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Thermal tolerance patterns across latitude and elevation

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Linking variation in species' traits to large-scale environmental gradients can lend insight into the evolutionary processes that have shaped functional diversity and future responses to environmental change. Here, we ask how heat and cold tolerance vary as a function of latitude, elevation and climate extremes, using an extensive global dataset of ectotherm and endotherm thermal tolerance limits, while accounting for methodological variation in acclimation temperature, ramping rate and duration of exposure among studies. We show that previously reported relationships between thermal limits and latitude in ectotherms are robust to variation in methods. Heat tolerance of terrestrial ectotherms declined marginally towards higher latitudes and did not vary with elevation, whereas heat tolerance of freshwater and marine ectotherms declined more steeply with latitude. By contrast, cold tolerance limits declined steeply with latitude in marine, intertidal, freshwater and terrestrial ectotherms, and towards higher elevations on land. In all realms, both upper and lower thermal tolerance limits increased with extreme daily temperature, suggesting that different experienced climate extremes across realms explain the patterns, as predicted under the *Climate Extremes Hypothesis*. Statistically accounting for methodological variation in acclimation temperature, ramping rate and exposure duration improved model fits, and increased slopes with extreme ambient temperature. Our results suggest that fundamentally different patterns of thermal limits found among the earth's realms may be largely explained by differences in episodic thermal extremes among realms, updating global macrophysiological 'rules'.

This article is part of the theme issue 'Physiological diversity, biodiversity patterns and global climate change: testing key hypotheses involving temperature and oxygen'.

1. Introduction

Describing large-scale patterns in functional diversity can help identify historical and eco-evolutionary processes that have shaped functional trait distributions, and lend meaningful information for predicting how biodiversity will respond to future environmental change [1]. For example, variation in thermal tolerance

is of particular interest, given rapid global warming [2,3]. Macrophysiological study of thermal tolerance limits has revealed striking patterns that conform to and/or challenge expectations based on our current understanding of physiological responses, and help to reveal mechanisms that underlie those patterns. For example, decreased latitudinal variation in upper compared to lower thermal limits in many ectotherm groups [2,4–7] suggests that evolution of heat tolerance is constrained [7,8], now known as *Brett's rule sensu* Gaston *et al.* [1]. Another repeated pattern is the increase in species' thermal tolerance breadth found at higher latitudes [2,5,9–13], named *Janzen's rule sensu* Gaston *et al.* [1], which is thought to arise from selection for wider thermal tolerance in regions with greater climate variability. However, later work by Brett [14] and further comparative study between marine and terrestrial organisms [15] has revealed a contrasting pattern in marine realms—marine ectotherms have shown a much steeper decline of heat tolerance with latitude compared to terrestrial ectotherms. These results suggest that marine and terrestrial ecosystems differ in the role of temperature on fitness, the relative importance of behavioural thermoregulation and/or the extreme temperatures that each experience across latitudes.

Understanding the extent to which thermal limits vary with experienced temperatures among realms is important before other mechanisms are invoked to explain their differences. For example, the *Climate Variability Hypothesis* has received considerable attention in explaining how thermal limits vary across latitude [1,9]. This hypothesis predicts that species will evolve wider thermal tolerance breadths in environments with more variable temperatures, and thermal specialization in thermally stable environments, and thus could be a mechanism that gives rise to *Janzen's rule* [1,9]. A corollary of the *Climate Variability Hypothesis* is that organisms' thermal limits are adapted to the climate extremes that they experience. However, estimation of variability is often taken as the difference between means of seasonal or monthly extremes (e.g. mean temperature of the warmest month minus mean temperature of the coldest month), and geographical patterns of such aggregated (averaged) metrics may differ from patterns of shorter-term (e.g. daily) extremes of biological significance [3,16]. The *Climate Extremes Hypothesis* [17] is a variant of the *Climate Variability Hypothesis*, which predicts that extreme thermal events, even if rare, are a key selective agent in the evolution of thermal tolerance. Indeed, there is increasing evidence that rare but extreme thermal events play important roles in selecting for thermal tolerance [17–21]. The climate extremes hypothesis predicts a positive relationship between thermal tolerance limits and extreme episodic temperatures for both cold and heat tolerance limits. If patterns of extreme temperatures vary between land and sea, this hypothesis could explain differences in latitudinal patterns between these realms.

Recently compiled global data on thermal tolerance limits enable us to test this prediction with increasing power; however, when comparing studies, patterns may be obscured by variation in trait measurements owing to methodological differences among studies. For example, an organism's ability to withstand temperatures during an assay is affected by several contextual factors, including the historical thermal environment to which it is acclimated, the rate of temperature change (e.g. during temperature ramping experiments) and the duration of exposure to extreme temperatures [22–25]. In multispecies comparisons of thermal tolerance limits, multivariate analyses can allow a relationship of interest to

be estimated while simultaneously controlling for and estimating the effects of methodological variables. This can reveal the extent to which methodological differences cause noise versus bias in the relationships of interest.

Here, we analyse a comprehensive global database of upper (heat) and lower (cold) thermal tolerance limits of ectotherms and endotherms [26], and summarize their relationships with latitude, elevation and extreme environmental temperatures. We separately analyse three thermal limit metrics. First, *critical thermal limits* are defined as the temperature at which loss of function is observed during a ramping up or down of environmental temperatures [27]. In animals, this is typically defined as a major loss of motor control evidenced by onset of spasms or loss of equilibrium or movement. Second, *lethal limits* are defined as the temperature resulting in the death of 50% (LT50) or 100% (LT100) of exposed individuals, typically measured using a static temperature and set exposure duration (e.g. 24, 72 h). Third, the *edges of the thermal neutral zone* are defined as the hot or cold environmental temperature at which an endotherm can no longer maintain its homeostatic body temperature without incurring changes in metabolic heat production or dissipation. These values are fundamentally different from thermal endpoints of ectotherms, as endotherms can function beyond these limits, but are included to broaden the taxonomic scope of our comparisons.

Using these data, we test for geographical patterns in thermal tolerance and assess support for the *Climate Extremes Hypothesis*. Based on previous across-species findings [28], we predicted that pre-assay acclimation temperature would positively affect upper and lower thermal limits, and have a greater effect in the ocean. We predicted that ramping rate and assay duration could either increase or decrease measured thermal tolerance limits. On the one hand, previous theoretical [29] and empirical [16,17] work suggests that faster ramping rates and shorter assay durations would lead to more extreme upper and lower thermal limits, because organisms spend less time at suboptimal temperatures. On the other hand, if organisms can acclimate to sublethal temperatures during a temperature-ramping experiment, slower ramping rates could lead to more extreme thermal limits owing to within-assay acclimation [23,29]. Finally, under the *Climate Extremes Hypothesis*, we predicted that upper and lower thermal limits would increase with extreme environmental temperature, regardless of the latitude and elevation. We compare results from analyses with and without methodological covariates; if patterns differ strongly, this would indicate that methodological differences are an important source of bias in meta-analyses of thermal tolerance.

The result is the most complete and robust description to date of global thermal tolerance variation with latitude, elevation and temperature extremes, to our knowledge. Our results indicate that climate extremes indeed explain a great deal of the observed variation in thermal limits when methodological covariates are taken into account, and potentially drive the contrasting geographical patterns in thermal limits among realms.

2. Methods

We used the recently published *Globtherm* database, which collates experimentally derived thermal tolerance limit data for more than 2000 species from marine, terrestrial, freshwater and intertidal realms [26]. Globtherm includes one representative

upper and/or lower temperature limit per species and several metrics describing methodological details associated with each value, including the acclimation temperature prior to the thermal limit assay and the temperature ramping rate (full description available in [26]). We considered ectothermic and endothermic animals, but excluded plants owing to small sample sizes within some realms and because response metrics were difficult to directly compare to those of animals. We refer to this as the 'full dataset'.

We created a second, smaller dataset that included only observations for which each methodological covariate of interest was available. For critical thermal limit studies, we included acclimation temperature and ramping rates, which were together available for 515 of 1075 thermal limits. The difference between the starting temperature and an organism's critical thermal limit may also affect estimation of the critical thermal limit [22,29], so we extracted starting temperature for 578 critical limits. However, starting temperature was highly correlated with the critical limit (electronic supplementary material, figure S1) and we found no obvious way to standardize it in a manner that would be independent of the critical thermal limit itself, so we dropped it from subsequent analyses. For lethal limit assays (static temperature), we returned to the original papers to extract exposure durations. We had acclimation temperature and duration of exposure for 130 of 293 lethal limit studies. For upper and lower edges of endotherm thermal neutral zones, we included acclimation information, available for 162 of 880 thermal limits, but we did not extract exposure duration as it was rarely reported and we had no strong expectation that exposure duration within the homeostatic range would affect the temperature at which homeostasis was lost. We refer to this dataset, in which all covariates of interest were available (acclimation temperature, ramping rate in critical limit studies, exposure duration in lethal limit studies), as the 'complete covariate dataset'.

To test whether extreme environmental temperatures predict thermal tolerance, we collected temperature data from the collection location of organisms for each reported thermal limit. For terrestrial and freshwater species, we extracted daily maximum and minimum air temperatures for the location at which species were collected, as defined by longitude and latitude coordinates in GlobTherm. These data were obtained from the Berkeley Earth data interface (berkeleyearth.org/data/; accessed on 15 December 2018) based on the gridded series of temperature data comprising the period 1950–2000. Extreme temperatures were characterized for each approximately 1° pixel as the mean across years in daily minimum and maximum temperature recorded over this period. We favoured the use of temperature data at this high temporal resolution (estimating daily extremes) rather than high spatial resolutions, because it captures variation in extreme temperatures more accurately [16]. For marine and intertidal species, we followed a similar procedure based on data from NOAA Optimum Interpolation (OI) Sea Surface Temperature (https://www.esrl.noaa.gov/psd/data/gridded/data. noaa.oisst.v2.html; accessed on 15 December 2018) at a weekly temporal resolution. Climate data extraction and processing were performed using R packages 'raster' [30] and 'ncdf4' [31]. Without information about the preferred microhabitats of each species, nor the portions of the year in which they are exposed to environmental extremes, we view this approach as a first-pass. We expect the actual temperatures experienced by organisms in their respective microsites to differ from these climatologies, and interpret our results in the light of this. The full updated database of thermal tolerance limits, including added experimental covariates, some corrections from Globtherm (as described in the electronic supplementary material) and the extracted climate variables, are available in the Dryad Digital Repository (doi:10.5061/dryad.5002200).

(a) Analysis

We fit a series of models to parallel subsets of data, grouped by realm (marine, intertidal, freshwater, terrestrial) and thermal metric type (critical, lethal, thermal neutral zone). We grouped the data in these ways to accommodate different intended model formulations, in which some realms had *elevation* as fixed effect (freshwater and terrestrial), and different methodological covariates for different thermal metrics. We only fit models to data groupings with more than 6 data points per model fitted effect, and all models were fit using the *nlme* package in R [32].

To explore how thermal tolerance varies geographically and with methodological differences, we fit two sets of geographical models. First, we fit models without methodological covariates (termed '*non-covariate models*') to the full dataset, and second, we fit more parametrized models (termed '*covariate models*') to the complete covariate dataset (covariate details below). All models included the absolute latitude of collection, thermal limit type (upper versus lower) and the interaction between the two. We also included elevation as a non-interacting fixed effect for terrestrial and freshwater ectotherm data. We excluded elevation from thermal neutral zone models owing to lack of data (elevation reported for only 22 of 880 limits) and excluded collection depth for marine species as it was generally not reported or unknown.

Covariate models included the following methods covariates. First was acclimation temperature prior to the thermal limit assay. However, because the choice of acclimation temperature across studies is expected to be correlated to the ambient temperature of the collection location, the relationship between acclimation temperatures and thermal tolerance limits could artificially include the effects of climate at the collection location itself. Instead, therefore, we compared acclimation temperatures that were high or low *relative* to the ambient extreme temperatures at the locations of collection, by standardizing acclimation temperature as the difference between the extreme environmental temperature of each collection location (maximum for upper limits and minimum for lower limits) and the acclimation temperature, which we call the 'acclimation offset'. This approach is somewhat similar to the methods used previously in which this same value (acclimation offset) was used to infer the thermal tolerance limits of organisms at the most appropriate acclimation temperature for their location of collection [33,34]. For lethal limit models, we next included log exposure duration [35], and for critical limit models, we included log ramping rate. In all models, we included a taxonomic hierarchical (nested) random effect from phylum to genus, to account for lack of taxonomic independence among species [15,36,37].

To explore how thermal tolerance varies directly with extreme temperatures at collection locations, we fit two sets models to temperature as above (non-covariate and covariate models) for each of the realm and thermal limit data groups. In non-covariate models, we fit thermal tolerance limits as a function of thermal limit type (upper versus lower), extreme temperature and their interaction as fixed effects (full dataset). Covariate models were fit to the complete covariate dataset and included acclimation temperature, ramping rate (critical limit models) or exposure duration (lethal limit models) and their interaction with thermal limit type. We compared Akaike information criteria (AIC) of all models in order to compare the model fits.

3. Results

We found relationships between thermal tolerance and latitude to generally follow expectations based on smaller subsets of data in previous studies. For all realms and metric types, cold
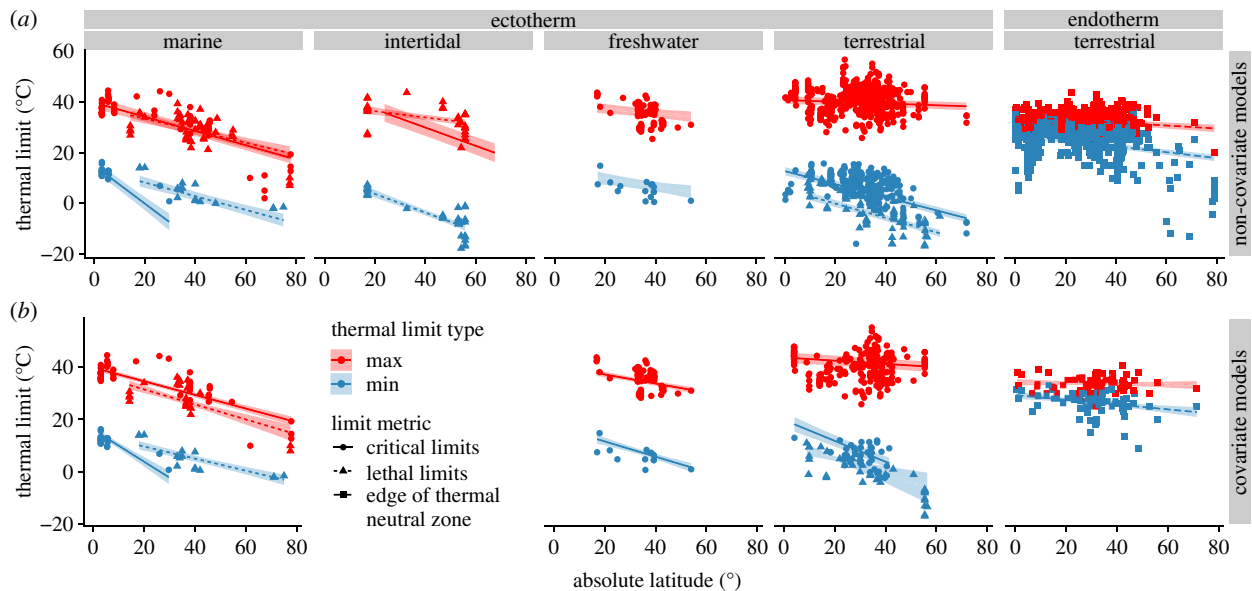
Figure 1. Thermal limits as a function of absolute latitude. Shaded areas indicate best-fit significant model relationships from linear mixed-effects models ± standard error, and points show raw data used to fit models. When shown, lines represent significant relationships. Data and results from (*a*) models that do not account for methodology ('non-covariate models' fit to full dataset: 2132 thermal limits); and (*b*) models that include methodological covariates ('covariate models' fit to complete covariate dataset: 719 thermal limits).

tolerance limits declined strongly with latitude (figure 1 and table 1). By contrast, heat tolerance limits declined weakly with latitude in terrestrial ectotherms and endotherms and moderately in freshwater and intertidal ectotherms, but declined strongly with latitude in marine ectotherms (figure 1). Relationships with elevation were similar to those with latitude; heat tolerance limits in terrestrial and freshwater ectotherms did not decline towards higher elevations, while cold tolerance limits did (figure 2 and table 1).

Geographical patterns in thermal limits were not strongly affected by covariates of acclimation temperature and ramping rate or exposure duration, although model fits were better when covariates were included (delta AIC > 77 for all model comparisons with and without covariates; electronic supplementary material, table S1), and small differences in slopes are perceptible (figure 3; see electronic supplementary material, table S2 for model results when covariates were not included). Higher acclimation offsets were associated with increased heat and cold tolerance limits, as expected, although the increase was not always significant (figure 4*a* and table 1). In lethal limit studies, longer exposure durations were associated with lower tolerance to heat as predicted, but had uncertain partial effects (i.e. confidence intervals crossed zero) on tolerance to cold (figure 4*b* and table 1). In critical limit studies, upper and lower thermal tolerance limits often (but not always) increased with ramping rate (figure 4*c* and table 1).

When we used extreme environmental temperatures to predict thermal tolerance instead of latitude and elevation, model fits of ectotherms were improved (delta AIC were all greater than 9; electronic supplementary material, table S1) and indicated that upper and lower thermal tolerance limits were generally well predicted by local temperature extremes (figures 3 and 5; electronic supplementary material, table S3 and S4). Relationships were weaker in freshwater and terrestrial habitats compared to marine habitats, but there was also less latitudinal variation in extreme heat in these realms (figure 5). In many cases, the relationship between

thermal limits and extreme environmental temperatures were more positive when models accounted for methodological variation (figures 3 and 5; electronic supplementary material, tables S3 and S4). Although there remained substantial variation in the upper thermal tolerance limits of terrestrial ectotherms around the model-fitted lines (figure 5), including methodological covariates and temperature extremes largely improved model fits in all groups (AICs of electronic supplementary material, table S1). In endotherms, by contrast, the edges of thermal neutral zones did not vary strongly with extreme environmental temperature.

4. Discussion

Using many more species from a broader set of habitat types, our results show that previously described relationships between thermal tolerance and latitude are robust to the effects of key methodological variables, and suggest temperature extremes as a common underlying mechanism for latitudinal patterns in thermal tolerance limits. In terrestrial ectotherms, upper thermal limits only declined marginally with latitude and not at all with elevation, whereas lower thermal limits declined with both latitude and elevation. In aquatic ectotherms, especially marine species, upper and lower thermal limits declined at a similar rate with latitude, as found previously [15], in contrast with both *Janzen's* and *Brett's rules*. Although we currently lack information on species' behavioural and microhabitat preferences, we found broad congruence between thermal limits and environmental temperature extremes, which suggests that latitudinal patterns in thermal tolerance and their difference among realms may be broadly explained by the extreme temperatures experienced in each realm. Below we expand on the possible mechanisms of these patterns, the role of methodological variation across studies and caveats to these findings.

When methodological variation is accounted for, both upper and lower thermal tolerance limits of ectotherms

Table 1. Model summaries for models of thermal tolerance limits as a function of latitude and elevation, when methodological covariates were included. For each fixed effect, contrast coefficients and diagnostics (t- and p-values) indicate the effect of each parameter level on the reference level. The reference level of thermal limit type was the upper thermal limit (denoted *upper*), and therefore contrasts with lower thermal limits are shown (*lower*) in all but one model in which only lower thermal limits were included. A single asterisk (*) signals a p-value less than 0.05, and double (**) signals a p-value less than 0.01.

|	fixed effects	effect type	estimate	s.e.	t-value	p-value
marine						
critical thermal limit	reference (upper)	intercept	47.64	1.44	33.04	<0.001**
$n = 74$	abs. latitude	slope	−0.26	0.03	−9.51	<0.001**
	thermal limit type: lower	intercept	−30.39	1.98	−15.33	<0.001**
	acclimation offset	slope	0.13	0.08	1.67	0.107
	log ramping rate	slope	1.87	0.30	6.19	<0.001**
	abs. latitude × type: lower	slope	−0.35	0.09	−3.76	0.001**
	log ramping rate × type: lower	slope	−1.52	0.54	−2.84	0.009**
lethal thermal limit	reference (upper)	intercept	48.64	3.32	14.64	<0.001**
$n = 41$	abs. latitude	slope	−0.29	0.07	−4.25	0.051
	thermal limit type: lower	intercept	−38.35	5.52	−6.95	0.006**
	acclimation offset	slope	0.80	0.30	2.64	0.078
	log duration	slope	−2.28	0.51	−4.49	0.021*
	abs. latitude × type: lower	slope	0.06	0.09	0.64	0.567
	log duration × type: lower	slope	2.57	0.73	3.50	0.039*
freshwater						
critical thermal limit	reference (upper)	intercept	42.51	1.86	22.82	<0.001**
$n = 99$	abs. latitude	slope	−0.17	0.04	−4.07	<0.001**
	thermal limit type: lower	intercept	−27.37	2.68	−10.22	<0.001**
	elevation	slope	0.00	0.00	0.72	0.477
	acclimation offset	slope	0.22	0.04	6.06	<0.001**
	log ramping rate	slope	−0.42	0.17	−2.44	0.019*
	abs. latitude × type: lower	slope	−0.12	0.08	−1.52	0.135
	elevation × type: lower	slope	−0.01	0.00	−5.19	<0.001**
	log ramping rate × type: lower	slope	1.54	0.41	3.73	0.001**
terrestrial						
critical thermal limit	reference (upper)	intercept	45.08	2.03	22.18	<0.001**
$n = 310$	abs. latitude	slope	−0.06	0.02	−3.77	<0.001**
	thermal limit type: lower	intercept	−25.80	2.26	−11.40	<0.001**
	elevation	slope	0.00	0.00	−1.84	0.067
	acclimation offset	slope	0.11	0.03	4.14	<0.001**
	log ramping rate	slope	0.85	0.41	2.08	0.039*
	abs. latitude × type: lower	slope	−0.34	0.07	−4.92	<0.001**
	elevation × type: lower	slope	0.00	0.00	−2.06	0.041*
	log ramping rate × type: lower	slope	2.24	0.63	3.58	<0.001**
lethal thermal limit	reference (lower)	intercept	24.74	17.64	1.40	0.181
$n = 41$ (lower only)	abs. latitude	slope	−0.31	0.16	−1.90	0.077
	elevation	slope	0.00	0.00	−2.82	0.013*
	acclimation offset	slope	0.09	0.19	0.49	0.632
	log duration	slope	−4.38	5.90	−0.74	0.593
edges of thermal neutral zone	reference (upper)	intercept	34.00	1.30	26.11	<0.001**
$n = 154$	abs. latitude	slope	−0.02	0.04	−0.43	0.669
	thermal limit type: lower	intercept	−4.08	1.45	−2.81	0.006**
	acclimation offset	slope	−0.03	0.05	−0.60	0.551
	abs. latitude × type: lower	slope	−0.08	0.04	−1.74	0.086
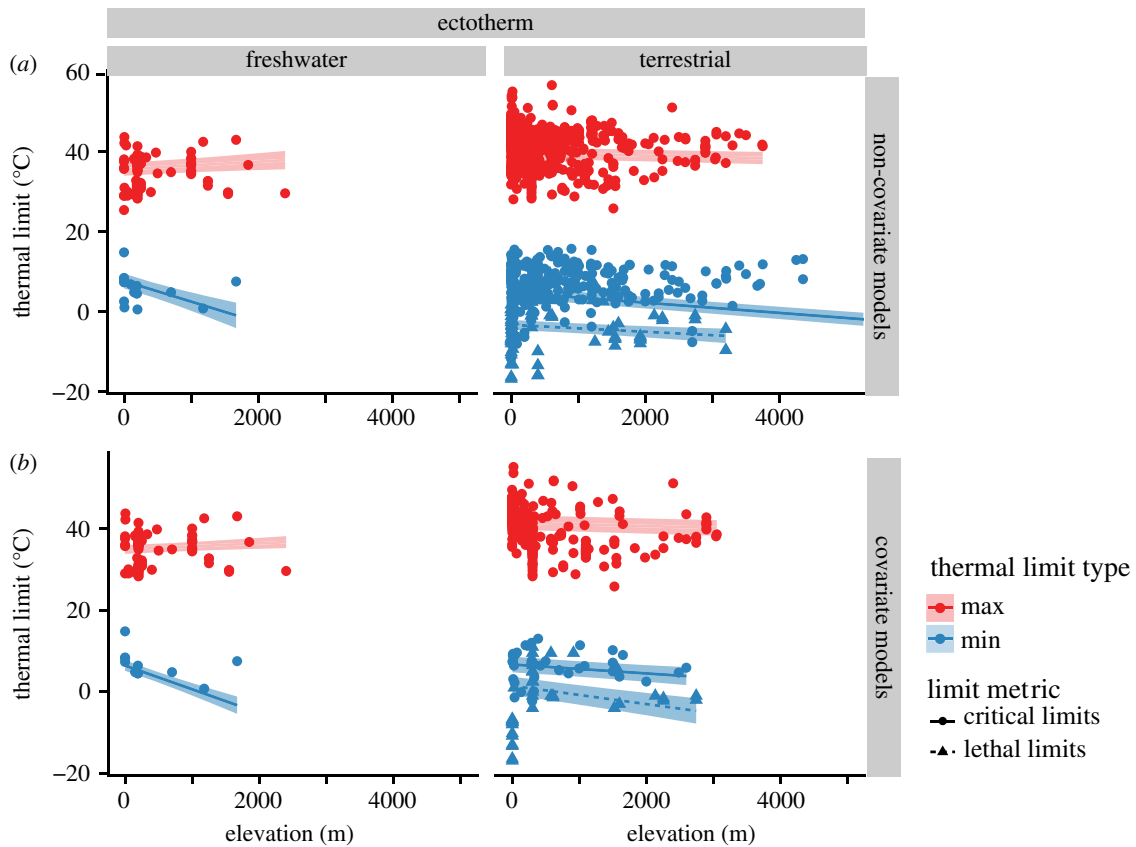
Figure 2. Thermal limits as a function of elevation. Shaded areas indicate best-fit significant model relationships from linear mixed-effects models ± standard error, and points show raw data used to fit models. When shown, lines represent significant relationships. Data and results from (*a*) 'non-covariate models' (full dataset), and (*b*) 'covariate models' that account for methodological variation (complete covariate dataset), as in figure 1 (table 1).
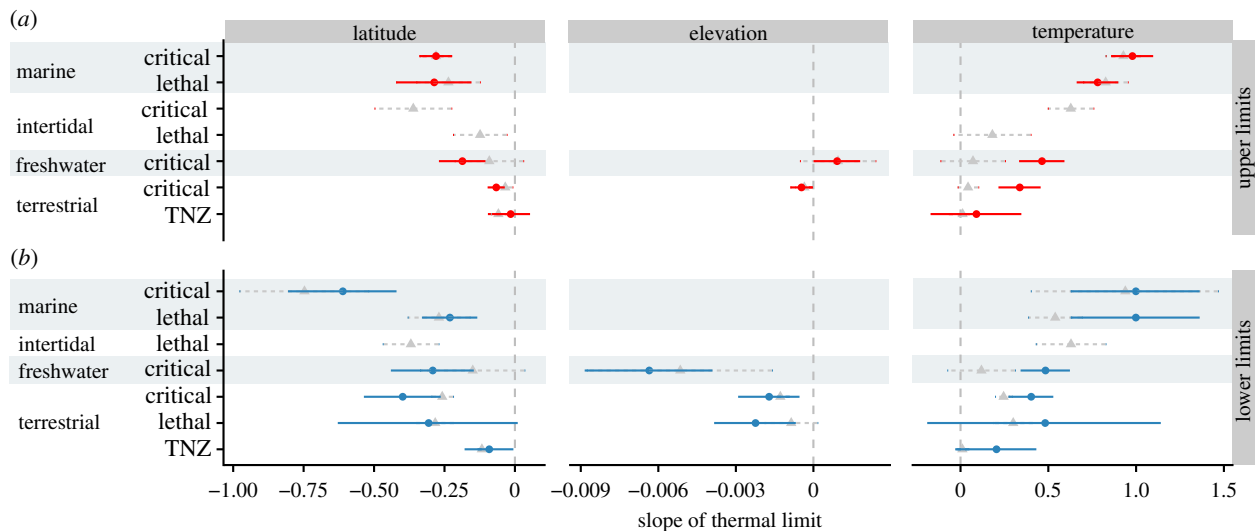

Figure 3. Slopes of relationships between thermal limits and the elevation, latitude and extreme temperature at the collection location for upper (*a*) and lower (*b*) thermal limits. Points and lines represent the mean coefficients and 95% confidence intervals from covariate models (methodological covariates accounted for, coloured circles and lines), red denotes upper and blue denotes lower thermal limits and non-covariate models (grey triangles and lines). Thermal limit types: critical limits, lethal limits, edges of the thermal neutral zone (TNZ).

show a positive relationship with extreme temperatures across realms. This suggests that extreme episodic temperatures represent a ubiquitous selective force on temperature limits, as expected under the *Climate Extremes Hypothesis* [17]. Less latitudinal variation in heat tolerance on land may be, at least partially, owing to the lower latitudinal variation in episodic extreme heat events (i.e. the small range of extreme maximum air temperatures on land in figure 5*b*).

The narrow variation in heat extremes across a wide breadth of latitude in North America was noted by MacArthur [38], who postulated that tolerance to extreme heat likely did not limit species' southern distributions. The shallower relationship between heat tolerance and heat extremes on land versus water could reflect greater spatial heterogeneity in terrestrial microhabitats or behavioural use of a wider variety of microhabitats; either can decouple maximum air
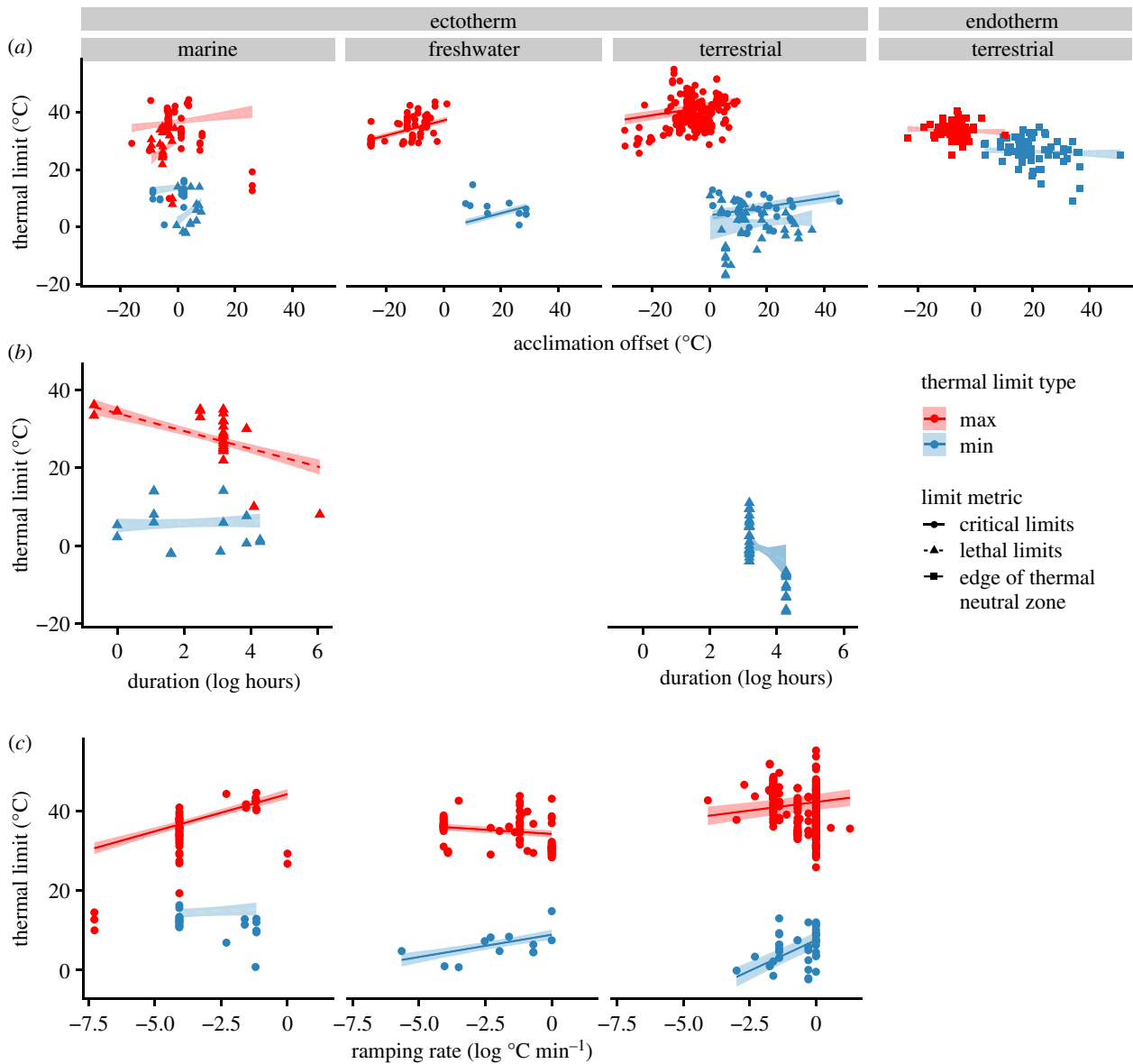
Figure 4. Thermal limits as a function of methodological variables (complete covariate dataset). Shading indicates best-fit partial regression relationships from full linear mixed-effects models ± standard error, indicating the partial effects of (*a*) acclimation offset for all experiments, (*b*) exposure duration for lethal limit experiments and (*c*) ramping rate for critical limit experiments. When shown, lines represent significant relationships. Points show raw data used to fit models.

temperature from maximum body temperature, leading to more stable body versus air temperatures [33]. Alternatively, higher episodic heat events on land may have selected for heat tolerance limits that have approached evolutionary constraints, leaving little room for further evolution [7,8].

In ectotherms, only lower thermal limits varied with elevation. This is consistent with some previous studies in terrestrial ectotherms ([29,39] although see [21]), and our results further show no change in heat tolerance of taxa collected at higher elevations in freshwater ectotherms (but see [39]). The lack of a trend in upper thermal limits with elevation may, in part, be attributable to the broad ranges of species in this dataset: many have Holarctic distributions with elevational ranges extending from the lowlands to the highlands, and their thermal tolerance limits may not be locally adapted to the precise elevation of collection (see [40,41]). In addition, thermal variability may increase at higher elevations, since thinner air results in high insolation and rapid warming during the day, but promotes cooling during the night [42]. Thus, although mean temperatures are cooler at higher elevations, increased variability around that mean may lead to similarly high

warm extremes at increasing elevations, to which species have adapted. For example, previous work has shown that above-ground-ants had no decline in upper thermal limits with elevation, while subterranean species had a decline in upper thermal limits with elevation, as expected if greater variability owing to insolation offsets the mean rate of adiabatic cooling [21]. For aquatic ecosystems, the water's higher thermal capacitance provides some thermal stability, but this is expected to vary with the size of the water body and its relative exposure, which can vary from highly temperature-exposed ponds to subterranean springs highly buffered to extremes. Knowledge of each organism's basic habitat requirements (e.g. aboveground or belowground, season of emergence) would be useful towards stronger tests. Additionally, oxygen availability declines steeply with increasing elevation in freshwater systems [43], thus experimental conditions in which oxygen is held constant may mask relevant variability in upper thermal limits that are mechanistically driven by variation in oxygen limitation [44].

The effects of methodological variation across studies were in directions consistent with predictions. When organisms were exposed to higher relative acclimation temperatures
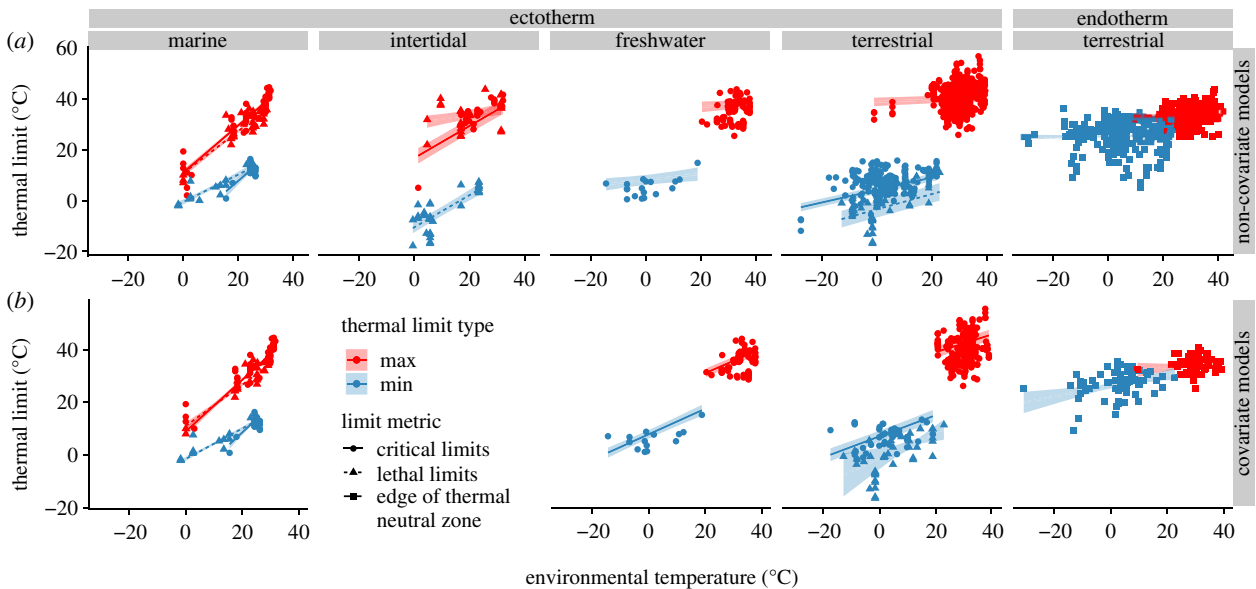
Figure 5. Thermal limits as a function of extreme environmental temperatures at collection locations. Shaded areas indicate best-fit significant model relationships from linear mixed-effects models ± standard error, and points show raw data used to fit models. When shown, lines represent significant relationships. Data and results from (a) models that do not account for methodology ('non-covariate models' fit to full dataset: 1807 thermal limits); and (b) models that include methodological covariates ('covariate models' fit to complete covariate dataset: 719 thermal limits).

(acclimation offset) before experimental trials, thermal tolerance limits were generally higher, as expected based on our understanding of acclimation responses in ectotherms [24,28,45]. For duration of lethal limit exposures, longer exposures to heat in marine ectotherms were associated with less extreme upper thermal tolerance, as predicted by Rezende *et al.* [35], in which the probability of death is an increasing function of exposure duration. However, there was no detectable increase in lower thermal limits with duration, potentially owing to a lack of power. For ramping rate, we found support for the failure rate model of Kingsolver & Umbanhowar [29], which predicts more extreme thermal limits when ramping rate is faster, for upper thermal limits of terrestrial and marine ectotherms. However, for lower thermal limits, we found that tolerance limits were either no different or more extreme (tolerating lower temperatures) when ramping rates were slower, suggesting that potentially acclimation, or cold-hardening, occurred [23,29]. Most of our estimated slopes for relationships between thermal tolerance and extreme environmental temperature were steeper in models that accounted for methodology, indicating that methodological variation was unevenly distributed across episodic extreme temperatures. In another recent study, the modelled relationships of thermal tolerance with body and genome size were also altered by accounting for methodological variation [46].

The rate at which upper thermal limits declined with latitude and increased with extreme temperatures was intermediate in freshwater ectotherms relative to marine and terrestrial ectotherms. An environmental mechanism could be at play in the latitudinal pattern, as freshwater systems are expected to have levels of environmental variability intermediate to marine and terrestrial systems [47]. Because we used air temperatures in place of water temperatures in the freshwater models, this possibility cannot be ruled out. The intermediate relationships may also be explained by a mixed assemblage of breathing modes among freshwater species, which may include organisms with obligate water gas exchange and organisms that can supplement or rely exclusively on aerial gas exchange (e.g. adult diving beetles

and water bugs). Oxygen-limited thermal tolerance may preclude water-breathing taxa from approaching the evolutionary constraints that limit heat tolerance in air-breathing (terrestrial) ectotherms [48], hence considering breathing mode of aquatic taxa in future work may help to elucidate mechanisms further underpinning the variation (see also [46]).

We discuss two major caveats to these findings. First, our database of extreme environmental temperatures only represents an approximation of the temperatures that an organism would encounter across its geographical range within its available or preferred microhabitats. Because we did not have estimates of habitat temperature in freshwater or intertidal realms, we used data from other realms (i.e. air temperatures for freshwater habitats and sea surface temperature for the intertidal), which are expected to be over- or underestimates, respectively. In addition, the temporal resolution of our extreme temperature data differed across datasets: we used daily temperature extremes from air temperature records, but weekly temperature extremes from sea surface temperature records. This difference probably did not have a large effect on our results, as sea temperatures are generally more temporally stable than air temperatures, and the alternative of using a longer resolution on land would risk averaging out the important extremes [16]. Nevertheless, one feasible near-term goal is to compare these estimates of extremes with temperature loggers. Additionally in all realms, and especially on land, the actual temperatures experienced by organisms are expected to differ greatly owing to microhabitat variation and behaviours [33,49,50]. One achievable near-term goal is to associate the thermal tolerance limits in Globtherm with information about species' microhabitat preferences, even at a coarse scale, as well as with their timing of emergence versus dormancy, so that differences in patterns among these groups can be more finely attributed to habitat-differences in temperature extremes (e.g. [21]). Nevertheless, the positive slopes between thermal limits and temperature extremes extracted even on this course scale suggest a role for the effect of regional temperature extremes, and perhaps

microhabitat variation can further account for unexplained variation among species. A second caveat is that our analyses assumed that the methodological variables modelled had similar effects on thermal tolerance limits among species within realms and thermal metric types. Species can differ in their acclimation capacity [43,19], and species-specific responses to the covariates may further explain some of the unaccounted variation, especially in upper thermal tolerance limits.

Nevertheless, the support for the *Climate Extremes Hypothesis* as found here has potential implications for how global warming and the associated increases in occurrence of extreme events [51] will have impacts across realms. Although marine ectotherms have upper thermal limits more closely related to extreme temperatures, and have greater responsiveness to climate warming than terrestrial ectotherms [3], our results suggest that there is a ubiquitous role of climate extremes in limiting performance in all realms. As such, we expect local extinctions of populations to be more closely tied to increases in heat extremes, rather than increases in mean temperatures, both on land in the ocean. Similarly, we expect reduction in cold climate extremes to relax selection on cold tolerance, and promote the persistence of individuals and species that previously could not tolerate cold.

5. Rules revisited

Considering that this work represents the broadest spatial and taxonomic comparative analysis of its kind conducted to date, these results lend strength to the three emerging empirical patterns. Our results formalize previous observations, first made by Brett himself [14], that *Brett's rule*

diminishes in freshwater habitats, and is non-existent in the marine realm, where upper thermal limits have rates of decline with latitude as great or greater than that of lower thermal limits. Our results also show that *Janzen's rule* is found in terrestrial habitats, but diminishes in freshwater habitats, and is not supported in the oceans. While the above two rules, then, appear to be realm-specific, our results lend cross-realm support for the *Climate Extremes Hypothesis* in ectotherms, providing evidence for a ubiquitous underlying mechanism when high temporal resolution climate extremes are considered.

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