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**Efecto de la temperatura ambiental en los rangos de tolerancia térmica de *Hetaerina americana* (Odonata: Calopterygidae)**

# TESIS

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# ÍNDICE

**RESUMEN**

La temperatura es un factor ambiental clave que restringe significativamente el comportamiento, la fisiología y la distribución de los organismos. Conocer la plasticidad en la fisiología térmica de las especies nos permite comprender su distribución geográfica y predecir las capacidades de respuesta en un contexto de cambio climático. Los insectos con ciclos de vida complejos en los que las larvas son acuáticas mientras que los adultos son terrestres, podrían aportar información nueva sobre los mecanismos subyacentes a las tolerancias térmicas, ya que enfrentan diferentes desafíos térmicos en cada estadio ontogenético. Los insectos del orden Odonata tienen capacidad limitada de regulación térmica, por lo que su capacidad para hacer frente a temperaturas desfavorables depende en gran medida de sus límites de tolerancia térmica, tanto en adultos como en etapas inmaduras. *Hetaerina americana* es la especie de género con la distribución geográfica más amplia (desde el sur de Canadá hasta Nicaragua), habita una gran diversidad de hábitats, desde tropical hasta templado y frío. Para evaluar si el amplio rango de condiciones térmicas que experimentan las poblaciones de esta especie en México determina la tolerancia térmica de la especie, medimos los límites térmicos críticos máximos (CTmax) y mínimos (CTmin) en machos, hembras y larvas en 3 poblaciones a lo largo de un gradiente latitudinal: La Huacana (sitio tropical) y La Mintzita (sitio templado) en el estado de Michoacán, y San Pedro Mártir en el estado de Baja California (sitio frío). Contrariamente a nuestras predicciones, encontramos que los adultos mostraron una gran plasticidad en CTmax, lo cual es consistente con las variaciones locales y estacionales en la temperatura ambiental, mientras que la variación en CTmin fue muy limitada y no refleja la temperatura ambiental. Para las larvas, la variación en los límites térmicos críticos tanto estacional

como entre las poblaciones también fue limitada, pero los límites CTmax y CTmin fueron más altos que en los adultos. La mayor amplitud de tolerancia térmica de las larvas podría promover el establecimiento y la persistencia de la especie en lugares con temperaturas extremas o desfavorables. Esto podría explicar la amplia distribución de la especie y proporcionar algunas pistas sobre los mecanismos de adaptación térmica.

**Palabras clave**: tolerancia térmica, Hetaerina americana, temperatura ambiental, plasticidad, distribución.

## ABSTRACT

Temperature is a key environmental factor that significantly restricts behavior, physiology and distribution of organisms. Knowing the plasticity in the thermal physiology of the species allows us to understand its geographical distribution and predict response capacities in a context of climate change. Insects with complex life cycles in which larvae are aquatic while adults are terrestrial, could bring brand new information about the mechanisms underlying thermal tolerances, since they face different thermal challenges in each ontogenetic state. Insects of the order Odonata have limited thermal regulation capabilities, so their ability to cope with unfavorable temperatures depends largely on their thermal tolerance limits, both adults and immature stages. *Hetaerina americana* is the gender species with the widest geographic distribution (from southern Canada to Nicaragua), inhabits a great diversity of habitat conditions from tropical to temperate and cold. To assess whether the wide thermal conditions experienced by populations of this species in México set their thermal tolerances, we measured the maximum (CTmax) and minimum (CTmin) critical thermal limits in males, females and larvae in 3 populations along a latitudinal gradient: La Huacana (tropical site) and La Mintzita (temperate site) in the state of Michoacán, and San Pedro Mártir in the state of Baja California (cold site). Contrary to our predictions, we found that adults showed great plasticity in CTmax that were consistent with local and seasonal variations in environmental temperature, while variation in CTmin were very narrow and did not track environmental temperature. For larvae, both seasonal and interpopulation variation in critical thermal limits were also narrow, but CTmax and CTmin limits were higher than in adults. The broader thermal breadth of larvae could promote the establishment and persistence of the species in places with extreme or

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unfavorable temperatures. This could explain the wide distribution of the species and provide some clues about thermal adaptation mechanisms.

## INTRODUCTION

Among the abiotic factors that affect the functionality of living organisms, temperature is one of the most important, since it affects biological systems at different levels of biological organization such as morphology, behavior and physiological processes of organisms (Angilletta, 2009 ; Bowler, 2008; MacMillan and Sinclair, 2011; Rezende et al, 2011; Allen et al, 2012; Dallas and Ketley, 2011). As a consequence of this, unfavorable temperature conditions can affect the adequacy and population density of a species (Ribeiro et al, 2012); since all organisms have an optimal temperature range to perform their activities (Sheldon and Tewksbury, 2014).

In insects, body temperature strongly depends on environmental conditions, so exposure to temperature extremes can have important physiological consequences. Low temperatures reduce insect capacity to maintain ionic homeostasis, resulting in a loss of excitability of nerves and muscles (Colinet *et al*, 2018; MacMillan and Sinclair, 2011). Very cold temperatures cause intra and extracellular freezing, while high temperatures can lead to protein denaturation or changes in the elements of cell membranes (Colinet *et al*, 2018; Williams *et al*, 2016). Physiological responses to extremes of temperature can be active (*i.e.* acclimatization) or passive (*i.e.* production of thermal shock proteins and antioxidant defenses) and can be expensive in terms of energetic resources, producing long-term effects from exposure to extreme temperatures (Williams et al. 2016). In general terms, the thermal sensitivity of insects and other ectothermal organisms may be the result of genetic adaptations, phenotypic plasticity or acclimation processes: studies in *Drosophila simulans* have suggested that genetic adaptations to heat tolerance can result from mutations (Barghi *et al*., 2019); plastic responses to extremes of temperature could promote or limit

evolutionary adaptations in insects, due to a relationship between seasonal plasticity and the gene expression variation, resulting in a limitation in the potential adaptative responses (Oostra *et al*., 2018); acclimation has been associated with the production of proteins capable of maintain and repair the structure of other damaged proteins in *D. melanogaster* (Kristensen *et al*., 2016).

To understand the processes associated with species distribution patterns, it is important to know the relationship between the physiological and behavioral responses of organisms and the environment in which they operate. In this sense, the altitudinal and latitudinal gradients have been used extensively to compare the different responses in thermal tolerance along temperature gradients (Bozinovic *et al*., 2011; Addo-Bediako *et al*., 2000; Calosi *et al*., 2010). On a macroecological scale, according to the climate variability hypothesis (CVH) as latitude increases organisms need to be able to withstand a greater variability in temperature conditions than at low latitudes (Addo-Bediako et al, 2000), for this reason, the physiological patterns in thermal tolerance in insects are shaped by the thermal environment in which they develop. It has been described a similar pattern at higher altitudes, where seasonal temperature should allow species to have a wide range of thermal tolerance than species of low altitudes (Shah *et al*, 2017) It has also been suggested that thermal tolerance in insects may be plastic, these plastic responses can be induced during development or in the adulthood and can be product of a short or long term exposure (Overgaard, 2011; González-Tokman *et al*., 2020). It has been described in insects that cold tolerance can be more plastic than heat tolerance, but there also is a positive relationship between increased latitudinal distribution range and phenotypic plasticity of thermal tolerance (Overgaard, 2011). The range of thermal tolerance in insects commonly shows a

macro-ecological pattern that matches with the extremes of temperature that organisms experience (Bozinovic et al, 2011; Sunday et al, 2012; Addo-Bediako et al, 2000; Araújo et al, 2013): species with a wider latitudinal distribution show a wider thermal breadth but, in general, the upper thermal limits show much less variation than do the lower thermal limits.

**Critical thermal limits**

To measure the effect of temperature on the performance of organisms, response factors to temperature changes such as locomotion, growth, development rate, fertility, survival, etc. can be evaluated. In order to determine the thermal tolerance of organisms based on these factors, there are parameters such as the critical thermal maximum (CTmax) and minimum (CTmin) that represent the extremes of temperature that individuals are able to tolerate before presenting a loss in motor functioning due to exposure to temperature changes (Terblanche et al. 2011, Oyen et al. 2016, MacMillan and Sinclair 2011). The difference between the ranges of the critical thermal limits (CTmax and CTmin) is defined as thermal tolerance and allows to establish the temperature breadth in which organisms have total control of their functions (Sheldon and Tewksbury, 2014).

**The Odonata order**

Among insects, the order Odonata has a complex life cycle, in which the egg and larvae states are aquatic while adults are terrestrial. The environmental conditions in which eggs and larvae develop can affect physiological performance of adults (Stoks and Córdoba-Aguilar, 2012), in addition, the environmental challenges faced by immature individuals are very different from those experienced by terrestrial adults since water temperature, in general, has less daily and seasonal variation than air temperature (Havird *et al,* 2019; Webb et al, 2003; Williams et al, 2016). In this sense, the complex life cycles of some

groups of insects may be relevant in thermal tolerance studies; since having different ecological needs at each stage of the life cycle, they allow to explore different patterns of response to environmental temperature conditions. The order Odonata could be an excellent study model to explore these patterns.

The larvae of odonates could have ecological relevance depending on the strategies they adopt based on their thermal tolerance, it has been suggested that in aquatic arthropods there may be a relationship between exposure to extremes of temperature and oxygen demand and availability (the oxygen and capacity limitation of thermal tolerance hypothesis, OCLTT) , high temperatures could be related to a limitation in the ability of individuals to carry out efficient oxygen transport (Verberk *et al*., 2015). The availability of oxygen is much lower in water than in air and decreases even more with the increase of temperature, in this sense, the larvae of odonates face thermal challenges that could have important energy consequences in terms of oxygen deficiency. The adults, on the other hand, face different conditions in which it has been suggested there is no such strong relationship between thermal tolerance and oxygen demand (Verberk *et al*., 2015).

**The genus *Hetaerina***

The genus *Hetaerina* (Calopterygidae) is widely distributed in the American Continent, males present wing pigmentation and territorial behavior (Córdoba-Aguilar and Cordero-Rivera, 2005). In territorial defense, males compete for territories in riparian vegetation through very complex and expensive flights while females arrive to territories only to mate. After copulation, the eggs are deposited in the submerged vegetation, while females remain submerged for several minutes. It is common that territorial defense be performed mainly in the sunniest hours of the day, and it has even been observed in some species such as *H.*

*vulnerata*, that males have no territorial activity in cloudy days (Isarrarás-Hernández, pers. Obs); which suggests the environmental temperature can play an important role for territorial behavior in this genus. Larvae develop in the submerged vegetation, so they will face different thermal challenges than adults. In immature states of aquatic insects the oxygen limitation associated with thermal tolerance is important, due to the oxygen solubility and diffusion capacity in water; this can be a limitation when metabolic demand increases with temperature (Chown et al, 2015).

Although the genus Hetaerina is widely distributed in the American continent, most species have a restricted distribution, some even restricted to a few locations (i.e. *H. infecta*, *H. pilula*, *H. rudis*) or small areas as well as to specific ecosystems (Vega-Sánchez, 2013). *Hetaerina americana* has the widest distribution, with populations from Nicaragua to southeastern Canada (Vega-Sánchez, 2016), this wide distribution suggests that this species has a high capacity to colonize a wide diversity of habitats, ranging from coniferous forests to rainforests, as well as a wide temperature gradient. When organisms experience a wide variation in environmental temperature, they are expected to be thermal generalists with a wide range of thermal tolerance (Sheldon and Tewksbury, 2014), since the thermal tolerance of organisms is associated to extreme temperatures they can experience on their habitat (Oyen et al, 2016). In addition, it has been described in insects that, although CTmax is higher in warm altitudinal gradients, tolerance to high temperatures is a trait with limited phenotypic plasticity (García-Robledo *et al*., 2015); because of this, the variation in thermal tolerance in insects has been related to a greater variation in the minimum thermal limits (CTmin) as latitude and altitude increases, while, in general, the maximum thermal limits (CTmax) remain less variable (Addo-Bediako et al, 2000).

**HIPÓTESIS Y OBJETIVOS**

This work aims to establish the relationship between the critical thermal limits of males, females and larvae of *H. americana* with the environmental temperature experienced by populations present in sites with contrasting temperature conditions. Consequently, we predict:

- Thermal tolerance of adults and larvae of *H. americana* will be related to the environmental temperature they experience in their wild populations. The CTmin will track minimum environmental temperatures; while CTmax will show a reduced interpopulation variation.

- Thermal tolerance of adults and larvae will also respond to seasonal (intrapopulation) temperature variation, showing wide phenotypic plasticity of CTmin and narrow for CTmax.

- Due to the greater temperature homogeneity that exists inside the water bodies throughout the day and the lower variation of accessible microclimates, the larvae will have a more restricted range of thermal tolerance than that of adults. Which will be reflected in a lower CTmax and a higher CTmin than that of males and females.

**MATERIALS AND METHODS**

To cover the full range of thermal conditions that individuals of *H. americana* could experience in Mexico, we selected the three most contrasting populations: La Huacana in the Tierra Caliente zone of the state of Michoacán 18°56'50.14"N, 101°53'34.10"O, 274 masl (warm site with average temperature of the coldest month/warmest month:

14°C/35°C), La Mintzita in the temperate zone of the state of Michoacán 19°38'55.19"N ,

101°16'15.18"O, 1892 masl (temperate site with average temperature of the coldest

month/warmest month 5°C/29°C) and San Pedro Mártir in the state of Baja California 31°

6'3.40"N, 115°37'19.70"O, 1227 masl (cold site, average temperature of the coldest

month/warmest month -16°C/23°C). To determine the *H. americana* critical thermal limits

of each population, both during warmest and coldest season of the year, we captured 30

middle-aged individuals of each sex and 30 larvae, to test for CTmin and CTmax. The air

and water temperature were taken on the days of collection (table 1).

Table 1. Environmental temperature of the collection sites.

|  | San Pedro Mártir (cold site) | La Mintzita (temperate site) | La Huacana (warm site) |
|---|---|---|---|
| Average temperature of the coldest month/warmest month | -16°/23°C | 5°/29°C | 14°/35°C |
| Average annual temperature | 18°C | 17.5°C | 24°C |
| Collection day air temperature cold season/warm season | 19°/31°C | 24°/25°C | 28.3/36°C |
| Collection day water temperature cold season/warm season | 14°/15°C | 19°/20° | 27.8°/29.4° |

In order to minimize acclimatzation, stress or drying effects, we measured thermal limits at

each sampling site by establishing a measuring station less than 8 km (or 10 minutes

transfer) from the river. Then, air and water temperature at the moment of the

measurements was the same as the individuals experienced at the time of capture (Shah *et

al*, 2016). To determine the *H. americana* critical thermal limits, we randomly selected 15

individuals (of each sex and larvae) from each population to test for either critical thermal

maximum (CTmax) or critical thermal minimum (CTmin). CTmax and CTmin were

measured using electric cups that allow us to increase or decrease the temperature to a rate

of 1°C/min$^{-1}$. The critical temperature was estimated when the individuals stopped having reaction to an external stimulus (being disturbed with a stick). In adults, when approaching the CTmax they presented movement of legs and wings, sometimes even without the external stimulus, the CTmax was established when these movements ceased; in larvae, when approaching the CTmax, they showed movement of legs and abdomen, so the critical temperature was established when these movements ceased, and generally in an upside-down position. When approaching the CTmin, adults gradually reduced their mobility and their reaction to the external stimulus became slower. CTmin was established when there was no reaction to the external stimulus which was accompanied by a flexion of the legs towards the ventral center of the thorax; larvae had a similar reaction than adults when approaching the CTmin. The recovery time of each individual was measured both for CTmax and CTmin by placing them at room temperature until they were incorporated again. Measurements of the total length of each individual were taken with a digital caliper.

**STATISTICAL ANALYSES**

To assess whether critical thermal limits of *H. americana* vary between populations, seasons and sex/stage (i.e. male, female, larvae) we performed independent generalized linear models (GLM) for CTmax and CTmin, models considered population, season, recovery time, total length and sex/stage as fixed factors, when evaluating the normality and heteroskedasticity of the variances, it was found that the response variable CTmax was non-normal and heteroskedastic, and CTmin was normal but also heteroskedastic; to deal with heteroskedasticity in both cases we used the gls function of the nlme library (Pinheiro *et al*., 2020) in Rstudio (RStudio Team, 2016). Thermal tolerance breadths from males, females and larvae collected at each population and season were estimated using the

difference between CTmin and CTmax. Statistical analyses were performed in the JMP and Rstudio software.

**RESULTS**

**CTmax**

We found significant effects for population (table 2), the highest CTmax values were reached at the warm place, La Huacana, both in warm and cold seasons, while only at the cold site (San Pedro Mártir) were seasonal differences in the CTmax, as individuals were able to tolerate higher temperatures in warm season than in cold season. When comparing the CTmax of males, females, and larvae, larvae reached significantly higher CTmax than adults in all three sites and in both seasons. Also, in San Pedro Mártir, the larvae tolerate higher temperatures in warm season than in cold season (figure 1). CTmax also showed an individual size effect, however, this size-CTmax effect was due to difference in size among males, females and larvae, since when comparing the CTmax of the same sex or stage no relationship was found between CTmax and body size.

**Table 2. Generalized linear models effects on CTmax and CTmin.** The complete models are: glm((CT min or max)~ (Population+Season+Sex/Ontogeny), family=gamma). The significant factor that influence the response are in bold.

| Parameters | Df | CTmax Residual Deviance | Pr(<$X^2$) | Df | CTmin Residual Deviance | Pr(<$X^2$) |
|---|---|---|---|---|---|---|
| Population | 2 | 705.09 | **2.2e-16** | 2 | 777.85 | **1.606e-06** |
| Season | 1 | 661.35 | **327e-08** | 1 | 773.84 | 0.183374 |
| Sex/Ontogeny | 2 | 529.37 | **2.2e-16** | 1 | 661.41 | **1.743e-11** |

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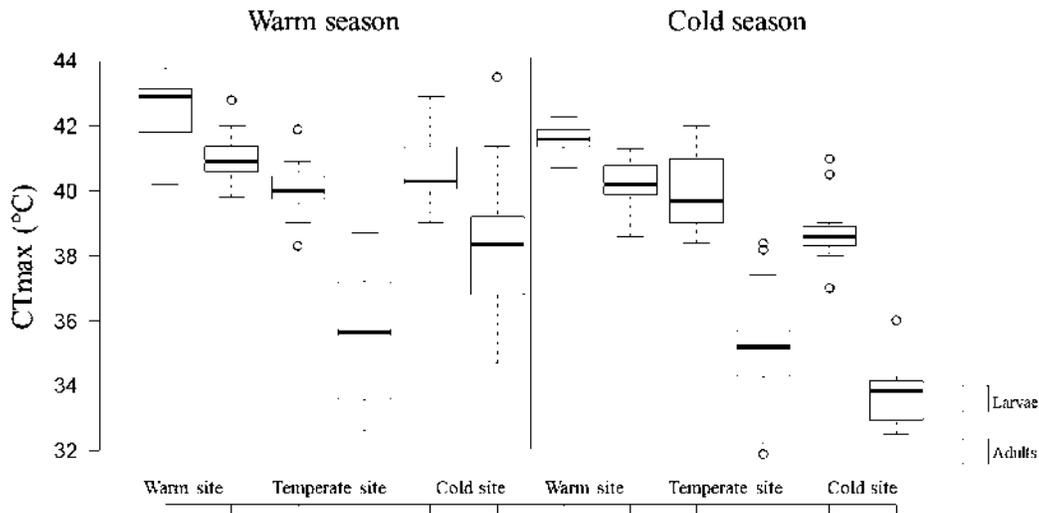| | | | | | | |
|---|---|---|---|---|---|---|
| Total length | 1 | 820.5 | **2.2e-16** | 1 | 838.4 | **2.2e-16** |
| Environmental temperature | 1 | 1798.62 | **2.2e-16** | 1 | 1888.34 | **2.2e-16** |



Figure 1. CTmax of adults and larvae of *Hetaerina americana* in the two seasons and three locations. The X axis shows the locality, while Y axis the temperature of CTmax.

## CTmin

The CTmin of males, females, and larvae also showed significant differences both between locations and seasons (table 2): in the coldest site, San Pedro Mártir, individuals reach significantly lower CTmin than in the temperate (La Mintzita) and the warm site (La Huacana). In the cold season the CTmin is also lower than the warm season. In the warm and temperate sites, adults show differences in CTmin between seasons, tolerating lower temperatures in cold than in the warm season. On the other hand, in the cold site, San Pedro Mártir, there is no difference in CTmin values between seasons. When comparing the CTmin between males, females and larvae, the larvae reached significantly lower CTmin temperatures than adults in all three sites regardless of the season (figure 2). It is also

interesting that in the larvae there is no difference in the values of CTmin between seasons within each site.
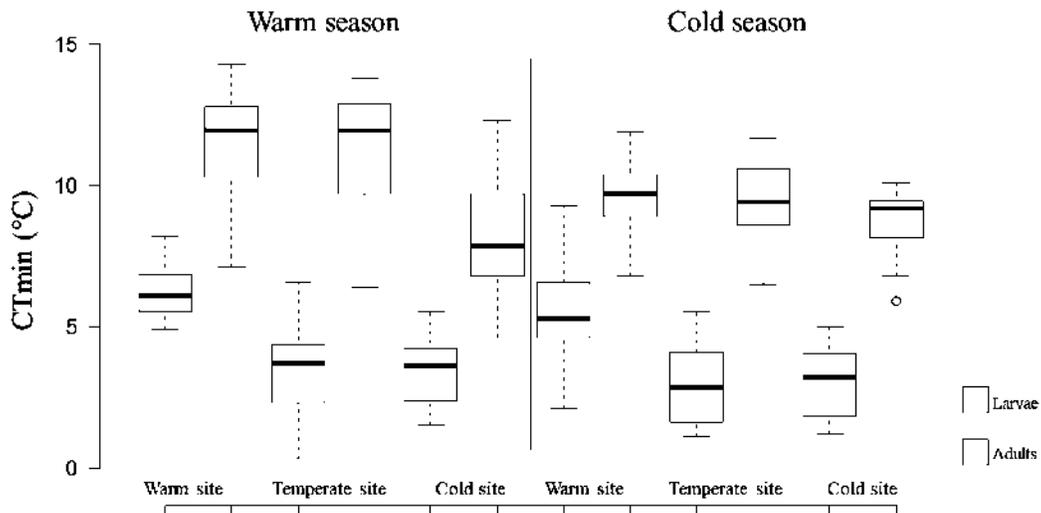


Figure 2. CTmin of adults and larvae of *Hetaerina americana* in the two seasons and three locations. The X axis shows the locality, while Y axis the temperature of CTmin.

## Thermal breadth

Because each individual was used only for one of the two treatments (CTmax or CTmin), thermal tolerance was estimated based on the difference in the means of CTmax and CTmin in each group (i.e. males, females, larvae, sites, seasons) (Shah *et al*, 2016).

In all sites and seasons, larvae presented a significantly wider thermal tolerance breadth than adults. A positive relationship was found between the thermal breadth of adults with the environmental temperature (F=11.73, $R^2$=0.53, p=0.0065) (figure 3). Larvae did not show this relationship (F=0.5051, $R^2$=0.11, p=0.5165).
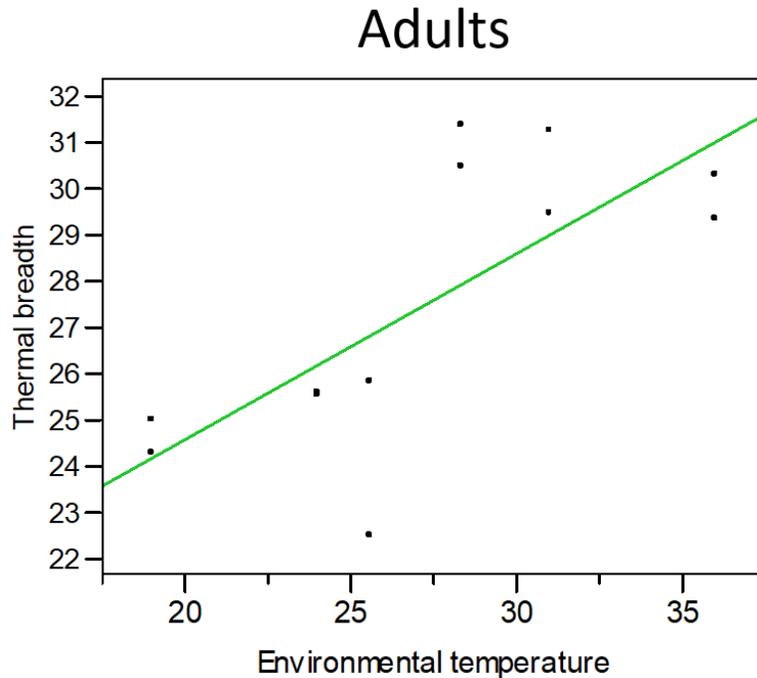
## Adults



Figure 3. Linear regression of the thermal breadth of adults (DF=1, F=11.73, p=0.0065) with the environmental temperature in the day they were collected.

## DISCUSSION

In adult and immature insects, thermal tolerance is closely related to environmental temperature (Andersen *et al*, 2018; Bjerregaard-Jørgensen *et al*, 2019; Calosi *et al*, 2010; Camus *et al*, 2017; Chown *et al*, 2015; Havird *et al*, 2019; Hoffman *et al*, 2013; Oyen *et al*, 2016; Shah *et al*, 2016). Although in ectotherms there is no capacity for regulating body temperature through endogenous homeostatic control, the thermal heterogeneity of natural environments allows the modification, up to certain limits, of body temperature through behavioral thermoregulation (Abram *et al*, 2016; Bale *et al*, 2002). The capacity of thermoregulation in insects has also been associated with heat production by movement of the wing muscles and as a product of metabolic activity (Heinrich, 1974), as a consequence, the ability to regulate body temperature in insects is limited. In ectothermal species, macro-

physiological patterns state that heat tolerance has lower range of variation as well as a higher phylogenetic inertia than cold tolerance, regardless of latitudinal or altitudinal distribution of their populations (Addo-Bediako et al, 2000). *H. americana* populations showed a similar pattern related with the thermal environment experienced by their individuals. Nevertheless, it is interesting that while differences in the critical thermal limits of adults had a great correspondence with the environmental temperature they experienced in their populations, larvae in all populations had the CTmax and CTmin well above and below than the water temperature of the streams they inhabit. This study is the first one, that we know, that compares thermal tolerance in adults and larvae of a single odonate species, and the fact that adults were way more sensitive to temperature in terms of a lower CTmax and higher CTmin than larvae, suggests that larvae could be playing an important ecological role for the wide distribution of the species and its permanence in sites where the thermal challenge is too big for the adults to withstand it.

Also, unlike what literature suggests, the critical thermal maximum (CTmax) exhibited ample variation for both populations and seasons. As expected, CTmax in *H. americana* reached its higher value at La Huacana population (i.e. tropical site), but without being affected by the season of the year, while at San Pedro Mártir population (i.e. cold site) CTmax levels were affected by the season and significantly reduced in cold season.  The critical thermal minimum (CTmin) also showed inverse patterns to those observed in other insects, being constrained to a relatively narrow range, and showing a less accurate relationship with the environmental temperature of each population than CTmax. In general, CTmin was lower in San Pedro Mártir, but individuals from warm and temperate populations had the same levels of CTmin. In addition, in the cold site *H. americana* almost

lacks of seasonal CTmin variations for both larvae and adults. Although the critical thermal limits, CTmax and CTmin, are determined similarly (by the temperature at which there is a loss of postural control), the physiological mechanisms inherent to CTmax and CTmin are not fully understood and could have different physiological implications.

This suggests that in *H. americana*, to have a wide range of thermal tolerance could be an important adaptation that would allow a wide range of distribution of the species, and, therefore, individuals would have to have the physiological mechanisms that allow this wide tolerance range. There seems to be a pattern in relation to the environmental temperature and the variation in the critical thermal limits: in the warm place, CTmax was no different between seasons for adults, while CTmin varied accordingly with the temperature reduction in winter, and could allow them to tolerate those changes; while in the cold site CTmin had no differences between seasons, so CTmax was significantly higher in warm season than in cold season. This suggests that places where the thermal challenge is imposed by high temperatures, populations present plasticity in their physiological responses that allow them to tolerate high environmental temperature conditions. On the contrary, when thermal challenge is imposed by low temperatures, the plasticity of physiological responses is reversed allowing greater tolerance to cold.

Critical thermal limits are not static within each population of *H. americana*, and vary depending not only ofthe site but year's season. The features that present changes in spatial and temporal scales within the same species, as in this case, are commonly attributable to phenotypic plasticity (Turcotte, 2016), in addition, the insects distribution is commonly related to variation in temperature extremes , which have been associated with differences in the plastic responses of populations to thermal challenges (Overgaard, 2011). In this

context, we can suggest that the wide distribution of *H. americana* could be related to phenotypic plasticity in thermal tolerance of populations, since ectothermal animals with wide latitudinal distribution must have a very wide range of thermal tolerance or have the capacity of altering tolerance based on the variation in temperature they experience (Overgaard, 2011).

The larvae had higher CTmax and lower CTmin than adults, regardless of site and season, this means larvae have a significantly wider thermal breadth, which contrasts with the prediction that , living in a less variable thermal environment, larvae would also have a more restricted thermal tolerance. However, precisely because of the lower temperature variability that occurs inside the water bodies with respect to the air temperature (Havird *et al,* 2019; Webb et al, 2003; Williams et al, 2016), which can fluctuate more throughout the day,  larvae will have a much more restricted amount of microenvironments to perform behavioral thermoregulation. In addition, during the adult emergence, larvae are exposed to a drastic change of temperature (i.e. stream *vs* air temperature) which in our populations was never less than 5ºC. Therefore, covering a wider range of thermal tolerance could be an adaptive response to cope with the variation in air temperature, regardless of the year's season. We have observed in *Hetaerina vulnerata* populations from cold sites  that adults die in winter but they are present again in spring, when the environmental temperature rises and the larvae emerge from the river (Isarrarás-Hernández, pers. obs.). In this sense, larvae could be playing a crucial role for the species permanence in places with extreme temperature conditions.

Because many physiological processes associated with CTmax can be lethal, it has been suggested that the critical thermal maximum is not very variable for species with wide

distributions, and as latitude increases there is a greater variation in CTmin (Addo-Bediako *et al*, 2000). However, in *H. americana* the critical thermal maximum increased significantly in the warm place as compared to temperate and cold sites. This increase in CTmax in the warm site could be due to a relationship between developmental plasticity and acclimatization. Some studies state that in animals with short life cycles, temperature conditions during development will be related to acclimatization capacity during adult life (Beaman et al, 2016), in *H. americana* could be different physiological mechanisms between adults and larvae, due to the different ecological needs of individuals in each development stage.

The critical thermal limits and the range of thermal tolerance in different populations of *H. americana* are not static, but rather show variations associated with the thermal environment in which they operate. The mechanisms associated with these variations could be related to the plasticity of the thermal tolerance of individuals, due to the relationship, in species of wide distribution, between the variability of the environmental temperature and the increase in phenotypic plasticity of the thermal tolerance limits (Overgaard, 2011). It is known that, in insects, exposure to extremes of temperature may increase tolerance to subsequent exposures (Hoffman et al 2002, Sinclair and Roberts 2005, Luo et al 2014), at sites with low temperature variation throughout the year. We could expect that traits allow not only tolerance, but the capacity of taking advantage of temperature conditions, could be being settled from one generation to another. In addition, our results suggest that large thermal breadths of larvae are playing a crucial role in the wide distribution of *H. americana*, which allow the species to face a wider range of thermal conditions, even air

temperature that adults cannot withstand, such as those associated with climate change predictions.

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