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Physiology

Linking critical thermal maximum to mortality from thermal stress in a cold-water frog

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Estimates of organismal thermal tolerance are frequently used to assess physiological risk from warming, yet the assumption that these estimates are predictive of mortality has been called into question. We tested this assumption in the cold-water-specialist frog, *Ascaphus montanus*. For seven populations, we used dynamic experimental assays to measure tadpole critical thermal maximum (CTmax) and measured mortality from chronic thermal stress for 3 days at different temperatures. We tested the relationship between previously estimated population CTmax and observed mortality, as well as the strength of CTmax as a predictor of mortality compared to local stream temperatures capturing varying timescales. Populations with higher CTmax experienced significantly less mortality in the warmest temperature treatment (25°C). We also found that population CTmax outperformed stream temperature metrics as the top predictor of observed mortality. These results demonstrate a clear link between CTmax and mortality from thermal stress, contributing evidence that CTmax is a relevant metric for physiological vulnerability assessments.

1. Introduction

Physiological traits provide insight into the initial response of organisms when their environment changes—a key aspect of quantifying vulnerability to climate change [1–5]. Thermal tolerance traits such as critical thermal maximum (CTmax—maximum temperature tolerated before the loss of function) indicate the temperature bounds of performance and mortality [6]. As such, these traits are increasingly used to partly assess organismal risk from warming [7–10]. For example, investigations of ectothermic warming tolerance—the difference between maximum environmental temperature and CTmax [4,7]—and related metrics have uncovered patterns of thermal physiological vulnerability (hereafter, vulnerability) at both interspecific and intraspecific scales [7,11–15]. Yet, the usefulness of these patterns and metrics in informing predicted responses to future warming events depends on the assumption that CTmax accurately predicts thermal tolerance in ecologically relevant conditions, resulting in uncertainty about its adequacy as a metric of thermal tolerance for inferring vulnerability [9,16–18].

Estimates of CTmax are sensitive to the experimental approach used to measure them due to the interacting effects of the magnitude and duration of thermal stress on tolerance [18–20]. CTmax is estimated using a dynamic experimental approach, which exposes organisms to gradually increasing temperatures (i.e. increasing magnitude of thermal stress) until physiological failure is reached (e.g. loss of righting response). The rate of temperature increase is positively related to estimates of CTmax as faster ramping rates shorten the duration of thermal stress experienced and thus allow for higher estimates of CTmax [19,21]. Furthermore, experimental ramping rates are often faster than warming rates experienced in nature, suggesting that experimental CTmax may overestimate organismal thermal tolerance in natural conditions [22,23]. However, recent conceptual frameworks suggest that CTmax measured using dynamic approaches quantifies the same additive accumulation of heat stress as metrics estimated using static/non-dynamic approaches, suggesting estimates from both approaches should be correlated if they follow the same failure rate function [18,24,25]. Thus, empirically connecting dynamic and static metrics of thermal tolerance would help resolve concerns that the sensitivity of CTmax to methodology limits its ecological significance and would bolster CTmax as an adequate proxy for estimating thermal tolerance [17,18,26].

The effectiveness of CTmax to infer vulnerability to climate change may also be limited by the perception that there is insufficient variation within and among taxa. Across broad geographical and taxonomic scales, several studies have found CTmax to be relatively invariant compared to metrics of other physiological traits [3,27–29]. Low levels of variation may be the outcome of physiological constraints [29,30] and/or thermoregulatory behaviours [27,31,32] and suggest limited ability for populations to evolve higher CTmax as a response to warming temperatures [22,28]. However, CTmax has been observed to vary among populations [14,33,34] which would suggest there is the potential for CTmax to evolve in response local temperature variation [35–37]. Directly linking variation in CTmax across populations to measures of fitness like mortality would provide necessary insight into among-population variation in vulnerability to future warming.

Here, we tested the assumption that CTmax is predictive of mortality from thermal stress among populations of a cold-water frog, *Ascaphus montanus* (Rocky Mountain tailed frog). *A. montanus* inhabits cold streams in the northern U.S. Rocky Mountains and extreme southern Rocky Mountains of Canada. This species is already facing population declines that are partly due warming stream temperatures [38], and low CTmax relative to other amphibians [39–41] suggests continued risk from warming. Previous research found population variation in CTmax [41], providing an ideal opportunity to investigate if natural population variation in CTmax estimated via the dynamic method and mortality at different ecologically relevant constant temperatures (i.e. static approach) in tadpoles are related. We expected these two approaches to be related, such that populations with higher CTmax experienced less mortality. We also tested the strength of CTmax as a predictor of mortality from thermal stress by comparing its performance against local thermal conditions, which are commonly related to physiological traits [15,42–44], including in this system [41].

2. Methods

We sampled tadpoles, a pre-reproductive life-history stage, to ensure that survival was the sole contributor to fitness during the experiments. For both dynamic and static experiments (detailed below), we used handheld fish nets to collect tadpoles from streams. Tadpoles were held in stream water in insulated containers during sampling with frequent water changes to maintain temperatures. We transported tadpoles to laboratory facilities (Fort Missoula, University of Montana) for experiments via the protocol outlined in [45].

(a) Dynamic CTmax experiments

See [41] for full details of CTmax experiments. Briefly, we sampled 10–24 tadpoles (developmental stages 26.5 to 44 [46]; electronic supplementary material, table S1) from each of seven populations of *A. montanus* in Montana. Due to the distance between these streams (7–224 km, electronic supplementary material, figure S1) and limited vagility of the species [47], we refer to these sampled sites ($n = 7$) as populations. We held tadpoles for 3 days at 8°C, a commonly experienced temperature among populations [41], without food to reduce the effects of natal stream temperature and feeding. For CTmax experiments, tadpoles were moved to an experimental tank and held in individual mesh containers. They were given 2 min to habituate before temperature ramping at 0.3°C min^{-1} began. CTmax was defined as the temperature at which tadpoles lost the ability to respond to tactile stimuli but fully recovered when placed in cooler temperatures. We used population median CTmax for analyses, hereafter referred to as CTmax_p, representing previously characterized CTmax.

(b) Static thermal stress experiments

Ascaphus montanus tadpoles overwinter in their natal streams for at least 1 year [48]. To collect tadpoles for static thermal stress experiments, we returned 2 years later (3 July–28 July 2019) to substantially decrease the probability of sampling the same cohort. Since we permanently removed tadpoles from the population for CTmax_p experiments, we did not resample the same individual. We collected approximately 60 tadpoles from each population (total $n = 420$).

Tadpoles from each population were evenly and randomly assigned to one of five holding temperature treatments: 5°C, 10°C, 15°C, 20°C, 25°C (electronic supplementary material, table S1). Temperatures were decided based on thermal regime data [41]: 5°C and 10°C are commonly experienced temperatures; 15°C and 20°C treatments are near maximum stream temperatures; 25°C is greater than current maximum stream temperatures but is ecologically relevant given current rates of warming [49]. Tadpoles were held in the temperature treatments for 3 days and fed *ad libitum* by placing rocks collected from their stream in the holding tanks, from which they graze algae. We maintained oxygenation using flowing water and bubblers. After 3 days, we counted surviving tadpoles.

(c) Local stream temperatures

We characterized stream temperatures experienced by the tadpole at three timescales: (i) immediate, (ii) annual and (iii) long-term/multi-generational. To characterize the immediate and annual stream temperature metrics, we used quality-controlled [50] logged temperature data at 4 h intervals from each stream (detailed in [41]). Using Water Year 2018 data, we calculated ‘immediate thermal experience’ by averaging maximum daily temperatures of the 3 days preceding sampling for the static mortality experiments. We calculated ‘annual thermal experience’ by measuring the maximum temperature experienced in a year for each population. Lastly, we used modelled temperature data of 40-year averages of the mean temperature during the warmest month (August) for each stream [49,51] to quantify ‘long-term thermal experience’. These metrics were uncorrelated with each other (electronic supplementary material, table S2).

(d) Data analysis

Analyses were performed in R v. 4.1.2 (R Core Team 2021). Statistical significance was evaluated using $\alpha = 0.05$. To test

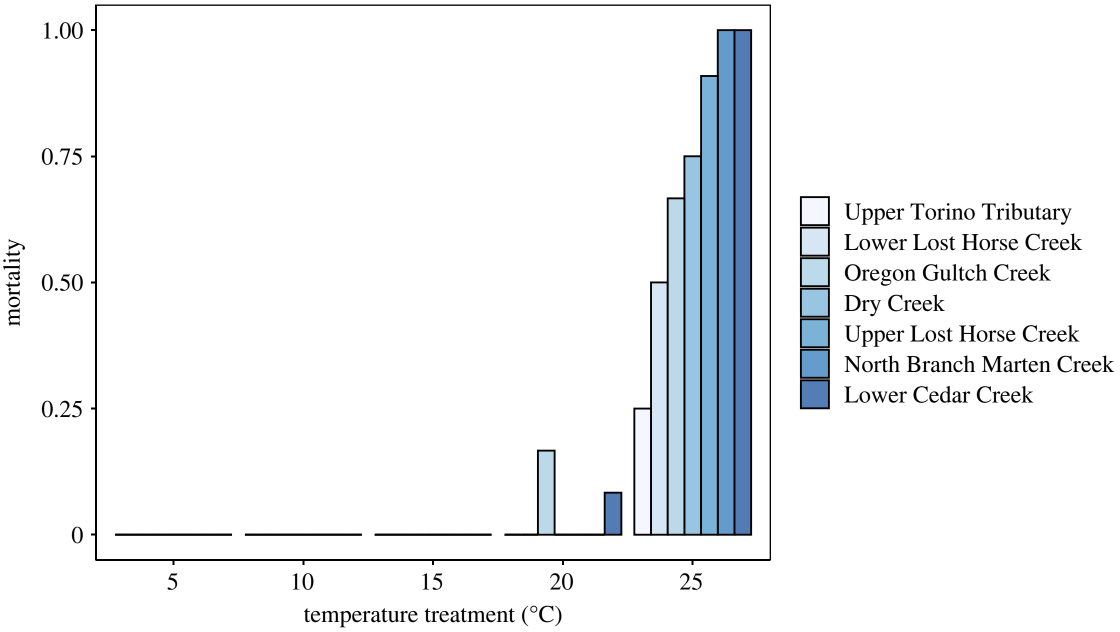

Figure 1. The proportion of tadpoles within populations that experienced mortality after being held for 3 days at one of five temperatures. Bars and colour values represent one population and are ordered by increasing mortality in the 25°C treatments.

differences in mortality among temperature treatments, we used a Fisher's exact test for count data. Due to limited variation in mortality in other temperature treatments, we only investigated the relationship between the probability of mortality and CTmax$_p$ in the 25°C treatments. For this and subsequent models, we used a logistic regression with a two-column matrix of number of deaths and number survived as the response variable. To assess the strength of CTmax$_p$ as a predictor of mortality, we compared the performance of this model against 10 other models: (1) null; (2–4) each thermal experience metric modelling separately as independent predictors, (5–7) additive predictors with CTmax$_p$ and (8–10) interactive predictors with CTmax$_p$. All models used a binomial distribution. We compared models using Akaike's information criterion [52] adjusted for sample size (AICc), AICc weights and evidence ratios with the top model.

3. Results

The probability of tadpole mortality was different among temperature treatments (Fisher's exact test, $p < 0.001$). There was no mortality in the 5°C, 10°C or 15°C temperature treatments. In the 20°C treatments, two populations experienced mortality (Lower Cedar Creek: one individual; Oregon Gultch Creek: two individuals). All populations experienced some degree of mortality in the 25°C treatment (figure 1), which was not related to the order of population sampling (electronic supplementary material, table S3).

CTmax$_p$ ranged from 29.4°C to 30.8°C (s.d. = 0.55). Results from the logistic regression with CTmax$_p$ as the sole predictor of mortality in 25°C demonstrated that a 1°C increase in CTmax$_p$ is associated with a multiplicative decrease in the predicted odds of mortality by 0.08 (odds ratio = 0.08, $p < 0.0005$; figure 2; see electronic supplementary material, table S4 for regression results and detailed interpretation). CTmax$_p$ was the best predictor of mortality from thermal stress when compared to the stream thermal environment at any temporal scale. The top model with CTmax$_p$ as the sole predictor was 8.5 times better supported than the next model (CTmax$_p$ and immediate thermal experience) and almost 25 times better supported than the top model without CTmax$_p$ (table 1).

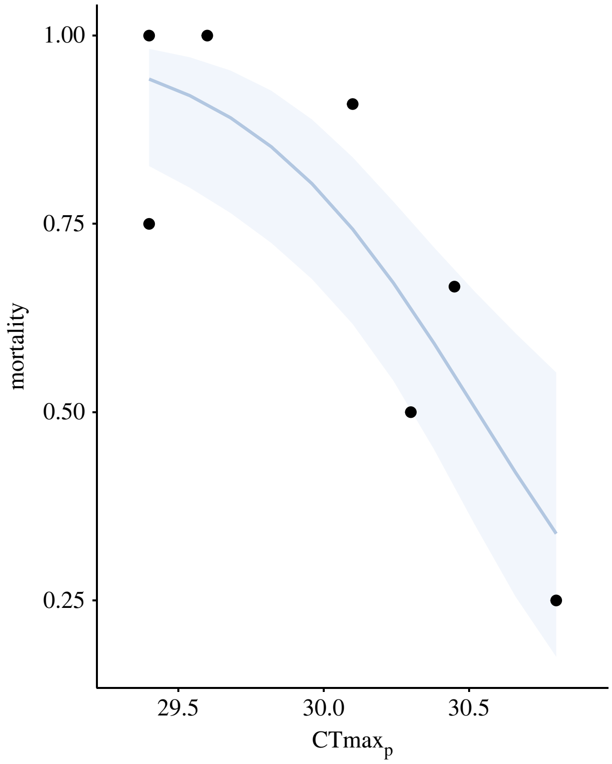

Figure 2. Mortality of *Ascaphus montanus* tadpoles when held at 25°C was related to past estimates of population CTmax (CTmax$_p$). The trend line shows the predicted probabilities and 95% confidence intervals as estimated by the logistic regression model with CTmax$_p$ as the sole predictor of mortality ($p < 0.0005$).

4. Discussion

Thermal tolerance traits are important for assessing vulnerability to climate change as they contribute to species'

Table 1. Model comparison results identifying the top predictors of variation in mortality at 25°C for models with weights > 0. AICc weights demonstrate the probability that a model is the best model; evidence ratios are in reference to the top model. See electronic supplementary material, table S5 for full results.

model predictors	AICc	ΔAICc	AICc weight	evidence ratio
$CTmax_p$	32.62	0	0.76	1
$CTmax_p$ + immediate thermal experience	36.90	4.28	0.09	8.5
$CTmax_p$ + long-term thermal experience	37.70	5.08	0.06	12.67
$CTmax_p$ + annual thermal experience	38.74	6.12	0.03	21.35
immediate thermal experience	39.04	6.42	0.03	24.73
annual thermal experience	39.39	6.77	0.02	29.55

capacity to cope with environmental change *in situ* and through movement to preferable conditions [53]. The strength of vulnerability assessments thus relies on the accuracy of experimental measurements of thermal tolerance, garnering much debate over dynamic versus static approaches [16,18,25,54,55]. Here, we show a clear relationship between CTmax and mortality from thermal stress among populations of *Ascaphus montanus* and demonstrate its strength as a predictor of mortality over environmental metrics. These results support the assumption that CTmax estimated by dynamic approaches is related to mortality from chronic thermal stress and is a relevant metric for vulnerability assessments.

We found that populations with greater CTmax experienced significantly less mortality when held in a stressful temperature for 3 days. This result aligns with previous studies showing a relationship between dynamically measured CTmax and thermal tolerance metrics from static approaches (e.g. subcritical limits [26] and lethal temperature [20,24,25,56]) and highlights that the methodological sensitivity of CTmax does not preclude the trait from assessing thermal tolerance. This result also supports the conceptual framework proposing that dynamic and static experimental approaches ultimately measure similar tolerance outcomes [18]. Under this framework, exposure to thermal stress causes a shift from the 'permissive temperature range', where growth and reproduction occur, to the 'stressful temperature range' [18,57], where cellular and molecular damages accumulate until heat failure [16,58–60]. The transition point between permissive and stressful temperatures is rarely estimated, yet here, the observed mortality in the 20°C and 25°C treatments suggests that it falls between 15 and 25°C and, importantly, varies among populations. These results also underscore that populations within a species can vary in their sensitivity to warming temperatures, demonstrating potential for heterogeneous responses to climate change across the landscape.

Reduced variation in CTmax relative to other physiological traits has suggested that CTmax may be evolutionarily constrained [22,28,29]. Here, we showed that relatively small differences in CTmax can have a large impact on mortality from thermal stress. We found that the predicted probability of mortality in 25°C after 3 days for a population with a $CTmax_p$ of 29.5°C was 93%, but a 1°C increase in $CTmax_p$ (30.5°C) reduced the predicted probability of mortality to 52%—a 44% reduction in predicted probability of mortality. $CTmax_p$ also outperformed any metric of local thermal environment as the strongest predictor of mortality despite having been collected 2 years before the mortality experiments. The degree to which variation in CTmax reflects underlying genetic variation and acclimation to an individual's thermal history will be critical to understanding its potential as an evolved response to warming temperatures.

Climate change is exposing species to both low magnitude–long duration thermal stress and high magnitude–short duration thermal stress through chronic warming and extreme temperature fluctuations [61], respectively. The connection we found between mortality from a static experimental approach (low magnitude–long duration thermal stress) and CTmax from a dynamic approach (high magnitude–short duration thermal stress) suggests that either metric can be used to understand how *A. montanus* populations may tolerate these different patterns of warming—a key but often missing characteristic of traits used in vulnerability assessments [62]. Importantly, our results provide critical evidence that CTmax can be used to understand the consequences of subcritical thermal stress, which will be experienced before CTmax is reached in nature [49].

Ethics. All experimental protocols were approved by Colorado State University IACUC (16-6667AA) and University of Montana IACUC (024-17WLDBS-042117). Collection was permitted by Montana Fish, Wildlife and Parks (permit 2017-060-W).

Data accessibility. Datasets for this study are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.sbcc2frbx [63]. R code for the analyses performed in this study are available from the Zenodo Repository: https://doi.org/10.5281/zenodo.7822020 [64].

The data are provided in the electronic supplementary material [65].

Authors' contributions. A.S.C.: conceptualization, data curation, formal analysis, investigation, writing—original draft and writing—review and editing; C.K.G.: funding acquisition and writing—review and editing; W.C.F.: conceptualization, funding acquisition, project administration, supervision and writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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