Life through a wider scope: Brook Trout (*Salvelinus fontinalis*) exhibit similar aerobic scope across a broad temperature range

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Abstract

Brook Trout (*Salvelinus fontinalis*) have been widely introduced throughout the world and are often considered as direct competitors with native salmonid species. Metabolic rate is one metric we can examine to improve our understanding of how well fish perform in different habitats, including across temperature gradients, as metabolism can be directly influenced by environmental temperatures in ectotherms. We estimated the standard metabolic rate, maximum metabolic rate and aerobic scope of lab-reared juvenile Brook Trout (~1 year) using intermittent-flow respirometry across a range of temperatures (5-23°C) likely experienced in the wild. We included a diurnal temperature cycle of ± 1.5°C for each treatment temperature to simulate temporal variation observed in natural waterbodies. Standard metabolic rate and maximum metabolic rate both increased with acclimation temperature before appearing to plateau around 20°C, while mass specific aerobic scope was found to increase from 287.25±13.03 mg O2·kg-1·h-1 at 5°C to a mean of 384.85±13.31 mg O2·kg-1·h-1 at 15°C before dropping at higher temperatures. Although a slight peak was found at 15°C, the generally flat thermal performance curve for aerobic scope suggests Brook Trout are capable of adjusting to a relatively wide range of thermal regimes, appearing to be eurythermal, or a thermal generalist at least for salmonids. The ability of this population to maintain similar physiological performance across a wide range of temperatures may help explain why Brook Trout succeed in a variety of different thermal habitats.

Keywords: Aerobic scope; Brook Trout; temperature variation; thermal generalist

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1.1 Introduction

Brook Trout (*Salvelinus fontinalis*) are a widely introduced species throughout the world and are often considered in direct competition with native trout species in areas of introduction (DeHaan et al., 2010; Gunckel et al., 2002; Isaak et al., 2015). In their native range of north-eastern North America, Brook Trout are considered a cold-water species, yet in many introduced areas, such as the Rocky Mountains in western North America, they are viewed as possessing a warmer water tolerance than native species. The perceived warmer water tolerance is based on evidence that shows Brook Trout are often found in the lower reaches of streams where water temperatures are typically warmest (Paul & Post, 2001). It is also assumed that Brook Trout possess a physiological advantage over native species in warmer waters (i.e., increased growth and food conversion efficiency; McMahon et al., 2007). Previous studies looking at the effects of temperature on Brook Trout and native salmonid species found that peak aerobic scope (Graham, 1949) and temperature preference (Macnaughton, Kovachik, et al., 2018) of Brook Trout both occur from 15–17°C, while their upper incipient lethal temperature (UILT) is ~25°C (Fry et al., 1946; McCormick et al., 1972). UILT is a plastic trait across life stages and populations and is defined as the upper temperature a species is able to tolerate without mortality (Fry et al., 1946). Many native species that Brook Trout co-occur with have lower UILTs, including Bull Trout (*Salvelinus confluentus*) – 20.9°C – (Selong et al., 2001) and Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*)– 19.6°C – (Bear et al., 2007). Since temperature affects growth, reproduction, and metabolic performance of fish, temperature tolerance can have a substantial influence on the habitats fishes occupy across watersheds (Isaak et al., 2017; McMahon et al., 2007; Selong et al., 2001).

Metabolic rate (MR) is an estimate of the amount of energy expended by an organism under a given condition (Fry, 1957; Treberg et al., 2016), and is most often measured indirectly in fish using techniques such as respirometry, which measures oxygen consumption over time. With ectotherms, there are three main metrics estimated to describe the aerobic MR: standard metabolic rate (SMR), maximum metabolic rate (MMR) and aerobic scope (AS). SMR is the minimal metabolic costs required by an ectotherm to maintain physiological functions in an unfed state and at rest, i.e., including homeostasis (Beamish, 1964; Brett & Groves, 1979; Fry, 1971; Treberg et al., 2016). Standard metabolic rate is comparable to basal metabolic rate in endotherms (BMR; mammals, birds, etc.), but unlike BMR, which should be measured within the organism's thermal neutral zone of environmental temperature, SMR is measured at a defined environmental temperature. MMR is the maximum aerobic metabolic rate of an organism (Brett & Groves, 1979; Fry, 1971; Treberg et al., 2016), often achieved during exhaustive exercise. Aerobic scope is the difference between SMR and MMR and can be used as a measurement of the amount of oxygen available for life processes beyond those required for basic existence (SMR). An organism's AS also sets a theoretical limit for the amount of aerobic energy that can be allocated to any additional energetically demanding processes, e.g., growth, reproduction, anti-predator behaviour (Eliason & Farrell, 2016). It is thought that with higher AS, an organism has the ability to perform more energy demanding processes simultaneously, conferring a competitive advantage due to a greater metabolic capacity (Eliason & Farrell, 2016).

As most fishes are ectotherms, temperature influences their metabolic rate, with SMR and MMR generally increasing as water temperatures increase, at least up to some upper threshold (Norin & Clark, 2016; Schulte, 2015; Szekeres et al., 2016). This makes temperature extremely important for the survival of fish species (Eliason & Farrell, 2016). Thermal physiology has two main contrasting views; the conservative view that species do not easily evolve and adapt to changes in temperature, whereas the labile view predicts that species can easily acclimate (short-term) or adapt (long-term/generational) to thermal changes, leading to long term evolution (Hertz et al., 1983). Under the labile view, evolutionary adaptation can occur slowly through many generations or more quickly through natural selection removing individuals who are unable to acclimate to a thermal change (Hertz et al., 1983). Since these opposing views were proposed by Hertz et al. (1983), empirical evidence supporting both arguments has been found because some species and populations are more readily able to adapt to changes in temperature and climate than others, as seen in several Pacific salmon species (T. D. Clark et al., 2011; Eliason & Farrell, 2016; Poletto et al., 2017).

Changes in performance traits, including MR, over a range of temperatures is often displayed graphically using a thermal performance curve (Schulte et al., 2011). It has been shown with many salmonid species that SMR and MMR have an exponential relationship with increasing water temperatures, until a certain point, before reaching a plateau or sharply declining near upper lethal temperatures (Fry, 1947; Lee, 2003; Macnaughton et al., 2018). Based on SMR and MMR relationships with temperature, AS thermal performance curves are often reported to increase as water temperatures increase until an optimum temperature and then decline as water temperatures continue to increase (Eliason & Farrell, 2016).

It is possible that different responses to temperature changes may be related to the breadth of a species', or a population's thermal performance curve. Thermal generalists possess flatter thermal performance curves, and, therefore, exhibit similar metabolic performance across a wide temperature range, whereas, thermal specialists have narrower thermal performance curves with a clearly defined peak in performance (Angilletta et al., 2002; Gilchrist, 1995). Possessing a similar AS across a broad range of temperatures may allow a species to better cope with temperature variation in their environment, and a recent review by Nati et al. (2016) showed that a broad AS range does not prevent having a peak AS at an optimal temperature for teleost fish, including several salmon, trout and sculpin species. Thermal generalists may benefit from a broader AS range in some situations, such as in habitats that experience large diurnal temperature variations, but be hindered in other situations, such as when living in sympatry with thermal specialists at their optimum temperature (Angilletta et al., 2002). Field studies investigating the thermal preference of Brook Trout across their native and introduced ranges have found fish across a wide range of water temperatures and have suggested the mean preferred temperature to be anywhere from 10.6 ± 0.96°C to 17.1 ± 0.31°C (Baird & Krueger, 2003; Goyer et al., 2014). Based on the wide range of assumed temperature preference, we hypothesized that Brook Trout are a thermal generalist, with a wide thermal performance curve and similar AS across our test temperature range.

When considering the effect temperature has on biological processes, such as metabolic rates in fish, Morash et al. (2018) indicated that previous studies ignored the inherent natural temperature fluctuations that fish experience in the wild. Failing to account for temporal variation in temperature when conducting laboratory experiments may incorrectly estimate results of physiological variables such as AS. Morash et al. (2018) showed that if fish experience a range of temperatures along the thermal performance curve, the value of their AS at the mean temperature will lie somewhere off the curve, between the lower and upper temperature AS values (also referred to as Jensen's inequality). Since fish experience daily thermal variation in their natural habitat, temperature variability should be considered when estimating physiological variables (i.e., AS) in the lab, especially if the intent is to compare with estimates obtained for wild fish, in-situ experiments, or when using lab-derived predictions to represent wild fish.

The goal of this study was to estimate SMR, MMR, and AS of juvenile Brook Trout across a range of acclimation temperatures experienced in the wild (5–23°C) using intermittent-flow respirometry. A daily thermal cycle of 3°C (treatment temperature ± 1.5°C) was used to simulate natural daily temperature variations experienced by fish in the wild. Results from this experiment may serve as a baseline for comparison with native congeneric species where Brook Trout have been introduced.

1.2 Materials and Methods

1.2.1 Animal Husbandry

Brook Trout used in this experiment were the second generation (F1) from a brood stock obtained from the Whiteshell Fish Hatchery in eastern Manitoba, Canada. This strain of Brook Trout originated from Gods Lake/Gods River in Northern Manitoba and was brought to the Whiteshell Fish Hatchery in the 1970s before being stocked into the South Duck River on the east slope of the Duck Mountains, Manitoba. Following stocking in the South Duck River, a new brood stock was established at the hatchery from this riverine source (Kevin Dyck, personal comm., 2018). Brook Trout were obtained from the hatchery in 2016 and bred in the Fish Holding Facility at the Freshwater Institute in the fall of 2017. 12 males and 8 females were used as brood stock from the P1 population of Brook Trout. Gravid fish were anaesthetized using MS-222 (concentration: 80 mg·l^{-1} (Syndel Laboratories Ltd., Vancouver, British Columbia, Canada), buffered with 160 mg l^{-1} of sodium bicarbonate) before eggs and milt were collected by gently squeezing and sliding a thumb along the underside of the fish towards the vent to encourage gamete release. Eggs and milt from all brood stock were combined in a bowl and gently mixed before being placed in a vertical incubator egg tray system. Eggs were held at 10°C throughout incubation and hatch.

The general population of Brook Trout was reared in two aerated 600 l circular flow-through tanks held at ~10°C and fed *ad libitum* once daily with commercial pellet fish food (EWOS Pacific: Complete Fish Feed for Salmonids, Cargill). Fish were maintained on a 12:12 h diurnal light cycle, with 20 min transition intervals of low light levels to simulate dawn and dusk periods.

1.2.2 Experimental Setup

From October 30, 2018 – February 6, 2019, a total of 275 juvenile fish of ~1 year of age (weight range = 4.6–74.5 g) were haphazardly selected from the general population tanks and transferred to one of two 200 l flow-through tanks for acclimation. As only two tanks were available for acclimation, fish were acclimated to the five treatment temperatures in a staggered order over the three month experimental period as tank space allowed. The order in which treatments were started was chosen randomly using a random number generator. The treatment order was 10, 23, 20, 15, 5°C to avoid growth and mass increasing with treatment temperature (i.e. smallest fish at 5°C, largest fish at 23°C). The tanks were held at 10°C for the initial day following transfer to allow fish to recover before being gradually acclimated to their treatment temperatures (5°, 15°, 20°, 23°C). Diurnal fluctuations were included during the first part of the acclimation period, which consisted of gradually warming or cooling each of the groups to their treatment temperature ± 1.5°C, at a rate of 1.5–2°C per day, using WitroxCTRL software (Loligo® Systems, Tjele Denmark). Once the treatment temperature was reached, a diurnal temperature cycle was maintained for three weeks by setting the tank temperature to ± 1.5°C of the treatment temperature (e.g., 13.5–16.5°C for the 15°C treatment group, Figure 3). To ensure comparable experimental manipulations between treatments, the 10°C treatment group was subjected to the same acclimation procedure as other groups, which included a three week 'acclimation period' in the acclimation tank set at 10 ± 1.5°C. The temperature fluctuations followed the daily thermal regime of streams from the Spray River watershed in Banff National Park, a system where Brook Trout were introduced nearly a century ago. Stream temperature data was recorded in the Spray River watershed for a separate set of experiments. Temperature fluctuations were based on the average daily temperature range from three streams recorded using HOBO Tidbit® v2 temperature loggers (ONSET Computer Corporation, Bourne, Massachusetts, USA) from mid-July to mid-September 2017. The lowest temperature in the cycle was from 08:00-09:00 h, warming throughout the day until peak temperatures at 17:00 h, before cooling again overnight. Fish were continued to be fed as described above for the duration of the experiment unless otherwise indicated.

Following a minimum of three weeks of acclimation (21-32 days total acclimation), n = 8 fish per temperature treatment were haphazardly selected from their acclimation tank at a time and subjected to intermittent-flow respirometry trials using AutoResp software (Loligo® Systems, Tjele Denmark), that maintained the diurnal temperature cycle (Figure 4). Intermittent respirometry was conducted at each treatment over a 9-11 day period. A respirometry trial for each individual proceeded as follows: fish were fasted for 24 h prior to experiments, weighed on a wetted scale and measured for fork length and total length before undergoing an exhaustive chase protocol as described in Mochnacz et al. (2017). Air exposure time for weighing and measuring was generally under 20 s. During the chase protocol, fish were encouraged to swim against a constant flow of water until exhaustion. The exhaustion end-point was determined when the fish was no longer able to maintain its position in the current and did not respond to a caudal tail pinch. Immediately following the chase protocol, fish were transferred to a respirometry chamber (volumes: 540, 655 ml + 61 -69 ml tube volumes; Loligo® Systems, Tjele Denmark), where three MMR estimates were taken for each fish (measurement cycle = Measure – 180 s, Flush – 300 s, Wait – 40 s) and the time to exhaustion was recorded. Following the estimation of MMR, SMR estimates were collected for a minimum of 24 h, with the same measurement cycle as MMR. Once SMR estimates were completed, fish were removed from the chambers and euthanized with a lethal dose of MS-222 (concentration: 300 $mg \cdot l^{-1}$, buffered with 600 mg l^{-1} of sodium bicarbonate), after which individuals were dissected to determine sex and maturity (immature vs mature gonad state). Background oxygen demand (BOD) of microbial growth in the water was estimated before and after each experimental trial by recording oxygen consumption in an empty chamber. BOD estimates were also taken in empty chambers during MMR estimates. To ensure BOD levels were kept to a minimum, the system was cleaned with a 10% hydrogen peroxide solution and thoroughly rinsed with fresh water after experiments. Dissolved oxygen sensors were calibrated between experiments using a two-point calibration in an anoxic solution of sodium sulfite (0% oxygen; 1 g Na_2SO_3:100 ml of water) and in water vapor-saturated air in an enclosed vessel.

We originally planned treatment temperatures of 5°, 10°, 15°, 20°, and 25°C (± 1.5°C), with 5°C being the lowest we were able to maintain water temperature with the experimental setup and 25°C the highest temperature tolerated by juvenile Brook Trout (upper thermal tolerance to be 25.3°C; Fry et al., 1946). The warmest treatment was intended to test for any potential decline in AS at the species' upper thermal limits. However, within a week of acclimation at the 25°C treatment, some fish were observed to have skin lesions and reduced feeding. Several mortalities also occurred over the following days, therefore, we ended this treatment after 11 days, and fish from this treatment were euthanized and not used for the current study. Instead a new group of fish was acclimated to 23°C, which became the new upper temperature treatment. No fish in the 23°C treatment demonstrated signs of poor health or issues like feeding hesitancy and there were no mortalities during the acclimation phase for this group. A total of 126 fish were used in the experiment (n = 24 for treatments at 5°, 10°, 15°C, n = 26 at 20°C and n = 28 at 23°C) due to some mortalities during experimentation at the highest temperature treatments (one at 20°C and four at 23°C). Additional fish were tested at 20 and 23°C to account for fish mortality and to ensure n = 24 estimates of SMR were completed for each treatment. Furthermore, one additional fish was tested at 20°C as the individual fish was already acclimated as a potential extra fish in case of mortalities. All procedures conducted were approved by Fisheries and Oceans Canada Animal Care Committee (FWI-ACC-AUP-2018-02/2019-02).

1.2.3 Data Analysis

Oxygen consumption of individual fish was measured using in-line oxygen probes (PreSens, Regensburg, Germany) inside the respirometry chambers and automatically calculated as $\dot{M}O_2$ estimates (mg $O_2 \cdot h^{-1}$) by the AutoResp software, based on the volume of the respirometry chamber and tubing in millilitres (minus the volume of the fish based on wet mass in grams). Goodness of fit of oxygen linear depletion rates (r^2 values) were automatically generated and were used to validate the quality of the estimate, where only r^2 values above 0.9 were used for the final analysis of SMR and MMR estimates. SMR was calculated using the lowest 20th quantile of $\dot{M}O_2$ estimates, after removing the first 10 h of measurements to ensure only estimates from when the fish returned to a resting state following the exhaustive chase and handling stress. $\dot{M}O_2$ estimates for SMR were further analysed visually using the 'FishMO2' package (Chabot et al., 2016) in R (R version 3.5.2, R Core Team, 2018) to verify the rate of $\dot{M}O_2$ decline for each measurement. MMR was calculated using the highest of the three $\dot{M}O_2$ estimates obtained immediately following the exhaustive chase. The average value of BOD of each experiment was subtracted from all SMR and MMR estimates. AS estimates were obtained by subtracting the SMR estimate from the MMR estimate for each fish. It is worth noting that

SMR and MMR estimates for each fish were obtained at slightly different temperatures due to the temperature cycling occurring within the experiment and the different time of day that each estimate was achieved. MMR was estimated between 10:00 and 14:00 h, while SMR was often found during the early hours of the morning (~04:00–10:00 h). This led to an average temperature difference of 0.87 ± 0.55 °C (0.00–2.13°C). To account for these differences in temperature when analysing AS, we used the average temperature difference between SMR and MMR for each fish. Although this averaging may introduce some inaccuracy depending on the shape of the thermal performance curve and the effect of Jensen's inequality, we assumed this inaccuracy will be small relative to the variation across treatment groups given the relatively small daily temperature differences relative to the range of acclimation temperatures used in the current study.

1.2.4 Statistical Analysis

We found substantial variation in fish size (fish body mass and fork length), 4.6–74.5g and 84-184mm respectively, within our sampled experimental fish and a large number of both male and female fish, some of which had already reached maturity (Table 1). The variability within our sampled fish allowed us to test for possible differences or interactions between several variables, including mass, sex, and time to exhaustion, and their effects on SMR, MMR and AS. The large range in fish mass made it necessary to mass correct the data for analysis to avoid mass confounding the analysis, due to the relationship between mass and MR.

To account for the large range of fish mass across treatments and the effect that mass had on metabolic rate (see section 1.3.1), whole body metabolic rate data (SMR, MMR, AS) were mass corrected to the average mass of all fish in the study (33.3 g) using multivariate polynomial predictive equations derived from the dataset. Multiple linear regression models were run using the MuMIn package in r to analyse changes in metabolic rates across treatment temperatures. A global model was developed for each metabolic rate metric (i.e., SMR, MMR, AS), which contained all of the variables thought to influence metabolic rates based on previous studies (Chabot et al., 2016; Fry, 1971; Treberg et al., 2016); MR = Temperature + Temperature^2 + Temperature^3 + Mass + Mass^2 + Mass^3 + Sex + Time to exhaustion + Maturity. Polynomial temperature and mass terms were included in the model to improve model fit, as preliminary evaluation of the data using only linear and quadratic terms did not fit our data appropriately and we prioritized fitting the model to the data over the biological intuitiveness of the model itself. The need for cubic terms is likely due to the fact that our 10°C treatment SMR and MMR estimates deviated from the expected quadratic relationship (higher for SMR and lower for MMR than 15°C treatment). Due to this difference, the expected quadratic relationship did not fit our dataset. Sex and time to exhaustion were found to be covariate factors of mass, with both variables being significant for whole body estimates of SMR, MMR and AS, but not for mass-corrected estimates, thus, excluding them from the final model. Fish maturity status was also found to be a significant variable, however, due to uneven variance across treatments we were unable to account for its effects, so mature fish were removed from our final analysis. Biological data and metabolic rate estimates that include mature fish can be found in Table A5 in the appendix. The best fit model was chosen from all models that included all dependencies for polynomial terms (i.e., any model with T^2 also needed to include T). AICc and AIC weight were both used to perform model averaging for SMR, MMR and AS on all models with a ΔAIC value within 2 of the model with the lowest AICc value. Full model selection steps and AICc values can be found in the Supplementary data (Tables A2, A3, and A4). Model-averaged coefficients from our best fit models were used to create equations for each MR. The equation was then run using temperature data and the standardized mass of 33.3 g to mass correct MR estimates for each fish. Residuals of the relationship for each fish were added to each estimate to account for individual variation (Guzzo et al., 2019; Poletto et al., 2017).

Mass corrected data was $\log_{10}$ transformed for analysis to test for effects of temperature on SMR, MMR, and AS using ANOVAs. Post-hoc testing was done using Tukey's honest significant difference test (Tukey HSD) on any significant variables found to identify differences in sex, mass, time to exhaustion and temperature within and across treatments. P values < 0.05 were deemed significant. Mass-corrected data are presented in mass specific values ($mg\ O_2^{-1} \cdot kg^{-1} \cdot h^{-1}$) for easier comparison to other studies.

Statistical analysis was performed in R and R Studio (version 1.1.383, RStudio, Inc., 2017) using the packages 'car' (Fox & S., 2019), 'caret' (Kuhn, 2008), 'dplyr' (Wickham et al., 2020), 'MASS' (Venables & Ripley, 2002), 'multcomp' (Hothorn et al., 2008), 'MuMin' (Barton, 2016), 'plotrix' (J, 2006), and 'tidyverse'(Wickham, 2019).

1.3 Results

1.3.1 Mass

Fish mass differed significantly across treatment temperature (ANOVA, $F_{(4,94)}$=31.89, P>0.001), which can affect subsequent analyses due to the relationship between mass and metabolic rate. Whole body $\log_{10}$ SMR, $\log_{10}$ MMR and $\log_{10}$ AS increased linearly against $\log_{10}$ mass within each temperature treatment (Table A1, Figure B1). The overall effect of mass on SMR ($F_{(1.93)}$=221.42, P<0.001), MMR ($F_{(1.93)}$=412.50, P<0.001), and AS ($F_{(1.93)}$=185.15, P<0.001) were all highly significant.

1.3.2 Time to Exhaustion

There was no effect of time to exhaustion (*E*) from the chase protocol on whole body SMR ($F_{(1,97)}$=0.09, P=0.76), MMR ($F_{(1,97)}$=0.62, P=0.43) or AS ($F_{(1,97)}$=0.03, P=0.87). Mass had a significant effect on time to exhaustion ($F_{(1,97)}$=6.94, P=0.01). Temperature did not have an effect on *E* ($F_{(1,97)}$=0.00, P=0.99); however as fish grew larger, their *E* increased regardless of the testing temperature (Figure 3), following a linear relationship represented by equation 1:

Eq.1

$$E = 825.39 + 5.25 \cdot M$$

where *M* is equal to mass in grams and *E* is measured in seconds.

1.3.3 Sex

There was no effect of sex on fish mass one mature fish were removed from the analysis (ANOVA, $F_{(1,97)}$=2.42, P=0.12), however male fish on average had a longer fork length and weighed more than female fish (143.7 mm, 35.8 g and 136.2 mm, 30.8 g respectively). There still was an effect of sex on whole body SMR estimates ($F_{(1,97)}$=6.94, P=0.01) but not on MMR ($F_{(1,97)}$=3.34, P=0.07) or AS ($F_{(1,97)}$=1.641, P=0.21). However, once MR estimates were mass corrected, sex was no longer found to be significant and for this reason, we did not further explore sex-dependent differences.

1.3.4 Metabolic Rate comparisons

Standard Metabolic Rate

Standard metabolic rate increased with treatment temperature (ANOVA; $F_{(4,93)}$=108.85, P<0.001) up to 20°C before appearing to plateau (Figure 4), with SMR estimates in the 20°C and 23°C treatments being statistically different from estimates at 5°, 10°, and 15°C (Tukey HSD). The response of Brook Trout SMR to increasing temperature and body mass can be predicted for whole body estimates using model equation 2:

Eq.2

$$\text{SMR } (\text{mg } O_2 \cdot h^{-1}) = -6.25+0.58 \cdot T-0.015 \cdot T^{2}+0.00013 \cdot T^{3}+0.21 \cdot M-0.0018 \cdot M^{2}+0.0000053 \cdot M^{3}$$

where T is temperature in °C and M is body mass in g. Mean mass specific SMR for Brook Trout was found to be 54.06±3.08 (mean ± S.E.) mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ at 5°C and increased to a mean of 190.60±11.35 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ at 20°C before dropping slightly to 178.34±5.86 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ at 23°C . Model selection steps for whole body SMR equations are presented in Table A2.

Maximum Metabolic Rate

Maximum metabolic rate also increased with treatment temperature (ANOVA; $F_{(4,93)}$=20.15, P<0.001), with values peaking at 15°C (Figure 4). MMR estimates at 5°C was lower than all other treatments, while 15°C was higher than the 20°C treatment, but not the 10° or 23°C treatments (Tukey HSD). The response of Brook Trout MMR to increasing temperature and body mass can be estimated using model equation 3:

Eq.3

$$\text{MMR } (\text{mg } O_2 \cdot h^{-1}) = -10.19+1.59 \cdot T-0.057 \cdot T^{2}+0.00044 \cdot T^{3}+0.33 \cdot M+0.0043 \cdot M^{2}-0.000057 \cdot M^{3}$$

where T is temperature in °C and M is body mass in g. Mean mass specific MMR for Brook Trout was found to be 330.70±13.89 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ at 5°C and increased to a mean of

504.32±15.55 mg O_2·kg^{-1}·h^{-1} at 15°C before dropping at higher temperatures. Model selection steps for whole body MMR equations are presented in Table A3.

Aerobic Scope

Aerobic Scope increased from 5–15°C (ANOVA; $F_{(4,93)}$=14.20, $P < 0.001$), after which it began to decrease (Figure 5). AS estimates at 15°C were found to be statistically different from all other treatments, and the 20°C treatment was different from the 10°C treatments (Tukey HSD). The response of Brook Trout AS to increasing temperature and body mass can be estimated using model equation 4:

Eq.4

$$\text{AS (mg } O_2\cdot h^{-1}) = -3.20+0.92\cdot T-0.034\cdot T^{2}+0.071\cdot M+0.0072\cdot M^{2}-0.000069\cdot M^{3}$$

where T is temperature in °C and M is body mass in g. Mean mass-specific AS for Brook Trout was found to be 287.25±13.03 mg O_2·kg^{-1}·h^{-1} at 5°C and increased to a mean of 384.85±13.31 mg O_2·kg^{-1}·h^{-1} at 15°C before dropping at higher temperatures. Model selection steps for whole body AS equations are presented in Table A4.

1.4 Discussion

Brook Trout used in the current study appear to be a thermal generalist, able to maintain a relatively stable AS across a range of temperatures and consequently, are well adapted to live in various thermal environments. The greatest AS occurred at 15°C, SMR increased with temperature, and a peak in MMR occurred between 15 and 20°C. Our results agree with results from a number of studies (see Smith and Ridgway, 2019), including Graham (1949), who found a peak in AS at 16°C and a peak in active MR at 19°C for Brook Trout. SMR estimates in both Graham's study and ours were similar across tested temperatures, with a mean SMR at 5°C of 54.06 mg O_2·kg^{-1}·h^{-1} and ~35 mg O_2·kg^{-1}·h^{-1} and increasing to 178.34 mg O_2·kg^{-1}·h^{-1} and ~200 mg O_2·kg^{-1}·h^{-1} around 23°C, respectively. Maximum metabolic rate and AS in the current study were both higher across temperatures than what Graham (1949) reported, leading to a flatter thermal performance curve. The difference in MMR and AS estimates may be due to population specific differences, differences in exhaustion techniques, or differences in equipment used to obtain MMR estimates. While Graham used a swim-tunnel style approach, which has been suggested to be the better method for eliciting MMR in fish species that are good at sustained swimming (Norin and Clark 2016; Raby et al. 2020), we used an exhaustive chase approach, and this method has been shown to be equally effective for obtaining MMR estimates (Little et al., 2020) and Zhang et al. (2020). A recent literature review conducted by Smith and Ridgway (2019) found the mean optimal temperature for maximised AS in Brook Trout from 24 laboratory studies, including the study by Graham (1949) compared above, to be ~15°C. Of these 24, studies that included an acclimation, as ours did, were the most consistent at finding 15°C as the optimal temperature for Brook Trout. Although most of the papers included in the review did not present full thermal performance curves, the review showed the inclusion of a proper acclimation period to allow the fish to adjust to the test temperature is crucial for getting reliable thermal performance data. The ability of these fish to adjust to such a broad range of acclimation temperatures could be due to strong phenotypic plasticity, and this plays into their success as an introduced species.

Brook Trout have thrived in many areas of introduction, including waters with different thermal regimes than their native ranges, which illustrates the wide thermal tolerance this species possesses. Comparisons of Brook Trout MR data to other salmonids illustrates differences in the shape of the thermal performance curve between thermal generalists and thermal specialists. As mentioned above, the thermal performance curves of thermal generalists tend to be flatter across a wide range of temperatures, exhibiting similar metabolic performance across a wide temperature range, whereas, thermal specialists have narrower thermal performance curves with a more pronounced peak at an optimum temperature. Although the thermal performance curves of Bull Trout and Westslope Cutthroat Trout, two potential thermal specialist species living with introduced Brook Trout in Western North America, have yet to be identified in the literature, studies have been done to assess the thermal niche occupied by both of these species. Field sampling has suggested juvenile Bull Trout rarely occupy waters where mean summer temperatures reach above 12°C (Isaak et al., 2015), have a maximum growth temperature of 13.2°C and limited survival above 20°C (Selong et al., 2001). This data suggests Bull Trout occupy a colder thermal niche in the wild than temperature preference indicates Brook Trout prefer. Alternatively, the optimal growth temperature for Westslope Cutthroat Trout has been found to be 13.6°C (Bear et al., 2007), approximately 1.5°C lower than Brook Trout, however the preferred temperature of young of the year and juvenile Westslope Cutthroat Trout has been estimated to range from 12-18°C (Bear et al., 2007; Macnaughton, Kovachik, et al., 2018) which overlaps the preferred temp of Brook Trout (~15°C; Smith & Ridgway, 2019). Although Bull Trout and Brook Trout populations co-occur in streams that possess a cold water temperature regime, the similar preferred temperatures of Westslope Cutthroat Trout and Brook Trout may indicate a higher likelihood of overlapping thermal niches in watershed where the preferred water temperatures are found. The thermal performance curve of Brook Trout in our study is similar to that seen in Chinook Salmon from Mokelumne River Hatchery in Clements, CA, USA, that possessed a relatively flat thermal performance curve across a wide range of temperatures (12–26°C; Poletto et al., 2017). A study by Eliason and Farrell (2016) illustrates differences in thermal performance curves between several species/populations of Pacific salmon (*Oncorhynchus spp.*). Different populations of Pacific salmon within the same species (*O. nerka*) display varying shapes and breadths of thermal performance curves across a similar range of temperatures. Many of the Pacific salmon thermal performance curves differ from the Brook Trout in our study, with several curves displaying a much more pronounced maximum AS peak over a smaller temperature range. The more pronounced thermal performance curves are consistent with a thermal specialist, whose physiological performance is high across a narrow range of temperatures. Comparing the Brook Trout thermal performance curve from our study to other populations of Brook Trout and other sympatric salmonid species, provides insight into intra- and inter-specific variation in AS, and in turn how species and populations have adapted to different habitats and temperature regimes.

Since it is known that temperature has an effect on MR, a daily thermal fluctuation likely also affects MR estimates to some degree. Although inclusion of a daily thermal fluctuation helps our study better reflect the natural thermal variation fish experience in the wild, it means comparisons of our data to previous studies (see below) cannot be taken directly without accepting the differences between the study designs. The thermal variation included in our study was done to better understand what MR in wild fish may be. But, physiological performance of lab populations of fish may not necessarily represent the performance but of their wild counterparts due to vastly different lifestyles, i.e., lab fish generally experience constant water speeds, daily high quality food, little to no seasonal changes. Therefore, it is important to conduct studies on populations in the wild to fully understand how the physiology and thermal preferences of wild fish compare to results of lab-based studies. Wythers et al. (2005) and Schulte et al. (2011) also suggest that accounting for environmental variation (i.e., diurnal temperature fluctuations), including providing sufficient time for acclimation to changes in temperature, is important when using thermal performance curves to understand potential effects of climate change on a species and for making more accurate predictions based on climate warming scenarios.

Acute temperature challenge testing representing the critical maximal temperature (CTmax) showed that Brook Trout are able to maintain normal swimming behaviour well beyond their UILT of 25.3°C (Fry et al., 1946), up to water temperatures of ~30°C before loss of equilibrium occurred (Morrison et al., 2020). Although this result does not reflect the species performance in the face of long term exposure to elevated temperatures, it shows Brook Trout can probably withstand brief forays into warmer waters for activities such as foraging, as seen in previous studies with Lake Trout (*Salvelinus namaychus*) (Guzzo et al., 2019). The high heat tolerance of Brook Trout, shown by Morrison et al. (2020), paired with the data from the current study show Brook Trout's ability to survive and maintain performance across a wide range of water temperatures. This wide range of temperature tolerance may prove beneficial to Brook Trout given that projected climate change scenarios for native streams in the eastern portions of Canada and the United States estimate increases in stream temperatures and major losses in suitable habitat (M. E. Clark et al., 2001; Flebbe et al., 2006; Isaak et al., 2018; Meisner, 1990; Meisner et al., 1988).

1.5 Conclusions

Understanding the physiological performance of Brook Trout can help us recognise possible competitive advantages that this species may have when living in sympatry with ecologically similar species. Further testing should be done on additional populations and life stages of Brook Trout to assess physiological performance across similar temperature gradients and confirm if other populations occupy similarly broad thermal niches. In addition, building thermal performance curves for native species that occupy similar thermal habitats (e.g., Bull Trout) will improve our understanding of interspecific variation in metabolic rates across ecologically relevant temperatures. Increasing our knowledge on the physiological performance and temperature tolerances of Brook Trout and sympatric salmonids will help conservation efforts, both in relation to assessing effects of increasing water temperatures on factors such as distribution and physiological performance, and risks introduced Brook Trout may pose for recovering imperiled native species.

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Table 1 – Biological data for experimental fish (immature only). Data are expressed as ranges or exact ratios.

	Treatment Temperature				
	5°C	10°C	15°C	20°C	23°C
n	24	20	21	14	20
Fork length (mm)	140–184	85–136	130–178	114–169	84–137
Total length (mm)	145–189	89–142	133–183	118–175	87–141
Mass (g)	30.1–70.4	6.9–27.9	22.7–67.4	17.7–64.2	4.6–31.7
Sex ratio (Male:Female)	12:12	10:9 (1 unknown)	9:12	8:6	9:11

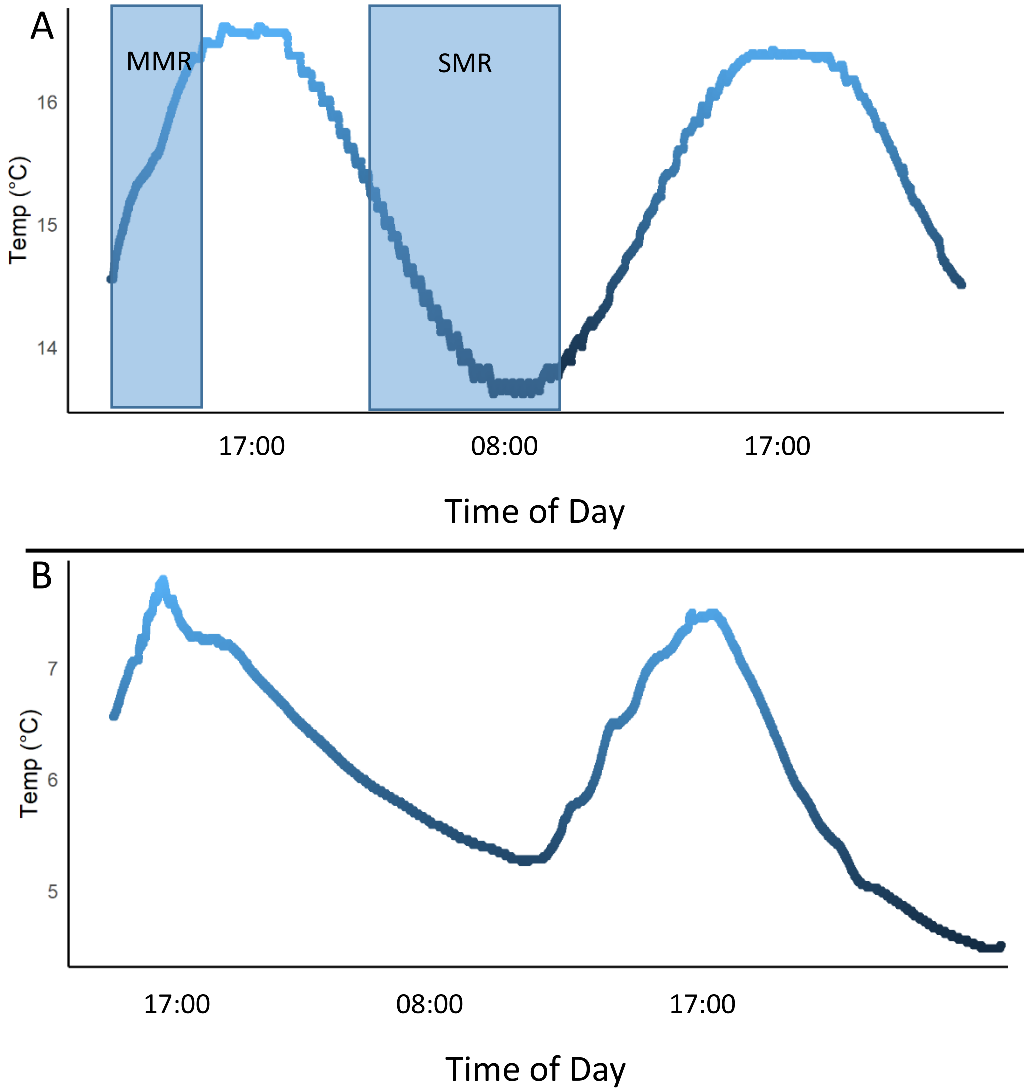


Figure 1 – Example diurnal temperature cycle experienced by Brook Trout. Plot A displays diurnal cycle fish experienced during acclimation and respirometry. Measurements used for calculation of maximum metabolic rate (MMR) estimates occurred during the warming section of the cycle (between 10:00-14:00), and standard metabolic rate (SMR) estimate generally fell within the lower end of the temperature cycle (between 4:00-10:00) as shown by blue boxes. The peak of the daily temperature cycle occurred at 17:00 h and the minimum temperature was reached at ~08:00 - 09:00 h. Treatment temperatures experienced ±1.5°C variation in diurnal temperature both during three-week acclimation and intermittent-flow respirometry. Plot B displays the daily thermal regime seen in a representative watershed where Brook Trout have been introduced in the Spray River watershed in Banff National Park, Alberta, Canada.

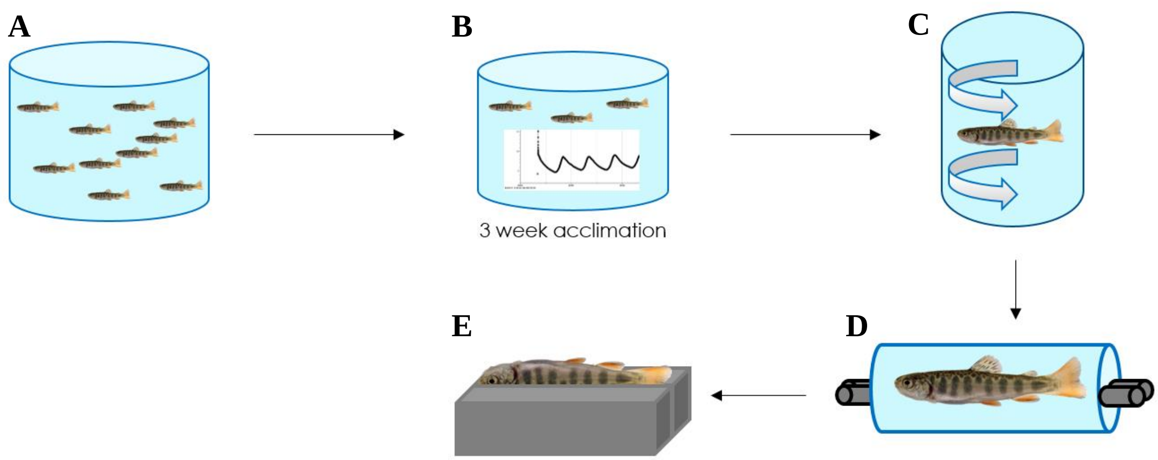


Figure 2 – Experimental design: Brook Trout were haphazardly netted from two general population tanks (A), and transferred to acclimation tanks where they experienced three weeks of diurnal temperature cycling (B), before being subjected to an exhaustive chase protocol to elicit MMR (C). Once exhaustion was reached, Brook Trout were transferred to intermittent respirometry chambers for 24 h to get SMR estimates (D). Following respirometry, Brook Trout were sacrificed and sex and maturity were recorded (E).

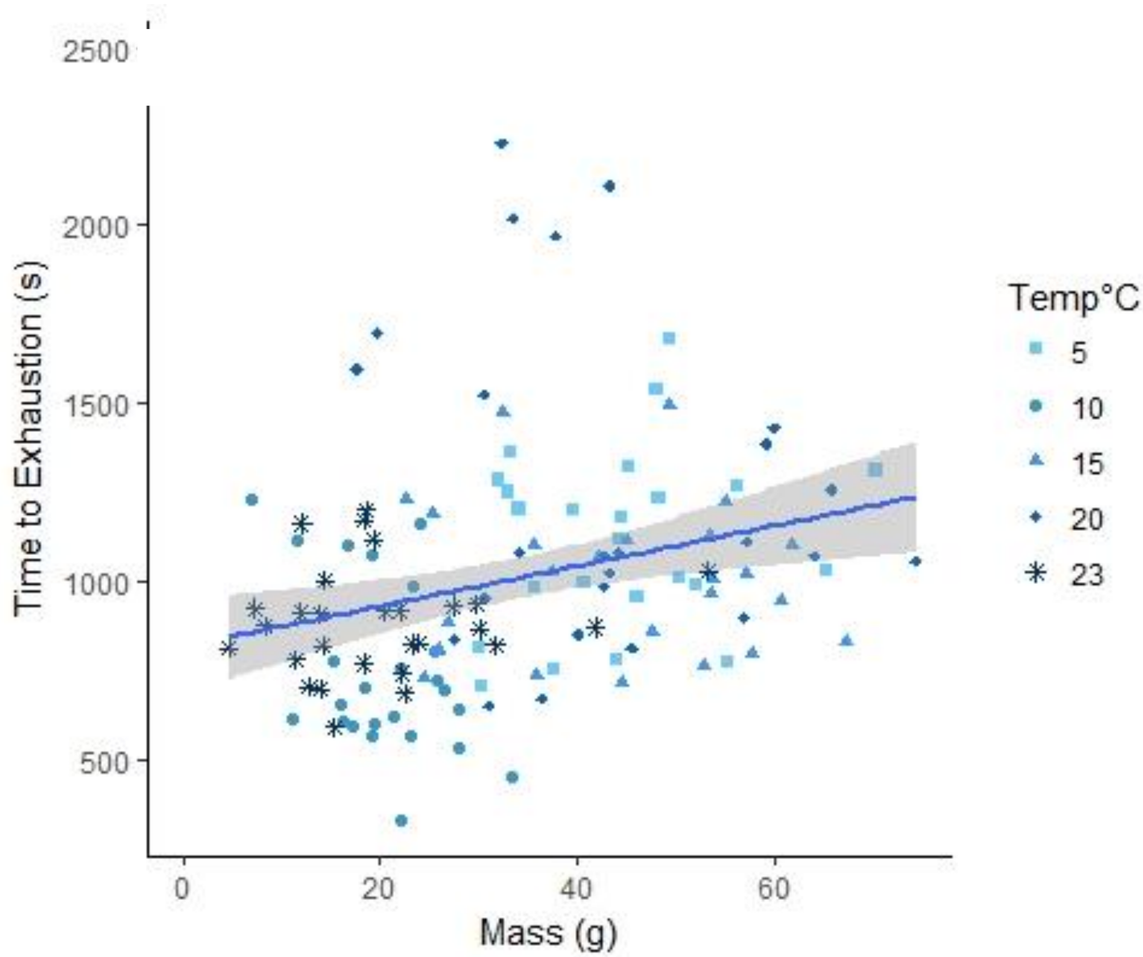


Figure 3 – Time to exhaustion of juvenile Brook Trout significantly increases as mass increases (linear model, $P = 0.002$), regardless of the temperature the fish is tested at. Each point represents an individual fish and the shape of the point indicates which treatment it is from. The line represents the fitted linear model to the data with shading around the line representing the standard error of the line. The equation for the line is given in the text. The equation of the trend line is: $E = 825.39 + 5.25 \cdot M$ where M is equal to mass in grams and E is measured in seconds.

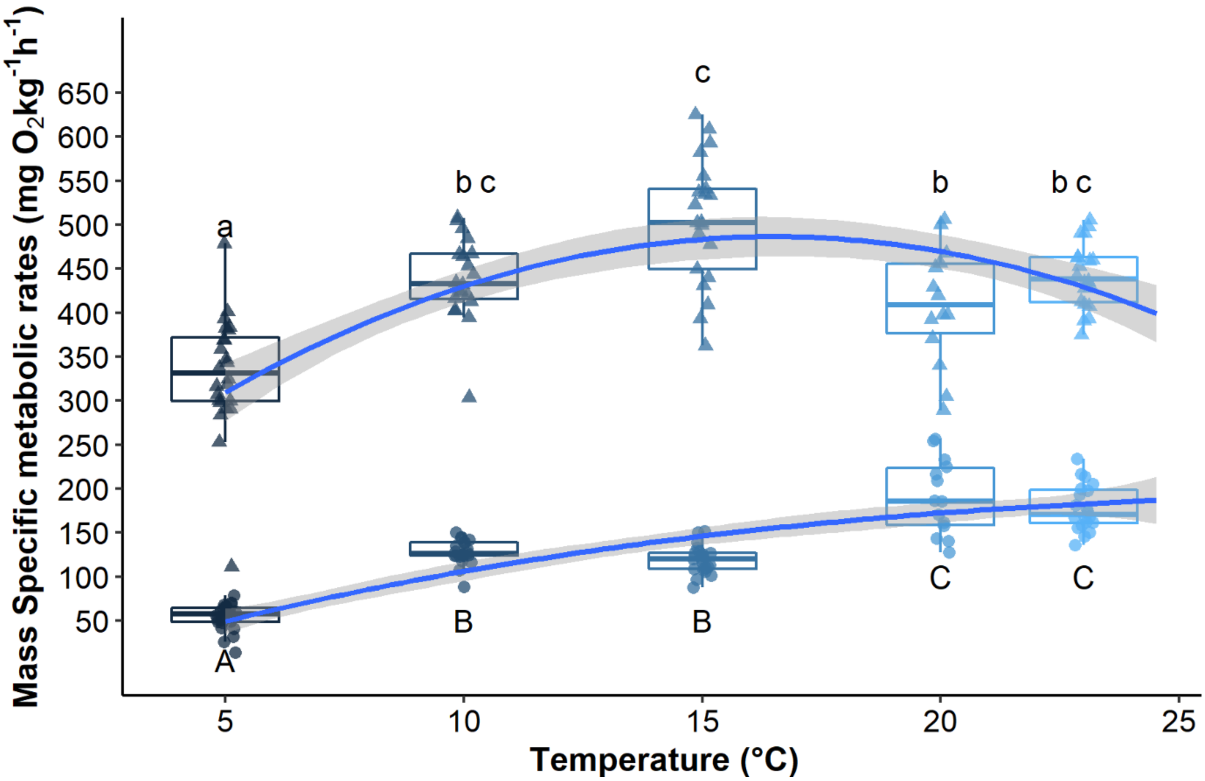


Figure 4 – Brook Trout mass corrected standard metabolic rate (SMR), circles, and maximum metabolic rate (MMR), triangles, thermal performance curves fitted with a 95% CI. n = 24 for each temperature treatment group. Variation within treatments on x-axis is due to temperature fluctuations during testing introduced by the diurnal temperature cycle. SMR treatments that do not share an uppercase letter are significantly different, MMR treatments that do not share a lowercase letter are significantly different. Boxplots show the median, 25th and 75th percentile values, with whiskers extending up to 1.5·IQR.

Trend line equation for SMR: $SMR = 0.0374T^3 - 1.7525T^2 + 31.235T - 60.259$

Trend line equation for MMR: $MMR = -1.25T^2 + 40.026T + 164.13$

Where T equals temperature.

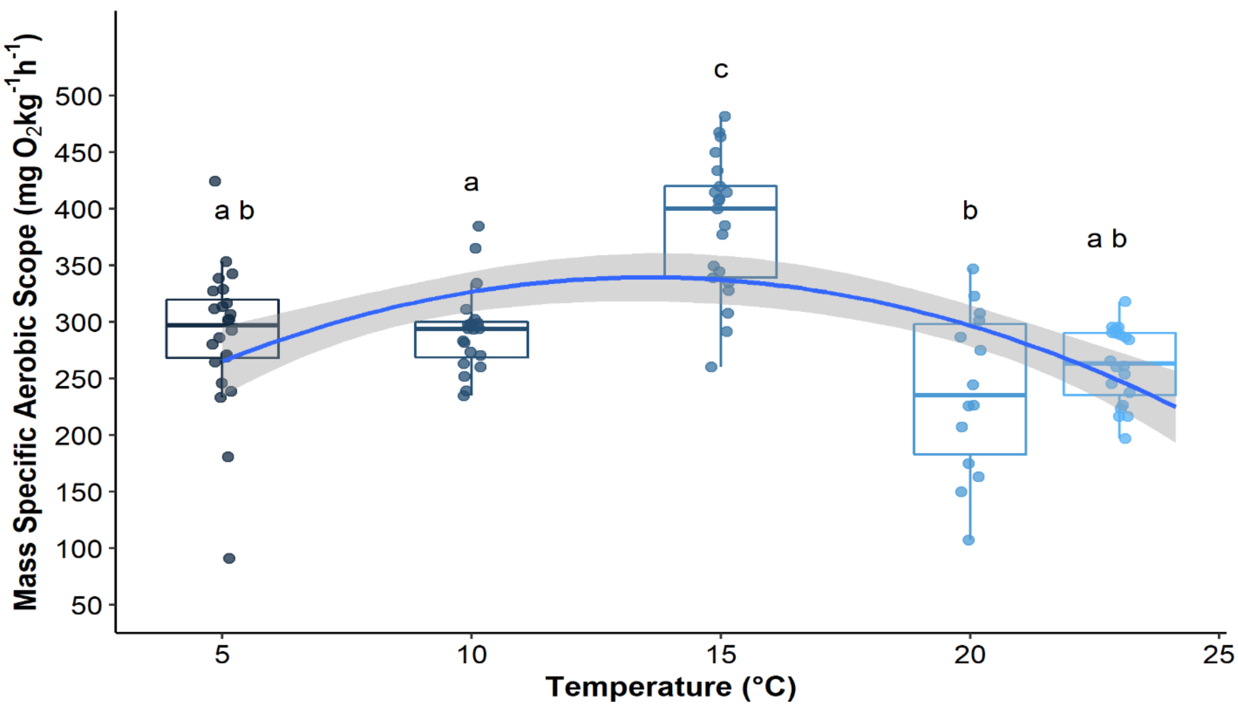


Figure 5 – Brook Trout mass specific aerobic scope curve fitted with a 95% CI. n=24 for each temperature treatment group. Variation on x-axis is due to temperature fluctuations during testing introduced by the diurnal temperature cycle. Treatments that do not share a lowercase letter are significantly different. Boxplots show the median, 25th and 75th percentile values, with whiskers extending up to 1.5·IQR. Trend line for AS: AS = $-0.91T^2 + 23.80T + 182.36$ Where T equals temperature.

Appendix A. Supplementary Data Tables

Table A1 – Whole body $\log_{10}$ Metabolic Rate x $\log_{10}$ mass statistical analysis (linear regression).

TEMP	METABOLIC	D.F.	*F* VALUE	*P* VALUE	R^2	INT
5	SMR	1,22	45.83	<0.001	0.68	-1.82
	MMR	1,22	61.06	<0.001	0.74	-0.30
	AS	1,22	30.26	<0.001	0.58	-0.26
10	SMR	1,22	92.20	<0.001	0.77	-1.12
	MMR	1,22	104.80	<0.001	0.80	-0.08
	AS	1,22	28.35	<0.001	0.50	-0.26
15	SMR	1,22	106.60	<0.001	0.87	-0.77
	MMR	1,22	138.10	<0.001	0.86	-0.13
	AS	1,22	75.49	<0.001	0.77	-0.31
20	SMR	1,23	177.50	<0.001	0.90	-0.97
	MMR	1,23	71.85	<0.001	0.85	-0.04
	AS	1,23	17.17	<0.001	0.62	-0.17
23	SMR	1,22	82.63	<0.001	0.71	-0.53
	MMR	1,22	473.50	<0.001	0.94	-0.09
	AS	1,22	46.92	<0.001	0.76	-0.71

Table A2 – Model selection table for whole body standard metabolic rate (SMR) based on AICc and AIC weight. *T* = temperature (°C) and *M* = mass (g). Intercept, T, T^2, T^3, M, M^2, and M^3 are parameter estimates for each model. Only models with all dependencies for polynomial terms were included in model selection. Models are listed in order of lowest AICc value to highest. All models with ΔAIC within 2 of the lowest AICc value were considered top models (bolded) and used to derive model averaged predictions. The final model with model-averaged coefficients is shown in italics.

	Intercept	*T*	T^2	T^3	*M*	M^2	M^3	*d.f.*	*Loglik*	*AICc*	*ΔAIC*	*AIC Weight*
SMR =	***-6.25***	***0.58***	***-0.015***	***0.00013***	***0.21***	***-0.0018***	***5.30e^{-06}***					
SMR =	**-5.64**	**0.50**	**-0.0086**		**0.18**	**-0.0012**		**6**	**-134.19**	**281.3**	**0**	**0.35**
SMR =	**-6.38**	**0.49**	**-0.0083**		**0.27**	**-0.0039**	**2.47e^{-05}**	**7**	**-133.44**	**282.1**	**0.83**	**0.23**
SMR =	**-8.44**	**1.15**	**-0.060**	**0.0012**	**0.20**	**-0.0013**		**7**	**-133.45**	**282.1**	**0.85**	**0.23**
SMR =	-8.69	1.06	-0.053	0.0010	0.27	-0.0037	2.13e^{-05}	8	-132.90	283.4	2.12	0.12
SMR =	-4.23	0.25			0.18	-0.0011		5	-138.05	286.8	5.47	0.02
SMR =	-5.12	0.25			0.28	-0.0043	2.85e^{-05}	6	-137.14	287.2	5.9	0.02
SMR =	-4.26	0.49	-0.0084		0.099			5	-138.77	288.2	6.89	0.01
SMR =	-4.54	0.55	-0.014	0.00013	0.099			6	-138.76	290.4	9.15	0.00
SMR =	-2.91	0.24			0.10			4	-142.16	292.7	11.47	0.00
SMR =	13.38	-3.29	0.29	-0.0070				5	-184.45	379.5	98.26	0.00
SMR =	1.56				0.069			3	-198.28	402.8	121.54	0.00
SMR =	-0.56	0.52	-0.012					4	-197.79	404	122.73	0.00
SMR =	1.37				0.082	-0.00018		4	-198.25	404.9	123.65	0.00
SMR =	1.55	0.16						3	-200.09	406.4	125.16	0.00
SMR =	0.39				0.19	-0.0036	3.11e^{-05}	5	-197.93	406.5	125.22	0.00
SMR =	3.86							2	-214.20	432.5	151.24	0.00

Table A3 – Model selection table for whole body maximum metabolic rate (MMR) based on AICc and AIC weight. *T* = temperature (°C) and *M* = mass (g). Intercept, *T*, T^2, T^3, *M*, M^2, and M^3 are parameter estimates for each model. Only models with all dependencies for polynomial terms were included in model selection. Models are listed in order of lowest AICc value to highest. All models with ΔAIC within 2 of the lowest AICc value were considered top models (bolded) and used to derive model averaged predictions. The final model with model-averaged coefficients is shown in italics.

	Intercept	*T*	*T²*	*T³*	*M*	*M²*	*M³*	*d.f.*	*Loglik*	*AICc*	*ΔAIC*	*AIC Weight*
MMR =	***-10.19***	***1.59***	***-0.057***	***0.00044***	***0.33***	***0.0043***	***-5.72e⁻⁵***					
MMR =	**-8.53**	**1.34**	**-0.038**		**0.26**	**0.0064**	**-7.59e⁻⁰⁵**	**7**	**-200.68**	**416.6**	**0**	**0.44**
MMR =	**-10.82**	**1.32**	**-0.037**		**0.53**	**-0.0020**		**6**	**-202.46**	**417.8**	**1.25**	**0.24**
MMR =	**-12.96**	**2.40**	**-0.12**	**0.0019**	**0.26**	**0.0069**	**-8.22e⁻⁰⁵**	**8**	**-200.24**	**418.1**	**1.48**	**0.21**
MMR =	-13.77	2.00	-0.089	0.0012	0.54	-0.0022		7	-202.28	419.8	3.21	0.09
MMR =	-8.40	1.29	-0.036		0.38			5	-206.00	422.6	6.05	0.02
MMR =	-7.13	0.98	-0.013	-0.00054	0.38			6	-205.96	424.8	8.24	0.01
MMR =	-4.46	0.23			0.53	-0.0019		5	-218.39	447.4	30.83	0.00
MMR =	-2.52	0.23			0.31	0.0049	-6.12e⁻⁰⁵	6	-217.55	448	31.43	0.00
MMR =	-2.28	0.22			0.39			4	-220.64	449.7	33.12	0.00
MMR =	1.71				0.36			3	-235.92	478.1	61.50	0.00
MMR =	0.68				0.43	-0.00098		4	-235.46	479.3	62.75	0.00
MMR =	2.66				0.21	0.0059	-6.25e⁻⁰⁵	5	-234.84	480.3	63.74	0.00
MMR =	65.52	-14.45	1.17	-0.028				5	-300.11	610.9	194.27	0.00
MMR =	4.80	1.60	-0.056					4	-319.46	647.3	230.76	0.00
MMR =	13.79							2	-323.66	651.5	234.86	0.00
MMR =	14.76	-0.067						3	-323.43	653.1	236.51	0.00

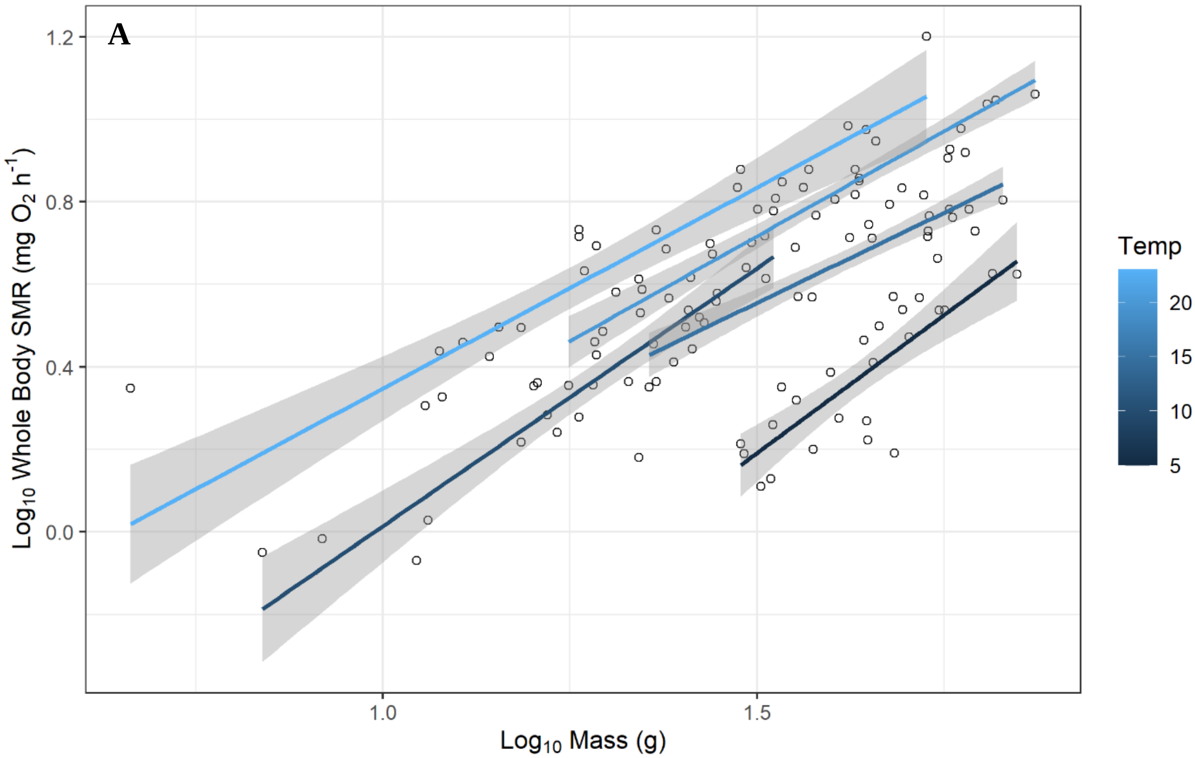
Table A4 – Model selection table for whole body aerobic scope (AS) based on AICc and AIC weight. *T* = temperature (°C) and *M* = mass (g). Intercept, T, T^2, T^3, M, M^2, and M^3 are parameter estimates for each model. Only models with all dependencies for polynomial terms were included in model selection. Models are listed in order of lowest AICc value to highest. All models with ΔAIC within 2 of the lowest AICc value were considered top models (bolded) and used to derive model averaged predictions. The final model with model-averaged coefficients is shown in italics.

	Intercept	*T*	T^2	T^3	*M*	M^2	M^3	*d.f.*	*Loglik*	*AICc*	*ΔAIC*	*AIC Weight*
AS =	***-3.20***	***0.92***	***-0.034***		***0.071***	***0.0072***	$\boldsymbol{-6.95e^{-5}}$					
AS =	**-2.38**	**0.93**	**-0.034**		**-0.044**	**0.011**	$\mathbf{-1.07e^{-04}}$	**7**	**-215.19**	**445.6**	**0**	**0.43**
AS =	**-4.73**	**0.89**	**-0.033**		**0.29**			**5**	**-218.11**	**446.9**	**1.25**	**0.23**
AS =	-4.46	1.44	-0.074	0.00093	-0.041	0.011	$-1.10e^{-04}$	8	-215.10	447.8	2.20	0.14
AS =	-5.55	0.90	-0.033		0.34	-0.00069		6	-217.79	448.5	2.88	0.10
AS =	-3.65	0.62	-0.012	-0.00050	0.28			6	-218.08	449.1	3.47	0.08
AS =	-5.74	0.95	-0.036	0.000081	0.34	-0.00070		7	-217.79	450.8	5.20	0.03
AS =	-0.46				0.30			3	-228.94	464.1	18.54	0.00
AS =	0.59	-0.057			0.29			4	-227.93	464.3	18.68	0.00
AS =	1.58				0.031	0.0093	$-9.17e^{-05}$	5	-227.04	464.7	19.12	0.00
AS =	2.74	-0.052			0.011	0.0095	$-9.11e^{-05}$	6	-226.18	465.3	19.67	0.00
AS =	-1.31				0.36	-0.00082		4	-228.58	465.6	19.98	0.00
AS =	-0.13	-0.053			0.34	-0.00061		5	-227.72	466.1	20.49	0.00
AS =	47.48	-10.34	0.85	-0.021				5	-279.89	570.4	124.83	0.00
AS =	6.00	1.0080	-0.045					4	-296.49	601.4	155.81	0.00
AS =	13.56	-0.29						3	-300.46	607.2	161.56	0.00
AS =	9.49							2	-306.72	617.6	171.95	0.00

Table A5 – Biological data for all experimental fish, including mature individuals. Data are expressed as ranges or exact ratios. All treatments had n = 24 fish, except 20°C, which had n = 25. Metabolic rates expressed as mean ± SE mass specific metabolic rate estimates (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$).

	Treatment Temperature				
	5°C	10°C	15°C	20°C	23°C
Fork length (mm)	140–184	85–154	130–182	114–181	84–160
Total length (mm)	145–189	89–161	133–189	118–185	87–165
Mass (g)	30.1–70.4	6.9–33.3	22.7–67.4	17.7–74.5	4.6–53.3
Sex ratio (Male:Female)	12:12	14:9 (1 unknown)	12:12	18:7	13:11
Maturity ratio (Immature:Mature)	24:0	20:4	21:3	14:11	20:4
Mass Specific SMR	47.50 ± 3.32	135.32 ± 3.48	115.97 ± 4.10	189.69 ± 5.89	200.80 ± 10.34
Mass Specific MMR	315.76 ± 13.36	413.82 ± 10.65	480.96 ± 14.19	351.16 ± 15.63	389.34 ± 9.89
Mass Specific AS	295.82 ± 12.17	279.27 ± 6.91	386.89 ± 12.69	227.49 ± 15.05	137.43 ± 12.27

Appendix B. Supplementary Data Figures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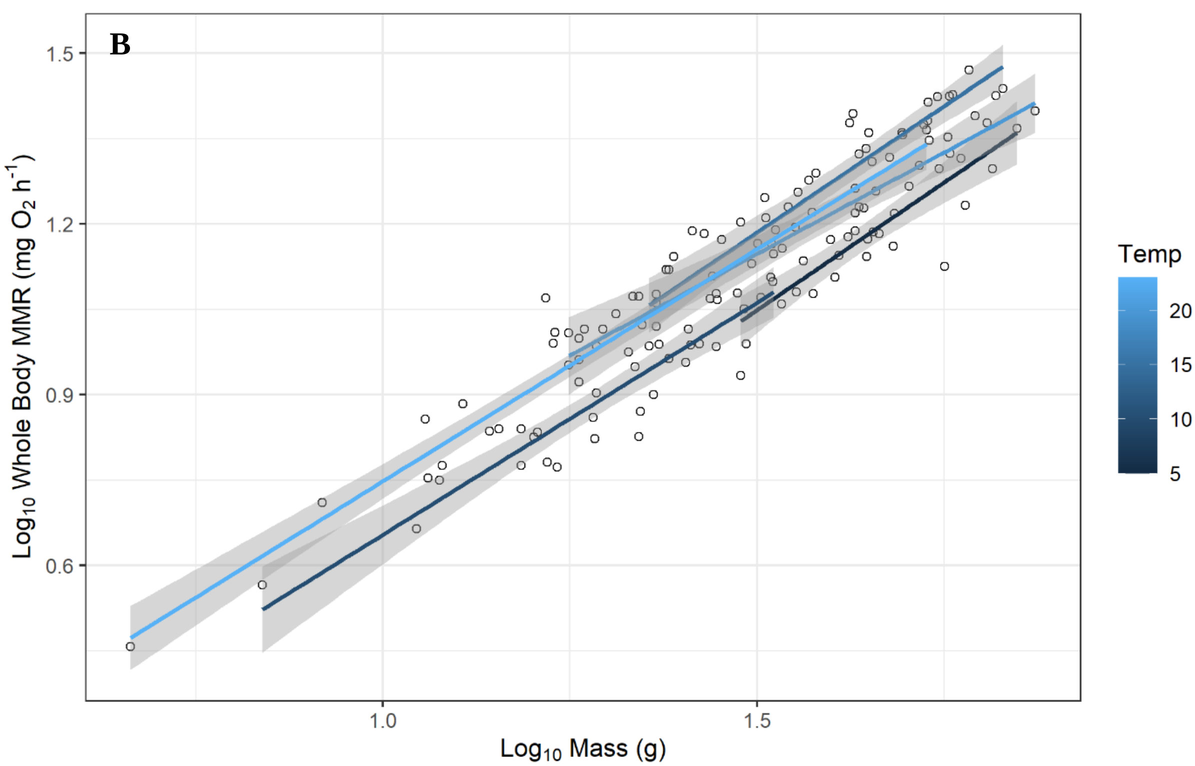
Log10 Mass (g)
Temp
20
15
10
5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Log10 Mass (g)
Temp
20
15
10
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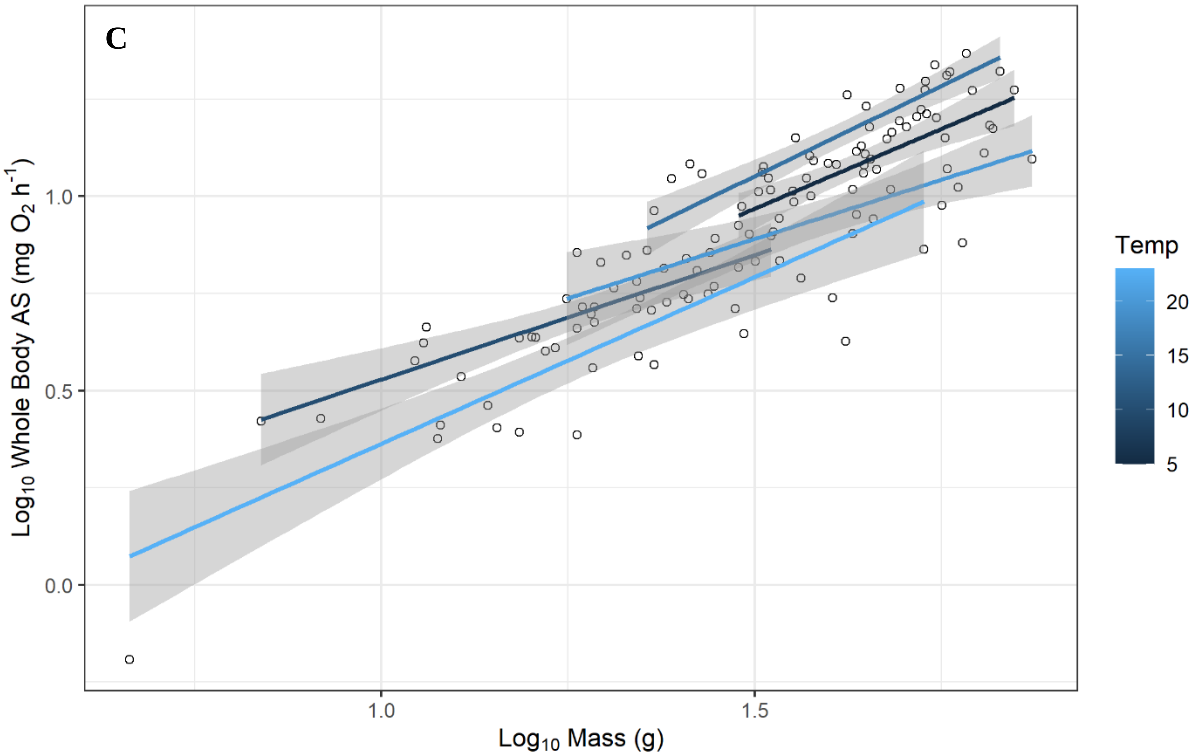

Figure B1: Allometric relationships between $\log_{10}$ whole body SMR (A), MMR (B), and AS (C) and $\log_{10}$ Mass (g). Scaling coefficients for each line can be found in Table A1.