



How repeatable is CT_{max} within individual brook trout over short- and long-time intervals?

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ABSTRACT

As stream temperatures increase due to factors such as heated runoff from impervious surfaces, deforestation, and climate change, fish species adapted to cold water streams are forced to move to more suitable habitat, acclimate or adapt to increased thermal regimes, or die. To estimate the potential for adaptation, a (within individual) repeatable metric of thermal tolerance is imperative. Critical thermal maximum (CT_{max}) is a dynamic test that is widely used to measure thermal tolerance across many taxa and has been used in fishes for decades, but its repeatability in most species is unknown. CT_{max} tests increase water temperature steadily over time until loss of equilibrium (LOE) is achieved. To determine if CT_{max} is a consistent metric within individual fish, we measured CT_{max} on the same lab-held individually-marked adult brook trout *Salvelinus fontinalis* at three different times (August & September 2016, September 2017). We found that CT_{max} is a repeatable trait (Repeatability \pm S.E.: 0.48 ± 0.14). CT_{max} of individuals males was consistent over time, but the CT_{max} of females increased slightly over time. This result indicates that CT_{max} is a robust, repeatable estimate of thermal tolerance in a cold-water adapted fish.

1. Introduction

Historically, the native range of cold-water adapted brook trout *Salvelinus fontinalis* extended from northern Quebec to Georgia, east from the Atlantic Ocean to Manitoba in the north, and along the Appalachian ridge in the south (Meisner, 1990). Contraction of this range has occurred (Hudy et al., 2008) and is expected to continue as a result of factors such as increasing stream temperatures and more variable stream flow regimes (Letcher et al., 2015; Xu et al., 2010). In fishes and other ectotherms, temperature is known to govern metabolism and behavior over short time periods (seconds to months) (Johnston and Dunn, 1987), and growth (Elliott, 1976; Selong et al., 2001), survival (Letcher et al., 2015), and fecundity in the long term (months to years) (Jobling, 1997; Xu et al., 2010). The long-term persistence of *S. fontinalis* and other cold-adapted species will likely depend on their ability to cope with and adapt to changing thermal conditions.

Within species, rearing conditions can influence temperature tolerance across populations. Whitney et al. (2013) found evidence of intra- and interpopulation survival differences for sockeye salmon *Oncorhynchus nerka* eggs collected from rivers with varying thermal regimes. They noted that when rearing temperature was increased there was more variation in survival rates of eggs collected from rivers with varying thermal histories, and that eggs collected from warmer rivers had higher survival than eggs collected from cooler rivers when reared in a common, warmer temperature. Similarly, Stitt et al. (2014)

demonstrated thermal tolerance variation of *S. fontinalis* over a large geographical area where fish from northern populations exhibited lower thermal tolerance than fish collected from southern, warmer populations. Stitt et al. (2014) also found increased intrapopulation variation of thermal tolerance at lower acclimation temperatures. Both Whitney et al. (2013) and Stitt et al. (2014) conclude that within-population variation in adaptive capacity to increasing thermal regimes could be a key mechanism for long term population persistence of cold-adapted species in warming environments.

To examine adaptive capacity within a population (for example, adaptation to increased thermal regimes in cold water fish) a trait that is potentially heritable needs to be identified, there needs to be phenotypic variation in that trait across the population, and there must be selection on that trait. Further, heritability is defined as the amount of additive genetic variance divided by the amount of phenotypic variance (Falconer and Mackay, 1996). Because measurement error can influence estimates of phenotype, the accuracy of heritability estimates is improved when variation of repeated trait measurements is low (Ge et al., 2017). Therefore, to determine the degree to which thermal tolerance is heritable, a measurement of thermal tolerance that can be measured repeatably within individuals will have the best potential to provide accurate estimates of heritability.

There are several methods used to measure thermal tolerance in fish which can be categorized as either static or dynamic, based on water temperature changes during test conditions. Static tests expose fish to a

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constant water temperature and typically measure upper and lower incipient lethal temperature. Dynamic tests expose fish to changing water temperature and typically end with the lack of righting response or the onset of muscular spasms (Lutterschmidt and Hutchison, 1997). While these tests may not accurately represent environmental conditions fish experience in the wild, they are intended to be a rapid measurement of a fish's ability to tolerate reduced or elevated temperatures (Bennett and Judd, 1992). Regardless of which method is utilized, it is important that measures of thermal tolerance are repeatable within individuals for the metric to be an effective indicator of thermal tolerance.

Critical thermal maximum (CT_{max}) is a dynamic test that is widely used to measure thermal tolerance across many taxa and has been used with fish for decades (e.g. Chen et al., 2017; Becker and Genoway, 1979; Lutterschmidt and Hutchison, 1997). The highest temperature at which a fish loses equilibrium under increasing temperature, but is not immediately lethal, is considered the CT_{max} (Beitinger et al., 2000; Cowles and Bogert, 1944). Typically, CT_{max} trials are done on groups of individuals and mean values for each group are reported, ignoring within-individual variation. Also, most studies using CT_{max} to measure thermal tolerance only test individual fish once, ignoring the potential for within-individual variation in CT_{max} over time. While it is expected that CT_{max} could vary seasonally (e.g. Houghton and Shoup, 2014; Scott, 1987), for CT_{max} to be considered a repeatable metric of thermal tolerance, individual ranks within groups should not change over time. Evidence exists indicating that CT_{max} is a repeatable metric within individual shortnose sturgeon *Acipenser brevirostrum* (Bard and Kieffer, 2019) and zebrafish *Danio rerio* (Morgan et al., 2018) over short time periods (days to weeks), but information regarding long term (months to years) is lacking.

To determine if CT_{max} is a repeatable metric of thermal tolerance within an individual *S. fontinalis*, we designed a study where individually-marked *S. fontinalis* were exposed to CT_{max} trials three times. The first two trials were one month apart, and the last trial was one year later. In addition to estimating within-individual repeatability of CT_{max} , we constructed linear mixed models to determine if CT_{max} , sex, and body size at one sample influenced CT_{max} at a subsequent sample within individual fish.

2. Materials and methods

2.1. Fish origin and rearing

Gametes were collected on November 21 and 22, 2013 from 3-year old, F1's whose parents were wild fish originating from two different streams. Since fertilization, the F1's had been reared for 3 years in a common environment at the Silvio O. Conte Anadromous Fish Research Laboratory where water temperature was adjusted biweekly to mimic average daily water temperature of a nearby *S. fontinalis* stream that is a long-term study site (Letcher et al., 2016) plus 2 °C. Fish were spawned in 10 batches, each batch consisting of eggs from two to four females fertilized by milt from two males. Fertilized eggs were combined to a single tray and incubated in Heath Trays with partially recirculated, dechlorinated municipal water and water temperature was adjusted biweekly to mimic average daily water temperature of a nearby brook trout stream that is a long-term study site (Letcher et al., 2016) plus 2 °C. Prior to yolk sac absorption, alevins were transferred to ~1-m diameter by 0.6-m-deep, circular fiberglass rearing tanks; the water source remained the same. Fry were fed *ad libitum* Bio Vita Starter (Bio-Oregon, Westbrook, ME) twice per day. As fish size increased, feed size was increased, fish were moved to larger 1.5 m circular rearing tanks, and water source was changed to filtered and ultraviolet (UV) treated Connecticut River water (temperature regime remained as described earlier). On July 6, 2016, 80 (3-year old; fork length range = 230–307 mm) *S. fontinalis* were randomly selected from the rearing tank and implanted with HPT12 passive integrated transponder (PIT) tags (12.5 mm long x 2.12 mm diameter; 134.2 kHz ISO FDXB,

Biomark Inc., Boise, ID) following the methods of Gries and Letcher (2002). Upon recovery, PIT tagged fish were placed in one of two rearing tanks with a final density of 40 *S. fontinalis* per tank.

2.2. Thermal challenge

Thermal challenge trials were conducted in flow through 0.6 m diameter exposure tanks supplied with 280 ml^{min} filtered and UV treated Connecticut River water at a temperature of approximately 16 °C. Programmable thermostats and temperature probes (1/32 DIN Ramp/Soak Controller CN7500 and PFA RTD Sensor Probe, Omega Engineering, Stamford, CT) controlled solenoid valves (McMaster-Carr, Atlanta, GA) to dispense ~40 °C filtered and UV treated Connecticut River water to increase the temperature in each exposure tank 2 °C^{hour}; controllers were programed to increase water temperature in the exposure tanks linearly over time. Within each exposure tank, airlifts were used to provide aeration and directional flow, and to ensure heated water was thoroughly mixed with tank water. Water temperature within each exposure tank was recorded every minute using a HOBO Water Temperature Pro v2 Data Logger (Onset Computer Corporation, Bourne, MA) cable tied to a standpipe in the center of the tank. Exposure tank water temperature was also monitored throughout the experiment with a Traceable Ultra Long-Stem Thermometer model 4352 (Control Company, Friendswood, TX). Oxygen concentration levels were monitored and ranged from 85% to 100%, which are above levels known to influence CT_{max} (Ern et al., 2016). On three occasions (August 2016, September 2016, and September 2017), PIT tagged *S. fontinalis* were randomly assigned to temperature exposure tanks at a density ≤10 fish/tank. Fish were transferred from rearing tanks (17 °C) to six exposure tanks (17 °C) for a one hour tank acclimation period prior to initiating the 2 °C^{hour} thermal ramp (Baroudy and Elliott, 1994; Elliott and Elliott, 1995). Transfers were made to each tank in 15-min intervals to allow for adequate observation and sampling time between each tank. Once the first tank reached 27 °C, each tank was scanned constantly for fish that lost equilibrium. Characteristics for determining loss of equilibrium (LOE) were consistent with other studies examining thermal stress in salmonids (e.g. Baroudy and Elliott, 1994; Becker and Genoway, 1979; Galbreath et al., 2006), and included sporadic, non-directed swimming followed by the inability of a fish to maintain dorso-ventral orientation for more than 10 s. Once a fish reached LOE, it was removed from the temperature exposure tank, scanned for its PIT number, and was then recovered in a tank containing 17 °C filtered and UV treated Connecticut River water. For each fish, the time of LOE was recorded along with PIT tag number and temperature from both the programmable controller and long-stem thermometer. Temperature was also obtained by aligning LOE time for a given fish with the time stamp from the HOBO Logger at the conclusion of the study. On November 9 and 10, 2016, all fish were measured (fork length ± 1 mm). All rearing and experiments were carried out in accordance with USGS - LSC, Standard Operating Procedures for the Acquisition, Care, and Handling of Laboratory Animals (2019).

2.3. Data analysis

Traditionally, maximum water temperature at LOE is the endpoint for CT_{max} (Lutterschmidt and Hutchison, 1997). In the current study the rate of temperature increase used was ecologically relevant, and relatively slow (2 °C^{hour}) compared to other rates which have been tested; (for example 0.0104–18 °C^{hour} (Elliott and Elliott, 1995)). Therefore it was possible for fish to experience LOE at the same or similar temperatures, but several minutes apart. Galbreath et al. (2004) note that utilizing time to LOE as a measure of CT_{max} not only accounted for imperfect heating rates within experiment tanks, but was also a more sensitive measure when comparing CT_{max} across different heating rates and that by using time to rather than temperature of LOE increased their ability to detect CT_{max} differences across groups of juvenile rainbow

trout *O. mykiss*, brown trout *Salmo trutta* (Linnaeus, 1758), and *S. fontinalis*. However, their experiment was conducted in a single tank where all fish were exposed to identical thermal conditions. In the current study, CT_{max} trials were conducted in multiple tanks where thermal conditions could vary slightly between tanks. To account for thermal variation between tanks we combined time with temperature and calculated cumulative degree minutes from the time a temperature ramp started to the time of LOE and used this measure as a more accurate measure of CT_{max}. Any fish that died during the study (N = 30 fish died in between CT_{max} trials) were removed from the analysis. Prior to analysis, CT_{max} data were standardized within each sample to a mean = 0 and standard deviation = 1.

To test for repeatability of CT_{max} within individual fish, linear mixed-effects models were constructed using the ‘lme4’ package (Bates et al., 2014) in R (R Core Team, 2017). All models in our model set included individual fish as the random effect; which allowed intercepts to vary based on individual fish. Akaike’s Information Criterion (AIC) (Burnham and Anderson, 1998) was used to evaluate support for models that contained combinations of variables (fixed effects; size, sex, and CT_{max} value at the beginning of the sample interval) which were likely predictors of CT_{max} at the end of the sample interval (dependent variable). Marginal R² (variation explained by fixed effects) and conditional R² (total model variation explained) were calculated for the top two models (Nakagawa and Schielzeth, 2013). Finally, adjusted repeatability (hereafter, repeatability) was calculated as the proportion of total variance in CT_{max} accounted for by an individual, controlling for fixed effects, using R package ‘rptR’ (Nakagawa and Schielzeth, 2010; Stoffel et al., 2017)

3. Results

There was relatively little inter-individual variation in temperature at LOE within each sampling period (<1 °C), but greater inter-individual variation in cumulative degree minutes in LOE across individuals within a sampling period (>400-degree minutes) (Table 1, Fig. 1). Despite variation in CT_{max} (as measured by cumulative degree minutes), there was no evidence that CT_{max} varied between samples or across sizes of *S. fontinalis*, but there was evidence that CT_{max} at the end of the sample interval was dependent on CT_{max} at the beginning of the sample interval and sex (Table 2). There was marginal evidence for an interaction between CT_{max} at the beginning of the sample interval and sex (AIC difference between model 1 and model 2 was 2.51), but a likelihood ratio test between the top two preferred models was insignificant (p = 0.47) and the log likelihood of each model were nearly identical (model 1 = -60.06 and model 2 = -59.62). Additionally, the amount of variation explained by the both the fixed effects (marginal R²) and the whole model (conditional R²) were almost identical for Model 1 and Model 2 (Table 2). This indicates that CT_{max} at the end of the sample interval is highly dependent on CT_{max} at the beginning of the sample interval, and that females had CT_{max} values in subsequent samples 0.26 standard deviations higher than males (Table 3).

CT_{max} was a repeatable measure within an individual *S. fontinalis*. An individual’s CT_{max} value at the beginning of a sample interval was the most important predictor of CT_{max} in a subsequent sample. Also, CT_{max} in females increased after the first CT_{max} trial. Model 1, which contained CT_{max} at the beginning of the sample interval and sex as predictors of

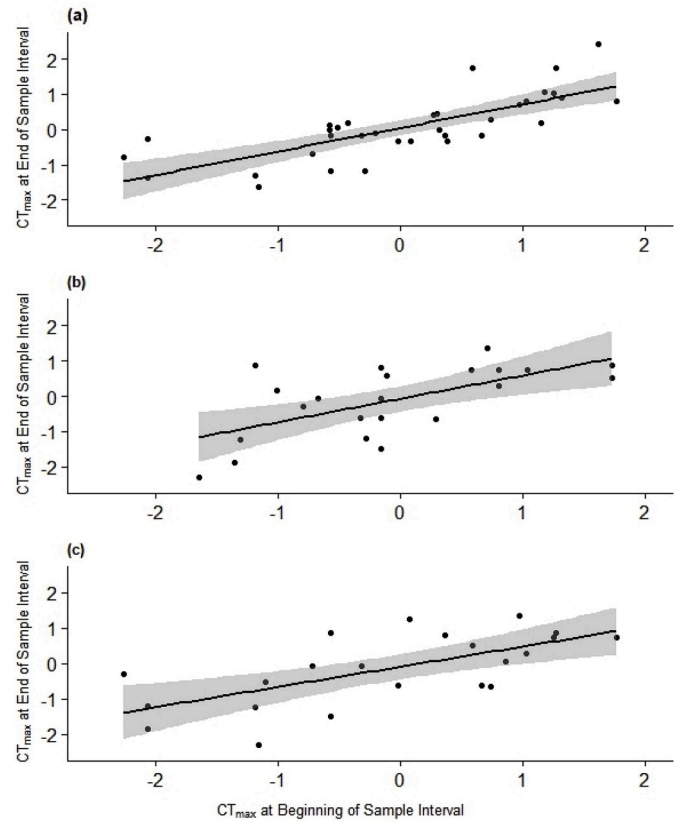


Fig. 1. Standardized CT_{max} of individually tagged adult *Salvelinus fontinalis* subjected to repeated thermal tolerance trials over different sample intervals (a) 1-month between samples (b) 11-months between samples, and (c) 12-months between samples.

Table 2

Model selection results for linear mixed-models constructed to examine the effect of CT_{max} at the beginning of the sample interval, sex, size, and sample number on subsequent CT_{max} value with individual *Salvelinus fontinalis* as random effect in all models.

	Model fixed effects	DF	AIC	Δ AIC	R ² _(m)	R ² _(c)
1)	CT _{max} at beginning of interval + sex	4	137.36		0.39	0.68
2)	CT _{max} at beginning of interval + sex + (CT _{max} at beginning of interval * sex)	5	139.87	2.51	0.38	0.69
3)	CT _{max} at beginning of interval + size	4	151.63	14.27		
4)	sex	3	165.84	28.48		
5)	CT _{max} beginning of interval	3	166.88	29.52		
6)	CT _{max} at beginning of interval + sample	6	178.45	41.09		

CT_{max} at the end of the sample interval, had an AIC value 28.48 lower than Model 4, which contained only sex as a predictor of CT_{max} at the end of the sample interval (Table 2). Additionally, in the most supported

Table 1

Mean ± SD, minimum, and maximum temperature(°C) and cumulative degree minutes at loss of equilibrium for individually tagged adult *Salvelinus fontinalis* subjected to three CT_{max} trials.

Sample	Temperature(°C)			Cumulative Temperature		
	Mean	Min	Max	Mean	Min.	Max
1	29.47 ± 0.14	29.25	29.75	8146.74 ± 100.44	7919.4	8323.71
2	29.63 ± 0.19	29.05	29.95	8244.03 ± 173.61	7716.61	8663.81
3	29.28 ± 0.23	28.74	29.67	8214.07 ± 197.6	7760.14	8523.19

Table 3

Parameter estimates from the most supported linear mixed model in Table 2; (a) random effect = individual *Salvelinus fontinalis*, and (b) fixed effects = CT_{max} at time 1 + sex.

(a)			
Parameter	Variance	Std. Dev.	
Individual	0.22	0.47	
Residual	0.24	0.49	
(b)			
Parameter	Estimate	Std. Error	t value
CT _{max} at Time 1	0.47	0.103	4.58
Sex	0.26	0.17	1.51

model, CT_{max} at the beginning of the sample interval explained almost twice as much variation as any other factor included in the model and the individual random effect accounted for 0.22 of the variance (Table 3). Further, a likelihood ratio test comparing a model with the random effect (individual fish) to a model without the random effect was significant ($p = 0.0036$). Finally, using 'rptR' we found that CT_{max} was repeatable within individual fish ($R \pm S.E.; 0.48 \pm 0.14$) when applied to the most supported model.

4. Discussion

Local adaptation to increased thermal regimes is not uncommon in fishes (e.g. Chen et al., 2017; Stitt et al., 2014; Whitney et al., 2013), yet the mechanisms responsible for adaption are not completely understood. Specifically for *S. fontinalis*, Stitt et al. (2014) documented latitudinal differences where thermal tolerance of southern hatchery strains was higher than that of hatchery strains from the northern extent of their range. Over a smaller spatial scale, Chadwick et al. (2015) found interpopulation variation in physiological response (gill heat shock protein-70, plasma glucose, and cortisol) of wild caught *S. fontinalis* from streams with varying thermal regimes. However, in these and other studies of thermal tolerance, a single measure of thermal tolerance per individual is often reported and the potential for intra-individual variation was outside the scope of the research. In this study, we repeatedly exposed individually marked *S. fontinalis* to CT_{max} trials and determined that CT_{max} is a repeatable measure of thermal tolerance within individual fish.

Chadwick et al. (2015) noted increased intrapopulation variation in physiological response parameters in streams where mean daily water temperatures met or exceeded 21.0 °C. In the current study fish only exceeded mean daily water temperatures of 21.0 °C on days where they were subject to CT_{max} trials; in rearing tanks our fish rarely experienced mean daily temperatures above 19.0 °C. However, we found a wide range in CT_{max} values (Table 1), indicating phenotypic variation even when temperature did not exceed 21.0 °C. Despite substantial variation in CT_{max} between individuals, we found that across short (1 month) and long (1 year) temporal scales, CT_{max} was a highly repeatable trait within individual *S. fontinalis*. Intra-individual repeatability was estimated to be 0.48, which is consistent with Morgan et al. (2018) who found that after an initial acclimation to high temperature between samples one and two, CT_{max} was a highly repeatable trait in warm-water adapted *D. rerio* that were subjected to weekly CT_{max} trials for 1-month. Our results are also within the range of repeatability estimates of other studies that used the same analytical technique and reported repeatable CT_{max} (e.g. Potier et al., 2015; Seaman and Briffa, 2015). Also consistent with Morgan et al. (2018) is our finding that fish size did not influence CT_{max}. However, contrary to their study, the inclusion of sex in our most supported model indicates that high heat acclimation occurred only within female *S. fontinalis* from one sample to the next. In previous studies of fruit flies *Drosophila pseudoobscura* (Coyne et al., 1983) and diamond-back watersnake *Nerodia rhombifer* (Winne and Keck, 2005), females

demonstrated increased thermal tolerance over male conspecifics. Palter et al. (1986) noted increased levels of heat shock congregate protein 70 in female fruit flies *Drosophila melanogaster* that may explain increased thermal tolerance among females, but in the current study the underlying mechanism remains unclear.

Narrow-sense trait heritability within a population is defined as additive genetic variation divided by phenotypic variation (Falconer and Mackay, 1996). When only a single measurement of a given trait is observed during the life of an individual, the resulting estimate of trait heritability can be misleading because a single value ignores the amount of phenotypic variation that could be attributed to within-individual variation over time, which could result from factors such as measurement error, environmental effects, and trait fluctuations due to naturally occurring cycles (Ge et al., 2017). In the current study, all fish were subjected to identical rearing conditions, thereby reducing any influence of environment on phenotype. Additionally, we found that intra-individual CT_{max} values were highly repeatable indicating that any measurement error was likely to have been small, and that as a trait measurement, CT_{max} is reliable over both short and long temporal scales.

There is also value in evidence that thermal tolerance traits are repeatable from a physiological perspective. Demonstrated repeatability provides validation that observed variation is not due to variation in the methods used in the present study, but to actual individual differences in the trait. In addition, consistent differences in thermal tolerance among individuals allows for investigation of the physiological mechanisms that underly these differences. Given the likely effects of climate change on cold water fishes such as Brook Trout, there is substantial interest in determining the physiological factors that limit thermal tolerance. Though controversial, the oxygen and capacity limitation of thermal tolerance (OCCLT) posits that limitation in the ability to deliver oxygen to tissues is critical to determining thermal tolerance of animals (Clark et al., 2013; Pörtner and Knust, 2007). Examining individual differences in gill, heart and circulatory function among individuals with known, repeatable difference in thermal tolerance should provide a useful means of investigating the physiological basis of thermal tolerance in fish.

To examine temporal variation in thermal tolerance within individual fish, we used cumulative degree minutes as a metric for maximum water temperature at LOE as the endpoint for CT_{max}. Traditionally, maximum water temperature at LOE is the endpoint for CT_{max} (Lutterschmidt and Hutchison, 1997). However, Galbreath et al. (2004) note that utilizing time to LOE as a measure of CT_{max} not only accounted for imperfect heating rates within experiment tanks, but was also a more sensitive measure when comparing CT_{max} of *S. fontinalis*, *O. mykiss*, and *S. trutta* across different heating rates. Here we demonstrate that cumulative degree minutes is a robust, more sensitive, measure of CT_{max} that allows for slight deviation in heating rates and differentiates between fish that experience LOE at similar temperatures, but several minutes apart.

It is important to be mindful that CT_{max} values obtained in the laboratory will likely be higher than maximal thermal values observed in the wild where warmer temperatures may interact with other biotic and abiotic factors to limit geographic distributions (Terblanche et al., 2011). For example, in the current study *S. fontinalis* did not reach CT_{max} until water temperatures were greater than 29 °C. However, Childress and Letcher (2017) reported that growth rate of *S. fontinalis* decreased when water temperatures exceeded 19.5 °C in the field (23.3 °C in the laboratory) and Wehrly et al. (2007) found that chronic exposures (60 days) to 21 °C water limited *S. fontinalis* distributions in Michigan and Wisconsin. Also, Terblanche et al. (2007) found that CT_{max} values of tsetse fly *Glossina pallidipes* varied with thermal ramp rate; with slower rates resulting in lower (yet, possibly more ecologically meaningful) CT_{max} values. In the current study, all fish were exposed to the same thermal ramp rate at each sample (2 °C per hour). Therefore, while it may not be expected that fish in the wild would survive to the extreme temperatures observed in this study, it is appropriate to compare CT_{max}

values across individuals and samples within this study.

In the present study we examined CT_{max} of individual fish within a relatively narrow seasonal window (August and September). Previous studies have shown that there are seasonal changes in CT_{max} in ectotherms (e.g. Houghton and Shoup, 2014; Scott, 1987) that are likely driven by elevated summer temperature that lead to an acclimation response. Although examining seasonal changes was outside of the scope of the present study, we are currently examining whether such seasonal changes occur in brook trout. It would also be of interest to examine whether there are seasonal differences that are independent of seasonal temperature, such as 'anticipatory' changes in thermal physiology that might be cued by photoperiod.

5. Conclusions

Most population models for cold-water fishes do not include evolutionary processes (Bassar et al., 2016). Population declines due to warming stream waters may not be as severe as suggested if populations can adapt to warming waters. Evolutionary adaptation requires a phenotypic trait that varies, that the trait is heritable, and that there is selection on that trait. Previous investigations by Whitney et al. (2013) and Stitt et al. (2014) documented variation in adaptive capacity to increasing thermal regimes of *O. nerka* and *S. fontinalis*, indicating phenotypic variation in thermal tolerance. In this study we demonstrated that cumulative degree minutes is a robust measure of CT_{max} that is highly repeatable within individuals over short and long temporal scales. Also, calculating cumulative degree minutes allows for slight deviation in heating rates and differentiates between fish that experience LOE at similar temperatures, but several minutes apart and enables a more accurate comparison between treatments and over time. Finally, we found that inter-individual variation in CT_{max} was most likely due to repeatably measured individual variation and not error introduced by the CT_{max} process used to evaluate thermal tolerance. This finding is an important first step in determining whether CT_{max} is a metric capable of estimating heritability of thermal tolerance in cold-water adapted *S. fontinalis*.

CRediT authorship contribution statement

M.J. O'Donnell: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Supervision, Project administration. **A.M. Regish:** Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing, Supervision, Project administration. **S.D. McCormick:** Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing. **B.H. Letcher:** Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2020.102559>.

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