch date and average daily growth rate regardless of river for (A) the first 25 days of life; (B) the time period of June 1st to 21st; and (C) for their entire life post-hatching (average mm/day, based otolith estimation) for Arctic charr (left) and brook trout (right) (Salvelinus alpinus and S. fontinalis) among five rivers in northern Labrador, Canada.

4.8 Appendix Chapter 4

Table 4.8.A1: Fish sampling locations (degrees decimal minutes) and temperature logger locations sampled June 24 to June 29th, 2013, in northern Labrador, Canada. Number of loggers at each location included in brackets. (TL = locations with temperature loggers).

Site	River name	Sampled	Coordinates		Logger location
			Latitude	Longitude	
1	Hebron River	Yes	57°N 51.96	63°W 32.37	
3	Kamanatsuk Brook	Yes	56°N 45.48	62°W 52.31	
2	Fraser River	Yes	56°N 42.34	63°W 32.90 (TL)	Spawning bed (2)
4	Anaktalik Brook	Yes	56°N 29.94 52°N 30.03 56°N 30.01	62°W 55.68 (TL) 62°W 56.79 62°W 55.37	River (1)
	Ikadlivik Brook	No	56°N 24.00	62°W 31.55 (TL)	River (1)
5	Igluvigaluk Brook	Yes	56°N 17.43 56°N 17.64 56°N 16.64	62°W 23.86 62°W 23.58 62°W 26.06	
	Konrad Brook	No	56°N 13.81	62°W 49.48	

Table 4.8.A2: Total number of Arctic charr and brook trout (*Salvelinus alpinus* and *S. fontinalis*) for each river caught June 24 to June 29th, 2013 in Northern Labrador, Canada and subsequently aged. Only species that had more than 10 individuals in a river were included in the analyses.

		Anaktalik	**Fraser**	**Hebron**	**Kamanatsuk**	**Igluvigaluk**	**Total**
Arctic charr	Caught	100	40	107	3	18	268
	Aged	92	19	83	0	12	206
Brook trout	Caught	5	3	4	129	27	168
	Aged	0	0	0	100	18	118
Total	Caught	105	43	111	132	45	436
	Aged	92	19	83	100	30	324

CHAPTER 5: IMPACTS OF INTRINSIC, EXTRINSIC AND TIMING FACTORS ON SIZE AND GROWTH RATES IN THREE SYMPATRIC SALMONIDS

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ABSTRACT

Intrinsic and extrinsic factors combined with hatch timing contribute to variation in early life history; however, their relative importance is often poorly understood. Hatch size is a function of maternal investment and hatch time and is an early indicator of competitive ability and subsequent survival in salmonids. This study had two objectives: 1) test whether the magnitude of species differences in hatch time, growth rate and subsequent late spring body size are greater than: a) within-species differences caused by maternal diet variation from freshwater or marine feeding (intrinsic) and b) within-species differences from different parts of the watershed (extrinsic); and 2) we test the prediction that late hatchers (individual differences) grow faster than early hatchers to compensate for a shorter growing season. Young of the year salmonids (Atlantic salmon, brown trout, and brook trout) were collected from Renew's River in Newfoundland, Canada in June and September. We compared body size at capture, hatch time, and growth rate among species and river sections. We could not adequately address objective 1a) because unexpectedly there was almost no intra-specific variability in maternal life history strategies in this watershed. However, there were differences in size at the end of the growing season and hatch dates among river sections. We found no relationship between size and age in any of the species, andthere was a strong relationship between hatch date and growth rate. Therefore we

suggest that increased growth rate in younger fish may be an adaptation to compensate for a shorter growing season.

5.1 INTRODUCTION

When coexisting species have overlapping habitat or resource requirements competition amongst them can have lasting impacts on fitness. One adaptation which allows individuals to reduce interspecific competition is niche segregation of food resources (Syrjänen *et al.*, 2011), breeding/ontogenetic timing (Werner and Gilliam, 1984; Sachet *et al.*, 2009), or space (Harwood *et al.*, 2002; Mäki-Petäys *et al.*, 2004; Young, 2004; Heggenes and Saltveit, 2007; Berg *et al.*, 2014). Yet how species segregation changes with ontogeny is often unknown, and generalizations may be problematic. Across a lifespan, characteristics of early life stages are typically the least understood. We do know that conditions early in life influence phenotypic trajectory and have effects on overall life history (Schlichting and Pigliucci, 1998; Taborsky, 2006; Clarke *et al.*, 2016). For example, early body size affects competitive ability (Johnsson *et al.*, 1999; Skoglund *et al.*, 2012), which has an additive, reciprocal, positive impact by contributing to fast growth (Metcalfe, 1986) thus producing a subsequently large body size (Huntingford *et al.*, 1990). Early life history traits are a function of maternal contributions (intrinsic), the environment (extrinsic), and timing (intrinsic and extrinsic influences), but their relative importance (Martin *et al.*, 2013), and how they influence species coexistence is unclear.

Intrinsic factors have a large impact on early development, but there can be a great deal of variation among individuals, populations and species. It is assumed that there is considerable interspecific variation in maternal contributions. However, in some cases

intraspecific variation can be greater than variation among species, particularly when there are differences in maternal life history (Berejikian *et al.*, 2014), size (Gagliano and McCormick, 2007), age (Jeuthe *et al.*, 2013), or diet (Gehman and Bingham, 2010). Maternal contributions impact several important aspects of early life history through spawning location (Franssen *et al.*, 2013; Gauthey *et al.*, 2015), yolk quality (Blount, 2004; Brown *et al.*, 2013), hatch or birth timing (Fagundes *et al.*, 2015), offspring body size (Pepin, 1991; Pess *et al.*, 2011), and parental care (Klug and Bonsall, 2009). All of which can affect growth, development, and likelihood of survival. However, such intrinsic factors do not act in isolation and interact with extrinsic forces.

Extrinsic factors are major contributors to intra- and interspecific variation by directly impacting metabolism (e.g., Enders and Boisclair 2016), yolk conversion efficiency (e.g., Brown et al. 2011), and behaviour (e.g., Biro et al. 2004, Leduc et al. 2009). Important interrelated extrinsic influences include abiotic factors such as temperature (Crisp, 1981; Benjamin *et al.*, 2013) and biotic factors such as intra- and interspecific interactions (e.g., predator-prey dynamics or competition; Morgan and Christy 1997, Christy 2003, Jones et al. 2003, Skoglund et al. 2011, 2012, Briga et al. 2017). The relative influence of extrinsic factors on development varies among individuals, populations and species, which makes it difficult to tease out relative contributions to life history. Co-existence of related species is thus predicted to be environmentally context dependent (extrinsic), and influenced by intraspecific variation (intrinsic).

Both intrinsic and extrinsic factors act in concert with life history timing to create an integrated phenotype (Pigliucci, 2003; West-Eberhard, 2003), which is subject to

selection. Specifically, intrinsic and extrinsic factors vary through space and time and affect growth (Michaud *et al.*, 2010), survival (Pess *et al.*, 2011), and reproduction (Varpe *et al.*, 2007). Previous work has shown that hatch time can affect growth rate where individuals that hatch later grow faster than early hatchers even after taking into consideration differences in abiotic conditions (e.g., warmer temperatures later in the season), which is hypothesized to be an adaption to a shorter growing season for late hatchers (Chapter 4). Body size is a strong predictor of competitive ability (Johnsson *et al.*, 1999), and survival (Pepin, 1991; Pess *et al.*, 2011). For example, breeding and development timing directly impact hatch or birth timing and thus determine conditions newborns experience such as temperature and food availability. Since early life history stages are often the most vulnerable to sub-optimal conditions and predation, reproductive timing is likely a key driver of species co-existence, but is poorly understood. The relative strengths of this three-way input of intrinsic factors, extrinsic factors and timing on phenotypic expression has not been addressed [to our knowledge] in the context of competing species.

One way to address questions regarding differences in early life history in coexisting species is to examine similar species that do not occur naturally together. Human introductions have facilitated the movement of many species. By examining interactions between native and non-native species, we can see how competition shapes early life history without the necessity of long time periods of co-evolution, assuming that there has been insufficient time for local adaptation. Fish, especially salmonids, are good for these comparisons due to large differences in life history (e.g., anadromy versus residency), historical records of stocking efforts, and human propensity to stock them around the world.

We examined interactions between two native salmonid species (Atlantic salmon, *Salmo salar* and brook trout, *Salvelinus fontinalis*) and one invasive species (brown trout, *Salmo trutta*), to answer questions about young of the year (YOY) differences in life history and the impacts of hatch timing, body size, and subsequent growth. Atlantic salmon overlap naturally with brook trout in North America, and brown trout in Europe. All three species now occur together in both places because brown trout were introduced to North America (Scott and Crossman, 1964; Hustins, 2007) and brook trout were introduced to Europe (Holčik, 1991) in the 1800s and have since become naturalized.

A great deal of research has been conducted on interactions among Atlantic salmon, brown trout, and brook trout. In Europe, brook trout have been shown to displace brown trout from habitats (Holčik, 1991), and conversely in North America brown trout can displace both Atlantic salmon and brook trout (Gibson and Cunjak, 1986). When all three species occur sympatrically it creates an opportunity to compare life histories within and among species. Early size impacts competitive ability which subsequently affects habitat use and the establishment and maintenance of feeding territories (Johnsson *et al.*, 1999). For example, in Europe larger Atlantic salmon cause smaller brown trout to choose shallower, sub-optimal habitats (interspecific competition; Berg et al. 2014). In another study, larger brown trout fry were more successful (i.e., had more food in their stomachs) after they established feeding territories, and significantly smaller fish could not compete and moved downstream from the natal site (intraspecific competition; Skoglund and Barlaup 2006). Additionally, it seems that individuals that established territories obtain a prior residence advantage where they are more likely to maintain a territory than be

displaced from it (Fausch, 1998), which may make earlier emergence important despite risking poor environmental conditions (Armstrong and Nislow, 2006). Therefore, early interactions can have lasting, additive impacts through intra- and inter- specific competition and subsequent likelihood of survival and growth.

All three species of interest in this study have substantial intra-specific variation in life history, including being facultative ocean migrators (anadromous). Anadromy can confer large benefits to the mother through quantity and quality of their diet. On average, females that go to sea are larger than residents at sexual maturity (Gross, 1987), and subsequently have larger and/or more offspring (Hendry *et al.*, 2004). However, there is a trade off because there is a much higher risk of predation in the marine environment (Hendry *et al.*, 2004). It follows that the differences in parental life history can impact intrinsic factors that fish will experience during early life history, so we cannot ignore the potential importance that intra-specific variation in life history will have on offspring. Early development is affected by a combination of intrinsic and extrinsic factors that interact with timing to produce particular phenotypes. This study had two objectives: 1) as size is a key influence on competitive ability, we test whether the magnitude of species differences in hatch time, growth rate and subsequent late spring body size are greater than: a) within-species differences caused by maternal diet variation from freshwater or marine feeding (intrinsic) and b) within-species differences from different parts of the watershed (extrinsic); and 2) assuming large body size is advantageous and given that development timing is a key component (intrinsic and extrinsic interaction), we test the prediction that late hatchers (individual differences) grow faster than early hatchers to compensate for a

shorter growing season as we found in two salmonid species (brook trout and Arctic charr – *Salvelinus alpinus*) in Chapter 4.

5.2 MATERIALS AND METHODS

5.2.1 Study site and fish collection:

Breeding populations of Atlantic salmon, brown trout and brook trout are present at our study site in Renew's River in Newfoundland, Canada (46°55'N, 52°56'W), which makes this an ideal system to address our hypotheses. The three species overlap in occurrence within the watershed but the recreational fishery focuses on brook trout in ponds, Atlantic salmon in the river, and brown trout in the estuary (Warner *et al.*, 2015).

Young of the year salmonids were collected (Atlantic salmon n=325; brown trout n=227; and brook trout n=153) using a Smith-Root LR24 backpack electrofisher from 16 sampling sites in a 10 km reach. The river has two waterfalls ~1.6 and ~6.2 km from salt water (named First Falls and Second Falls), which are partial barriers to fish movement. For sampling and analyses purposes the 10 kilometer reach of the watershed was divided into three sections: downstream of First Falls (DS), midstream between First and Second Falls (MS), and upstream of Second Falls (US). Each section was sampled during two one-week time periods: after spring ice melt when fry had emerged (June; n=338), and near the end of the growing season (September; n=367; Table 5.6.1). We acknowledge that emergence time and growing season can vary spatially and temporally (Klemetsen *et al.*, 2003; Öhlund *et al.*, 2008); however, due to constraints of electrofishing season we could only fish between June 15th and September 15th therefore we make the assumption that our

sampling times represent emergence and end of the growing season. The recorded numbers of captured fish (Table 5.6.1) conservatively bias impressions of relative abundance among species in a given area because the original sampling design had a cut-off of 30 individuals per species per river section, and if subsequent fish were captured they were released while we continued to fish 30 of the other species. Post-capture, retained fish were euthanized using an overdose of clove oil (for further details see Warner 2013).

5.2.2 Fish metrics

After extraction, both sagittal otoliths from each fish were cleaned and allowed to dry. A subset of the otoliths were used for microchemistry to determine the mother's life history (anadromous or resident – to address the objective (1a) about differences in offspring based on maternal life history), and another subset of the otoliths were used for aging (see below).

Otolith microchemistry:

[The microchemistry section is not to be examined because it constitutes previously completed work on this project by another student, see Appendix A]

The clean otoliths were attached, sulcus side down, to glass slides using two sided tape and stored in sealed polypropylene containers. The otoliths were randomly arranged on the slides to ensure that analyses were un-biased with respect to the site or date they were collected. A total of 420 individuals were selected for otolith chemistry, which included up to 30 random fish (if captured) from each species, from each river section, from each sampling event (June & September).

Otoliths were analyzed using laser ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS). Concentrations of strontium (Sr) 88 were determined using a Finnigan ELEMENT XR high resolution double focusing magnetic sector inductively coupled plasma mass spectrometer (HR-ICPMS) coupled to a GEOLAS 193 nm excimer laser system. A helium flow rate of 0.9 to 1.0 l/min was used to carry ablated material from the ablation cell to the ICP, with an additional 0.75 l/min argon make up gas added after the ablation cell. Stationary laser spots were used. A 60 μm laser beam was stationed over the otolith to produce depth profiles; from the top surface of the otolith into and through the otolith core. Laser energy was 3 J/cm^2 and the laser repetition rate was 10 Hz. Time resolved intensity data were acquired by peak-jumping in a combination of pulse-counting and analogue modes, depending on signal strength, with one point measured per mass peak. Approximately 30 seconds of gas background data were collected prior to each laser ablation of both standards and unknowns.

To determine the Sr concentration in the otoliths a data acquisition methodology of an analytical sequence of two analyses of the NIST 612 standard and one analysis of MACS1 reference material with analyses of up to 14 unknown otoliths, closing with a repetition of the same standards, was used. The NIST 612 standard was used to correct for instrument drift and changes in daily tuning. The MACS1 reference material has a similar matrix to the otoliths and was treated as an unknown. These data were acquired to allow the monitoring of accuracy and precision of the technique in general. Data were reduced using Memorial University's in-house CONVERT and LAMTRACE spreadsheet

programs, which employ procedures described by (Longerich *et al.*, 1996). For further detail, including testing on fish of known maternal origin see Warner 2013.

Aging:

Due to a decline in accuracy in daily aging of older fish (Campana and Neilson, 1985) we aged available otoliths from the June (post-emergence) but not the September sampling period (end of the growing season). Otoliths were mounted on a glass slide and polished using 30 and 3 μm lapping film. Salmonids deposit daily calcium carbonate rings which can be used to age fish (see Radtke 1989, 1996, and Adams et al. 1992 for methods). Otoliths form checks in the form of a thicker ring, due to stress, lack of food, environmental changes, hatch and emergence from the gravel nest. During times of stress growth slows or stops, which makes two rings appear together and they form the check (Adams *et al.*, 1992). See Chapter 4 for in-depth description of hatch checks (Campana and Neilson, 1985; Campana, 2001). Hatch date can be determined by counting the number of daily rings from the hatch check and then back calculating from capture date. When analyzing age, we used a back-corrected age to the date of first capture in order to compare fish on the same day (June 15th).

Otoliths were photographed under a compound microscope at 100x magnification. To make ring visualizations easier, each photograph was adjusted using Photoshop (cropped, grey-scaled, colour range adjustments). There were originally 214 fish in the June sample, of those some otoliths were lost due to cracking or human error, and some were used in the microchemistry analysis (see above), therefore we daily aged 110 individuals

(Atlantic salmon n=44; brown trout n=32; and brook trout n=34). Each otolith was assigned a blind code and read without knowledge of species or river section. All otoliths were aged by the same reader (HDP). A random subset of 30 otoliths (27.5%) were aged twice to get a precision estimate based on coefficient of variation (CV) which provided an estimate of repeatability. A review found that a CV of less than 7.6% is generally acceptable for aging studies (Campana, 2001). The aging precision estimate for this study was 4.5%. We determined an average daily growth rate (mm/day) for the fish with the following calculation:

$$\text{Growth rate} = \frac{(\text{Fork length} - \text{hatch length})}{\text{Age}}$$

For simplicity brook trout hatch length was estimated to be 18 mm based on hatch size of another population in the region (Penney *et al.*, 2018). Atlantic salmon and brown trout were estimated to be 20 mm based on other work at 4°C (brown trout: Réalis-Doyelle *et al.*, 2016; Atlantic salmon: Peterson *et al.*, 1977). Work on Atlantic salmon otoliths has shown that the relationship between otolith size at hatch and body length at hatch can vary by ~2 mm (Meekan *et al.*, 1998) therefore obtaining precise estimates of fish length based on otolith size is difficult. For a sensitivity analysis, we ran the models on the growth rate with ±2 mm of their assumed hatch size, and found only very minor differences in the p values, and it did not change the conclusions. We acknowledge that there was no inter-individual variation in hatch size considered here, though we know that there is a relationship between egg size and hatch size, and that can vary among individuals (Einum and Fleming, 1999), therefore this is one source of error for our study.

5.2.3 Data analyses and statistics:

Unfortunately, there was not enough intraspecific variation in maternal life history strategies (Table 5.6.1) to address objective 1a (see Results 5.3.1). Therefore, the statistics below only address how extrinsic factors affect hatch time, juvenile body size and growth rate (objective 1b) and how growth rate depends on hatch time (objective 2).

General approach:

All statistics were performed in R version 3.3.3 (R Development Core Team, 2015; using packages car, ggpmisc, lme4, lubridate, and raster), and all graphs were created using ggplot2. For all tests significance was set at $\alpha = 0.05$, and model residuals were examined to ensure that assumptions were not violated. Statistics were performed on model 1 for each dependent variable (DV): size (fork length and dry weight), hatch date (using age as a proxy), and growth rate (average).

Main model:

We used the same model (model 1) for the statistics on each dependent variable for objective 1b.

$$DV \sim Sp + R + Sp \times R + error$$

[model 1]

Where DV is each dependent variable, and species (Sp) and river section (R) and their interaction are the explanatory variables. We conducted an Analysis of Variance (ANOVA,

Type II, in the 'car' package in R v.3.3.3) on fork length and dry weight for June and September separately. Data from June and September were analyzed in separate models because during data exploration when month was included it was very significant ($p<0.00001$) in the 2-way interactions with both species and river section. Therefore, the decision was made to split the data apart to draw conclusions about body size among species and river sections. Only the June fish were aged, therefore we conducted an ANOVA on hatch date (using age at capture as a proxy in the model) and growth rate in post-emergence fish. We completed Tukey tests to compare 1) among species; 2) among river section; and 3) among species across river sections.

To test the hypothesis that late hatchers grow faster than early hatchers to compensate for a shorter growing season (objective 2) we conducted linear regressions for each species (combined river sections) and river section (combined species) to determine if there was a relationship between age (hatch date) and: 1) fork length; and 2) growth rate.

5.3 RESULTS

5.3.1 Residency and anadromy:

We determined through microchemistry analysis that in this watershed almost all the Atlantic salmon (99.97%) and brown trout (98.55%) came from anadromous mothers, and most brook trout (78.89%) came from resident mothers (see Warner 2013 for details). Intrinsic comparisons based on variation in maternal diet within species were therefore not possible.

5.3.2 Size at capture:

For post-emergent fish (June; Table 5.6.1) there were significant body size differences in fork length and dry weight among species and river sections but neither of the interactions were significant (Table 5.6.2). There were minor differences in length and weight (pooling river section) among Atlantic salmon, brown trout, and brook trout (Table 5.6.1), where brown trout were the longest and brook trout were the heaviest. When pooling species, fish tend to get longer and heavier from upstream to midstream to downstream (Table 5.6.1, Figure 5.7.1). The paired Tukey tests showed these results were significant for length (upstream compared to downstream: $p=0.013$) and weight (upstream compared to downstream: $p=0.006$ and upstream compared to midstream $p=0.06$).

For size at the end of growing season (September) there was a significant difference among river sections, but species and the interaction between species and river section were not significant (Table 5.6.2). Atlantic salmon, brown trout, and brook trout were similar in lengths and weights (Table 5.6.1, Figure 5.7.1). When comparing river sections, upstream fish were shorter and lighter than midstream or downstream (Table 5.6.1). The paired Tukey tests showed these results were significant for length (upstream compared to midstream: $p<0.001$, upstream compared to downstream: $p<0.001$) and weight (upstream compared to midstream: $p<0.001$, upstream compared to downstream: $p<0.001$).

5.3.3 Hatch date and growth rate:

Overall, hatch date for post-emergent fish (June) and the interaction between species and river section and the main effects of river section and species were not significant (Table 5.6.2, Figure 5.7.3). A Tukey test showed that upstream fish were

significantly older than downstream fish in age (p=0.002), but neither was significantly different from midstream (see Table 5.6.1 for summary).

For growth rate in post-emergent fish (June) the interaction between species and river section was not significant, but there was a significant difference in growth rate among river sections and species (Table 5.6.2; Figure 5.7.2). When we examined species regardless of river section we found that Atlantic salmon grew significantly slower than brown trout (Tukey p=0.004) or brook trout (Tukey p=0.005). We examined river sections regardless of species and found that downstream fish grew faster than fish from midstream (Tukey p=0.047) and upstream sections (Tukey p=0.0004).

5.3.4 Linear relationships

There was no relationship between body size and age at capture in any of the species when combining river sections or among any of the river sections (Figure 5.7.3), which means that in all cases older fish were not larger. However, we did determine that the relationship between growth rate and hatch date were correlated for each species when combining river sections and among river sections when combining species (Figure 5.7.4). Fish that hatched later grew much faster than those that hatched early, and this relationship was consistent across species and river sections.

5.4 DISCUSSION

Early life history can have lasting impacts on individuals; however, there is a dearth of information available on how it affects species' interactions. For this chapter we compared young of the year brook trout and Atlantic salmon (native species) with brown

trout (invasive but naturalized). Our first objective was to test the hypothesis that the magnitude of species differences in hatch time, growth rate and subsequent late spring body size would be greater than within-species intrinsic differences caused by maternal diet variation from freshwater or marine feeding (intrinsic impacts). Previous work has shown that partial migration often occurs in all three of these species and that both forms occur sympatrically and allopatrically in many populations (Hendry *et al.*, 2004; Dodson *et al.*, 2013). Surprisingly, we found that there was nearly no intra-specific variation in life history strategies in fish from our sample site (Renew's River) because almost all of the brown trout and Atlantic salmon mothers (>97%) were anadromous, and almost all of the brook trout mothers (78.9%) were residents (as shown in Warner, 2013).

Therefore, we could only test for within-species environmental differences from different parts of the watershed (extrinsic impacts). We determined that species and river section affected body size (length and weight) of post-emergence (June) fish by the end of the growing season (September), there was a significant effect of river section on body size but there was no longer a difference among species. In both June and September, fish were biggest (length and weight) in the downstream section and smallest in the upstream section. This means that river section played a large part in affecting the body size of the juvenile fish, in fact there was more of a difference among river sections than among species. The difference in size was not age related, downstream fish were bigger because they grew faster, not because they were older, and this extrinsic force was bigger than intrinsic differences among species. Perhaps this is unsurprising given that salmonids have a high degree of phenotypic plasticity and are strongly influenced by their environment

(Klemetsen, 2013). We suspect that there may be a combination of systematic differences in temperature, quantity and quality of prey, and competition in different sections of the river, which resulted in differences in size. However, we did not install temperature loggers, examine stomach contents, or examine behavioural interactions among fish, which would have allowed us to test for these factors.

Our second objective was to test the prediction that late hatchers would grow faster than early hatchers to compensate for a shorter growing season. We confirmed our prediction for a strong relationship between hatch time and growth rate, in that fish that hatched late grew faster. This pattern held for all species and river sections that we studied; however, we did not have otoliths from all species in all three river sections (no brown trout in the upstream section or brook trout in the downstream section) but it is likely that they would show the same pattern. It is possible that this is evidence of growth compensation in that individuals that are disadvantaged by timing of their parents spawning, or temperature profiles during development may be able to overcome this issue and catch up to individuals in their cohort that hatched at a more optimal time. Age-based growth compensation can be difficult to tease apart from other confounding factors such as growth compensation based on food availability (e.g., Zhu et al. 2003, Carlson, et al. 2004). Therefore, due to the nature of the field data we do not know if this is true growth compensation, or if it can be explained by differences in temperature at important developmental time points, differences in diet, or differences in hatch characteristics such as hatch size. However, this pattern held in Chapter 4 when we controlled for age and abiotic conditions, so we have assumed that the relationship would be the same here. Lastly, we do not know if this pattern

will continue throughout the whole season and what impact it has on final size at the end of the first growing season.

Brown trout have naturalized in Newfoundland, and seem to be coexisting with the native salmonids. In our study we found that there was a difference in hatch phenology between brown trout and the two native salmonid species, and that Atlantic salmon grew the slowest. Observed declines in brook trout and Atlantic salmon populations in Newfoundland may be at least partially a result of brown trout competition in early life and predation by brown trout adults on juveniles (Öhlund *et al.*, 2008; Westley and Fleming, 2011; Warner *et al.*, 2015; DFO, 2016). It is unclear if the populations in Renew's River are stable or if they will continue to decline, and as such we should ensure that they continue to be monitored, especially considering the recent issues with Atlantic salmon declines elsewhere in Newfoundland and Labrador (Veinott *et al.*, 2018). It is clear that extrinsic and intrinsic factors both play a key role in early life history of salmonids particularly through differences in timing, unfortunately we were unable to test for other intrinsic factors such as differences in maternal life history.

We know that early development is a fundamental aspect of life history and has lasting impacts on life time development and future reproductive success (Schlichting and Pigliucci, 1998; Taborsky, 2006). Brown trout, Atlantic salmon, and brook trout all co-occur in some systems, but most studies have focused on interactions between pairs of species. Here we have an important geographical area that we can study multi-species interactions, such as competition for redd sites, food and refugia, and trophic interactions between adults and juveniles. Adding to the knowledge of early growth, development and

competitive ability in these populations, can give insight into future salmonid conservation and management.

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5.6 TABLES

Table 5.6.1. Descriptive statistics for each sampling month for Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) in the three river sections. Included are the percent of young of the year that are from resident (non-anadromous) mothers, mean length, and mean dry weight, and also mean hatch date, age (on June 15th), and growth rate for June samples (N= sample size, %Res= percent of embryos that came from resident mothers, SD= standard deviation). Note: SD is the same for age and hatch date so was only reported for age. Note: NA is not applicable.

Time	Species	Section	N	% Res	Length (mean ±SD)	Dry weight (mean ±SD)	N	Hatch date (mean)	Age (mean±SD)	Growth rate
June	*Salmo salar*	Upstream	30	13.3	31.7±2.4	0.053±0.016	8	Mar 28	78.3±13.7	0.14±0.04
		Midstream	30	0.0	32.7±2.1	0.058±0.017	18	Mar 31	75.7±18.0	0.17±0.04
		Downstream	30	0.0	36.0±3.5	0.086±0.030	18	Mar 30	76.3±12.0	0.20±0.05
		Combined	90	4.44	33.4±3.3	0.066±0.026	44	Mar 30	76.9±15.0	0.16±0.04
	Salmo trutta	Upstream	1	0.0	38.1	0.095	0	NA	NA	NA
		Midstream	30	3.3	33.5±4.0	0.071±0.030	14	Apr 8	67.5±14.4	0.19±0.07
		Downstream	30	0.0	34.0±3.5	0.071±0.025	17	Apr 14	61.8±13.2	0.23±0.05
		Combined	61	1.64	34.8±4.8	0.071±0.027	31	Apr 11	64.4±13.9	0.21±0.06
	Salvelinus fontinalis	Upstream	30	83.3	34.4±4.6	0.070±0.035	19	Mar 25	81.5±15.5	0.20±0.08
		Midstream	30	80.0	34.9±5.2	0.074±0.043	15	Apr 3	72.4±16.7	0.22±0.09
		Downstream	3	100.0	37.8±3.0	0.091±0.027	0	NA	NA	NA
		Combined	63	82.5	33.8±3.7	0.073±0.039	34	Mar 29	77.5±16.4	0.21±0.08

	Species combined	Upstream	61		33.1±3.6	0.062±0.029	37	Mar 30	80.0±14.5	0.17±0.07
		Midstream	90		33.7±4.0	0.068±0.032	47	Apr 3	72.2±16.6	0.19±0.06
		Downstream	63		35.1±3.9	0.079±0.028	25	Apr 9	66.5±14.4	0.22±0.07
Sept	*Salmo salar*	Upstream	30	3.3	44.6±4.4	0.20±0.07				
		Midstream	30	0.0	50.2±7.8	0.30±0.14				
		Downstream	30	0.0	53.3±6.3	0.35±0.13				
		Combined	90	2.2	49.4±7.2	0.28±0.13				
	Salmo trutta	Upstream	17	5.9	46.3±3.8	0.23±0.10				
		Midstream	30	0.0	51.0±6.1	0.27±0.13				
		Downstream	30	0.0	52.8±3.8	0.30±0.07				
		Combined	77	1.3	50.7±5.4	0.26±0.09				
	Salvelinus fontinalis	Upstream	30	66.7	48.7±6.6	0.20±0.05				
		Midstream	12	100.0	51.3±6.7	0.27±0.10				
		Downstream	4	50.0	52.5±4.0	0.30±0.09				
		Combined	46	73.9	49.7±6.5	0.25±0.11				
	Species combined	Upstream	77		46.6±5.5	0.21±0.08				
		Midstream	72		50.7±6.8	0.28±0.12				
		Downstream	64		53.0±5.1	0.32±0.11				

Table 5.6.2. Results of ANOVAs for fork length (FL) and dry weight (DW) for June and September and the ANOVAs for hatch date (HD; using age as a proxy) and growth rate (GR). All tests had the same main effects: river section (RS), and species (SP), and their interaction.

Month	Dependent variable	Main effects	F-value	df	p	
June	FL	SP	6.39	2,205	0.002	**
		RS	8.63	2,205	0.0003	***
		SP*RS	2.01	4,205	0.09	
	DW	SP	3.26	2,204	0.04	*
		RS	7.26	2,204	0.0009	***
		SP*RS	2.34	4,204	0.06	
	HD	SP	3.02	2,102	0.05	
		RS	1.34	2,102	0.27	
		SP*RS	0.86	2,102	0.43	
	GR	SP	7.33	2,102	0.001	*
		RS	3.94	2,102	0.02	*
		SP*RS	0.09	2,102	0.91	
Sept	FL	SP	2.36	2,204	0.10	
		RS	23.88	2,204	<0.00001	***
		SP*RS	0.79	4,204	0.53	
	DW	SP	1.89	2,204	0.15	
		RS	21.19	2,204	<0.00001	***
		SP*RS	0.98	4,204	0.42	

Note: * $p<0.05$ **$p<0.01$ *** $p<0.001$

5.7 FIGURES

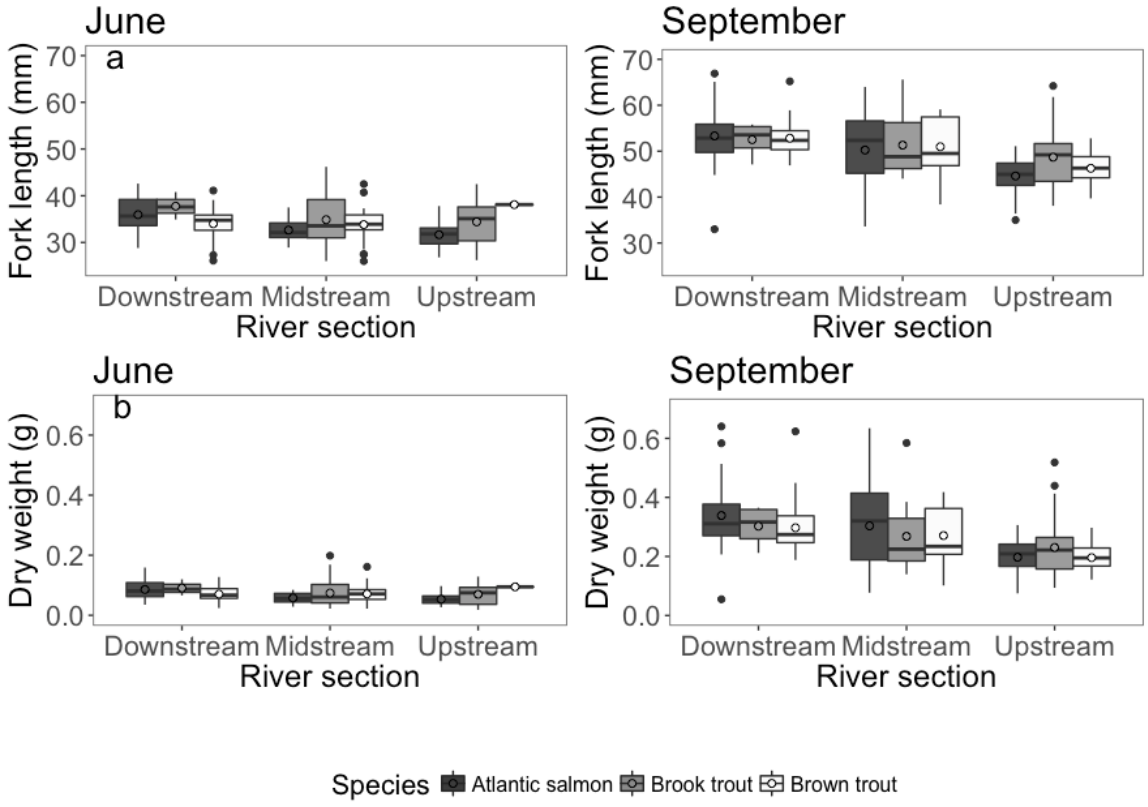

Figure 5.7.1. A) Fork length (mm) and B) dry weight (g) among river sections and species in June and September. The box plot represents the interquartile range (IQR, 25 and 75%), and the horizontal line represents the median value for fork length. Open circle represents the mean. Whiskers represent the next quartile (1.5 x IQR).

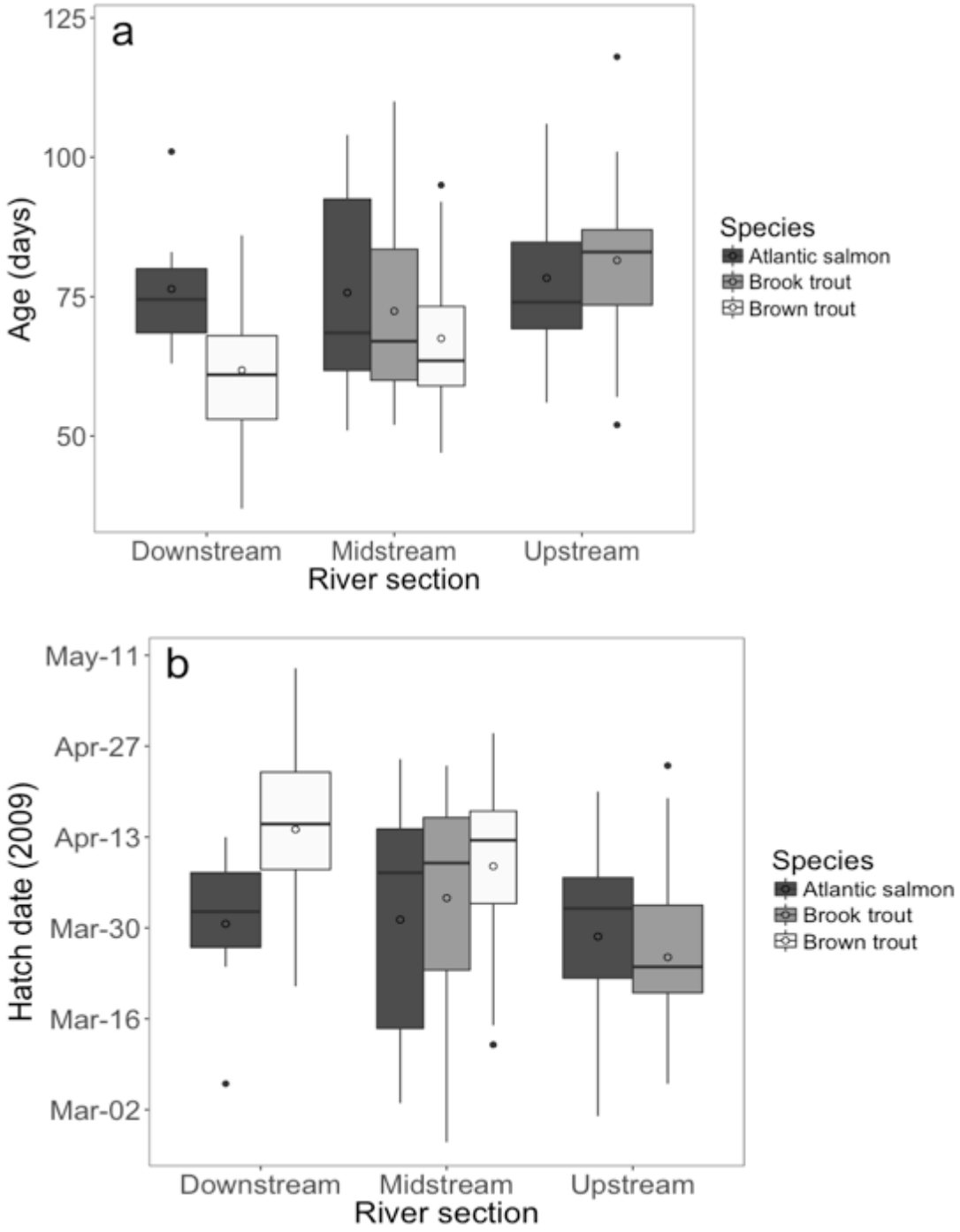

Figure 5.7.2. A) Age (corrected to earliest capture date) and B) hatch date within river sections and among species in June. The box plot represents the interquartile range (IQR, 25 and 75%), and the horizontal line represents the median value for fork length. Open circle represents the mean. Whiskers represent the next quartile (1.5 x IQR).

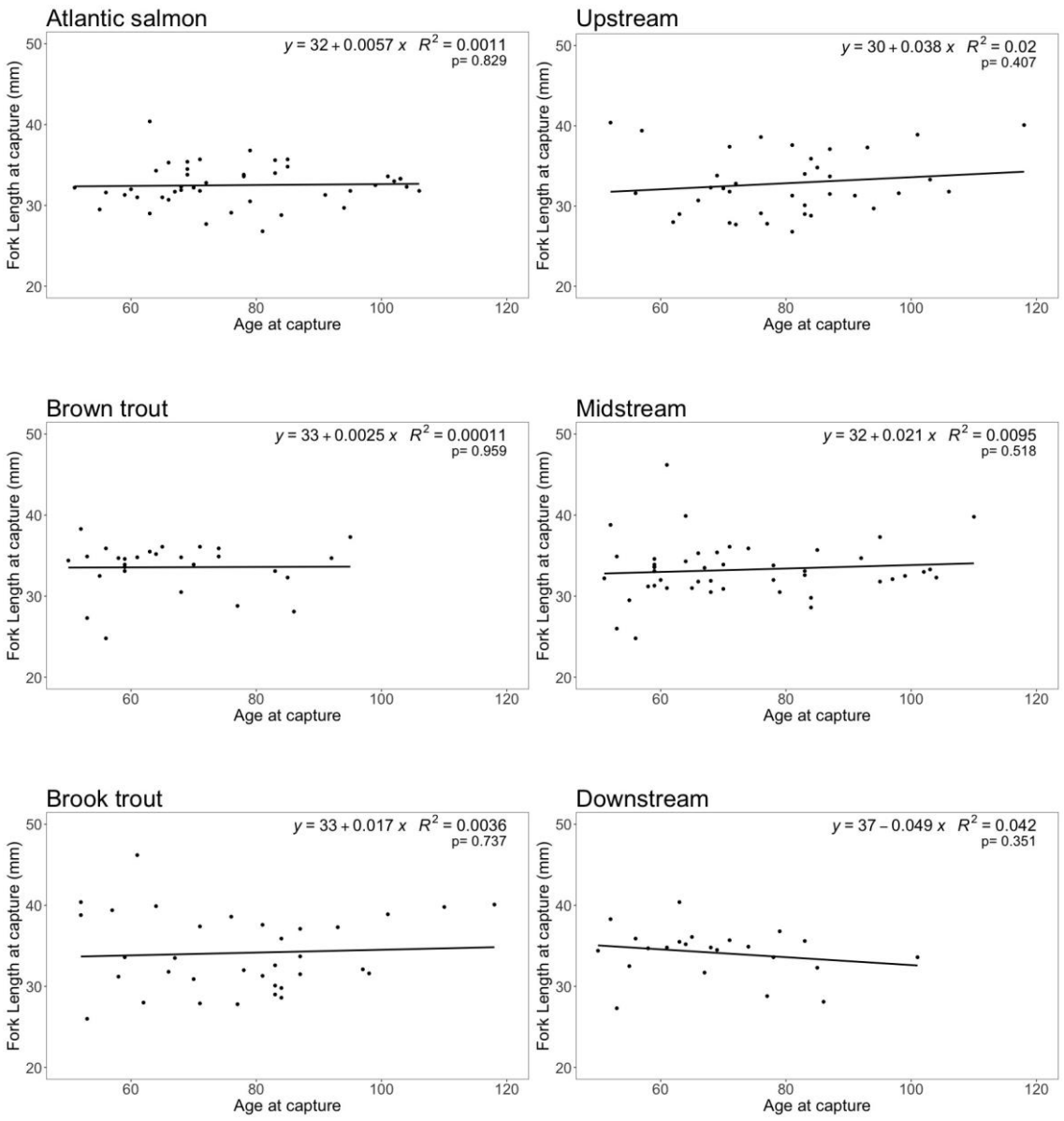

Figure 5.7.3. Age (days) and fork length (mm) at capture for Atlantic salmon, brown trout, and brook trout with combined river sections (left), and (right) upstream, midstream and downstream with combined species.

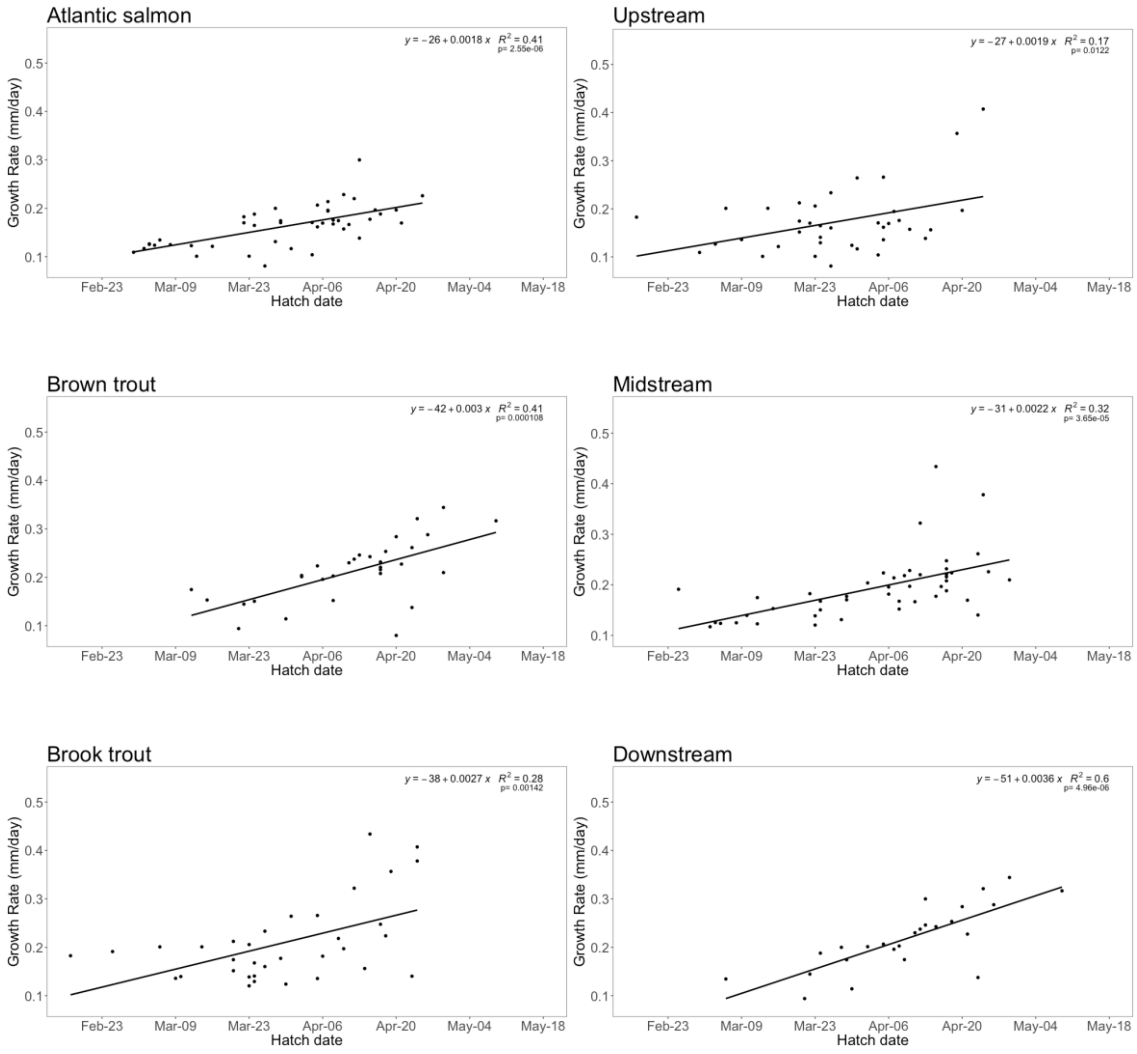

Figure 5.7.4. Age (days) and growth rate (mm/day) for Atlantic salmon, brown trout, and brook trout with combined river sections (left), and (right) upstream, midstream and downstream with combined species.

5.8 Appendix Chapter 5

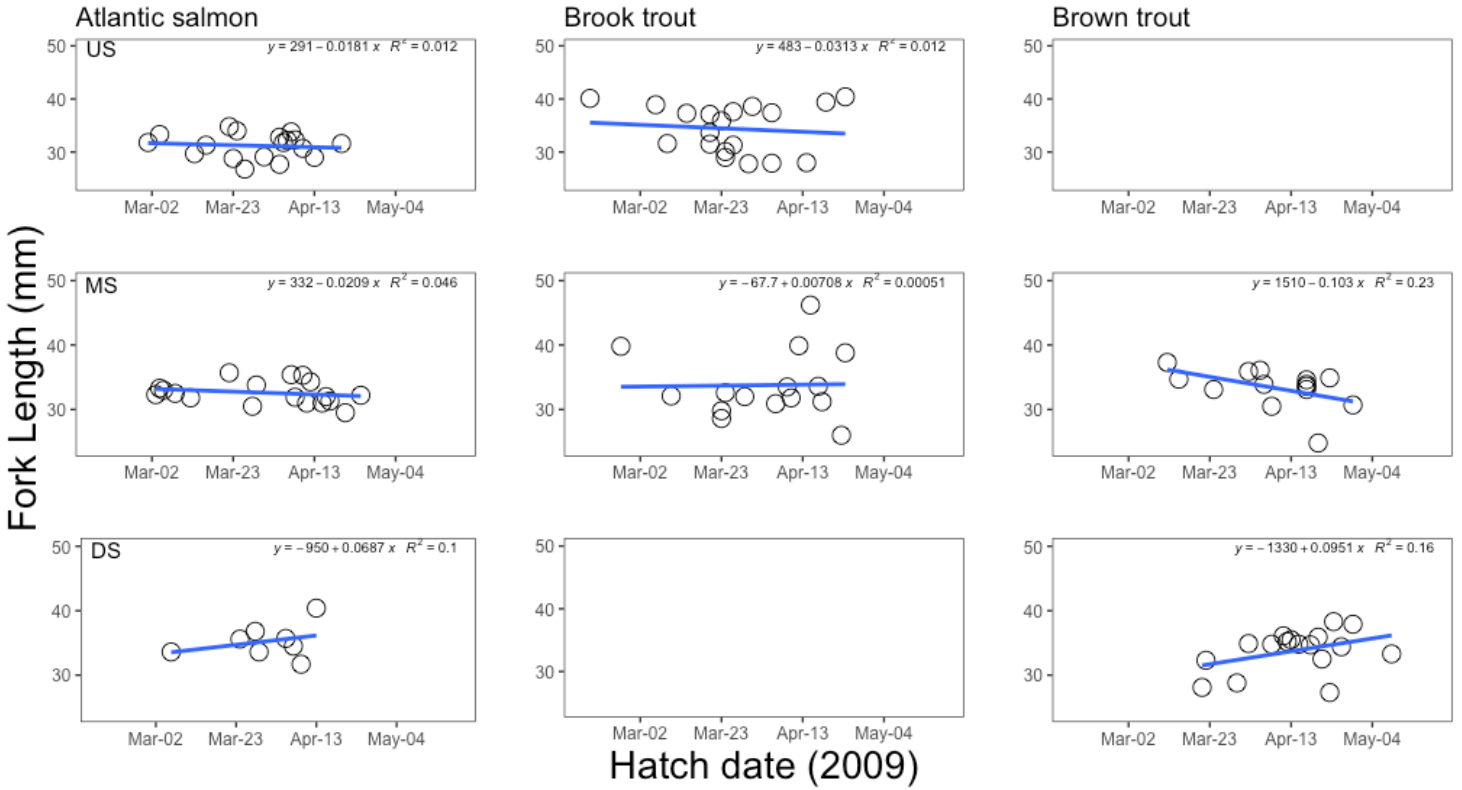

Figure 5.8.A1: Relationships between hatch date and fork length at capture (mm) for Atlantic salmon, brook trout, and brown trout (left to right) for the upstream (US), midstream (MS) and downstream (DS) river sections (top to bottom). Note: there were no brown trout aged from the upstream section and no brook trout aged from the downstream section.

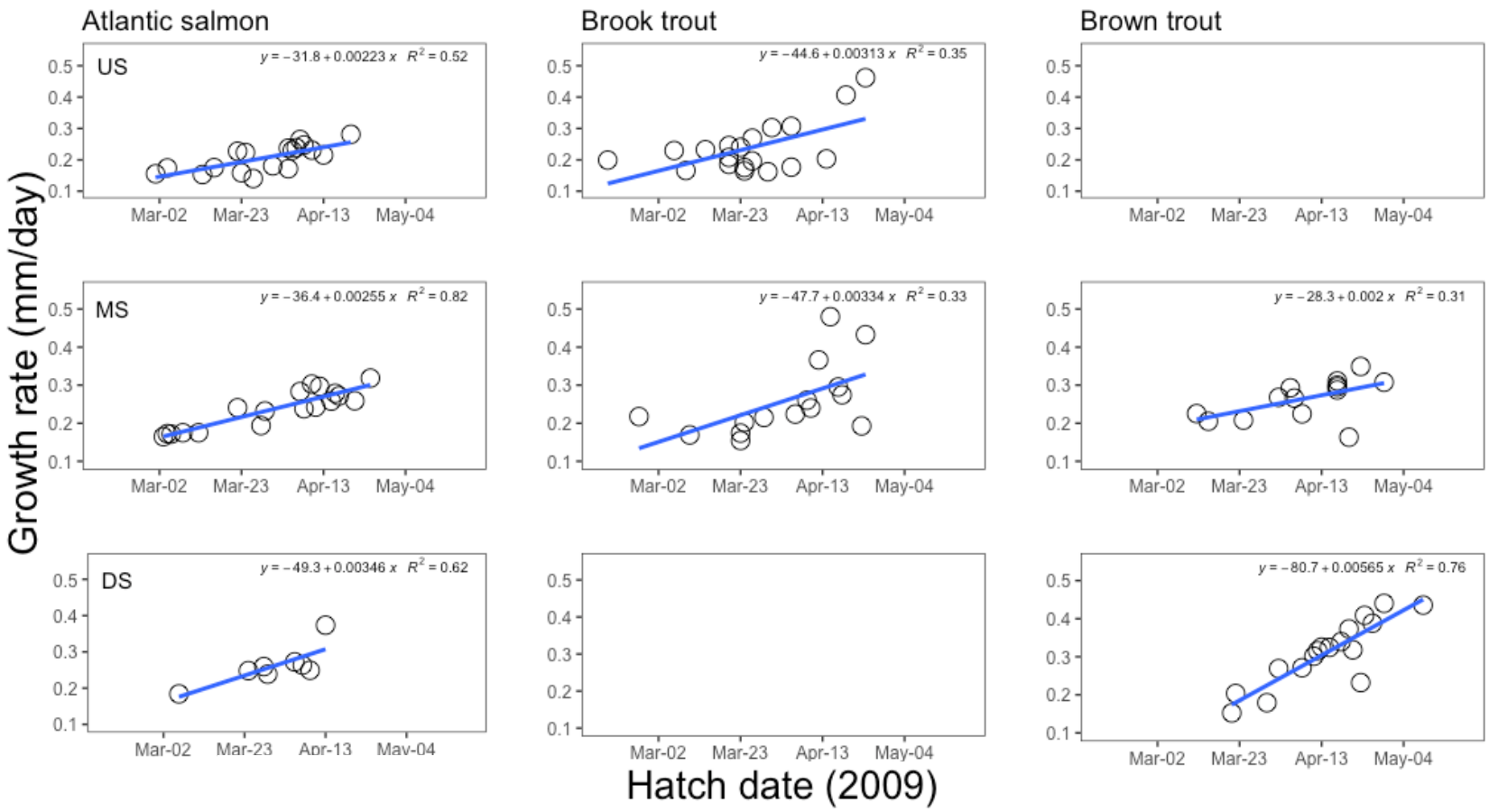

Figure 5.8.A2: Relationships between hatch date and growth rate (mm/day) for Atlantic salmon, brook trout and brown trout (left to right) for the upstream (US), midstream (MS) and downstream (DS) river sections (top to bottom). Note: there were no brown trout aged from the upstream section and no brook trout aged from the downstream section.

CHAPTER 6: DISCUSSION

6.1 RESEARCH QUESTIONS AND RESULT OF THE FOUR DATA CHAPTERS

The research questions for my thesis focused on two adaptations for early life history: phenotypic plasticity and age-based growth compensation.

1. **How do extrinsic (environmental) factors affect early development?**

While it has been long established that environmental impacts affect embryonic development, in reality multiple factors can have additive, compounding, or synergistic impacts on development. In Chapter 2 I examined how two variables (temperature and conductivity) at a range edge of a species' distribution impacted embryonic development, hatch time and body size in the banded killifish. I found that temperature affected hatch success, whereby hatch success was higher and individuals hatched faster at warmer than at cooler temperatures. In this chapter, the conductivity levels were well within the range experienced naturally in the wild, whereas temperature had a much larger range that encompassed very stressful conditions. Therefore most of the variation in early developmental traits was explained by temperature. When a factor approaches range limits it most likely will overshadow the effects of other variables because the organism is dealing with the metabolic stress of a sub-optimal factor.

In Chapter 3, I showed that hatch synchrony was affected by an interaction between temperature variability and acidity. Hatch time is an important early life history characteristic that is often under strong selective pressure for organisms to reproduce within an optimal timing window such as: synching spawning to lunar cycles to increase fertilization success (Babcock et al. 1986); timing of spawning or breeding to coincide with

ideal conditions for offspring's hatch or birth (Morgan and Christy, 1994; Dickerson *et al.*, 2005). The optimal timing window can differ depending on species, population, and spatiotemporal factors and is much easier to predict in homeotherms (i.e., gestation period doesn't vary much). Organisms rely on cues to signal that an optimal timing window is beginning, which works in conjunction with constraining factors such as developmental age which dictates whether the individual can reproduce, hatch, or emerge at a particular time (McNamara *et al.*, 2011). Missing an optimal time window can decrease fitness because: adults may miss out on optimal mating opportunities (e.g., Dickerson et al. 2005), fertilization does not take place (Babcock *et al.*, 1986), and/or offspring are disadvantaged by poor timing (Morin *et al.*, 1990; Christy, 2003; Varpe *et al.*, 2007).

Chapter 3 showed that multiple factors affected hatch timing, but Chapter 2 showed that it can depend on the nuances of the factors that are chosen. In Chapter 3 I saw a difference in hatch synchrony among treatment combinations however, the levels of the treatments were not particularly stressful, as evidenced by the lack of size differences among treatments. However, in Chapter 2 the importance of temperature far outweighed the conductivity effects because two of the temperatures were well below the optimal thermal range for reproduction. This shows that there are subtleties that should be considered when designing experiments to examine environmental effects.

2. **How do intrinsic (maternal effects) and extrinsic factors (environmental conditions) interact during development?**

Chapter 3 also examined the relative contributions of maternal and environmental impacts on embryonic development (hatch time and body size), and how maternal effects

influenced the degree of phenotypic plasticity. Overall maternal factors were more important for early life history characteristics and the degree of phenotypic plasticity that the embryos expressed. Larger eggs, and thus higher maternal investment, produced longer hatchlings, and offspring with greater plasticity. A large body size has been shown to be advantageous (Pepin, 1991; Pess *et al.*, 2011). This novel finding could show that if embryos from larger eggs are more plastic, they may have potential for greater range of expression which may provide advantages to fit their environment.

It is unsurprising that maternal impacts were influential during early life history. However, here we were able to show a relationship between maternal effects and the degree of plasticity. It is unclear if the differences in plasticity in hatch size will also impact juvenile growth rates, and if this can be affected by growth compensation, as shown in Chapters 4 and 5. We do know that predicting the integrated phenotype of salmonids is difficult due to the high degree of plasticity (individual phenotypic flexibility) and local adaptation (population's specialization for a particular environment).

3. **Are delays in hatch phenology enough to induce growth compensation in late hatching individuals?**

Both chapters 4 and 5 examined the relationship between growth rate and hatch timing. We tested this question with two species in Labrador (Arctic charr, and brook trout; Chapter 4) and three species in Newfoundland (Atlantic salmon, brown trout, and brook trout; Chapter 5). We analyzed both chapters in similar ways and found support for the hypothesis that later hatchers grow faster than early hatchers in 4 species from 6 different locations. This compensatory effect may be an adaptation for a shorter growing season. In

Chapter 4 and 5 there was no relationship between age and fork length, which means that older fish were not necessarily bigger. This result suggested that the growth rates were different between early hatchlings and late hatchlings. In Chapter 4, I tracked daily growth rate, so I was able to compare different time periods to look at 1) time since hatch (first 3 weeks of life) where the individuals would be experiencing different abiotic conditions; 2) abiotic conditions (same 3 week period in June) but fish were different ages; and 3) for the whole life. Chapter 5 shows the same pattern; however, I examined life time growth rate rather than growth each day.

I found that there is a relationship between hatch time and growth rate that cannot be explained by changes in environmental conditions or age alone which may point to a within-population adaptation to a short growing season. Among-population differences in growth rates often exist due to latitudinal and altitudinal gradients in temperature and photoperiod, with individuals in more extreme regions experiencing shorter growing seasons (Campos *et al.*, 2009; Sinnatamby *et al.*, 2014). Populations often adapt to such conditions in a pattern deemed counter-gradient variation in growth rates. Populations experiencing shorter growing seasons evolve greater capacity for growth, which can mitigate some negative environmental effects on size (Arendt and Wilson, 1999; Purchase and Brown, 2000). The possible link between counter-gradient variation in growth rate and growth compensation is an area to be explored but was outside the scope of this thesis.

6.2 CONTRIBUTIONS TO THE FIELD

In this thesis I have presented phenotypic plasticity and growth compensation separately; however, they are not mutually exclusive. Salmonids are incredibly plastic, and

this impacts many aspects of their early life history including hatch timing, hatch size and growth rate. Chapter 3 showed how maternal impacts can overshadow environmental impacts when the environment is not stressful. Chapters 4 and 5 showed that there is a relationship between hatch time and growth rate. It would be interesting to see if the pattern of growth compensation we saw in the wild populations could be replicated under laboratory conditions to begin the process of understanding how hatch and emergence timing impact growth rate in a more controlled setting. From a big picture perspective, I am left with several questions including: Will these patterns of growth and plasticity hold in other populations? Would the importance of maternal impacts change with different levels of environmental stressors? Salmonids are a very well-studied group of fishes, and it would be very interesting to start trying to answer some of these questions with other fishes, for example can we find evidence of age-based growth compensation in other taxa?

The study species that I used in my thesis included several salmonids and the banded killifish. Many populations of salmonids are struggling and have shown declining numbers (Veinott *et al.*, 2018), and the banded killifish populations in Newfoundland are considered 'of concern' (COSEWIC, 2014). Understanding early life history can be key to predicting future trends; however, in practise it becomes quite difficult to tease apart all the contributing factors. Salmonids are socioeconomically and ecologically important and a fundamental understanding of their evolution and ecology is key to learning how to properly manage and conserve populations. However, what I have learned through the process of my PhD is that predicting specifics of early life history is incredibly challenging because there are a myriad of factors that are of importance.

6.3 ENVIRONMENTAL CHANGE AND THE FUTURE

Understanding how the natural world functions is an important aspect of science, particularly with the great changes occurring in the Anthropocene. We are seeing an unprecedented number of complex issues including pollution, micro-plastics, over-harvesting, and habitat degradation. However, perhaps one of the main driving factors of change in the foreseeable future is climate change. The global impacts of climate change are predicted to be extensive and far reaching. Global air and water temperature will increase, oceans will acidify, there will be changes in ocean salinity, sea ice and glaciers will decline or disappear, sea levels will rise, changes in precipitation will occur, and there will be an increase in the frequency and intensity of severe weather events such as droughts and floods (I.P.C.C., 2018).

While impacts of climate change will occur in both marine and terrestrial systems, freshwater ecosystems are likely to be particularly affected. In freshwater systems it is predicted that climate change will impact various abiotic factors, through increases in: temperature (mean and variability; Jeppesen *et al.*, 2012), the frequency and duration of extreme weather events (Reist *et al.*, 2006), and increased run off due to increased precipitation (Wenger *et al.*, 2011). Climate change may lead to lower light intensity (due to particulates in the water and changes in ice cover), and lower ice thickness and cover (Jonsson and Jonsson, 2009). Additionally, there will be changes in water level (Jeppesen *et al.*, 2012), water flow regime (Wenger *et al.*, 2011), and possible acidification (Collier *et al.*, 1990), increases in hypoxia (Jenny *et al.*, 2016), eutrophication, stratification and/or salinization of rivers, lakes and streams (Jeppesen *et al.*, 2012). These abiotic changes are simply examples of predicted impacts of global climate change. Changes in abiotic factors

due to climate change will have impacts on biological interactions such as intra- and inter-specific competition (Hein *et al.*, 2012), disease and parasites (Jonsson and Jonsson 2009), as well as changes in metabolism, growth, development (Linton *et al.*, 1998), reproduction (Lahnsteiner *et al.*, 2012) and can affect predator-prey interactions in freshwater systems (Reist *et al.*, 2006).

Salmonids in particular are likely to be strongly impacted by changes in climate. Because salmonids have a relatively low upper thermal maxima, particularly for early life history stages, increasing water temperatures in freshwater may cause populations and species of salmonids to move further north, into deep lakes, or to higher altitudes at a greater rate than other more heat-tolerant fishes. The total effects of these changes are complicated, and the cascading impacts on populations through changes in trophic dynamics are difficult to predict. However, salmonids are also incredibly versatile and variable so it is possible they will respond in ways we have not considered. Even small changes that lead to sub-optimal conditions can have large, far-reaching impacts. For example, a review found that a 0.15 to 0.3°C annual increase was enough to affect species assemblage composition, and change body size and age structure (Jeppesen *et al.*, 2012). In the northern hemisphere's marine systems species distributions tend to move further north with warming temperatures (I.P.C.C., 2018); however, freshwater systems are generally smaller and bound by land or sea, leaving less available habitat for displaced species to redistribute to.

6.4 CONCLUSION

The results of this thesis have supported the previous work that has shown that abiotic factors are particularly important during early development. I found that both environmental and maternal factors can impact hatch success, and hatch size, and that

phenology of hatching can impact subsequent growth rates in the first season. This is important because, small changes in growth and survival resulting from environmental changes in early life history can have far reaching implications for populations. For example, if early conditions are warmer than usual it may result in faster growth rate, which in turn may lead to earlier age at sexual maturity. Early maturity and smaller size at maturity affects the number and size of eggs that a mother can produce. These cascading effects are difficult to study, and when coupled with plasticity and local adaptations, are incredibly difficult to predict.

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Appendix A: correspondence regarding coauthorship

Chapter 2 was analyzed and written by Heather Penney; however, the fish were collected and measured as part of Lucas Warner's (2013) Master's thesis. As such, we have gone through the appropriate channels to ensure proper copyright with the Associate Dean of Graduate studies and Patrick Gamsby from the QEII Library (see below).

MEMORIAL UNIVERSITY

Penney, Heather Dawn <p52hdp@mun.ca>

follow-up from our meeting

Danine Farquharson <daninef@mun.ca> Tue, Aug 1, 2017 at 3:52 PM
To: Heather Penney <p52hdp@mun.ca>, Craig Purchase <cfpurchase@mun.ca>

Hello Heather and Craig,

It was a pleasure to meet you today and have such an enlightened and considerate discussion about copyright, co-authorship, and academic integrity. As I mentioned, because both of you are asking all the right questions about these issues, it is a sure sign that integrity is being upheld. I appreciate that if you were submitting this work to a peer-review journal for possible publication, the process might just be more clear than a dissertation for examination.

To summarize my recommendations: please do as you planned and include a co-authorship statement for the chapter of your dissertation (manuscript format) that draws on or uses information from the previously completed Master's thesis. Be as specific as possible in that statement about what information from the Master's thesis you are working with AND what is your contribution (and if you can quantify it, all the better). Additionally, make a reference to any/all co-authorship statements in the Acknowledgments section of the dissertation to ensure that your examiners' are made aware of the situation in more than one place.

Finally, please contact Patrick Gamsby in the QEII Library with your questions about the Copyright form; he is the appropriate person to advise you on whether that is necessary and how to proceed.
Feel free to forward/copy/include this email in any submissions as it makes clear that you've consulted with SGS and that these concerns have been foremost in your minds.

Best wishes,
Danine Farquharson
****
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Gamsby,Patrick <pgamsby@mun.ca> 8/2/17
to me, Craig

Hi, Heather,

Based on your description, I don't see a copyright issue here. As long as you cite Lucas' thesis and/or data, you should be fine.

Best,
Patrick