

Gill morphology as an indicator of thermal adaptation and phenotypic plasticity in Atlantic salmon (*Salmo salar*)

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1 | Abstract

Climate change is a growing problem facing freshwater ecosystems, as water conditions continue to fluctuate with increasing intensity, and this is having major impacts on the biodiversity of these habitats. For many fish species, including Atlantic salmon (*Salmo salar*), adaptations to this environmental variability will be critical to their long-term outlook. This study explores how Atlantic salmon may respond to climate change and whether there is evidence that they can adapt to warming water conditions. To accomplish this, I examined whether gill morphometrics are an indicator of thermal tolerance in Atlantic salmon. Two potential drivers of thermal tolerance were assessed: thermal adaptation and phenotypic plasticity. Thermal adaptation is driven by genetic responses to the environment, which may evolve over generations, while phenotypic plasticity can occur over a much shorter period of time (i.e. within an individual's life span). Using three geographically separate populations that span a thermal gradient, Atlantic salmon gill metrics were compared and related to CT_{max} data to thereby assess thermal adaptation. To assess phenotypic plasticity, gills from one population were compared before and after exposure to prolonged warm temperatures. We found no significant evidence of thermal adaptation in gill morphology between populations and no relationship between CT_{max} temperatures and gill morphology, as well as no evidence of any phenotypic plasticity occurring over the course of our experimental temperature challenge. However, there were plenty of interesting trends in the data which will be discussed in depth later in this thesis. Overall, this study serves to help predict both future population-level and individual-level responses to climate change in Atlantic salmon in Newfoundland.

2 | *Introduction*

The Atlantic salmon (*Salmo salar*) is a species of anadromous fish in the Salmonidae family with native populations in the North Atlantic Ocean, Barents Sea, and Baltic Sea (Thorstad et al., 2021). These fish have a complex anadromous life history that involves spending time in North American or European river catchments, before migrating to the ocean for one to several years and then returning to freshwater for spawning (Corey et al., 2019; Mills et al., 2013; Thorstad et al., 2021). Understanding their performance during both the freshwater and saltwater phases is therefore crucial for this species' long-term survival. However, owing to the substantial impediments to managing oceanic threats, more focus should be placed on freshwater ecosystems when it comes to salmon conservation (Thorstad et al. 2021). Freshwater ecosystems will hereby be the main focus of our study.

Additionally, Atlantic salmon is one of the most economically valuable fish species in the world, contributing to recreational fisheries, commercial fisheries, and aquaculture (Hvas et al., 2020; Gallagher et al., 2022; Thorstad et al., 2021). Moreover, this fish is of considerable social and cultural importance, particularly for Indigenous peoples who have long used salmon for food and ceremonial purposes (Hvas et al., 2020; Gallagher et al., 2022; Thorstad et al., 2021). This is one of the many reasons that Atlantic salmon need to be protected from potential threats to their environment and their well-being. Declining populations have been observed in many regions throughout its native range and the impact has been detrimental to the livelihoods of many (Gallagher et al., 2022; Lehnert et al., 2019; Mills et al., 2013). These declines, combined with the food value of this fish and the decline of wild fish stocks of other capture fisheries, have sparked a large investment in salmon aquaculture (Hvas et al., 2020). Aquaculture may be an

important response to economic losses, but it does not provide a solution for the loss of the recreational fishery and the other cultural and social values of wild Atlantic salmon. This highlights why the Atlantic salmon has been chosen as the focal species of our study. We seek to support wild salmon populations by understanding responses to temperature changes and predicting how these responses will shape the future of this species under climate change.

The earth's climate is in flux and this is a growing problem for global biodiversity (Corey et al., 2019; Gallagher et al., 2022; Lehnert et al., 2019; Potts et al., 2021). Water and air temperatures are slowly climbing, thus it is critical to understand how organisms, such as Atlantic salmon, are responding to these changes (Jutfelt, 2020; Mills et al., 2013; Potts et al., 2021). It is necessary to evaluate the potential for thermal adaptation and to use that information to better understand future population dynamics (Corey et al., 2019; Gallagher et al., 2022; Jutfelt, 2020; Lehnert et al., 2019). Over the past several years, many species worldwide, including the Atlantic salmon, have experienced population declines or in some cases, extinctions (Gallagher et al., 2022; Lehnert et al., 2019; Mills et al., 2013; Thorstad et al., 2021). For example, North American populations of Atlantic salmon declined considerably in the early 1990s and have remained in low abundances ever since (Mills et al., 2013). Poor feeding conditions and warm water temperatures have constrained the potential recovery of these populations (Corey et al., 2019; Mills et al., 2013). One of the goals of this study is to connect the decline in Atlantic salmon to the ever-changing climate of the Earth and to understand how this species will respond to these altered water conditions.

To study responses to climate change, this project investigates thermal tolerance in Atlantic salmon. Thermal tolerance in Atlantic salmon can be driven by both thermal adaptation and phenotypic plasticity. Thermal adaptations related to genetic differences are often documented at both individual and population levels (Anttila et al., 2013; Debes et al., 2021; Potts et al., 2021). These differences are driven by adaptations to a fish's environment that occur over a long period of time, likely many generations (Anttila et al., 2013; Debes et al., 2021). The expectation is that populations from colder environments will have lower thermal tolerance, whereas populations adapted to warmer environments will have higher thermal tolerance (Anttila et al., 2013; Bigman et al., 2021; Debes et al., 2021; Muir, 2022). On the other hand, phenotypic plasticity refers to non-genetic differences in response to environmental change, which is primarily seen at an individual level though population-level differences do occur (Muir, 2022). This happens in a much shorter time frame than genetic adaptation, often within an individual's life span (Muir, 2022; Potts et al., 2021). Plastic responses to thermal stress, such as exposure to prolonged periods of increased or decreased temperatures, are common in Atlantic salmon (Corey et al., 2019; Jutfelt, 2020; Muir, 2022; Thorstad et al., 2021).

In our study, gill morphometrics were investigated as a potential indicator of thermal tolerance in Atlantic salmon to investigate questions related to thermal adaptation and phenotypic plasticity. Gill morphometrics were chosen because the gills are the main respiratory organs for fishes and can limit the amount of oxygen a fish can uptake (Bigman et al., 2021; Muir, 2022). Therefore, the gills may limit the thermal tolerance of Atlantic salmon. During this project, I compared differences in gill morphometrics between three populations of Atlantic salmon and examined the relationship between gill metrics and thermal tolerance. There is strong

evidence in the literature of a correlation between respiratory surface area and metabolic rate, in Atlantic salmon specifically as well as 63 other teleost fish species (Bigman et al., 2021; Muir, 2022). Fish with larger respiratory (gill) surface areas have a higher metabolic rate (Bigman et al., 2021), likely due to exposure to higher temperatures (Anttila et al., 2013; Muir, 2022; Potts et al., 2021). Fish with smaller respiratory (gill) surface areas have a lower metabolic rate (Bigman et al., 2021), likely due to exposure to lower temperatures (Anttila et al. 2013; Muir, 2022; Potts et al., 2021). Therefore, in terms of thermal adaptation, we may expect that fish adapted to warmer environments may have larger gills compared to fish adapted to colder environments. Additionally, plastic responses to temperature have also been identified in gill morphology. The expectation in the literature is that the gills of fish that are exposed to warm conditions for a prolonged period will increase in size in response to the warm water, compared to the gills of fish that are not exposed to these warm conditions (Bigman et al., 2021; Jutfelt, 2020; Muir, 2022; Potts et al. 2021). One study that focused on pugnose shiner (*Notropis anogenus*) is a particularly compelling example of plasticity in response to temperature (Potts et al., 2021). In that study, gill morphometrics of pugnose shiner indicated evidence of phenotypic plasticity, where fish acclimated to warmer temperatures showed an increase in gill size compared to fish acclimated to cooler temperatures (Potts et al., 2021). This increase in gill size was also shown to be related to high thermal tolerance measured by a higher critical thermal maximum (i.e., CT_{max}) (Potts et al., 2021).

Thermal tolerance, measured as CT_{max} , is an important metric that can be used to study the effects of temperature and climate on salmon (Debes et al., 2021; Gallagher et al., 2022; Potts et al., 2021). CT_{max} is the maximum temperature that an ectotherm can reach before losing its

equilibrium (Potts et al., 2021), and essentially, represents the upper limit of a fish's thermal niche (Debes et al., 2021; Potts et al., 2021). In a recent Atlantic salmon study, the mean values of CT_{max} for these fish were shown to be between $23.9 \pm 0.3^{\circ}C$ and $26.1 \pm 0.2^{\circ}C$ (Anttila et al., 2013), whereas other studies have documented CT_{max} values $> 28^{\circ}C$ (Debes et al., 2021). In my study, gill morphometrics are also compared to a measurement of thermal tolerance using CT_{max} experiments.

As alluded to above, the overarching goal of this project is to answer specific questions and hypotheses that relate to the ability of Atlantic salmon to respond to climate change in Newfoundland. Here, to address these hypotheses, I compare the gill morphometrics and thermal tolerance (CT_{max} data) of Atlantic salmon from different thermal regimes. Three key questions were posed: (a) Does gill morphology differ among Atlantic salmon populations from different thermal regimes? (b) Is gill morphology associated with a measure of thermal tolerance (CT_{max})? And (c) Does gill morphology show plastic response to prolonged temperature stress? I predict that we will see significant differences in gill morphology amongst the populations and that the results will also correlate positively with CT_{max} . As a result of this presumption, I also expect that there will be strong evidence of thermal adaptation and phenotypic plasticity in our sampled fish and this would likely indicate potential mechanisms for salmon to respond to climate change. This project seeks to address these questions while providing invaluable data to support Atlantic salmon research worldwide, which in turn will help us understand whether salmon can respond to future climate change through genetic and/or plastic responses. Moreover, in a world where water temperatures keep rising and many salmon populations continue to decline,

understanding thermal tolerance in Atlantic salmon will be crucial to any future conservation or recovery of this species.

3 | *Materials and Methods*

3.1 | Fish Collection and Experimental Set-Up

For this study, approximately 300 age 1+ juvenile Atlantic salmon were collected by electrofishing from three distinct locations on the island of Newfoundland: Western Arm Brook on the Northern Peninsula, Campbellton River on the north central coast, and Garnish River on the south coast (Figure 1). These three geographically separate populations span a thermal gradient and thus salmon from these rivers are expected to be adapted to different temperatures (Figure 2). Data from 2020 and 2021 highlight that Western Arm Brook has the coldest average water temperatures and Garnish River has the highest average temperatures, with Campbellton River falling somewhere in the middle (Figure 2). After capture, salmon parr from all three rivers were transported to the Ocean Sciences Center in August 2022. They were initially kept in separate tanks based on their sampling location for approximately one month and then were uniquely tagged to population with visual implant elastomer, before being placed into mixed population groups of ~100 fish for the remainder of the experiments. Each tank contained approximately 160-L of freshwater maintained at a stable temperature of 15°C, which is an optimal temperature for Atlantic salmon growth (Debes et al. 2021). Fish were checked and fed daily with a diet of frozen blood worms and commercial pellets. These tank conditions and animal care protocols (protocol 22-01-LS) were maintained until the cessation of experiments.

3.1.1 | CT_{max} Trials (for assessment of thermal adaptation)

To assess thermal adaptation in Atlantic salmon, critical thermal maximum (CT_{max}) experiments were performed between October 21st and November 3rd, 2022. For each CT_{max} trial, approximately 42 fish were acclimated to an experimental tank for 24 hours and then exposed to an increasing temperature at a rate of 2°C/hour, starting at 15 °C. When a fish lost equilibrium, the temperature was recorded (i.e., CT_{max}). In total, 418 fish were used for the CT_{max} trials with approximately equal numbers from each population (Western Arm Brook, Campbellton River, and Garnish River). After CT_{max} was recorded, each fish was humanely euthanized in a cold-water bath containing MS-222, as approved by the animal care protocol number 22-01-LS. The euthanized fish were then assigned an identification number in ascending order (1- 418) and were measured for length (± 0.1 cm) and weight (± 0.01 g). Additionally, the population of origin of each fish was identified based on their implant elastomer tag. A red elastomer tag corresponded to the Western Arm Brook population, a blue elastomer tag corresponded to the Campbelltown River population, and a yellow elastomer tag corresponded to the Garnish River population.

3.1.2 | Prolonged Temperature Trial (for assessment of phenotypic plasticity)

To assess phenotypic plasticity in Atlantic salmon, a prolonged temperature experiment was conducted to discern how these fish might respond to exposure to warm water conditions. Two subsets of fish exposed to two different water temperature conditions were used for the

experiment. One subset was kept in water at the optimal temperature for Atlantic salmon growth (15°C), while the other subset was kept in warmer water above the optimal temperature for growth (20°C) (Debes et al., 2021; Elliott & Elliott, 2010). After the CT_{max} trials were completed, the remaining fish were subjected to increased water temperature for approximately one month. On November 10th, 2022, the temperature of the water in each tank was slowly increased from 15°C (the optimal temperature for Atlantic salmon growth) to 20°C within a few hours. The only factor that changed for the salmon was the temperature of the water, as normal feeding and animal care protocols were maintained over the course of the experiment. Due to having the coldest maximum temperatures and thus a higher likelihood of fish being less adaptable to warm water conditions, Western Arm Brook was selected to evaluate plasticity for this experiment. Therefore, on December 8th, 2022, after one-month exposure to 20°C, ten fish from the Western Arm Brook population were randomly selected from the warm water tanks. The selected fish were humanely euthanized with MS-222 following the same procedure as mentioned above and assigned an identification (CHR1- CHR10), with CHR used to emphasize that these fish were chronically exposed to high temperatures.

3.2 | Gill Sampling

The gill sampling method was identical for both the thermal adaptation and the phenotypic plasticity portions of the study. A subset of fish had their gills dissected and preserved after both the CT_{max} trials and the prolonged temperature exposure trial. Each gill sample was placed in a vial of 10% neutral buffered formalin solution and the vials were then labelled with the corresponding fish identification number. Subsequently, for the thermal

adaptation portion of the study, ten gill samples from each of the three populations (n=30 total) were selected. These selections were based on the CT_{max} data and were made in the interest of gill morphology measurement. These thirty samples were chosen to match the CT_{max} distribution of the respective populations (Figure 3). For the phenotypic plasticity portion of the study, ten gill samples from one population (Western Arm Brook) were selected and sampled after exposure to warm water (20°C) for one month, and compared with ten gill samples from Western Arm Brook from the CT_{max} trials (acclimated to 15°C). All gill samples were transferred to a vial of distilled H₂O, a day prior to dissection, in order to separate the gill arches. For consistency, the second gill arch on the right side of the fish was chosen for measurement and removed from the rest of the gill arches using a scalpel. The second gill arch was chosen due to the fact it is likely to be more stable and less affected by external factors than the first gill arch. The second gill arch was also an ideal candidate because it was unlikely to be accidentally damaged during dissection. After removal from the branchial basket, the gill arch of interest was dried using paper towel, placed on a camera stand next to a ruler for scale, and photographed using a Sony a600 camera with a 50 mm macro lens (Figure 5). Afterwards, the gill of interest was placed in a small tube of 10% formalin solution, labelled, and set aside for potential future reference.

3.3 | Gill Morphology and Gill Surface Area Measurements

The gill morphology was analyzed for any evidence of thermal adaptation and phenotypic plasticity using the ImageJ program (National Institutes of Health, n.d.). Gill surface area is the ideal measurement for this project and so was calculated for each of the 40 samples (Figure 4). However, due to the fact it can be difficult to quantify gill surface area accurately, three

additional measurements, correlated closely with surface area (Potts et al., 2021), were taken as well. These included total gill filament length, average gill filament length, and the total number of gill filaments (Figure 4). All four morphometric traits were measured following the methodology of Potts et al., (2021). To quantify the gill surface area, the area around the gill filaments of each gill arch was estimated (Figure 4). The total number of filaments was quantified by simply counting the filaments on each gill arch. To simplify this step, filaments were measured and counted in groups of five at a time as opposed to individually. The average gill filament length and total gill filament length were then calculated by measuring the length of every fifth filament and then using the average of two subsequent measurements to estimate the length of the four filaments between them. Total filament length was the sum of all measured filament lengths, whereas average length was the total divided by the number of filaments.

3.4 | Image J Analysis

ImageJ is an image processing program (National Institutes of Health, n.d.) that was used for the analysis of the Atlantic salmon gill arches. The gill photos were uploaded to the program and using the ruler visible in each photo, the scale was set to 1.0 cm. The total filament length, average filament length, the total number of filaments, and the gill surface area were then determined for each gill photo using the methods described above (Figure 4). To measure the surface area of the gill arch, the polygon tool was used to trace the outline of all gill filaments and then an area measurement in cm^2 was produced. On the same gill arch, the segmented line tool was then used to draw a line along the length of every fifth gill filament. As the gill filaments were not often perfectly straight, the segmented line tool offered the ability to curve the

measurement lines as needed. These lines produced lengths in cm which were used to quantify average filament length, total filament length, and total number of filaments.

3.5 | Controlling for Body Size

After the quantification of the gill arches using ImageJ, the results needed to be controlled for the body size of the fish before statistical analyses. All gill morphology data were adjusted for body size using the following formula:

$$\text{Trait}_{\text{adj}} = \text{Trait}_{\text{raw}} \times \left(\frac{\text{mass}_{\text{mean}}}{\text{mass}_{\text{obs}}} \right)^b$$

where $\text{Trait}_{\text{adj}}$ is the adjusted gill trait value, $\text{Trait}_{\text{raw}}$ is the raw gill trait value of the individual, $\text{mass}_{\text{mean}}$ is the mean mass of all fish in the comparison (i.e. among populations or 15°C vs 20°C plasticity), mass_{obs} is the observed mass of the individual, and b is the common slope between the gill trait and the mass of all fish in the comparison. To calculate the common slope b , an ANCOVA was performed using \log_{10} transformed data (trait and mass) to assess the interaction between body size (mass) and population, with the specific gill trait serving as the response variable (Figure 6). As no significant interactions were found, a common slope b (Table 1) was derived from the regression relationship and used in the equation. With the common slope calculated, the rest of the variables in the equation were obtained from the non-transformed data. This is because the non-transformed trait value is what is being adjusted, with the common slope b representing how body size and trait value should vary in non-transformed space. The common slope b was different for each gill trait, as shown in Table 1. After controlling for body size in all fish for all four gill traits, the adjusted data were then used for analyses and interpretation. The

above formula used for body size control is known to be mathematically sound and has been used in many previous studies (Fleming & Gross, 1989; Hendry & Taylor, 2004; Ihssen et al., 1981; Potts et al., 2021; Reist, 1986). Furthermore, the ANCOVA results supported both the use of a common slope b for each gill trait and the validity of the resulting data that were calculated using the aforementioned body size adjustment formula (see Results).

3.6 | Data Analyses

3.6.1 | Thermal Adaptation

Adjusted gill morphology data from Garnish River, Campbellton River, and Western Arm Brook were analyzed and compared across the populations, as well as compared to individual-specific measurements of thermal tolerance (CT_{max}). An ANOVA model was used to make comparisons among the populations and regression was used to examine the relationship between individual values for gill morphology and CT_{max} . The resulting p-values and R^2 values from these inter-population analyses presented the opportunity to assess the evidence of thermal adaptation in these juvenile Atlantic salmon. In addition, a principal component analysis (PCA) was performed comparing all gill metrics, which was accomplished using the `prcomp` function in R.

3.6.2 | Phenotypic Plasticity

Adjusted gill morphology data from Western Arm Brook were analyzed in this portion of the study. The ten gills from the CT_{max} trials that were kept in tanks with optimal water temperatures (15°C) were compared to the ten gills from the prolonged warm temperature trial

(20°C) using size-adjusted data and a t-test analysis. A t-value and a p-value were generated for each comparison to understand the relationship between temperature and gill size and evaluate evidence for phenotypic plasticity in these juvenile Atlantic salmon.

4 | Results

4.1 | Thermal Adaptation Trials

4.1.1 | Relationship Between Log₁₀ Body Size and Log₁₀ Gill Trait

In controlling for body size, it was important to analyze the relationship between log-transformed body size and log-transformed gill trait. Initially, an analysis of covariance (ANCOVA) was used to determine whether population and body size showed any significant interaction effects for each gill trait. For all four gill metrics, population and log body size were shown to have no significant interaction effects (Table 2), and thus the interaction term was removed from the model. Using the new model without the interaction term, both the covariate (log body size) and the treatment (population) were assessed separately for significant effects on the response variable (log gill trait). Body size had a significant positive effect on total gill surface area, average gill filament length, and total gill filament length, but not on total number of gill filaments (Table 3). On the other hand, population did not have any significant effect on any of the gill traits (Table 3). The regression plots for each individual gill trait show a positive linear relationship with body size, although the relationship is weak and not significant for total number of filaments (Figure 6). The R² values for total gill surface area, average gill filament length, and total gill filament length are near or above 50% and the p-values were highly

significant ($p < 0.001$; see Table 4), whereas the R^2 value for total number of filaments is only 8% and the p-value is > 0.05 (Table 4). Moreover, there was no evidence of differences in slope amongst the populations (Figure 6), and therefore a common slope was confidently used for each gill trait to adjust for body size.

4.1.2 | Inter-Population Gill Trait Comparisons

Inter-population comparisons were made for each mass-adjusted gill trait to assess whether significant differences were present among populations. ANOVA showed that there were no significant differences among the populations for any of the four gill traits (Table 5, Figure 7). Therefore, the population of origin appears to have no meaningful effect on gill morphometrics.

A principal component analysis (PCA) was used in order to more broadly examine among population differences in the combination of all gill traits. It revealed no statistically important differences in patterns of gill morphometrics among the populations (Figure 8), as all three populations clustered together with no obvious distinctions, although a couple of outliers were visible. Principal components 1 and 2 account for 94.7% of the variance, which indicates the PCA is highly representative of the full data set.

4.1.3 | CT_{max} Comparisons

The relationship between CT_{max} and mass-adjusted gill trait was compared by regressing each gill metric against CT_{max} data. The trait data from all three populations were pooled together

for this analysis and compared to the individual CT_{max} measurements ($n=30$). There was no significant regression relationship between CT_{max} values and any of the gill traits (Figure 9). The regression lines for all four traits were relatively flat and even slightly negative. Despite the use of a representative CT_{max} distribution for each individual population (Figure 3), no meaningful relationship between gill metric and CT_{max} was detected. Total gill surface area, average gill filament length, total gill filament length, and total number of gill filaments all displayed minuscule R^2 values and non-significant p-values (Table 6). Overall, the thermal tolerance (measured as critical thermal maximum) of the studied Atlantic salmon showed no relationship with gill morphology.

4.2 Phenotypic Plasticity Trials

4.2.1 | Intra-Population Temperature Comparison

An intra-population comparison was made between the gill metrics of fish from Western Arm Brook before and after prolonged exposure to increased temperature. This was done to assess whether there was significant evidence of phenotypic plasticity when comparing fish of the same population exposed to optimal temperature conditions ($\sim 15^\circ\text{C}$) versus high temperature conditions ($\sim 20^\circ\text{C}$). For all gill traits, except total number of filaments, there was a relationship between the gill metric and body size (mass) (Figure 10, Table 7). Using only the data relevant to this portion of the study (Western Arm Brook measurements), new common slopes and average body weight values were used to calculate size-adjusted values for each gill trait (Table 1). There was little difference in gill metrics between the two temperature scenarios (Figure 11). Fish exposed to optimal temperatures ($\sim 15^\circ\text{C}$) showed slightly larger gill surface areas, total filament

lengths, and total number of filaments, while fish exposed to high temperatures ($\sim 20^{\circ}\text{C}$) showed slightly larger average filament lengths (Figure 11). The differences for total gill surface area ($t = 0.96$, $df = 9$, $p = 0.361$), total number of gill filaments ($t = 0.62$, $df = 9$, $p = 0.551$), average gill filament length ($t = -0.637$, $df = 9$, $p = 0.540$), and total gill filament length ($t = 0.113$, $df = 9$, $p = 0.913$) were all non-significant. In the end, there was little evidence of intra-population phenotypic plasticity over the one-month period.

5 | *Discussion*

5.1 | Background

The earth's climate is warming, and global water temperatures are rising as a result (Corey et al., 2019; Gallagher et al., 2022; Lehnert et al., 2019; Potts et al., 2021). Less ice cover and warmer temperature conditions in the winter months are met by high temperature conditions and fewer cold-water refuge opportunities in the summer months (Corey et al., 2017; Elliott, 1991; Gallant et al., 2017; Jonsson & Jonsson, 2009). This begs the question: How are anadromous fish species, such as Atlantic salmon, coping with these changing water conditions? The goal of this study was to answer this question using gill morphology as the main indicator. The gills are the main respiratory organs for fishes and limit oxygen uptake; thus, the gills potentially provide a unique window into the capacity of fish to adaptively respond to changing environmental conditions (Bigman et al., 2021; Muir, 2022). Given that the gills limit the amount of oxygen a fish can take up, they may also limit how thermally tolerant a fish can be when exposed to different water conditions (Bigman et al., 2021; Muir, 2022). Warmer temperatures result in increased aerobic metabolism, and consequently, increased oxygen demand (Bigman et

al., 2021; Jutfelt, 2020; Muir, 2022). The scientific assumption is that Atlantic salmon populations from colder environments have lower thermal tolerance and correspondingly, smaller gill trait measurements, whereas populations from warmer environments have larger gill trait measurements and higher thermal tolerance (Anttila et al., 2013; Bigman et al., 2021; Debes et al., 2021; Muir, 2022; Potts et al., 2021). Theoretically, these differences can be driven by both genetic (thermal adaptation) and non-genetic (phenotypic plasticity) responses to the environment (Anttila et al., 2013; Debes et al., 2021; Muir, 2022). Notwithstanding the assumptions, are these trends being observed in Atlantic salmon specifically?

5.2 | Thermal Adaptation Results and Connections to Literature

In the first part of our study, we focused on thermal adaptation and how it drives thermal tolerance in the focal species. The idea of thermal adaptation encompassed population-level genetic differences among Atlantic salmon that are likely to have evolved over many generations (Anttila et al., 2013; Debes et al., 2021; Muir, 2022). Our initial hypothesis was that the fish would show significant differences in gill morphology amongst populations and that the gill morphometrics would correlate positively with CT_{max} temperatures. The sampled fish are from three geographically separated populations with differing thermal regimes and differing CT_{max} tolerances, thus this seemed the likely outcome. Recent results have shown definitively that thermal tolerance (CT_{max}) does differ among these populations, with Western Arm Brook having significantly lower thermal tolerance than both Campbellton River and Garnish River, which did not differ from one another (Heath, 2023). Therefore, with this in mind, we also evaluated whether there may be evidence of thermal adaptation in terms of gill morphometrics in our sampled fish. After experiments were completed and the resulting data were analyzed, our predictions were rejected. There were no significant differences in gill morphology among

populations and there was no relationship between gill morphometrics and CT_{max} data. Overall, there was no meaningful evidence of thermal adaptation in gill morphometrics across any of our sampled fish, although it is worth noting that certain trends seemed to be present. The inter-population differences may not be significant, but each population did exhibit unique affinities for certain gill traits over others. The subset of fish from Campbellton River and Western Arm Brook both appear to make up for having slightly fewer gill filaments than Garnish River fish by having slightly longer gill filaments on average, resulting in a slightly larger overall gill surface area (Figures 7A, B, and D). This effect is slightly greater for the Campbellton River population. These trends are inversed for the Garnish River population, with these fish seemingly having a smaller overall gill surface area, despite having a larger number of gill filaments (Figures 7A and 8D).

Despite these intriguing trends, the results from this part of the study are largely unexpected and mostly disagree with the expectations presented by related studies. The literature suggests that significant differences should be seen among populations with differing water temperature conditions (Anttila et al., 2013; Corey et al., 2019; Dabruzzi & Bennett 2013; Jutfelt, 2020; Potts et al., 2021; Sollid et al., 2006), however, this was not what was observed in our results. As oxygen levels are strongly correlated with temperature, species reliant on gills for oxygen uptake are required to adapt their respiration to match the conditions they are experiencing in their environment (Bigman et al., 2021; Muir, 2022). Gill surface area changes in size in response to environmental conditions and often reflects the metabolic needs of a specific fish at any given time (Bigman et al., 2021; Dabruzzi & Bennett, 2013; Jutfelt, 2020; Muir, 2022; Sandblom et al. 2016). Essentially, when needed, gill surface area would likely change in

order to meet species-specific oxygen requirements (Bigman et al., 2021; Dabruzzi & Bennett, 2013; Muir, 2022; Sandblom et al. 2016; Sollid et al., 2005; Vinagre et al., 2015). Warmer temperatures often result in lower dissolved oxygen concentrations, while also resulting in increasing aerobic demands on fish, and in many similar studies, this results in gill surface areas increasing in size (Dabruzzi & Bennett, 2013; Muir, 2022; Potts et al., 2021; Sollid et al., 2005; Vinagre et al., 2015; Zhang & Kieffer, 2014). Therefore, in regions where water temperatures are colder and oxygen levels are higher (i.e. Western Arm Brook), gill surface area and other related gill metrics of juvenile Atlantic salmon should be significantly smaller in size when compared to fish in areas experiencing higher temperatures. The vice versa should also be true, with warmer water conditions (i.e. Garnish River) promoting the growth of larger gill morphometrics in juvenile Atlantic salmon. It is possible that the disconnect between our results and the expected results from the literature can be attributed to small sample size, measurement error, slight age variation, or even avoidance behaviour by the fish themselves. This will be discussed further below. One thing these results do seem to confirm is that gill morphology is closely related to species-specific oxygen demand and maximum possible uptake, as opposed to local oxygen conditions and routine consumption (Bigman et al., 2021; Elliott & Elliott, 2010; Muir, 2022; Laurent & Perry, 1991).

In terms of CT_{max} , the literature suggests that gill morphology and CT_{max} data should be positively correlated (Bartlett et al., 2022; Corey et al., 2017; Gallant et al., 2017; Jonsson & Jonsson, 2009; Potts et al., 2021; Vinagre et al., 2015), although our results show no significant relationship between the two. CT_{max} values often increase when fish are acclimated to warmer temperatures and decrease in response to cooler temperatures (Bartlett et al., 2022; Corey et al.,

2017; Gallant et al., 2017; Jonsson & Jonsson, 2009; Leclair et al., 2020; Potts et al., 2021; Vinagre et al., 2015). Interestingly, one study focusing on seasonal CT_{max} in redbreast dace (*Clinostomus elongatus*) found that CT_{max} increased in the summer when temperatures were high and decreased in the fall when temperatures were low (Leclair et al., 2020). While this suggests some plasticity in CT_{max} , there is also clear evidence that CT_{max} is heritable (Anttila et al., 2013; Debes et al., 2021) and this was supported by recent work using the populations studied here (Heath, 2023). The overall expectation is that fish with larger gill morphometrics would have a higher CT_{max} temperature and would therefore display a higher temperature tolerance, and vice versa (Anttila et al., 2013; Bigman et al., 2021; Debes et al., 2021; Muir, 2022; Potts et al., 2021). This type of response has been observed in other studies with different focal species (e.g., shortnose sturgeons, various tropical reef fishes, frillfin goby, Atlantic shrimp) (Ospina & Mora, 2004; Potts et al., 2021; Vinagre et al., 2015; Zhang & Kieffer, 2014). However, in our results, no such significant response was discerned, as each of the R^2 values explained less than one percent of the variance in the data.

5.3 | Phenotypic Plasticity Results and Connections to Literature

In the second part of our study, we shifted our attention to the relationship between phenotypic plasticity and thermal tolerance in the focal species. The idea of phenotypic plasticity comprised individual-level non-genetic plastic responses among Atlantic salmon that are likely to have developed within an individual's lifetime (Anttila et al., 2013; Debes et al., 2021; Muir, 2022). Our hypothesis was that after exposure to higher water temperatures for a month-long period, there would be evidence of phenotypic plasticity among our sampled fish from the

chosen population (Western Arm Brook). We predicted that significant gill morphology differences would be observed between the fish in optimal water temperature conditions (~15°C) and the fish in high water temperature conditions (~20°C). Conversely to this prediction, the results indicated no significant intra-population gill trait differences between the two temperature conditions and subsequently, there was no evidence of phenotypic plasticity seen in the fish after one month of increased temperature exposure. These results were unexpected and seem to disagree with the general consensus in the literature, which is that periods of high temperature exposure should cause fish to plastically respond and enlarge their gills (Bigman et al., 2021; Dabruzzi & Bennett, 2013; Elliott, 1991; Muir, 2022; Potts et al., 2021; Sollid et al., 2005; Thorstad et al., 2021; Vinagre et al., 2015). Exposure to warmer, more hypoxic conditions generally seems to induce organisms to lengthen their gill lamellae and increase the total surface area of their gill filaments (Dabruzzi & Bennett, 2013; Muir, 2022; Potts et al., 2021; Sollid et al., 2005; Sollid & Nilsson, 2006; Vinagre et al., 2015). Several other studies have performed similar temperature challenges on various focal species and each one has reported significant plastic changes. Potts et al. (2021) reported significant plastic responses in the gills of pugnose shiner (*Notropis anogenus*) across three different rearing temperatures (16, 25, and 28°C) over four months. There was a significant increase in surface area, filament length, and number of filaments in the gills of this species between the lower temperature and the higher temperature (Potts et al., 2021). Similarly, another study examining Atlantic stingrays (*Dasyatis sabina*) found that gill surface area increased 1.7-fold when exposed to extended hypoxic conditions (Dabruzzi & Bennett, 2013). Crucian carp (*Carassius carassius*) and goldfish (*C. auratus*) have also been shown to lengthen their gill filaments and increase their overall respiratory surface area in response to higher-than-normal temperatures (Sollid et al., 2005). The reported differences in

the Atlantic stingrays, Crucian carp, and goldfish were all observed over about a one-month period (Dabruzzi & Bennett, 2013; Sollid et al., 2005). This supports our decision to employ a one-month temperature challenge in our study, as significant gill morphology differences have previously been reported in this time frame. In essence, all these studies suggest that plastic responses in gill morphology are common and are triggered at a species-specific temperature. Therefore, it is expected that Atlantic salmon would follow this same trend, though the temperature that triggers their gill changes may be slightly different.

5.4 | Importance of the Study and Key Findings

This study provides important data describing the relationship between gill morphology and temperature in juvenile Atlantic salmon. Studies on this specific relationship in juvenile Atlantic salmon are non-existent and so our study will provide valuable data to inform future research on the topic. One key finding of this study, which is present in the results of both the thermal adaptation trials and the phenotypic plasticity trials, is that gill surface area and gill filament length both increase with body size, whereas gill filament number does not. This can be seen in the regression relationship between logarithmically transformed body mass and logarithmically transformed gill metrics, as well as in the regression relationship between non-adjusted body mass and gill metrics. The numbers are slightly different, but the result is the same. This seems to suggest that, in juvenile Atlantic salmon, the number of filaments might be fixed early in life or less plastic, whereas filament length and thus surface area, are more plastic. It appears any changes in gill surface area as a fish grows are a direct result of the adjustments it makes to the lengths of its gill filaments. This seems to be a novel discovery in terms of the gill morphology of Atlantic salmon and warrants further study to firmly establish its significance.

The fixation of filament number at an early age and the dependence on filament length adjustments is a very interesting life-history strategy that could prove to be unique to Atlantic salmon.

5.5 | Potential Limitations and Improvements

Our study faced several potential limitations, either acting independently or in tandem, which may have led to the non-significant results. The disconnect in my results regarding thermal adaptation in gill morphometrics among populations and the expected results from the literature might be attributed to small sample size, measurement error, slight age variation among the sampled fish, and/or avoidance behaviour exhibited by the fish themselves. Most related studies used large sample sizes, with some even comparing multiple species over different temperature conditions, so it is possible that increasing the sample size of my study may have led to more significant results. In terms of potential measurement error, it is quite possible that there were a few mistakes made during the gill photo analysis. This human error could be reduced by having two or more people analyze the gill photos, or even by having one person do measurements in triplicate. In addition, there is also an age variation found across much of the literature (i.e. juveniles vs. adults) that may contribute to these differences as well. For example, Potts et al. (2021) worked with young-of-the-year fish for their 2021 study, whereas my study worked with 1+ fish. Different ages mean different stages of development and potentially different degrees of gill morphology plasticity, which could also explain some of the observed differences between our results and the results of other studies (e.g. Potts et al., 2021). The age variation among the three populations in our study may not be meaningful, although it is still

worth considering due to the differing CT_{max} values seen among these populations (Heath, 2023). Most interestingly though, it is possible that the sampled fish from any or all of the three observed populations could have exhibited avoidance behaviour that prevented the development of thermal adaptations. Atlantic salmon parr are known to seek cold water refuges when exposed to high water temperatures and even to conceal themselves from sunlight in order to remain as cool as possible (Breau et al., 2007; Breau, 2013; Gallant et al., 2017; Jonsson & Jonsson, 2009). From that information, it is reasonable to infer that the lack of thermal adaptation in gill morphology seen in these fish could be a result of this avoidance behaviour, as avoiding heat reduces the necessity for heat adaptations (i.e. gill enlargement). Further to this point, there is strong evidence that 1+ and 2+ fish do aggregate in cool water when exposed to high temperatures, although smaller fish (like 1+ parr) may not experience as much thermal stress in rivers as fish in later life stages (such as adult salmon) (Breau et al., 2007; Breau, 2013).

In terms of the phenotypic plasticity results, it is possible that small sample size coupled with a relatively short period of increased temperature exposure may have been the downfall of this portion of the study. Human error, as described above, is another potential factor that could and should be addressed in any further studies involving gill morphology. In terms of the sample size, increasing it may also increase the likelihood of attaining significant results, as other studies often had sample sizes much larger than the $n=20$ of my study. Nonetheless, the length (time) and degree (temperature range) of the temperature exposure provide a better explanation, as the high temperature exposure only lasted for one month and was just 5°C above the optimal temperature. In an ideal scenario, a 4–6-month temperature challenge would have been used and likely would have yielded more significant results, as showcased by the pugnose shiner study

(Potts et al., 2021). Potts et al. (2021) exposed their focal species to a temperature increase of 12°C for a four-month period and found a plastic response in all gill metrics, except filament density. Thus, one month and a 5°C temperature increase may not be sufficient to elicit changes in gill morphology. Future studies might aim for a longer time window and/or a higher temperature change in order to observe a response.

Oxygen limitation is another potential influence on the results of our study and it applies to both the thermal adaptation and plasticity aspects alike. Oxygen was not limited in these experiments whatsoever, as dissolved oxygen concentrations were at or above 100% saturation for the duration of the experiments, and thus no stress conditions were provided in regard to oxygen limitation. It is possible that experiments performed under different oxygen conditions (such as hypoxic conditions) may have provided different results. Future studies might choose to limit oxygen during CT_{max} trials or during temperature challenges to see how oxygen limitation affects the relationship between thermal tolerance and gill morphology.

5.6 | Ideas for Future Study

Future studies that explore the relationship between gill morphology and thermal stress in Atlantic salmon in Newfoundland would prove highly beneficial both to us as salmon consumers and to the health and future prospects of the species itself. Gill morphology goes hand in hand with many other physiological processes, so a larger-scale study that incorporates more research parameters would be highly beneficial. Investigating temperature-affected processes such as the amount of blood flow allocated to the gills during respiration, the cellular changes that occur during gill remodeling, or the creation of heat shock proteins in response to high temperatures

would all offer unique insights. Investigated independently, these examples would all contribute massively to the literature, but if observed together in a single study, I believe this would push our understanding of Atlantic salmon responses to climate change forward immensely. A study that compared laboratory observations of Atlantic salmon with in-situ observations, taking into account the natural complexities of a true Atlantic salmon habitat, would also be strongly advised. Observations under controlled conditions can be valuable, but if those observations do not correlate with what is happening in the wild, the research is largely uninformative. Finally, I would also like to advocate for further studies that focus on the relationship between gill morphology and body size. It would be interesting to see how the body size of Atlantic salmon parr either confirms or refutes the temperature-body size rule, which states that an organism's body size is dependent on the temperature in which it is reared (Walters & Hassall, 2006). In the same vein, investigating how Atlantic salmon fit into the Gill Oxygen Limitation Theory would be intriguing, which essentially, asserts that in water-breathing ectotherms the 2D nature of gill surface area means it cannot supply sufficient oxygen to the ever-growing 3D bodies of these organisms (Pauly, 2021).

5.7 | Conclusion

Overall, understanding how gill morphometrics relate to thermal tolerance is an important first step in being able to accurately predict future Atlantic salmon population responses to climate change in Newfoundland, while also providing a strong reference point for future studies at a broader scale. Our study may not have identified significant effects of gill morphology on thermal tolerance, but the observed thermal tolerance trends are intriguing and warrant further investigation. As mentioned, investigating gill morphometrics in combination with other

temperature-affected parameters (i.e. heat shock protein production or altered blood flow in the cardiovascular system) may unlock the potential predictive ability for future temperature-driven changes that this study was striving to reveal. Fully grasping the degree of variation in thermal tolerance that exists across Newfoundland Atlantic salmon populations, as well as predicting the risk that climate change poses for these fish remain major priorities that can hopefully be fully addressed soon.

6 | *Acknowledgements*

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8 | *Tables*

Table 1: Common slope b and mean body size values used to adjust gill metrics for body size (full equation shown in text).

Gill Metric	Common Slope b (Thermal Adaptation)	Common Slope b (Phenotypic Plasticity)	Mean Body Size (Thermal Adaptation) (g)	Mean Body Size (Phenotypic Plasticity) (g)
Total Gill Surface Area	0.60249	0.71882	8.108	11.197
Average Gill Filament Length	0.32465	0.35427	8.108	11.197
Total Gill Filament Length	0.41417	0.40464	8.108	11.197
Total Number of Filaments	0.07451	0.05041	8.108	11.197

Table 2: Interaction term results of Analysis of Covariance (ANCOVA) models used to test for differences in slope among three populations (Campbellton River, Western Arm Brook, Garnish River) and the corresponding log-transformed body sizes (in grams) on four log-transformed gill metrics of juvenile Atlantic salmon.

Effect	Gill Metric	F-stat	d.f.	P-value
Population x Body Size	Total Gill Surface Area	0.054	2	0.851
Population x Body Size	Average Gill Filament length	0.005	2	0.995
Population x Body Size	Total Gill Filament Length	0.109	2	0.897
Population x Body Size	Total Number of Gill Filaments	0.184	2	0.833

Table 3: Results of Analysis of Covariance (ANCOVA) models, after the removal of the interaction term from the model, used to test for differences in slope among three populations (Campbellton River, Western Arm Brook, Garnish River) and the corresponding log-transformed body sizes (in grams) on four log-transformed gill metrics of juvenile Atlantic salmon.

Effect	Gill Metric	F-stat	d.f.	P-value
Body Size	Total Gill Surface Area	54.949	1	7.16e-08
Body Size	Average Gill Filament Length	66.670	1	1.2e-08
Body Size	Total Gill Filament Length	24.64	1	3.69e-05
Body Size	Total Number of Gill Filaments	2.339	1	0.138
Population	Total Gill Surface Area	0.162	2	0.851
Population	Average Gill Filament Length	0.924	2	0.41
Population	Total Gill Filament Length	0.36	2	0.701
Population	Total Number of Gill Filaments	0.529	2	0.596

Table 4: Results of regression analyses used to test the relationship between four log-transformed gill metrics and their corresponding log-transformed body size (population was removed from this model, as it had no effect on either body size or gill trait).

Gill Metric	Slope b	R squared	P-value
Total Gill Surface Area	0.60249	0.6761	2.503e-08
Average Gill Filament Length	0.32465	0.7054	6.521e-09
Total Gill Filament Length	0.41417	0.4798	2.218e-05
Total Number of Filaments	0.07451	0.07958	0.131

Table 5: Results of analysis of variance (ANOVA) models used to test for differences among three populations (Campbellton River, Western Arm Brook, and Garnish River) on four mass-adjusted gill metrics of juvenile Atlantic salmon.

Gill Metric	F-stat	d.f.	P-value
Total Gill Surface Area	0.2265	2	0.7988
Average Gill Filament Length	0.7697	2	0.4731
Total Gill Filament Length	0.3923	2	0.6793
Total Number of Filaments	0.5471	2	0.5849

Table 6: Results of regression analyses used to test the relationship between four mass-adjusted gill metrics and their corresponding critical thermal maximum (CT_{max}) temperatures (in °C).

Gill Metric	Slope b	R squared	P-value
Total Gill Surface Area	1.0333	0.0095	0.6092
Average Gill Filament Length	0.3762	0.0051	0.7066
Total Gill Filament Length	30.021	0.0009	0.8755
Total Number of Filaments	177.27	0.0095	0.6078

Table 7: Results of regression analyses used to test the relationship between four gill metrics and their corresponding body sizes (in grams).

Gill Metric	Slope b	R Squared	P-value
Total Gill Surface Area	0.1218	0.9607	4.20e-14
Average Gill Filament Length	0.1566	0.9081	9.04e-11
Total Gill Filament Length	12.565	0.7954	1.29e-07
Total Number of Filaments	80.145	0.0199	0.553

9 | *Figures*

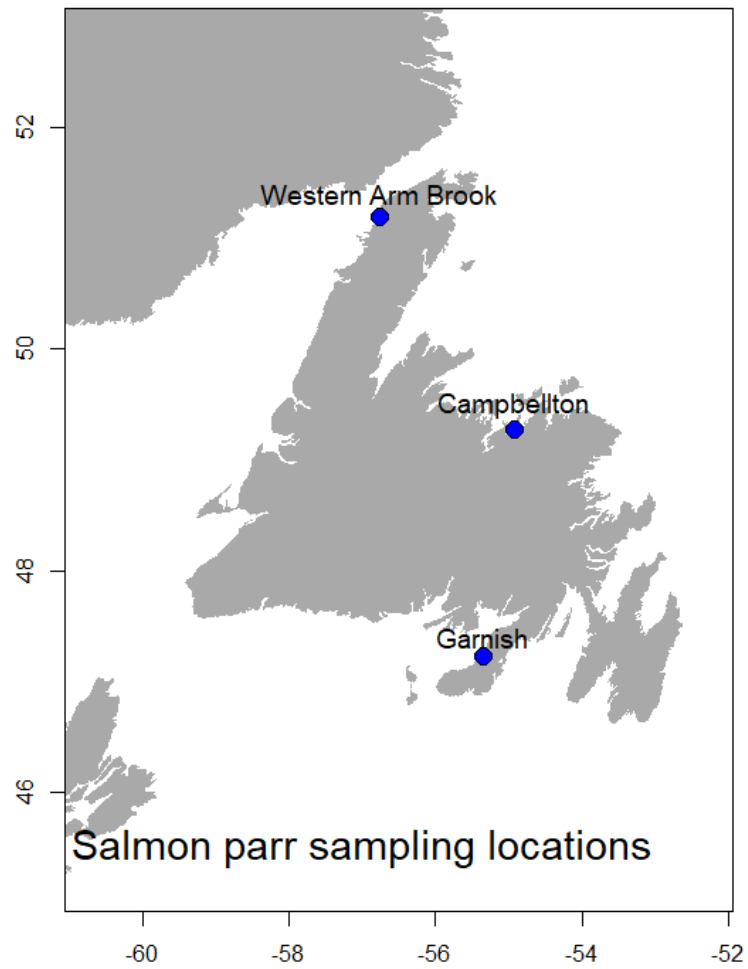


Figure 1. Map showing the three sampling locations of Atlantic salmon parr in Newfoundland.

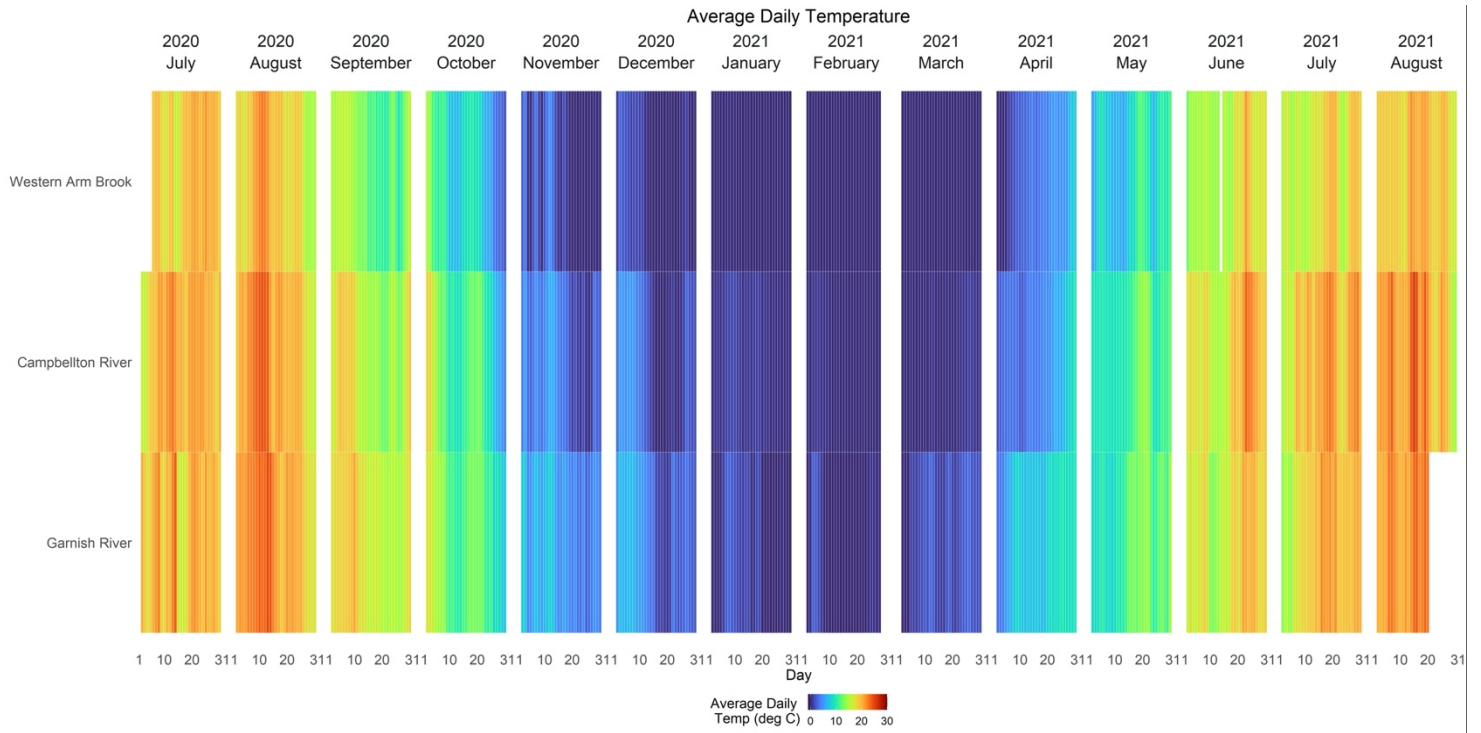


Figure 2. Average daily water temperatures between July 2020 and August 2021 at each river (Campbellton River, Western Arm Brook, Garnish River). Data and figure provided by Nicholas Kelly (Biologist) from DFO Salmonids Section.

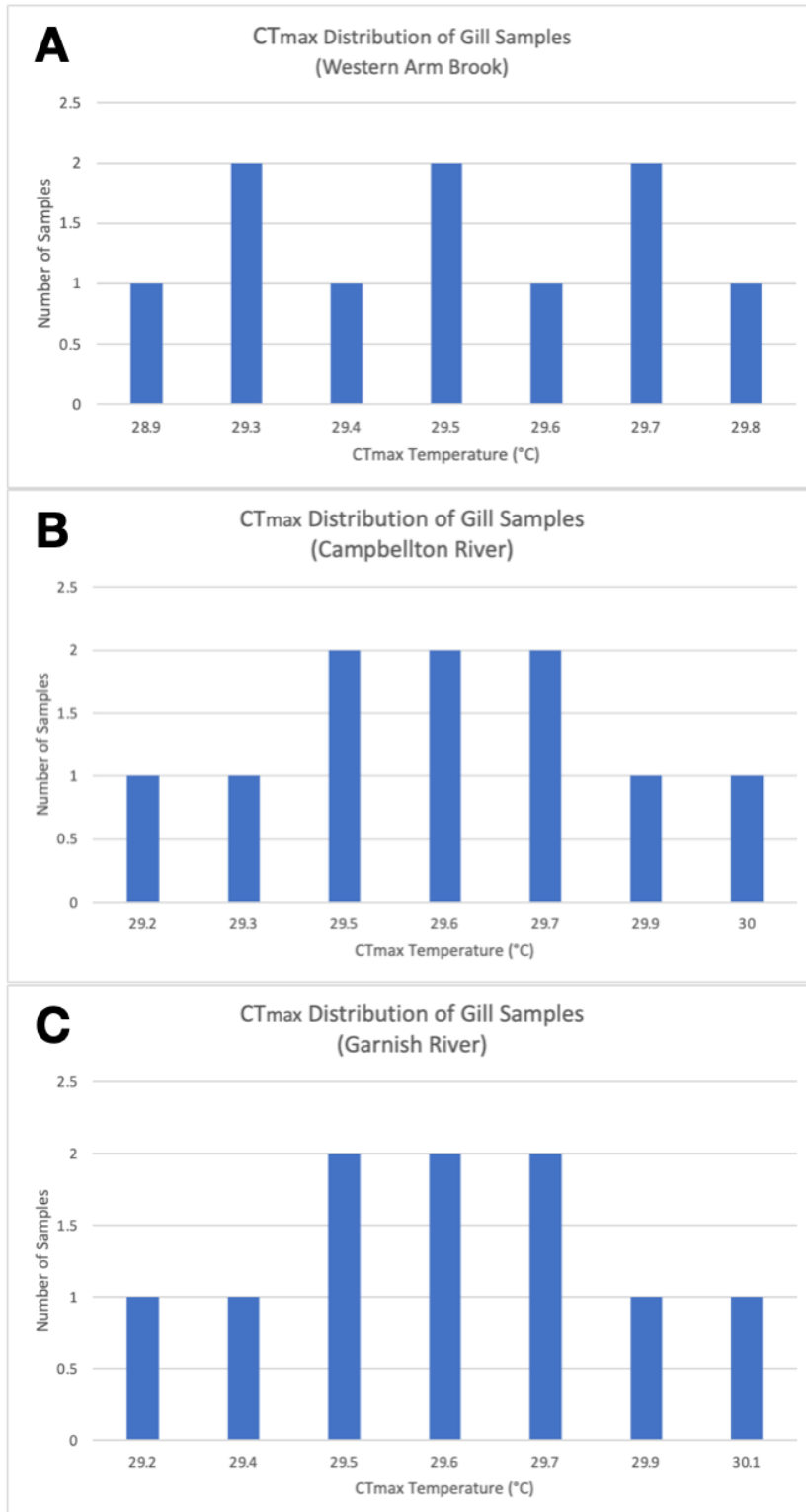


Figure 3. Graphs showing the CT_{max} distribution of gill samples from the three different populations, including **A.** Western Arm Brook, **B.** Campbellton River, and **C.** Garnish River.

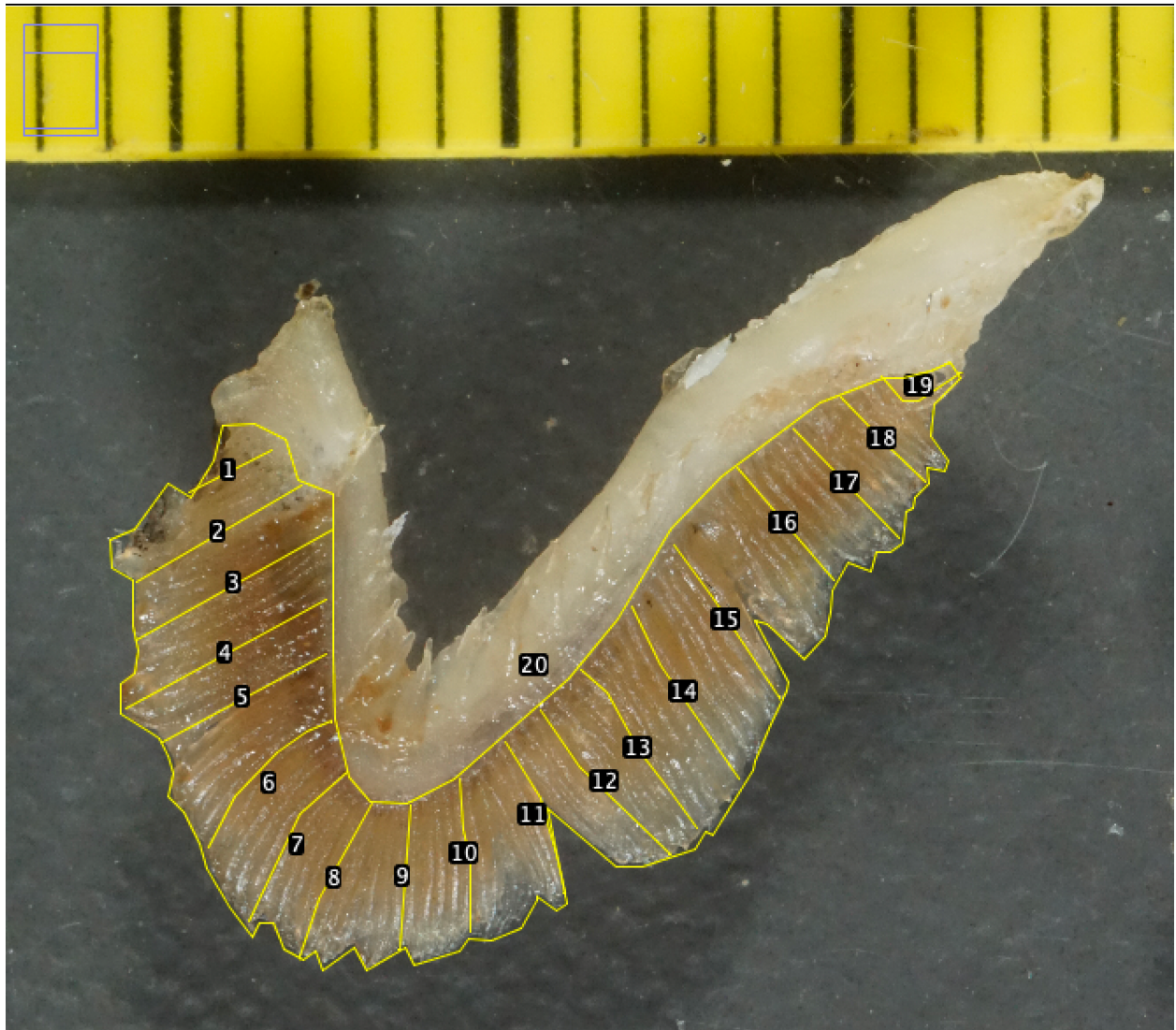


Figure 4. Image showing how total gill surface area, total gill filament length, average filament length, and total number of filaments are calculated using ImageJ software. Numbers 1-19 indicate measurements used to calculate gill filament length (average and total) and total number of gill filaments. Number 20 indicates the measurement used to calculate total gill surface area. See text for full details.

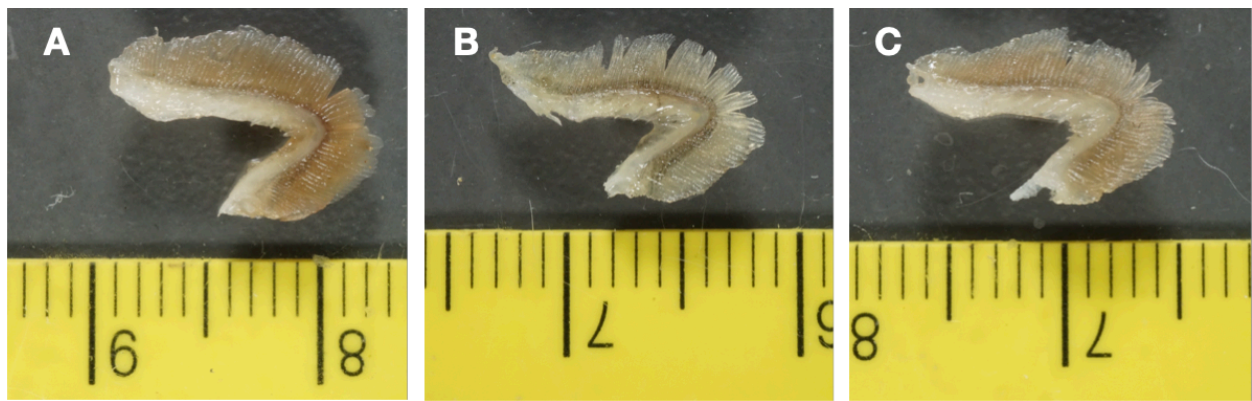


Figure 5. Examples of gill photos from the three populations, including **A.** Western Arm Brook (Sample 406), **B.** Campbellton River (Sample 211), and **C.** Garnish River (Sample 328).

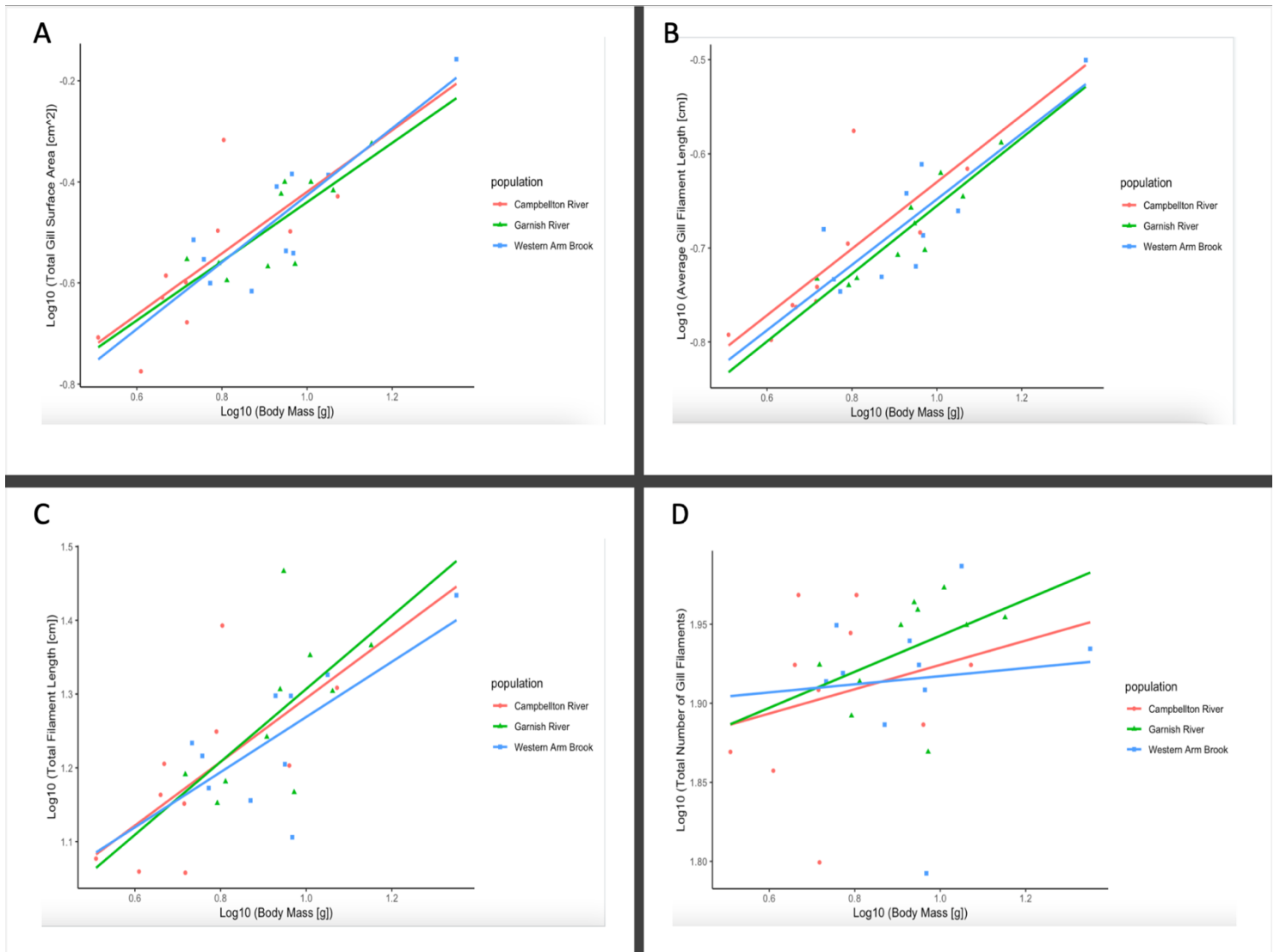


Figure 6. Relationship between logarithmically transformed gill trait and logarithmically transformed body size, coloured by population (Campbellton River, Western Arm Brook, Garnish River). Panels show **A.** Total gill surface area, **B.** Average gill filament length, **C.** Total gill filament length, and **D.** Total number of gill filaments.

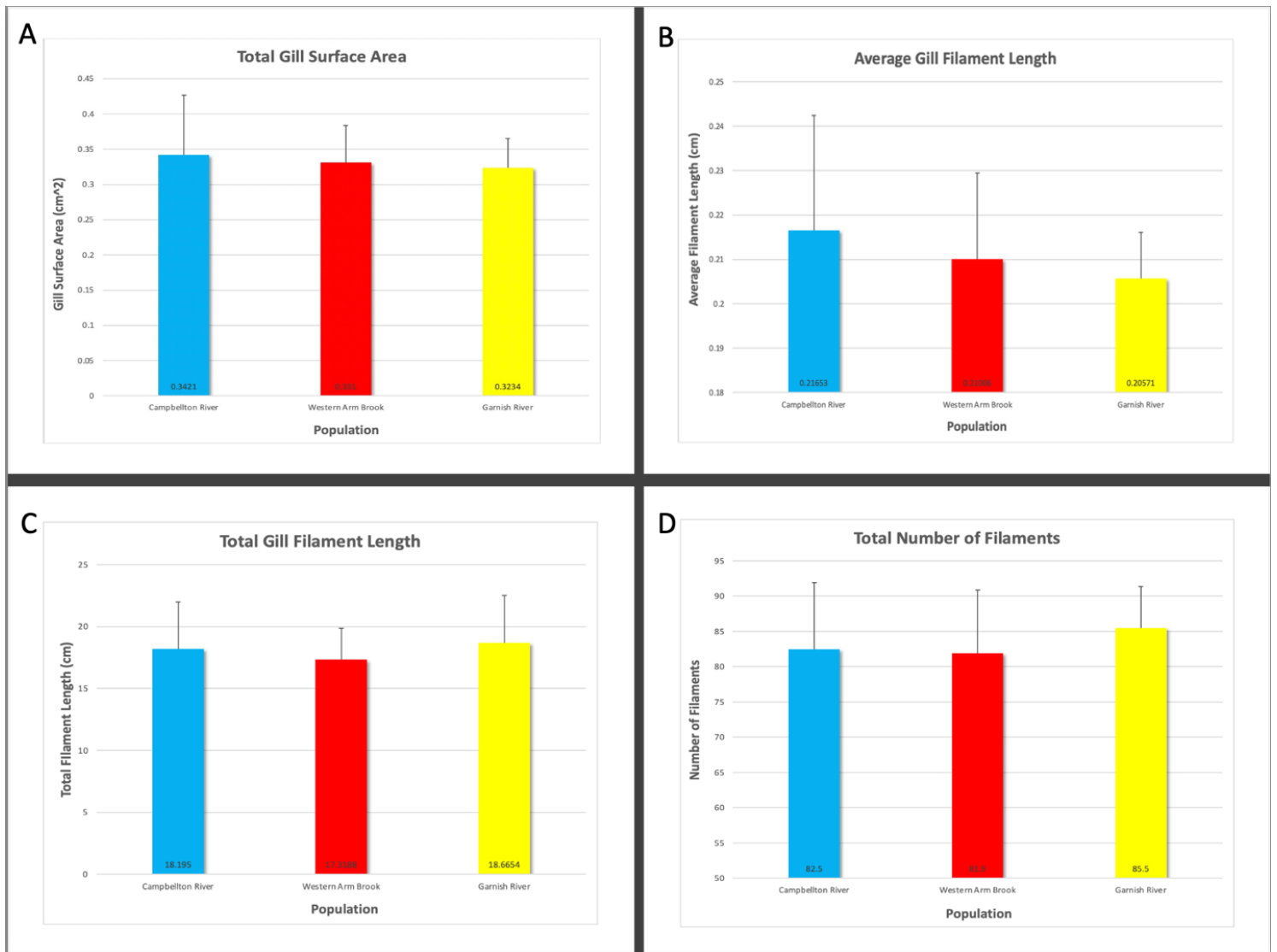


Figure 7. Mean values (+ standard deviation; SD) of four mass-adjusted gill metrics compared across three populations (Campbellton River, Western Arm Brook, Garnish River) of juvenile Atlantic salmon to illustrate inter-population differences. Panels show **A.** Total Gill Surface Area, **B.** Average Gill Filament Length, **C.** Total Gill Filament Length, and **D.** Total Number of Filaments.

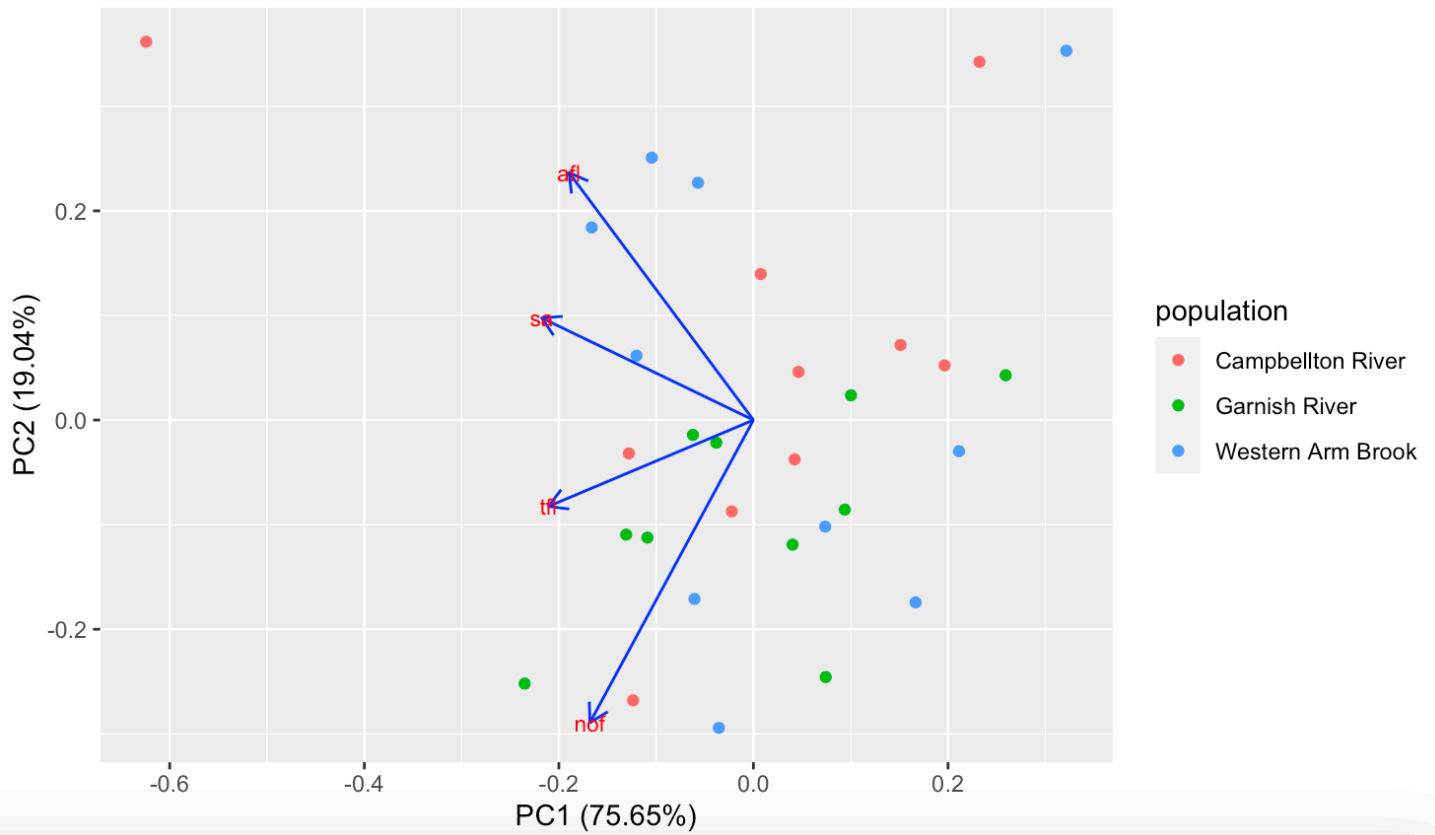


Figure 8. Principal component analysis (PCA) comparing four mass-adjusted gill metrics across three distinct populations (Campbellton River, Western Arm Brook, Garnish River) of juvenile Atlantic salmon. (sa = total surface area, afl = average filament length, tfl = total filament length, nof = total number of filaments).

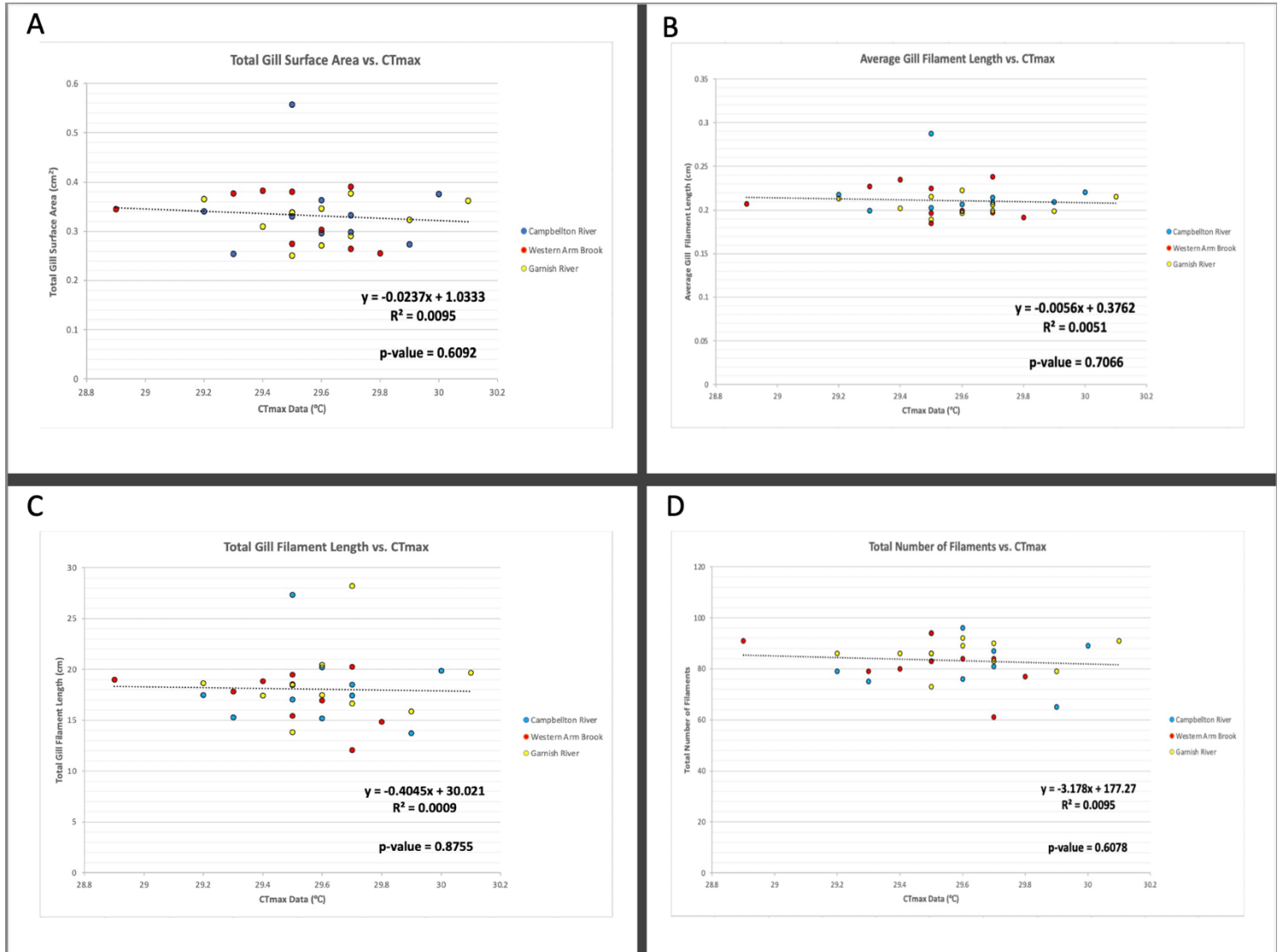


Figure 9. Relationship between four mass-adjusted gill metrics and CT_{max} temperatures across three distinct populations (Campbellton River, Western Arm Brook, Garnish River) of juvenile Atlantic salmon. Panels show **A.** Total gill surface area, **B.** Average gill filament length, **C.** Total gill filament length, and **D.** Total number of gill filaments.

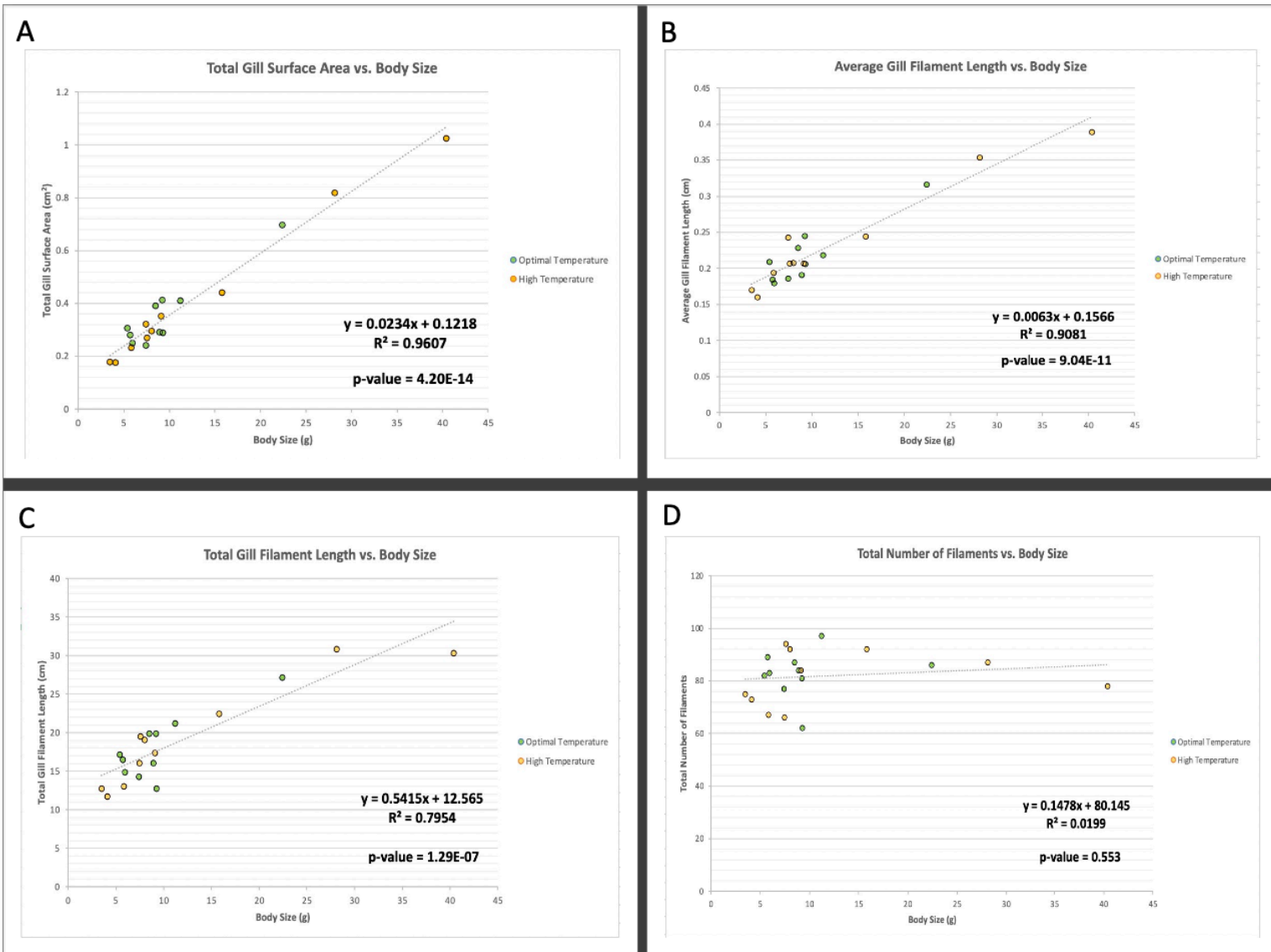


Figure 10. Relationship between four gill metrics (not mass-adjusted) and body size in juvenile Atlantic salmon from the Western Arm Brook population across both temperature conditions. Panels show **A.** Total gill surface area, **B.** Average gill filament length, **C.** Total gill filament length, and **D.** Total number of gill filaments.

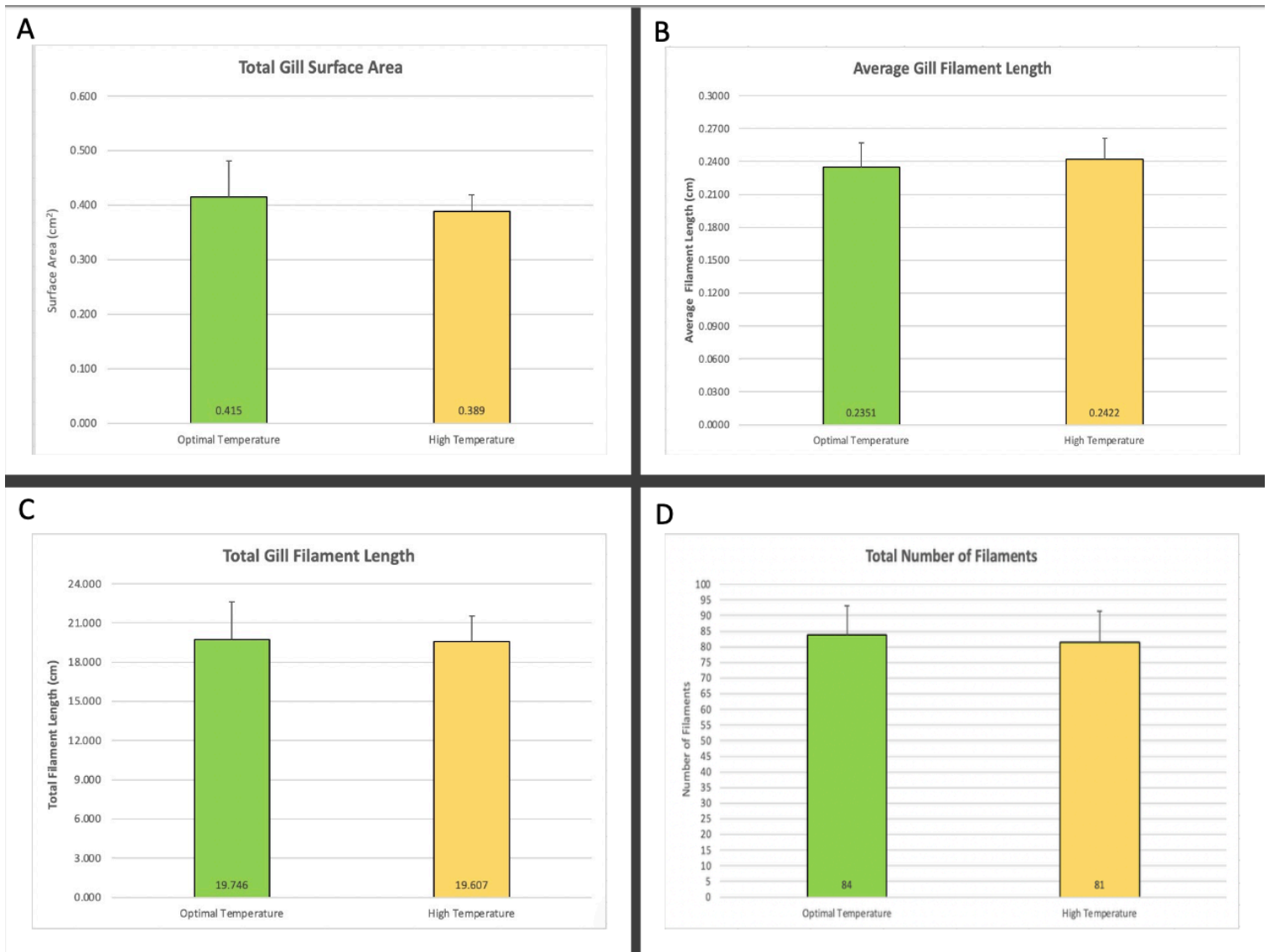


Figure 11. Intra-population (Western Arm Brook) comparison of juvenile Atlantic salmon using mean values (+ standard deviation; SD) of four mass-adjusted gill metrics from two different temperature conditions (optimal temperature (~15°C) and high temperature (~20°C)). Panels show **A.** Total gill surface area, **B.** Average gill filament length, **C.** Total gill filament length, and **D.** Total number of gill filaments.

10 | Appendix

Table 8: Raw data from thermal adaptation trials. (TSA = total surface area, AFL = average filament length, TFL = total filament length, TNF = total number of filaments).

Fish ID	Population	Body Size (g)	CT _{max} (°C)	TSA (adjusted) (cm ²)	AFL (adjusted) (cm)	TFL (adjusted) (cm)	TNF (adjusted) (cm)
405	Campbellton River	6.37	29.5	0.557	0.2873	27.308	94
412	Campbellton River	4.66	29.6	0.363	0.2066	20.187	96
126	Campbellton River	5.22	29.9	0.274	0.2092	13.708	65
309	Campbellton River	9.13	29.6	0.296	0.1995	15.197	76
324	Campbellton River	4.57	29.7	0.332	0.2089	18.474	87
85	Campbellton River	3.23	29.2	0.341	0.2175	17.477	79
211	Campbellton River	6.17	30	0.376	0.2204	19.874	89
285	Campbellton River	11.8	29.7	0.298	0.2144	17.419	81
298	Campbellton River	4.07	29.3	0.254	0.1992	15.254	75
407	Campbellton River	5.19	29.5	0.33	0.2023	17.052	83
406	Western Arm Brook	8.48	29.5	0.38	0.2248	19.479	86
88	Western Arm Brook	8.92	29.5	0.275	0.185	15.408	83
162	Western Arm Brook	7.41	29.8	0.255	0.1914	14.859	77
280	Western Arm Brook	9.28	29.7	0.265	0.197	12.068	61
176	Western Arm Brook	9.21	29.4	0.382	0.235	18.816	80
308	Western Arm Brook	11.22	29.5	0.338	0.1966	18.527	94
313	Western Arm Brook	5.92	29.6	0.303	0.1986	16.949	84
323	Western Arm Brook	5.41	29.7	0.39	0.2382	20.251	84
379	Western Arm Brook	5.72	28.9	0.345	0.207	19.009	91
390	Western Arm Brook	22.43	29.3	0.377	0.227	17.822	79
410	Garnish River	14.18	29.5	0.338	0.2153	18.426	86
404	Garnish River	11.52	29.4	0.31	0.2018	17.41	86
212	Garnish River	8.69	30.1	0.362	0.2152	19.673	91
380	Garnish River	5.22	29.2	0.365	0.2133	18.634	86
316	Garnish River	10.21	29.6	0.346	0.2223	20.467	92

328	Garnish River	8.86	29.7	0.377	0.2058	28.22	90
125	Garnish River	6.2	29.9	0.323	0.1986	15.864	79
185	Garnish River	8.09	29.6	0.271	0.1962	17.467	89
278	Garnish River	6.48	29.7	0.291	0.1992	16.661	83
305	Garnish River	9.37	29.5	0.251	0.1894	13.832	73

Table 9: Raw data from phenotypic plasticity trials. (TSA = total surface area, AFL = average filament length, TFL = total filament length, TNF = total number of filaments).

Fish ID	Population	Body Size (g)	Temperature Conditions	TSA (adjusted) (cm ²)	AFL (adjusted) (cm)	TFL (adjusted) (cm)	TNF (adjusted) (cm)
406	Western Arm Brook	8.48	Optimal (~15°C)	0.476	0.2517	22.207	88
88	Western Arm Brook	8.92	Optimal (~15°C)	0.343	0.2068	17.575	85
162	Western Arm Brook	7.41	Optimal (~15°C)	0.326	0.2152	16.918	79
280	Western Arm Brook	9.28	Optimal (~15°C)	0.330	0.2200	13.770	63
176	Western Arm Brook	9.21	Optimal (~15°C)	0.475	0.2624	21.468	82
308	Western Arm Brook	11.22	Optimal (~15°C)	0.410	0.2183	21.177	97
313	Western Arm Brook	5.92	Optimal (~15°C)	0.397	0.2247	19.257	86
323	Western Arm Brook	5.41	Optimal (~15°C)	0.516	0.2703	22.988	85
379	Western Arm Brook	5.72	Optimal (~15°C)	0.454	0.2344	21.589	92
390	Western Arm Brook	22.43	Optimal (~15°C)	0.422	0.2470	20.507	83
CHR1	Western Arm Brook	15.81	High (~20°C)	0.344	0.2160	19.529	90
CHR2	Western Arm Brook	28.13	High (~20°C)	0.422	0.2554	21.215	83
CHR3	Western Arm Brook	7.58	High (~20°C)	0.357	0.2378	22.801	96
CHR4	Western Arm Brook	7.45	High (~20°C)	0.430	0.2804	18.891	67
CHR5	Western Arm Brook	8.02	High (~20°C)	0.375	0.2335	21.849	94
CHR6	Western Arm Brook	5.85	High (~20°C)	0.372	0.2443	16.908	69
CHR7	Western Arm Brook	9.11	High (~20°C)	0.408	0.2226	18.897	85
CHR8	Western Arm Brook	4.12	High (~20°C)	0.361	0.2280	17.500	77
CHR9	Western Arm Brook	3.48	High (~20°C)	0.412	0.2567	20.428	80
CHR10	Western Arm Brook	40.39	High (~20°C)	0.407	0.2469	18.049	73