Temperature-dependent performance scales with maximum heat tolerance across ectotherms

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Significance

Climate warming is increasingly reshaping the thermal environments that govern species persistence worldwide. Predicting vulnerability requires integrating multiple aspects of thermal biology, yet relationships among widely used thermal traits remain poorly understood. By synthesizing data from more than 100 ectotherm species, we quantify links between acute heat tolerance and traits describing sustained biological function across temperatures. We show that performance at relatively benign temperatures and performance at thermal extremes are coupled, but this coupling is strongly process and timescale dependent, with close correspondence for short term locomotion but weaker coupling for metabolic processes. Our results link the processes that maintain performance across temperatures with those that cause failure, fundamentally advancing our projections of species performance in a warming world.

Abstract

Projecting species' responses to changing temperatures remains a major challenge in ecology. Central to this effort is harnessing our understanding of species' thermal physiological traits, which underlie ectotherm fitness. These traits are typically characterized by describing performance across temperatures (thermal performance curve, TPC), and/or tolerance limits, which capture endpoints of biological failure. Despite their importance, we still lack an understanding of the functional relationship between these traits, limiting our ability to integrate them into comprehensive vulnerability assessments. Using a synthesized dataset of >100 ectotherms, we tested how heat tolerance (CTmax) relates to key TPC traits: thermal optima, thermal maxima, and the supra-optimal range of temperatures where performance is positive. Across taxa, TPC traits were positively related to CTmax, demonstrating a link between heat tolerance and temperature-dependent performance at sub-critical temperatures. While acute locomotor performance scaled proportionally with CTmax, metabolic processes and sustained locomotion scaled sub-proportionally, suggesting decoupling of CTmax and performance among high-CTmax species. This suggests that using CTmax as a comparative metric may overestimate thermal safety margins for metabolic processes critical to growth. Our results indicate that while CTmax and TPCs reflect shared underlying constraints—particularly in acute neuro-muscular traits—their relationship is dependent on timescale and the TPC response trait. Our findings connect our understanding of the processes that maintain performance over thermal gradients with those that cause performance to fail, improving our ability to project species persistence in a warming world.

Introduction

For the vast majority of Earth's species—the ectotherms—temperature fundamentally underlies biochemical, cellular, and physiological processes that shape fitness and population growth (1–4). As such, thermally sensitive traits have long been central to understanding the ecology and evolution of ectotherms (5, 6) and are increasingly used to predict species' vulnerability to warming by relating trait variation to current and projected habitat temperature (e.g.,(7–15)). Two commonly used experimental approaches to quantify biological responses to temperature include estimating thermal performance curves (TPCs) and thermal tolerance limits (Figure 1). While TPCs describe performance across the range of sublethal temperatures (7–9), thermal tolerance limits represent the critical thresholds of biological failure (10). Despite their widespread use, it remains unclear how TPCs and thermal tolerance limits are related and whether the processes governing sublethal performance are fundamentally shared with, or distinct from, those underlying failure at thermal extremes (11). Developing an integrative understanding of biological responses to temperature is critical to characterizing the fundamental ecology of ectotherms and for informing predictions of species performance in the context of global change.

Temperature-dependent performance likely arises from the integration of several distinct mechanistic processes, including kinetic effects on biochemical reaction rates (12, 13), the balance between cellular and molecular damage and repair (7, 14–16), and constraints imposed by oxygen supply and demand (17, 18). Although a unifying framework has yet to be established, the near universality of TPC shape across ectotherms suggests common underlying constraints (12, 13) that may extend to heat tolerance limits (12, 13, 19). In this context, thermal tolerance limits may reflect the functional endpoint where the same processes constraining sublethal performance propagate to whole-organismal failure. Within the damage–repair framework, for instance, performance begins to decline as damage accumulation begins to outpace repair capacity and thermal tolerance limits would represent the point at which cumulative damage effects propagate to organismal failure (13, 19). Similarly, under the oxygen supply/demand framework, performance decreases when metabolic demands outpace the capacity to deliver oxygen (17, 18, 20); thermal tolerance limits may capture the endpoint when transport capacity is fully exhausted, triggering a transition to anaerobic respiration and eventual systemic collapse (21).

Alternatively, thermal tolerance limits may reflect functional endpoints that arise from different processes than sublethal performance. TPCs primarily encompass temperatures where organismal function is maintained, reflecting the balance of metabolism, resource allocation, and damage repair. In contrast, thermal tolerance limits may emerge from the abrupt breakdown of critical physiological systems. At high temperatures, organisms may experience loss of neural function (22), disruption of cardiac electrical excitability (23), destabilization of membrane and ion channel regulation (24), or oxygen limitation (12, 22), with CTmax representing the first system to fail. As a result, thermal tolerance limits may be better characterized by functional

endpoints rather than as outcomes of continuous, rate-dependent processes like those captured in TPCs, and may operate on a different timescale than the steady-state dynamics shaping performance (i.e., “stressful range” versus “permissive range”) (13, 19). These differences are potentially reinforced by experimental design: thermal tolerance limits often capture responses to rapid, acute thermal stress, whereas TPCs can be quantified across a range of exposure durations. Longer exposure times can allow plasticity and bioenergetic constraints to influence performance (25), potentially integrating processes that are not captured by the rapid temperature exposure typical of tolerance assays. Consistent with this decoupling, the molecular processes that prepare or maintain function have been found to differ from those that determine failure under acute stress (26). Together, these differences suggest that temperature-dependent performance and thermal tolerance limits may represent partially or wholly independent traits, governed by distinct cellular, molecular, and genetic mechanisms (27, 28), and capable of evolving independently.

Despite their foundational role in thermal biology, the degree to which thermal performance curves and thermal tolerance limits reflect shared underlying constraints across species remains unclear, motivating explicit tests of their covariation among taxa. Here, we synthesize a novel dataset of TPCs and thermal tolerance limits across ectotherms to test the extent to which these thermal traits are related across species. Given the increasing use of thermal traits in assessing ectotherm risk from warming (11), we focus our analyses on TPC traits and thermal tolerance limits that are relevant at high temperatures: the optimal temperature for performance (Topt), the maximum temperature of performance (TPCmax), the supra-optimal range (SOR; the difference between TPCmax and Topt), and critical thermal maximum (CTmax) (Figure 1). If the processes shaping TPCs also govern CTmax, then TPC traits and CTmax should co-vary across species. However, the strength of these relationships may depend on the performance response type used to quantify TPCs, the biological scale at which performance is measured, and the duration of thermal exposure captured (detailed in Table 1). Our null expectation is that TPCs are unrelated to CTmax because they reflect distinct processes. Positive relationships between TPCs and CTmax would indicate that these measures share underlying mechanistic processes, and proportional (1:1) positive relationships would suggest a high degree of conserved overlap between these underlying processes. We therefore evaluate support for relationships between TPCs and CTmax based on their proximity to a 1:1 relationship. By characterizing the shape of the relationship between performance at thermal extremes and permissive temperatures, we advance our understanding of how these thermal traits are related across species.

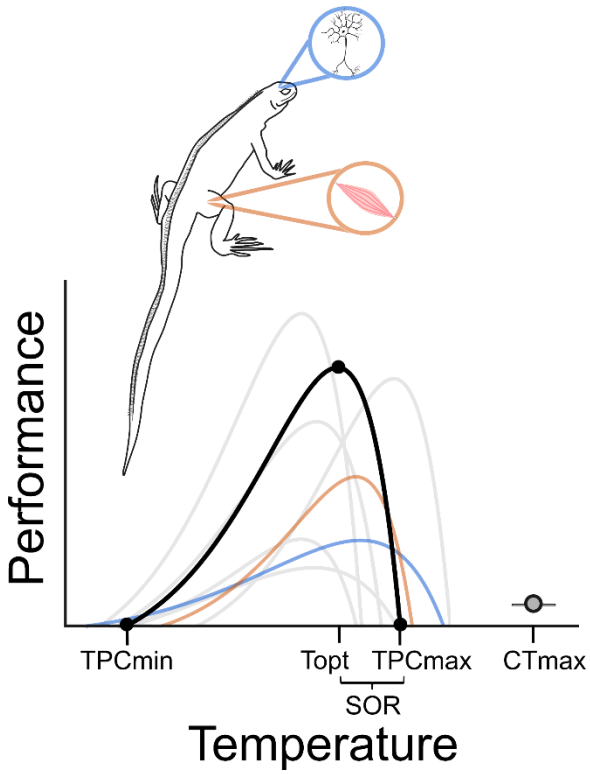
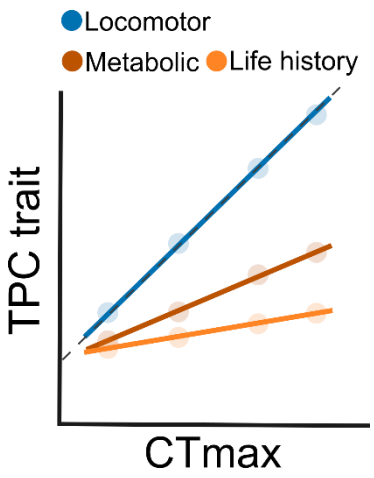
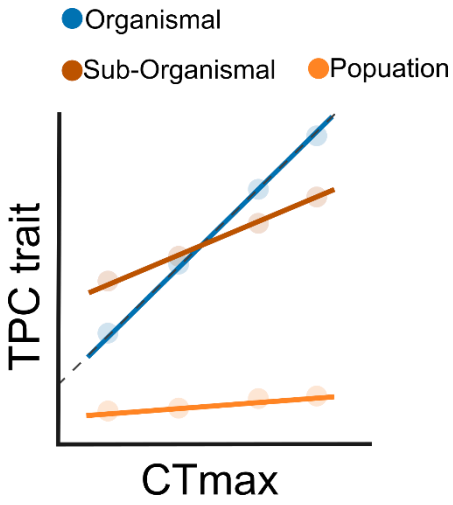
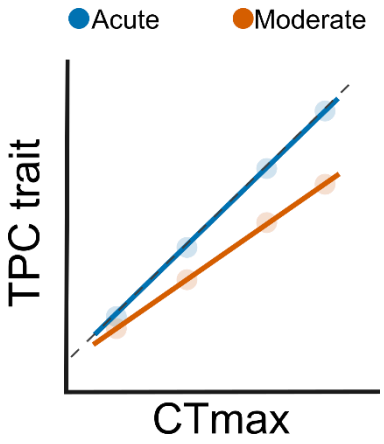


Figure 1. Conceptual illustration of a thermal performance curve (TPC) in black depicting key TPC traits, including the minimum and maximum performance temperature (TPCmin, TPCmax, respectively), the optimal temperature (Topt), and the supra-optimal range (SOR: the range between TPCmax and Topt). The critical thermal maximum (CTmax) is indicated for reference and typically occurs at temperatures exceeding those associated with optimal or maximal performance**.** The aggregation of underlying temperature-dependent processes results in the emerging TPC for a single integrated measure of performance (7). For example, the TPC for running speed (shown in black) reflects the effects of contributing physiological processes (grey), including muscular (orange) and neuronal (blue) performance. TPCs were drawn using hypothetical parameters and the Thomas 2012 model (29) of thermal performance.

Table 1. Descriptions and predicted outcomes for three non-mutually exclusive hypotheses tested here explaining variation in the relationships between thermal performance curve (TPC) traits (Topt and TPCmax) and heat tolerance (CTmax). Here and in the results, we use color to indicate whether the relationship is proportional (blue) or sub-proportional (shades of orange). We interpret proportional scaling as the strongest support for the corresponding response being shaped by similar temperature-dependent processes as CTmax.

	Description & Prediction	Prediction Figure
Response Type Hypothesis	TPC-CTmax relationships depend on the degree to which measured TPC responses integrate physiological processes that overlap with those determining CTmax. Because CTmax in animals is often measured as a neuromuscular response (e.g., loss of righting response), we expect that locomotor TPC traits will reflect many of the same integrated physiological processes as CTmax and thus scale near-proportionally with CTmax, while other traits, such as metabolism or life history traits, will demonstrate sub-proportional relationships with CTmax.	
Biological Scale Hypothesis	TPC-CTmax relationships vary across levels of biological organization, reflecting aggregation of processes across biological scales. Because CTmax is measured at the organismal level, we expect proportional scaling between organismal TPCs and CTmax. However, we expect that TPCs measured at other biological scales will show sub-proportional relationships with CTmax, reflecting aggregation that is constrained by biological scale. We expect mean TPC trait values to differ across levels of biological organization, with the highest values for sub-organismal TPCs and the lowest values for population TPCs (30). This is demonstrated as different intercepts in the prediction panel.	

Exposure Duration Hypothesis	We hypothesize that the experimental timescale of TPC measurement influences its relationship with CTmax. Because CTmax represents an acute thermal endpoint, we predict that acutely measured TPCs will exhibit closer-to-proportional relationships, whereas TPCs measured over longer (moderate) timescales will scale sub-proportionally with CTmax.	

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Materials and Methods

To test our hypotheses about the relationships between thermal performance curves (TPC) and maximum temperature tolerance (CTmax), we assembled a global dataset of TPCs and CTmax from terrestrial, marine, and freshwater ectotherms. We compiled thermal performance data used to fit TPCs, from which we derived the temperature of peak performance (thermal optimum, Topt), the maximum temperature of positive performance (TPCmax), and the supra-optimal range (SOR; the range of temperatures between Topt and TPCmax). We then used hierarchical mixed-effects models to quantify the strength and proportionality of relationships between TPC traits and CTmax, and to test for variation in these relationships across the biological response category captured in the TPC, the biological scale of the TPC, and TPC assay duration.

Data compilation and trait classification

We compiled data to fit TPCs from published studies and existing datasets (47, 48), retaining studies that had performance values across at least five temperatures. We standardized performance values to common units where necessary (e.g., durations were converted to rates) to ensure comparability across studies. We compiled CTmax values from published thermal-limit databases (49–52) and targeted searches for species with TPC data (*SI Appendix, Part A.1.i*). We included all endpoints of maximum thermal limits (e.g., onset of muscle spasms, loss of righting response, 50% mortality) and refer to them collectively as CTmax.

To account for the effects of acclimation temperature on CTmax (53–55), we compiled CTmax acclimation response ratios (ARR), which quantify the change in CTmax per degree increase in acclimation temperature (*SI Appendix*, Equation S1), from published studies (52, 56–59). We standardized CTmax values to a common acclimation temperature (*SI Appendix, Equation S2*) using taxon-summarised ARRs. Because standardized and unstandardized CTmax estimates were highly correlated across taxonomic levels (*Table S1*), we used phylum-level mean ARRs for all estimates. We present our results using CTmax values ARR-standardized to 15°C, the mean global surface temperature (60), although our conclusions were robust to the choice of standardization temperature (*Figure S1*).

To support our hypothesis testing, we classified performance values by response type (metabolic, locomotion, life history; *SI Appendix, List 2*) and level of biological organization (sub-organismal, organismal, population). We classified thermal performance assays by exposure duration to capture the potential for plasticity and bioenergetic constraints to influence performance during the experiment. Assays lasting ≥ 30 minutes—long enough for physiological responses such as the upregulation of heat-shock proteins (61)—were classified as “moderate” in duration, while assays < 30 minutes were classified as “acute”.

TPC fitting and trait estimation

We fit thermal performance curves (TPCs) using non-linear least squares (nlsLM, *minpack.lm* in R) and the Norberg model (62) as modified by (29) (referred to as the Norberg-Thomas model) (Equation 1). We modeled temperature-dependent performance as:

$$f(T) = ae^{bT}\left[1 - \left(\frac{T - T_{ref}}{\frac{c}{2}}\right)^2\right] \quad (1)$$

where performance, f, varies as a function of temperature, T (°C), a acts as a scaling parameter, b determines the slope of the exponential rise and the direction of the curve skew, c captures the breadth of positive performance, and T_{ref} is the temperature at which the decay function peaks. We chose the Norberg-Thomas model because it accommodates both left- and right-skewed curves, requires only four parameters, and is suitable for datasets with ≥ 5 temperatures.

To quantify uncertainty in TPC-derived traits, we determined confidence intervals around our fitted TPCs using non-parametric bootstrapping of residuals using the Boot function in the *boot* package in R (63) with 10,000 iterations per TPC, and calculated TPC traits (Topt, TPCmax, SOR) from each bootstrapped fit. We calculated Topt via numerical optimization, TPCmax directly from model parameters (TPCmax = $T_{ref} + c/2$; Equation 1), and SOR as the difference between TPCmax and Topt. We applied quality control filters to exclude poor fits, including those with high uncertainty in trait estimates or parameter estimates near model bounds (*SI Appendix, Part A.1.ii*). We assessed data quality independently for each trait to ensure that low-confidence estimates for one trait did not lead to the unnecessary exclusion of robust data for another trait.

Statistical Analyses

We conducted all analyses in R v4.5.1 (64). We tested for relationships between TPC traits (TPCmax, Topt, SOR) and CTmax using multilevel meta-regressions fitted using restricted maximum likelihood using the *rma.mv* function in the *metafor* package (65). This approach incorporates known sampling variances, accommodates unbalanced data, and allows flexible random-effects structures. For each model, we used the mean of 1,000 random bootstrap-derived TPC trait estimate as the response variable and their variance as weights in our multilevel meta-regressions. To account for phylogenetic non-independence among TPCs, we included taxonomic classification as a random intercept in all models. We selected Class as the most appropriate taxonomic level intercept following model comparison via Akaike Information Criterion (AIC, (66), *Table S2*).

As an initial step, we tested for overall relationships between each TPC trait and CTmax using models with CTmax as a fixed effect. For Topt and TPCmax models, we standardized (z-scored) CTmax by subtracting the dataset mean and dividing by the standard deviation (sd) (Topt mean =

37.42°C, sd = 9.17; TPCmax mean = 38.12°C, sd = 9.69), allowing effect size comparison across models. For interpretability, figures are presented in the main text on the original temperature scale whereas analyses were conducted on standardized scales; corresponding figures on the standardized scale are provided in the *SI Appendix*. These baseline models established the direction, strength, and scaling of TPC–CTmax relationships in the absence of additional co-variates.

We then tested hypotheses predicting variation in TPC–CTmax relationships across response type, biological scale, and exposure duration. For each hypothesis, we fitted meta-regression models with the hypothesized predictor as a main effect and in interaction with CTmax. Because predictor categories were not always mutually exclusive and some predictor combinations were unevenly represented (*Figure S3*), we first evaluated each hypothesis using separate models to avoid overparameterization. To identify which predictors best explained variation in TPC traits, we constructed a global model informed by the results from hypothesis-specific analyses and compared it with all possible subsets using AIC.

We assessed model support using AIC, with comparisons to intercept-only models including Class as a random intercept, and *pseudo*-R^2, calculated as the reduction in among-Class variance relative to the intercept-only model. We evaluated support for predictors using moderator tests (Omnibus test of moderators, QM), Wald-type tests, and 95% confidence intervals. To evaluate support for our hypotheses (Table 1) we interpreted the magnitude and direction of predictor-CTmax relationships (i.e., the interaction term of the model) relative to the expectation that TPC traits scale proportionally with CTmax.

Results

TPC traits were positively related to CTmax across ectotherms.

Across ectotherms, all TPC traits we estimated were consistently positively related to CTmax (Figure 2). Both Topt and TPCmax increased with CTmax but scaled sub-proportionally relative to the proportional expectation, with positive slopes, confidence intervals (CIs) above zero, and a clear reduction in AIC relative to the null model (Table S3). The slope of the relationship between TPCmax and CTmax was greater than that of Topt and CTmax (Topt: slope = 6.34, 95% CI [6.27, 6.41], $p < 0.001$, (proportional slope expectation = 9.17)); TPCmax: slope = 7.87, 95% CI [7.77, 7.97], $p < 0.001$, (proportional slope expectation = 9.69)), indicating stronger coupling between thermal performance maxima and CTmax. Species with higher CTmax also exhibited broader supra-optimal ranges (SOR) (Figure 2); this relationship was similarly sub-proportional (slope = 0.42 [0.40, 0.44], $p < 0.001$, Table S4). These results indicate that while TPC traits increase with CTmax, heat tolerant (i.e., high CTmax) species exhibit performance declines at increasingly cooler temperatures relative to their CTmax.

The magnitude of variation in TPC traits and CTmax was remarkedly consistent (Figure 2B). Bootstrapped mean standard deviation and 95% CIs revealed nearly identical levels of variation for TPCmax (sd = 9.41 [8.54, 10.3]) and CTmax (sd = 9.69 [8.55, 10.9]), suggesting these traits are similarly evolutionarily constrained. While Topt demonstrated slightly lower variation (sd = 8.14 [7.30, 8.94]), the values remained similar in magnitude. Variation was lowest for SOR (sd = 4.76 [4.01, 5.54]), indicating that the maintenance of performance after Topt is the most evolutionarily constrained thermal trait measured here.

Response type hypothesis – Consistent with our expectations, TPC-CTmax relationships differed based on the biological response measured (Figure 3A). Our multi-level regression models for animal ectotherms ($N_{Topt} = 171$; $N_{TPCmax} = 111$)—which excluded plants which lacked locomotor TPCs (N = 26) and life history TPCs (N = 5)—revealed that locomotion TPCs exhibited stronger relationships with CTmax than metabolic TPCs (Topt: locomotion slope = 8.91 [8.61, 9.22], metabolic slope = 5.43 [5.31, 5.55], $p_{interaction} < 0.001$; TPCmax: locomotion slope = 9.16 [8.82, 9.49], metabolic slope = 5.77 [5.64, 5.90], $p_{interaction} < 0.001$; Table S5). Notably, the locomotor Topt slope CIs overlapped the proportional expectation (9.17), indicating that locomotor thermal optima increased proportionally with CTmax. While the locomotion TPCmax relationship was slightly less than the proportional expectation (9.69), it was the closest to proportionality for TPCmax among all hypotheses tested here.

Biological scale hypothesis – We did not find support for our hypothesis that TPC-CTmax relationships depend on the level of biological organization at which TPCs are measured. Multi-level regression models to fitted Topt data ($N_{Topt} = 202$) exhibited a non-significant interaction term (Table S6), indicating that Topt from sub-organismal and organismal TPCs demonstrated

the same sub-proportional relationship with CTmax (slope coefficient = 6.26 [6.19, 6.33], $p < 0.001$) (Figure 3B). Note that we excluded population-levels TPCs from this analysis because our final dataset only contained two population-level TPCs. Topt values were generally lower for sub-organismal-level TPCs than for organismal-level TPCs (sub-organismal intercept in reference to organismal intercept -2.69 [-2.93, -2.44], $p < 0.001$). Sample size imbalances ($N_{population} = 2$, $N_{organismal} = 136$, $N_{suborganismal} = 21$) and an over-representation of plant species in the sub-organismal category (19 of 21) precluded robust tests for biological scale effects on the TPCmax–CTmax relationship.

Exposure duration hypothesis – We found mixed support for the hypothesis that TPC-CTmax relationships vary with TPC assay duration (Figure 3C). Multi-level regression models ($N_{Topt} = 204$, $N_{TPCmax} = 159$) indicated sub-proportional scaling with CTmax across both acute and moderate duration TPCs. Specifically, Topt slopes were 6.84 [6.69, 6.98] for acute and 7.13 [6.83, 7.43] for moderate exposures ($p_{interaction} = 0.002$). For TPCmax, acute and moderate slopes were statistically indistinguishable (acute slope = 6.84 [6.67, 7.01], moderate slope = 7.00 [6.63 7.37], $p_{interaction} = 0.12$; Table S7). Contrary to our prediction that acute-exposure slopes would be closer to proportional expectations, slopes were closer to proportional when TPCs reflected longer temperature exposure assays ('moderate' duration). However, although the interaction term for the Topt-CTmax model was statistically significant, the substantial overlap in 95% confidence intervals suggest that the functional differences between these categories are minimal. Overall, these relationships indicate that TPC-CTmax relationships are consistently sub-proportional and largely similar across assay durations tested here.

Topt for acute locomotion processes increased proportionally with CTmax across animal ectotherms.

We used a model-comparison approach to identify the predictors that best explained variation between Topt and CTmax. Because our hypothesis-specific test indicated that biological scale had little impact on TPC–CTmax relationships and introduced data imbalances, we restricted this analysis to organismal-level TPCs (N = 162 TPCs, 85 species). Additionally, because plants lacked locomotion TPCs, we focused on animal ectotherms to allow direct comparison of physiological processes. Data limitations precluded a model comparison approach for TPCmax due to sample size imbalances that prevented reliable estimation of interaction effects (Figure S3).

Our top-ranked model included response type and exposure duration as independent and interacting variables with CTmax. Results indicated that Topt for acute locomotion scaled proportionally with CTmax across species (slope = 8.97 [8.76, 9.19], $p < 0.001$; Table S9), whereas Topt for moderate-duration locomotion traits increased sub-proportionally with CTmax (slope = 6.93 [6.75, 7.11], $p < 0.001$). In contrast, Topt for metabolic traits scaled sub-proportionally with CTmax regardless of assay duration ($slope_{acute} = 5.72$ [5.45, 5.99],

313 $slope_{moderate}$ = 3.68 [3.55, 3.80], $p < 0.001$). Consistent with our initial predictions, slopes for
314 moderate-duration assays were lower than those for acute assays in this integrated model (Figure
315 3D). The apparent reversal of duration effects compared to the independent hypothesis model is
316 likely attributable to the exclusion of plants or variation interacting with response types. Indeed,
317 when the single-hypothesis model for exposure duration described above was restricted to
318 animals, Topt from moderate-duration assays increased with CTmax at a lower rate than Topt
319 from acute TPC assays (Table S10).

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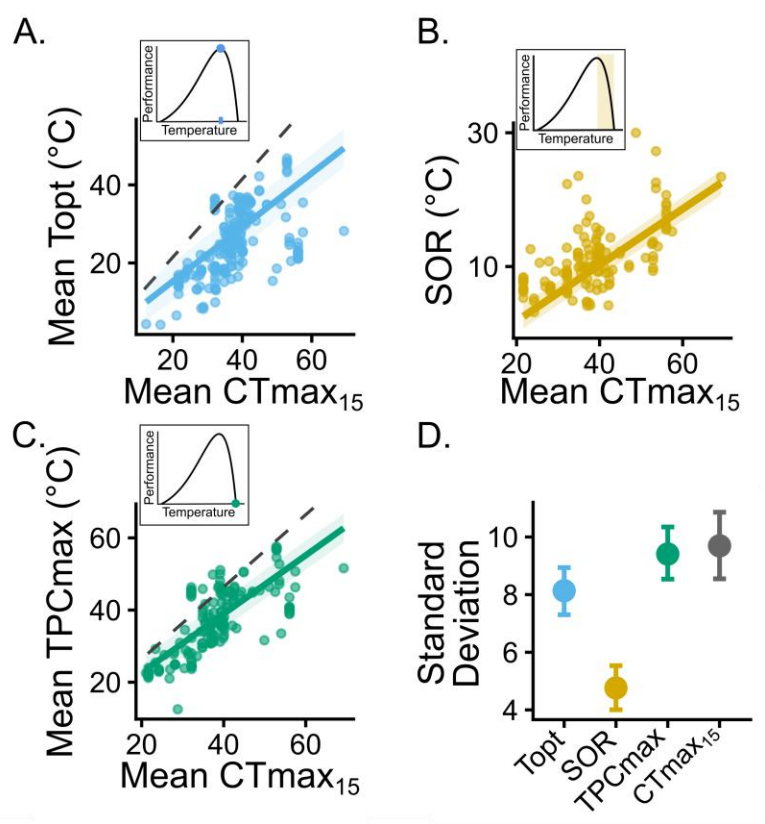

Figure 2. Relationships between thermal performance traits and heat tolerance. (A) Topt, (B) the supra-optimal range (SOR), and (C) TPCmax increased with CTmax across species. Points represent the mean of 1,000 bootstrapped estimates per TPC. CTmax values were ARR-standardized to 15°C (Eq. S2) and scaled and mean-centered in our analyses (Figure S4); axes here show back-transformed values on the original temperature scale for interpretability. Solid lines show model-predicted relationships with 95% CIs (shaded); the grey dashed line indicates the proportionality expectation. (D**)** Comparison of trait variation. Points and bars represent the mean standard deviation and 95% CI of 1,000 bootstrapped estimates for each trait. Sample sizes: Topt: N = 204, 102 species; SOR: N = 136 TPCs, 68 species; TPCmax: N = 159, 79 species.

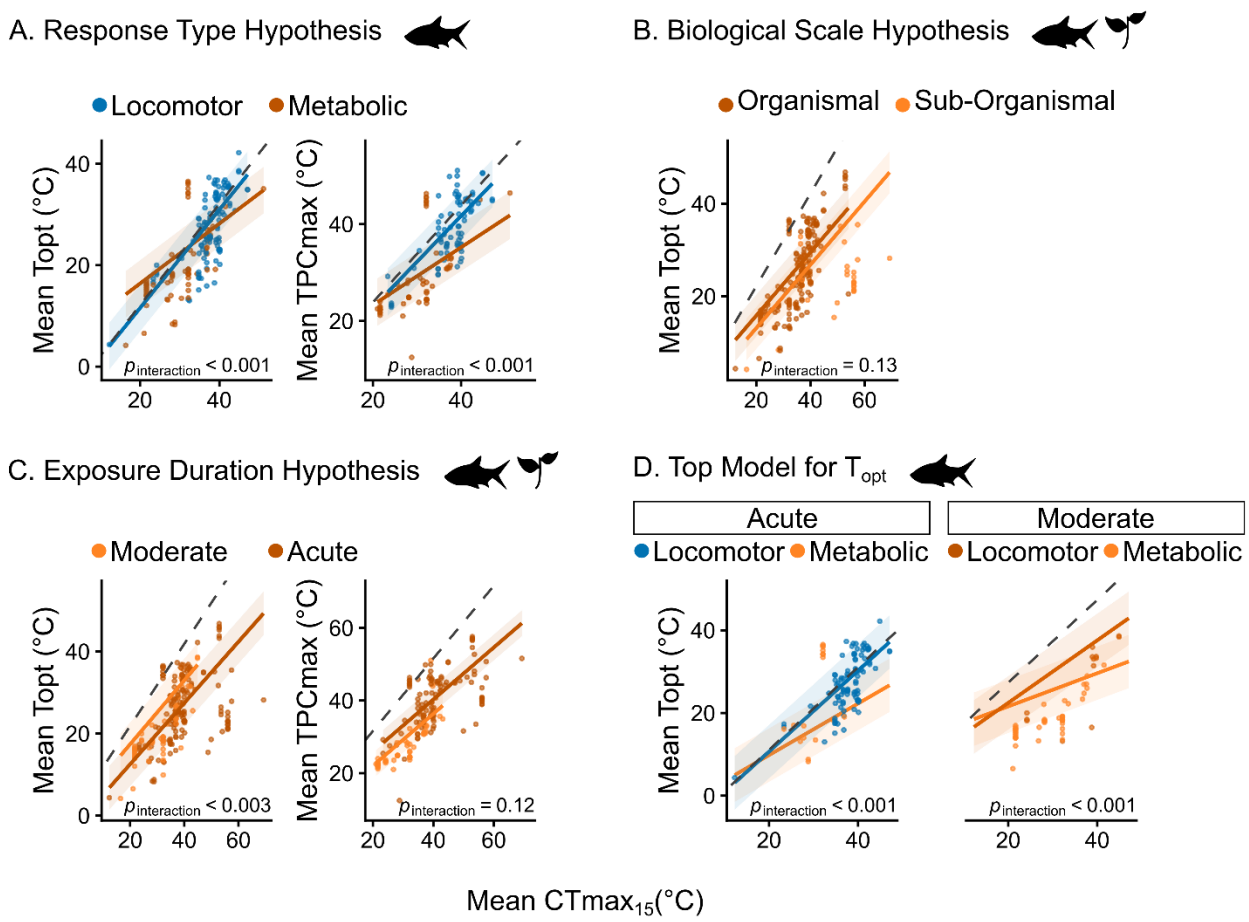

Figure 3. Scaling relationships between thermal performance curve (TPC) traits and CTmax. Results from single hypothesis models (A-C) testing (A) response type, (B) biological scale, and (C) exposure duration hypothesis. (D) The top-ranked model for Topt, which includes exposure duration and response type. Points represent mean trait estimates derived from 1,000 bootstrap fits; solid lines indicate model-predicted relationships with 95% CIs (shaded). Dashed grey lines denote proportionality. Blue lines indicate slopes overlapping proportionality; orange lines (both dark orange and light orange) denote sub-proportionality. Model projections are back-transformed to the original temperature scale and exclude random Class effects (see Figure S5 for analogous plots on scaled axes used in our analysis). $CTmax_{15}$ refers to species CTmax when standardized to an acclimation temperature of 15°C using phylum-averaged acclimation response ratios (Eq S2). Icons indicate inclusion of animals (fish) or plants (leaf). Sample sizes: (A) Topt: $N_{metabolic}$ = 64, $N_{locomotion}$ = 107; TPCmax: $N_{metabolic}$ = 53, $N_{locomotion}$ = 75); (B) Topt: $N_{organismal}$ = 174, $N_{suborganismal}$ = 28; (C) Topt: N_{acute} = 141, $N_{moderate}$ = 63; TPCmax N_{acute} = 108, $N_{moderate}$ = 51; (D) N = 162.

Discussion

Our analysis of over 100 ectotherm species reveals that maximum heat tolerance (CTmax) is not an independent threshold of acute failure at high temperature extremes but rather is fundamentally coupled with performance at sublethal temperatures. We found that species with higher CTmax consistently exhibited higher thermal optima (Topt) and thermal maxima (TPCmax), as well as a wider supra-optimal range of performance (SOR). These results provide empirical support for the hypothesis that the mechanistic processes governing temperature-dependent performance, such as biochemical reaction rates and the balance of cellular damage and repair, are related to the processes that ultimately propagate to organismal failure at extreme temperatures (13, 19). By demonstrating that CTmax contains meaningful information about variation in TPCs, including the breadth of performance decline (SOR), our results suggest that heat tolerance and temperature-dependent performance may be governed by shared, rather than decoupled, physiological processes.

While broad positive relationships suggest shared mechanisms, the form of the relationship provides insight into the strength of coupling between TPCs and CTmax. Our results suggest that the strength of coupling is driven by both the type of biological response being measured and the timescale of the assay. Specifically, proportional (1:1) scaling was restricted to acute locomotor responses, suggesting that the locomotor system is fundamentally related to tolerance limits captured by CTmax. Because CTmax assays for animals define failure as the loss of coordinated movement, CTmax may reflect the terminal endpoint of the same mechanistic processes governing locomotor Topt. As the timescale of the locomotor assay increased to moderate durations, this relationship became sub-proportional. This shift suggests that while the underlying neuromuscular mechanisms may remain consistent, time-dependent constraints— such as bioenergetic depletion and plasticity (15)— may begin to decouple Topt from organismal failure.

Unlike the proportional scaling observed for acute locomotor traits, metabolic responses consistently exhibited sub-proportional relationships with CTmax. Consequently, in heat tolerant species, sub-proportional relationships led to a widening gap between metabolic thermal optima and absolute thermal tolerance. This divergence suggests that metabolic responses (e.g., somatic growth rate, consumption rate, respiration rate) are driven by partially independent processes than the ones captured by CTmax. While locomotion may be related to CTmax through a strong overlap in mechanistic similarity, metabolic performance may reflect a broader array of cellular and systemic functions that reach their limits at different endpoints. Furthermore, these divergent scaling patterns may reflect difference in the selective landscape for these traits. While the processes underlying performance and failure in locomotion may be under similar selection pressures, metabolic responses may be subject to different multivariate selection pressures due to their involvement in many aspects of organismal growth, maintenance, and reproduction.

Given that Topt and TPCmax capture different aspects of thermal performance and ecological contexts (6, 31–33), the consistency of their relationships with CTmax is striking. Prior macro-physiological evidence suggests that heat tolerance limits like CTmax are more evolutionarily constrained than cold tolerance limits across species (34–37), leading to the expectation that performance traits capturing non-maximal heat tolerance (e.g., Topt and to a lesser extent TPCmax) should exhibit more variation than CTmax. While we found slightly lower variation in Topt compared to CTmax and TPCmax, the magnitudes of variation were remarkably similar across these traits. The similarity in trait standard deviations and the correlations we found among TPCs and CTmax suggest that temperature-dependent performance and tolerance may reflect an integrated evolutionary module. Whether this is the outcome of a fundamental mechanistic constraint or correlational selection across the thermal niche remains to be tested. Nonetheless, a similar magnitude of variation does not imply a similar biological consequence. Due to the non-linear, asymmetric shape of the TPC (Figure 1)(38), small shifts in relative performance at the warm-end of the curve and upper tolerance limits can have disproportionate impacts on fitness relative to equivalent shifts in Topt (39, 40). For example, minor increases in CTmax have been shown to significantly decrease the probability of mortality during heating events (41). Conversely, in environments where temperatures rarely approach CTmax, selection may act more strongly on temperatures that maximize daily performance, such as Topt and SOR. Thus, while CTmax, Topt, and TPCmax may exhibit similar amounts of variation, the relative fitness value of that variation is dependent on the frequency and intensity of thermal stress in the environment.

The patterns uncovered here help reconcile a long-standing debate about the utility of CTmax in studies of relative vulnerability to warming temperatures (11, 42). Although CTmax captures failure endpoints that may rarely be experienced in nature (36, 43), its strong positive relationships with TPC traits provide an empirical link to thermal traits relevant to temperatures frequently experienced in nature and directly tied to fitness. This link is particularly evident within the supra-optimal range (SOR), the temperatures capturing declining performance before failure. We found that species with higher CTmax had wider SORs, suggesting that the processes enabling performance at high temperatures may be related to those underlying absolute tolerance. Consequently, in the absence of TPCs, CTmax may provide a practical, *comparative* metric for identifying species capable of maintaining functional performance across a range of warm temperatures, even if fitness begins to decline before CTmax is reached. However, the consistently sub-proportional relationship with metabolic responses suggests that vulnerability insights from CTmax may overestimate safety margins for processes such as growth and maintenance. While the relative ease of experimentally estimating CTmax, and its widespread availability in the literature makes it an indispensable first step in large-scale assessments of thermal risk across many taxa, accurately forecasting population persistence still requires TPCs to capture the non-linear cumulative performance impact of temperature over broad temperature ranges.

Ultimately, our results provide an empirical basis for incorporating organismal physiology into population-level forecasting models (15, 16, 44–46). We show that while CTmax and TPCs are strongly related, the relationships among them are frequently non-proportional, a critical distinction for predicting responses to both extreme heatwaves and chronic, sub-lethal thermal stress (16). While CTmax captures meaningful variation in acute locomotor performance, its sub-proportional scaling with metabolic traits underlying growth suggests that relying solely on tolerance limits risks missing other effects of temperature causing organisms and populations fail. Moving forward, resolving the phylogenetic structure of these relationships, and their genetic and plastic bases, will be critical for refining our understanding of how thermal traits vary across ectotherms. By providing an explicitly multivariate understanding of temperature-dependent traits, we connect our understanding of the processes that maintain performance over temperatures with those that cause organismal performance to fail, improving our ability to project species persistence in a warming world.

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Data Availability

All data and code can be accessed from [zenodo link].

Author Contributions

Conceptualization, methodology, Writing - Review & Editing: All authors
Investigation: ASC, JC, JRB
Validation: ASC, AR, JRB
Data Curation Visualization, Writing - Original Draft: ASC
Project administration, Funding acquisition, Formal analysis: ASC, JRB
Supervision: JRB

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