How much warming can mosquito vectors tolerate?

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Abstract

While climate warming is expected to substantially impact the global landscape of mosquito-borne disease, impacts will vary across disease systems and regions. Understanding which diseases, and where within their distributions, these impacts are most likely to occur is critical for preparing public health interventions. While research has centered on potential warming-driven expansions in vector transmission, less is known about the potential for vectors to experience warming-driven stress or even local extirpations. In conservation biology, species risk from climate warming is often quantified through vulnerability indices such as thermal safety margins – the difference between an organism's upper thermal limit and its habitat temperature. Here, we estimated thermal safety margins for 12 major mosquito species (including *Aedes aegypti* and *Anopheles gambiae*) that are the major vectors of malaria, dengue, chikungunya, Zika, West Nile and other major arboviruses, across their known ranges to investigate which mosquitoes and regions are most and least vulnerable to climate warming. We find that most mosquito vector species have large positive thermal safety margins across the majority of their range, when realistic assumptions of mosquito behavioral thermoregulation are incorporated. For species with distributions spanning both hemispheres, we find the lowest climate vulnerability, in terms of both the magnitude and duration of thermal safety, just south of the equator, as well as at their northern temperate range edges, and the highest climate vulnerability in the subtropics. Underlying these broad scale patterns, we find clear biogeographical differences in vector thermal safety with regions such as the Middle East, India, northwestern Africa, southeastern Australia, and the southwestern U.S., and desert and xeric shrubland biomes having the highest climate vulnerability across vector species.

Introduction

Climate warming is poised to cause large shifts in the distribution and burden of mosquito-borne diseases, as temperature is a fundamental driver of mosquito range limits. Near cool range edges, warming is widely expected to promote mosquito population growth and disease transmission (Chen *et al.* 2010; Medlock & Leach 2015; Mordecai *et al.* 2019; Parmesan & Yohe 2003; Ryan *et al.* 2019). However, the impacts of warming across other portions of mosquito ranges are less clear and will depend on the extent of warming (*e.g.*, the magnitude and duration of high temperatures), as well as species physiological limits and response strategies. In particular, ectotherm physiology theory and empirical work predict that upper thermal limits may restrict species persistence in currently suitable ranges under excessive warming (Deutsch *et al.* 2008; Huey & Berrigan 2001; Kearney *et al.* 2009a; Kellermann *et al.* 2012; Pinsky *et al.* 2019; Pörtner *et al.* 2006; Sunday *et al.* 2014). Understanding which mosquito species and regions will be most impacted by warming is critical for preparing targeted vector control and disease

prevention strategies.

The potential impacts of climate change across species and broad spatial scales are often quantified through vulnerability indices such as 'thermal safety margins'—the difference between an organism's critical thermal maximum and its habitat temperature—(often used interchangeably with 'warming tolerance'; Angilletta 2009; Clusella-Trullas *et al.* 2021; Deutsch *et al.* 2008). Thermal safety margins, which reflect the amount of additional warming an organism could experience before trait performance is inhibited, have been estimated for hundreds of species of ectotherms including lizards, snakes, fish, and insects (Deutsch *et al.* 2008; Diamond *et al.* 2011; Sunday *et al.* 2019, 2014; Vinagre *et al.* 2019). These prior studies have often estimated the lowest thermal safety margins, and thus the highest susceptibility to warming, for species in the tropics. From a conservation standpoint, this finding has raised concern given the high biodiversity contained in the tropics. However, whether this pattern applies for species that threaten human health, such as mosquitoes that vector pathogens, has not been rigorously evaluated.

Thermal safety margins have been extensively used to describe species vulnerability to climate change (*e.g.*, Pinsky *et al.* 2019; Sunday *et al.* 2014), but can provide biased estimates if the estimated critical thermal maximum and/or environmental temperatures do not reflect those relevant to the organism (Clusella-Trullas *et al.* 2021). For example, air temperature in full sun, as is often captured by weather station data sources, can differ drastically from an organism's body temperatures (Kearney *et al.* 2009b; Sunday *et al.* 2014). Similarly, environmental temperature estimates with coarse temporal resolution (*e.g.,* daily or monthly scales) may fail to capture short-term thermal extremes, which can have major impacts on organismal fitness and population growth (Buckley & Huey 2016a, b; Dowd *et al.* 2015; Vasseur *et al.* 2014). To address these limitations, recent advances in climate monitoring and microclimate and biophysical modeling have enabled increasingly accurate estimates of realized environmental temperatures on time scales relevant to short ectotherm life cycles (Hersbach *et al.* 2020; Kearney & Porter 2017, 2020).

Here, we estimate thermal safety margins for 12 major mosquito vector species across their ranges to identify the disease systems and locations that are most and least vulnerable to climate warming. We specifically ask: how much buffering do mosquitoes have from thermal extremes in their current ranges? We define thermal safety margins as the difference between a species critical thermal maximum, estimated in the laboratory for a range of life history traits under constant temperature, and the hottest hourly body temperature experienced in the coolest microhabitat available (Clusella-Trullas *et al.* 2021; Pinsky *et al.* 2019). To ensure that our estimates provide a meaningful metric of climate vulnerability, we explicitly: 1) incorporate the realistic potential for mosquito movement to cooler, fully-shaded microhabitats (*i.e.*, 'behavioral thermoregulation'); 2) estimate operative mosquito body temperature rather than air temperature, as these can differ markedly (Kearney *et al.* 2009b; Sunday *et al.* 2014; Woods *et al.* 2015); and 3) use hourly temperature data to capture the impact of short-term thermal extremes that may be missed with more temporally coarse temperature estimates (*e.g.*, daily, monthly). Further, as

mosquito climate vulnerability will depend not only on the difference in critical thermal maxima and body temperatures, but also *how often* and for *how long* critical thermal maxima are exceeded, we also estimated the time spent in thermal danger (here, the number of consecutive hours or days when body temperatures exceed critical thermal maxima).

Methods

Mosquito thermal tolerance data

We included the following 12 mosquito species in our investigation as these constituted major disease vectors with available thermal tolerance estimates: *Aedes aegypti, Ae. albopictus, Ae. camptorhynchus, Ae. triseriatus, Ae vexans, Anopheles gambiae, An. stephensi, Culex annulirostris, Cx. pipiens, Cx. quinquefasciatus, Culex tarsalis, and Cx. theileri* (Table 1). As our measure of the estimated critical thermal maximum ($\mathrm{CT_{max}}$) for each species, we used the estimated thermal maxima ($\mathrm{T_{max}}$) calculated for individual life history traits and synthesized in Mordecai *et al.* (2019) and Villena *et al.* (2022) (Table 1, Supplemental Table S1). Specifically, when thermal performance estimates were available for multiple life history traits for a given species, we used the trait with the warmest critical thermal maximum for that species (*i.e.*, biting rate for *Ae. aegypti*; $\mathrm{CT_{max}}$: 40.00). This estimate, as well as the critical thermal minima ($\mathrm{CT_{min}}$) and optima ($\mathrm{T_{opt}}$) were derived from Bayesian thermal response models fit to empirical data on mosquito trait performance at different constant temperatures. We note that there is no consensus methodology for estimating organismal thermal tolerance, but rather a wide range of methods including static and dynamic heat tolerance assays (*e.g.*, 'thermal knockdowns') of varying durations (Bates & Morley 2020; Clusella-Trullas *et al.* 2021; Hoffmann *et al.* 2013; Jørgensen *et al.* 2019; Lutterschmidt & Hutchison 1997; Rezende *et al.* 2014; Terblanche *et al.* 2007). Notably, the experimental methodology can impact the estimated $\mathrm{CT_{max}}$ (*e.g.*, Terblanche *et al.* 2007; Woods *et al.* 2018), with longer duration heat stress assays – as in the constant temperature lab exposures from which our $\mathrm{CT_{max}}$ estimates are derived –typically yielding lower estimated $\mathrm{CT_{max}}$than short duration assays (Bates & Morley 2020; Peck *et al.* 2009; Woods *et al.* 2018). Thus, our estimate of mosquito species' $\mathrm{CT_{max}}$ may be lower than that made by a thermal knockdown assay, and we may be biased towards under-estimating thermal safety margins and therefore over-estimating mosquito climate vulnerability. However, thermal knockdown assays have not been frequently or consistently conducted across mosquito species, thus we chose to use an estimate of $\mathrm{CT_{max}}$ that could be more reliably compared between species. We note that, for a given species, empirical estimates were often made on a lab colony representing a single population. Thus, although mosquito thermal tolerance can vary between populations, we applied the given thermal tolerance estimate to the species across its range as data was only available for a single population.

Table 1. Species-specific trait values used in biophysical models of mosquito body temperature. For each species, the thermal limit values ($\mathrm{CT_{max}}$, $\mathrm{CT_{min}}$, and $\mathrm{T_{opt}}$) are from the trait with the warmest critical thermal maximum for that species (noted in the 'Warmest trait' column).

Supplemental Table S1 contains the CT_{max}, CT_{min}, and T_{opt} for each trait and species. MDR: mosquito immature development rate. Note the following abbreviations are used below: YF for yellow fever, RRV for Ross river, WNV for West Nile fever, RV for Rift valley ever, MVE for Murray Valley encephalitis, JE for Japanese encephalitis, SLE for St. Louis encephalitis, and WEE for Western equine encephalitis.

Species	**Human diseases vectored**	**CT_{min} (°C)**	**T_{opt} (°C)**	**CT_{max} (°C)**	**Warmest trait**	**Activity pattern** (diurnal, nocturnal, crepuscular)
Aedes aegypti	dengue, chikungunya, Zika, YF	13.8	33.8	40.0	Biting rate	Diurnal, crepuscular
Ae. albopictus	dengue, chikungunya, Zika, YF	8.7	32.6	39.6	MDR	Diurnal, crepuscular
Ae. camptorhynchus	RRV	9.3	32.2	38.8	MDR	Diurnal, crepuscular
Ae. triseriatus	La Crosse encephalitis	0.8	29.3	36.5	MDR	Diurnal, crepuscular
Ae vexans	RVF, WNV, Zika	9.1	25.0	40.8	Immature survival	Diurnal, nocturnal, crepuscular
An. gambiae	malaria, lymphatic filariasis	34.6	16.95	43.55	Biting rate	Nocturnal, crepuscular
Anopheles stephensi	malaria	19.1	36.05	42.25	Biting rate	Nocturnal, crepuscular
Culex annulirostris	RRV, MVE, Barmah forest, Kunjin virus, JE	11.1	32.8	39.2	MDR	Nocturnal, crepuscular
Cx. pipiens	WNV	3.2	23.0	42.6	Egg viability	Nocturnal, crepuscular
Cx. quinquefasciatus	WNV, lymphatic filariasis, SLE, WEE	3.1	31.9	39.3	Biting rate	Nocturnal, crepuscular
Cx. tarsalis	WNV, SLE, WEE	5.9	24.6	43.1	Immature survival	Nocturnal, crepuscular
Cx. theileri	RVF	5.5	23.6	45.4	Egg viability	Nocturnal, crepuscular

Mosquito occurrence data

To determine the distribution over which to estimate thermal vulnerability, we used published and/or publicly available occurrence records from the Global Biodiversity Information Facility (GBIF; *i.e.*, a collection with an accompanying latitude and longitude) for each species

(Supplemental Table S2; Supplemental Figure S1). For species with hundreds of occurrence records (*i.e., Ae. aegypti*), we randomly selected 20-30 records from each 10° latitudinal band on which to estimate thermal safety margins. While our approach was not designed to comprehensively cover the entire range of a given species, in cases of clear data missingness in GBIF (*i.e., Aedes vexans* occurrences in Africa, *Anopheles stephensi* in Kenya), we further supplemented these occurrence records with those from published data sources through targeted literature searches. We used the associated latitude and longitude to classify occurrence records into one of 14 biomes (Olson *et al.* 2001) and as 'tropical' (0-23.5°), 'subtropical' (23.5-35.0°), and 'temperate' (35.0-66.5°).

Estimating mosquito body temperatures

For each vector species and collection location, we estimated mosquito body temperatures using NicheMapR: a suite of R programs for microclimate and biophysical modeling (Kearney & Porter 2020). In the first step, we used the microclimate model with hourly ERA5 weather data as input, to estimate downscaled microclimate conditions (*e.g.*, temperature, wind speed, humidity, radiation) in both full shade and full sun. This microclimate data is then used as the input in the biophysical model, along with information on organismal functional traits such as body size and thermal limits, to estimate steady-state body temperatures. We supplied species-specific parameter values for activity patterns (*e.g.*, diurnal, nocturnal, crepuscular) and thermal limits (*e.g.*, point estimates and 95% credible intervals for T_{opt}, CT_{max}, CT_{min}). In the biophysical model, T_{opt} is considered the organisms' preferred body temperature (*i.e.*, the temperature that it will attempt to maintain), while CT_{max} and CT_{min} impact the organisms' thermoregulatory behaviors (Kearney & Porter 2020). For species with critical thermal limits estimated for multiple life history traits, we used the values from the trait with the highest critical thermal maximum (Table 1; Supplemental Table S1). We assumed all mosquito species had a body size of approximately 3 mg with 85% solar absorptivity (Brust 1967). Using these parameter values, we estimated species' thermal safety margins *with* and *without* behavioral thermoregulation (*i.e.*, the ability to move across fully exposed to fully shaded microhabitats; main results and Supplemental Figure S2, respectively). These models have been extensively validated in a wide range of field conditions (Kearney *et al.* 2014; Kearney & Maino 2018; Kearney & Porter 2017).

Incorporating impacts of drought on vector life cycles

In environments with highly seasonal precipitation, mosquito populations may reduce activity or aestivate during drought (Adamou *et al.* 2011; Lehmann *et al.* 2010), and thus avoid exposure to high temperature extremes experienced during that time. To incorporate potential seasonal and aestivation responses driven by low moisture availability, we masked out any periods in which the prior 30 or more days had soil moisture below 5%. That is, if the prior 30+ days each had soil surface wetness <5%, then hourly thermal conditions from any subsequent days were excluded from our thermal vulnerability calculations until soil moisture rose above 5% again. This soil

moisture metric roughly corresponded to precipitation events (Supplemental Figure S3). Although some mosquito species may persist during drought by using artificial water sources (*e.g.*, *Aedes aegypti*; Trpiš 1972), we used this drought mask for all species for consistency, as comprehensive information on the drought responses of mosquito populations across their ranges is not available. For all species, incorporating this drought mask had minimal impact on the thermal safety estimates (Supplemental Tables S4-S5, Figure S4).

Estimating thermal safety margins

To quantify mosquito vulnerability to climate warming, we calculated thermal safety margins: the difference in an organism's critical thermal maximum (CT_{max}) and the warmest temperature the organism experiences (T_e) in the coolest microhabitat available (Deutsch *et al.* 2008; also referred to as 'warming tolerance'). By using highly resolved microclimate data, and estimating T_e both with and without behavioral thermoregulation, we believe the thermal safety margins calculated here provide a relevant metric of thermal vulnerability. To capture both the magnitude and duration of thermal safety, we specifically calculated three related indices: 1) thermal safety margins, 2) the longest continuous period (in hours) that T_e exceed CT_{max} (*i.e.*, 'thermal danger'), and 3) the longest streak of consecutive days in thermal danger (where a day is counted if estimated mosquito body temperatures exceeded species' critical thermal maxima for at least one hour). Together, these capture both average thermal safety and seasonal and diurnal variation in thermal safety. After estimating these indices at each collection location for each species, we used generalized additive models (GAM) to estimate latitudinal patterns of thermal vulnerability. For the main results presented here, we subset occurrence records to those collected between 0 – 3000 feet of elevation to focus on latitudinal variation on temperature and avoid confounding, regionally-varying effects of elevation. However, we also present gam models for each species fit using all occurrence points and a random intercept for low (<3000 ft) and high (>3000 ft) elevation records (Supplemental Figure S5). We fit all models using up to 8 knots estimated using restricted maximum likelihood with the 'mgcv' R package (Wood 2017).

Identifying maxima and minima in thermal safety

To identify the latitudes with highest and lowest in thermal safety for each species, we searched for global and local maxima and minima in thermal safety margins, respectively, in the GAM fits. That is, we searched for latitudes in which the first derivative of the fitted function switched from positive to negative, and in which the estimated thermal safety margins were the highest (or lowest) of their neighboring latitudes (*i.e.*, highest or lowest in a sequence of three). We further confirmed the locations of maximum and minimum thermal safety by visually inspecting the plotted GAM fits for each species. To estimate uncertainty, we drew 1,000 samples from the posterior-fitted GAM parameter vector, multiplied by the linear predictors, and detected maxima and minima in each sampled fit, as in Pinsky *et al.* (2019) (Wood 2017; Supplemental Figure S6). We performed this analysis on the low elevation occurrence records only (*i.e.*, <3000 ft), as there were not sufficient high elevation occurrence records for robust estimation.

Results

Most vector species had positive thermal safety margins across the majority of their distributions (Figure 1). The only species and locations experiencing thermal danger when the ability to behaviorally thermoregulate was incorporated (as in the main model specification) were *An. stephensi* (>30°N), *An. gambiae* (>15°N), *Cx. annulirostris* (27°S - 37°S), and *Cx. quinquefasciatus* (25°N - 35°N and 25°S - 35°S). Across all species, the average thermal safety margins were 4.16 ± 2.34°C (mean ± 1 s.d.) under this scenario. These results were highly similar when excluding the drought mask or when including occurrence records from higher elevations (Supplemental Figures S4-S5, Supplemental Table S4-S5). However, when the ability to behaviorally thermoregulate was removed (*i.e.*, when vectors were restricted to fully exposed habitats), no species were thermally safe across their entire range (Supplemental Figure S2), highlighting the importance of access to thermal refugia during high temperature extremes. In particular, without behavioral thermoregulation, thermal safety margins were 4.78 ± 0.73°C lower (Supplemental Table S4), putting most species in thermal danger across large portions of their range. However, we note we may be biased towards under-estimating thermal safety margins and over-estimating thermal danger by using $\mathrm{CT_{max}}$ estimates made under constant temperatures (as described in the *Methods: Mosquito thermal tolerance data* and Discussion).

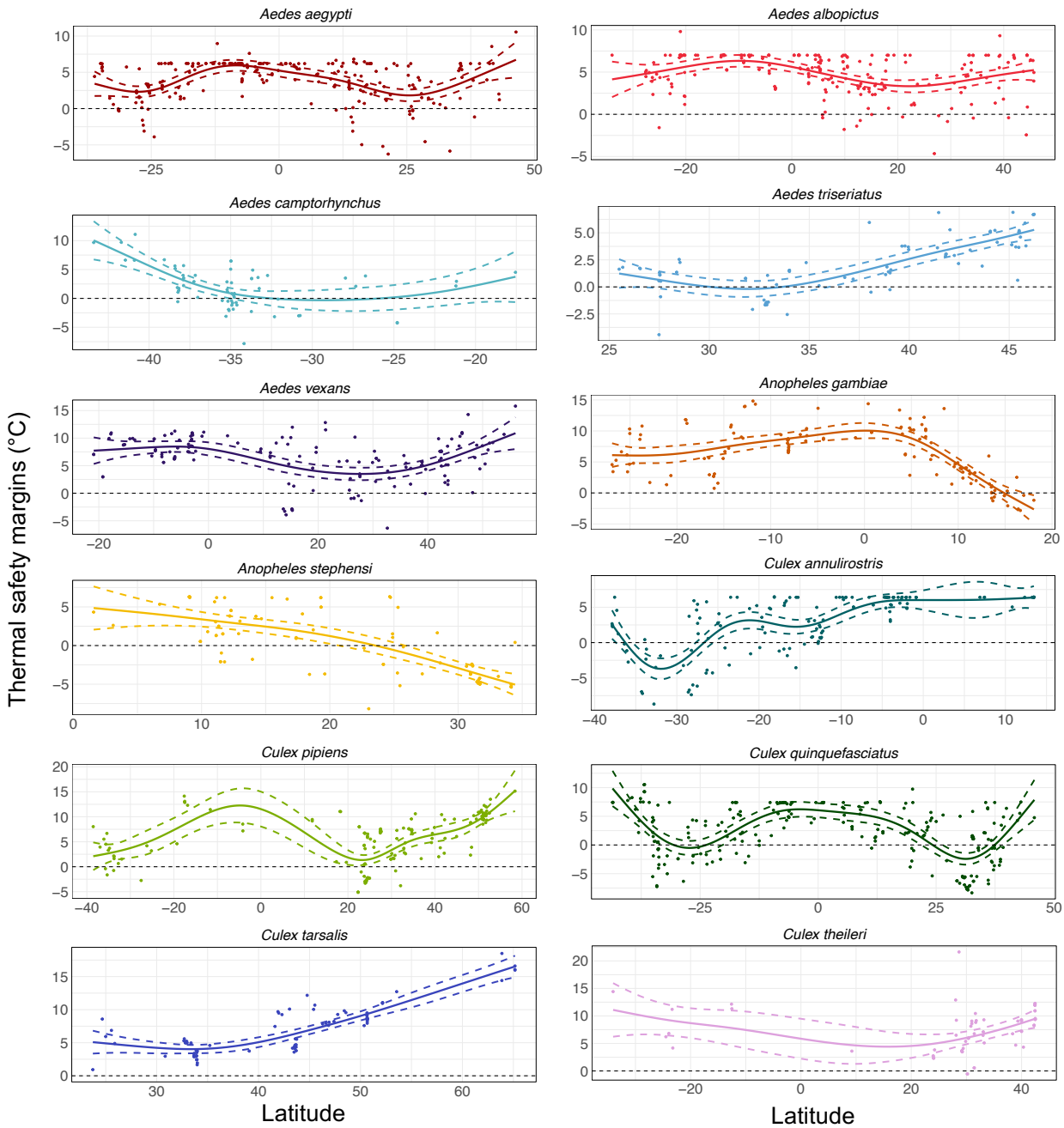

Figure 1. Thermal safety margins across latitude for individual mosquito vectors. Points are estimates at individual occurrence records for a given species; solid and dotted lines show the mean and 95% confidence intervals from the GAM fits. These are estimated with behavioral thermoregulation and with the drought mask (see Supplemental Figures S2, S4, and S5 for corresponding plots under alternative assumptions).

For vector species with distributions spanning both hemispheres, thermal safety margins typically peaked just south of the equator and at the northern temperate range edges (Figure 2,

Supplemental Figures S7-S9). For these species, thermal safety margins were lowest between 23 – 35° (N or S)—the latitudinal extent of the subtropics—with the exception of *Cx. theileri,* for which thermal safety was lowest in the northern tropics (~18°N). For species with distributions restricted to the northern hemisphere, thermal safety either increased *(Ae. triseriatus, Cx. tarsalis*) or decreased (*An. stephensi*) towards the poles. For all species, these patterns of thermal safety were mostly driven by variation in experienced temperature in fully shaded habitats across latitude (Supplemental Figure S10). That is, the highest hourly mosquito body temperatures were typically estimated for species occurrences in the subtropics, with relatively cooler maximum body temperatures around the equator and northern temperate range edges. These patterns held true when excluding the drought mask (Supplemental Figure S4, Supplemental Table S4).

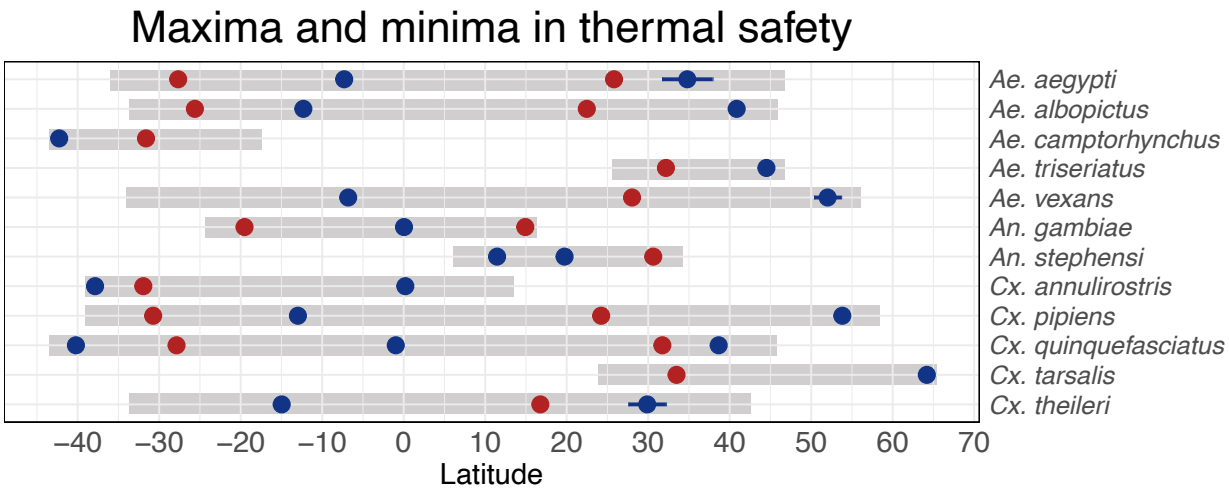

Figure 2. Latitude of maxima (blue points) and minima (red points) in thermal safety margins across the latitudinal ranges (gray rectangles) for each species. See 'Methods: *Identifying maxima and minima in thermal safety'* for details on how locations were estimated. See Supplemental Table S1 and Supplemental Figure S6 for estimates and uncertainties.

In addition to thermal safety margins typically being lowest in the subtropics, variation in thermal safety showed clear biogeographical differences (Figure 3). In particular, regions in the Middle East, India, northwestern Africa, southeastern Australia, and the southwestern U.S. had the lowest thermal safety margins across vector species (Figure 3, top panel). That is, these regions typically experience the hottest single-hour temperatures, although not necessarily the hottest mean temperatures across the year (Supplemental Figure S11). Variation in biogeography may underlie these regional patterns as we found that thermal safety was typically lowest in deserts and xeric shrublands and flooded grasslands and savannas—biomes that constitute the majority of these regions (Olson et al. 2001; Figure 3, bottom panel).

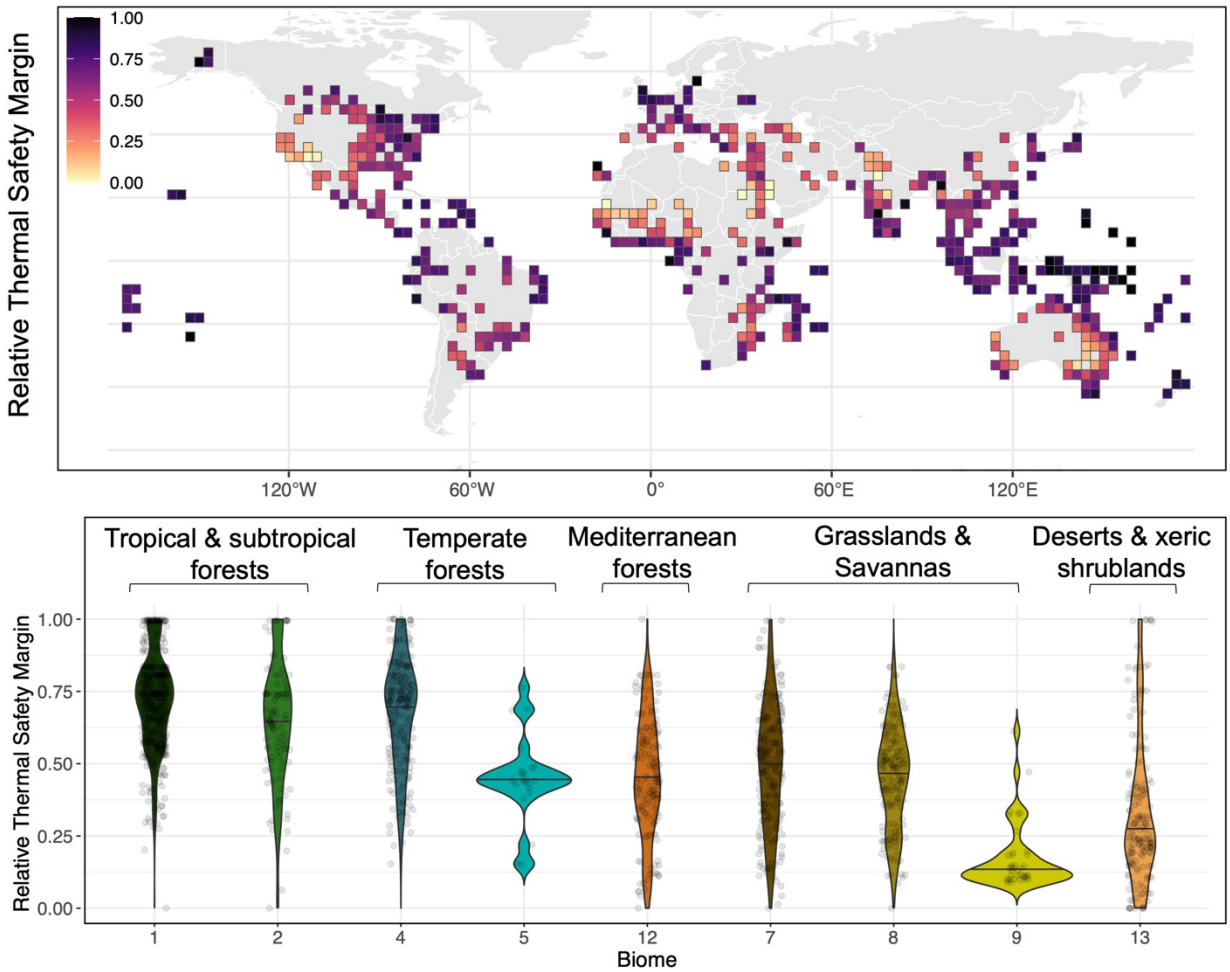

Figure 3. Vector thermal safety margins by grid cell (top) and biome (bottom). For each vector species, thermal safety margins were scaled from 0 to 1 before being combined (by taking the average of each grid cell) as shown here. In the top panel, grid cells are 3°x3°. In the bottom panel, biomes refer to the 14 biomes classified by the World Wildlife Fund. The biome numbers listed on the x-axis correspond to 1) Tropical & Subtropical Moist Broadleaf Forests, 2) Tropical & Subtropical Dry Broadleaf Forests, 4) Temperate Broadleaf & Mixed Forests, 5) Temperate Conifer Forests, 7) Tropical & Subtropical Grasslands, Savannas & Shrublands, 8) Temperate Grasslands, Savannas & Shrublands, 9) Flooded Grasslands & Savannas, 12) Mediterranean Forests, Woodlands & Scrub, 13) Deserts & Xeric Shrublands. Lines within each violin plot denote the median relative TSM for that biome. Points are scaled estimates of TSMs for individual occurrence points within each biome (jittered to improve visibility). See Supplemental Figures S7-S9 for scaled TSMs for each individual vector species.

When thermal danger occurred (*i.e.*, when estimated mosquito body temperatures exceed species' critical thermal maxima), it typically lasted fewer than five hours (Figure 4). In particular, the longest continuous period in thermal danger throughout the year ranged from an average of 3.1 ± 2.4 hours (*Ae. albopictus*) to 7.6 ± 2.4 hours (*An. stephensi*) (mean ± s.d. across occurrence points; Supplemental Figure S12, Supplemental Table S5). Similarly, species typically did not experience thermal danger on many consecutive days: the longest consecutive streak in thermal danger ranged from 1.0 ± 0.0 days (*Cx. theileri*) to 6.5 ± 7.9 days (*Cx.*

quinquefasciatus) (Supplemental Figure S12, Supplemental Table S6). *Cx. tarsalis* did not have any occurrence records where thermal danger was estimated to occur, so it was excluded from these metrics. For both measures, the longest streaks of thermal danger typically occurred in subtropical regions (Figure 4), matching patterns observed in thermal safety margins. These results were highly similar when removing the drought mask with a few exceptions. Without the drought mask, the average longest streak of consecutive days in thermal danger increased from 5.3 to 7.4 days for *Ae. aegypti*, 3.6 to 6.3 days for *Ae. vexans*, and 5.5 to 6.6 for *An. stephensi* (Supplemental Table S6). When the ability to behaviorally thermoregulate was removed, these streaks of thermal danger substantially increased for most species (Supplemental Table S5-S6). That is, the longest streaks of consecutive days in thermal danger increased for all species and doubled or tripled for all but *Ae. aegypti, Ae. albopictus, Ae. camptorhynchus* and *Ae. triseriatus*. Notably, a single species—*Culex quinquefasciatus*—accounted for the majority of instances of thermal danger occurring on >20 consecutive days (Figure 4, Supplemental Figure S12).

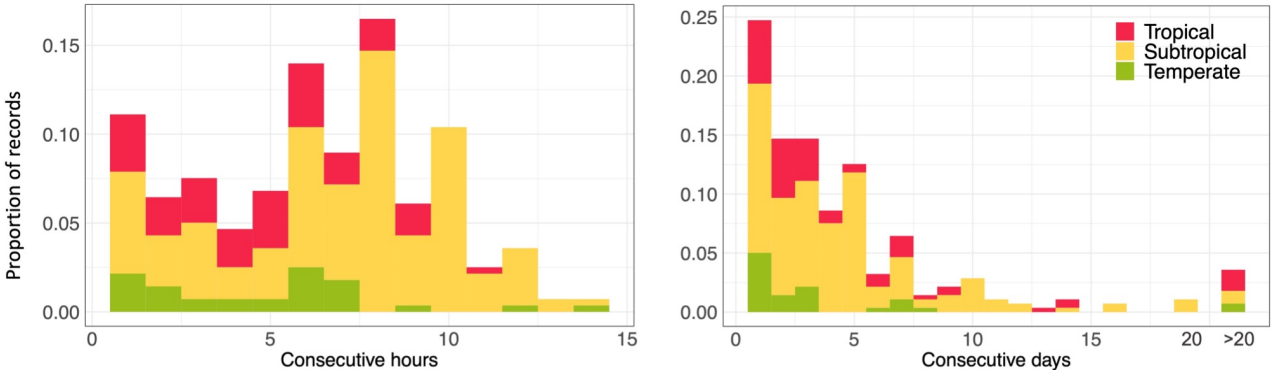

Figure 4. Longest periods in thermal danger by region for all vector species combined. X-axes show the longest streak of consecutive hours (left) or days (right) in thermal danger. Note that the y-axis refers to the proportion of records for which thermal safety margins are negative, not all possible records, most of which are not in thermal danger. See Supplemental Figure S12 and Table S5-S6 for species-specific values of these metrics.

Discussion

We investigated the risk posed by climate warming for 12 major mosquito vector species by estimating thermal safety margins—here, the difference between a species' critical thermal maxima and the hottest hourly body temperature it experiences. We found that, when able to access shaded microhabitats, most species had wide safety margins across the majority of their ranges, suggesting low risk from warming (Figure 1). Conversely, when limited to exposed habitats, thermal safety margins were approximately 5°C lower, placing most species in thermal danger (*i.e.,* body temperature would exceed critical thermal maxima) across large portions of their range (Figure 1). Our results thus suggest that behavioral thermoregulation is likely already a pervasive strategy for buffering mosquitoes from high temperature extremes. The reliance on behavior and access to cooler microhabitats to avoid thermal danger has been well documented

in other ectotherm species (Kearney *et al.* 2009b; Pinsky *et al.* 2019; Sunday *et al.* 2014). For mosquitoes, behavioral avoidance of high temperatures in laboratory settings has been observed in *Aedes, Anopheles*, and *Culex* species (Blanford *et al.* 2009; Thomson 1938; Verhulst *et al.* 2020), and preference for cooler, shaded oviposition sites in field settings has been documented for *Aedes* (Barrera *et al.* 2006; Vezzani & Albicócco 2009) and *Culex* (Vezzani & Albicócco 2009) species. However, the extent and potential fitness costs of mosquito behavioral thermoregulation remain largely unknown. Our results highlight the importance of understanding mosquito behavioral thermoregulation and for incorporating microhabitat availability when investigating mosquito responses to warming, as behavior may lessen projected negative impacts of future warming.

We find that buffering against climate warming for most vector species was highest around the equator and at their northern temperate range edges, and lowest around the subtropics (*i.e.*, 23-35° N or S) (Figures 1-2). While this finding contradicts the expectation that tropical species are most vulnerable to climate warming from seminal studies such as Deutsch et al. 2008 and Tewksbury et al. 2008, this same pattern was found in a recent meta-analysis including over 400 ectotherm species (Pinsky *et al.* 2019). Similarly, studies of ectotherm range shifts in response to warming, reflecting evidence of climate vulnerability, have found the fastest range shifts at higher latitudes (Ramalho *et al.* 2023), as well as more nuanced responses including east-west and equator-ward shifts (Lenoir & Svenning 2015; Pinsky *et al.* 2013; VanDerWal *et al.* 2013). Our findings thus contribute to a growing body of evidence that species risk and responses to climate warming do not vary unidirectionally with latitude, and current risk may be highest in subtropical, rather than tropical, regions because of their higher thermal extremes (Johansson *et al.* 2020; Kingsolver *et al.* 2013; Pinsky *et al.* 2019; Ramalho *et al.* 2023).

We also find that short-term temperature extremes drive the heightened risk from warming in the subtropics. That is, across species we estimated the highest mosquito body temperatures experienced for one or more hours occurred around 29°N (Supplemental Figure S10). This is consistent with expectations from climatology that, although average daily mean temperatures typically peak at the equator and decrease monotonically towards the poles, average daily maximum temperatures typically peak around the subtropics, and are relatively lower at the equator and higher-latitude temperate regions (Buckley & Huey 2016a; Hoffmann 2010; Kingsolver & Buckley 2017; Supplemental Figure S11). These short-term thermal extremes, even if rare, are known to cause major declines in individual fitness, scaling up to drive population and species-level impacts on demographic rates in other ectotherm taxa (Buckley & Huey 2016a, b; Ma *et al.* 2015). Our findings thus suggest that subtropical mosquito populations may be under the greatest pressure to shift their ranges, seasonality, and/or adapt to warming in coming decades, with relatively higher stability in tropical and temperate populations. However, this will depend on the relative fitness costs of exposure to high short-term thermal extremes versus high mean temperatures, which is not well understood and constitutes a key future research direction (Bates & Morley 2020)

While we describe patterns of mosquito climate vulnerability across broad spatial scales (*i.e.,* between tropical, subtropical, and temperate regions) and latitude, regional-scale biogeographical differences clearly drive variation in vulnerability (Figure 3). We found that thermal safety margins were the lowest in specific regions including the Middle East, India, northwestern Africa, southeastern Australia, and the southwestern U.S., which are largely classified as desert and xeric shrubland biomes (Olson *et al.* 2001), but are highly ecologically and climatologically distinct from each other. As described for the subtropics broadly, these regions each experience the highest short-term thermal extremes, although not necessarily the warmest annual mean temperatures (Supplemental Figure S11).

As climate vulnerability depends not only on the magnitude of temperature extremes, but also on their duration, we estimated the longest continuous period that mosquito species spent in thermal danger. As before, we found that the longest stretches of thermal danger, in terms of both consecutive hours and days, occurred in subtropical regions (Figure 4), followed by tropical then temperate regions. Comparing across species, we found that thermal danger, when it occurred, typically lasted no more than 3 to 7.6 hours (for *Ae. albopictus* and *An. stephensi*, respectively, Supplemental Table S5, Figure S12). At these time scales, rapid hardening or heat shock responses, which have been well documented in other ectotherm species, could substantially increase short-term critical thermal maxima (Kellermann *et al.* 2017; King & MacRae 2015; Ma *et al.* 2021). As heat shock responses are highly conserved across ectotherm species (Lindquist & Craig 1988), this may be a pervasive strategy for mosquitoes mitigating thermal danger under current and future climatic extremes. However, the precise time scales over which they operate and the overall fitness costs of inducing these short-term heat shock responses remain poorly understood. In other ectotherm species, the production of heat shock proteins has been associated with decreased critical thermal maxima on subsequent days (Bai *et al.* 2019) and overall decreases in development and reproduction (Feder & Hofmann 1999; McMillan *et al.* 2005; Sørensen *et al.* 2003). In our study, thermal danger typically occurred on no more than 1 to 6.5 consecutive days (for *Cx. theileri* and *Cx. quinquefasciatus*, respectively, Supplemental Table S6, Figure S12), which could be sufficiently short to minimize heat damage and fitness costs. Understanding the impacts of heat stress on mosquitoes at these time scales and frequencies will improve estimates of mosquito climate vulnerability and is an important direction for future research.

The time dimension of heat stress is also important for accurately estimating species critical thermal maxima (Bates & Morley 2020). In this study, we used estimates of critical thermal maxima derived from laboratory experiments, which measured mosquito life history traits (including immature and adult survival and development as well as fecundity, biting rate, and egg viability) under constant temperatures, typically occurring for several hours, days, or weeks (Supplemental Table S1; summarized in Mordecai et al. 2019 and Villena et al. 2022). Prior work has demonstrated that the duration of heat exposure and the rate of temperature change used in heat tolerance assays, as well as the thermal history of the organism itself, including cross-generational effects, can all impact the estimated critical thermal maxima

(Heerwaarden & Kellermann 2020; Kingsolver *et al.* 2011, 2015; Schiffer *et al.* 2013; Terblanche *et al.* 2007; Waite & Sorte 2022). As a result, there is no single definitive critical thermal maximum for a given taxon or individual, but instead a continuum depending on temporal dynamics of heat exposure and the specific trait of interest (Bates & Morley 2020; Clusella-Trullas *et al.* 2021; Hoffmann *et al.* 2013; Jørgensen *et al.* 2019; Kellermann *et al.* 2012; Lutterschmidt & Hutchison 1997). In general, critical thermal maxima estimated from longer duration heat stress assays, as used here, are lower than those estimated from acute heat exposure assays such as thermal knockdowns (Bates & Morley 2020; Peck *et al.* 2009; Woods *et al.* 2018). Thus, we may have been biased towards calculating smaller thermal safety margins, and thus over-estimating the extent of thermal danger and mosquito climate vulnerability. We used estimates of critical thermal maxima derived from constant-temperature laboratory experiments because they are widely available across the focal taxa, they are consistent in their temporal dimension, and they have relevance to life history traits that govern fitness in the field (Mordecai *et al.* 2019, 2020; Shocket *et al.* 2018, 2020; Tesla *et al.* 2018). Further, we chose the most thermally tolerant life history trait as our measure of critical thermal maximum in order to be conservative in choosing temperature values that truly present thermal danger, but if positive population growth requires all life history traits to be within the thermal limits, we could be under-estimating thermal danger (Table 1). Other measures that could be more locally relevant (*e.g.*, responses to short-term heat exposures following more realistic daily temperature variation) have not been conducted systematically across taxa and would likely not be broadly representative and comparable across species entire ranges, as we have examined here.

While we sought to provide an ecologically realistic estimate of mosquito risk to climate warming, there are several additional limitations in our risk metric. First, mosquito populations may suffer declines before thermal safety falls to zero, given the typically steep drop-off in thermal performance between a species' thermal optima and maxima (Mordecai *et al.* 2019), and potential trade-offs in mosquito life history traits. Further, although mosquito thermal tolerance can vary between populations (*e.g.*, Couper *et al.* 2023; Reisen 1995; Ruybal *et al.* 2016), we applied a single estimate of mosquito critical thermal maxima to a species across its range, as population-level estimates are not yet available. Additionally, seasonality in mosquito life cycles and prolonged periods of dormancy may buffer populations from thermal extremes (Lehmann *et al.* 2010). As mosquito seasonality and dormancy is largely driven by precipitation and moisture conditions (Huestis & Lehmann 2014; Lehmann *et al.* 2010; Shocket *et al.* 2021), we attempted to account for this by applying a drought mask, in which periods following 30 or more days of dry surface soils were excluded from our thermal safety margin estimates (this drought mask had minimal impact on our findings). Incorporating regionally-specific, population-level mosquito responses would enable more accurate estimates of climate vulnerability but are not available for most species and locations. In addition to these limitations, the ultimate impacts of climate warming on mosquito species will depend not only on current thermal vulnerability, but on rates of warming, which are expected to be spatially heterogeneous but generally greatest around the poles (Clem *et al.* 2020); as well as changes in other abiotic and biotic drivers (*e.g.*, drought,

urbanization, vector control measures; Franklinos *et al.* 2019). For example, urbanization or land clearing could compound the impacts of climate warming by eliminating thermal refugia provided by canopy cover (Alkama & Cescatti 2016). Ongoing work on mosquito population responses to temperature in the face of concurrent global changes will help to further refine estimates of warming-associated risk.

Despite these complexities, our findings are largely in agreement with patterns of ectotherm range shifts in response to warming, suggesting our metric provides meaningful information on mosquito climate vulnerability. Our results suggest that the subtropics may be the region most likely to experience shifts in the seasonality and/or intensity of mosquito-borne disease transmission in coming decades, potentially necessitating shifts in the types and timing of vector control strategies for effective disease prevention. Further, the tropics—which currently experience the highest mosquito-borne disease burden—could remain relatively favorable for transmission under near-term climate warming, highlighting the need for sustained vector control in this region.

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