Running head – Seasonality in mosquito thermal tolerances

Community-wide seasonal shifts in thermal tolerances of mosquitoes

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Abstract

The broadening in species' thermal tolerance limits and breadth from tropical to temperate latitudes is proposed to reflect spatial gradients in temperature seasonality, but the importance of seasonal shifts in thermal tolerances within and across locations is much less appreciated. We performed thermal assays to examine the maximum and minimum critical temperatures (CT_{max} and CT_{min}, respectively) of a mosquito community across their active seasons. Mosquito CT_{min} tracked seasonal shifts in temperature, whereas CT_{max} tracked a counter-gradient pattern with lowest heat tolerances in summer. Mosquito thermal breadth decreased from spring to summer and then increased from summer to autumn. We show a temporal dichotomy in thermal tolerances with thermal breadths of temperate organisms in summer reflecting those of the tropics ('tropicalization') that is sandwiched between a spring and autumn 'temperatization'. Therefore, our tolerance patterns at a single temperate latitude recapitulate classical patterns across latitude. These findings highlight the need to better understand the temporal and spatial components of thermotolerance variation, including plasticity and rapid seasonal selection, and the potential for this variation to affect species responses to climate change. With summers becoming longer and increasing winter nighttime temperatures, we expect increasing tropicalization of species thermal tolerances in both space and time.

Keywords

Acclimation, hardening, physiological plasticity, climate change, vector-borne disease, ecophysiology.

Introduction

Thermal tolerance limits reflect the environments in which species can persist, thus influencing species potential geographic distributions (Addo-Bediako et al. 2000, Gaston et al. 2009, Overgaard and MacMillan 2017). Environmental variability represents an important selection pressure that can influence species' thermal tolerance limits (Chevin et al. 2010, Hoffmann and Sgrò 2011, Seebacher et al. 2015), particularly for ectotherms. For instance, thermal tolerances broaden towards higher latitudes following greater seasonal variation in temperature, whereas there is a constriction in thermal tolerances towards the more thermally stable tropics (Ghalambor 2006, Sunday et al. 2011). The climate variability hypothesis states that broadened thermal limits in temperate zones result from exposure to wide ranging temperatures (Janzen 1967), including both increased basal thermal breadth and greater ability to adjust thermal traits to fluctuating environmental conditions via acclimation plasticity (Addo-Bediako et al. 2000, Overgaard et al. 2014, Phillips et al. 2016, Bacigalupe et al. 2018). Broad thermal limits might allow species to cope with increasing and more variable temperature regimes, dictating their vulnerability to current global climate change (Overgaard et al. 2011, Seebacher et al. 2015, Gunderson and Stillman 2015).

Some species can quickly adjust their thermal traits to climatic exposure, especially small-bodied organisms with short life cycles (Rohr et al. 2018) as illustrated in many acclimation studies carried out under controlled laboratory conditions (Chown et al. 2009, Schulte et al. 2011). Similarly, there is a substantial literature on ectotherm overwintering biology that shows individuals sampled from the field during winter dormancy have substantially lower thermal limits than individuals sampled from the active season (Angilletta 2009, Denlinger and Lee 2010). However, many studies predicting organismal responses to climate change treat high and low temperature tolerances as though they are locally adapted, but phenotypically fixed (Addo-Bediako et al. 2000). Furthermore, although some authors have suggested that phenotypic plasticity in high and low temperature tolerances may be able to buffer ectotherms from climate change (Sgrò, C. M. et al. 2016, Rohr et al. 2018), the data have not always been consistent with this view and the topic is currently under debate (Gunderson and Stillman 2015).

Environmental thermal variability is particularly evident in sub-tropical and temperate systems, where organisms experience a greater range of temperatures across seasons relative to tropical systems at similar elevations. If organismal thermal limits reflect climatic exposure, seasonal changes in temperature should yield predictable phenotypic responses—either plastic (i.e., acclimation) or rapid seasonal selection (Layne et al. 1987, Sanabria et al. 2012, Bergland et al. 2014, Sharma et al. 2015, Anderson et al. 2018). Latitudinal patterns of thermal tolerances have been observed across multiple taxonomic groups, with relatively narrow thermal breadths in the tropics and broadened thermal breadths towards higher latitudes (Addo-Bediako et al. 2000, Sunday et al. 2011). Whether this pattern broadly plays out in time as temperature changes from spring (cold-warm) to summer (warm-hot) to autumn (hot-cold) remains largely unexplored in the field (Figure 1 and 2a).

As a field, we are currently limited in our knowledge of the prevalence of shifts in both upper and lower thermal limits of organisms during their active season under ecologically realistic settings, as well as the magnitude of these phenotypic shifts in thermotolerances (Nussey et al. 2007, Valladares et al. 2007, Forsman 2015), particularly in response to seasonal variation in environmental temperature (Brown and Feldmeth 1971, Hu and Appel 2004, Arnan et al. 2015). For example, it is often assumed that upper thermal limits are fixed with little variance from tropical to temperate regions (Sunday et al. 2011) and thus this invariance in upper thermal limits will limit the ability of ectotherms to respond to climate change. Yet, we know that both upper and lower thermal limits can change throughout the year either as a product of thermally dependent phenotypic plasticity (i.e., acclimation, Rohr et al. 2018) or, in ectotherms with short generation times, rapid seasonal selection (Bergland et al. 2014). For example, Bujan et al. (2020) recently showed that upper thermal limits were several degrees higher in late summer than in early spring in multiple species within a community of ants in a highly thermally seasonal site in Oklahoma, USA. Understanding the potential for organisms to adjust their thermotolerance distributions through space and time requires repeated measures of upper and lower thermal traits collected over a range of environmental conditions experienced by natural populations, species, and even whole communities across seasons.

Here, we test the extent to which the distribution of thermal tolerances change predictably across seasons in an entire mosquito community, akin to spatial patterns in thermal tolerance limits and breadth across latitude (Addo-Bediako et al. 2000, Sunday et al. 2011). Although some evidence exists showing that thermal parameters can change seasonally in the field (Brown and Feldmeth 1971, Houghton and Shoup 2014, Anderson et al. 2018), to our knowledge none of these studies have tracked shifts in both upper and lower thermal limits across time or taken a whole-community approach. We chose mosquitoes as our model system because they are a speciose group of insects with life cycles and activity that span across seasons, as well as providing an important cross-disciplinary opportunity to assess disease vector responses to climate change because they are responsible for a large number of diseases transmitted to humans and other animals. Furthermore, temperature is known to affect both population dynamics and vector competency of mosquito species (Paaijmans et al. 2009, Lambrechts et al. 2011, Sternberg and Thomas 2014, Mordecai et al. 2019). It is therefore crucial to understand spatial and temporal dynamics of mosquito populations both within and across species (Li et al. 2014), and in response to changes in their environment (Sgrò et al. 2016).

We show clear shifts in upper and lower thermal tolerances and thermal breadth across the mosquito community over the timespan of one active season. Mosquitos shifted from more temperate-like thermal tolerance breadths in the spring, to more tropical patterns of narrow thermal breadth in summer, back to broader thermal patterns in the autumn. It is beyond the scope of this study to distinguish the extent to which these predictable shifts in thermal phenotypic distributions are due to selection on basal tolerances across time, due to plasticity (i.e., acclimation), or some combination of both. However, we suggest that explicitly incorporating predictable seasonal variation in thermotolerances nested within and among sites along latitudinal and altitudinal gradients should improve our ability to accurately predict how species ranges and other responses, like activity, may shift given future climate scenarios.

Methods

Study area

We sampled mosquitoes across a heterogeneous landscape in northern Florida, USA between April-October 2017. Our study area consisted of variable levels of forest cover, from low density, high solar radiation forests (e.g., *Pinus palustris, Pinus echinate, Pinus glabra*), to high density, low solar radiation forests (e.g., *Quercus virginia, Quercus alba, Quercus laevis*). The survey area spanned ~34 km from the city of Gainesville (29°39’N, 82°19’E) to the Ordway-Swisher Biological Station (OSBS) (29°41’N and 82°W), an National Ecological Observatory Network site.

Sampling design

We used John Hock New standard miniature light traps (Model 1012, 6VDC associated with an Insulated Dry-ice container; http://johnwhock.com/) to capture mosquitoes. Mosquitoes were attracted by a standardized release of CO_2 gas from melting dry ice and were collected by a mesh bag attached to a fan. Each trap was filled with approximately 2.5 kg of dry ice pellets. Once deployed, a light sensor on the trap triggered gas release at a standardized starting time of 18:00 and all traps continued to collect mosquitos until they were retrieved the following day at 08:00 (10 hours sampling effort per trap). We chose this sampling period because mosquitoes are most active during crepuscular and nocturnal hours (Lumsden 1952). All collected mosquitoes were immediately transported to an experimental facility for critical temperature measurements.

To account for diverse species-specific habitat preferences, we attempted to always place mosquito traps in two sites per day per location (i.e., location between Gainesville and OSBS). On each day of sampling, we attempted to always pair one survey site with limited vegetation (open, low canopy cover) with a second survey site with complex vegetation (closed, high canopy cover). In total, across 47 survey days, we sampled 71 sites (30 open and 41 closed sites) from which we placed a mosquito trap at ground height and a second trap in the canopy to sample different microhabitats (noting that not all open habitats contained a tree for canopy sampling) (Donoso et al. 2010). Each site was sampled one time. In total, we sampled 131 microhabitats (ground or canopy) over the course of this study (61 microhabitat samples in Gainesville and 70 microhabitat samples in OSBS). Ground traps were placed at < 1.5 m above-ground, whereas canopy traps heights ranged from 5 to 18 m above-ground. Each tree used to deploy a canopy trap was randomly selected, but we ensured it was within 20 m of the ground trap and that each tree had a sufficiently strong branch to hold the weight of a light trap suspended by paracord.

Environmental data

We characterized microclimatic conditions at each site by monitoring air temperature every 15 minutes during sampling hours using a HOBO pendant data logger (model #U23-002). Each mosquito trap contained a logger and each logger was installed below a weather shield to block direct solar radiation and precipitation. We further characterized macroclimatic conditions using mean daily temperature extracted from nearby weather stations. For samples taken in and around the city of Gainesville, we used temperature data from a weather station located at Gainesville's regional airport. For samples taken at the OSBS, we used temperature data from a weather station located at OSBS. For simplicity, we refer to microclimatic conditions as microhabitat temperature and macroclimate conditions as environmental temperature.

Thermal tolerance trials

The critical thermal minimum and maximum of ectotherms are commonly used to infer organismal thermal constraints (Hutchison 1961). Here, we follow Hazell and Bale (Hazell and Bale 2011) and use the same response across individuals—the temperature at which coordination and response is lost during a ramped exposure to low or high temperatures to indicate CT_{min} and CT_{max} respectively (Scheffers et al. 2014, Overgaard and MacMillan 2017).

In total, our sampling across 71 sites yielded 28,406 total individual mosquitoes collected representing 18 species. From each trap, we haphazardly selected up to 10 individuals (not knowing species identity) and obtained their critical thermal limits (10 individuals for CT_{min} and

10 for CT_{max} respectively, for a total of 20 individuals phenotyped per trap). Mosquitoes were removed from mesh bag traps using an aspirator and each individual was placed into its own glass vial (3 cm diameter and 10 cm long). Each vial was sealed with a piece of cotton followed by a lid, placed in a temperature-controlled water bath (Lauda Eco Silver RE 1050) and floated on its side. We had a total of 10 vials in the water bath per experimental trial. Critical thermal limit trials consisted of exposing mosquitoes to a constant ramping rate of 0.5 °C/min and we frequently rotated each vial to elicit a response from mosquitoes. No response from an individual indicated that it reached its thermal limit. For all experiments, we held a starting temperature of 22°C for 15 min after which the ramping began. This step assured that all runs started at the exact same temperature and all mosquitoes were able to acclimate to that starting temperature. Our experimental design was the same for critical thermal minima and maxima. All experiments were completed within a four-hour period each morning.

We recorded within-vial air temperature for four of the ten vials in each heating or cooling trial using a copper-constantan thermocouple (data recorded by a Sable Systems TC-2000 and Expedata Software) and water bath temperature using a built-in water bath thermister (data recorded by LAUDA Wintherm Plus software). We then calculated the within-vial air temperatures across all 10 vials by modeling the relationship between vial and water bath temperature using linear regression. We used this analysis to convert water bath CT_{min} and CT_{max} temperatures to vial air temperatures ($Y_{min}=0.804x+5.044$ and $Y_{max}=0.878x+3.380$). Importantly, the CT_{min} and CT_{max} reported herein represent the relative hardiness to low and high temperatures of mosquito species, not the environmental temperature at which populations go extinct. We determined species thermal breadths as the difference between the mean CT_{min} and the mean CT_{max} measured from individuals of a species collected on the same day.

Statistical analyses

Organisms can acclimate to changes in their environment and short-lived organisms can also undergo rapid seasonal selection, thereby adjusting their physiology to maintain performance across a range of environmental conditions (Allen et al. 2016). However, acclimation (or rapid seasonal selection) can occur at a variety of timescales from days to weeks to months. Thus, a measure of organismal thermal tolerance may reflect environmental conditions experienced prior to their collection from the field. To more accurately assess the time scale of how quickly changes in thermal tolerances occurred in our study, we examined the relationship between a mosquito's environmental exposure in time prior to its capture and its thermal tolerance limits. To do this, we derived a mean temperature across a range of exposure intervals ranging from 1 to 30 days prior to each individual date of capture. Using ordinary least square models, we compared the fit of models that correlated mosquitoes' thermal tolerances against air temperatures derived from these exposure intervals. The number of days prior to capture that yielded the strongest fit between air temperature and thermal limits indicates a plausible period in the wild where thermal exposure best influences thermal tolerance limits. We used these air temperature values in subsequent analyses.

We used a linear mixed effect (LME) model framework to assess whether mosquitos' thermal phenotypes (CT_{min}, $CT_{max,}$ and thermal breadth) track changes in temperature through time. The structure of our models included a random intercept and random slope term within species to account for differences in thermal phenotypes arising from different lineages (e.g., when phylogenetic information is lacking for fully considering species relatedness).

First, we assessed whether mosquito thermal tolerance limits and breadth change throughout time using Julian day as a predictor variable (fixed-effect). Given that temperature increases from spring to summer and decreases from summer to autumn (Fig. 2a), we added a quadratic term to Julian day using the model formula:

$y \sim \mathrm{poly}(Julian, 2)$, Random = $\sim \mathrm{poly}(Julian,2)|Species$

Next, we assessed whether mosquito thermal tolerance limits and breadth track changes in temperature. Here, we fitted two separate models based on two aspects of thermal exposure. The first model used environmental temperature (captured by macroclimate/regional air temperatures) as the predictor variable, whereas the second model used microclimatic temperature (representing exposure to air temperature at the site and time of collection) as the predictor variable. For each temperature type, we adopted the following model formula:

$y \sim Temperature$, Random = $\sim Temperature|Species$)

We investigated species level differences in thermal tolerance limits and breadth across seasons by fitting species-specific linear models. These models include the same fixed terms as in the above-mentioned models but without the random effect term of species. We ran these linear models for each species with five or more observations as smaller sample sizes preclude the explanatory power of our models.

We performed additional analyses to assess the potential effect of body mass on mosquitos' thermal tolerances. Body mass may influence species thermal tolerances via its effect on acclimation potential (Rohr et al. 2018). As such, we included body mass as a fixed effect term to the above aforementioned CT_{min} and CT_{max} models. Adding this variable did not dramatically improve the fit of any model, and thus we excluded body mass from further consideration (Supporting Information Table S1).

Model performance was accessed using Akaike information criterion (AICc) and adjusted R^2 following Nagelkerke's formula (Nagelkerke 1991). Predictor variables were scaled by one standard deviation before analysis to produce standardized coefficients (slopes). All analyses were performed in R using the package 'nlme' (Pinheiro et al. 2018) and 'MuMIn' (Bartoń 2017).

We calculated thermal safety margins for each species by subtracting the thermal tolerance (CT_{min} or CT_{max}) from the minimum or maximum temperature of the previous 11 days (see above methods for details), respectively. A negative thermal-safety margin indicates that the environmental temperature has surpassed a species' thermal tolerance limit and should cause physiological failure and/or death. We fitted a linear model and a polynomial model to display and visualize general patterns of thermal-safety margins changing in response to the mean minimum and maximum temperature of prior 11 days and Julian day, respectively.

Results

Temperature tracked a hump-shaped path through time, increasing from spring to summer (slope = 0.81, P-value < 0.001) and decreasing from summer to autumn (slope = -2.13, P-value < 0.001) (Figure 2a and Supporting Information Figure S1). The minimum temperature recorded was 9.5°C, and the maximum temperature recorded was 36.1°C.

We measured thermal tolerance for 962 individuals (CT_{min} = 520, CT_{max} = 442) from 18 species (Figure 2b). We calculated thermal breadth for 13 out of 18 mosquito species, from which we had measures of both CT_{min} and CT_{max} from the same sampling day. The most abundant species were from the genus *Culex* (e.g., *C. erraticus*, *C. nigripapus*, and *C.*

quinquefasciatus) and *Aedes* (e.g., *A. infirmatus* and *A. vexans*) (Figure 2b). Most genera remained present over the entire sampling season, meaning that community composition did not vary considerably over the study period (Figure 2b).

Mosquito CT_{min} was best approximated by temperature in the last 11 days from when they were collected from the field (Supporting Information Figure S2). The correlation between CT_{max} and temperature was largely invariant to increments in days before sampling (R^2 always < 0.1). Qualitatively similar results were obtained using either micro-climate temperature or environmental temperature, but the strongest correlations were obtained using environmental temperature as a predictor of mosquitoes' CTs (Supporting Information Figure S2). For consistency, we adopted the same exposure interval of 11 days for CT_{min} and CT_{max}.

Mosquitoes' CT_{min} values were lower than the minimum environmental temperature recorded during our sampling period, and their CT_{max} values were higher than the maximum-recorded temperature (Figure 3). This indicates mosquitoes in our study area operate in a thermal safe zone relative to exposure to environmental temperature (Figure 3). The same pattern is evident at the microclimate scale, with most species showing CT_{min} values smaller than the minimum-recorded temperature (11 out of 15 species) and CT_{max} values higher than the maximum-recorded temperature (14 out of 15 species) (Supporting Information Figure S4). However, instances of thermal danger did occur during cool autumn (4 species) and warm summer conditions (1 species) (Supporting Information Figure S5).

Mosquito CT_{min} increased with time during the first period of our sampling season following the increase in environmental temperature from spring to summer and decreased towards the end of our sampling season following the decrease in environmental temperature from summer to autumn (Fig 4C, Supporting Information Table S2). The CT_{max} values for mosquitoes decreased from spring to summer and then increased from summer to autumn (Fig 4A), although the slope and variance explained of the polynomial model for CT_{max} over time was nearly half of those observed for CT_{min} (Supporting Information Table S2). As a result, community-wide, mosquito thermal breadth decreased in the summer period, returning to spring levels in autumn (Fig 4E; Supporting Information Table S2).

Mosquito's CT_{min} followed seasonal changes in environmental temperature (Fig 4D, Fig 5). The effect of environmental temperature on mosquito's CT_{min} was positive, with 7 of the 9 modeled species showing a significant increase in their CT_{min} in response to temperature (Fig 4D, Fig 5). In contrast to expectations, mosquito's CT_{max} declined with increases in environmental temperature (Fig 4B). The effect of environmental temperature on CT_{max} was not as strong as its effect on CT_{min} (~2.45 times smaller, Fig 5), with only 2 of 10 modeled species showing a significant response between environmental temperature and CT_{max}. Thermal breadth of mosquitoes also decreased with increasing environmental temperature (Fig 4F, Fig 5). The reduction observed in mosquito's thermal breadth resulted primarily from increases in CT_{min}, as CT_{max} showed little change with temperature. Environmental temperature was a better predictor of thermal tolerance than microhabitat temperature, but similar trends hold for microhabitat climate data (Supporting Information Figure S6 and S7).

Discussion

An increase in thermal breadth with the spatial gradient of latitude from tropical to temperate locations is considered a macrophysiological rule (Gaston et al. 2009, Sunday et al. 2011, Franks et al. 2014, Rezende et al. 2014). Our study shows clear shifts in both high and low thermal tolerances, as well as in thermal breadth of an entire mosquito community across time at a single latitude that recapitulates the vast majority of the larger-scale latitudinal pattern generalized across diverse taxa. Specifically, mosquito thermal breadth decreased from spring (cold) to summer (hot) and then increased towards the autumn (cold). This constriction of thermal breadth from spring to summer and re-expansion from summer to autumn at our single site suggests that latitudinal patterns in organismal thermal biology may result dynamically from seasonal shifts in thermal traits (e.g., compare Figures 1 and 4c).

Spatiotemporal patterns in thermal tolerances appear to be highly nested, whereby seasonal shifts in thermal breadth within a temperate latitude band reflect inter-latitudinal patterns in thermal biology. As noted by Sunday et al. (2011), considerable inter-specific variation in thermal tolerances exists at sub-tropical and temperate latitudes. Our study on mosquitos suggests that the thermal variation observed by macroecological studies within each latitudinal band may not simply be random variability, but rather predictably structured variation that may result from thermal traits shifting with seasons both within and across species, at least in some organisms (Figure 1).

We show thermal breadth waxes and wanes with seasonal changes in temperature at our single latitude rather than staying static in time (Figure 4). Although our study was not designed to distinguish the extent to which these shifts in thermal tolerances are due to rapid selection on basal tolerances across time (e.g., Bergland et al. (2014)) or due to acclimation plasticity, the response we observed is consistent with both mechanisms. We found that species' thermal tolerances during the summer resemble 'classical' tropical species whereas during spring and autumn thermal tolerances resemble 'classical' temperate species (Figure 1). This manifests in a community-wide summertime physiological *tropicalization* of animals (thermal breadths of temperate organisms that reflect those of the tropics) that is sandwiched between a spring and autumn *temperatization* owing to overall greater climate variability. Under climate change, communities are becoming increasingly thermophilic (often referenced as *tropicalization* or *borealization*) whereby cold-adapted species are being replaced by warm-adapted species in non-tropical ecosystems (De Frenne et al. 2013). As such, with summers expected to become longer due to climate change and with nights on the seasonal shoulders expected to become warmer, we might expect an increasing *tropicalization* of temperate species with wide ranging implications from species persistence to activity times to whole community interactions.

Evidence for thermal acclimation via laboratory experiments (Hoffmann et al. 2005, Rohr et al. 2018), as well as spatial shifts in thermal traits (Sunday et al. 2014, Seebacher et al. 2015, Sgrò et al. 2016), are widespread. For example, thermal limits have been shown to shift across climate gradients formed by habitat (e.g., forest fragmentation; (Phillips et al. 2016), elevation (Sheldon and Tewksbury 2014, Slatyer et al. 2016) and latitude (Addo-Bediako et al. 2000, Sunday et al. 2014). Conversely, temporal shifts in thermal breadth are poorly documented in nature within species, much less across entire communities. Our study indicates that a diverse community of mosquitoes seasonally adjust their thermal tolerance parameters and do so relatively quickly (~11 days) in response to changes in temperature. The observed seasonal changes in tolerance occur mainly in lower thermal limits (Terblanche et al. 2007), agreeing with previous studies suggesting stronger physiological constraints on upper thermal limits (Vorhees et al. 2013, Gunderson and Stillman 2015). That said, we observed a counter-gradient pattern in CT_{max} as tolerances to heat were lowest in summer and highest during spring and autumn; with the most pronounced counter-gradient pattern occurring from summer to autumn. There is a growing literature showing support of counter-gradient patterns between CT_{max} and environmental temperatures in lizards (e.g., Llewelyn et al 2016 and Hodgson and Schwanz

2019) and our study extends this phenomenon to invertebrates. Counter-gradient patterns may be triggered by exhaustive exposure to high temperatures (e.g., during summer) that drives behavioral thermoregulatory shifts towards cooler microhabitats. Under such a scenario, behavioral avoidance of very warm temperatures when the risks of high-temperature stress are greatest, can then lead to lowering of CT_{max} through less exposure to warm temperatures and thus less high-temperature acclimation or less selection for high-temperature tolerance. While reports of counter-gradient patterns in CT_{max} are few at this point, to our knowledge our study is the first to show such a pattern in a seasonal context. Given the importance of CT_{max} for forecasting potential negative effects of climate change, and particularly impacts of extreme high temperature events, more consideration of this phenomenon in natural populations under seasonal cycles is warranted.

The lifespan of a mosquito can range from a few days up to two months or so, depending on weather conditions and food availability. Due to the short timescale involved in the response of CT_{min} and CT_{max} to recent environmental temperatures, 11 days, we expect that acclimation plasticity plays an important role in shaping the observed patterns of summer *tropicalization* and *temperatization* in spring and fall in our mosquito community. However, owing to the relatively short lifespan of many mosquito species, it is also possible that shifts in thermal tolerances are due to rapid seasonal selection, as has been shown to occur in field populations of the short-lived fly *Drosophila melanogaster* (Bergland et al. 2014). Partitioning the relative importance of selection, acclimation plasticity, and even selection for acclimation plasticity should be the focus of future work. Understanding the contributions of each of these mechanisms for shaping thermal tolerances would allow better prediction of the effects of exposure to thermal variation on species in a changing world.

One potential systematic bias in sampling that has not been well explored in previous studies on thermal limits or thermal breadth is how the timing of seasonal sampling may affect these parameters. By sampling across seasons in which mosquitos are active, our temporal span and resolution allowed us to capture a larger range of thermal responses present across the whole community, which is fundamental for multivoltine species with rapid generation times (Nadeau et al. 2017). Similarly, Bujan et al. (2020) recently showed that CT_{max} increased from spring to summer in several species within an ant community at a temperate site. Furthermore, both (Ragland and Kingsolver 2008) and Sheldon and Tewksbury (2014) show that temperature variation restricted to the months species are active was a better predictor of thermal tolerances in mosquitoes and beetles than was annual seasonality. Together, these studies suggest that consideration of time within a year when animals are sampled in the field is equally important as the latitude at which they are sampled to avoid methodological bias in tolerance estimates. For example, without a temporal consideration, macro-ecological studies exploring thermal tolerance limits at temperate latitudes within the bounds of summer, will likely observe a dampening of thermal breadth (i.e., thermal breadths of temperate species that are more equivalent to tropical species). This may not only lead to investigators missing important predictable variability in tolerance limits that exists within populations, but it may also explain some of the commonly observed overfilling of niche space in species distribution models (Sunday et al. 2014). Specifically, species frequently occur in areas that are colder than they are expected to given their cold tolerance limits. If the lower thermal tolerances for these species were estimated from locally *tropicalized* summer individuals rather than locally *temperatized* individuals from spring or fall, the estimates for lowered thermal tolerances may be systematically biased upwards indicating overfilling of the expected species range. Overall, we argue that more detailed understanding of the scale of predictable seasonal variation in thermal tolerances can have wider-ranging implications for predicting a range of climate impacts from specific populations to community interactions and ecosystem functioning.

Mosquitoes are prominent disease vectors and species from the genera *Aedes*, *Anopheles, Mansonia,* and *Culex*, all of which we observed in our study, can carry viruses that can induce human and/or wildlife diseases such as St. Louis encephalitis, Eastern equine encephalitis, and West Nile virus. Our findings suggest that in the near-term, increases in temperature due to climate change will not surpass conditions for mosquito activity in North Florida – their thermal tolerances consistently surpassed the recorded extreme temperatures in our study both at fine and coarse temperature sampling (Figure 3, Supporting Information S3, S4 and S5). We note that although the minimum recorded temperature during our study was 9.5 °C, we did not sample in the coldest months of winter and across years temperatures can reach sub-zero in our region, which would exceed the CT_{min} of some species in our study. However, species can invoke diapause or other seasonal dormancy responses to cope with seasonally predictable cold exposure. These adaptations place them into a physiologically protective state whereby high and low temperature tolerances can be substantially different than animals in the active season (Werner and Gilliam 1984, Hahn and Denlinger 2007, Ragland and Kingsolver 2008, Ragland and Keep 2017). Species can also avoid extreme temperatures by seeking shelter within buffered microhabitats (Williams et al. 2015). Winter temperatures are considered the major factor limiting species distributions in our study region, which occurs at the southern extent of the North American freeze line. Thus, with climate change, mosquitoes may increase their annual activity time as well as expand their distribution Northward or upward in altitude, both of which can lead to greater host-vector interactions (Siraj et al. 2014). Temperature, and particularly daily and seasonal thermal variation, are emerging as important predictors of vector competency for mosquito-borne diseases (Lambrechts et al. 2011, Mordecai et al. 2019). Yet, our understanding of what internal physiological factors of individual mosquitoes may combine with mosquito population dynamics to affect disease risk is still developing (Sternberg and Thomas 2014, Thomas et al. 2018). Our work on community-wide patterns of variation in thermal breadth for activity lays a foundation for understanding seasonal patterns of disease risk within and across latitudes. Additionally, our findings were relatively consistent within species and across species (community level) suggesting that our results are likely broadly applicable to mosquito species distributed across North America, as well as likely applicable to many other species of ectotherms, from insects to amphibians (Rohr et al. 2018).

Conclusions

We show that through time at a single latitude, thermal breadth shrinks and expands with seasonal changes in temperature—predictable seasonal shifts in thermal traits that recapitulate broader latitudinal patterns. Importantly, our results suggest that to more accurately understand species vulnerability and responses to climate change, studies of species sensitivity and exposure over time as well as space are required to fully characterize a species' phenotypic range. Considering the variability in thermal limits expressed across seasons may help explain the overfilling of thermal tolerances at the cold boundary of species ranges (Sunday et al. 2014). Such understanding can inform species distribution models and can more broadly inform predictions of climate impacts from specific populations to community interactions and ecosystem functioning. Filling this knowledge gap is critical for understanding general ecological patterns across scale, intrinsic resilience to climate change (Sunday et al. 2014, Seebacher et al. 2015, Sgrò et al. 2016), and how common disease vectors might impact society in the future. In the case of our study system, mosquitos as disease vectors may phenotypically become more tropical-like in pattern as a reflection of summers becoming longer and winter night-time temperatures becoming warmer, with likely consequence for their future distribution, activity, and vector competency.

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Figures legends

Figure 1. Conceptual figure highlighting our main hypothesis in terms of spatio-temporal temperature variation and its impact on mosquito thermal tolerance. Sunday et al. 2011 suggests increasing thermal breadth with latitude due to largely fixed CT_{max} and substantial lowering of CT_{min}. In addition to space, temperature also changes seasonally through time. We predict lower CT_{min} during Spring and Autumn and higher CT_{min} during the summer. CT_{max} is predicted to stay constant. As a result, thermal breadth should reflect the same pattern in time at a single location (latitude) as it does across locations separated by latitude. As a result, temperate latitudes are ‘tropical’ during the summer and ‘temperate’ during the spring and autumn.

Figure 2. Variation in environmental temperature and species composition through time. Environmental temperature varied considerably through time over the sampling season (A). Most species occurred over the entire sampling season (B). In (A), black circles represent mean daily environmental temperature and the black line represents the fit of a polynomial function. In (B), circles represent species occurrence. For seasonal variation in microclimate temperature refer to Supporting Information Figure S3.

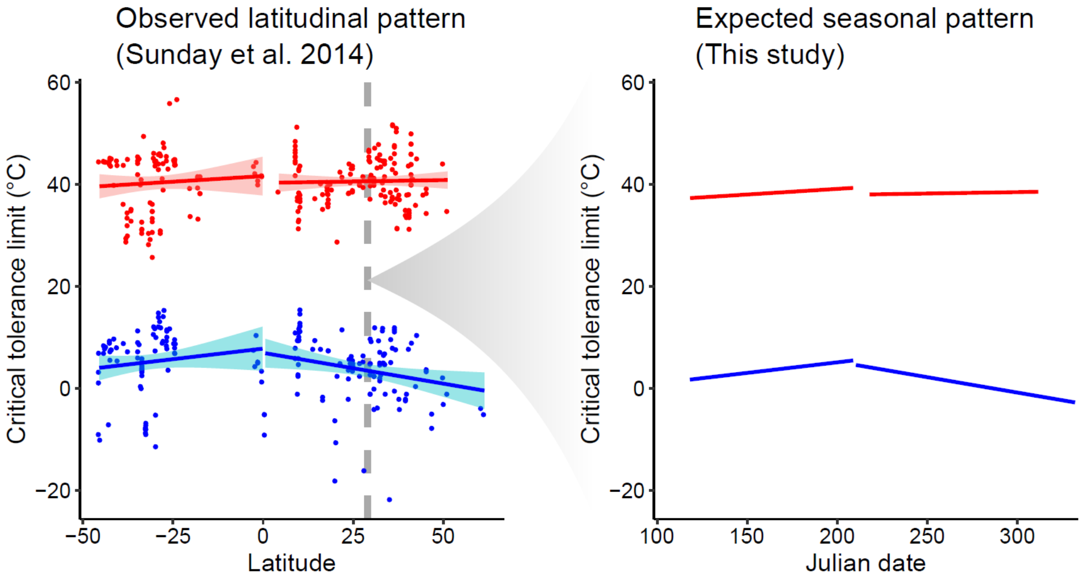
Figure 3. Thermal safety margins of mosquitoes in North Florida. The white areas of the diagrams (A) and (B) show where species have a physiological thermal-safety margin to the mean minimum temperatures (A) or to the mean maximum temperatures (B) of the previous 11 days. Diagrams (C) and (D) show thermal-safety margins of species as a function of Julian day based on the difference between the mean minimum environmental temperature observed and their CT_{min} (C) and the difference between their CT_{max} and the mean maximum environmental temperature observed (D). Similar trends hold for thermal safety margins measured at the scale of the microclimate (Supporting Information Figure S5). Lines are derived from a linear model and a polynomical model to visualize general patterns of thermal-safety margins changing in response to temperature (A and B) and Julian day (C and D), respectively

Figure 4. Responses of mosquito thermal tolerances to time (Julian day) and environmental temperature. Observations (dots) and regression lines are color coded by species. Black lines represent general patterns regardless of species. The responses of mosquito thermal tolerances to time (A, C and E) were modeled using 2nd ordered polynomial regression model within a mixed-effect framework [y ~ poly(x,2)] whereas the responses of mosquito thermal tolerances to

environmental temperature (B, D and F) were modeled using linear regressions. Similar trends hold for relationships of mosquito thermal tolerances and temperature measured at the scale of the microclimate (Supporting Information Figure S6). Lines are connecting fitted values from the linear mixed-effects model.

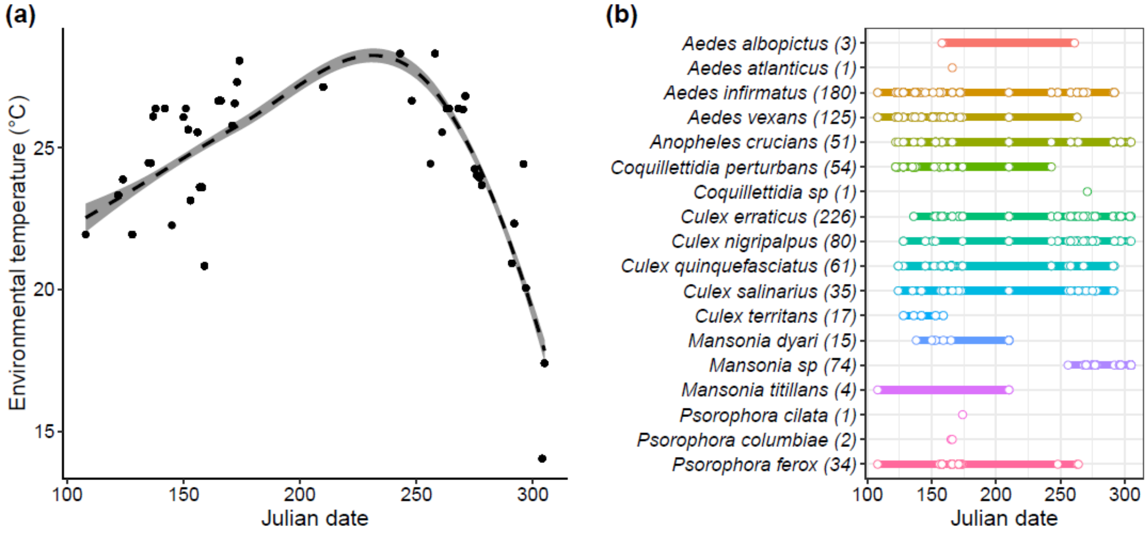
Figure 5. Effect of environmental temperature on mosquito thermal tolerance. Environmental temperature had a positive effect on mosquito CT_{min}, but a negative effect on their CT_{max} and thermal breadth. Most species showed seasonal shifts in their CT_{min} (7 out of 9), and thermal breadth (5 out of 8), but only a few showed seasonal shifts in their CT_{max} (2 out of 10). Refer to Supporting Information Figure S4 for the relationships between mosquito thermal tolerance and temperature measured at the scale of the microhabitat. Similar trends hold for relationships between mosquito thermal tolerance and temperature measured at the scale of the microhabitat (Supporting Information Figure S6 and S7).

Figure 1

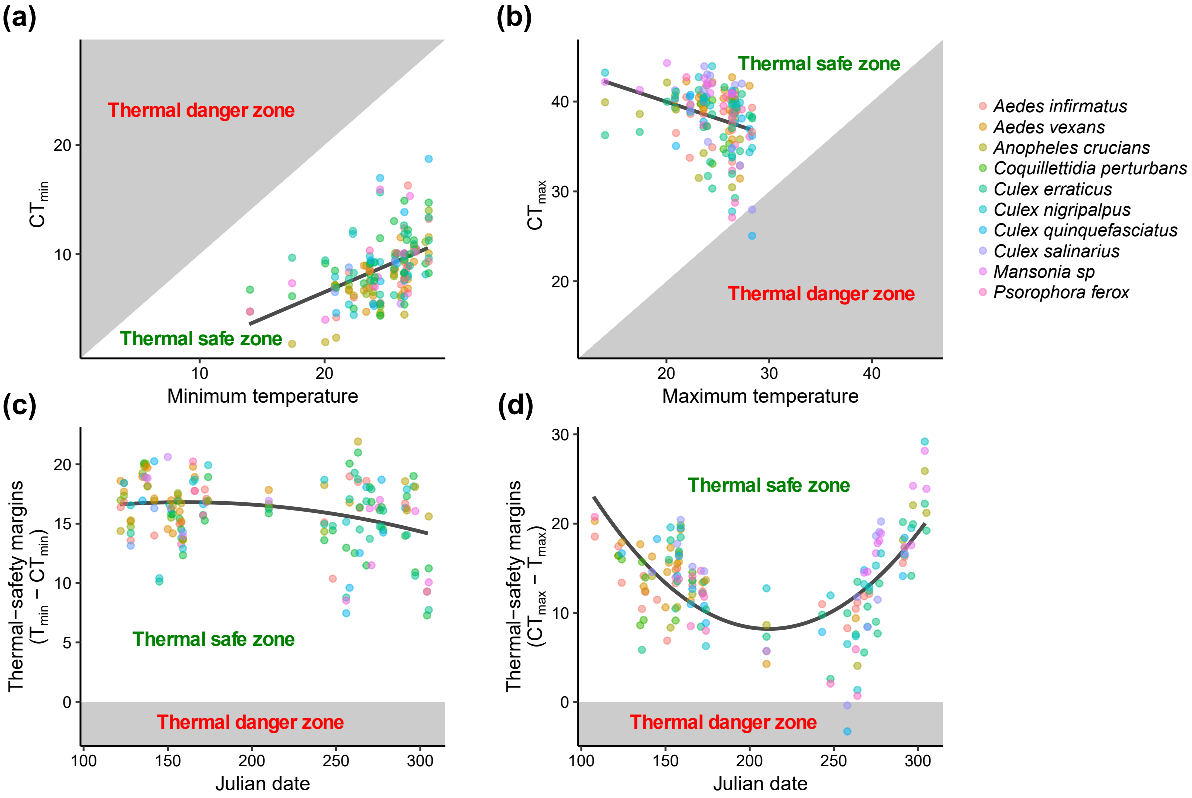


Observed latitudinal pattern
(Sunday et al. 2014)
Expected seasonal pattern
(This study)
Critical tolerance limit (°C)
60
40
20
0
−20
−50
−25
0
25
50
Latitude
Critical tolerance limit (°C)
60
40
20
0
−20
100
150
200
250
300
Julian date

Figure 2


(a)
Environmental temperature (°C)
15
20
25
100
150
200
250
300
Julian date
(b)
Aedes albopictus (3)
Aedes atlanticus (1)
Aedes infirmatus (180)
Aedes vexans (125)
Anopheles crucians (51)
Coquillettidia perturbans (54)
Coquillettidia sp (1)
Culex erraticus (226)
Culex nigripalpus (80)
Culex quinquefasciatus (61)
Culex salinarius (35)
Culex territans (17)
Mansonia dyari (15)
Mansonia sp (74)
Mansonia titillans (4)
Psorophora cilata (1)
Psorophora columbiae (2)
Psorophora ferox (34)
100
150
200
250
300
Julian date

715 Figure 3



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717
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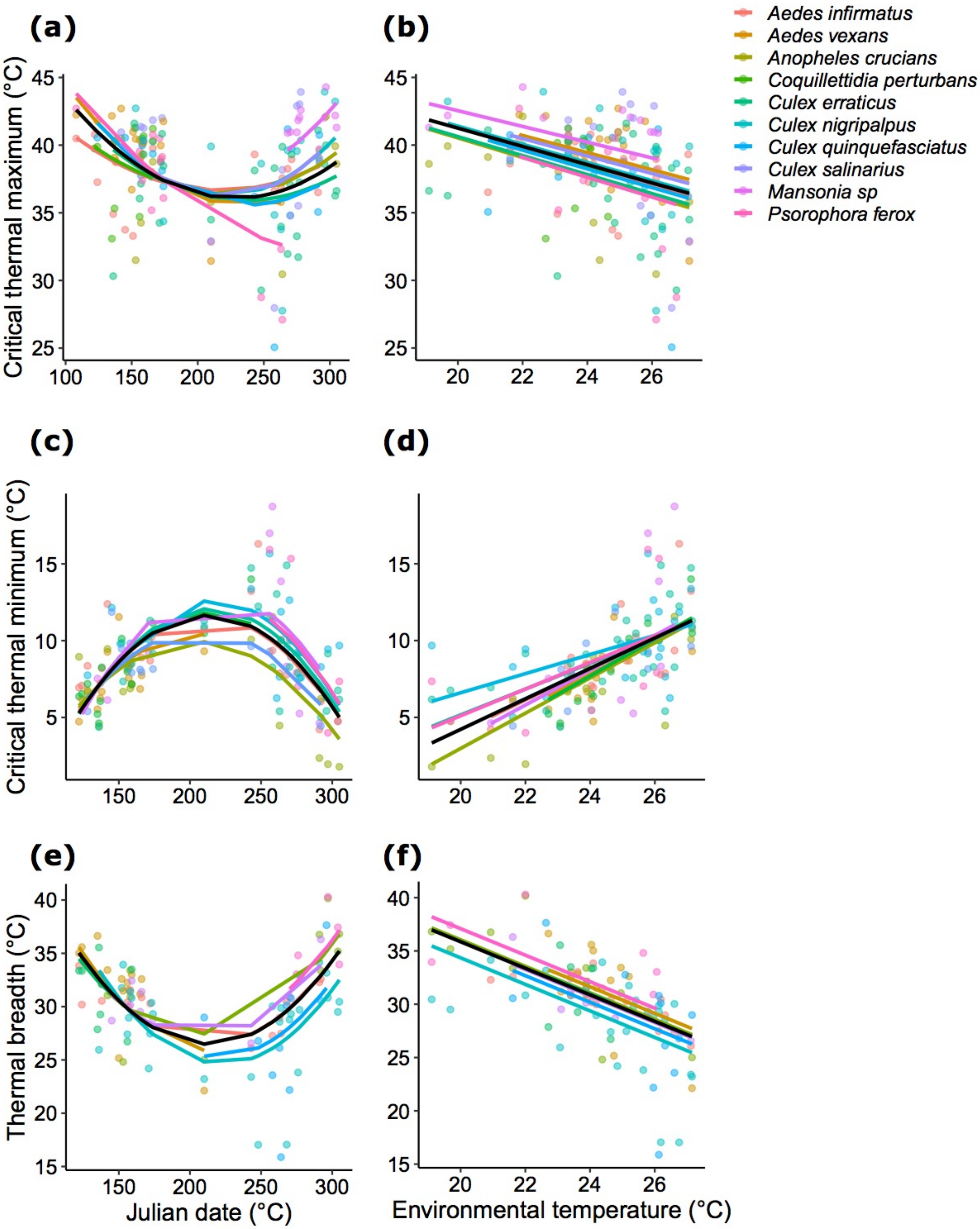
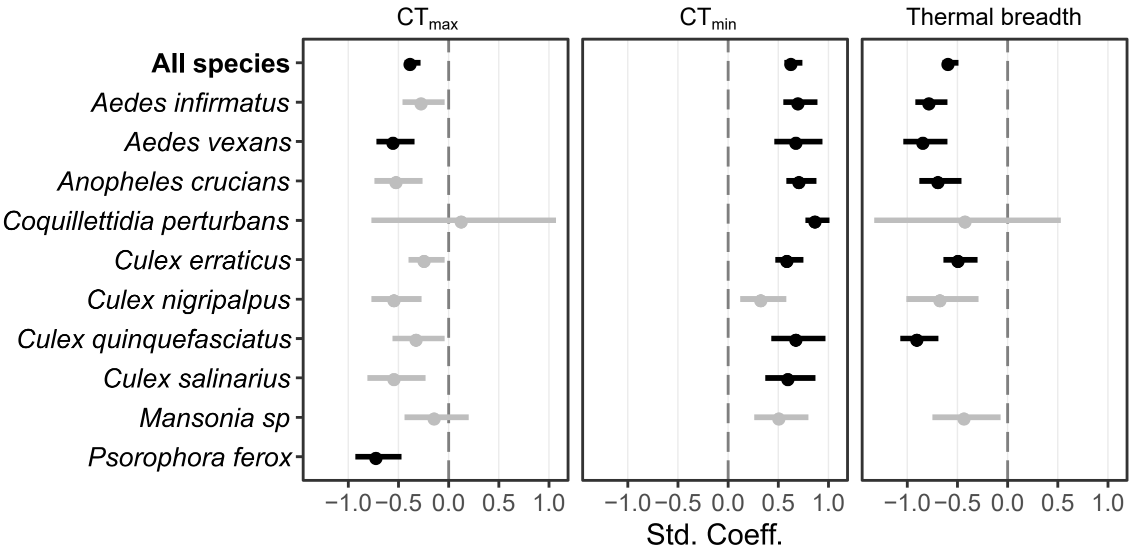
Figure 4.



(a)
(b)
(c)
(d)
(e)
(f)
Aedes infirmatus
Aedes vexans
Anopheles crucians
Coquillettidia perturbans
Culex erraticus
Culex nigripalpus
Culex quinquefasciatus
Culex salinarius
Mansonia sp
Psorophora ferox
Critical thermal maximum (°C)
Critical thermal minimum (°C)
Thermal breadth (°C)
Julian date (°C)
Environmental temperature (°C)
45
40
35
30
25
15
10
5
20
100
150
200
250
300
20
22
24
26

Figure 5.



CT_{max}
CT_{min}
Thermal breadth
All species
Aedes infirmatus
Aedes vexans
Anopheles crucians
Coquillettidia perturbans
Culex erraticus
Culex nigripalpus
Culex quinquefasciatus
Culex salinarius
Mansonia sp
Psorophora ferox
−1.0
−0.5
0.0
0.5
1.0
Std. Coeff.