Impacts of embryo incubation temperature on ontogenic thermal tolerance of brook

trout (*Salvelinus fontinalis*)

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ABSTRACT

Impacts of embryo incubation temperature on ontogenic thermal tolerance of brook trout (*Salvelinus fontinalis*)

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I investigated the climate vulnerability of a cold-water salmonid by observing the upper thermal tolerance of brook trout (*Salvelinus fontinalis*) across multiple life stages. Using hatchery- and wild-ancestry fish, I assessed variation in thermal tolerances and carryover effects within and among brook trout life stages and populations that were reared under varying winter thermal regimes as embryos. Embryonic, fry, and gravid adult upper thermal tolerance were tested via CT_{max}. Warm acclimation was associated with an increase in embryonic upper thermal tolerance. CT_{max} variability was markedly higher in embryos than juvenile or adult salmonids. Effects of thermal incubation treatments varied by life stage, with incubation temperature and life stage both influencing body size and thermal tolerance. Collectively, these results suggest that brook trout only exhibit short-term carryover effects from thermal stressors during embryo incubation, with no lasting effects on phenotype beyond the first few months (10 weeks) after hatch.

Keywords: Salmonid, ontogeny, climate change, stress, acclimation response, thermal plasticity, carryover effects

Acknowledgments

PREFACE

All research presented in this M.Sc. thesis was conducted in accordance with animal care protocols approved by the animal care committees of Trent University in accordance with guidelines set by the Canadian Council on Animal Care.

Thesis Format

This M.Sc. thesis is written in a manuscript-based format. There is modification throughout the titles and content of the manuscripts that I use as thesis chapters. Redundancy does occur, although an effort was made to reduce repetition within the introduction and methods in each chapter. Additionally, acknowledgements from each manuscript are compiled at the start of the thesis, and references are combined at the end of the thesis. The two data chapters (manuscripts) are ordered in logical progression.

Co-Authorship

This thesis contains results of my own empirical research. However, I conducted this research with the support and guidance of several co-authors. The thesis (manuscript) chapters are either published or under journal peer-review and were co-authored. As such, chapters frequently contain "we" and "our" and I have left it in that format throughout. See below for a list of manuscripts used as thesis chapters and the contribution of authors on each paper.

Chapter 2: Lechner, E. R., Stewart, E. M. C., Wilson, C. C., & Raby, G. D. (2023). CT_{max} in brook trout (*Salvelinus fontinalis*) embryos shows an acclimation response to developmental temperatures but is more variable than in later life stages. *Journal of Fish Biology*, *104,* 901-905.

The study was designed by Lechner, Raby, and Wilson, using equipment and facilities provided by Raby and Wilson. Lechner analyzed the data and wrote the paper with support from Stewart, Raby, and Wilson. Experimental set-up and animal husbandry was supported by Stewart.

Chapter 3: Lechner, E. R., Stewart E.M.C., Frasca, V. R., Jeffries, K.M., Wilson, C.C., & Raby, G.D. Thermal stressors during embryo incubation have limited ontogenic carryover effects in brook trout. *Journal of Thermal Biology*, 103880.

This study was designed by Lechner, Raby, Jeffries, and Wilson, using equipment and facilities provided by Wilson, and Raby. Experimental set-up and animal husbandry was supported by Stewart and Frasca. Lechner analyzed the data and wrote the paper with support from Stewart, Raby, Jeffries, and Wilson.

List of Abbreviations

CT$_{max}$ Critical Thermal Maximum

LT$_{50}$ Median Lethal Temperature

s.d. Standard Deviation

DTU Daily Thermal Units

GLM General Linear Models (Analysis)

AIC Akaike's Information Criterion

K Fulton's Condition Factor

List of Definitions

Acclimation: The change in phenotype of an organism that occurs in response to a change in the environment (modified from Borowitzka, 2018).

Adaptation: The process by which organisms evolve to increase fitness following natural selection (Miller and Stillman, 2012).

Adaptive Phenotypic Plasticity: The evolution of plasticity in response to environmental variability or unpredictability that in turn confers a fitness advantage (via natural selection) under variable or unpredictable environmental conditions.

Carryover Effects: Events or conditions in an animal's life that influence their phenotype in subsequent life stages or situations.

Critical Thermal Maximum: The upper temperature at which locomotor activity becomes disorganized, causing organisms to lose their ability to escape from conditions that will promptly lead to their death (modified from Hutchison, 1961).

Fitness (a) Ecological fitness: Effectiveness of an organism in interacting with its environment, encompassing not just its ability to survive and reproduce, but also its role and performance within the ecosystem. This includes how well an organism exploits available resources, competes with other organisms, and adapts to biotic and abiotic factors in its habitat. Ecological fitness considers the broader context of an organism's interactions and its contribution to the stability and functioning of its ecosystem.

(b) Evolutionary fitness: The ability of an organism to survive and reproduce in its environment. It is measured by the number of offspring an individual contributes to the next generation, relative to other individuals in the population. Higher evolutionary fitness means an organism has traits that are better suited to ensuring its survival and reproductive success.

Resilience: The ability of an organism or population to sustain under differing environmental conditions

Selection: The process in which certain environmental conditions influence the rates of survival and reproduction among individuals within a population. These pressures create differential survival advantages or disadvantages, leading to changes in the frequency of genotypes in the population over generations. This process drives the evolution.

Thermal History: Environmental temperatures an organism was previously exposed to in a given timespan.

Pejus Temperature: The upper or lower thermal limit of optimal physiological performance; the temperature at which organisms begin to experience reduced physiological performance.

List of Figures

List of Tables

1.1 General Introduction

Temperature has a dominant role in shaping metabolism, lifespan, and population dynamics for ectotherms (Burraco et al., 2020). An individual's thermal niche is characterized by a thermal performance curve, which illustrates the relationship between fitness and temperature. The extremes on the curve include the critical thermal maximum (CT_{max}) and minimum (CT_{min}) which represent temperatures that an individual cannot withstand for a long period of time, while the peak represents peak performance and thermal optimum (Payne et al., 2016; Figure 1 modified from Krenek et al., 2011). In some organisms, this thermal optimum can be wide (eurythermal, a thermal generalist) or narrow (stenothermal, a thermal specialist) depending on their biological and physiological constraints (Logan and Buckley, 2015). We can measure upper thermal tolerance by using the CT_{max} which aids in the identification of thermal niche, behaviour, and fitness under varying climates.
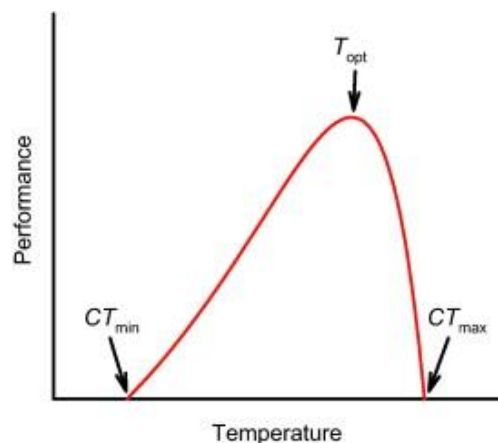
Figure 2. General shape of a thermal performance curve describing the relationship between temperature and ectotherm performance (illustration modified from Krenek et al., 2011). Peak performance is defined by the thermal optimum (T_{opt}), while

1

the extremes on either side of the curve represent where performance reaches zero (CT_{max} = critical thermal maximum and CT_{min} = critical thermal minimum).

Many ectotherms exhibit thermal plasticity (reversible, developmental, or transgenerational; McKenzie et al., 2021) to cope with sub- or supra-optimal temperatures (Cook et al., 2018). Thermal and other environmental conditions can influence future traits (and subsequently fitness) in new or similar environments, a phenomenon known as "carryover effects" (Moore and Martin, 2019). Carryover effects differ from transgenerational effects (parental effects) due to their occurrence within an animal's lifespan; they can be thought of as "downstream consequences" that "carry over" from one season to the next (Harrison et al., 2011), a form of adaptive phenotypic plasticity. Carryover effects can be evident between life-history stages, physiological states, or social situations and include any non-lethal change occurring from a broad range of events or experimental settings (O'Connor et al., 2014). The term 'carryover effect' originates from repeated measures within clinical experiments and has since then been applied to ecological and evolutionary studies with a focus on migratory systems, such as avian ecology (O'Connor et al., 2014). These carryover effects are plastic responses that can occur by faster, reversible, and more flexible mechanisms that help species cope with climate change when compared with adaptation, which is a long-term and heritable method (Waite and Sorte, 2022).

Many species, including ectotherms, can exhibit phenotypic plasticity (a short-term buffering) which can provide thermal resilience (an increased ability to withstand

warmer temperatures) and can strengthen the potential for thermal adaptation in future

generations from direct offspring (Morgan et al., 2022). However, the mechanisms of

phenotypic plasticity may be energetically costly if prolonged throughout the course of an

individual's lifetime (Morgan et al., 2022). There may be individual (intraspecific

variation), population, and species-specific (interspecific variation) thermal tolerance that

can cause differing in acclimation or adaptation across individuals and populations

(Cooke et al., 2022; McDermid et al., 2012). Additionally, thermal conditions during

early rearing stages (such as embryos and fry) have the potential to influence thermal

physiology in later life stages (Jonsson and Jonsson, 2014), which is currently an area of

focus in thermal biology.

1.2 Critical Thermal Maximum (CT_{max})

Critical thermal maximum (CT_{max}) is a plastic trait subject to acclimation and is

used as a standardized metric of upper thermal tolerance in ectotherms. This test has been

used across many taxa (shrimp – Vinagre et al., 2013; crabs – Hopkin et al., 2006;

amphibians – Duarte et al., 2012; molluscs – McMahon, 1990; and insects – Terblanche

et al., 2005) and has been used for decades on fish species (O'Donnell et al., 2020). The

framework was originally tested on trout by Brett (1941) and Fry et al. (1946). The CT_{max}

test typically involves assessing the temperature at which fish lose equilibrium (righting

response), defined as a loss of equilibrium (LOE) (Lutterschmidt and Hutchison, 1997).

The tests are typically done on individuals only once to avoid variation over time

(O'Donnell et al., 2020), heat hardening (Bilyk et al., 2012), and learned behaviour. The

temperatures reached during the tests are usually warmer than what can be tolerated long-

term for wild populations (Morrison et al., 2020), however, it is intended to be a rapid measurement of a fish's ability to tolerate temperature change (O'Donnell et al., 2020). Values acquired from CT_{max} tests are usually higher than chronic survival temperatures (upper incipient lethal level) which is defined as the temperature beyond which 50% of the population can no longer survive on a long-term timeframe (Fry et al., 1942). Regardless, studying upper thermal tolerance via CT_{max} (acute temperature warming) is critical in defining the fundamental niche, thermal limits, predictors for biogeographical distribution, and species responses under climate change (Ørsted et al., 2022).

CT_{max} measurements are predominately used on adult and juvenile fish that actively swim as the endpoints [loss of equilibrium (LOE, i.e., belly up and lack of righting response)] can be properly observed (Recsetar et al., 2012; Smith and Ridgway, 2019). Median lethal temperature (LT_{50}) experimental protocols have been favoured for embryonic thermal studies (Ineno et al., 2005; Recsetar and Bonar, 2013) and are similar to a median lethal time protocol, but instead of time we look at temperature. LT_{50} refers to the temperature at which 50% of a test population dies and is not ideal for determining upper thermal tolerance as the lethality makes it difficult to compare against other life stages where CT_{max} is more commonly used (i.e., post-hatch stages), and is traditionally non-lethal.

1.3 Brook Trout (*Salvelinus fontinalis*)

Brook trout are a cold-water freshwater fish native to eastern North America that prefer well-oxygenated, clear flowing streams or stratified freshwater lakes. They occupy

thermal habitats between 10–19°C in summer (Smith and Ridgway, 2019; Morrison et al., 2020), and their embryos incubate in temperatures of 3-8°C from fall to spring (Noakes and Curry, 1995; Curry et al., 1997). Brook trout are sensitive to increases in water temperature (Cook et al., 2018) and climate change is already impacting their population sizes and distribution by reducing preferred thermal and abiotic habitats (Stanfield et al., 2006). Brook trout are both an economically and culturally important freshwater fish as they support recreational fishing and indigenous harvest. As a result of their importance for recreational fisheries, fishery management agencies invest into brook trout culture for stocking lakes and streams. For example, brook trout make up approximately 70% of fish production intended for stocking in Quebec (Banousse et al., 2024). As a result of their cultural and economic value, brook trout have long been the subject of research, including research on their thermal biology (review in Smith and Ridgway, 2019). As a result, there is a robust base of knowledge available in thermal biology for both wild and hatchery populations, including on Ontario brook trout (review in Smith and Ridgway, 2019).

1.4 Objectives

The goal of my thesis was to generate useful new data on the climate vulnerability of brook trout by quantifying the thermal tolerance and thermal plasticity of brook trout across multiple life stages (embryo, fry, and gravid adults). The upper thermal tolerance and acclimation responses of early life stages and reproductively mature adults are underappreciated in thermal biology, with minimal studies looking at the life stages we have chosen to study, specifically for brook trout (Dahlke et al., 2020). Additionally,

embryos and reproductive adults are considered the most thermally vulnerable life stages across both freshwater and marine fish species (Dahlke et al., 2020). Embryonic, fry, and gravid adult upper thermal tolerance will be tested via CT_{max}.

The objective of my thesis is to produce novel, critical information for studying the variation of thermal tolerance among brook trout that were reared as embryos under several winter thermal regimes by examining acclimation responses and thermal plasticity across brook trout life stages. My two chapter-specific objectives that my research will address are to investigate the acclimation response and heat tolerance in embryonic brook trout, and to look at the effects of embryonic rearing temperatures and how they impact the upper thermal tolerances across subsequent life stages. This knowledge will be applicable to fisheries science, conservation policies, and to improving our understanding of climate change resiliency among salmonids and other freshwater fishes.

Chapter 2: Acclimation response and heat tolerance in embryonic brook trout (*Salvelinus fontinalis*)

2.1 Abstract

Critical thermal maximum (CT_{max}) is widely used to measure upper thermal tolerance in fish but is rarely examined in embryos. Upper thermal limits generally depend on an individual's thermal history, which molds plasticity. We examined how thermal acclimation affects thermal tolerance of brook trout (*Salvelinus fontinalis*) embryos using a novel method to assess CT_{max} in embryos incubated under four thermal regimes. Warm acclimation was associated with an increase in embryonic upper thermal tolerance. However, CT_{max} variability was markedly higher than is typical for juvenile or adult salmonids.

2.2 Introduction

Thermal tolerance, acclimation capacity, and adaptation jointly determine whether species and populations can cope with a warming and more variable climate (Buckley et al., 2013; Morgan et al., 2018). An individual's response to environmental changes is shaped by the interaction between acclimation capacity and developmental plasticity (Beaman et al., 2016), as fish thermal niches are influenced by environmental stressors and oxygen limitation (Ellis et al., 2013; Ern et al., 2023). Thermal acclimation can increase upper thermal thresholds in ectotherms, a form of thermal plasticity (Seebacher et al., 2014), but early developmental stages such as embryos and larvae, where there is a critical window of sensitivity to warming (Angilletta, 2009), may have less acclimation capacity.

In fishes, it has recently been proposed that embryos have narrower thermal limits than do juveniles and adults, creating a 'thermal bottleneck' in the life cycle (Dahlke et al., 2020), at a life stage where natural mortality in most fish species is greatest (Bogstad et al., 2016). If true, the thermal bottleneck hypothesis suggests we should refocus our resources to study the more vulnerable life stages, such as embryos and gravid adults (Dahlke et al., 2020). If we are to compare the upper thermal limits of different life stages, it would be best to do so using comparable endpoints (Pottier et al., 2022). Dahlke et al. (2020) used long-term rates of survival for embryos to determine upper thermal limits but used CT_{max} for juvenile and adult fish (Pottier et al., 2022).

In this study, we assessed the CT_{max} response in brook trout (*Salvelinus fontinalis*) embryos acclimated to different thermal regimes, with a methodology adapted from Cowan et al. (2023). Brook trout are also a well-studied species in thermal biology, with ample published literature on adult and subadult CT_{max} (Carline and Machung, 2001; Stitt et al., 2014; Wells et al., 2016; O'Donnell et al., 2020), but no recent thermal tolerance studies have used fish younger than sub-adults, with only one project from 1972 looking at newly hatched and swim-up alevin (McCormick et al., 1972). We predicted that embryos acclimated to (incubated in) higher temperatures would reach a higher CT_{max}. In juvenile and adult fish, CT_{max} responds reliably to acclimation temperature (Chatterjee et al., 2004), but embryos are thought to be less physiologically flexible than later life stages (McCormick et al., 1972), so a reasonable alternative prediction might have been that their CT_{max} would not significantly change with acclimation. To our knowledge, this

study is the first of its kind to examine the effects of acclimation on upper thermal tolerance of the embryo life stage, though others have quantified CT_{max} of embryos (acclimation effects in Del Rio et al., 2019; CT_{max} in Cowan et al., 2023). To do so, we acclimated brook trout embryos to four controlled thermal regimes: a) ambient, b) mid-winter cold shock brought on by a controlled snowmelt, c) short-term warming during the final ~5-20 days of incubation to stimulate an early spring, and d) chronic warming (+2°C above ambient). We then conducted CT_{max} 1-3 days prior to anticipated hatching dates (Piper, 1982) to quantify embryonic upper thermal tolerance. Hatching dates were predicted for each population and treatment separately based on accumulated daily thermal units (Piper, 1982).

2.3 Material and Methods

The research was conducted at the Ontario Ministry of Natural Resources and Forestry Fisheries Research Facility in Codrington, ON, Canada (-44.15°N, 77.80°W) between November 2022 and March 2023. All animal husbandry and handling procedures were approved by the Trent University Animal Care Committee following guidance set by the Canadian Council on Animal Care (Trent University AUP # 26398).

2.3.1 Brook Trout Populations, Gamete Collection and Husbandry

Brook trout gametes were taken from spawn-ready fish in the hatchery from four wild-origin populations founded from spawn collections in Algonquin Provincial Park, Ontario. The four populations differ in their genetic ancestry due to historical introgression from supplemental stocking. Inferred introgression based on previous

9

genetic work (AlShamlih, 2014) showed considerable differences in native vs. hatchery ancestry of the different populations: Scott Lake (100% hatchery ancestry), Stringer Lake (90% hatchery ancestry), Shallnot Lake (16% hatchery ancestry), Westward Lake (0% hatchery ancestry).

To collect and fertilize eggs, reproductive adults were anaesthetized using buffered tricaine methanesulfonate (MS-222; 50 mg L^{-1}, Syndel, Nanaimo, BC, Canada) and dry-spawned by gentle hand-stripping to avoid water activation of the gametes. Single-pair crosses (1:1 female and male mated unrelated pairs, with individuals used only once to avoid inbreeding) were made from each of the four populations, for a total of 16 families (four Scott, three Stringer, four Shallnot, and five Westward families). Embryo families were reared separately in partitioned egg baskets (9cm x 9 cm x 7.5 cm; see Appendix A1) within six separate 170 L incubation tanks such that families and populations could be tracked separately. In all, each incubation temperature treatment (see below) had embryos from 16 unique families of brook trout.

2.3.2 Thermal Regimes (Incubation Treatments)

Fertilized eggs from all 16 families were split between four thermal regimes (temperature treatments), ambient groundwater (minimum of 4°C, i.e., typical wild conditions), a mid-winter cold shock (brought on by a controlled snowmelt), chronic warming (2°C above ambient), and a short-term warming at late-stage embryogenesis (ambient, then ramped to 10°C from February 7th–9th until all hatched). Ambient rearing temperatures ranged from 4°C to 9.25°C across the study period (Figure 2a). The average

rearing temperatures (°C) from fertilization to hatch were 6.25 ± 1.03 (mean ± s.d.), 6.16 ± 1.15, 8.1 ± 0.83, and 7.57 ± 1.94 for the ambient, cold shock, chronic and short-term warming, respectively (Figure 2a). We used two replicate incubation tanks per treatment, with no significant differences between replicates. Approximately 10–12 mL of fertilized embryos from each family were randomly sorted into one egg basket (one hour after fertilization) in each of the tanks at the onset of the experiment. Embryo survival was checked thrice weekly, and mortalities were recorded to look at survivorship across thermal regimes. There were no statistically significant differences in embryo survival (to hatch) among the thermal treatments (Stewart et al., 2024).

Temperatures were logged every ten minutes throughout incubation using RBRsolo temperature loggers (RBR Ltd., Ottawa, ON; https://rbr-global.com/, accuracy ± 0.002°C), and tanks were heated (as needed) using 800 W titanium rod heaters controlled with thermostats. Tank water was filtered and recirculated (90% recirculation) using Eheim Type 2217 cannister filters, and fresh creek water was flushed through the tanks at *ca*. 1 L min^{-1} to maintain water quality and desired temperatures.

2.3.3 CT$_{max}$ Protocol

Embryos were randomly selected from each of the four populations for each CT$_{max}$ trial, and the observer (E.L. for all trials) was blinded to which treatment and population were in each trial. A CT$_{max}$ experiment (temperature ramping of 0.3°C min^{-1}) was conducted on up to nine embryos at a time (N=393) following a 10–20-minute acclimation period at ambient temperature. Embryos were placed in a petri dish sectioned

with a grid to keep each embryo separated and were filmed with a Logitech Capture

USB-camera. A new, factory calibrated ThermoWorks Precision & Reference

Thermometer (ThermoWorks, Inc., Utah, USA; https://thermoworks.com, accuracy ±

0.03°C) placed in the petri dish was used to confirm the thermal ramping rate and an

RBRsolo temperature logger was used to assign a temperature at CT_{max}. Embryonic

CT_{max} was marked based on the temperature at which the final movement occurred for

each embryo. Endpoints were taken from video recordings for accuracy. CT_{max} trials

began at a mean temperature (± s.d.) of 9.23 ± 1.12°C regardless of their acclimation

treatment, to ensure the total thermal stress, or accumulated thermal units, during the

CT_{max} trial itself would be as consistent as possible (Ørsted et al., 2022). Embryos were

not removed the moment their endpoints were reached as it would disturb the arena. The

trial ended one to five minutes after the last clear endpoint with no other embryonic

movements. Embryos were recovered within the chronically warmed tanks (to reduce

cold shock) and were monitored for hatching and responsiveness (signs of life) until 24

hours post-trial. Survival rates were 75% based on the subsample of embryos for which

we monitored survival (n = 138).

Cumulative daily thermal units (DTU = mean daily temperature °C) were

calculated based on daily mean temperatures from the temperature loggers (Piper, 1982).

Embryo DTUs on the day they were tested ranged from 356.01–635.86°C and expected

hatching was approximately 438.33°C (Piper, 1982), but this varied among families and

treatments. DTUs and average temperature were calculated for each embryo from

fertilization date to date of CT_{max} trial to test embryos immediately prior to hatch. Short-

term thermal acclimation windows were calculated by averaging the daily temperatures for each embryo for 5-, 10-, and 15-day windows prior to the day of its CT_{max} trial. Acclimation was expected to be a predictor of CT_{max}, so acclimation windows were calculated to assess what length of window was most relevant for thermal acclimation.

2.3.4 Statistical Analyses

All fish were treated as individual data points in all statistical analyses. Statistical analyses were conducted in R for Mac OS X (R Core Team 2020, Version Code 4.2.1). General linear models (GLMs) were used to model CT_{max} and assess the effects of incubation/rearing treatment (ambient, cold shock, chronic and short-term warming), genetic effects, and tank effects. CT_{max} temperature was the response variable, and fixed effects were treatment, tank, and genetic population. Treatment and tank were not modelled together because they were likely to cause model over-fitting due to multicollinearity. A secondary set of GLMs were used to model CT_{max} and assess which thermal acclimation window (5, 10, or 15 days prior to CT_{max} trial) best predicted CT_{max}. Embryos that did not move or that hatched during the CT_{max} trials were excluded from analyses (n = 26, 8%). Statistical assumptions were assessed visually with plots of residuals.

GLMs were created for all possible combinations of factors and were assessed for best fit using Akaike's Information Criterion (AIC) using the package *AICcmodavg* (Mazerolle, 2020). GLMs were compared using ΔAIC, AIC cumulative weights, and Pseudo-R^2. McFadden Pseudo-R^2 values were calculated with the *rcompanion*

(Mangiafico, 2022) package and the nagelkerke function. A Tukey pairwise comparison

post-hoc analysis was conducted using the *emmeans* (Lenth, 2023) package to estimate

the marginal means of the best-fit model. Raw data are publicly available on Figshare at:

https://figshare.com/s/24b5a8377ee098740a95.

2.4 Results

CT$_{max}$ was best predicted by the thermal regime of embryo incubation (treatment;

Table 1). Specifically, the embryos from both elevated temperature treatments (chronic

and short-term warming) had higher CT$_{max}$ compared to embryos from the ambient

treatment (Figure 2b). There were significant *post hoc* differences in mean ± s.d. CT$_{max}$

between ambient (23.15 ± 3.44°C, n = 101) and short-term warming (25.5 ± 2.38°C, $p <$

0.001, n = 51), such that short-term warming conditions increased CT$_{max}$ by *ca.* 2.3°C.

This was also true between the ambient and chronic warming treatments (25.42 ± 2.31°C,

$p < 0.001$, n = 133), such that chronic warming increased CT$_{max}$ by *ca.* 2°C ($p < 0.001$).

There were no significant *post hoc* differences between short-term warming and chronic

warming ($p > 0.05$). We found that there were no differences in embryonic CT$_{max}$

between the ambient and cold shock treatments (22.88°C ± 3.37, $p > 0.05$, n = 108).

Thus, as with the ambient treatment, mean CT$_{max}$ for embryos from the cold shock

treatment was 2.62°C and 2.54°C lower than the short-term warming and chronically

warmed treatments (Tukey post hoc $p < 0.001$), respectively. However, there was

substantial variation in the data, with a range of *ca.* 10°C in CT$_{max}$ values in each

treatment group. Given that variation and the fact that two warmer treatments were not

statistically different, our linear model with treatment only explained 3% of the variation

in CT_{max}. Short-term acclimation temperatures for each embryo (5 day, 10 day, 15 day) to predict CT_{max} were not useful for explaining the variation in the data, with no differences found across treatment or genetic population.

Table 1. Summary of Akaike's Information Criterion (AIC) models for predicting CT_{max} of brook trout (*Salvelinus fontinalis*) embryos under varying thermal regimes (°C). Models are listed in order of best-fit for each set of candidate models, although CT_{max} was best predicted by thermal regime. Treatment = thermal regime incubation treatment. Tank = tank. Strain = genetic population. Roman numerals indicate the 5 models.

Model	*K*	*AIC*	*ΔAIC*	*CWt*	*Pseudo R^2*
i. Tank	7	1392.08	0	0.68	0.00351
ii. Treatment	4	1395.52	3.44	0.80	0.0293
iii. Treatment + Strain	7	1395.71	3.63	0.91	0.0335
iv. Tank + Strain	10	1396.09	4.01	1.00	0.0378
v. Strain	5	1438.52	46.44	1.00	0.000679
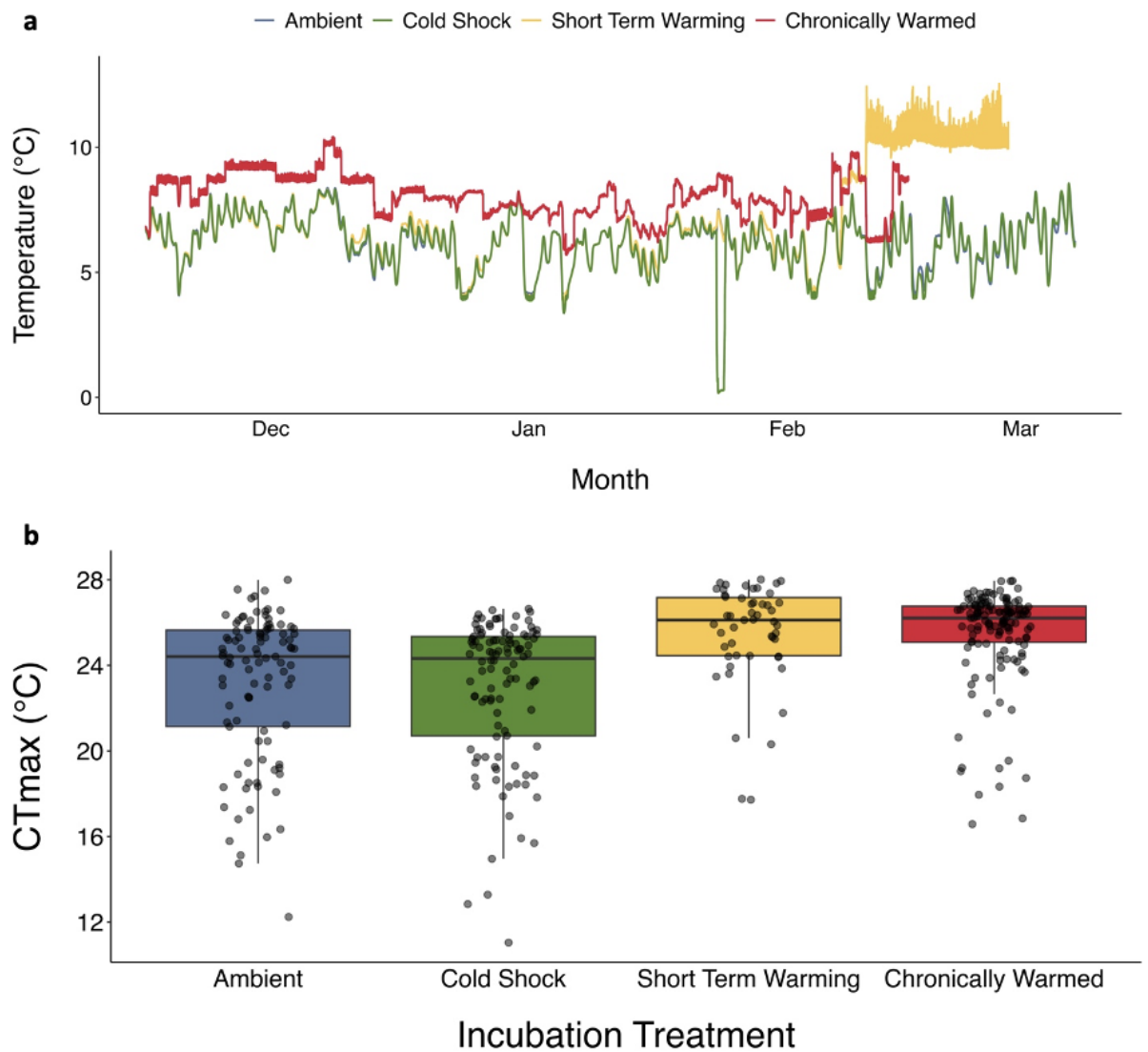
Figure 3. (a) Incubation temperature (°C) of each treatment from fertilization to final CT$_{max}$. CT$_{max}$ data was collected between January 3rd and March 7th, 2023. Temperature was controlled to never drop below 4°C. Mean (°C) ± s.d. for thermal regimes were 6.25 ± 1.03, 6.16 ± 1.15, 8.1 ± 0.83, and 7.57 ± 1.94 for the ambient, chronic, and short-term warming treatments, respectively. **(b) Median CT$_{max}$ (°C) for brook trout (*Salvelinus fontinalis*) embryos reared under ambient, cold shock, short-term warming, and chronically elevated rearing temperatures.** Median (thick middle

line) shown with boxes extending across the Interquartile Range (IQR), whiskers to 1.5*(IQR) and outliers shown beyond the whiskers. Outliers are identified by an open diamond. Each point represents an individual fish.

2.5 Discussion

While CT_{max} has been widely used in many fishes and is known to respond quickly to acclimation (Zhang and Kieffer, 2014; Stewart et al., 2023), acclimation of CT_{max} in fish embryos has not been an area of focus. Our findings provide evidence that fish embryos have some short-term thermal plasticity (i.e., can acclimate); embryo CT_{max} changed in response to rearing temperatures. The extent of variability in CT_{max} we observed, with values spanning *ca.* 10°C in each treatment, was far higher than in juveniles or adults for this species (Ellis et al., 2013; Morrison et al., 2020). Salmonids, including brook trout, typically produce a range of *ca.* 2°C for a given group of animals (e.g., same life stage and acclimation, e.g., Anttila et al., 2015; Morrison et al., 2020; Debes et al., 2021; Stewart et al., 2023). That variability perhaps highlights a challenge with this approach. The high variation we observed was also noted in black gobies (*Gobius niger*), where embryos exhibit greater variation than larvae and adults (Cowan et al., 2023). Unexplained, additional variation in embryos would necessitate relatively larger sample sizes to tease apart treatment effects. That variability also suggests that a) embryos are more phenotypically/physiologically diverse in terms of thermal tolerance than juveniles and adults, and/or b) that using the temperature of final movement, as we did here, is a less precise measure of thermal physiology than loss of equilibrium in

swimming fishes. In either case, the intraspecific variation we observed provides interesting substrate for future study.

In juvenile and adult brook trout, CT_{max} varies intraspecifically but typically falls between 27–30°C across studies with different methods (acclimation temperature 10°C, CT_{max} 28.74°C ± 0.67°C in Lee and Rinne 1980; acclimation temperature *ca*. 11°C, CT_{max} 27.88°C in Stewart et al., 2023). The embryos in this study in the two warmed treatments reached CT_{max} values slightly lower than the 27-30°C range for later life stages (Figure 2b) yet were acclimated to relatively cold temperatures compared to most studies on adults and juveniles. Thus, it appears that if there is any difference in upper thermal tolerance between embryos and juveniles/adults for this species, the difference is marginal after accounting for acclimation; additional experiments and data could help clarify the magnitude of the differences in thermal tolerance among life stages.

Plasticity may be limited in key life stages, such as embryos, resulting in a narrower tolerance range and limited thermal safety margin (Dahlke et al., 2020) in comparison to juveniles and adults (O'Connor and Heasman, 1998; Sokolova et al., 2012). Thermal tolerance and acclimation responses should be examined at all developmental stages to generate comprehensive profiles of thermal safety margins and pinpoint windows of sensitivity (i.e., bottlenecks, Pottier et al., 2022) while also accounting for oxygen limitation (Ellis et al., 2013; Ern et al., 2023). Using methods that are comparable across life stages is important for quantifying plasticity, vulnerability, and variability across ontogeny.

Chapter 3: Effects of embryonic rearing temperatures and their impacts on upper thermal tolerances across subsequent life stages of brook trout (*Salvelinus fontinalis*)

3.1 Abstract

Winter climate is changing rapidly in northern latitudes, and these temperature events have effects on salmonid thermal biology. Stressors during winter egg incubation could reduce hatching success and physiological performance of fall-spawning fishes. Here we quantified the potential for ontogenic carryover effects from embryonic thermal stress in multiple wild and hatchery-origin populations of brook trout (*Salvelinus fontinalis*), a temperate ectotherm native to northeastern North America. Fertilized eggs from four populations were incubated over the winter in the laboratory in four differing thermal regimes: ambient stream-fed water, chronically warmed (+2°C), ambient with a mid-winter cold shock, and short-term warming late during embryogenesis (to stimulate early spring). We examined body size and upper thermal tolerance at the fry (10 weeks post-hatch and 27-30 weeks post-hatch) and gravid adult (age 2+) life stages (overall N = 1,482). Our thermal treatments during incubation had effects that varied by life stage, with incubation temperature and life stage both influencing body size and thermal tolerance. Ten weeks after hatch, fry from the ambient and cold shock treatment groups had higher and less variable thermal tolerance than did the warmer treatment groups. At 27-30 post-hatch and beyond, differences in thermal tolerance among treatment groups were negligible, indicating that the effects of thermal acclimation were short-lived. Collectively, our study suggests that brook trout only exhibit short-term carryover effects from thermal stressors during embryo incubation, with no lasting effects on phenotype beyond the first few months after hatch.

3.2 Introduction

Although many studies on ectotherms have focused on the effects of high
temperatures in summer, the impact of climate change on winters is especially dramatic
at north temperate latitudes (Shuter et al., 2012). Animals that reproduce in the fall, like
most salmonids, may be particularly vulnerable in cases in which embryos develop
slowly over winter and hatch in spring (Kim et al., 2019). Co-occurrence of thermal
stress with other stressors during winter, such as abrupt runoff (premature melt) events or
hypoxia, can reduce hatching success and physiological performance (Del Rio et al.,
2021). Temperature differences during embryogenesis can affect fitness-relevant traits
like growth, reproduction, and migratory behaviour (Jonsson and Jonsson 2014, 2019;
Jonsson and Greenberg, 2022). Understanding how temperatures during early life
experiences shape an individual's future fitness is key to predicting how wild populations
will fare under changing conditions (Birnie-Gauvin et al., 2021).

In this study we examined how developmental temperatures and altered rearing
conditions as embryos influence thermal tolerance (heat resistance) and growth rates
across life stages of brook trout (*Salvelinus fontinalis*). We were also interested in
examining the degree of thermal acclimation and possible carryover effects across brook
trout ontogeny. We hypothesized that upper thermal tolerance (CT_{max}) would change
throughout ontogeny and that elevated rearing conditions would impact growth rate and
upper thermal tolerance. We predicted that individuals exposed to warm temperatures
during incubation as embryos would have greater thermal tolerance (higher CT_{max}) and

larger body sizes in comparison to those exposed to cooler temperatures as embryos. We also predicted that acclimation responses would be evident across ontogeny in the form of carryover effects. We expanded on a previous study (Stewart et al., 2024) that examined the survivorship of brook trout embryos under varying winter thermal regimes in 2020 (ambient and chronic warming) and conducted CT_{max} on these survivors at age 2+. We reared brook trout embryos under four thermal regimes in 2022 (ambient, chronic warming, ambient with a mid-winter cold shock that simulated a snowmelt event, and short-term warming late during embryogenesis). We measured CT_{max} at two developmental periods for the 2022 cohort: 1) 10 weeks post-hatch, and 2) 27-30 weeks post-hatch. In addition to assessing the effects of our incubation treatments on post-hatch CT_{max}, we quantified body size in all life stages across the experiments. Transient thermal stress in embryos, and especially cold shocks during winter months are underappreciated in fish thermal biology. The effects of these fluctuating winter temperature events on salmonid thermal biology are essential components to understanding resilience to near-term climate change.

3.3 Methods

Brook trout were similarly raised as **Chapter 2** between Fall 2022 to Spring 2023 and followed the same guidelines and animal care. This chapter follows the surviving fry from **Chapter 2** and adult brook trout from Stewart et al. (2024) that experienced varying thermal regimes as embryos during the 2020 fall season.

10 weeks post-hatch fry (2022)

Once the yolk sacs of the 2022 fish (**Chapter 2**) were fully absorbed (between March 24th and April 4th, 2023), fry were moved by family to subdivided 170 L flow-through holding tanks and reared in common conditions (ambient groundwater, ranging from 4-7°C and fed 3-4 times daily). Fry were held at common garden conditions for 10 weeks. All fish were fed EWOS crumble pellets in varying sizes appropriate to the life stage and body size of the fish (https://www.ewos.com/ca/). At 10 weeks post-hatch (hatch dates were specific to each genetic population and treatment; post-hatch refers to post-full-hatch), fry were moved to Trent University (44.36°N, 78.29°W) in an aerated and insulated transport tank. Fry were acclimated at Trent University for 2-3 days in holding tanks (12 cm x 10 cm x 15 cm) at 10.42°C $\pm$ 0.41 (mean $\pm$ s.d.) prior to CT_{max} trials, fed 3-4 times a day and were fasted on the days of the trials. Fry were randomly selected for each of the 16 combinations of family and incubation treatment (Table 2, 2022a). We conducted 49 CT_{max} trials between March 29th - April 28th, 2023, with a warming rate of 0.3°C min^{-1} on 2-12 fry at a time (N = 885). The CT_{max} arena was partitioned so that family-treatment combinations could remain separated (see Appendix A2). Fry were allowed to habituate to the CT_{max} arena for 10-15 min. After warming began, fish were monitored continuously (all by the same observer, E.L.); the endpoint for CT_{max} was loss of equilibrium. The moment fish lost equilibrium, they were removed (with the temperature recorded) and individually recovered in 10°C water for 10-35 minutes. Once the CT_{max} trial was complete and all fish had regained equilibrium, they were euthanized with tricaine methanesulfonate (MS-222, 0.1g mL^{-1}, buffered with 0.2 g mL^{-1} of sodium bicarbonate) and weighed (nearest 0.001g) and measured (total length and body depth, nearest 0.01mm).

27-30 weeks post-hatch (2022)

Once fry reached 27-30 weeks post-hatch, families were pooled due to limited husbandry space at the research hatchery. Post family pooling, a random subsample of the fry were transported to Trent University, where we ran 27 CT_{max} trials between October 23rd – November 3rd, 2023. Fry were acclimated for 4-8 days in holding tanks held at 8.06°C ± 0.9 (mean ± s.d.) and fed 2-3 times a day. Fry were fasted for 16-26 hours before the trials. Fish were held separately by treatment (Table 2, 2022b). CT_{max} trials were conducted on 14-31 fry at a time (N = 522) in a non-partitioned cooler (Appendix Figure A3). CT_{max} methodology was the same as above, but with individual fry were weighed to the nearest 0.1g and measured for total and fork length (mm).

Gravid adults (2020)

Egg families were established in fall of 2020 using 16 parental crosses (15 females and 16 males for 14 full-sibling and two half-sibling families; 2020 cohort in Table 2; Stewart et al., 2024) from four genetically-distinct populations (Scott Lake, Stringer Lake, Shallnot Lake, and Westward Lake) and reared in the same design as **Chapter 2** (2022 fish) but including only the ambient (control) and chronically warmed treatment groups. Embryos hatched in spring of 2021 and were held in common garden conditions (ambient groundwater from Marsh Creek) until October 11th – October 12th, 2023, when we conducted six CT_{max} trials. Adult fish were fed once daily but were not fed the day of trials. The observer was blinded to which treatment was in each trial as the individual fish were PIT tagged. CT_{max} trials were conducted on 10-14 fish at a time (N =

72). The methodology was the same as described above except that fish recovered in 8°C water for 30-60 minutes before being weighed (nearest 0.1g) and measured for fork length (mm).

Table 2. Brook trout (*Salvelinus fontinalis*) life stage information for Chapter 2 and Chapter 3. 2020 refers to the gravid adults, 2022a refers to the 10 weeks post-hatch fry, 2022b refers to the 27-30 weeks post-hatch fry, 2022c refers to the seven-day heat shock experiment. Families describe unique family crosses (i.e., no shared parents across families), except for in 2020, when two families were created using eggs from a single female, meaning two of the sixteen families were half-siblings. Response variables were used in general linear models (GLMs). Response variables included CT_{max}, length and weight.

Treatment	Year	Genetic population	N
Ambient	2020	Scott Lake (16 families)	36
	2022a	Scott (4 families)	178
		Stringer (3 families)	
		Westward (5 families)	
		Shallnot (4 families)	
	2022b	Pooled populations and families from 2022a	160
Chronically warmed (+2°C above ambient)	2020	Scott (16 families)	36
	2022a	Scott (4 families)	287
		Stringer (3 families)	
		Westward (5 families)	
		Shallnot (4 families)	
	2022b	Pooled populations and families from 2022a	116
Short-term warming (late-stage embryogenesis)	2022a	Scott (4 families)	240
		Stringer (3 families)	
		Westward (5 families)	
		Shallnot (4 families)	
	2022b	Pooled populations and families from 2022a	111

Mid-winter cold shock	2022a	Scott (4 families)	179
		Stringer (3 families)	
		Westward (5 families)	
		Shallnot (4 families)	
	2022b	Pooled populations and families from 2022a	149

3.3.1 Statistical Analyses

Statistics were run on the same software as **Chapter 2**. GLMs were used to model the effects of incubation treatment, genetic effects (treatment levels), and body index (Fulton's condition factor; $K = 10^5 W/L^3$ where W is weight in grams, and L is length in mm) on each life stage's CT_{max}. These models can be seen in Table A1, A2, and A3, for 10-week post-hatch fry, 27-30 weeks post-hatch fry, and adult fry (respectively). Fry (10 weeks post-hatch) that did not recover from CT_{max} trials were excluded from analyses (n = 26, 0.03%). Statistical assumptions were assessed visually with plots of residuals for GLMs, and linearity, homoscedasticity, independence, and normality for linear regressions. See **Chapter 2** for all packages and functions used. Raw data are publicly available on Figshare at: https://figshare.com/s/ed15d6a13483a86b4664 (fry and gravid adult datasheets).

3.4 Results

The 10-week post-hatch fry from both warm treatment groups (chronic and short-term warming, but then reared at common ambient temperatures post-hatch) had lower CT_{max} in comparison to both ambient and cold shock treatments, the opposite of what we saw for embryo CT_{max} in **Chapter 2** (Figure 3). At 10 weeks post-hatch, ambient (28.68°C ± 0.29, n = 178) and cold shock (28.64°C ± 0.36, n = 179) treatment groups had

higher mean CT_{max} (± s.d.) in comparison to the chronic and short-term warming treatment groups (chronic: 28.35°C ± 0.52, $p < 0.05$, n = 287, and short-term: 28.16°C ± 0.72, $p < 0.05$, n = 240). At 27-30 weeks post-hatch, the ambient (28.14°C ± 0.35, n = 160) treatment group CT_{max} was not significantly different to the cold shock (28.10°C ± 0.33, n = 149) treatments, with no other difference in CT_{max} between ambient, chronic, and short-term warming treatment groups (chronic warming 28.13°C ± 0.37, n = 116; short-term warming 28.15°C ± 0.35, n = 111; Figure 3).

Thermal tolerance of age 2+ adult treatment groups did not differ based on the temperature treatment in which they were incubated as embryos (Figure 3). The mean CT_{max} of ambient was 27.37°C ± 0.68, n = 36, while the mean CT_{max} for the chronic warming treatment group was 27.20°C ± 0.86, n = 36 (Tukey post hoc $p > 0.05$).

Figure 3. Mean CT$_{max}$ (°C) for brook trout (*Salvelinus fontinalis*) life stages incubated under varying winter thermal regimes as embryos. Error bars represent standard deviation (s.d.).

The 10 weeks post-hatch fry exposed as embryos to chronic warming conditions and short-term warming as embryos were shorter (mean total length for chronic warming = 27.04 ± 2.27 mm, and for short-term warming 28.40 mm ± 3.59) than the ambient (30.07 mm ± 4.49) and cold treatments (30.55 mm ± 4.37), respectively (Figure 4; Tukey post hoc $p < 0.05$ across all treatments). No differences were detected between the ambient and cold treatments. The 10-week post-hatch fry exposed to elevated temperatures as embryos were smaller in mass in comparison to both ambient and cold treatment groups (chronic warming: 0.17g ± 0.05; short-term warming: 0.21g ± 0.08; ambient: 0.24g ± 0.12; cold-shock: 0.25g ± 0.11; $p < 0.05$), however, there were no statistical significance between ambient and cold shock treatment groups ($p > 0.05$).

At 27-30 weeks of being reared in common garden conditions, embryos incubated in warmed conditions (chronic and short-term warming) were on average 8.7mm (mean total length = 94.05 ± 8.68 mm) and 7.8mm longer (93.14 ± 8.01 mm) than the ambient treatment group (85.38 ± 9.25 mm), respectively (Tukey post hoc $p < 0.05$). The cold shock treatment group was on average 5.9 mm longer (91.26 ± 9.25 mm) than the ambient treatment group (Tukey post hoc $p < 0.05$). At 27-30 weeks post-hatch, the fry who were reared in elevated treatment groups as embryos were larger than the ambient

and cold-shock treatment groups (chronic warming: 7.9g ± 2.2; short-term warming: 7.83g ± 2.0; ambient: 6.1g ± 2.0; cold-shock: 7.4g ± 2.2; Tukey post hoc p < 0.05).

The age 2+ adult also had similar body sizes between treatment groups (Tukey post hoc p > 0.05, with no statistical significance between total length (mm) and mass (g) across treatment groups. Gravid adults from the elevated treatment group weighed approximately 35g less (chronic warming: 440.75g ± 72.7; ambient: 476.08g ± 76.16) and were approximately 6mm shorter than the ambient treatment group (chronic warming: 348.47mm ± 39.9; ambient: 355.17 ± 18).
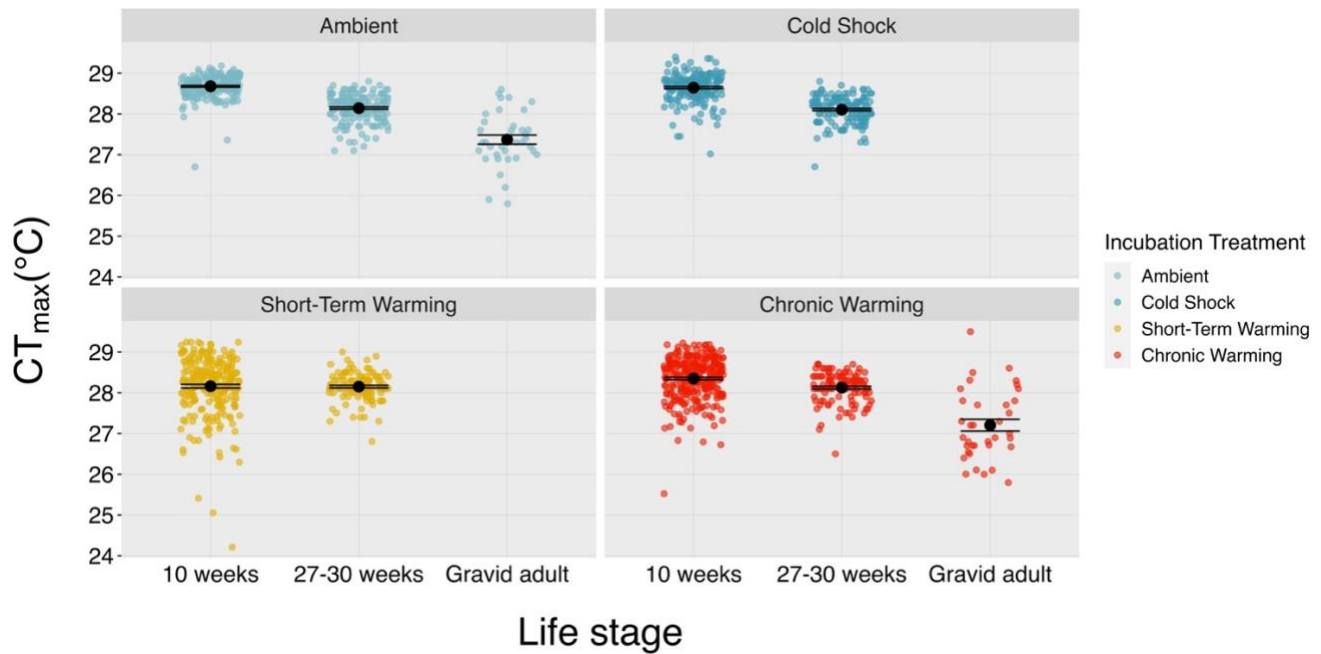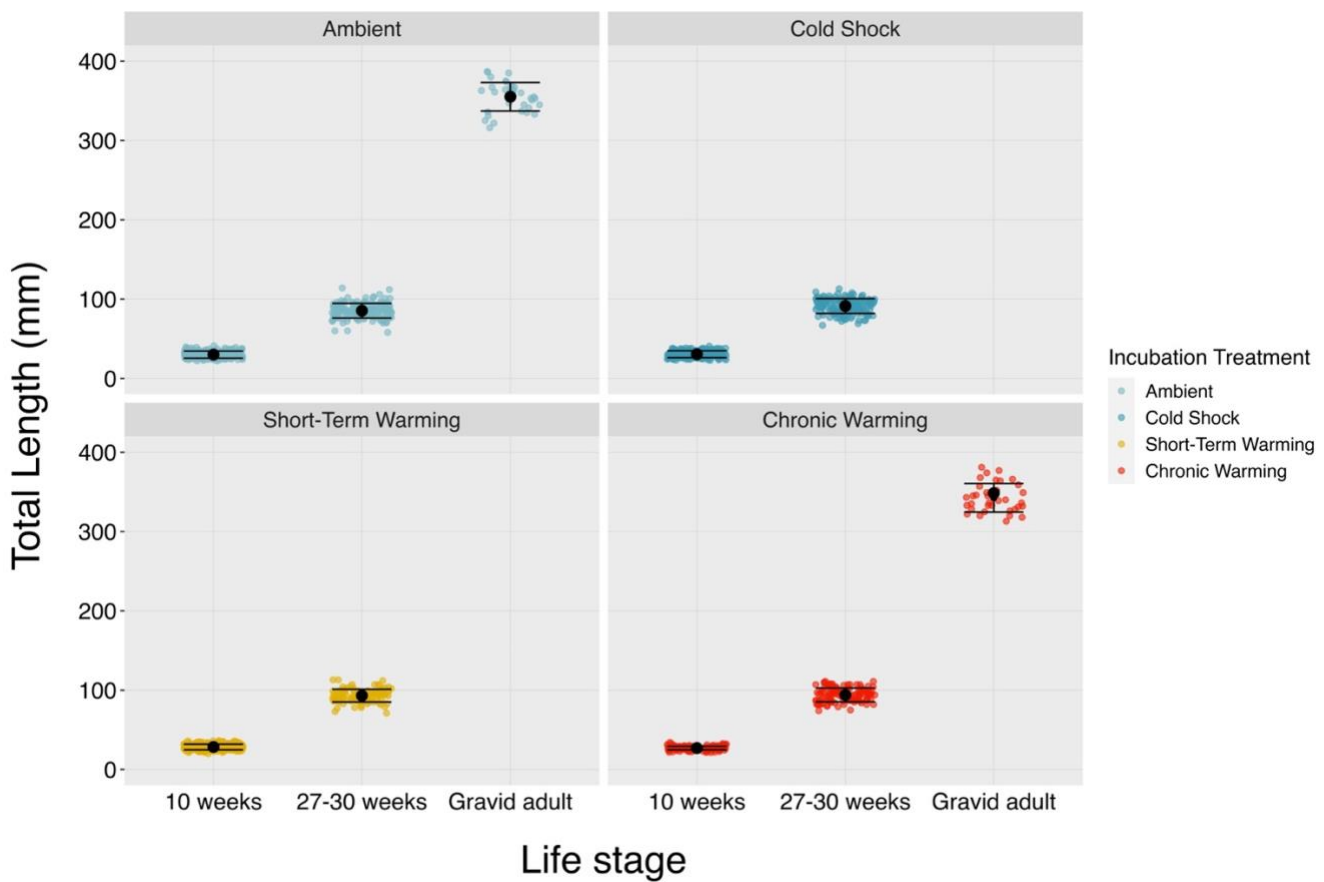
Figure 4. Mean total length (in mm) for brook trout (*Salvelinus fontinalis*) life stages incubated in varying winter thermal regimes as embryos. Error bars represent standard deviation (s.d.). There were significant differences in mean total length for both 10- and 27-30-weeks post-hatch fry. Rearing in elevated treatment groups as embryos significantly reduced the total length at 10 weeks post-hatch, and significantly improved the total length at 27-30 weeks post-hatch in brook trout.

For body condition of 10 weeks post hatch fry (Fulton's K), no differences were detected among treatments (Tukey post hoc $p > 0.05$), although K differed among populations (Tukey post hoc $p < 0.05$). Almost all population pairs differed significantly, except for Westward Lake and Shallnot Lake (Figure 5). For the same fry cohort (10 weeks post-hatch), populations with substantial hatchery introgression were longer (Scott mean total length $\pm$ s.d. 33.14 ± 3.65 mm and Stringer 32.14 ± 2.46 mm; 5.2-7.2mm range increase) than fish from the primarily native-ancestry populations (Shallnot 25.90 ± 1.55 and Westward 26.90 ± 1.38; Tukey post hoc $p < 0.001$; Figure 5). Genetic populations varied with ancestry and thermal tolerance (CT_{max}) in 10-week post-hatch fry (Figure 4). The heavily-introgressed lakes [Scott Lake (100% hatchery ancestry, mean CT_{max} $28.29°C \pm 0.66$, n = 226)] and Stringer Lake (90% hatchery ancestry, mean CT_{max} $28.28°C \pm 0.59$, n = 222) did not differ between one another, and the largely-native ancestry lakes [Shallnot Lake (16% hatchery ancestry, mean CT_{max} $28.51°C \pm 0.45$, n = 214) and Westward Lake (0% hatchery ancestry, mean CT_{max} $28.62°C \pm 0.41$, n = 222)] did not differ between one another, however there were significant differences between

hatchery introgressed and wildly-ancestry lakes (Tukey post hoc p < 0.001), where wild-ancestry lakes were more thermally tolerant.

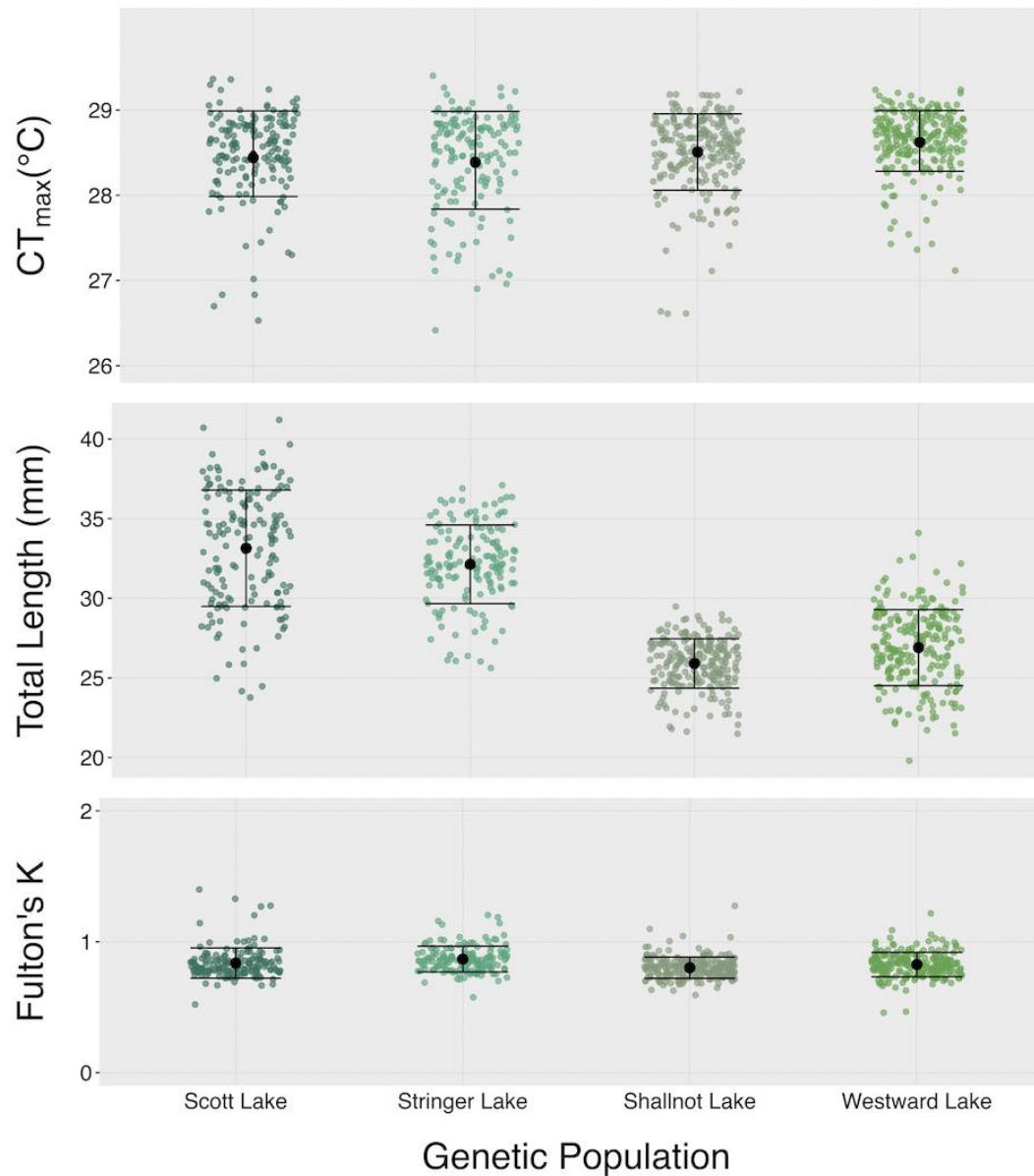**Figure 5. Mean CT$_{max}$ (°C), total length (in mm), and Fulton's K condition factor (Fulton's K) for brook trout (*Salvelinus fontinalis*) 10-week post-hatch fry across four genetically distinct populations originating from lakes in Algonquin Provincial Park.** Genetic populations differ with percent hatchery-introgression: Scott Lake (100% hatchery ancestry), Stringer Lake (90% hatchery ancestry), Shallnot Lake (16% hatchery

ancestry), Westward Lake (0% hatchery ancestry) (Alshamlih, 2014). Error bars represent standard deviation (s.d.).

3.5 Discussion

Contrary to our prediction, incubation of brook trout embryos in higher temperatures did not increase their upper thermal tolerance in subsequent life stages. Each free-swimming life stage exhibited higher thermal tolerance in comparison to the embryos from **Chapter 2**, and thermal carryover effects were limited in duration and scope. Although brook trout embryos exhibited a positive acclimation effect (i.e., improved CT_{max} when exposed to warmer water during incubation) for increased thermal tolerance (**Chapter 2**), that benefit was not only lost, but reversed by 10 weeks post-hatch. Differences in thermal tolerance due to carryover effects from incubation temperatures were undetectable at 27-30 weeks post-hatch and in age 2+ adults. A possible explanation for the lack of positive acclimation effects in the fry life stages could be due to the metabolic costs and constraints associated with prolonged thermal plasticity (Pottier et al., 2022). Thus, the effects of elevated rearing temperatures were most prominent at the embryonic life stage and within the first few months after hatching.

Elevated temperatures during incubation can cause early life mortalities, and both physiological and behavioural abnormalities (Chen et al., 2013). Our 10 weeks post-hatch fry were smaller and had poorer performance when incubated in elevated water conditions in comparison to ambient and cold shock conditions. Our 27-30 weeks post-hatch fry that were incubated in elevated temperatures ended up being larger (possibly

due to increased metabolism at warmer temperatures) and performed the same as those at

cooler temperatures, illustrating that the fry exhibited life stage specific physiological

constraints. In sockeye salmon (*Oncorhynchus nerka*), smaller fish exhibited a greater

increase in thermal tolerance during early development even when reared at common

garden conditions, illustrating the ability for fry to compensate for previously

experienced depressive conditions (Chen et al., 2013). However, smaller fry will likely be

more susceptible to thermal changes until they gain body mass (Chen et al., 2013). More

recent findings suggest that embryonic exposure to unstable but nonlethal conditions may

have relatively little effect on fry performance (Del Rio et al., 2019). Del Rio et al. (2019)

reared developing Chinook salmon (*Oncorhynchus tshawytscha*) from fertilization to fry

in four treatments with differing temperature and oxygen saturation levels. The high

temperature treatment with normal oxygen conditions increased rate of development but

upon reaching the fry life stage there was no significant difference in body condition

factor (Fulton's K condition factor) (Del Rio et al., 2019).

The highly introgressed populations (Scott Lake and Stringer Lake) in our study

had lower CT_{max} and longer total lengths in comparison to the wild-type populations.

Highly introgressed (with the Hills Lake strain, i.e., Scott and Stringer Lake) populations

are known to have greater thermal tolerance than other Ontario wild and hatchery

populations (McDermid et al., 2012; Stitt et al., 2014), which our study failed to support.

In addition, previous work showed that 0+ juveniles from a brook trout lineage that had

been selected for larger body condition, faster growth, and later sexual maturity, had

higher thermal resistance than control juveniles that were from random lineages, and that

the most sensitive fish were of lower mass and length (Gourtay et al., 2023). Here, we found that the larger fish were more sensitive to thermal stress and were less resilient. Other studies have found similar results to Gourtay et al. (2023) in that salmonids of lesser mass and length were more sensitive to thermal stressors (sockeye salmon in Chen et al., 2013; chinook salmon in Clark et al., 2008). However, in some species, such as cutthroat trout (*Oncorhynchus clarkii*), Caribbean neon goby (*Elacatinus lobeli*), neon goby (*Elacatinus oceanops*), and leopard coral grouper (*Plectropomus leapardus*), CT_{max} (upper thermal tolerance) declines with body size and/or mass (McKenzie et al., 2021). Additional factors that can lead to thermal tolerance differences among introgressed and native populations of brook trout are transgenerational effects from parents and grandparents (i.e., parental effects) (Wahl, 1974). Carline and Machung (2011) and Vincent (1960) found that wild populations of brook trout had higher CT_{max} than those of domesticated populations when reared under identical conditions, and their reasoning related to genetic divergence and prior temperature exposure of each population within their natural or artificial (hatchery) habitats.

Studying how fluctuating winter temperatures affect fall-spawning fish is crucial in understanding thermal resilience and population persistence under near-term climate change. Embryos and reproductively mature adults are considered the most vulnerable life stages as they have the smallest thermal safety margins in both marine and freshwater fish species (Dahlke et al., 2020). Our study confirmed embryos as a thermally sensitive and vulnerable life stage for brook trout, as predicted by Dahlke et al. (2020). The contrast in thermal performance of hatchery-based reproductive adults compared with

33

wild populations (Dahlke et al. 2020) suggests that hatchery-based studies may underestimate potential effects of climate change on natural populations (Smith and Ridgway 2019). This potential mismatch could be addressed using field-based respirometry and bioenergetic studies to parameterize thermal niches and environmental pressures more accurately in natural populations, our research provides novel information regarding brook trout thermal biology as we investigated thermal tolerance and thermal plasticity in embryos and subsequent post-hatch life stages further adding to the field of salmonid thermal biology.

Chapter 4: General Discussion

4.1. Summary of Findings

The embryos from both elevated temperature treatments (chronic and short-term warming) had higher CT_{max} compared to embryos from the ambient and cold-shock treatments. However, there was substantial variation in the data, with a range of *ca*. 10°C in CT_{max} values in each treatment group. Incubation of brook trout embryos in higher temperatures did not increase their upper thermal tolerance in subsequent life stages. Although each free-swimming life stage exhibited higher thermal tolerance in comparison to the embryos, thermal carryover effects were limited in duration and scope. Although brook trout embryos exhibited a positive acclimation effect (i.e., improved CT_{max} when exposed to warmer water during incubation) that benefit was not only lost but reversed by 10 weeks post-hatch. Differences in thermal tolerance due to carryover effects from incubation temperatures were undetectable at 27-30 weeks post-hatch and in age 2+ adults. Thus, the effects of elevated rearing temperatures were most prominent at the embryonic life stage and within the first few months after hatching. Our 10 weeks post-hatch fry were smaller and had lower thermal tolerance when incubated in elevated water conditions in comparison to ambient and cold shock conditions. Our 27-30 weeks post-hatch fish that were incubated in elevated temperatures ended up being larger and performed the same as those at cooler temperatures, illustrating that the fry exhibited life stage specific physiological constraints. Additionally, the hatchery-introgressed populations (populations highly introgressed with Hills Lake; Scott Lake and Stringer Lake) in our study had lower CT_{max} and higher body condition in comparison to the wild-type native populations (Shallnot Lake and Westward Lake).

4.2 General Discussion

Ectotherms cope with climate change and seasonal changes by acclimation (short-term, i.e., plastic responses), adaptation (long-term and over generations), or relocation to more favourable areas (i.e., thermal refuge or a new habitat). For freshwater fishes that cannot relocate, adaptation and acclimation are the only routes available that avoid extirpation (localized extinction). Fortunately, brook trout are quite flexible at cold water temperatures and are known to acclimate (Stewart et al., 2023) and exhibit thermal plasticity (Stitt et al., 2014). Brook trout also exhibit intraspecific variation across and within populations in differing geographical areas in respect to their thermal tolerance (Stitt et al., 2014; McDermid et al., 2012). The intraspecific variation in thermal tolerance is driven by phenotypic plasticity, heritable traits, and ontological changes across body size, condition, and life stages (McKenzie et al., 2021). However, maintaining a buffer capacity for thermal plasticity can be energetically costly (Angilletta et al., 2003; Auld et al., 2010; Norin et al., 2024), especially for young fry that have little to no energy reserves and are primarily focused on growth and survival.

Our finding that fry thermal tolerance decreased with increasing incubation temperature concurs with data from Chen et al. (2013), who conducted CT_{max} at 90 days post-hatch on sockeye salmon from eggs incubated at 10, 14, and 16°C, and found that elevated incubation temperature decreased CT_{max}. By contrast, our findings differ with a previous study by Del Rio et al. (2021). The Del Rio et al. (2021) study incubated Chinook salmon embryos under multiple rearing temperature and conditions, post hatch

36

they were reared in common garden conditions (similar to our study) and post-hatch life stages were then investigated. Del Rio et al. (2021) found that early, late, and chronic warming (chronic warming and hypoxia) conditions increased CT_{max} in Chinook salmon (*Oncorhynchus tshawytscha*) fry at 32-35 days post-hatch. Our results may differ from Del Rio et al. (2021) due to their manipulation of oxygen, and differences in rearing season and temperature at common garden conditions among the two studies.

Cold stress may increase the mortality of developing fish due to limited energy reserves and temperatures possibly falling outside thermal niches (Pörtner and Peck, 2010). We found no downstream consequences for those fish that were exposed to cooler incubation conditions, and they performed equally or better at subsequent warmer temperatures. The salmonids in Del Rio et al. (2021) are from California and encompass the southern range of that species, while both ours and those of Chen et al. (2013) are from populations in the middle part of their respective species' ranges. Although brook trout are cold-water adapted fish, populations of brook trout in the southern ranges will be exposed to warmer water temperatures in comparison to those from more northern populations, this may impact upper thermal tolerance as they are adapted to different environmental temperatures and have differing thermal experience. Our cold stress (the simulated snowmelt) was a singular event. However, it is possible that repeated cold shock stress would have had clearer, negative cumulative effects on phenotypes.

Although young fry are exposed to considerable variation in temperature based on geographical location, behaviour and habitat selection (Biro et al., 1996; Biro et al.,

2008), there may be no benefit for fitness tradeoffs in order to prolong thermal plasticity. This puts early life stages of brook trout at risk, which could in turn impact population persistence. The possible mechanism behind the penalty in thermal tolerance could be related to body condition or nutrition deficiency (Harrison et al., 2011), which is supported by our finding that body size was negatively impacted by elevated rearing conditions. Our suggestion for this is that at 10 weeks post-hatch, the fry that were exposed to elevated temperatures as embryos were both thermally sensitive (expressed a lower thermal tolerance as a lower CT_{max}) and smaller (expressed shorter total lengths). When comparing strictly total length at 10 weeks post-hatch it was clear that the embryo incubation in warmed treatments had negative effects. As environmental temperatures increase, so does individual energy expenditure (Brownscombe et al., 2022). If energy reserves cannot be replenished, individuals may experience fitness related constraints, such as decreased body condition due to the high metabolic costs at warmer temperatures. Not only does incubation at warmer temperatures result in physiological impacts during the initial period after hatching, but it can also influence the thermal sensitivity of the fry, subsequently impacting fitness, growth, and reproduction in adulthood.

The data shown here represent the first experimental comparison of thermal tolerance across life stages of brook trout, although there are review papers that compare results across life stages from separate studies (e.g., Smith and Ridgway, 2019). For the early life stages (young fry and earlier), there has been especially little research on thermal tolerance in brook trout, and no research conducted on carryover effects. One study examined the upper temperature thresholds (upper incipient lethal temperature) of

newly hatched (20°C) and swim-up alevin (24.5°C) (McCormick et al., 1972). The current work fills in that gap by not only examining CT_{max} and acclimation responses in embryos but also examining carryover effects of subsequent post-hatch life stages. Additionally, we discovered a clear effect of life stage on CT_{max} as upper thermal tolerance differed across ontogeny (sub-adults in Wells et al., 2016; adults in O'Donnell et al., 2020; review in Smith and Ridgway, 2019). When looking at the mean upper thermal tolerance of each life stage (embryos, fry, and gravid adults) from the ambient treatment groups they were 23°C, 28°C, and 27 °C, respectively. Embryos exhibited the lowest CT_{max} in comparison to all subsequent life stages, while the 10-week post-hatch fry were the most thermal tolerant (exhibited the highest CT_{max}) in comparison to the older fry and gravid adults (see Figure 2). Our data partially supports that of Dahlke et al. (2020) as brook trout embryos were the most thermally vulnerable life stage. By contrast, reproductively mature adults in our study were more thermally tolerant than anticipated as per Dahlke et al. (2020). This could be due to the thermal variation in hatchery versus wild populations, as our study used hatchery reared fish with little to no bioenergetic limitations such as foraging costs, food availability, predator avoidance, etc., whereas Dahlke et al. (2020) used wild populations. Further work is required to assess thermal tolerance at the life stages between embryos and swimming fry (i.e., alevin and larval stages).

A possible limitation to this study was the variation in the density of fish in CT_{max} trials. Past and present social experiences can impact the behavioural response of fish to high temperatures (Riepe et al., 2023; Melanson et al., 2023). Although high densities of

bluehead suckers (*Catostomus discobolus*) during CT_{max} trials significantly decreased upper thermal tolerances by 5.9°C (Riepe et al., 2023), a short period of prior social experience for mangrove rivulus fish (*Kryptolebias marmoratus*) led to more thermally tolerant individuals who emerged at warmer temperature than socially naïve fish, suggesting an increase in thermal tolerance with social experience (Melanson et al., 2023). Additionally, chronic social stress due to established dominant/subordinate relationships in rainbow trout (*Oncorhynchus mykiss*) can lead to decreases in the thermal tolerance of subordinate fish on a cellular and tissue-specific scale (LeBlanc et al., 2011). Our fish were habituated to living in high densities in a hatchery setting, so it seems unlikely that modest variation in density among our CT_{max} trials had a meaningful systematic effect on our findings. However, further research assessing the effect of density and social stress on CT_{max} in brook trout and other fishes would be useful.

Future research should focus on a wider range of incubation temperatures and thermal tolerance metrics to examine repeatability and reliability and alter captive rearing and holding conditions to promote natural conditions. Future incubation temperatures should include stable versus variable temperatures, such as seasonality and daily fluctuations (Wehrly et al., 2011; Zeigler et al., 2013), and additional winter-thermal regimes including multiple cold-shock events (brought on by freeze thaw events). Different estimates of upper thermal tolerance besides CT_{max} should be conducted on all life stages, such as upper/lower incipient lethal temperatures (Rajaguru and Ramachandran, 2001), to have a more thorough comparison to strengthen our knowledge of the thermal niche of brook trout. Additionally, altering captive rearing and holding

conditions to better imitate natural conditions and natural life spans would help to refine hatchery and facility research in order to obtain research that is comparable to wild populations.

4.3 Conclusions and Significance

A significant unknown for wild fish populations is how changing thermal regimes during early development may influence the thermal performance at later life stages. Developmental conditions can potentially shift thermal performance curves (Cavieres et al., 2019) and other fitness related traits, such as metabolism, oxygen consumption, behaviour, and size in relation to fecundity throughout life history. It is also important to consider these parameters when studying cool- and cold-water species residing in northern climates. It is important to note that our study focused on responses at the whole-organism level but there is the potential for early rearing temperatures to affect subsequent developmental and phenotypic processes (Jonsson and Jonsson, 2014, 2019). Further examination of potential carryover effects of thermal stress during incubation and early development of cold-water fishes may be valuable for informing their sustainable management and conservation.

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Appendix

Appendix A – Figures and Tables

Figure A1. Stainless steel mesh egg baskets (9cm x 9 cm x 7.5 cm) used for embryo rearing at the Ontario Ministry of Natural Resources and Forestry Research Facility in Codrington, Ontario. Labelled 1-64 with A and B sections to partition families and populations within a thermal treatment (Lechner et al., 2023; Stewart et al., 2024). Each tank had a separate thermal treatment.

Figure A2. Partitioned cooler modified for fry CT$_{max}$ arena. Pumps and heaters were separated from the fish by a mesh screen allowing flow through. An additional pump filtered warm water evenly throughout the sections. An airstone was used to maintain 100% oxygen saturation (monitored using a YSI; YSI ProSolo ODO Optical Dissolved Oxygen Meter https://www.ysi.com/prosolo-odo), and temperature was recorded using an RBR ProSolo Temperature logger (https://rbr-global.com/).

Figure A3. Non-partitioned cooler modified for fry CT_max arena. Pumps and heaters were separated from the fish by a mesh screen allowing flow through. An airstone was used to maintain 100% oxygen saturation (monitored using a YSI; YSI ProSolo ODO Optical Dissolved Oxygen Meter https://www.ysi.com/prosolo-odo). Temperature was recorded using a factory calibrated ThermoWorks Precision & Reference Thermometer (ThermoWorks, Inc., Utah, USA; https://thermoworks.com, accuracy ± 0.03°C). This set-up was used for 27+ weeks post-hatch fry from both Experiment 1 and 2.

Table A1. Summary of AIC models for predicting CT_max of brook trout (*Salvelinus fontinalis*) fry at 10 weeks post-hatch reared under varying thermal regimes as embryos. Models are listed in order of best-fit for each set of candidate models.

Treatment = thermal regime incubation treatment as embryos. Population = genetic

population. Roman numerals indicate the eleven models.

Model	K	AIC	ΔAIC	CWt	Pseudo_R2
i. Treatment + Population + Total Length	9	1028.71	0	1	0.32
ii. Treatment + Population + Mass	9	1047.83	19.12	1	0.30
iii. Treatment + Mass	6	1068.72	40.01	1	0.29
iv. Population + Total Length	6	1118.19	89.48	1	0.25
v. Population + Mass	6	1154.56	125.85	1	0.23
vi. Fulton's K	3	1179.66	150.95	1	0.21
vii. Total Length	3	1184.48	155.76	1	0.20
viii. Mass	3	1187.97	159.26	1	0.2
viv. Treatment + Population	8	1279.21	250.49	1	0.15
x. Treatment	5	1356.26	327.55	1	0.09
xi. Population	5	1426.12	397.42	1	0.04

Table A2. Summary of AIC models for predicting CT~max~ of brook trout (*Salvelinus fontinalis*) fry at 27-30 weeks post-hatch reared under varying thermal regimes as embryos. Models are listed in order of best-fit for each set of candidate models. Treatment = thermal regime incubation treatment as embryos. Population = genetic population. Roman numerals indicate the seven models.

Model	K	AIC	ΔAIC	CWt	Pseudo_R2
i. Treatment + Total Length	7	355.99	0	0.82	0.13
ii. Total Length	3	359.14	3.16	0.99	0.103
iii. Treatment + Mass	7	365.19	9.21	0.99	0.11
iv. Mass	3	365.91	9.92	1	0.086
v. Fulton's K	3	399.63	43.65	1	0.00033
vi. Treatment	6	403.04	47.06	1	0.0072
vii. Treatment + Fulton's K	7	404.98	49	1	0.0075

Table A3. Summary of AIC models for predicting CT~max~ of 2+ year adult brook trout (*Salvelinus fontinalis*) reared under varying thermal regimes as embryos. Models are listed in order of best-fit for each set of candidate models. Treatment = thermal regime incubation treatment as embryos. Roman numerals indicate the four models.

Model	K	AIC	ΔAIC	CWt	Pseudo_R2

i. Treatment + Mass	4	171.86	0.0	0.36	0.019
ii. Treatment	3	171.96	0.10	0.71	0.005
iii. Treatment + Fork Length	4	173.64	1.77	0.86	0.0085
iv. Treatment + Total Length	4	173.78	1.92	1.0	0.008

Appendix B – Abstracts of Non-Thesis Publications

Publication #1 Stewart, E. M., **Lechner, E. R**., Frasca, V. R., Wilson, C. C., & Raby, G. D. (2024). Differences in embryo survivorship among populations of brook trout (*Salvelinus fontinalis*) under variable winter thermal regimes. *Environmental Biology of Fishes*, *107*, 189-202.

The vulnerability of fish populations to climate change is driven primarily by impacts on their most vulnerable life stages. The earliest life stages may, in many cases, be more thermally sensitive than juvenile and adult fish, as developing embryos cannot behaviourally thermoregulate. We quantified the survivorship of brook trout (*Salvelinus fontinalis*) embryos under a range of winter thermal regimes. In the laboratory, embryos from four populations with varying amounts of native or hatchery-introgressed ancestry were reared under four simulated winter thermal regimes relevant to the central portion of the species' range: ambient stream temperatures, ambient + 2 °C, ambient with a simulated winter snowmelt event, and ambient followed by an early rise to spring temperatures. Embryo survival (hatching success) differed significantly among populations across all thermal regimes, with the pure native population having the lowest survival. No consistent differences in survivorship across thermal regimes were detected, although native populations exhibited more among-family variability in response to thermal stress. Our study does not align closely with others that found reduced embryo survival under thermal stress but concurs with observations of substantial intraspecific variation in thermal performance in coldwater fishes. Our results suggest that brook trout

embryos may be somewhat resilient in the face of warmer and more variable incubation conditions, but it is likely that their resiliency varies among populations. Future experiments that include more extreme and variable conditions to clarify environmental limits of early life stages of brook trout would be useful for conservation planning.

Publication #2 Bihun, J, C., Stewart, E. M. C, **Lechner, E. R**., Brownscomb, J. W., and Raby, G. D. (2024). Thermal performance curves for aerobic scope and specific dynamic action in a sexually dimorphic piscivore: implications for a warming climate. (Accepted, *Journal of Experimental Biology*).

Digestion can make up a substantial proportion of animal energy budgets, yet our understanding of how it varies with sex, body mass, and ration size is limited. A warming climate may have consequences on animal growth and consumption that will differentially impact individuals and their ability to efficiently acquire and assimilate meals. Many species, such as walleye (*Sander vitreus*), exhibit sexual size dimorphism, whereby one sex is larger than the other, suggesting sex-differences in energy acquisition and/or expenditure. Here we present the first thorough estimates of specific dynamic action (SDA) in adult walleye using intermittent-flow respirometry. We fed male (n = 15) and female (n = 9) walleye two ration sizes; 2% and 4% of individual body weight, over a range of temperatures from 2 – 20°C. SDA was shorter in duration and reached higher peak rates of oxygen consumption with increasing temperatures. Peak SDA increased with ration size and decreased with body mass. The proportion of digestible energy lost to SDA (i.e., the SDA coefficient) was consistent at 6% and

was unrelated to temperature, body mass, sex, or ration size. Our findings suggest that sex has a negligible role in shaping SDA. Standard metabolic rates were similar between sexes, but maximum metabolic rate and aerobic scope was ~13% higher in males across temperatures. Large female fish, which are important for population growth due to reproductive hyperallometry, may therefore face a bioenergetic disadvantage and struggle most to perform optimally in future, warmer waters.