THE INFLUENCE OF TEMPERATURE VARIABILITY ON METRICS OF LIFE
HISTORY IN BROOK TROUT (*SALVELINUS FONTINALIS*)

by

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Abstract

Increased mean annual temperatures and temperature variability associated with climate change is expected to have detrimental effects on the distribution, growth, survival, and phenology of many commercially and recreationally important cold-water fish. Using a common-garden experiment, we investigated the phenotypically plastic responses of two populations of brook trout (*Salvelinus fontinalis*) to bidirectional temperature variability. Specifically, we examined the effects of four temperature variability treatments (temperature constancy, periodic variability, low stochasticity, and high stochasticity) on mortality and average total fish length over a 41-day period. Fish populations were then monitored for a subsequent 47 days at a normal, consistent temperature. Trout exposed to the high stochasticity treatment (Treatment 4) consistently experienced the lowest survival and had significantly more deaths than fish in all other treatments in two of the three time-period cycles analysed. Seventeen days following the experiment, the fish exposed to the high stochasticity treatment (Treatment 4) continued to experience significantly more deaths than all the other treatments. While there were no significant differences between treatments observed at the end of the monitoring period, a reversal of the general trends was observed, in which survival was found to decrease with decreasing temperature variability. Trout from the WN population experienced significantly more deaths than the OB population throughout the monitoring period. Average total fish length was significantly higher in the temperature constancy (Treatment 1) and low stochasticity (Treatment 3) treatments than in the periodic variability (Treatment 2) and high stochasticity (Treatment 4) treatments. Trout from the WN population were significantly smaller when compared to the OB population. There was no statistical relationship observed between survival and constancy at any time during the study, while a significant, positive relationship between average total length and constancy was found for the OB population. While a portion of our results could be attributed to issues during the experimental period, our findings generally suggest that high temperature variability has a significant, negative effect on metrics of fitness in brook trout. Additionally, brook trout originating from smaller populations potentially exhibit adaptive plasticity with regards to survival and growth when faced with environmental stressors, such as temperature variability.

List of Abbreviations Used

Abbreviation/Symbol	Definition
ANOVA	Analysis of Variance
AR5	The fifth IPCC Assessment Report
CI	Confidence Interval
c.v.	Coefficient of Variation
DY	Ditchy
FW	Freshwater River
GLM	Generalized Linear Model
GLMM	Generalized Linear Mixed-Effects Model
IPCC	Intergovernmental Panel on Climate Change
LM	Linear Model
OB	Lower Ouananiche Beck
RCP2.6	Representative Concentration Pathway + 2.6 W/m^2
RCP8.5	Representative Concentration Pathway + 8.5 W/m^2
WN	Watern Cove

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Chapter 1

Introduction

Within the current century, the growing threat of climate change is expected to increase global air temperatures while accelerating the retreat of mountain glaciers, reducing Arctic sea ice, and increasing global sea levels (McGinn 2002). Each of the last three decades has been increasingly warmer at the Earth's surface, with the 30-year period from 1983 to 2012 likely being the warmest period of the last 1400 years in the Northern Hemisphere (IPCC 2014). The fifth Intergovernmental Panel on Climate Change (IPCC) assessment report (AR5) predicts that the global mean surface temperature change will likely be between 0.3°C to 0.7°C by 2035, and likely between 0.3°C to 1.7°C under the lowest emissions scenario (RCP2.6) and 2.6°C to 4.8°C under the highest emissions scenario (RCP8.5) by 2100. The AR5 also predicts increases in mean annual temperature over all land areas of North America in the mid- and late-21st century periods under both RCP2.6 and RCP8.5. The largest changes in mean annual temperatures are expected to occur over the high latitudes of the United States and Canada, along with most of eastern Canada, where changes greater than 6°C are expected in the late-21st century under RCP8.5. In addition, projections also indicate substantial increases in the occurrence of extremely hot weather events over North America in all periods of the 21st century (Romero-Lankao et al. 2014).

The effects of climate change on bodies of fresh water have already been observed. For example, in the Experimental Lakes Area of northwestern Ontario, mean annual air temperatures increased by 2°C and evaporation rates increased by 30% from the late 1960s to the mid 1980s (Schindler et al. 1990). In addition to rising temperatures, human population densities are also expected to increase in North America, with the population expected to grow and reach between 531.8 and 660.1 million by 2050 (Statistics Canada 2001; IIASA 2007). The continued growth of the North American population will no doubt increase pressure on freshwater resources, ultimately resulting in detrimental effects on the supply and quality of freshwater lakes and rivers across the continent (Chu et al. 2005).

Thermal regimes in freshwater ecosystems are extremely important to fish and other ectothermic aquatic organisms, as their physiological processes are directly controlled by

the conditions of the surrounding environment (Neuheimer and Taggart 2007; Buisson et al. 2008; Pörtner and Farrell 2008; Durance and Ormerod 2009). As such, temperature strongly influences the distribution and presence of individual species across spatial and temporal scales (Brannon et al. 2004; Rieman et al. 2007; Wenger et al. 2011a). Determining the impacts of climate change on aquatic resources is of particular concern for commercially and recreationally important cold-water fish, such as trout and salmon, for which some populations are experiencing stress as a result of unsuitably high temperatures and concomitant reduction of habitat (Mohseni et al. 2003; Chu et al. 2005; Rieman et al. 2007; Isaak et al. 2010; Wenger et al. 2011b). Increased temperatures associated with climate change are known to have a variety of impacts on freshwater fishes, including changes in bioenergetics and growth (Brandt et al. 2002), geographical shifts in distribution (Rahel 2002), and altered trophic dynamics (Jackson and Mandrak 2002). Climate change has also been hypothesized to have consequences for overall biodiversity, community structure, and phenology (Walther et al. 2002).

It has been argued that phenology (e.g. reproducing at the optimal time of year) is one of the most essential factors in adaptation to climate change in poikilotherms. As such, evolutionary change is likely to occur for traits and genes related to phenology, physiology, and behavior as compared to thermotolerance (Bradshaw and Holzapfel 2008; Jensen et al. 2008). Poikilotherms also generally respond to unfavorable environmental conditions with changes in behavior (Coutant 1987). This is particularly relevant to phases of the life cycle during which individuals are unable to escape or avoid suboptimal environmental conditions by movement, such as during immobile juvenile life stages (Bradshaw and Holzapfel 2008; Jensen et al. 2008).

In salmonid fishes, adaptation to changing temperature conditions would most likely be important during the incubation of eggs and post-fry emergence. The timing of fry emergence as it relates to exogenous food availability and the amount of remaining endogenous yolk reserves is highly affected by temperature conditions and is fundamental to fry survival (Jensen et al. 2008). The critical phase that occurs following fry emergence is typically characterized by strong, density-dependent competition for food and territory in addition to high predation. As such, mortality rates during the early life stages of salmonids are typically close to 90% (Elliott 1994). However, mortality during this period

is considered to be size selective (Einum and Fleming 2000), such that larger fry are more resistant to starvation (Einum and Fleming 1999), more resistant to size-limited predators (Werner and Gilliam 1984), and likely able to exploit larger food items (Wankowski 1979). Temperature conditions may dramatically impact the early development and growth of salmonid fishes, thus potentially limiting individual survival and success (Jensen et al. 2008).

Climate change will also likely impact the quality and quantity of habitats available to anadromous salmonid species that migrate back to fresh water each year to spawn. These species undertake their migrations during the summer period, when high average temperatures and extreme hot weather events are now occurring more frequently (Isaak et al. 2012). These migrations have occasionally become disrupted during warmer weather periods, as the fish need to pause to congregate near cooler water sources (Goneia et al. 2006; Sutton et al. 2007; Keefer et al. 2009), and the fish that do migrate during these warmer weather periods are often not as successful in returning to their spawning areas (Cooke et al. 2004; Keefer et al. 2008). Thermal "events" have also been recently documented (Lynch and Risley 2003; Doremus and Tarlock 2008; Keefer et al. 2010), manifest by the simultaneous deaths of hundreds or thousands of adult salmon in environmental conditions that exceeded their thermal tolerances. Therefore, while temperature may play an important role in the survival and growth of a fish post-emergence, it may also negatively inhibit spawning events.

The persistence of a species depends on its ability to resist and respond to anthropogenic and natural environmental change, as well as the spatial scale at which adaptation to this environmental change occurs. The ability of a population to respond to environmental change relates in part to the level of phenotypic plasticity expressed within that population, while the spatial scale of adaptation to this change relates to genetic differences in plasticity between populations (Hutchings et al. 2007). Several species occur across temperature and seasonality clines along a variety of gradients, and typically exhibit adaptive variation in morphological and physiological traits (Endler 1986). Spatial climate gradients may, therefore, be useful in identifying mechanisms of adaptation indicative of evolutionary responses to climate change (Baumann and Conover 2011).

Phenotypic plasticity can be described as the ability of a genotype to produce different phenotypes across an environmental gradient (Schlichting and Pigliucci 1998; Sultan and Stearns 2005). Adaptive phenotypic plasticity in salmonid fishes might evolve in response to environmental changes experienced in early life. Plasticity can be adaptive given that: (1) environmental variation has a substantial influence on fitness; (2) traits respond in a predictable way to different environments; and (3) the phenotypic response to environmental change has a genetic basis and is capable of responding to natural selection (Hutchings 1996). Norms of reaction are linear or nonlinear functions that describe how the phenotypic value of a trait for a given genotype changes with the environment. At the population level, they can be used to describe how individuals will, on average, respond to changes in an environmental variable (Hutchings et al. 2007).

One of the most effective means of assessing the genetic basis of phenotypic variation is to conduct a common-garden experiment in which individuals from different groups are reared under the same environmental conditions (Imsland and Jónsdóttir 2003; Conover et al. 2006). As such, group-level differences in a trait of interest, or a norm of reaction, can be indicate that these differences have a genetic and potentially adaptive basis (Hutchings et al. 2007).

Brook trout (*Salvelinus fontinalis*) are coldwater obligates that occur in clear, cool, well-oxygenated streams and lakes in a native range that extends from northern Quebec, Canada, south to the U.S. state of Georgia (Meisner 1990; Keleher and Rahel 1996; Scott and Crossman 1998). These fish actively seek out groundwater upwellings of cold water as a means of thermal refuge (Biro 1998), and are dependent on these seeps in several developmental events and life stages (Ridgway and Blanchfield 1998; Ridgway 2008). However, there is evidence to suggest that brook trout have upper temperature tolerances between 28° and 30°C (Lee and Rinne 1980; Benfey et al. 1997; Selong et al. 2001).

The present study examines the effects of thermal correlates of climate change on metrics of fitness in a widely distributed fish. It aims to evaluate the genetic basis of responses by brook trout to the bidirectional variability in temperature forecast to be associated with climate change (Jensen et al. 2008). It also aims to quantify population-genetic differences in phenotypically plastic responses to bidirectional temperature variability associated with climate change, particularly at small spatial scales.

Chapter 2

Materials and Methods

2.1 Source Populations

Brook trout inhabit streams on Cape Race, Newfoundland, Canada (bounded by 53°16' W, 46°45' N, 53°04' E, and 46°38' S; Hutchings 1990; Figure 2.1). This small, barren, coastal region is characterized by extensive areas of heath moss and patches of stunted boreal forest. The region is traversed by a parallel series of short (0.27-8.10 km), low-order streams, with most streams containing resident trout populations (Wood et al. 2014). These populations offer several appealing attributes for studies of life-history variability and phenotypic plasticity across a small spatial scale: (1) populations are pristine and largely unexploited due to the small average size of individuals (commonly <15 cm); (2) several populations do not differ significantly in fish density and many do not experience interspecific predation or competition; (3) most streams end in 30-50 m waterfalls emptying directly into the sea, which effectively eliminates gene flow among populations; and (4) populations exhibit substantial differences in life histories, which is thought to be the result of changes to environmental selective regimes following habitat fragmentation in the region (Hutchings 1993; Belmar-Lucero et al. 2012; Wood et al. 2014). Phylogeographic work on Cape Race suggests that the trout populations originated from a common ancestor and have been isolated since the Wisconsin deglaciation (10 000-12 000 years before present; Danzmann et al. 1998).
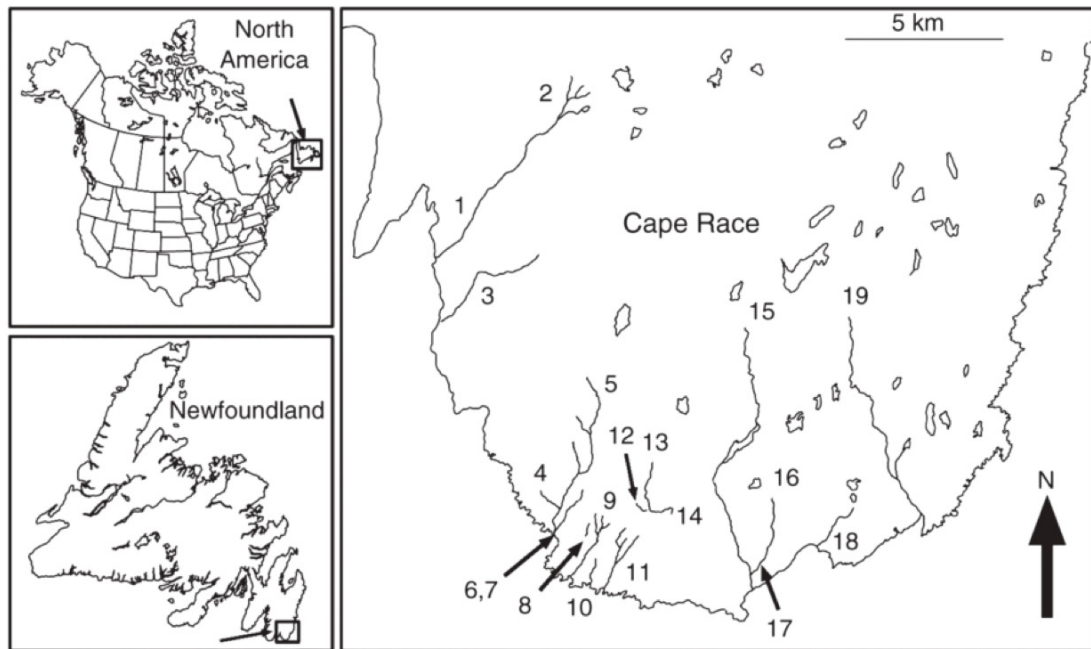
Figure 2.1 Geographic locations of all previously analyzed study streams in Cape Race, Newfoundland, Canada. Numbers on the inset correspond to the names of the study streams, in which the two streams examined are designated by: (14) Lower Ouananiche Beck (OB) and (15) Watern Cove (WN). Figure retrieved from Wood et al. 2014.

2.2 Experimental Design

Mature adult trout randomly sampled from four Cape Race populations (Ditchy (DY; 46° 38.940' N, 53° 11.424' W), Watern Cove (WN; 46° 37.942' N, 53° 09.546' W), Lower Ouananiche Beck (OB; 46° 38.971' N, 53° 10.992' W), and Freshwater River (FW; 46° 38.760' N, 53° 13.304' W)) were reared in flow-through, 90-litre aquaria at Dalhousie University, beginning in March 2015. Although the aquaria could hold 90 litres, the aquaria were only filled with 40 litres of water. Groups of brook trout of unequal densities were non-randomly assigned to aquaria on either the top or bottom shelf of an aquarium rack. Brook trout from only one of the four populations was present in any given aquarium. All aquaria experienced the same fluctuations in water temperature and photoperiod, and were provided with the same amount of food, namely dry Corey Aquafeeds 3 mm pellets. Within the experimental laboratory, light intensity was held constant. However, aquaria on the top shelf of the rack likely received more light than aquaria on the bottom shelf.

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On November 2, 2015, the water temperature experienced by all aquaria was reduced to induce spawning. The four populations were spawned on different dates in December 2015 because of difficulties in obtaining adequate quantities of sperm and eggs. The FW population was spawned on December 10th, the DY population was spawned on December 17th, and the OB and WN populations were spawned on December 20th. All fish were culled following spawning due to the spread of cotton-wool disease in the aquaria. Cotton-wool disease is characterized by grey-white filaments emerging from lesions throughout the body surface, giving the appearance of fungus or cotton, most notably caused by the presence of the pathogen *Flavobacterium columnare* (Decostere et al. 1998). Fertilized eggs from each population were collected and placed in separate 9-litre, flow-through aquaria to acclimate to laboratory conditions at a water temperature of approximately 7°C. Initial fertilization attempts were only successful for the WN and DY populations, who each produced several hundred viable offspring. The OB and FW populations each produced fewer than fifty viable offspring. Due to a high number of mortalities shortly after egg fertilization, the number of surviving individuals in each population was insufficient for the intended experiment.

Mature adult brook trout from the same four Cape Race populations were being reared and spawned simultaneously at Concordia University in Montreal, Quebec, Canada. Once again, initial fertilization attempts were successful for only two of the four populations. On February 1, 2016, fertilized eggs and alevins from the WN and OB populations were transported to Dalhousie University. Nineteen tubes of OBxOB brook trout and fourteen tubes of WNxWN brook trout were acclimated to laboratory conditions in small, 2.8-liter flow-through aquaria and exposed to the same mean temperature of approximately 5°C.

In this common-garden experiment, brook trout fry were subjected to one of four temperature variability scenarios, namely: (1) a constant temperature of 10°C; (2) a consistent, cyclical fluctuation in temperature of 6°C every two days, with temperatures ranging from 7° to 13°C; (3) a stochastic or random fluctuation in temperature of 6°C every

two days, with temperatures ranging from 7° to 13°C; and (4) a treatment analogous to (3) but with the stochastic temperature change occurring every day.

There were five replicates of each treatment for the WN population (i.e. 20 tanks), and seven replicates of each treatment for the OB population (i.e. 28 tanks). Fourteen tanks were also established to quantify tank effects.

Each replicate tank contained twenty-seven fry at the beginning of the experimental period. Fish were randomly selected for each tank from a pool of all available fish in a given population. The individuals were placed in their experimental tanks one week prior to the start of the experiment to allow sufficient time to acclimate. All tanks were subjected to the same environmental conditions. As such, all fish were reared in identical tanks and experienced the same photoperiod, light intensity, water flow, and food.

The temperatures associated with treatments 3 and 4 were chosen randomly by means of a dice roll. Each of the three temperatures was randomly associated with two numbers on a six-sided dice. For each temperature change that occurred in the experiment, the dice was rolled to determine the next temperature. Treatment 2 followed a cyclical temperature pattern of 7°C-10°C-13°C-10°C-7°C. This pattern was repeated throughout the experiment. The temperatures that each treatment were subjected to were determined prior to the start of the experiment (Appendix A).

On April 26, 2016, three aquaria racks, each able to support sixty 2.8-litre, flow-through tanks, were established at one of the three experimental temperatures (Rack A: 7°C, Rack B: 10°C, and Rack C: 13°C) in the laboratory. Tank-effect tanks were also added to the 7°C and 13°C racks, with seven of these tanks on each rack. These tanks were randomly allocated to one of seven positions on the rack, and kept in the same positions throughout the experiment to account for potential tank effects (see Figure 2.2 for a schematic of positions).

The experimental tanks were separated by rack, or temperature, and randomly allocated to a position. Temperature changes (i.e. movements between racks) occurred every two days for the tanks associated with treatments 1-3, and every day for the tanks associated with treatment 4. Tanks were randomly allocated to a position on a rack every

time a temperature change occurred. Any tank not being moved to a different rack on a given day (i.e. staying at the same temperature) was nevertheless randomly re-allocated to a different position on the rack in order to avoid tank-position bias. The tanks associated with treatment 4 were only re-distributed on a given rack if they were subjected to the same temperature for more than two days.

All fish were fed daily with an identical mixture of live shrimp (*Artemia* sp.) and dry Corey Aquafeeds 0.7 mm pellets in each tank. The experiment ended on June 5, 2016. Over the course of the experiment, Rack A experienced an average temperature of 7.3°C±0.13, Rack B averaged 11°C±0.08, and Rack C averaged 13°C±0.07. On June 6, 2016, the experimental setup was disassembled. In order to maintain the integrity of the experiment, replicates for a given treatment and population combination were combined where possible, and all tanks were moved to the 7°C rack.

(a)

X	2	3	4	5	6	7	8	9	10	11	**X**
13	14	15	16	17	18	19	20	21	22	23	24
25	26	**X**	28	29	**X**	31	32	33	**X**	35	36
37	38	39	40	41	42	43	44	45	46	47	48
X	50	51	52	53	54	55	56	57	58	59	**X**

(b)

1	2	3	4	5	6	7	8	9	10	11	12
13	14	15	16	17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32	33	34	35	36
37	38	39	40	41	42	43	44	45	46	47	48
49	50	51	52	53	54	55	56	57	58	59	60

(c)

X	2	3	4	5	6	7	8	9	10	11	**X**
13	14	15	16	17	18	19	20	21	22	23	24
25	26	**X**	28	29	**X**	31	32	33	**X**	35	36
37	38	39	40	41	42	43	44	45	46	47	48
X	50	51	52	53	54	55	56	57	58	59	**X**

Figure 2.2 Available tank positions on (a) Rack A at 7°C, (b) B at 10°C, and (c) C at 13°C, with "X"s in bold indicating the presence of a tank-effect tank, for the main temperature variability experiment (April 26, 2016 – June 6, 2016). Tank-effect tanks remained in the same tank positions for the duration of the experiment, while all other experimental tanks were randomly allocated to a position on the rack with every temperature change.

2.3 Post-Treatment Monitoring Experiment

On June 23, 2016, a monitoring experiment was initiated. Three replicates per treatment for each of the two populations (24 tanks in total) were created from the existing tanks. There were two replicates of the 13°C tank-effect tanks and four replicates of the 7°C tank-effect tanks (6 tanks in total). To accommodate the growth of the fish, identical 9-litre, flow-through aquaria were used. The number of fish in each tank was not equal, as the number of available fish for each treatment and population combination was not the same (Appendix B). The water temperature was held constant for all tanks. Fish were fed daily with an identical mixture of dry Corey Aquafeeds 0.7 mm pellets and dry 1.2 mm pellets. The tank-effect tanks were allocated to specific positions on the rack, while the experimental tanks were randomly allocated to fill the other positions on the rack (see Figure 2.3 for a schematic of the positions). The position of all tanks remained constant until the end of the experiment on August 11, 2016. Due to an unforeseen logistical issue, the data from tanks containing fish from the WN population subjected to treatment 2 were omitted from all statistical analyses that were part of the monitoring experiment.

X	2	3	4	5	**X**
7	8	9	10	11	12
13	14	**X**	**X**	17	18
19	20	21	22	23	24
X	26	27	28	29	**X**

Figure 2.3 Available tank positions on Rack A, with "X"s in bold indicating the presence of a tank-effect tank, for the monitoring experiment (June 23, 2016-August 11, 2016). Tank-effect tanks remained in the same tank positions for the duration of the experiment. All other experimental tanks were randomly allocated to a position on the rack, but remained in the same position for the duration of the experiment.

2.4 Data Collection

Mortality data were recorded daily for each tank throughout the temperature-variability experiment and the subsequent monitoring experiment. Deaths that were

considered accidental (i.e. fish found on the floor or stuck in the tank sponge) were recorded, but were omitted from all statistical analyses.

The total length of *S. fontinalis* individuals was first measured on April 18, 2016, approximately one week prior to the start of the temperature-variability experiment. Twenty fish from each of the WN, OB, and tank-effect tanks (i.e. 60 fish in total) were measured to the nearest mm, using a ruler. Measurements were rounded to the nearest whole number. During the experiment, the total length of the *S. fontinalis* individuals was measured every eight days (i.e. one "Cycle") for a total of five measurements (measurement dates: May 5th, May 13th, May 22nd, May 29th, June 6th). Four individuals per replicate were measured for the WN population, while three or two individuals per replicate were measured for the OB population and tank-effect tanks, respectively, such that twenty fish per treatment and population combination were measured (200 fish in total). Length measurements were estimated by analyzing photographs of each fish, using ImageJ 1.50i software. A ruler (in mm) was used as a reference in each photograph, and measurements were taken between the two longest points on the body of each fish. Measurements were rounded to the nearest 0.1 mm. A final set of measurements was taken 107 days after the start of the experiment (November 8, 2016) for the WN and OB populations only. While the methodology remained the same, the sample sizes were considerably smaller, with between 6-12 fish measured per treatment and population combination (68 fish in total).

2.5 Analyses

2.5.1 Temperature Variability Experiment (2016)

We analyzed the data in a number of ways: (1) we examined the effect of population (WN, OB; fixed factor), treatment (1, 2, 3, 4; fixed factor), and replicate (random factor) on mortality (total number of deaths) in relation to the number of survivors (binomial, paired response) using generalized linear mixed-effects models; (2) we examined the effect of population (WN, OB; fixed factor), constancy (numeric fixed effect), and replicate (random factor) on mortality (total number of deaths) in relation to the number of survivors (binomial, paired response) using generalized linear mixed-effects models; (3) we

examined the effect of population (WN, OB; fixed factor), treatment (1, 2, 3, 4; fixed factor), the total number of surviving fish in each tank (numeric fixed effect), and replicate (random factor) on total length using linear mixed-effects models; and (4) we examined the effect of population (WN, OB; fixed factor), constancy (numeric fixed effect), the total number of surviving fish in each tank (non-factor, numerical), and replicate (random factor) on total length using linear mixed-effects models.

Constancy in this study is a parameter designed to account for the stochasticity within each temperature-variability treatment, defined as the number of the days where the temperature at day $x+1$ is equal to the temperature at day x. Tanks subjected to Treatment 1 had a constancy value of 40, tanks in Treatment 2 had a constancy value of 20, tanks in Treatment 3 had a constancy value of 26, and tanks in Treatment 4 had a constancy value of 14.

Mortality analyses were conducted for day 17 to 24 (Cycle 3), day 25 to 32 (Cycle 4), and day 33 to 41 (Cycle 5) separately, while total length analyses were conducted only for day 33 to 41 (Cycle 5). Model fitting and stepwise reduction of the models were performed with ANOVA. Treatment levels were combined where possible by pooling levels with similar intercept values. This was performed as a means of post-hoc analysis in order to determine the statistical relationships present between treatments.

The relationship between average total fish length (response) and constancy (numeric fixed effect) was assessed for each population using linear models. The relationship between survival at the end of cycles 3, 4, and 5, and constancy (numeric fixed effect) was also assessed; however, the survival data was transformed using an arcsine, or arcsine square root, transformation before completing the analysis.

2.5.2 Post-Treatment Monitoring Experiment

We analyzed the data in two ways: (5) we examined the effect of population (WN, OB; fixed factor), treatment (1, 2, 3, 4; fixed factor), and replicate (random factor) on mortality (total number of deaths) in relation to the number of survivors (binomial, paired response) using generalized linear mixed effects models; and (6) we examined the effect of population (WN, OB; fixed factor), constancy (non-factor, numerical), and replicate

(random factor) on mortality (total number of deaths) in relation to the number of survivors (binomial, paired response) using generalized linear mixed effects models. In order to account for the change in the total number of experimental tanks and the change in the densities of each tank, the dataset was analyzed in two parts: June 23rd to July 12th (Part 1), and July 13th to August 11th (Part 2). On July 12, 2016, the fish were re-distributed within the tanks and three replicates were removed from the experiment. Model fitting and stepwise reduction of the models were performed with ANOVA. Treatment levels were combined where possible by pooling levels with similar intercept values.

The relationship between survival at the end of Part 1 and Part 2, and constancy (numeric fixed effect) was assessed in the same manner as described above, using the same data transformations.

All analyses were conducted using the lme4 (Bates et al. 2016) package in R (Version 3.3.2; The R Foundation for Statistical Computing 2015).

Chapter 3

Results

3.1 Temperature Variability Experiment

3.1.1 Mortality

Survival decreased in all four treatments throughout the experimental period (Figure 3.1, Figure 3.2). For the WN population, Treatments 1, 2, and 3 demonstrated similar decreasing trends for most of the experimental time frame, while Treatment 4 displayed a more dramatic and noticeable decrease in survival, relative to the other three treatments, beginning on day 19 (Figure 3.2). While the fluctuations in survival of trout in Treatment 1 were similar to what was experienced by trout in Treatments 2 and 3 for much of the experiment, there was a sudden, sharp decrease in survival observed at day 39. At the end of the experiment, trout in Treatment 1 had experienced the lowest survival. However, survival in this treatment was only ~1% lower than Treatment 4, ~7.3% lower than Treatment 2, and ~11.4% lower than Treatment 3. As such, Treatment 3 experienced the highest survival at the end of the experiment, followed by Treatment 2 and then Treatment 4 (Figure 3.3).

For the OB population, all four treatments displayed similar decreasing trends throughout the experiment, with no one treatment experiencing more dramatic or noticeable decreases than the other (Figure 3.1). At the end of the experiment, trout in Treatment 4 had experienced the lowest survival. However, survival in this treatment was only ~3.5% lower than Treatment 1, ~9.7% lower than Treatment 2, and ~11.6% lower than Treatment 3. As such, Treatment 3 experienced the highest survival at the end of the experiment, followed by Treatment 2 and then Treatment 1 (Figure 3.4).
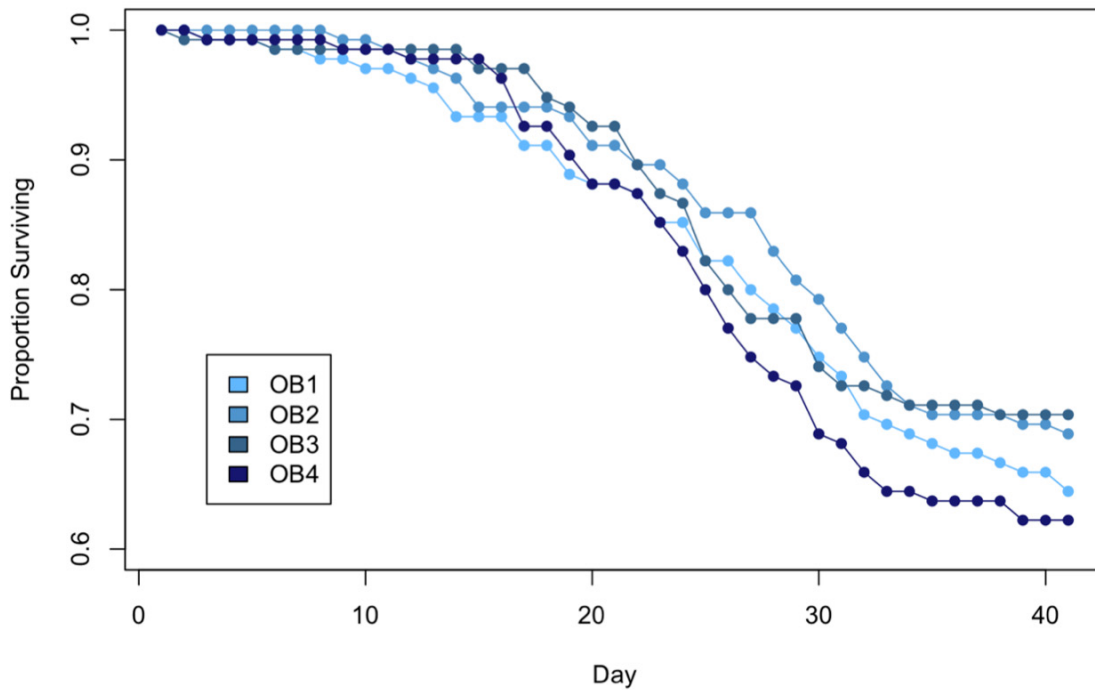
Figure 3.1 Proportion of surviving brook trout (*Salvelinus fontinalis*) from the OB population in four temperature variability treatments during the 41 days of the main temperature variability experiment (April 26, 2016 – June 6, 2016). Treatment 1 represents temperature constancy (a constant temperature of 10°C), Treatment 2 represents periodic variability, Treatment 3 represents low stochasticity, and Treatment 4 represents high stochasticity. Data collected at Dalhousie University in Halifax, Canada.

Figure 3.2 Proportion of surviving brook trout (*Salvelinus fontinalis*) from the WN population in four temperature variability treatments during the 41 days of the main temperature variability experiment (April 26, 2016 – June 6, 2016). Treatment 1 represents temperature constancy (a constant temperature of 10°C), Treatment 2 represents periodic variability, Treatment 3 represents low stochasticity, and Treatment 4 represents high stochasticity. Data collected at Dalhousie University in Halifax, Canada.

Figure 3.3 Proportion of surviving brook trout (*Salvelinus fontinalis*; ± 95% CI) from the WN population in four temperature variability treatments at the end of the main temperature variability experiment (Cycle 5; June 6, 2016). Treatment 1 represents temperature constancy (a constant temperature of 10°C), Treatment 2 represents periodic variability, Treatment 3 represents low stochasticity, and Treatment 4 represents high stochasticity. Data collected at Dalhousie University in Halifax, Canada.

Figure 3.4 Proportion of surviving brook trout (*Salvelinus fontinalis*; ± 95% CI) from the OB population in four temperature variability treatments at the end of the main temperature variability experiment (Cycle 5; June 6, 2016). Treatment 1 represents temperature constancy (a constant temperature of 10°C), Treatment 2 represents periodic variability, Treatment 3 represents low stochasticity, and Treatment 4 represents high stochasticity. Data collected at Dalhousie University in Halifax, Canada.

At the end of Cycle 3, trout in Treatment 4 had experienced significantly higher overall mortality than the combination of Treatments 1, 2, and 3 (Table 3.1). Interactions between Population and Treatment (df=40,43, p=0.2064), and Population as a fixed effect (df=45,46, p=0.1054) were not found be relevant, and were removed from the analysis. The model was first simplified by combining treatment levels 1 and 3 together (df=43,44, p=0.5625), followed by combining treatment levels 1+3 and 2 together (df=44,45, p=0.6075).

At the end of Cycle 4, trout in Treatment 4 had significantly more deaths (relative to survivorship) than the combination of Treatments 1, 2, and 3; the WN population was found to have significantly fewer deaths than the OB population (Table 3.2). Interactions between Population and Treatment were not found to be relevant, and were removed from the analysis (df=40,43, p=0.3417). The model was first simplified by combining treatment levels 1 and 3 together (df=43,44, p=0.9162), followed by combining treatment levels 1+3 and 2 together (df=44,45, p=0.7593).

At the end of Cycle 5, there was no significant difference in the number of deaths (relative to survivorship) between treatments (Table 3.3). Interactions between Population and Treatment (df=40,43, p=0.8294), and Population as a fixed effect (df=45,46, p=0.2289) were not found to be a relevant, and were removed from the analysis. The model was first simplified by combining treatment levels 1 and 4 together (df=43,44, p=0.8571), followed by combining treatment levels 1+4 and 2 together (df=44,45, p=0.1084).

Replicate as a random factor was not found to be relevant, and was removed from the analysis in Cycle 3 (accounted for 0%), 4 (accounted for 1.9%), and 5 (accounted for 3.8%).

Table 3.1 Results of the final, reduced GLM models, separated with a horizontal line, examining the effects of treatment (1, 2, 3, 4), and constancy on brook trout (*Salvelinus fontinalis*) mortality (total number of deaths) in relation to the number of survivors at the end of Cycle 3 of the main temperature variability experiment (May 21, 2016). Significant results are provided in bold.

Variable	DF	T-value	P-value
Treatment	47	2.59	**0.0128**
Constancy	47	-1.178	0.2450

Table 3.2 Results of the final, reduced GLM models, separated with a horizontal line, examining the effects of population (WN, OB), treatment (1, 2, 3, 4), and constancy on brook trout (*Salvelinus fontinalis*) mortality (total number of deaths) in relation to the number of survivors at the end of Cycle 4 of the main temperature variability experiment (May 29, 2016). Significant results are provided in bold.

Variable	DF	T-value	P-value
Population	47	-2.613	**0.0122**
Treatment	47	3.078	**0.0035**
Population	47	-2.433	**0.0190**
Constancy	47	-1.696	0.0968

Table 3.3 Results of the final, reduced GLM models, separated with a horizontal line, examining the effects of treatment (1, 2, 3, 4), and constancy on brook trout (*Salvelinus fontinalis*) mortality (total number of deaths) in relation to the number of survivors at the end of the main temperature variability experiment (Cycle 5; June 6, 2016). Significant results are provided in bold.

Variable	DF	T-value	P-value
Treatment	47	-1.945	0.0579
Constancy	47	0.178	0.8600

There is no relationship between survival at the end of the experimental period and constancy for the WN ($F_{1,2}=0.0617$, p=0.8270) and OB ($F_{1,2}=0.0066$, p=0.9429) populations (Figure 3.5). For both populations, survival appears to increase with increasing constancy, but then decreases sharply at the highest constancy value. There is no relationship between survival at the end of Cycle 4 and constancy for the WN population ($F_{1,2}=3.2940$, p=0.2112), despite survival being shown to increase with increasing constancy (Figure 3.6). There is also no relationship between survival at the end of Cycle 4 and constancy for the OB population ($F_{1,2}=0.0922$, p=0.7900). For this population, survival increases and then decreases with increasing constancy (Figure 3.6). There is no relationship between survival at the end of Cycle 3 and constancy for the WN ($F_{1,2}=0.9466$, p=0.4332) and OB ($F_{1,2}=0.0331$, p=0.8724) populations (Figure 3.7). Survival in the WN population increases with increasing constancy, but then decreases at the highest constancy value. Survival in the OB population, similar to Cycle 4, increases and then decreases with increasing constancy. In Cycle 3, 4, and 5, constancy was found to have no significant effect on the number of deaths (relative to survivorship; Table 3.1; Table 3.2; Table 3.3).
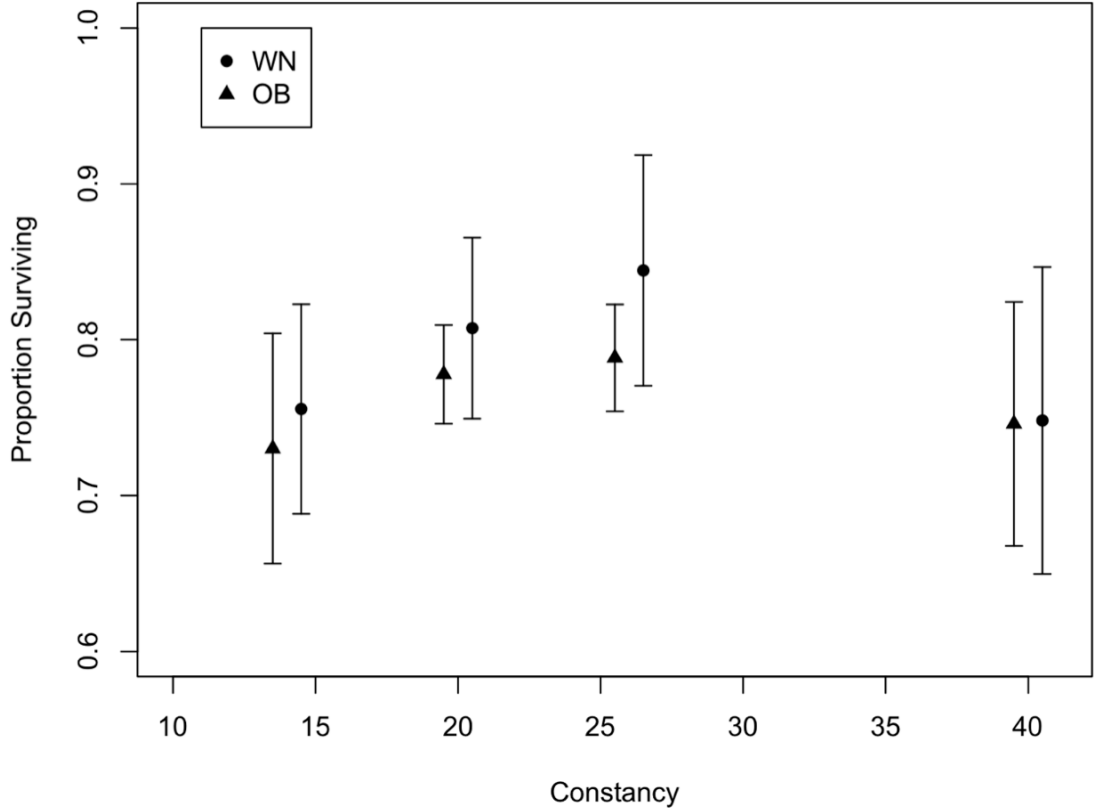
Figure 3.5 Relationship between the proportion of surviving brook trout (*Salvelinus fontinalis*; ± 95% CI) from the WN and OB populations and constancy at the end of the main temperature variability experiment (Cycle 5; June 6, 2016). Treatment 1 (temperature constancy) has a constancy value of 40, Treatment 2 (periodic variability) has a constancy value of 20, Treatment 3 (low stochasticity) has a constancy value of 26, and Treatment 4 (high stochasticity) has a constancy value of 14. Values on the x-axis are staggered by ± 0.5 in order to effectively present all trends. Data collected at Dalhousie University in Halifax, Canada.
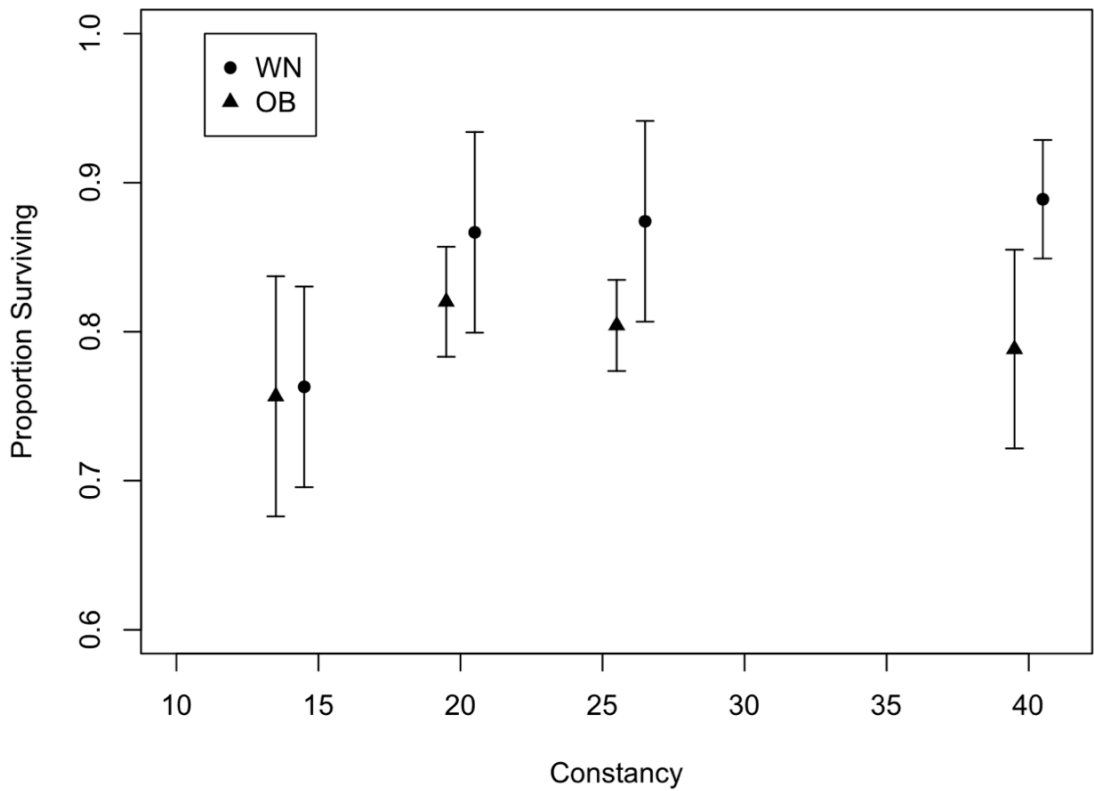
Figure 3.6 Relationship between the proportion of surviving brook trout (*Salvelinus fontinalis*; ± 95% CI) from the WN and OB populations and constancy at the end of Cycle 4 of the main temperature variability experiment (May 29, 2016). Treatment 1 (temperature constancy) has a constancy value of 40, Treatment 2 (periodic variability) has a constancy value of 20, Treatment 3 (low stochasticity) has a constancy value of 26, and Treatment 4 (high stochasticity) has a constancy value of 14. Values on the x-axis are staggered by ± 0.5 in order to effectively present all trends. Data collected at Dalhousie University in Halifax, Canada.
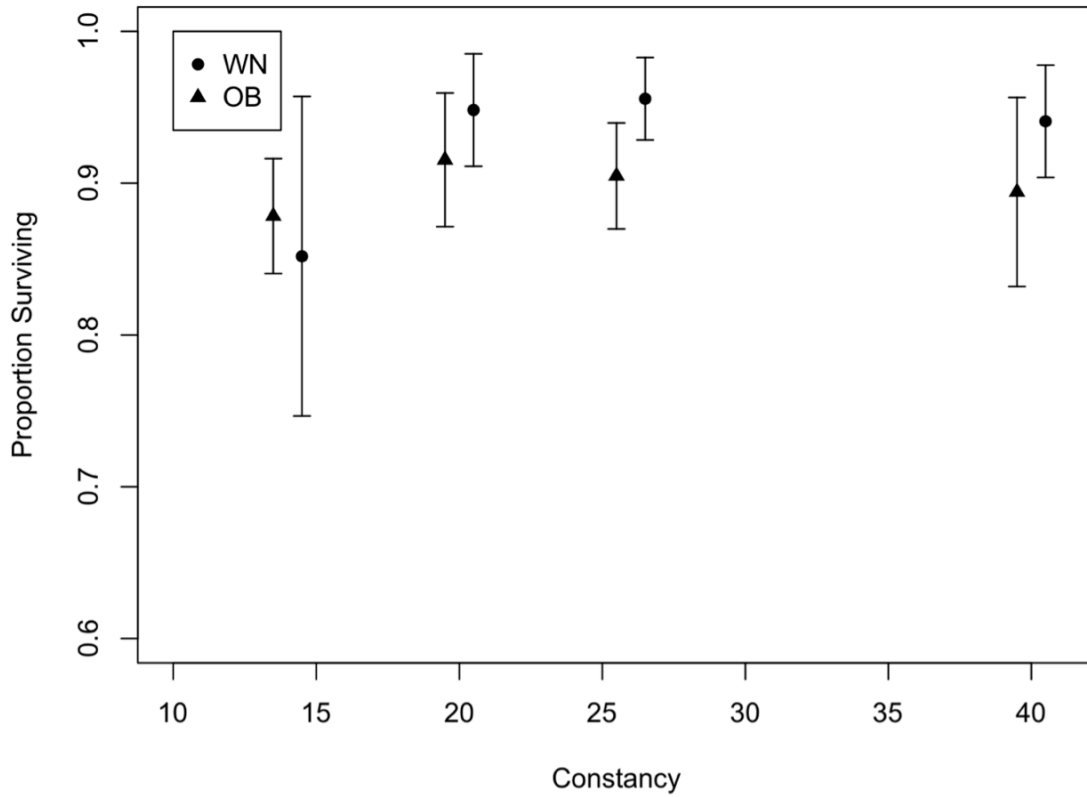
Figure 3.7 Relationship between the proportion of surviving brook trout (*Salvelinus fontinalis*; ± 95% CI) from the WN and OB populations and constancy at the end of Cycle 3 of the main temperature variability experiment (May 21, 2016). Treatment 1 (temperature constancy) has a constancy value of 40, Treatment 2 (periodic variability) has a constancy value of 20, Treatment 3 (low stochasticity) has a constancy value of 26, and Treatment 4 (high stochasticity) has a constancy value of 14. Values on the x-axis are staggered by ± 0.5 in order to effectively present all trends. Data collected at Dalhousie University in Halifax, Canada.

3.1.2 Total Length

Total fish length for the WN population at the end of the experiment was lowest in Treatment 2. Total fish length in this treatment was ~1.6% lower than Treatment 4, ~3.8% lower than Treatment 3, and ~5.6% lower than Treatment 1. As such, Treatment 1 experienced the highest total fish length at the end of the experiment, followed by Treatment 3 and then Treatment 4 (Figure 3.8). For the OB population, total fish length was lowest in Treatment 4. Total fish length in this treatment was ~5.3% lower than Treatment 2, ~6.8% lower than Treatment 3, and ~10.8% lower than Treatment 1. As such, Treatment 1 experienced the highest total fish length, followed by Treatment 3 and then Treatment 2 (Figure 3.9).
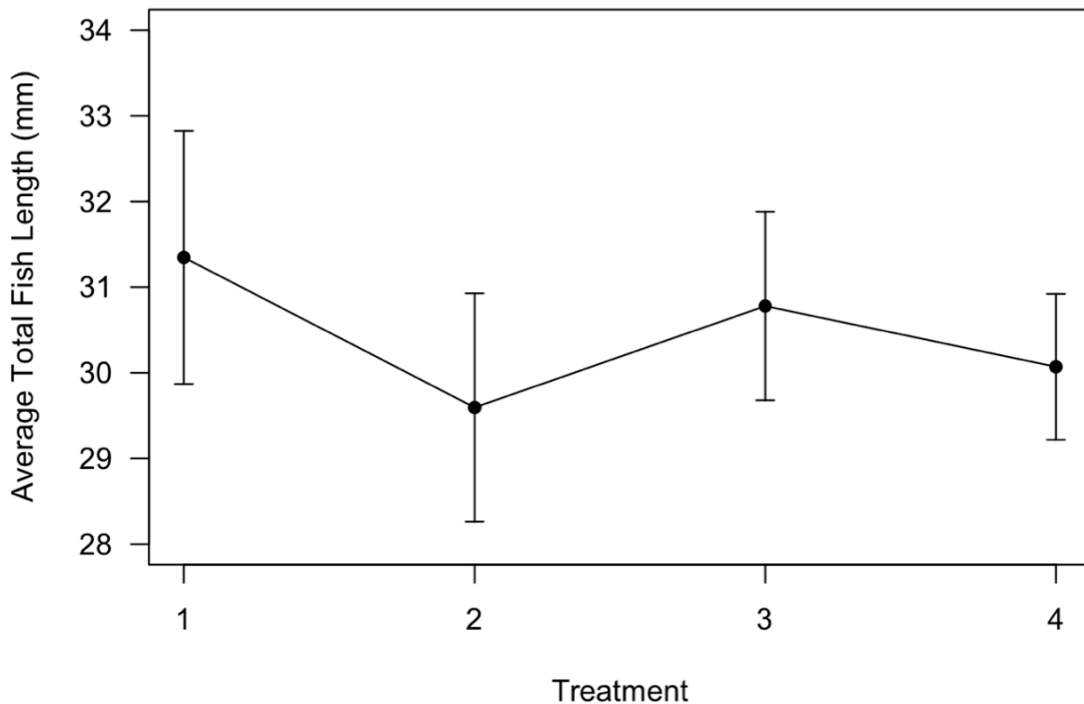
Figure 3.8 Average total length (mm) of brook trout (*Salvelinus fontinalis*; ± 95% CI) from the WN population in four temperature variability treatments at the end of the main temperature variability experiment (Cycle 5; June 6, 2016). Treatment 1 represents temperature constancy (a constant temperature of 10°C), Treatment 2 represents periodic variability, Treatment 3 represents low stochasticity, and Treatment 4 represents high stochasticity. Data collected at Dalhousie University in Halifax, Canada.
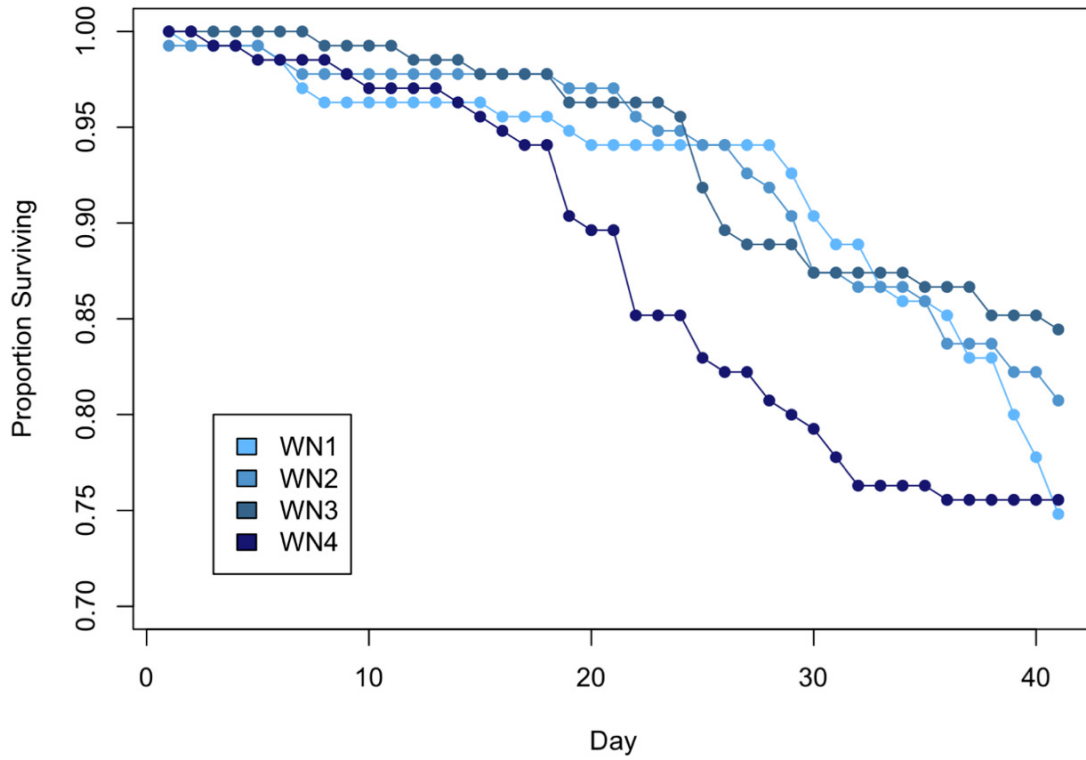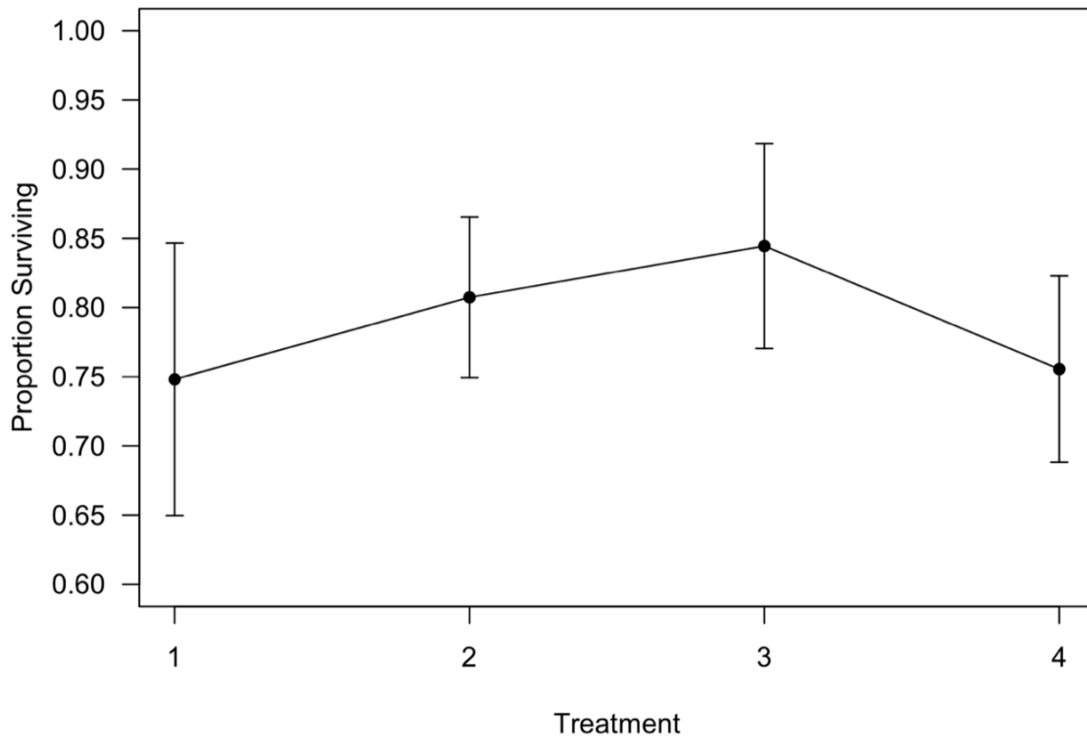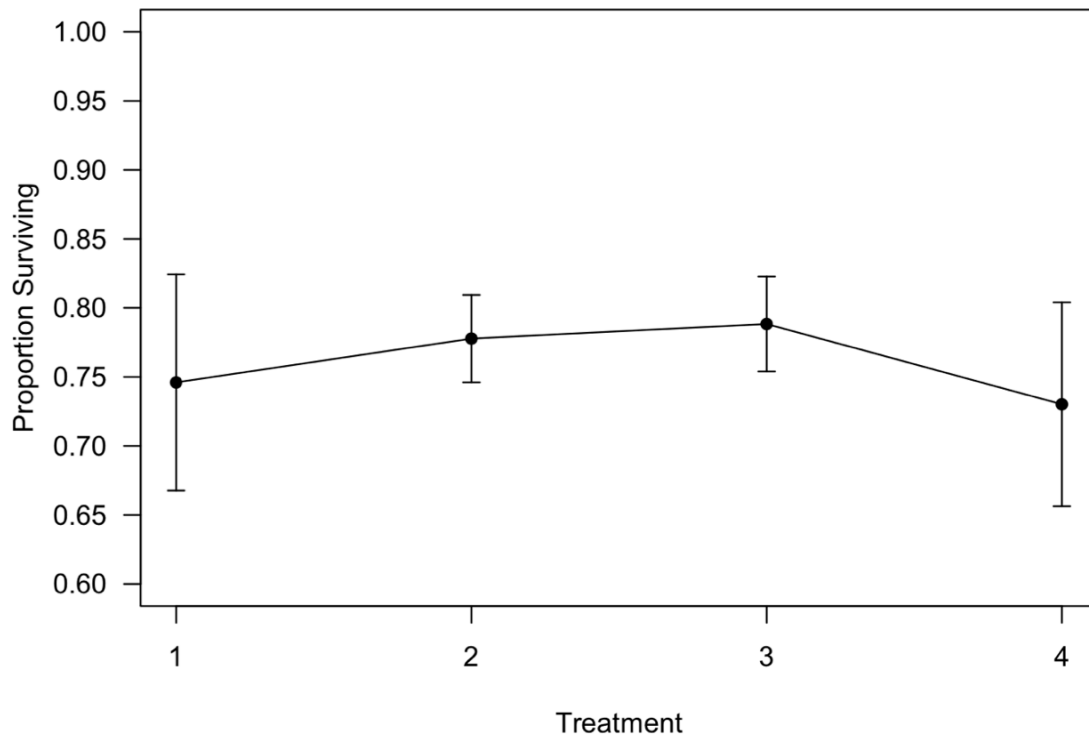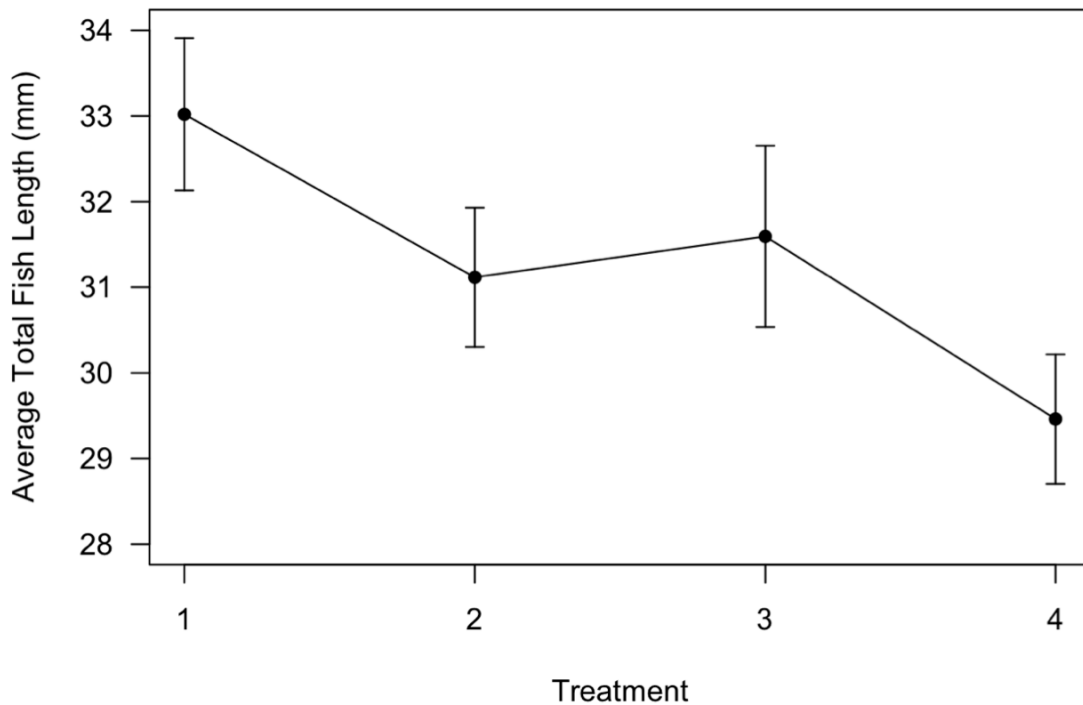
Figure 3.9 Average total length (mm) of brook trout (*Salvelinus fontinalis*; ± 95% CI) from the OB population in four temperature variability treatments at the end of the main temperature variability experiment (Cycle 5; June 6, 2016). Treatment 1 represents temperature constancy (a constant temperature of 10°C), Treatment 2 represents periodic variability, Treatment 3 represents low stochasticity, and Treatment 4 represents high stochasticity. Data collected at Dalhousie University in Halifax, Canada.

At the end of Cycle 5, the total fish length was significantly smaller in the combination of Treatment 2 and 4 compared to the combination of Treatment 1 and 3. Total fish length was also significantly smaller in the WN population compared to the OB population (Table 3.4). Interactions between Population and Treatment (df=149,152, p=0.1298), and the number of survivors (df=154,155, p=0.5362) were not found to be relevant and were removed from the analysis. The model was first simplified by combining treatment levels 1 and 3 together (df=152,153, p=0.0997), followed by combining treatment levels 2 and 4 together (df=153,154, p=0.2203).

Replicate as a random factor was not found to be relevant, and was removed from the analysis (accounted for 1.7%).

Table 3.4 Results of the final, reduced LM models, separated with a horizontal line, examining the effects of population (WN, OB), treatment (1, 2, 3, 4), and constancy on the average total length of brook trout (*Salvelinus fontinalis*) at the end of the main temperature variability experiment (Cycle 5; June 6, 2016). Significant results are provided in bold.

Variable	DF	T-value	P-value
Population	155	-2.168	**0.0317**
Treatment	155	-4.199	**<0.001**
Population	155	-2.188	**0.0301**
Constancy	155	4.717	**<0.001**

There is no relationship between total length and constancy for the WN population ($F_{1,2}$=5.793, p=0.1378). While survival initially decreases with increasing constancy, it does then continuously increase following the initial decline (Figure 3.10). There is a positive relationship between total length and constancy for the OB population ($F_{1,2}$=25.99, p=0.0364), with survival increasing with increasing constancy (Figure 3.10). At the end of Cycle 5, constancy was found to have a significant effect on total fish length (Table 3.4).
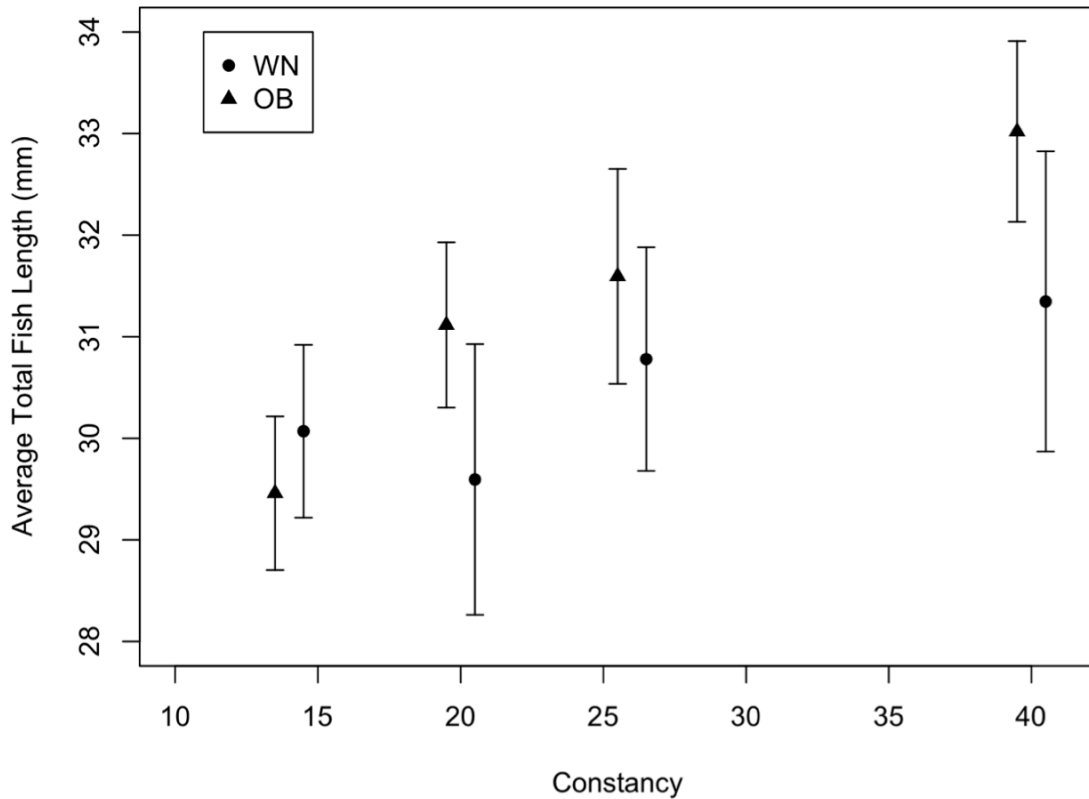
Figure 3.10 Relationship between the average total length (mm) of brook trout (*Salvelinus fontinalis*; ± 95% CI) from the WN and OB populations and constancy at the end of the main temperature variability experiment (Cycle 5; June 6, 2016). Treatment 1 (temperature constancy) has a constancy value of 40, Treatment 2 (periodic variability) has a constancy value of 20, Treatment 3 (low stochasticity) has a constancy value of 26, and Treatment 4 (high stochasticity) has a constancy value of 14. Values on the x-axis are staggered by ± 0.5 in order to effectively present all trends. Data collected at Dalhousie University in Halifax, Canada.

3.2 Post-Treatment Monitoring Experiment

3.2.1 Part 1

Survival decreased in all four treatments over the course of 17 days for both the WN and OB populations (Figure 3.11; Figure 3.12). For the WN population, it appears that the decreasing trends for all three treatments remained relatively similar until day 13, where following this point in the monitoring experiment, the survival of Treatment 1 appears to plateau and is dramatically higher than Treatment 3 and 4 (Figure 3.11). After 17 days, survival for the WN population was the lowest in Treatment 3. Survival in this treatment

was ~10.2% lower than Treatment 4, and ~24.6% lower than Treatment 1. As such, Treatment 1 experienced the highest survival after 17 days, followed by Treatment 4 (Figure 3.13).

For the OB population, the decreasing trends for all three treatments were once again relatively similar until day 13, after which the survival of Treatment 4 is lower than Treatment 2, and dramatically lower than Treatment 1 and 3 (Figure 3.12). After 17 days, survival for the OB population was lowest in Treatment 4. Survival in this treatment was ~11% lower than Treatment 2, 19.4% lower than Treatment 3, and ~19.8% lower than Treatment 1. As such, Treatment 1 experienced the highest survival after 17 days, followed closely by Treatment 3 and then Treatment 2 (Figure 3.14).

Figure 3.11 Proportion of surviving brook trout (*Salvelinus fontinalis*) from the WN population in three temperature variability treatments during the 17 days of Part 1 of the monitoring experiment (June 23, 2016 - July 12, 2016). Treatment 1 represents temperature constancy (a constant temperature of 10°C), Treatment 2 represents periodic variability, Treatment 3 represents low stochasticity, and Treatment 4 represents high stochasticity. The values for Treatment 2 were omitted due to an unforeseen logistical issue. Data collected at Dalhousie University in Halifax, Canada.

Figure 3.12 Proportion of surviving brook trout (*Salvelinus fontinalis*) from the OB population in four temperature variability treatments during the 17 days of Part 1 of the monitoring experiment (June 23, 2016 - July 12, 2016). Treatment 1 represents temperature constancy (a constant temperature of 10°C), Treatment 2 represents periodic variability, Treatment 3 represents low stochasticity, and Treatment 4 represents high stochasticity. Data collected at Dalhousie University in Halifax, Canada.
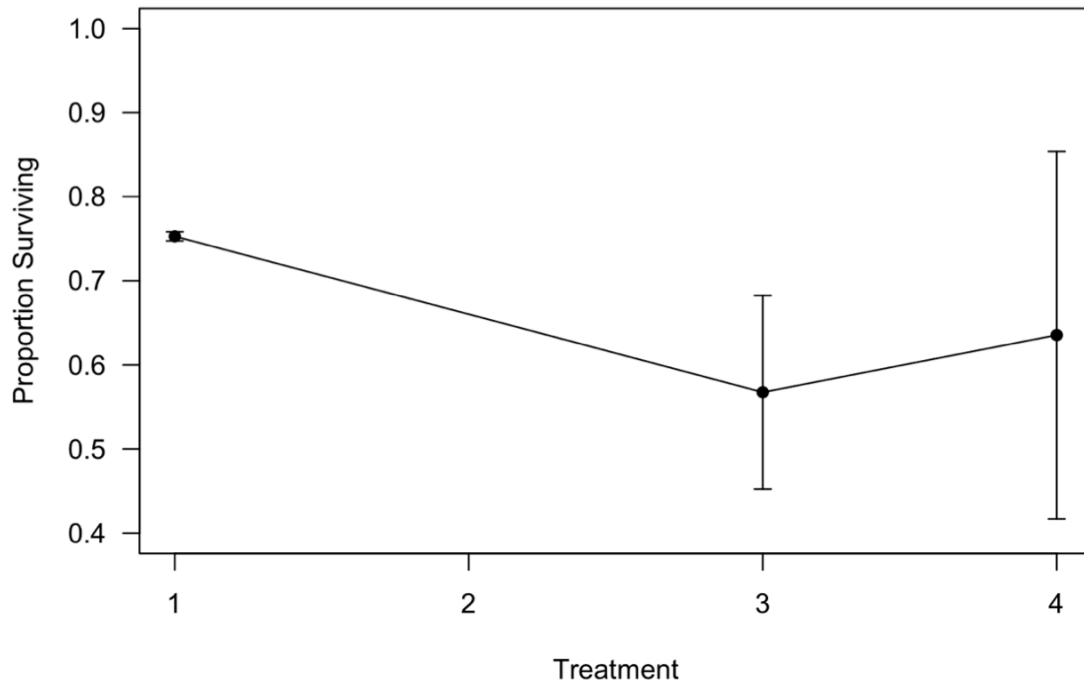
Figure 3.13 Proportion of surviving brook trout (*Salvelinus fontinalis*; ± 95% CI) from the WN population in three temperature variability treatments at the end of Part 1 of the monitoring experiment (July 13, 2016). Treatment 1 represents temperature constancy (a constant temperature of 10°C), Treatment 2 represents periodic variability, Treatment 3 represents low stochasticity, and Treatment 4 represents high stochasticity. The value for Treatment 2 was omitted due to an unforeseen logistical issue. Data collected at Dalhousie University in Halifax, Canada.
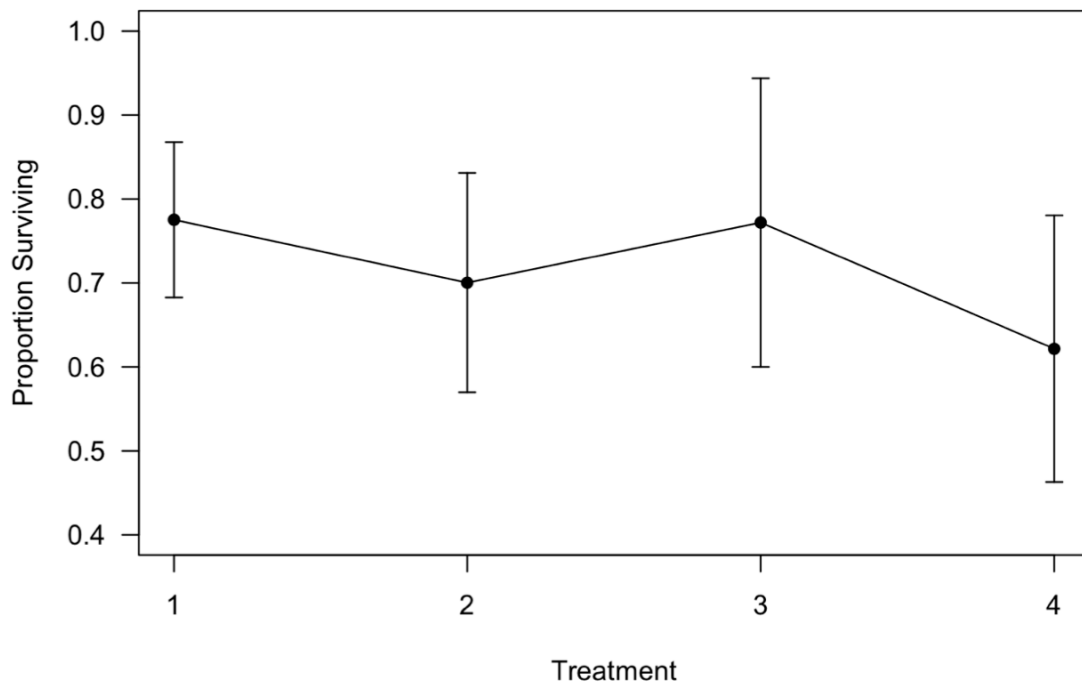
Figure 3.14 Proportion of surviving brook trout (*Salvelinus fontinalis*; ± 95% CI) from the OB population in four temperature variability treatments at the end of Part 1 of the monitoring experiment (July 13, 2016). Treatment 1 represents temperature constancy (a constant temperature of 10°C), Treatment 2 represents periodic variability, Treatment 3 represents low stochasticity, and Treatment 4 represents high stochasticity. Data collected at Dalhousie University in Halifax, Canada.

After 17 days, the WN population had significantly more deaths (relative to survivorship) than the OB population. In addition, Treatment 4 had significantly more deaths than the combination of Treatments 1, 2, and 3. The interaction between the WN population and Treatment 4 was not found to be significant (Table 3.5). The model was first simplified by combining treatment levels 1 and 3 together (df=6,8, p=0.112), followed by combining treatment levels 1+3 and 2 together (df=5,6, p=0.2582). Replicate as a random factor was found to be minimally relevant, and was not removed from the analysis (accounted for 6.1%).

Table 3.5 Results of the final, reduced GLMM and GLM models, separated with a horizontal line, examining the effects of population (WN, OB), treatment (1, 2, 3, 4), the interaction between population and treatment, and constancy on brook trout (*Salvelinus fontinalis*) mortality (total number of deaths) in relation to the number of survivors at the end of Part 1 of the monitoring experiment (July 13, 2016). Significant results are provided in bold.

Variable	DF	Z-value/T-value	P-value
Population	15	2.702	**0.0069**
Treatment	15	2.001	**0.0454**
PopulationWN:Treatment4	15	-1.765	0.0776
Population	19	1.399	0.1799
Constancy	19	-1.959	0.0668

There is no relationship between survival and constancy for the WN population ($F_{1,1}$=0.8633, p=0.5234), as survival decreases, then increases with increasing constancy (Figure 3.15). There is no relationship between survival and constancy for the OB population ($F_{1,2}$=5.475, p=0.1442), despite survival being shown to increase with increasing constancy (Figure 3.15). After 17 days, constancy was found to have no significant effect on the number of deaths (relative to survivorship; Table 3.5).
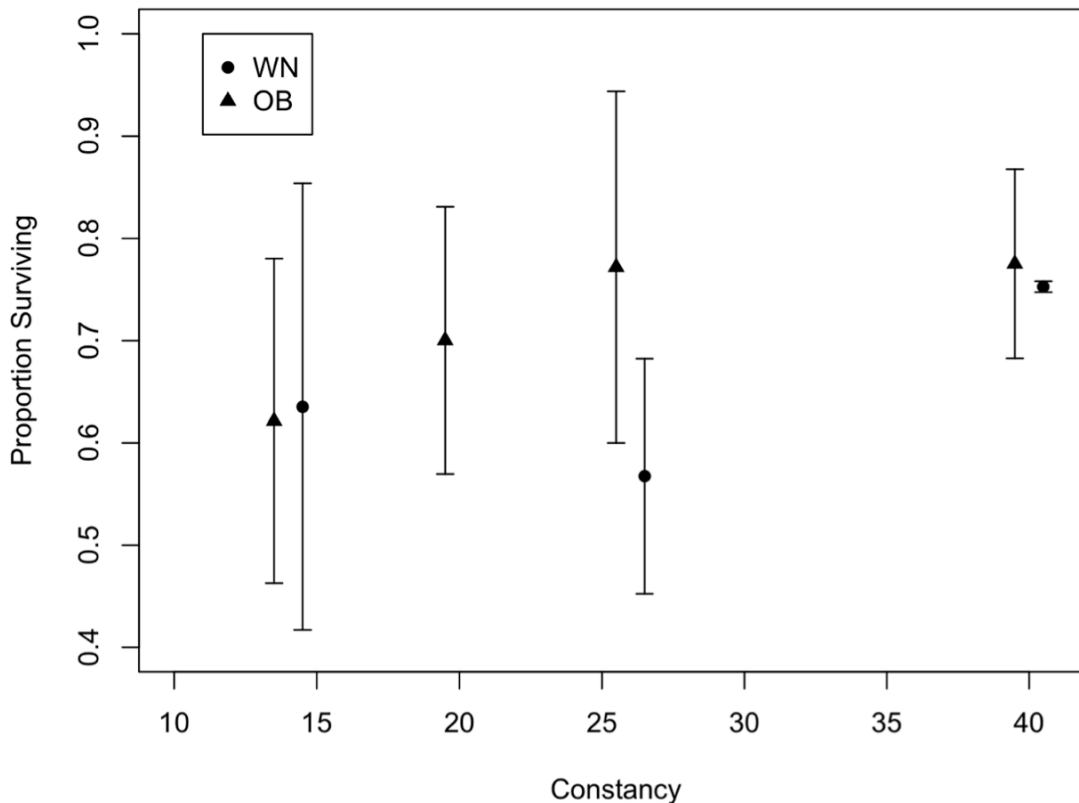
Figure 3.15 Relationship between the proportion of surviving brook trout (*Salvelinus fontinalis*; ± 95% CI) from the WN and OB populations and constancy at the end of Part 1 of the monitoring experiment (July 13, 2016). Treatment 1 (temperature constancy) has a constancy value of 40, Treatment 2 (periodic variability) has a constancy value of 20, Treatment 3 (low stochasticity) has a constancy value of 26, and Treatment 4 (high stochasticity) has a constancy value of 14. The value for the WN population in Treatment 2 was omitted due to an unforeseen logistical issue. Values on the x-axis are staggered by ± 0.5 in order to effectively present all trends. Data collected at Dalhousie University in Halifax, Canada.

3.2.2 Part 2

Survival decreased in all four treatments over the course of 30 days for both the WN and OB populations (Figure 3.16; Figure 3.17). For the WN population, starting on day 3, Treatment 1 had consistently lower survival than Treatment 3 and 4. Starting on day 6, Treatment 4 had consistently higher survival than Treatment 1 and 3 (Figure 3.16. After 30 days, survival for the WN population was the lowest in Treatment 1. Survival in this treatment was ~13% lower than Treatment 3, and ~16% lower than Treatment 4. As such, Treatment 4 experienced the highest survival after 30 days, followed by Treatment 3 (Figure 3.18).

For the OB population, the decreasing trends for all four treatments are relatively similar; however, starting on day 3, Treatment 3 had consistently lower survival than the other three treatments (Figure 3.17). Following day 27, Treatment 1 experiences a dramatic decline in survival, bringing its value close to that of Treatment 4 by day 29. There are also periods in which each treatment experiences no mortality for several consecutive days. After 30 days, survival for the OB population was the lowest in Treatment 3. Survival in this treatment was ~0.4% lower than Treatment 1, ~9% lower than Treatment 2, and ~14.3% lower than Treatment 4. As such, Treatment 4 experienced the highest survival after 30 days, followed by Treatment 2 and then Treatment 1 (Figure 3.19).
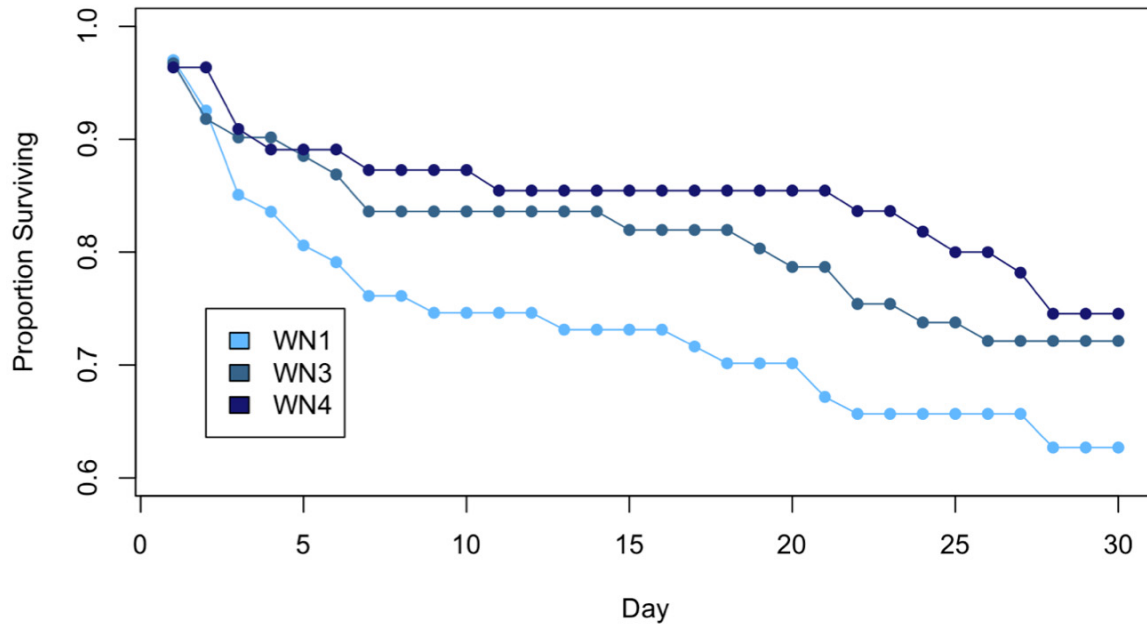
Figure 3.16 Proportion of surviving brook trout (*Salvelinus fontinalis*) from the WN population in three temperature variability treatments during the 30 days of Part 2 of the monitoring experiment (July 13, 2016 – August 11, 2016). Treatment 1 represents temperature constancy (a constant temperature of 10°C), Treatment 2 represents periodic variability, Treatment 3 represents low stochasticity, and Treatment 4 represents high stochasticity. The values for Treatment 2 were omitted due to an unforeseen logistical issue. Data collected at Dalhousie University in Halifax, Canada.
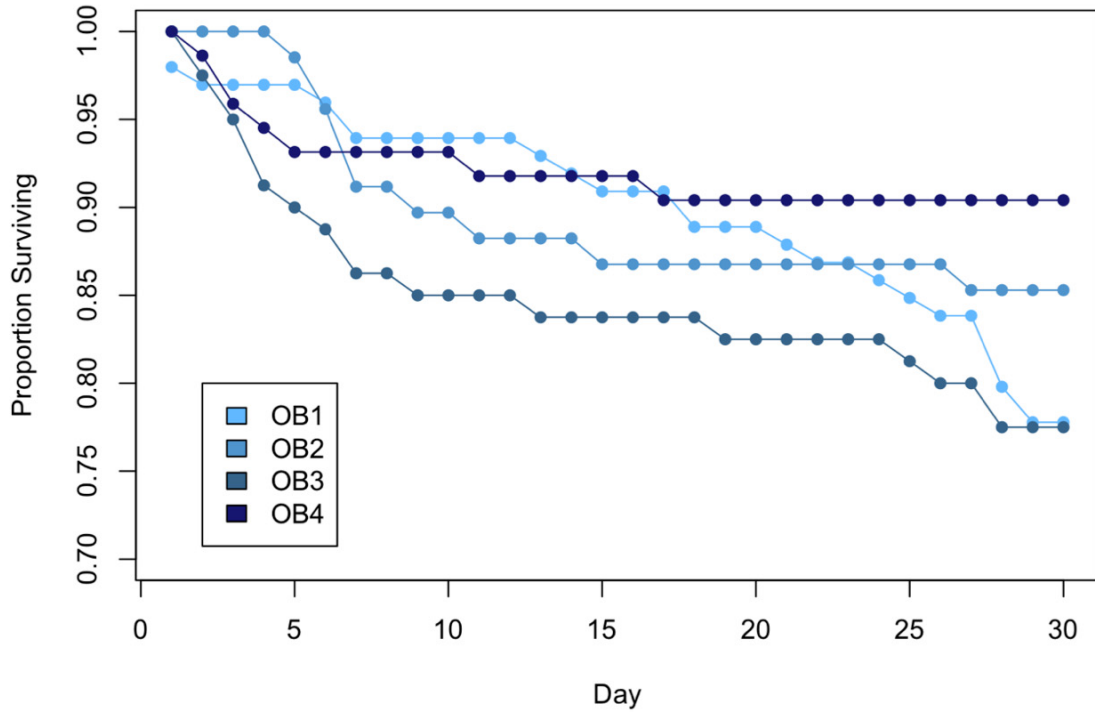
Figure 3.17 Proportion of surviving brook trout (*Salvelinus fontinalis*) from the OB population in four temperature variability treatments during the 30 days of Part 2 of the monitoring experiment (July 13, 2016 – August 11, 2016). Treatment 1 represents temperature constancy (a constant temperature of 10°C), Treatment 2 represents periodic variability, Treatment 3 represents low stochasticity, and Treatment 4 represents high stochasticity. Data collected at Dalhousie University in Halifax, Canada.
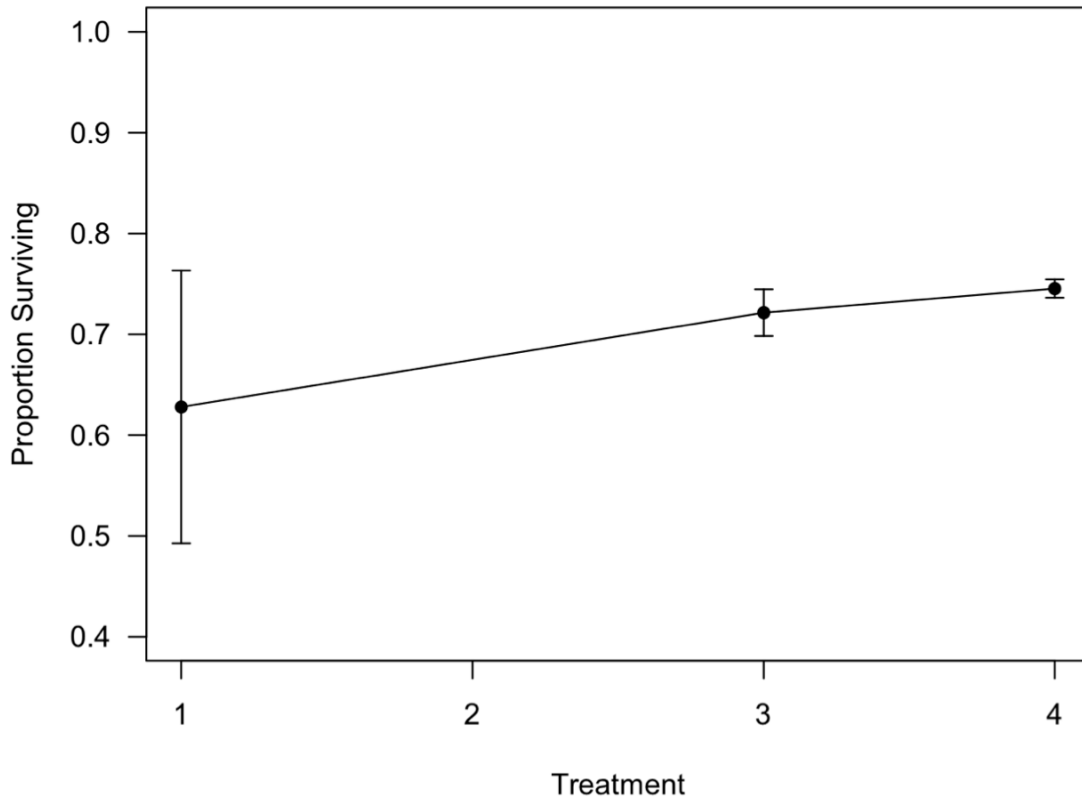
Figure 3.18 Proportion of surviving brook trout (*Salvelinus fontinalis*; ± 95% CI) from the WN population in three temperature variability treatments at the end of Part 2 of the monitoring experiment (August 12, 2016). Treatment 1 represents temperature constancy (a constant temperature of 10°C), Treatment 2 represents periodic variability, Treatment 3 represents low stochasticity, and Treatment 4 represents high stochasticity. The value for Treatment 2 was omitted due to an unforeseen logistical issue. Data collected at Dalhousie University in Halifax, Canada.
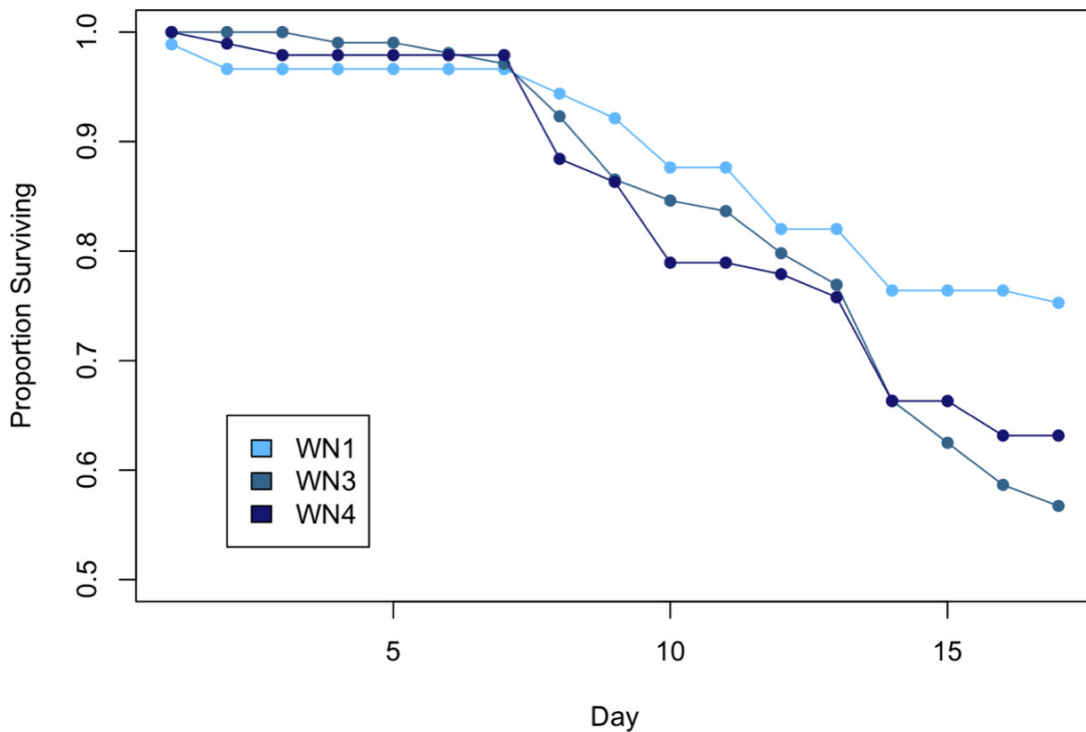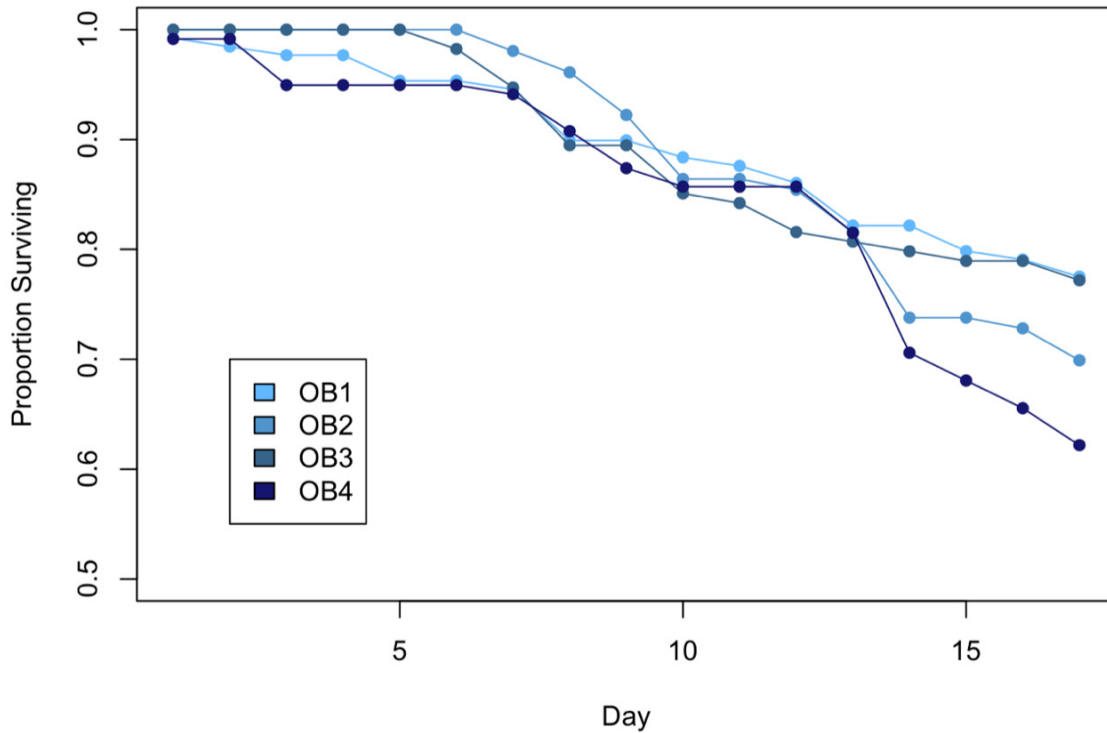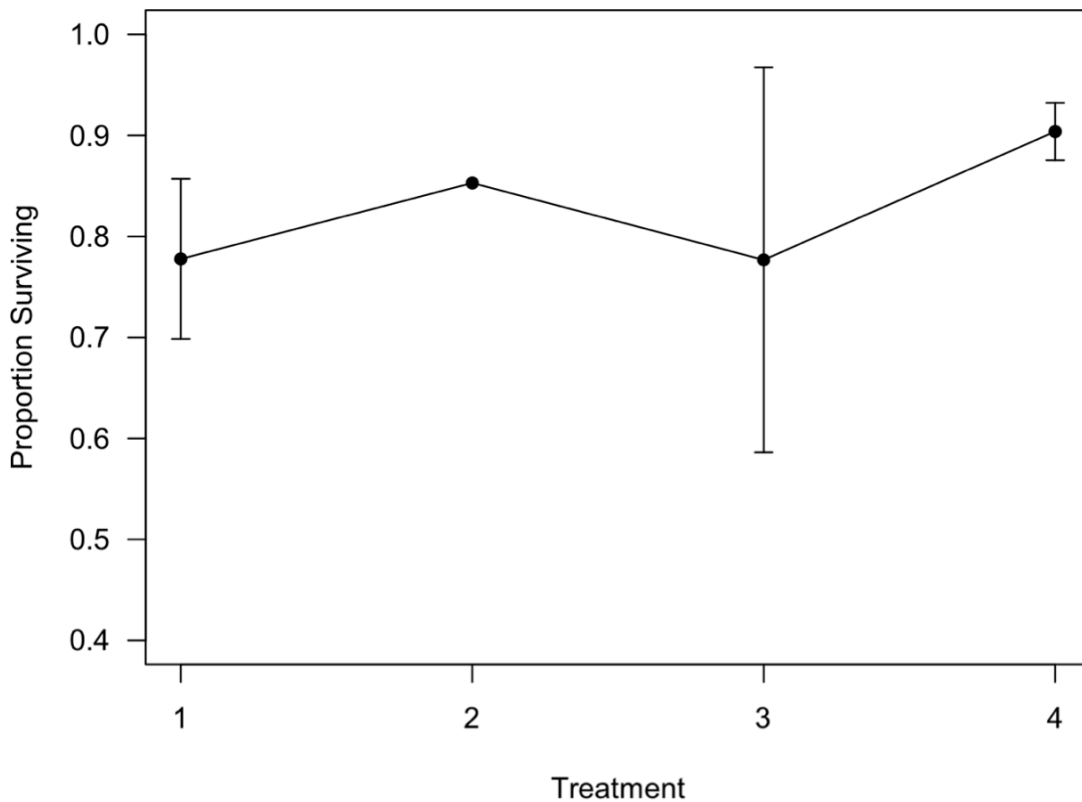
Figure 3.19 Proportion of surviving brook trout (*Salvelinus fontinalis*; ± 95% CI) from the OB population in four temperature variability treatments at the end of Part 2 of the monitoring experiment (August 12, 2016). Treatment 1 represents temperature constancy (a constant temperature of 10°C), Treatment 2 represents periodic variability, Treatment 3 represents low stochasticity, and Treatment 4 represents high stochasticity. Data collected at Dalhousie University in Halifax, Canada.

After 30 days, the WN population had significantly more deaths (relative to survivorship) than the OB population. However, there was no significant difference observed between treatments (Table 3.6). Interactions between Population and Treatment (df=10,12, p=0.3658) were not found to be a relevant and were removed from the analysis. The model was first simplified by combining treatment levels 1 and 3 together (df=12,13, p=0.4387), followed by combining treatment levels 1+3 and 2 together (df=13,14 p=0.2324).

Replicate as a random factor was not found to be relevant, and was removed from the analysis (accounted for 0.9%).

Table 3.6 Results of the final, reduced GLM models, separated with a horizontal line, examining the effects of population (WN, OB), treatment (1, 2, 3, 4), and constancy on brook trout (*Salvelinus fontinalis*) mortality (total number of deaths) in relation to the number of survivors at the end of Part 2 of the monitoring experiment (August 12, 2016). Significant results are provided in bold.

Variable	DF	T-value	P-value
Population	16	3.099	**0.0079**
Treatment	16	-1.941	0.0727
Population	16	2.877	**0.0122**
Constancy	16	2.318	**0.0361**

There is no relationship between survival and constancy for the WN ($F_{1,1}=12.85$, p=0.1732) population, despite survival being shown to decrease with increasing constancy (Figure 3.20). There is also no relationship between survival and constancy for the OB ($F_{1,2}=5.13$, p=0.1518) population (Figure 3.20). While survival initially decreases with increasing constancy, it increases slightly at the highest constancy value. After 30 days, constancy was found to have a significant effect on the number of deaths (relative to survivorship; Table 3.6).
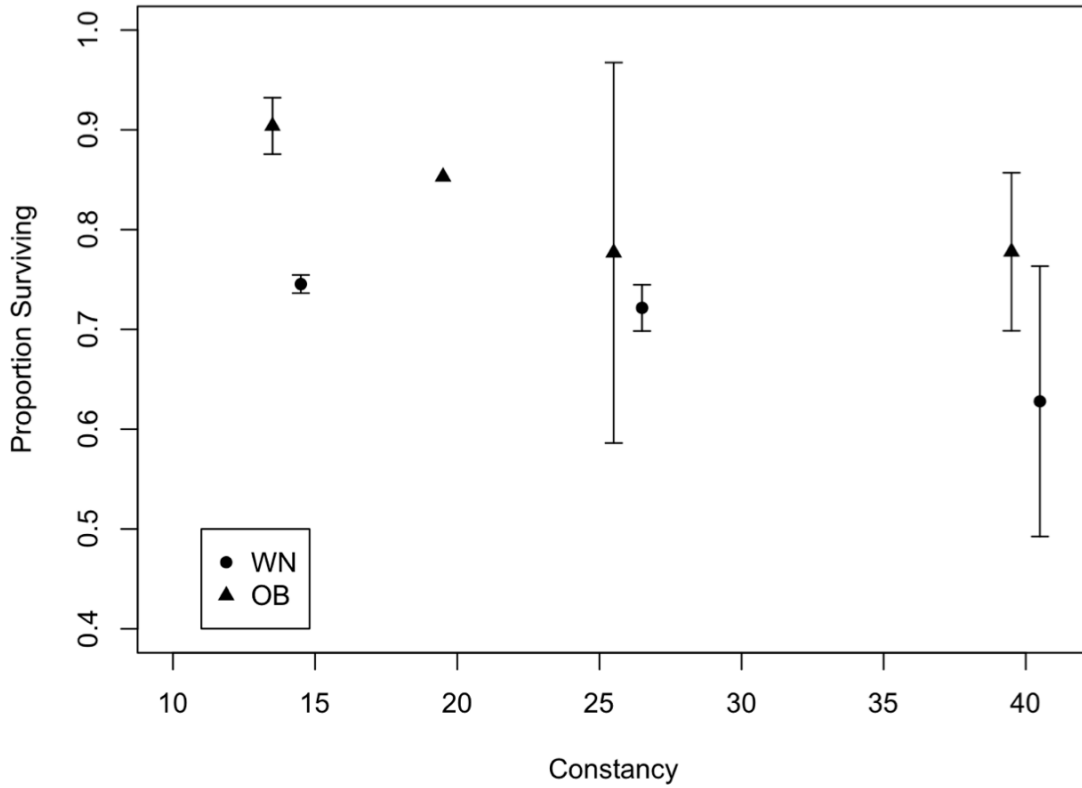
Figure 3.20 Relationship between the proportion of surviving brook trout (*Salvelinus fontinalis*; ± 95% CI) from the WN and OB populations and constancy at the end of Part 2 of the monitoring experiment (August 12, 2016). Treatment 1 (temperature constancy) has a constancy value of 40, Treatment 2 (periodic variability) has a constancy value of 20, Treatment 3 (low stochasticity) has a constancy value of 26, and Treatment 4 (high stochasticity) has a constancy value of 14. The value for the WN population in Treatment 2 was omitted due to an unforeseen logistical issue. Values on the x-axis are staggered by ± 0.5 in order to effectively present all trends. Data collected at Dalhousie University in Halifax, Canada.

Chapter 4

Discussion

Responses by two populations of brook trout to four temperature-variability treatments were examined in a common-garden experiment to determine the effect of thermal variability, or stochasticity, on two metrics of fitness. We find evidence for population-genetic differences in length-at-age (a metric of growth rate) and mortality between the two populations, as well as significant negative effects on these two metrics when exposed to conditions of high temperature variability.

4. 1 The Effects of Temperature Variability on Mortality – Main Experiment

Brook trout exposed to the high stochastic experimental treatment (Treatment 4) consistently experienced the lowest survival. These fish experienced significantly more deaths than fish in all other treatments in two of the three time-period cycles analyzed. Few studies have examined the effects of temperature variability on metrics of fitness in fish, although several have documented an inverse relationship between survival and mean temperature (McCairns 2004; Xu et al. 2010; Wood and Fraser 2015).

In contrast to our work, Xu et al. (2010) reported that survival in trout increases with variation in stream temperature. The contradictory nature of these results could be due to the parameter used to characterize thermal variability. Xu et al. (2010) used the coefficient of variation (c.v.) to characterize the variation in stream temperature. We could not use this parameter to effectively capture the variation within each treatment in our study, as the standard deviation was almost identical for each treatment. As such, we created an alternative measure of temperature variation, defined as the number of days where the temperature at day $x+1$ is equal to the temperature at day x. It is possible that this parameter does not adequately capture the variation present within each treatment, and thus may have biased the interpretation of the presented findings. In addition, Xu et al. (2010) analyzed data on adults while our study focused on the fry/alevin developmental stage. A final consideration is that the data collected in the study performed by Xu et al. (2010) was taken from wild populations, and was not collected from a common-garden or controlled

experiment. Hokanson et al. (1977) showed that temperature variability influenced the growth and survival of rainbow trout (*Oncorhynchus mykiss*) positively or negatively depending on whether the mean temperature was higher or lower than a threshold of 16°C. If the mean temperature was less than 16°C, temperature variability positively affected growth and survival. Otherwise, the effects were negative. While the mean temperatures of all our treatments were below 16°C, our results are still contradictory. This might be attributable to a difference between species, as brook trout may be characterized by a different mean temperature threshold.

No significant difference between treatments was observed at the end of the experiment (Cycle 5), likely due to a sudden increase in mortality of the WN population tanks exposed to Treatment 1 (temperature constancy) in the last few days of the experiment. The mortality was to such a degree that overall survival for this treatment decreased to a level just slightly below that of Treatment 4. The cause of this sudden increase in mortality of only one subset of tanks is unclear; the affected tanks were not isolated to a particular row or side of the rack, and no other subset of tanks on the same rack experienced mortality to the same degree in this time frame. Had this sudden increase in mortality not occurred, it is likely that we would have reported a significant difference once again between Treatment 4 and the other three treatments at the end of the experiment.

For both populations, fish exposed to Treatment 1 (temperature constancy) and Treatment 4 (high stochasticity) were found to have the lowest survival at the end of the experiment, while fish exposed to Treatment 2 (periodic variability) and 3 (low stochasticity) experienced the highest survival. This result is unusual, especially given that Treatment 1 represented no temperature variability and is responsible for either the lowest, or the second lowest proportion of survival. The sudden increase in mortality of the WN tanks exposed to Treatment 1 in the last few days of the experiment is the most reasonable explanation for WN fish in Treatment 1 experiencing the lowest proportion of survival. The explanation for the results obtained for the OB population is not as clear; however, it could be due to issues that arose during the experiment. The three temperatures experienced by trout in this experiment were 7°C, 10°C, and 13°C. Due to abnormally high outside temperatures in the months of April, May and June of 2016, the RAS system used to control the water temperature of the aquaria struggled to maintain constant temperatures. By the

end of the experiment, the rack that was supposed to remain at 10°C was shown to have experienced an average temperature of 11°C±0.08. As such, it is possible that mortality was higher than expected for Treatment 1 due to temperatures averaging a full degree higher than planned. Finally, it is also possible that 40 days is not long enough to obtain clear data on the effects of temperature variability on survival in brook trout.

The origin stream for the WN population is a large, deep stream that is 8062 m in length and that averages 4.48 m in width, and 20 cm in depth (Wood et al. 2014; Appendix A). It is a third-order stream with a large drainage area. As such, this stream is likely a slower-moving body of water with decreased substrate size and canopy cover (Quinn 2005). Assuming that the drainage area is a reasonable predictor of trout biomass (Allan and Castillo 2009), one may deduce that this stream can support a large population of brook trout. As a consequence, it is likely to be less vulnerable to environmental stochasticity (Lande 1993) and more likely to contain a variety of habitat types that would be utilized by salmonids at different life stages (Wood et al. 2014; Appendix A). Large streams also generally contain more pool habitats and complex physical structures, which provide shelter for fish from high temperatures, environmental stress, and predation (Power 1987; Lonzarich and Quinn 1995). Oppositely, the origin stream for the OB population is a short stream that is 208 m in length, averaging 2.51 m in width and 18 cm in depth (Wood et al. 2014; Appendix A). It is a first-order stream with a small drainage area. As such, this stream is likely a fast-moving body of water at a moderate elevation that can only support a small population of brook trout (Quinn 2005). This stream also has a low sinuosity coefficient, at 1.07, confirming that this stream is characterized by fast-water habitats and is likely to have a lower diversity of habitat and fauna (Gordon et al. 1992; Wood et al. 2014).

Population-genetic differences in mortality were observed only once during the main experiment. In Cycle 4, the WN population was found to have significantly fewer deaths than the OB population. However, no differences were observed in Cycle 3 or Cycle 5. Wood and Fraser (2015) found that smaller populations of brook trout from Cape Race had higher survival during the embryonic period (fertilization to hatch) across three different temperatures. These findings are not in conjunction with the results from Cycle 4, which suggest instead that larger populations of brook trout are more likely to survive conditions of environmental variability and stress. As this result was found only once

during the main experiment and is not consistent with the literature, it might not be reflective of the true population-genetic differences that occur under these circumstances. It is possible that this result is simply a reflection of the short duration of time that had elapsed following the start of the experiment (32 days), in which results typically vary tremendously. Even at this point during the experiment, the fish were likely still acclimating to the treatment conditions.

4.2 The Effects of Temperature Variability on Mortality – Monitoring Experiment

When temperature variability was eliminated following the experiment, we observed that brook trout exposed to Treatment 4 (high stochasticity) continue to experience significantly higher mortality than all the other treatments. For the OB population, fish exposed to Treatment 1 (constancy) and 3 (low stochasticity) had the highest survival, while fish exposed to Treatment 2 (periodic variability) and 4 experienced the lowest survival. As such, survival was found to increase with decreasing temperature variability, or increasing constancy, for the OB population. This suggests that OB fish exposed to higher levels of temperature variability experience greater mortality than those exposed to lower levels of temperature variability. The findings for the WN population were less clear and did not follow the same patterns. However, we did observe that the WN population had significantly more deaths than the OB population. These findings differ considerably from those found during the main experiment, but are more in conjunction with our hypothesized relationship between survival and the degree of thermal constancy.

During the first 17 days of the monitoring experiment, we experienced several unexpected events that may explain our findings. The purpose of the monitoring experiment was to examine the survival of fish populations under more stable and natural temperature conditions following a period of high environmental stress. However, this is not what occurred due to circumstances beyond our control. Three days into the monitoring experiment, a power failure occurred and affected the cooling system, leading to considerable increases in water temperature in all tanks. Issues controlling the water temperature and water flow persisted from this date for another 17 days. Water temperatures on some days increased to 10-11°C from 7°C in conjunction with water flow

shutting off for certain tanks on the rack. On occasion, an entire row was found to have no water flow and, as a result, was subjected to much higher temperatures than the rest of the tanks on the rack. This unintentional introduction of apparent environmental stress resulted in many mortalities across tanks and treatments, with the number of deaths generally ranging between 20 and 70 individuals per day. There was also one occasion when none of the fish was fed for the day. Given that the first part of the monitoring experiment was unintentionally an extension of the environmental stress experiment, obtaining a result that matches what was found during the main experiment is reasonable. The overall results were potentially more favorable to our hypothesized relationship between survival and constancy due to the additional time spent under temperature variability conditions in conjunction with other environmental stressors. In other words, the fish had more time to acclimate to the environmental stochasticity treatments, and were able to respond in a manner that is more representative of the true changes in plasticity that would occur with increasing thermal stochasticity.

Forty-seven days following the experiment, we found no significant differences between the treatments. We also observed a complete reversal of the general trends, in which survival was found to decrease with decreasing temperature variability, or increasing constancy. Once again, we observed that the WN population had significantly more deaths than the OB population. These findings represent the complete reverse of the findings collected 17 days after the experiment. These results might be suggestive of adaptive plasticity. Fish exposed to conditions of higher temperature variability in the original experiment had greater survival, and therefore had a distinct advantage over the fish exposed to conditions of low temperature variability in the original experiment. Once the temperatures returned to what would normally be experienced by these populations in their natural setting, the fish exposed to low temperature variability conditions, that did not need to undergo as much plasticity or acclimation, were outperformed by the fish that did respond to environmental stress. These findings are consistent with the hypothesis that brook trout that plastically respond to environmental stressors, such as temperature variability, outperform fish that did not undergo these changes when experiencing normal environmental conditions.

The results from 17 and 47 days following the experiment are consistent with findings by Wood and Fraser (2015); namely, that smaller populations of brook trout from Cape Race had higher survival during the embryonic period (fertilization to hatch) across three different temperatures. The OB population, which originates from a stream likely supporting a small brook trout population, was seen to have significantly fewer deaths than the WN population. However, measurements of survival in our study were completed following the embryonic period.

These results may indicate that smaller populations may be better adapted to, and can maintain higher fitness, under sub-optimal conditions and extreme variability in temperature associated with future climate change (see also Wood and Fraser 2015). Studies on brook trout in the Cape Race area found increased spatial variability in the mean and coefficients of variation of several habitat parameters in small populations, providing further evidence that small populations in the area may experience a wider range of selective pressures (Fraser et al. 2014; Wood et al. 2014; Wood and Fraser 2015). There are no long-term environmental data available for the Cape Race streams (Wood and Fraser 2015), with measurements of stream-level habit characteristics for 19 different streams only available for 2010 and 2011 (Wood et al. 2014; Appendix A). Based on the available data, it has been further hypothesized that temporal variability in environmental conditions may be higher in smaller populations, and that plasticity at key life history traits may be favored as a means of adapting to temporal environmental change (van Kleunan et al. 2000; Paschke et al. 2003; Wood and Fraser 2015). Larger populations are usually found in larger streams that are characterized by greater habitat and environmental heterogeneity, such as the origin stream for the WN population. In such cases, genotypes with varying patterns of plasticity may occur simultaneously (Sultan 1995; Wood and Fraser 2015). These findings may serve as potential explanations for the significant success of the OB population following considerable environmental stress and thermal variability.

Xu et al. (2010) found that there was a negative relationship between body size and survival in small tributaries (~0.3 km long), while there was a positive relationship in larger tributaries (~1 km long). The results from our main experiment do not support these findings, as the WN population, which comes from a large stream (~8 km long),

experienced significantly shorter lengths (i.e., slower growth) in conjunction with significantly less mortality in Cycle 4 of the experiment. In addition, the OB population, which comes from a much smaller stream (~0.2 km long), experienced significantly longer lengths (faster growth) in conjunction with significantly greater mortality in the same cycle. However, given the assumption that the WN population remained significantly shorter, on average, than the OB population throughout the monitoring experiment, the results from this experiment do support these findings. At both 17 and 47 days following the main experiment, the WN population experienced significantly greater mortality. Yet, we cannot conclude for certain that this relationship exists for the monitoring experiment, as length data was not collected during this time.

4.3 The Effects of Temperature Variability on Total Fish Length

Fish length in both populations was significantly higher in Treatments 1 and 3 than in Treatments 2 and 4. This indicates that fish exposed to lower levels of temperature variability experience faster growth than fish exposed to higher levels of temperature variability. Indeed, we find a significant statistical relationship between total fish length and constancy for one of the two populations observed, in which fish length increases with decreasing temperature variability, or increasing constancy. Once again, there are very few studies that examine the effects of temperature variability on life-history traits in fish. However, a study by Wood and Fraser (2015) found that brook trout fry had significantly higher lengths at emergence at 7°C compared to 9°C. In addition, a study by Beacham and Murray (1985) on chum salmon (*Oncorhynchus keta*) found that the length of emerging fry declined significantly with increasing incubation temperature. A study by Jensen et al. (2008) on brown trout (*Salmo trutta*) also found that increasing temperature had a significant negative effect on alevin length. A study by Kelly et al. (2014) on thermal acclimation in lake trout (*Salvelinus namaycush*) found that fish acclimated to 15°C were significantly smaller than fish acclimated to 8° or 11°C, and fish acclimated to 19°C were significantly smaller than fish acclimated to 8°C.

Brook trout subjected to unduly high temperatures can experience stress, reflected by changes such as slower growth (Carlson et al. 2007) and reduced prey-consumption

rates (Sotiropoulos et al. 2006). While our study did not examine changes in temperature itself, our research suggests that brook trout also experience stress when faced with high temperature variability and respond, predictably, with slowed growth.

Population-genetic differences were also observed for total fish length, in which fish in the WN population were significantly smaller when compared to the OB population. These results may also be explained by the theory proposed by Wood and Fraser (2015); namely, that smaller populations can maintain higher fitness, and therefore increased growth, under sub-optimal conditions and extreme variability in temperature associated with future climate change. As temporal variability in environmental conditions may be higher in smaller populations, plasticity at key life history traits, such as growth, may be favored as a means of adapting to temporal environmental change (van Kleunan et al. 2000; Paschke et al. 2003; Wood and Fraser 2015).

These results are also in concordance with findings obtained from thermal acclimation studies in brook trout (see McDermid et al. 2012), which provide evidence for intraspecific variation in thermal performance attributable to heritable differences between strains. In one study performed by McDermid et al. (2012), it was suggested that the differences observed between strains of brook trout could be due to ancestral, local adaptations for temperature tolerance. While our study did not examine thermal acclimation, it is still possible that the differing physiological responses of the WN and OB populations to thermal stress can be explained by their respective ancestries, especially given that the populations have been isolated since a deglaciation event 10 000-12 000 years before present and exhibit substantial differences in life histories.

4.4 Conclusions and Implications

Overall, our results suggest that brook trout originating from smaller populations exhibit possibly adaptive plasticity with regards to survival and growth when faced with environmental stressors, such as temperature variability. Fish originating from larger populations take advantage of greater environmental heterogeneity within their habitat and do not need to engage as readily in plastic responses. High temperature variability has significant, negative repercussions on both brook trout survival and growth.

Climate change model projections for the future anticipate increases in temperature, as well as increases in climatic variability. Specifically, temperature variability around the mean and the frequency of extreme climatic events are predicted to increase (Jensen et al. 2008). Phenotypic plasticity in traits able to respond instantly to environmental change, such as physiological and behavioral traits, is favored under conditions of increased variability in environmental variables (Scheiner 1993). However, the degree of environmental stochasticity may alter this, as certain phenotypes could become maladaptive if the degree of environmental unpredictability is high (Hard 1995; Pertoldi and Bach 2007; Kristensen et al. 2008).

A growing number of studies have found that the distribution (Dunham et al. 2003), survival (Schrank et al. 2003), growth (Meeuwig et al. 2004), and general health of stream-dwelling fish populations (Cairns et al. 2005) can be negatively affected by high temperatures (Mather et al. 2008). Salmonids in streams are generally drift-feeding predators, acquiring energy by ingesting the invertebrates present in the water column (Keeley and Grant 1995; Macneale et al. 2010; Gunnarsson and Steingrímsson 2011). As such, increases in metabolic demands at sub-lethal temperatures may have negative effects on survival. These effects may be particularly detrimental during the summer season, when prey abundance in streams is generally low (Cada et al. 1987; Nislow et al. 2004; Sotiropoulos et al. 2006). This is especially likely, as the frequency of thermal impacts to brook trout populations, especially during the summer periods, is likely to increase in the future (Jentsch et al. 2007; Mantua et al. 2010). Furthermore, climate models predict a 49% decrease in brook trout distribution in Canada by the year 2050 (Chu et al. 2005) and a loss in range of 44% in the 2040s and 77% in the 2080s for the interior western United States (Wenger et al. 2011b).

As previously mentioned, the phase that follows fry emergence is typically characterized by strong competition for food and territory in addition to high predation. Mortality rates during this critical phase are typically close to 90% (Elliott 1994). However, larger fry have a substantial advantage over their smaller counterparts, as their increased size allows them to exploit larger food items (Wankowski 1979) and avoid size-limited predators (Werner and Gilliam 1984). As such, the size of salmonid fry is likely a significant indicator of subsequent growth and survival, with larger fry experiencing higher

survival (Bams 1969; Fowler 1972). High temperature variability was shown to significantly reduce total fish length in this experiment. In a natural setting, increasing thermal stochasticity would further limit individual survival and success, resulting in even higher mortality rates for early life stages of brook trout. Higher fry/alevin mortality, combined with higher adult mortality, could result in the collapse of brook trout populations under more extreme environmental conditions.

In the face of growing, worldwide environmental uncertainty, understanding the conditions that lead to brook trout survival and success is paramount in securing their future as a species. Our results suggest that smaller populations can adapt under conditions of high environmental stochasticity, and outperform fish from larger populations when exposed to normal conditions. However, small populations are still susceptible to episodic catastrophic events and an "extinction vortex", in which mutually reinforcing environmental and demographic stochasticity, as well as inbreeding, can drive a declining population further downward to extinction (Fagan and Holmes 2006; Wood et al. 2014). It has also been proposed that small populations may respond more unpredictably to environmental change in the future (Wood et al. 2014). While our results suggest that larger populations are most in need of conservation and management initiatives in the face of environmental stochasticity, it is important to note that the fate of smaller populations cannot be entirely ignored. Populations of all sizes require strong conservation and management effects, so that they may persist in the long term (Wood et al. 2014). As was suggested by Wood et al. (2014), developing criteria to distinguish between small populations that are successful and those in danger of extinction would be extremely beneficial in narrowing the scope of future conservation initiatives.

An understanding of fishes' ability to respond and adapt to climate change remains a significant knowledge gap (Wilson and Mandrak 2004; McCullough et al. 2009). The effects of temperature variability on metrics of fitness in fish is a vastly understudied topic that we have shown has important implications for the survival and success of brook trout populations in Cape Race. We anticipate that further study will provide critical information that may benefit a variety of economically important, cold-water fish in the face of an unpredictable and turbulent environmental future.

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Appendix A

Temperature Variability Experiment Treatment Conditions

Table A1 Temperature conditions experienced by each of the four temperature variability treatments during the main experiment (April 26, 2016 – June 5, 2016). Treatment 1 represents temperature constancy (a constant temperature of 10°C), Treatment 2 represents periodic variability, Treatment 3 represents low stochasticity, and Treatment 4 represents high stochasticity. Horizontal lines are included to differentiate between the five 8-day cycles.

Day of Experiment	Treatment 1	Treatment 2	Treatment 3	Treatment 4
0	10	7	10	10
1	10	7	10	10
2	10	10	13	13
3	10	10	13	13
4	10	13	7	13
5	10	13	7	13
6	10	10	7	7
7	10	10	7	7
8	10	7	10	13
9	10	7	10	13
10	10	10	10	7
11	10	10	10	10
12	10	13	13	10
13	10	13	13	7
14	10	10	13	13
15	10	10	13	10
16	10	7	13	10
17	10	7	13	7
18	10	10	13	10
19	10	10	13	7
20	10	13	10	7
21	10	13	10	7
22	10	10	7	13
23	10	10	7	10
24	10	7	10	13
25	10	7	10	13
26	10	10	7	7
27	10	10	7	10
28	10	13	7	13
29	10	13	7	7
30	10	10	13	10
31	10	10	13	10
32	10	7	7	10
33	10	7	7	13
34	10	10	10	10
35	10	10	10	7
36	10	13	7	13
37	10	13	7	7
38	10	10	10	13
39	10	10	10	7
40	10	7	13	7

Appendix B

Fish densities during Part 1 and Part 2 of the Monitoring Experiment

Table B1 Brook trout (*Salvelinus fontinalis*) densities in each tank for the WN population, OB population, and tank-effect (TE) tanks at the beginning of Part 1 (June 23, 2016) and Part 2 (July 12, 2016) of the monitoring experiment. The term "NA" indicates that the replicate did not contain any fish and was not included in the experiment.

Tank	Part 1 Densities	Part 2 Densities
WN-1-1	44	34
WN-1-2	45	33
WN-1-3	NA	NA
WN-2-1	40	26
WN-2-2	40	25
WN-2-3	41	26
WN-3-1	35	30
WN-3-2	35	31
WN-3-3	34	NA
WN-4-1	31	NA
WN-4-2	31	27
WN-4-3	33	28
OB-1-1	43	33
OB-1-2	43	33
OB-1-3	43	33
OB-2-1	34	34
OB-2-2	34	34
OB-2-3	35	NA
OB-3-1	38	27
OB-3-2	38	27
OB-3-3	38	26
OB-4-1	40	25
OB-4-2	40	24
OB-4-3	39	24
TE-1	37	31
TE-2	40	30
TE-3	37	31
TE-4	39	31
TE-5	39	30
TE-6	40	30