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RESEARCH ARTICLE

Effects of repeated daily acute heat challenge on the growth and metabolism of a cold water stenothermal fish

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ABSTRACT

Temperature is an important environmental factor influencing fish physiology that varies both spatially and temporally in ecosystems. In small north temperate zone lakes, cold water piscivores rely on nearshore prey; however, this region exceeds the optimal temperature of the foraging species during summer. To cope, piscivores make short excursions into the nearshore to feed and return to cold water to digest their meal, but the physiological impacts of these repeated acute exposures to warm water are not well understood. We exposed juvenile lake trout (*Salvelinus namaycush*) to treatments where they were held at ∼10°C and exposed to either 17 or 22°C for 5–10 min daily for 53 days mimicking warm-water forays. Control fish, held at an average temperature of ∼10°C but not exposed to thermal variation, consumed more food and grew slightly faster than heat challenged fish, with no clear differences in body condition, hepatosomatic index, ventricle mass, or muscle concentrations of lactate dehydrogenase and cytochrome c oxidase. Aerobic metabolic rates measured at 10°C indicated that standard metabolic rates (SMR) were similar among treatments; however, fish that were repeatedly exposed to 17°C had higher maximum metabolic rates (MMR) and aerobic scopes (AS) than control fish and those repeatedly exposed to 22°C. There were no differences in MMR or AS between fish exposed to 22°C and control fish. These results suggest that although SMR of fish are robust to repeated forays into warmer environments, MMR displays plasticity, allowing fish to be less constrained aerobically in cold water after briefly occupying warmer waters.

KEY WORDS: Metabolic rate, Aerobic scope, Behavioural thermoregulation, Lake trout (*Salvelinus namaycush*), Physiology, Temperature, Climate change

INTRODUCTION

Ecosystems are spatially and temporally complex, comprising dynamic habitat mosaics that animals navigate to acquire energy for survival, growth and reproduction. This is especially true for north temperate zone lakes, which contain discrete habitat types (Schindler and Scheuerell, 2002) and exhibit seasonal cycles in water temperature (Wetzel, 2001). The nearshore littoral zone has been shown to be important to the structure and function of north temperate lake ecosystems (Vadeboncoeur et al., 2002; Sierszen et al., 2003; Babler et al., 2008) and disproportionately (relative to its low proportion of total lake area) contributes to the energy sources of fish within these systems (Hampton et al., 2011; Vander Zanden et al., 2011). Although the temperature of the littoral zone is cool for most of the year (autumn–winter–spring), this region exceeds the preferred range for many cold water fish species during summer months (Gibson and Fry, 1954; Guzzo and Blanchfield, 2017; Magnuson et al., 1979; Morbey et al., 2006). As a result, many cold water fishes are known to behaviourally thermoregulate during summer by making rapid excursions into the warm littoral zone to feed on abundant or preferred prey and then return to cool deep water to digest their food (Cott et al., 2015; Guzzo et al., 2017; Sellers et al., 1998). Although increased access to energy-dense food may make this behaviour bioenergetically beneficial, repeated acute exposures to warm water may have negative physiological consequences. In fact, most research on the impacts of temperature on physiological performance and growth have compared changes in average (i.e. constant) temperatures, and therefore, relatively little is known on how exposure to thermal variation impacts the physiological performance and growth of fish (Carey, 1979; Morash et al., 2018).

Aerobic metabolism uses oxygen to convert food into more usable forms of energy. Therefore, the aerobic metabolic rate is the pace at which resources are converted into energy that animals use to carry out key activities, such as reproduction, foraging and locomotion (Brown et al., 2004; Sibly et al., 2012). Maximum metabolic rate (MMR) not only defines the upper boundary to aerobic capacity, which is related to several important physiological traits (e.g. swimming capacity) (Metcalfe et al., 2016), but together with standard metabolic rate (SMR; the minimum oxygen consumption required to maintain homeostasis) also determines an organism's aerobic scope (AS). AS is the absolute difference between MMR and SMR and is a measure of an animal's capacity to deliver oxygen to tissues to carry out simultaneous metabolic processes (e.g. growth, locomotion, reproduction) above maintenance metabolic requirements (Fry, 1971). As the metabolic rate of most fish is directly influenced by ambient temperatures, repeated acute exposures to warm water may affect the AS of fish.

Rapid excursions by fish between warm and cool habitats have been interpreted as flexible behaviours to maximize growth efficiency (Neverman and Wurtsbaugh, 1994; Armstrong et al., 2013), as energy-saving strategies (Sims et al., 2006) or as a strategy to exploit food resources during long periods of unfavourable thermal conditions in the feeding environment (Pepino et al., 2015). For example, if the duration of a foraging bout is enough to acutely elevate tissue temperatures by 5–10°C this could lead to oxidative stress or redox imbalance in ectotherms (Heise, 2006; Kaur et al., 2005; Leggatt et al., 2007; Lushchak and Bagnyukova, 2006;

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Received 11 December 2018; Accepted 14 May 2019

Parihar and Dubey, 1995). However, there is only limited evidence that diel thermal cycles can alter energy metabolism and growth dynamics in fish. For example, Morash et al. (2018) showed that Atlantic salmon (*Salmo salar*) parr exposed to fluctuating temperatures displayed reduced SMR and MMR compared with fish at a stable acclimation temperature equal to the mean value of the fluctuations. AS was also decreased in the fish exposed to cycling temperature. Atlantic salmon parr exposed to either a stable daily average temperature (based on expected seasonal daily averages) or daily thermal fluctuations that mimicked the April–October growing season (>7°C diurnal fluctuations) also showed small reductions in growth rate, and this decline was independent of ration size (1 or 3% body mass daily) (Imholt et al., 2011). While growth penalties may occur if SMR increases and resource availability stays constant and are limiting (Burton et al., 2011), the effect of daily fluctuations in temperature on Atlantic salmon SMR is a function of acclimation temperature and provenance (Oligny-Hébert et al., 2015), making inferences on the mechanism of reduced growth difficult.

Notably, much of the short-term thermal fluctuation experiments with fish have used species like Atlantic salmon that have relatively high thermal tolerances compared to cold water species and more stenothermal species such as the lake trout (*Salvelinus namaycush*), which are also known to make shallow water foraging bouts in small stratified lakes (Guzzo et al., 2017; Mackenzie-Grieve and Post, 2006; Morbey et al., 2006; Sellers et al., 1998). However, if the adjustments to acute changes in temperature – both warming and cooling upon return to deeper water – alter growth efficiency or bioenergetics, then cold water stenothermal species may be especially sensitive to disruptions to thermal environments. Understanding the metabolic responses of fish to repeated acute exposures to warm water, such as those experienced while making forays into nearshore water, is important as climate warming is expected to result in lakes having warmer surface waters (O'Reilly et al., 2015) and longer thermally stratified periods (De Stasio et al., 1996; Robertson and Ragotzkie, 1990). Additionally, warming of Arctic lakes that currently do not thermally stratify in summer may create novel thermal environments to which cold water fish will be exposed.

Even though most animals experience fluctuations in temperature in the natural habitats, we still possess limited knowledge of the effects of thermal variability on organismal physiology (Carey, 1979; Morash et al., 2018). Lake trout occupying small lakes provide an extraordinary example whereby individuals voluntarily expose themselves to acute thermal shifts while foraging (as opposed to the diel thermal changes passively experienced by many other aquatic organisms), suggesting a possible trade-off between resource acquisition and the physiological costs of repeated and abrupt thermal changes. The native distribution of lake trout covers north temperate and Arctic regions of North America. Lake trout have optimal growth at 10±2°C and a maximum AS at 15°C (Gibson and Fry, 1954; Christie and Regier, 1988; Evans, 2007; Kelly et al., 2014; McDermid et al., 2013). Like other cold-water piscivores, lake trout rely heavily on nearshore littoral energy in small lakes, particularly those that do not have deep water prey fish (Vander Zanden and Rasmussen, 1996).

In this study, we tested the hypothesis that excursions into warm water would lead to altered bioenergetics and metabolism in a cold water fish as a plastic response to cope with repeated acute exposures to water temperatures above their optimal for growth and AS. We exposed juvenile lake trout to two levels of a daily acute temperature challenge over a 2 month period, to simulate the conditions that these cold water piscivores are exposed to when making rapid forays into the nearshore to feed in small thermally stratifying lakes during summer. Specifically, we compared how the growth, relative size of energy-demanding tissues (liver and heart), metabolic rates (SMR, MMR, AS), and white muscle concentrations of lactate dehydrogenase (LDH) and cytochrome c oxidase (CCO) of fish differed among experimental treatments: control treatment where fish were held at mean temperatures of ∼10°C for the entire experiment; and exposure treatments where fish were also held at ∼10°C, but acutely exposed to warm water (17°C or 22°C) daily for 53 days to simulate foraging excursions from cold water. The total daily exposure to water above control lasted for 10–11 min, including 2.5–3 min to heat up to the exposure temperature, 5 min at the exposure temperatures and then 2.5–3 min to cool water back to optimal. The exposure duration was based on the results of an *in situ* tracking study of lake trout which found the median duration of warm water forays in Lake Opeongo, a dimitic Boreal Shield lake in Canada, to be between 5.6 and 15.7 min (Morbey et al., 2006).

MATERIALS AND METHODS

Fish collection and husbandry

Lake trout [*Salvelinus namaycush* (Walbaum 1792)] were reared from gametes collected from adult fish captured in Clearwater Lake, MB, Canada (54.05°N, 101.05°W) on 30 September 2013 with 95% of eggs hatching within 2 days of 1 January 2014. Fish were held at a temperature mirroring those recorded at their natal lake (https://www.hydro.mb.ca/hydrologicalData/static/) up to a maximum of 10°C and fed commercial trout feed (EWOS Canada Ltd, Surrey, BC, CA) once a day to satiation. For details on rearing conditions, see Kissinger et al. (2017). Prior to our experiment, fish were held in a large general population tank in round flow-through tanks (190 cm in diameter, 76 cm water depth, 2155 litre water volume). The water temperature in the general population tank was raised gradually by 1°C day^{-1} until it reached 10°C and fish were left to acclimate to this temperature for 8 weeks before our experiment began. This acclimation temperature was selected because it is within the optimal temperature range for growth of lake trout (Christie and Regier, 1988; McDermid et al., 2013). All procedures were approved by the University of Manitoba Animal Care Committee (Animal use protocol #F13-029).

Experimental design

Our experimental approach consisted of six round flow-through tanks (61 cm in diameter, 53 cm water depth, 152 litre water volume) that had water temperatures held constant at 10°C. There were three duplicated experimental treatments: control, where tanks were held at 10°C for the entire study; treatment 17°C (T17°C), where tanks were held at 10°C but were heated to 17°C for 5 min each day; and treatment 22°C (22°C), where tanks were held at 10°C but were heated to 22°C for 5 min each day (Fig. 1). A total of 42 fish were randomly selected from the general population, sorted by size, and placed into each of the six tanks in equal groups (n=7). Fish were offered a ration of ∼1.5% body mass daily with remaining food collected about 1 h after feeding. Feeding occurred 0.5–2 h before daily heat exposures. Water temperatures in each tank were monitored at 1 min intervals over the entire study using data loggers (HOBO TidbiT v2, Onset Computer Corp., Bourne, MA, USA). We increased the temperature of each treatment tank daily to mimic fish rapidly swimming up through the lake's mixed layer and into warm nearshore water to feed by draining roughly half the water in each tank and mixing in water heated with electric kettles to ∼50°C until tanks reached our treatment temperatures of 17°C or 22°C. We then let the tanks remain at these temperatures for the 5 min exposure,

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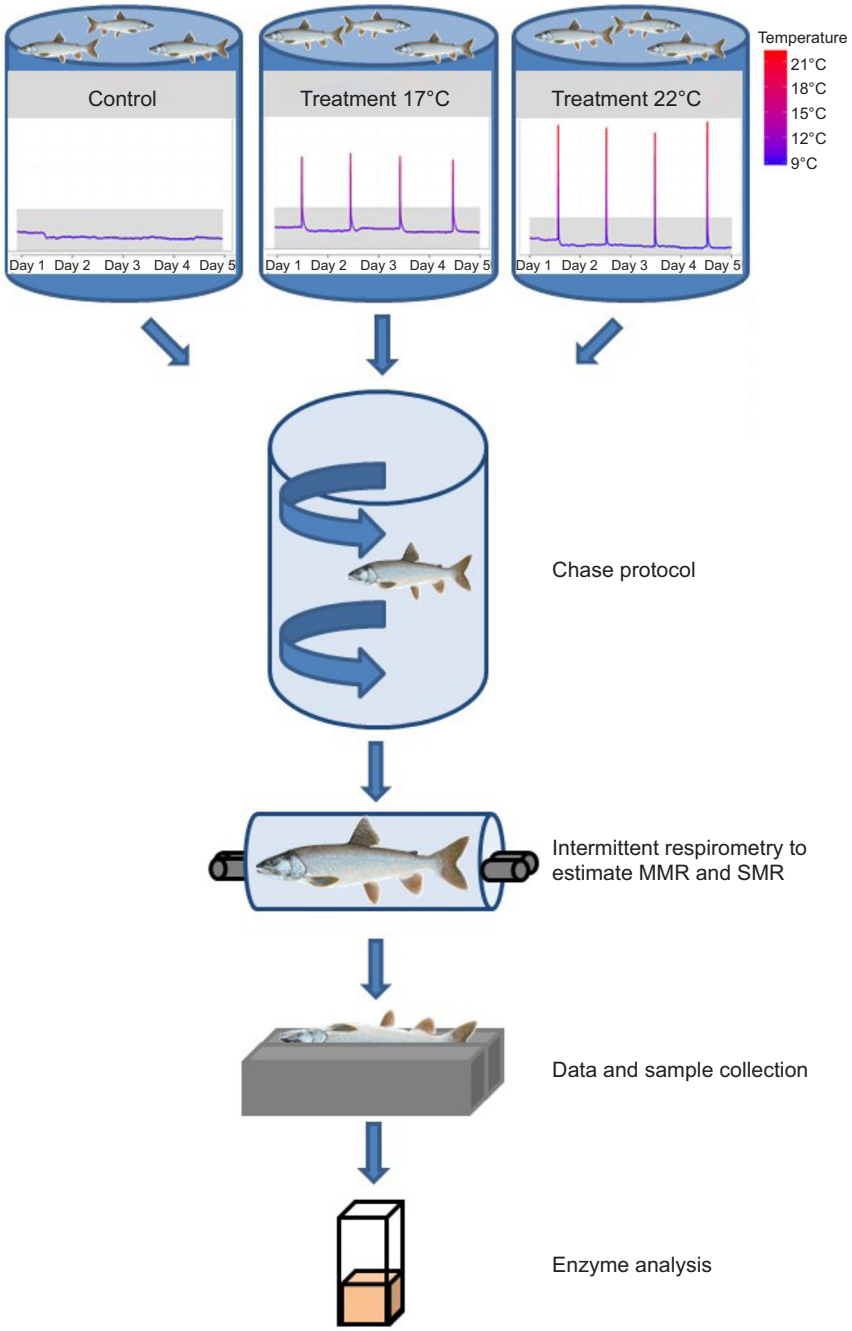

Fig. 1. Summary of experimental design. Size-matched lake trout were placed in one of three types of tank: control tank at constant 10°C or experimental tanks maintained at 10°C but with daily rises in temperature to 17 or 22°C for 5 min. The exposure period lasted for 57 days with 53 days of heat exposure. Two weeks after the exposure period, fish underwent a chase protocol to ensure exhaustion and excess post-exercise oxygen consumption. Fish were then placed in 10°C chambers for intermittent flow respirometry to immediately estimate MMR followed by 24 h of measurements to estimate SMR. Then, fish were euthanized, weighed, measured and livers and white muscle removed and frozen at −80°C. (5) White muscle samples were then quantified for lactate dehydrogenase (LDH) and cytochrome c oxidase (CCO) activity by spectrophotometry.

after which we mixed in 4°C water until each tank reached their original temperatures of ∼10°C. It took roughly 2–3 min to heat and cool treatments from their baseline temperature so, overall, treatment fish were in temperatures >10°C (i.e. above control levels) for about 9–11 min each day. To ensure that control treatments received the same handling stress as exposure treatments, but without the temperature effects, we performed sham treatments, where control tanks were half drained and 10°C water was mixed into the tanks in the same manner as treatment tanks. During each daily heating and cooling process, instantaneous water temperatures were monitored using a handheld probe (Pro ODO, YSI Inc., Yellow Springs OH, USA) to ensure temperatures in each treatment tank achieved set endpoints.

Prior to the start of the experiment (26 November 2015), fish were size-selected so that the mean fork lengths and masses of fish assigned to each treatment did not differ (fork length: $F_{2,38}$=1.17, P=0.32; mass: $F_{2,38}$=0.64, P=0.53). Mean (±s.d.) fork lengths were 181.5±7.0 mm, 180.6±9.8 mm and 185.0±7.2 mm and average masses were 59.7±9.4 g, 58.3±10.3 g, 62.3±8.8 g, for control, T17°C and T22°C, respectively. Following size selection, fish were elastomer tagged to allow individual identification and placed into their respective tanks. We then allowed them to acclimate to their new groups and tanks for 2 months at 10°C. Following social acclimation, fish were weighed and measured on 21 January 2016, at which point the mean fork lengths ($F_{2,37}$=0.34, P=0.71) and masses ($F_{2,37}$=0.78, P=0.46) of fish in each treatment still did not differ. At this point, mean (±s.d.) fork lengths were 194.2±8.1 mm, 191.8±13.2 mm, and 195.0±10.0 mm and average masses were 73.3±11.0 g, 67.6±15.5 g, 72.9±13.0 g, for control, T17°C and T22°C, respectively. Fish were left to recover without feeding for

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3 days, at which point feeding recommenced. The exposure period began 7 days later, on 28 January 2016 and lasted 57 days. Fish were weighed and measured approximately half-way through the exposure period (26 February 2016) and were not heated on that day and 3 days following to let them recover. Heating then recommenced and continued until the end of the experiment (23 March 2016), at which point final masses and lengths were recorded to determine growth dynamics. Therefore, the total experimental period was 64 days (21 January 2016–24 March 2016), with a total of 53 days of heat exposure. Fish were then allowed to recover for 13–19 days after the final day of heat exposure while being fed a maintenance ration of 0.5% body mass before metabolic rates were measured to determine if the repeated acute thermal exposures had a prolonged effect on metabolism. After measurement of metabolic rate, fish were euthanized by an overdose of tricaine methanesulfonate, followed by pithing and cervical severing. Fish were then weighed, fork length measured and white muscle was sampled and immediately frozen in liquid nitrogen before being placed in a −80°C freezer for enzyme analysis (Fig. 1). The heart ventricle was also removed and weighed following euthanasia to determine if differences in ventricle size independent of fish size existed among control and treatments, while controlling for fish mass.

The mean (±s.d.) temperatures of the tanks during the exposure period ranged from 9.4±1.0°C to 9.6±0.7°C. There were few occasions when maximum daily temperatures in all tanks, including control tanks, exceeded 10±2°C, which were not due to experimental spiking of water temperatures. These incidents were caused by interruptions in the fresh water supply due to facility maintenance. Control tank 1 water exceeded 10±2°C as follows: 1–2 February for 612 min (T_{max}=13.2°C), 19 February for 40 min (T_{max}=12.7°C) and 27 February for 472 min (T_{max}=12.5°C). Control tank 2 water exceeded 10±2°C as follows: 30–31 January for 1065 min (T_{max}=15.8°C) and 19 February for 57 min (T_{max}=13.0°C). Additionally, the mean daily temperature in T17°C tank 2 was 7.3°C on 2–3 March, as water temperature in this tank fell to 4.9°C during the evening between these two dates.

Metabolic rates

We used intermittent-flow respirometry (Loligo® Systems, Viborg, Denmark) to estimate whole-animal aerobic metabolic rates (Svendsen et al., 2016). Four acrylic cylindrical respirometry chambers (75 mm in diameter×250 or 270 mm in length, 1100 or 1200 ml in volume) were submerged in 10°C aerated freshwater controlled by a temperature regulator (TMP-Reg, Loligo® Systems). Each respirometry chamber was connected to two pumps (EHEIM GmbH & Co KG, Deizisau, Germany) with a flow rate of 5.0 l min^{-1} each. One pump recirculated water through the respirometry chamber and through an in-line oxygen probe holder during the measurement period of the intermittent respirometry cycle, while the other pump was used to bring oxygenated water from the water bath back to the respirometry chamber to restore oxygen content during the flush period. To estimate the rate of oxygen uptake ($\dot{M}_{O_2}$), we used a respirometry cycle that was 320 s in duration; this included a 140 s flush period, a 40 s wait period to achieve steady state after the end of flushing and a 140 s measurement period. We chose these durations based on pre-experiment trial runs to ensure that dissolved oxygen would return to a safe level (i.e. >9.0 mg O_2 l^{-1}; Evans, 2007) during each flush period and to ensure that the measurement period began after the linear decline of oxygen began (Svendsen et al., 2016). The mean R^2 of the all $\dot{M}_{O_2}$ measurements was 0.99 (n=7146, s.d.=0.01, range=0.9–1.0). We used Autoresp™ software (Loligo® Systems) to control the flush pumps during the experiment, monitor changes in oxygen concentration within each chamber, and calculate the slopes of oxygen decline during each measurement period. Blank measurements (i.e. without fish in the respirometry chambers) were performed between each run to estimate bacterial oxygen demand (BOD), which was negligible. Prior to the experiment, oxygen sensors (DP-PSt3-L2.5-ST10-YOP; precision±0.05 mg O_2 l^{-1}, PreSens, Regensburg Germany) were calibrated using a two-point calibration method as outlined in the AutoResp™ Software User Manual (Loligo® Systems).

MMR was calculated as the highest $\dot{M}_{O_2}$ measurement from the first three measurement periods following an exhaustive chase protocol (Norin and Clark, 2016; Killen et al., 2017). The chase protocol involved chasing fish in a round tank against a constant current. The fish were encouraged to burst forward against the current by tapping on the side of the tank with a net until they were unresponsive to a caudal pinch (typically 7–10 min) (Kissinger et al., 2017; Mochnacz et al., 2017; Roche et al., 2013). Fish were then immediately placed into a respirometry chamber and $\dot{M}_{O_2}$ measurements began directly after the chamber was closed, which took approximately 20-30 s. Following MMR measurement, fish were left in the respirometry chambers and $\dot{M}_{O_2}$ was measured for ∼24 h to estimate SMR – calculated as the lower 20th percentile of all $\dot{M}_{O_2}$ measurements during the 24 h post MMR measurements (Chabot et al., 2016). For each fish, AS was calculated as the differences between MMR and SMR. Metabolic rates were calculated both as whole-animal values (mg O_2 h^{-1}) and mass-specific values (mg O_2 kg^{-1} h^{-1}), but we focus on the former for graphical presentation and analysis in this paper. Summary data for metabolic rates in both units are presented in the Results.

White muscle enzymes

LDH and CCO activity were measured in white muscle tissue sampled from the fish following respirometry. Muscle was homogenized in 19 volumes of 50 mmol l^{-1} imidazole buffer (7.4 pH) using an IKA T25 digital ULTRA-TURRAX® homogenizer. Owing to high activity, homogenates for LDH assays were further diluted by 9 volumes of 50 mmol l^{-1} imidazole buffer (7.4 pH) to allow for linear rates of enzyme activity. Enzyme activities were measured at the same temperature as the fish were held at outside of heat challenges (10±0.1°C) in an Agilent Technologies Cary Series UV-Vis spectrophotometer equipped with a thermally controlled jacketed cell holder. Assays used were based on previous work performed on fish muscle enzymes. For LDH (E.C. 1.1.1.27), 50 mmol l^{-1} imidazole (pH 7.4) and 0.2 mmol l^{-1} NADH, tissue homogenate, 1 mmol l^{-1} sodium pyruvate (omitted for control rates of absorbance change) (Moon, 1987; Walsh et al., 1990). For CCO (E.C. 1.9.3.1), 50 mmol l^{-1} potassium phosphate buffer (pH 7.0) with 0.05% (w/v) of lauryl maltoside as a detergent, tissue homogenate, 60 µmol l^{-1} cytochrome c reduced with sodium dithionite to initiate the reaction and 300 µmol l^{-1} KCN was used to measure any CCO-independent rate of absorbance change (Spinazzi et al., 2011). Assays had a total volume of 1 ml. Each enzyme was measured at two different volumes of homogenate, which were pre-determined to ensure sufficiently linear reaction rates as well as proportionality with sample added. Measurements were made by recording the absorbance at 340 nm for LDH and at 550 nm for CCO. Control measurements were taken during the first 2–3 min of each assay before the reaction substrate was added for LDH and for the final 2–3 min for CCO after the addition of KCN to the cuvette. Control readings were subtracted from the reaction readings during analysis and results are expressed per gram of

tissue as µmol min^{-1} g^{-1}. All chemicals/reagents were purchased from Sigma-Aldrich, Canada.

Internal body temperatures

We estimated the internal body temperature that the average lake trout (based on both starting and final masses) in our study would have achieved by the end of the 5 min exposure period in each treatment using the results of Pepino et al. (2015), who quantified heat transfer in brook trout (*Salvelinus fontinalis*), a close relative to lake trout. We used a stepwise approach to estimate the internal body temperature of our fish because we rapidly increased ambient temperatures, whereas Pepino et al. (2015) moved fish directly from cool to warm temperatures when estimating rates. We first estimated how much the internal temperature would increase from 10°C to 13.5°C over 2.5 min, and then used that internal temperature estimate for 2.5 min at 13.5°C as a starting temperature and assumed fish would be in 17°C or 22°C for 7.5 min (2.5 min to heat and 5 min at the treatment temperature). We assumed fish would be at target temperatures for 7.5 min rather than 5 min for modelling internal temperatures because temperature increased rapidly in tanks and then slowed as we approached target temperatures to ensure we did not overshoot temperature endpoints.

Data analysis

We used a combination of linear (LMM) and generalized (GLMM) mixed-effects models to test for differences in biological metrics, metabolic rates and white muscle enzyme activity among our experimental treatments. In each model, we treated 'replicate tank' as a random intercept nested within treatment group to account for any unanticipated tank effects. The assumptions of models were tested following the methods of Zuur et al. (2009, 2010). Tukey pairwise *post hoc* multiple comparisons tests were used for among-treatment comparisons when treatment was found to be an influential fixed effect. For LMMs, marginal (R^2m) and conditional (R^2c) coefficients of determination were used to determine the proportion of variance explained by only fixed factors and fixed and random factors, respectively. For the gamma GLMMs (see below), R^2m and R^2c are not estimable, so the proportion of variance explained by random effects was calculated using intra-class correlation coefficients (ICC), where ICC=variance of random effects/total variance. All analyses and figures were completed in R v.3.5.2 (https://www.R-project.org). Analyses were performed using the following R packages: Tukey tests with emmeans (https://cran.r-project.org), LMM with nlme (Pinheiro and Bates, 2000; https://cran.r-project.org/web/packages/nlme), GLMM with lme4 (https://cran.r-project.org/web/packages/lme4), and R^2m and R^2c with MuMIn (https://cran.r-project.org/web/packages/MuMIn).

Average daily food consumption rates per fish in each tank were estimated by subtracting the amount of food that was remaining from the total amount of food offered to the tank and dividing by the number of fish in that tank. We then used a generalized linear mixed effect model (GLMM) with a gamma distribution and treatment treated as a fixed factor to model daily consumption rates of lake trout. A chi-squared test was used to test if daily consumption differed across treatments. We chose a gamma distribution because the amount of food offered to each tank increased over the study to maintain ∼1.5% by mass feeding, thus consumption increased over the study period. Examination of residuals confirmed that the gamma GLMM fitted the data well. The total food consumed by the average fish in each treatment over the entire study was estimated as the sum of the average daily consumption rates for each tank divided by two.

Specific growth rates (% of body size per day) for individual fish were calculated for both mass (SGR_M) and length (SGR_L) by fitting the exponential model: $y=ae^{bx}$, where y is the mass (g) or fork length (mm) on a given day (x) and b is the specific growth rate. Differences in SGR-M and SGR-L among treatments were tested using a LMM with response variables cube-root transformed prior to analyses to meet model assumptions. Condition was estimated using Fulton's K: body mass×(10,000/fork length^3). Condition factors were estimated for both the start (21 January 2015) and end of the exposure periods (24 March 2016), and the change in condition over this period (Δ condition) was calculated. The final hepatosomatic index (HSI) of each fish was estimated as: liver mass/body mass×100. The SGR-M, SGR-L, Δ condition, HSI and ventricle masses of lake trout were modelled using LMMs with treatment treated as a fixed factor and no data transformations required to meet assumptions.

Among-treatment differences in whole-animal metabolic rates (SMR, MMR and AS), ventricle masses and white muscle enzyme activity (LDH and CCO) were quantified using LMMs with final fish mass (continuous), treatment (factor) and their interaction (treatment×fish mass) included as fixed effects. Model selection for LMMs were performed using backwards stepwise regression with marginal F tests. For LMMs, metabolic rates, ventricle masses, enzymes and fish masses were $\log_{10}$ transformed for analysis. For graphics illustrating variation in metabolic rate and enzymes among treatments, we centred the residuals of the linear $\log_{10}$–$\log_{10}$ relationships between each of metabolic rate and enzyme around the mean value predicted from the model for a fish of 160 g, which was the average final mass of lake trout in our study. If mass was not found to influence one of the metabolic rate or enzymes, then the means and raw data for each treatment are presented.

RESULTS

Tank water temperatures

All fish experienced average daily water temperatures of ∼10°C during the 57 day treatment period, with fish in control tanks held at mean water temperatures of 10°C for the entire duration of the experiment (Fig. 1). On average, treatment tanks had temperatures exceeding the optimal 10±2°C for 10.86±4.61 min day^{-1} – roughly 5–6 min to heat and cool the water, plus the 5 min heat exposure. In all, treatment fish were acutely heated for 53 days, which equated to them being in elevated temperatures (>10±2°C) for ∼397 min and in treatment temperatures of 17°C or 22°C for ∼265 min during the exposure period.

Food consumption

We found evidence that food consumption by lake trout differed among treatments ($\chi^2_{2,312}$=7.36, P=0.03), with fish in control tanks eating slightly more food each day than those exposed to 17°C (Tukey test: Z=2.58, P=0.03) but similar amounts to fish exposed to 22°C (Tukey test: Z=2.01, P=0.10). We also found no difference in consumption between fish exposed to 17°C and 22°C (Tukey test: Z=−0.57, P=0.83). The average daily food consumption by fish in control tanks was (mean±s.d.) 1.30±0.23 g compared to 1.20±0.25 g and 1.22±0.24 g consumed on average per fish in 17°C and 22°C treatments, respectively (Fig. 2A). Overall, these daily differences in consumption translated into an average total food consumption of fish in control tanks to be roughly 135 g; about 10 g more than in the 17°C (∼125 g) and 22°C (∼127 g) treatments (Fig. 2B). ICCs indicated that differences in consumption between replicate tanks (within a treatment) accounted for <1% of the total variance explained in daily food consumption.

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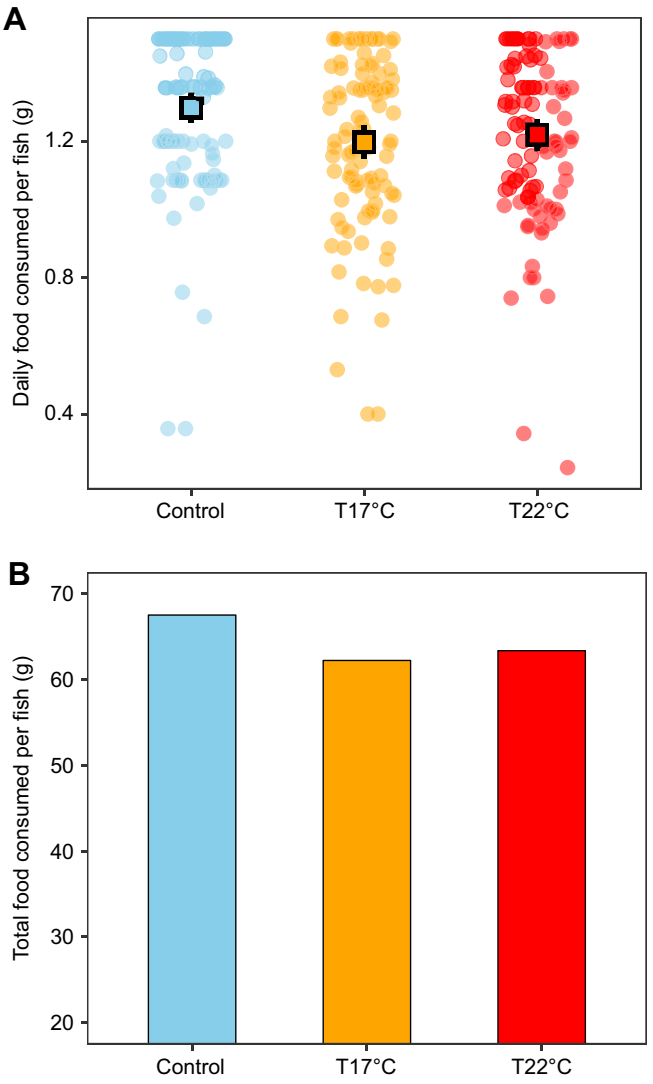

Fig. 2. Daily and total food consumption by lake trout (*Salvelinus namaycush*) in each experimental treatment. (A) For daily consumption, each dot represents the estimated average daily consumption per fish in each treatment (n=104 per treatment) and the square with error bar represents the treatment-level mean±95 CI average daily consumption for the fish in each treatment over the entire study. (B) Each bar represents the total food consumed by the average fish in each experimental group over the study, assuming all fish ate equal portions each day.

Fish growth and condition

We found evidence that SGR_M of lake trout differed among treatments ($F_{2,32}$=3.75, P=0.03, R^2m=0.18, R^2c=0.18; Table 1, Fig. 3A), with control fish (mean±s.d.; 0.015±0.011% g day^{-1}) having higher SGR_M than T17°C fish (0.014±0.022% g day^{-1}) and 22°C (0.012±0.023% g day^{-1}). SGR_M did not differ between treatments (Table 1, Fig. 3A). Similarly, the SGR_L of lake trout also differed among treatments ($F_{2,32}$=4.78, P=0.02, R^2m=0.21, R^2c=0.24), with fish in control tanks (0.0034±0.0003% mm day^{-1}) having higher SGR_L than T17°C fish (0.0029±0.0007% mm day^{-1}) and T22°C fish (0.0028±0.0004% mm day^{-1}), but no difference between T17°C and T22°C (Table 1, Fig. 3B). Halfway through the exposure period (26 February), mean (±s.d.) fork lengths were 218.4±11.4 mm, 214.4±16.8 mm and 218.4±14.5 mm, while mean body mass was 121.9±21.0 g, 109.0±31.2 g and 117.5±22.1 g, for control, T17°C exposed and T22°C fish, respectively. At the end of the experimental period (24 March), mean (±s.d.) fork lengths were 236.2±15.5 mm, 232.1±20.7 mm and 232.1±16.7 mm, while masses were 175.6±38.1 g, 153.9±51.1 g and 162.5±35.7 g, for control, T17°C and T22°C fish, respectively.

We found weak evidence that the change in condition of lake trout (Δ condition) over the exposure period differed among treatments ($F_{2,32}$=2.83, P=0.07; Table 1, Fig. 3C), with control fish (0.32±0.05) having greater Δ condition than T17°C fish (0.25±0.11) but not T22°C fish (0.31±0.06). No differences in Δ condition were evident between T17°C and T22°C fish (Table 1, Fig. 3C). Lake trout HSI following respirometry did not differ among treatments ($F_{2,30}$=0.02, P=0.98; control: 1.11±0.10; T17°C: 1.12±0.11; T22°C: 1.11±0.14; Fig. 3D). In general, we found little evidence for tank effects on biological metrics, with differences between R^2m and R^2c values being ≤5% for all metrics.

Metabolic rates

SMR increased with mass (mass: $F_{1,27}$=107.3, P<0.01, R^2m=0.80, R^2c=0.80; $\log_{10}$ SMR=0.87×$\log_{10}$ mass–0.98), but the slope of this relationship did not differ among treatments (treatment×mass: $F_{2,23}$=1.44, P=0.26). Mean SMR estimates also did not differ among treatments when accounting for mass (treatment: $F_{2,25}$=0.68, P=0.52) (Tables 2 and 3, Fig. 4A). MMR also increased with mass (mass: $F_{1,27}$=57.77, P<0.01, R^2m=0.66, R^2c=0.66; $\log_{10}$ MMR=0.51×$\log_{10}$ mass+0.40), with the slope of this relationship not differing among treatments (treatment×mass: $F_{2,23}$=0.47, P=0.63). However, mean MMR estimates did differ among treatments when accounting for mass (treatment: $F_{2,25}$=6.92, P<0.01, R^2m=0.78, R^2c=0.78), with fish from T17°C having greater MMR than those from control tanks and T22°C. No differences in MMR were observed between control fish and T22°C fish (Tables 2 and 3, Fig. 4B). AS also increased with mass (mass: $F_{1,28}$=30.52, P<0.01, R^2m=0.52, R^2c=0.52; $\log_{10}$ AS=0.49×$\log_{10}$ mass+0.35), with the slope of this relationship not differing among treatments (treatment×mass: $F_{2,23}$=0.20, P=0.82). Mean AS estimates did differ among treatments when controlling for the effect of mass (treatment: $F_{2,25}$=7.19, P=0.03, R^2m=0.63, R^2c=0.63), with fish from T17°C having greater AS than those from control tanks and T22°C, but no differences between control and T22°C (Tables 2 and 3, Fig. 4C). No tank effects on metabolic rates were evident based on R^2m and R^2c values. Summary data for both whole-animal and mass-specific metabolic rates can be found in Table 2.

Ventricle mass

Ventricle mass increased with fish mass (mass: $F_{1,30}$=81.06, P<0.01, R^2m=0.72, R^2c=0.72; $\log_{10}$ ventricle mass=1.02×$\log_{10}$ mass–3.12), but the slope of this relationship did not differ among treatments (treatment×mass: $F_{2,26}$=0.25, P=0.78). Mean ventricle mass also did not differ among treatments when accounting for mass (treatment: $F_{2,28}$=0.73, P=0.49) (Table 3).

White muscle enzymes

LDH activity was not related to the interaction of treatment and mass (treatment×mass: $F_{2,26}$=0.09, P=0.91) and did not differ among treatments (treatment: $F_{2,28}$=1.10, P=0.35) (Fig. 5A), but did increase with mass (mass: $F_{1,30}$=3.86, P=0.06, R^2m=0.11, R^2c=0.16; $\log_{10}$ LDH=0.27×$\log_{10}$ mass+2.25) (Fig. 5A). CCO activity was also not influenced by the interaction of treatment and mass (treatment×mass: $F_{2,26}$=0.06, P=0.94) and did not differ by treatment (treatment: $F_{2,28}$=1.44, P=0.26) (Fig. 5A). CCO activity showed a weak increase with mass (mass: $F_{1,30}$=2.93, P=0.09, R^2m=0.09, R^2c=0.10; $\log_{10}$ CCO=0.58×$\log_{10}$ mass–0.66). Mean

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Table 1. Pairwise Tukey tests of linear mixed effects models testing for the effect of treatment on specific growth rates based on mass (SGR-M) and fork length (SGR-L), the change in condition factor (Δ condition), final hepatosomatic index (HSI) and final ventricle mass of lake trout in control tanks and those exposed to repeated acute temperature stress in Treatment 17°C (T17°C) and Treatment 22°C (T22°C)

		Tukey test (treatment)			
Response	Treatment	Pairwise comparison	d.f.	*t*	*P*
SGR_M	$F_{2,32}$=3.75, *P*=0.03, R^2m=0.18, R^2c=0.18	Control–T17°C	32	2.58	0.04
		Control–T22°C	32	2.16	0.09
		T17°C–T22°C	32	0.48	0.88
SGR_L	$F_{2,32}$=4.78, *P*=0.02, R^2c=0.21, R^2m=0.24	Control–T17°C	32	2.28	0.07
		Control–T22°C	32	2.98	0.02
		T17°C–T22°C	32	0.68	0.78
Δ Condition	$F_{2,32}$=2.83, *P*=0.07, R^2c=0.13, R^2m=0.18	Control–T17°C	32	2.19	0.09
		Control–T22°C	32	0.38	0.92
		T17°C–T22°C	32	–1.89	0.16
HSI	$F_{2,30}$=0.02, *P*=0.98, R^2c=0.00, R^2m=0.00	–	–	–	–

Tukey tests were only performed when 'treatment' was included in the top model chosen by model selection. Mass specific growth rate (SGR_M) and length specific growth rate (SGR_L) were square-root and cubic-root transformed, respectively, for analysis. R^2m and R^2c are the marginal and conditional coefficients of determination, which represent the proportion of variance explained by only fixed effects (R^2m) and fixed and random effects of tank (R^2c). Note the residuals of the relationship between $\log_{10}$ ventricle mass and fish mass were used as a response variable to account for the effect of fish size when testing for differences among treatments. HSI, hepatosomatic index.

(±s.d.) CCO activities were 5.38±2.31 µmol min^{-1} g^{-1}, 4.36 ±1.99 µmol min^{-1} g^{-1} and 3.57±1.18 µmol min^{-1} g^{-1}, for control, T17°C and T22°C fish, respectively (Fig. 5B).

Internal body temperatures

We estimated that the average-sized fish at the start (68.4 g) and end (151.0 g) of the 57 day exposure period would have achieved

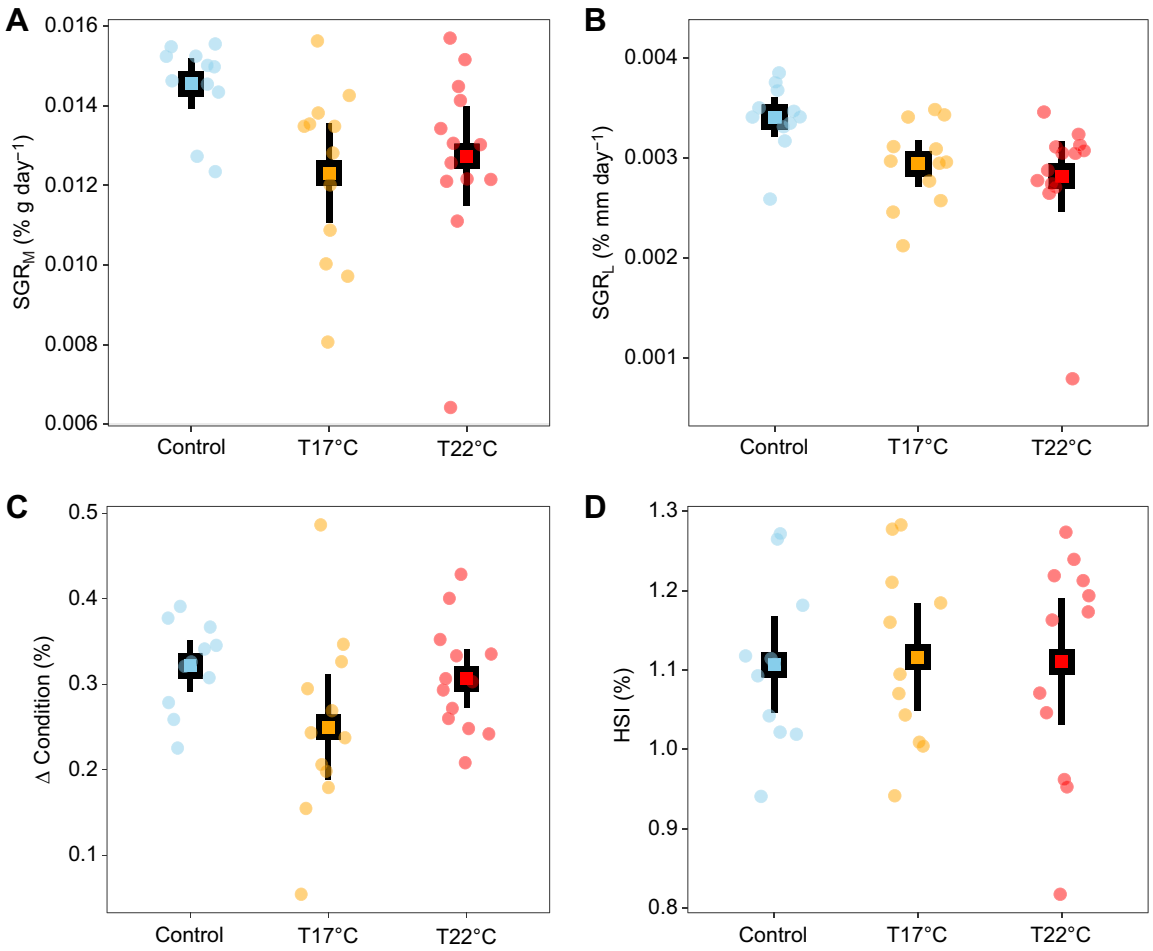

Fig. 3. Growth and condition of lake trout in each experimental treatment. Individual (points) and treatment-level mean±95 CI (squares and error bars) values of (A) mass specific growth rate (SGR_M), (B) length specific growth rate (SGR_L), (C) change in condition factor between the start and end of the exposure period (Δ condition), and (D) final hepatosomatic index (HSI) of lake trout from each treatment. SGR_M, SGR_L and Δ condition: control, *n*=11; T17°C, *n*=12; T22°C, *n*=12. HSI: control, *n*=10; T17°C, *n*=11; T22°C, *n*=12.

Table 2. Mean (±s.d.) whole-animal and mass-specific metabolic rates and body size during respirometry for each experimental treatment

			Whole animal (mg O_2 h^{-1})			Mass specific (mg O_2 kg^{-1} h^{-1})		
Treatment	*n*	Fish mass (g)	SMR	MMR	AS	SMR	MMR	AS
Control	9	164±17.9	9.04±0.99	33.2±2.57	24.1±2.30	55.2±5.02	203±18.5	148±17.3
T17°C	10	145±41.3	8.09±1.82	34.6±6.18	26.5±4.73	56.8±6.74	247±41.2	190±38.7
T22°C	11	146±25.8	7.85±1.49	32.0±4.06	24.1±2.83	54.0±5.72	223±22.2	169±21.4

SMR, standard metabolic rate; MMR, maximum metabolic rate; AS, aerobic scope.

internal body temperatures of 14.4°C (start) and 14.3°C (end) for T17°C, and 19.8°C (start) and 19.7°C (end) for T22°C, respectively. Thus, the increase in body mass experienced over the study had minimal effects on the rate of heat transfer (0.1°C difference in internal temperature regardless of treatment).

DISCUSSION

We found that fish repeatedly given acute exposure to 17°C showed a higher MMR and AS by the end of the study compared with control fish and those exposed to 22°C, but there were no differences in SMR among treatments. In temperature-acclimated lake trout, AS appears to be optimized (maximal) at approximately 15°C (Evans, 2007; Gibson and Fry, 1954; Kelly et al., 2014), and our results suggest that even brief, repetitive exposures to warm temperatures that are close to this optimal temperature can cause lake trout to experience a plastic increase in MMR. Fish exposed to an acute temperature increase – such as that experienced by fish while foraying into warmer water – display a sharp increase in SMR during the exposure that eventually decreases with acclimation during thermal compensation (Hazel and Prosser, 1974; Steffensen, 2005). If this spike in SMR occurs similarly across all individuals (given that we found no differences), a plastic increase in MMR by individuals that underwent repeated exposures to warm water may have more AS available before and after warm water excursions, relative to control fish. However, our results suggest that acute exposures to water well above the optimal for AS may not be beneficial.

It is interesting that plasticity in MMR is induced by relatively short but daily exposures to increased temperatures (i.e. ∼10 min per day). Notably, similarly short intermittent periods of strenuous activity can have a dramatic impact on physiological traits in vertebrates, including increased maximal oxygen uptake rate in humans during daily periods of physical training (Bacon et al., 2013; Sloth et al., 2013). The plasticity in MMR displayed by lake trout in response to intermittent warming may be an analogous response, preparing individuals for future foraging bouts in suboptimal thermal environments. Increasing the proportion of AS available may be especially important given that lake trout will not only experience a temperature-induced increase in baseline metabolism during forays into shallow water during summer (Guzzo et al., 2017; Morbey et al., 2006), but they will also be performing physically strenuous activities during their relentless pursuit and capture of prey, which will further consume a portion of their available AS (Norin and Clark, 2016; Norin and Clark, 2017).

Even with a plastic increase in MMR, lake trout can likely only tolerate brief exposures to warm littoral environments while foraging. Indeed, even after complete thermal acclimation, the AS of lake trout decreases drastically at 19°C compared with 15°C (Evans, 2007; Kelly et al., 2014). It has also been observed that lake trout vastly reduce their movements into the littoral zone as temperatures rise above 15°C during seasonal warming (Guzzo et al., 2017; Plumb and Blanchfield, 2009; Snucins and Gunn, 1995). The increase in MMR observed in this study in response to acute warming (Table 4) may also come with associated physiological trade-offs; for example, we found a weak correlation between mass-corrected SMR and MMR among individuals across all treatments (r=0.30). Plastic increases in MMR could cause an associated elevation in SMR, and thus also a rise in foraging requirements. Indeed, at the interspecific level, a strong positive correlation between SMR and MMR in fish is modulated by factors such as organ size and tissue composition (Killen et al., 2016). In addition, fish in control tanks in the current study consumed more food overall than fish in the temperature-increased treatment tanks (Table 4), suggesting that the fish with a higher MMR had a decreased appetite relative to control fish. It is possible that our protocol, which involved feeding in the hours before the temperature increase, may have had a suppressive effect on fish appetite over time, if fish became habituated to the timing of the temperature increase in relation to the feeding period. Such an effect could

Table 3. Pairwise Tukey tests for top models resulting from backwards stepwise model selection of linear mixed effects models testing for the effect of experimental group and mass on whole-animal SMR, MMR and AS, as well as ventricle mass, lactate dehydrogenase (LDH) and cytochrome c oxidase (CCO) activity of lake trout in control tanks and those exposed to repeated acute temperature stress at T17°C and T22°C

		Tukey test (treatment)			
Response	Top model	Pairwise comparison	d.f.	*t*	*P*
SMR	Mass (R^2m=0.80, R^2c=0.80)	–	–	–	–
MMR	Experimental group+mass (R^2m=0.78, R^2c=0.78)	Control–T17°C	25	−3.51	<0.01
		Control–T22°C	25	−1.00	0.58
		T17°C–T22°C	25	2.79	0.03
AS	Experimental group+mass (R^2m=0.63, R^2c=0.63)	Control–T17°C	25	−3.71	<0.01
		Control–T22°C	25	−1.47	0.32
		T17°C–T22°C	25	2.51	0.04
Ventricle mass	Mass (R^2m=0.72, R^2c=0.72)	–	–	–	–
LDH	Mass (R^2m=0.11, R^2c=0.16)	–	–	–	–
CCO	Mass (R^2m=0.09, R^2c=0.10)	–	–	–	–

R^2m and R^2c are the marginal and conditional coefficients of determination, which represent the proportion of variance explained by only fixed effects (R^2m) and fixed and random effects (R^2c). Tukey tests were only performed when 'treatment' was included in the top model chosen by model selection. See Results for model selection. SMR, MMR, AS, ventricle mass, LDH, CCO and mass were all $\log_{10}$ transformed for analysis

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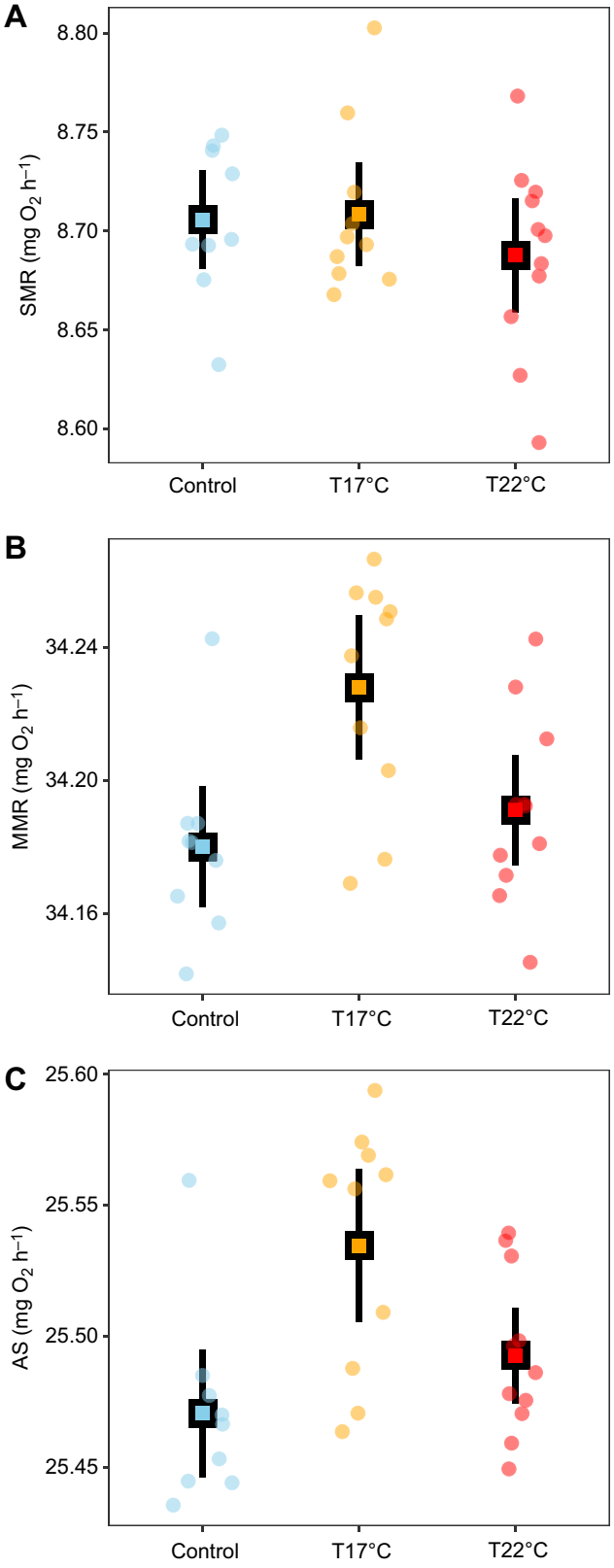

Fig. 4. Aerobic metabolic rates of lake trout in each experimental treatment. Individual (points) (control, n=9; T17°C, n=10; T22°C, n=11) and treatment-level mean±95 CI (squares and error bars) values of whole body (A) standard metabolic rate (SMR), (B) maximum metabolic rate (MMR) and (C) aerobic scope (AS), predicted for a 160 g lake trout from each treatment. Residual whole body SMR, MMR and AS values were obtained from the relationship of each variable with fish mass on a $\log_{10}$–$\log_{10}$ scale.

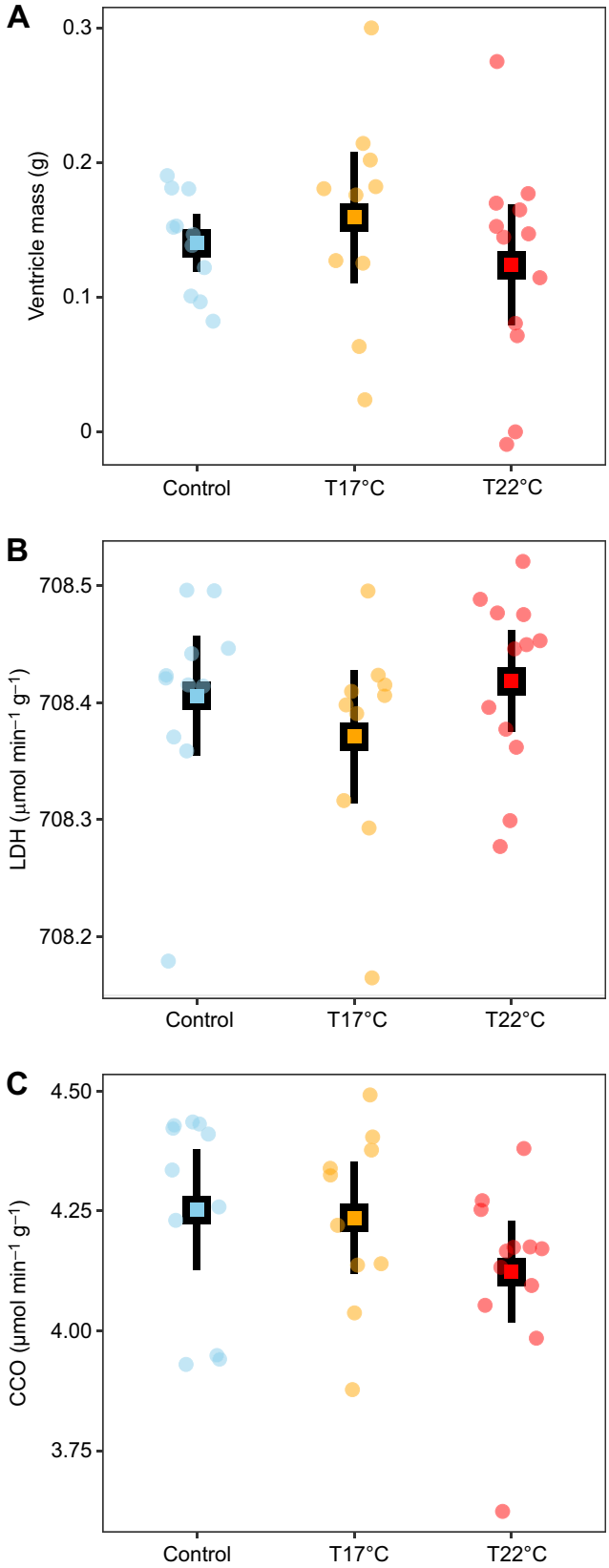

Fig. 5. White muscle enzyme activity measured in lake trout from each experimental treatment. Individual (points) (control, n=11; T17°C, n=10; T22°C, n=12) and treatment-level mean±95 CI (squares and error bars) values of (A) ventricle mass, (B) white muscle lactate dehydrogenase activity (LDH) and (C) white muscle cytochrome c oxidase activity (CCO) predicted for a 160 g lake trout from each treatment fish mass on a $\log_{10}$–$\log_{10}$ scale.

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Table 4. Mean response of each metric used in this study in treatment fish that were acutely exposed to warm (T17°C or T22°C) water relative to the control (constant 10°C).

	Response variable										
Treatment	Food consumption	SGR_M	SGR_L	Condition	HSI	Ventricle	SMR	MMR	AS	LDH	CCO
T17°C	↓	↓	↓	↓	–	–	–	↑	↑	–	–
T22°C	↓	↓	↓	–	–	–	–	–	–	–	–

Dashes indicate no differences found, and up or down arrows indicate if that treatment was higher or lower than control levels.

obscure the ability to detect differences in food demand as a potential consequence of an increased MMR and associated maintenance costs. Alternatively, the lower growth rates in exposed fish could also equate to lower protein synthesis and in turn, reduced feeding (Rosenfeld et al., 2015).

Based on average growth and food consumption, it appears that overall food conversion efficiency was on average highest for control fish. This suggests that decreased conversion efficiency could be another trade-off to phenotypic changes for coping with acute warming. However, there are several reasons why the effect of repeated and brief warming on conversion efficiency may require further study. Firstly, we could only quantify conversion efficiency at the level of the holding tanks, as it was not possible to quantify individual food consumption. Individual fish may show different patterns given that the fish from temperature treatments ate less food overall but displayed a statistically similar growth rate. Furthermore, the exact amount of energy required to digest food in fish adapted to fluctuating thermal regimes remains unknown. Temperature has complex effects on the energy required to digest and assimilate a meal in ectotherms and the subsequent activation of anabolic processes (termed specific dynamic action; SDA), but at least in some cool-water teleosts, warmer acclimation temperatures may decrease the ratio spent on digestion and assimilation relative to the amount of energy extracted from a meal (Tirsgaard et al., 2015). The physiological changes induced by brief, but frequent, foraging forays into warmer waters could alter either the time taken to digest a meal, or the total amount of energy spent on SDA, but this requires further study.

Although we measured the metabolic rates of all fish at 10°C, the temperature that fish were held at for most (exposed fish) or all (control fish) of the experiment (including the 2–3 weeks between the exposure period and measurement of metabolic rates), we still found differences in MMR and AS among treatments (Table 4). Interestingly, the pattern observed in MMR and AS across treatments closely resembles that of the temperature versus metabolic rate curves previously developed for lake trout based on acclimation experiments, which also have maximum AS at ∼15°C (Gibson and Fry, 1954; Evans, 2007). This is especially noticeable when taking into consideration that, on average, maximum daily internal body temperatures of lake trout in our experiment were 10°C (control), 14.3°C (17°C exposed fish) and 19.7°C (22°C exposed fish). Our results differ from those of Gibson and Fry (1954) in that SMR of their acclimated fish showed an exponential increase in response to temperature, while SMR in our study was constant across treatments. Our results also differ from those of Morash et al. (2018) who found that Atlantic salmon parr exposed to fluctuating temperatures had reduced SMR, MMR and AS compared with levels in fish acclimated to stable conditions. However, our finding of an increase in MMR after acute exposure to warm water are partially in line with those of Sandblom et al. (2016), who found that European perch (*Perca fluviatilis*) exposed to a 5°C temperature increase for 24 h had elevated MMR, but also an elevated SMR. The contrasting results for SMR could be because their acute exposure with perch was much longer in duration than in our study, and that metabolic rates were measured at the elevated temperature rather than the pre-exposure temperature. In the context of forays into warm water, the results of Sandblom et al. (2016) suggests that MMR of our fish exposed to 17°C may remain elevated relative to control fish while in warm water, as well as before and after the foray.

While differences were observed in some traits assessed in the present study, a lack of difference in SMR among treatments (Table 4) is not completely surprising, as for lake trout, SMR has been documented to be less influenced by temperature when compared with MMR at acclimation temperatures up to 15°C (Gibson and Fry, 1954; Evans, 2007). Results from Gibson and Fry (1954) indicate that for a 100 g lake trout, a rise in acclimation temperatures from 10 to 14.3°C (the internal temperature reached in our T17°C) would result in an increase in whole-animal SMR of only 2.3 mg O_2 h^{-1} (4.4–6.7 mg O_2 h^{-1}) compared with an increase in MMR of 10.6 mg O_2 h^{-1} (24.6–35.4 mg O_2 h^{-1}). For comparison, the average whole-animal SMR predicted for a 100 g lake trout in our study was 5.8 mg O_2 h^{-1} (across all treatments), while MMR was 3.1 mg O_2 h^{-1} greater for T17°C fish (28.2 mg O_2 h^{-1}) relative to control fish (25.1 mg O_2 h^{-1}). The contrasting findings between the acclimation work of Gibson and Fry (1954) and our study could be because the temperatures experienced during the acute heating events did not affect biochemical and membrane-associated processes that set SMR (Rolfe and Brown, 1997).

Our finding that MMR is more plastic to acute thermal exposure than SMR is also in line with results of a previous study that used lake trout from the same parental cross and found that MMR and AS responded to exposures to saltwater transfers (0, 5 and 20 ppt) while SMR did not (Kissinger et al., 2017). This study also found that some key enzymes (Na^+/K^+-ATPase), their regulatory genes (Na^+/K^+-ATPase α1a and α1b) and resulting plasma osmolality differed with saltwater exposure (Kissinger et al., 2017). The reduced sensitivity of SMR to abiotic heterogeneity highlights the ability of lake trout to acutely and chronically respond to a range of heterogeneous environments. Minimizing increases in SMR due to changes in the abiotic environment is extremely important to lake trout because existing in a state nearing SMR minimizes energy expenditure, which is essential when existing in low-productive oligotrophic lakes. The resilience of SMR in lake trout to acute thermal exposure and other environmental heterogeneity may be linked to evolutionary tactics that have allowed lake trout to survive and exploit a variety of low-productive environments across North America (Kelly et al., 2014; Martin and Olver, 1980; Muir et al., 2016). While a combination of plasticity and local adaptation has allowed this species to colonize a range of environments, changes in climate suggest that the physiological adaptations of this genus will be put to the test (Reist et al., 2013).

Acknowledgements

The authors thank Terry Smith and the rest of the University of Manitoba Animal Care Facility for their help throughout all stages of the experiment; Eva Enders for lending

respirometry equipment; Laura Murray and Rex Yoon for help setting up the respirometry system; and Tommy Norin for providing feedback on an earlier version of the paper.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: M.M.G., N.J.M., J.R.T.; Methodology: M.M.G., N.J.M., T.D., B.C.K., J.R.T.; Formal analysis: M.M.G., T.D., S.S.K.; Resources: B.C.K.; Data curation: M.M.G., T.D.; Writing - original draft: M.M.G., S.S.K., J.R.T.; Writing - review & editing: M.M.G., N.J.M., T.D., B.C.K., S.S.K., J.R.T.; Supervision: N.J.M., J.R.T.; Project administration: N.J.M., J.R.T.; Funding acquisition: N.J.M., J.R.T.

Funding
This work was funded by Fisheries and Oceans Canada, Species-at-Risk program; a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant (J.R.T.; grant no. 418503); an NSERC Canada Graduate Scholarship (M.M.G.), and a W. Garfield Weston Foundation award in Northern Research (M.M.G.). J.R.T. is the Canada Research Chair in Environment Dynamics and Metabolism (grant no. 223744).

Data availability
The data reported in this paper have been deposited in the open source database Zenodo: http://doi.org/10.5281/zenodo.3232882.

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