



The costs of living in a thermal fluctuating environment for the tropical haematophagous bug, *Rhodnius prolixus*

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ARTICLE INFO

Keywords:

Rhodnius prolixus

Survival time

Chagas disease

Constant temperature

Fluctuating temperature

Phenotypic plasticity

ABSTRACT

Environmental temperature is an abiotic factor with great influence on biological processes of living beings. Jensen's inequality states that for non-linear processes, such as most biological phenomena, the effects of thermal fluctuations cannot be predicted from mean constant temperatures. We studied the effect of daily temperature fluctuation (DTF) on *Rhodnius prolixus*, a model organism in insect physiology, and an important vector of Chagas disease. We measured development time from egg to adult, fecundity, fertility, body mass reduction rate (indirect measurement of nutrient consumption rates) and survival after a single blood meal. Insects were reared at constant temperature (24 °C), or with a DTF (17–32 °C; mean = 24 °C). Taking into account Jensen's inequality as well as the species tropical distribution, we predict that living in a variable thermal environment will have higher costs than inhabiting a stable one. Development time and fertility were not affected by DTF. However, fecundity was lower in females reared at DTF than at constant temperature, and males had higher body mass reduction rate and lower survival in the DTF regime, suggesting higher costs associated to fluctuating thermal environments. At a population and epidemiological level, higher energetic costs would imply an increase in nutrient consumption rate, biting frequency, and, consequently increasing disease transmission from infected insects. On the contrary, lower fecundity could be associated with a decrease in population growth. This knowledge will not only provide basic information to the field of insect ecophysiology, but also could be a useful background to develop population and disease transmission models.

1. Introduction

Terrestrial organisms inhabit thermally heterogeneous environments. This heterogeneity is given as a consequence of spatial and temporal variations, which at a spatial level comprise large scales such as latitude and altitude, and at smaller scales, variations between microclimates (Gaston and Blackburn, 2000). Simultaneously, temporal variations occur along years, months (seasonally), and during a day. It is widely known that temperature affects biological processes, and while this is so for all living beings, the impact of temperature changes on ectothermic animals is higher because of the close association between environmental and body temperatures. The thermal dependence of biological processes can be represented through performance curves. The performance or rate of a certain process fits a unimodal asymmetric function, having an optimal temperature between a lower and upper temperature limit (Martin and Huey, 2008). The way in which

performance increases and decreases is non-linear, and as a result, mean performance in a thermally fluctuating environment will be different from performance at the same average constant temperature. This phenomena is described by Jensen's inequality, a mathematical property of non-linear functions (Ruel and Ayres, 1999). Thus, ectothermic organisms inhabit a thermally fluctuating environment, where they constantly sense temperature and try to adjust to those variations through a plethora of mechanisms. For example, when insects are exposed to extreme temperatures (i.e., temperatures at which organisms lose motor functions, and approach lethal temperatures) responses may involve shelter-seeking behaviors (Hölldobler and Wilson, 1990), evaporative cooling (Prange, 1996), expression of heat shock proteins (King and MacRae, 2015), and production of free amino acids and cryoprotector polyols (Clark and Worland, 2008) among others. Exposure to temperatures within the thermal safe zone (between the upper and lower limits) is on the other hand, a less explored area. As

Abbreviations: DTF, Daily temperature fluctuation

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<https://doi.org/10.1016/j.jtherbio.2018.03.022>

Received 30 November 2017; Received in revised form 21 February 2018; Accepted 18 March 2018

Available online 20 March 2018

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Jensen's inequality states, temperature effect on performance will be given not only by its mean temperature, but also by its variance. If the range of fluctuation only includes ascending portions of the curve, mean performance will be higher than performance at constant temperature (Colinet et al., 2014).

Although traditionally thermal dependence of biological processes has been mostly studied in controlled constant temperature conditions, over the past decade thermal variance is becoming a central topic in eco-physiology (e.g., Terblanche et al., 2010; Folguera et al., 2011; Colinet et al., 2014; Kingsolver et al., 2015, among others), mostly associated with future thermal scenarios (Estay et al., 2014). The growing number of studies confirms that the effects of thermal fluctuations in insect's ecologically relevant traits have to be empirically determined. It is important to note that the effect of daily thermal fluctuations will depend not only on the shape of performance curve, mean and variance of environmental temperature, but also on the phenotypic plasticity within a population, or species (Bozinovic et al., 2016, 2011; Foray et al., 2013; Terblanche et al., 2009). Phenotypic plasticity is the property that allows a particular genotype to produce different phenotypes in response to environmental conditions (Pigliucci, 2001). Plasticity can occur by acclimation during rearing (developmental plasticity), or by acclimation during a period of time within a life stage (phenotypic flexibility) (Terblanche and Chown, 2006). A thermally fluctuating environment can modify temperature-performance curves. As is the case of metabolic rate thermal sensitivity, which decreases in overwintering butterflies as a consequence of thermal variability and thus, decreasing energy expenditure (Williams et al., 2012). Acclimation to fluctuating environments has also been observed in other traits such as hydric balance, and fecundity (Terblanche et al., 2010). Empirical information is important for performing accurate forecasts of insect responses to climate change. In particular, there are many studies that focus on insects that transmit diseases. *Aedes aegypti* adults live longer, and are more susceptible to dengue virus infection in moderate temperature fluctuations (Lambrechts et al., 2011). Something similar is observed in mosquitoes from *Anopheles* and *Aedes* genera, whose optimal conditions for development, and disease transmission occur in environments with moderate fluctuations, and low mean temperatures (Carrington et al., 2013; Lyons et al., 2013; Paaijmans et al., 2010). Consequently an increase in mean temperatures in areas affected by this disease would turn out as a disadvantage for mosquito and parasite proliferation (Paaijmans et al., 2010).

Triatomines are haematophagous bugs, vectors of Chagas disease, and although the effect of temperature on various traits has been widely studied across species (Asin and Catalá, 1995; Guarneri et al., 2003; Lehané et al., 1992; Schilman and Lazzari, 2004), there are few studies that account for the effects of daily thermal fluctuations (Luz et al., 1998, 1999; Damborsky et al., 2005). Triatomines can sense environmental temperature, and their thermo-preference changes with starvation and time of the day (Lazzari, 1991; Minoli and Lazzari, 2003; Pires et al., 2002; Schilman and Lazzari, 2004). At least for *Triatoma infestans*, it was demonstrated that the daily thermo-preference variation is a true circadian rhythm, which is endogenous controlled (Minoli and Lazzari, 2003). In this study, we use *Rhodnius prolixus* as a model organism. This species is found in the north of South America (Schofield, 1994), and is one of the most important vectors in that region. Besides from being highly relevant for medical reasons, this species is also a classic model in insect physiology (Schilman, 2017; Wigglesworth, 1972). For *R. prolixus*, the effect of temperature and humidity fluctuations on development time and survival was measured only within each life stage (Luz et al., 1998, 1999).

Here we test how daily thermal variations will affect ecologically relevant traits associated to life history, such as development time, fertility, fecundity, and starvation tolerance. In addition, we will test for the existence of developmental plasticity, or phenotypic flexibility favoring survival in a food deprived state. Phenotypic plasticity has been

described as an adaptation to variable environments (Beaman et al., 2016; Huey and Kingsolver, 1989). Therefore it is expected for species that have evolved in habitats with less temperature variation (such as the tropics) to have little or no plastic responses. Taking into account Jensen's inequality as well as the species tropical distribution, we predict that inhabiting a variable thermal environment will have higher costs than inhabiting a stable one.

2. Materials and methods

2.1. Insects

Eggs and insects used throughout this study were collected from a *Rhodnius prolixus* colony established in the laboratory. Insects were reared at 28 °C with a 12:12 light-dark cycle, and they were fed weekly on live hens.

2.2. Thermal regimes

Insects were subjected to two thermal treatments: 1) a constant temperature at 24 °C, and 2) a daily temperature fluctuation (DTF) with a mean temperature of 24 °C, a minima of 17 °C before the light phase begins at 8:00 a.m., and a maxima of 32 °C at 3:00 p.m.

Temperature variation simulated a natural daily variation, and it was achieved by fitting a model described by Parton and Logan (1981). Briefly, during the day temperature fits a sinusoidal function reaching its maxima around 3:00 p.m., and during the night fits a negative exponential where temperature decreases until it reaches a minimum temperature before the light phase begins (8:00 a.m.) (Fig. 1). This thermal profile was remotely sent using the software Pelt-C3 (Sable Systems International (SSI), Las Vegas, NV, USA) through a laptop's serial port to a Pelt-5 temperature controller (SSI) attached to a PTC-1 temperature control cabinet (SSI). Constant temperature chamber was obtained by using a modified PTC-1 temperature control cabinet (SSI) controlled with a custom designed Arduino temperature controller (see Rolandi, 2016 and <https://figshare.com/s/d554647c4ab348beac52> for hardware and software details).

Temperature and humidity of both cabinets were recorded with HOBO data-loggers (Onset Computer Corporation, Bourne, MA, USA) every minute for the whole duration of the experiments (Fig. 1).

2.3. Development time

We analyzed the effect of DTF during development. To do so, we collected eggs less than 24 h after being laid from rearing containers. Three replicates of 100 eggs were placed at constant and fluctuating

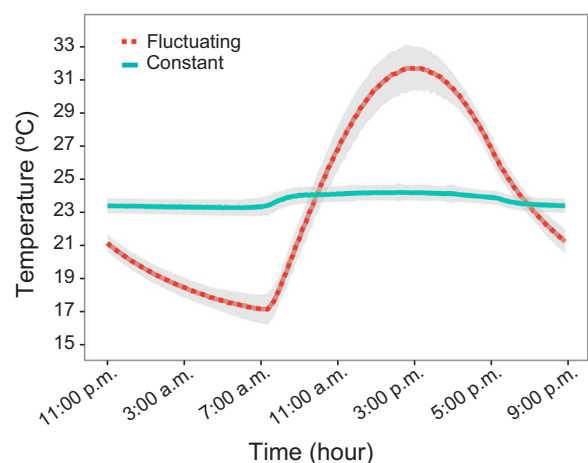


Fig. 1. Measured temperatures inside the temperature controlled cabinets throughout the experiments. Solid lines are mean temperatures, while grey contours are standard deviations.

thermal regimes. Once nymphs emerged insects were offered weekly for 1 h the possibility of feeding on chickens. Insects were kept in their initial thermal regimes through all development stages until moulting to adults. As adults began emerging experimental containers were checked every other day. Development time was registered as the number of days from egg-laid to adult emergence. In addition, the proportion of eggs that successfully reached the adult stage, and proportion of sexes was calculated.

2.4. Fecundity and fertility

We measured fecundity as the number of eggs laid in a 35 days period after feeding, and fertility as the hatching success of those eggs. These assays were performed with females and males reared under constant, and fluctuating temperatures. We used insects between 2 and 3 weeks after moulting as adults, which were individualized with an acrylic color code painted on their legs, and weighed before and three hours after feeding. For each thermal treatment, two experimental groups were made with the insects that consumed more blood than 60% of their initial weight: i) mated females: 1 female and 1 male, and ii) virgin females: 1 female. We used acrylic cylindrical containers (height: 3.5 cm; width: 3 cm) with a similar piece of filter paper inside acting as oviposition substrate in order to avoid differences in fecundity due to the substrate (Schilman et al., 1996). Insects were placed in the containers 3 h after feeding, and kept there for 35 days.

Every other day containers were checked for laid eggs (fecundity) and spermatophores (as indicators of copula). Eggs were then transferred into a 24-multiwell culture plate to register hatching success (fertility).

For each female we estimated the E value that is a measure of the conversion efficiency from blood to eggs (Chiang and Chiang, 1995):

$$E = \frac{\text{Number of eggs laid}}{\text{initial mass} \times \text{mass of ingested blood}} \times 1000 \quad (4.1)$$

2.5. Effect of thermal variability on body mass loss and tolerance to starvation

We measured mass loss and starvation tolerance, in individualized males of 2–3 weeks post-ecdysis. Prior to the assays insects were fed for the first time in that life stage on live hens, weighed before and after feeding, and then placed in each thermal regime. After 2 weeks, we performed weekly mass measurements until death. Tolerance to starvation was measured as time from feeding to death. To discern between developmental plasticity and phenotypic flexibility we used males that developed at constant, or fluctuating thermal regimes (mean = 24 °C) (developmental plasticity), as well as males reared at constant 28 °C and transferred into constant (24 °C), or fluctuating chamber after their first meal (phenotypic flexibility).

2.6. Data analysis

Statistical analysis were performed using R software (R Core Team, 2017). Normal data was analyzed by fitting linear models with *glm* function, and when the experimental design had random factors, with mixed effects linear model using *lme* function from “nlme” library (Pinheiro et al., 2017). Mean values are informed with their standard error (mean ± S.E.) unless otherwise noted. All data can be accessed through figshare (<https://figshare.com/s/7468a158cc5e7dd59de5>)

Development time was compared between thermal regimes and sex using experimental series as a random factor. Fertility and fecundity were analyzed by fitting a linear model.

Body mass loss as a function of starvation was measured repeatedly on the same individuals; hence we analyzed the data with a mixed effects model using each subject as a random factor, and thermal regime as well as plasticity type as fixed factors. The death of these insects was

recorded weekly and survival data was analyzed using a Cox proportional hazards model. We used *coxph* function of the “survival” package (Therneau, 2015) using the same fixed factors as before.

3. Results

3.1. Thermal regimes

Mean temperature of fluctuating regime was 23.73 ± 5.20 °C (± S.D.) and for the constant regime, 23.76 ± 0.59 °C (Fig. 1). Humidity was not controlled, but it was monitored: relative humidity ranged between 23% and 90% for both treatments. Mean relative humidity was $42.90 \pm 14.30\%$ (± S.D.) for the fluctuating regime and $50.59 \pm 12.73\%$ for the constant regime.

3.2. Development time from egg to adult

Development time for insects reared at a mean temperature of 24 °C was 130.05 ± 0.78 days (N = 245) at constant temperature, and 133.09 ± 0.91 (N = 247) at fluctuating temperature. There was no significant difference between them ($F_{1,487} = 0.10$; $P = 0.75$), and development time was homogeneous between sexes ($F_{1,487} = 0.11$; $P = 0.74$).

Although the proportion of eggs that reached the adult stage cannot be statistically tested because of the low number of cohorts, the percentage of success in reaching adult stage was similar and high for both thermal treatments ($82.33 \pm 1.11\%$ and $81.66 \pm 1.84\%$ for fluctuating and constant environment, respectively). The percentage of females and males that emerged was $52.51 \pm 2.84\%$ females, and $47.48 \pm 2.84\%$ males for the fluctuating thermal regime, and $50.83 \pm 2.84\%$ females, and $49.17 \pm 2.85\%$ males for the constant one.

Body mass of emerged adults was measured two weeks after moulting (before fecundity assays, see next section). There was a significant interaction between sex and thermal treatment ($F_{1,180} = 3.90$; $P = 0.049$). A *post hoc* Tukey test of the interaction revealed that females reared at constant temperature attained a significant ($t = -3.348$; $P = 0.005$) higher mass (82.22 ± 1.80 mg), than females reared at fluctuating temperature (73.51 ± 3.09 mg). On the other hand, there were no differences in body mass between males reared in both thermal treatments ($m_{\text{constant}} = 66.53 \pm 2.41$ mg; $m_{\text{fluctuating}} = 66.35 \pm 2.45$ mg; $t = -0.06$; $P = 0.99$).

3.3. Fecundity and fertility

The effect of mating status and rearing thermal regime was tested through a linear model (*glm* function). The mass of ingested blood (Buxton, 1930; Friend et al., 1965) and, for the first ovarian cycle, the initial body mass (Davey, 2007) play an important role in fecundity. Hence the model also had as explanatory variables the initial body mass and the ingested blood mass. As expected, egg production was higher in mated compared to virgin females (Fig. 2). There is a significant interaction between mating status and rearing temperature (Table 1); mated females reared at constant temperature laid significantly more eggs than mated females reared at fluctuating temperature ($t = 3.06$, $P = 0.0028$), while there was no effect of thermal regime on fecundity for virgin females ($t = 0.65$, $P = 0.52$) (Table 1, Fig. 2).

Egg production efficiency, estimated as E-value was not affected by development temperature ($F_{1,109} = 0.63$; $P = 0.43$), nor its interaction with reproductive status ($F_{1,109} = 3.35$; $P = 0.07$). Mated females are more efficient in blood to eggs conversion; E-value of mated females (4.13 ± 0.22) was significantly higher than of virgin females (1.64 ± 0.19) ($F_{1,109} = 18.26$; $P < 0.0001$).

We did not observe an effect of thermal regime in fertility (generalized mixed binomial model, $P = 0.17$); hatching success was high with a mean proportion of 0.90 ± 0.18 .

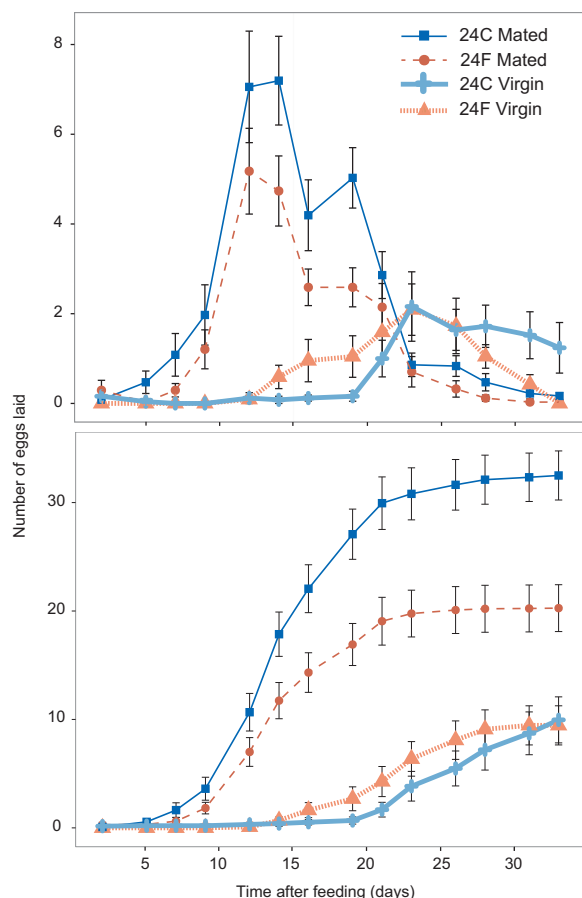


Fig. 2. Oviposition dynamics for mated and virgin females A) Average number of eggs laid per female B) Average cumulative number of eggs laid per female.

Table 1
Statistics of the effect of mating status and rearing thermal regime (*gls* function).

	D.F.	F	P
Intercept	1,107	3.49	0.06
T	1,107	2.39	0.13
R.S	1,107	24.56	< 0.0001
T x R.S	1,107	5.21	< 0.05
Mi	1,107	38.22	< 0.0001
Mb	1,107	5.82	< 0.05

T: rearing temperature, R.S: reproductive status, *mi*: initial mass, *mb*: ingested mass of blood. For both models variance structure was fitted with the function *varExp*.

The mean number of accumulated copulas in 35 days per experimental container (1 female and 1 male) was significantly higher for insects reared at constant (9.92 ± 0.68) than fluctuating temperature (7.23 ± 0.88) ($F_{1, 59} = 6.70$; $P = 0.01$). Initial mass, and ingested blood mass had a positive effect in the frequency of copula ($F_{1, 62} = 4.78$; $P = 0.03$ y $F_{1, 62} = 8.30$; $P = 0.006$, respectively). Nonetheless the number of laid eggs by mated females was not affected by the number of spermatophores ($F_{1, 60} = 0.62$; $P = 0.43$)

3.4. Effect of thermal variability on body mass loss and tolerance to starvation

Initial body mass was significantly higher in males that developed at mean temperature of 24 °C, both constant and fluctuating (62.50 ± 1.55 mg), compared to constant mean temperature of 28 °C (49.42 ± 1.35 mg) ($F_{1, 144} = 71.87$; $P < 0.00001$). This relation reverses when analyzing the mass of ingested blood: insects reared 28 °C

Table 2
Output of mixed effects linear model for body mass loss rate.

Variable	Coefficient	S.E.	D.F.	t	P
Intercept	108.435	2.06	845	52.64	< 0.00001
Days	- 2.177	0.07	845	- 30.40	< 0.00001
Constant temperature	- 2.247	2.78	143	- 0.80	0.42
Development Plasticity	8.66	2.92	143	2.96	< 0.01
Days ²	0.015	0.001	845	13.33	< 0.00001
Days x Dev. Plasticity	- 0.199	0.099	845	- 2.00	< 0.05
Days x Constant Temp	.470	0.090	845	5.20	< 0.00001
Dev. plasticity x Constant temp	.746	4.048	143	0.184	0.85
Days ² x Dev. Plasticity	0.0038	0.0015	845	2.54	< 0.05
Days ² x Constant Temp	- 0.0043	0.0013	832	- 3.25	< 0.01
Days x Dev. Plasticity x Constant temp	- 0.125	0.129	845	- 0.97	0.33
Days ² x Dev. Plasticity x Constant temp	- 0.0002	0.0018	845	0.10	0.92

ingested significantly more blood (61.80 ± 1.64 mg) than insects reared at fluctuating and constant mean 24 °C (57.65 ± 1.87 mg) ($F_{1, 144} = 4.54$; $P < 0.05$). After feeding, the effect of exposure to fluctuating temperatures during development or adult stage on body mass was analyzed with a 2nd order linear mixed effects model using *lme* function (Table 2, Fig. 3). There is a significant effect of plasticity type, which might be directly related to the difference in initial body and ingested blood masses. Triple interactions are not significant, nonetheless there are significant interactions between both time-associated terms of the model (days and days²), and plasticity type and thermal regime (Table 2). Regardless of plasticity type, insects at fluctuating temperatures have a higher decrease rate of body mass than insects in constant temperature (Table 2, Fig. 3). Probably as a consequence, survival time was shorter in males exposed to fluctuating temperature than those exposed to constant temperature. Median survival time was 49 days at fluctuating temperature, and 63 days at constant temperature (Table 3, Fig. 4).

4. Discussion

In this work, we have studied the effect of a DTF on different parameters with incidence on fitness of the haematophagous bug, *R.*

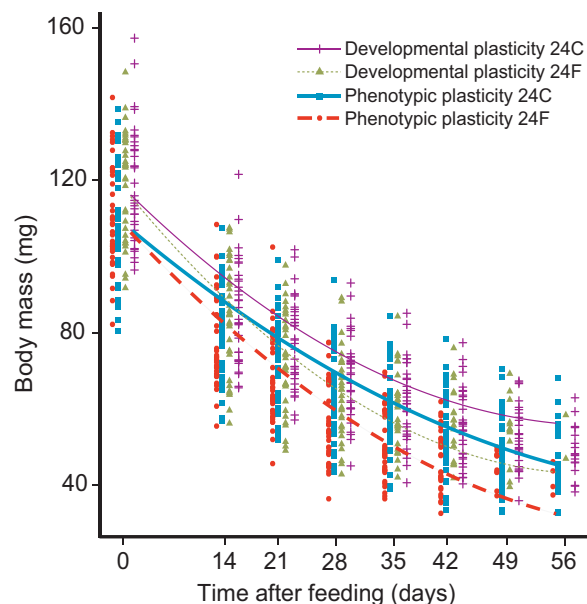


Fig. 3. Body mass loss rate as an indirect measure of blood consumption. Insects were measured weekly until 60 days after feeding. Lines show model fit.

Table 3
Analysis of deviance table for Cox's proportional hazards survival regression of starvation tolerance (days) with respect to plasticity type and thermal regime.

Variable	χ^2	D.F.	P
Plasticity type	0.06	1	0.80
Temperature	60.19	1	< 0.00001
Plasticity type x Temperature	2.22	1	0.14

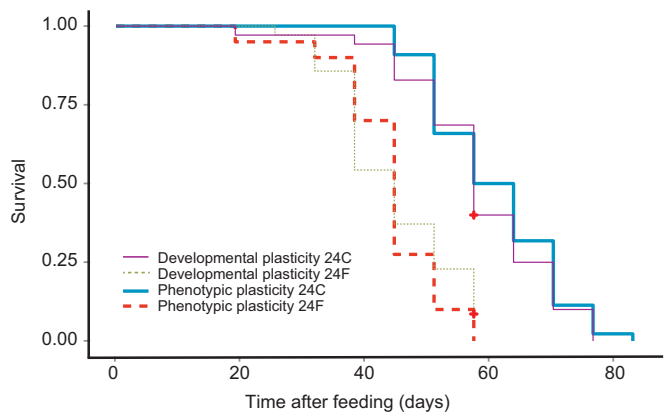


Fig. 4. Survival time for insects reared and acclimated to constant and fluctuating thermal regimes. Survival decreases in fluctuating thermal environments. Crosses show censored data of an experimental series that was terminated on day 63.

prolixus, as well as the plasticity of some of them. Although development time and starvation tolerance were previously measured on *R. prolixus* exposed to variable temperatures and humidity (Luz et al., 1998, 1999), there is no clear correlate between fluctuating and constant temperature treatments in those experiments. Thus the effect of fluctuation cannot be separated from the effect of temperature itself. To avoid this issue we chose to work with one fluctuating temperature and a constant temperature regime, both with the same mean temperature. In addition, the fluctuating regime mimicked natural thermal variations, contrasting with Luz et al. (1999) where fluctuations were obtained by switching insects from one temperature extreme to another. Although there are few studies with ecologically relevant thermal variation rates, there is evidence that they play an important role in insects' thermal response (Kelty and Lee, 2001). Moreover, the range of temperatures used in our experiments was chosen taking into account thermal extremes of the geographic distribution of this species (de la Vega et al., 2015). Although inside shelters thermal variation can be buffered compared to open areas (Heger et al., 2006), the fluctuation range chosen lies well-within the species thermo-tolerance range (de la Vega et al., 2015; de la Vega and Schilman, 2018). Taking into account *R. prolixus* high CTmax (~46 °C) and the asymmetric nature of performance curves, we expect that this temperature range lies within the ascending part of performance curves. Thus, based on Jensen's hypothesis as well as previous studies in other insects, we predicted that for *R. prolixus* a variable environment would increase the rate of the measured variables as opposed to a constant environment.

DTF did not affect development time, and at a mean temperature of 24 °C the development time, i.e., time from egg to adult, is 130 days. This result is against our prediction and there are several possible explanations for that. One possibility is that temperature oscillates around a portion of the development rate-temperature curve that fits a linear function. However, the thermal extremes of the development rate-temperature curve were observed at constant 15 °C and 35 °C and the optimal constant development temperature at 28 °C (Luz et al., 1999). Thus, another possible explanation is that the temperature range used here comprises a region of the descending part of the development rate-temperature curve slowing development rate (Ragland and Kingsolver,

2008; Worner, 1992). If this were the case, insects at the fluctuating regime would have longer development time than insects at constant regime, as occur for the yellow dung fly exposed to a DTF of 12–24 °C (Kjaersgaard et al., 2013), or equal in the case that the rates of the processes are exactly compensated. The latter scenario is rather unlikely because development rate involves various and complex underlying processes with different thermal performance curves. In *Drosophila melanogaster* closely related parameters such as reproductive rate and intrinsic rate of increase differ in their response to different temperature regimes, and much of the difference is mediated by generation time (Clavijo-Baquet et al., 2014).

For this species moulting process is controlled hormonally, and is triggered by feeding (Wigglesworth, 1972), moreover the size of the blood meal has to be above a certain threshold (Saenz et al., 2017). In our experimental design, insects were allowed to feed once a week triggering the moulting process in a synchronous way for both treatments, thus resetting development time, and not allowing the accumulation of small differences in development time across life stages. In contrast, Luz et al. (1999) measured development time separately within each life stage and found a low effect of fluctuating thermal regimes, thus allowing to accumulate the small differences within each instar. These differences could be also associated with the abrupt shifting of the insects from one temperature and humidity to another.

In relation to fecundity, the number and pattern of eggs laid is related to the amount of ingested blood (Buxton, 1930), the occurrence of copula (Davey, 1965), characteristics of the oviposition substrate (Rolandi and Schilman, 2017; Schilman et al., 1996). A cycle of egg production is triggered after feeding, and copula induces the release of a myotropic factor which increases motility of the oviduct muscles (Davey, 1965), causing mated females to lay more eggs than virgin ones. On the other hand, virgin females retain mature eggs in the ovary, causing the secretion of an antigonadotrophic factor that blocks vitellogenesis of younger eggs (Davey and Kuster, 1981). As expected, virgin females laid fewer eggs than mated ones. While DTF did not affect fecundity of virgin females, there is a negative effect of this treatment on fecundity of mated females. Mated females reared at constant temperature lay significantly more eggs than females reared at the fluctuating regime. This difference could be associated to a direct effect of the treatment, and to an indirect effect of initial body mass and ingested blood mass. An effect of initial body mass is expected on the first ovaric cycle since females emerge with remaining blood in their crops from their last meal as nymphs and thus, influencing egg production (Davey, 2007). Even after accounting for initial body mass and ingested blood mass, the effect of thermal treatment remains significant suggesting also a direct influence on fecundity. Initial body mass is lower on females reared at fluctuating temperature thus, indirectly affecting the number of eggs laid. Lower fecundity, and initial body and ingested blood mass in females reared at fluctuating temperatures support the hypothesis that this fluctuation regime may imply higher basal costs, thus decreasing energy available for egg production. The equal fecundity of virgin females under both thermal regimes, suggests that even under a putative unfavorable energetic situation they are not able to further down modulate the number of laid-eggs. Regardless of thermal regime, efficiency of blood to egg conversion showed, similar to Davey (1967) that virgin females have lower egg production efficiency. DTF might be affecting sperm production or viability. For males of a related species, *Triatoma infestans*, exposure to low temperatures (10 days at 12 °C during 5th nymphal stage) produce a decrease in blood consumption, size of spermatophores and fertility (Giojalas and Catalá, 1993). We did not quantify sperm production, but although the number of copula (estimated as number of spermatophores) was significantly higher in constant temperature regime, fertility (estimated as the percentage of successful eggs hatched) does not show an effect of DTF.

In order to test the predictions based on Jensen's inequality, we also measured variables associated with energetic balance such as starvation

tolerance, and body mass variation (as a proxy for blood consumption). In *R. prolixus*, starvation tolerance varies across life stages (first and second instars and adults are more susceptible to starvation) (Felicangeli et al., 1980), and as a consequence of temperature (Schilman and Lazzari, 2004), humidity and their fluctuation (Luz et al., 1998). First, third and fifth instars are more sensitive to starvation at fluctuating temperatures around higher (25–35 °C and 25–28 °C) than lower (20–25 °C and 15–28 °C) mean temperatures (Luz et al., 1998). Nonetheless, the effect of fluctuation cannot be isolated from the effects of different mean temperatures since starvation tolerance was not measured at the corresponding mean temperatures (Luz et al., 1998). In our study, males exposed to fluctuating temperatures have higher rate of body mass reduction compared to males from constant temperature. Although we cannot completely discard that this difference could be partially explained by differences in water loss rate, the cuticular permeability of this species is very low, and does not increase with temperature up to 35 °C ($CP \sim 2.5 \mu\text{g h}^{-1} \cdot \text{cm}^{-2} \cdot \text{Torr}^{-1}$; Rolandi et al., 2014); thus this variation might be associated with a higher metabolic rate, and consequently blood utilization rate under DTF. The shorter survival time of insects exposed to fluctuating temperature support our previous explanation. However, the combined effects of means and variance of environmental temperatures produced different biological responses (Bozinovic et al., 2016, 2012), thus, future research considering this interaction will deepen our understanding for *R. prolixus* under heterogeneous thermal landscapes. Our results agree with predictions of Jensen's inequality since the DTF range lies within the ascending portion of the metabolic rate-temperature curve (de la Vega et al., 2015; de la Vega and Schilman, 2018; Rolandi, 2016). Hence, the increase of metabolic rate with natural daily thermal variations will be higher than what is predicted at mean constant temperature. The magnitude of this effect will be given by the thermal sensibility of metabolic rate. In the caterpillar of the butterfly *Erynnis propertius* there is a modulation of metabolic rate; as a consequence of variable environments thermal sensibility decreases, favoring energy budget during winter, when thermal variability is high and food availability is low (Williams et al., 2012). A similar modulation of metabolic rate is observed in *Tenebrio molitor*, where increases in DTF range ($\delta = 8^\circ\text{C}$) cause reductions in maximum resting metabolic rate (Bozinovic et al., 2013). *R. prolixus*' tropical distribution raises a question about the evolution of the mechanisms for metabolic modulation. Although the latter remains to be measured, our results of body mass loss and starvation tolerance do not show evidence of developmental, or phenotypic plasticity of thermal sensitivity (or metabolic modulation is not big enough to counteract the effect of this fluctuation). Different acclimation responses to thermal fluctuation have been observed in traits such as expression of heat shock proteins between an invasive and native *Drosophila* species suggesting the importance of the biogeographic origin on the biochemical responses to DTFs (Boher et al., 2016). More comparative studies across species from different climates will further deepen the understanding of the implications of thermally heterogeneous environments.

Our results sum to the growing number of studies that account for heterogeneous thermal environments. *R. prolixus* exposed to a variable thermal environment have higher rates of mass loss and mortality, which can be associated to higher energetic costs compared to constant environment. Higher energetic costs could imply an increase in biting rate, and consequently an increase of disease transmission from infected insects (Kovats et al., 2001; Rolandi and Schilman, 2012). Assuming that *R. prolixus* takes a blood-meal after consuming 100% of the previous meal, we can estimate that males of *R. prolixus*, will feed in a constant environment (24 °C) every ca. 34 days while in a fluctuating environment (mean 24 °C, min = 17 °C, max = 32 °C) every 29 days. Considering a fixed probability of infection per bite, this increase in nutrient conversion rate with fluctuating temperature would mean a 17% increase in the biting and Chagas disease transmission rate. On the contrary, the lower fecundity observed for insects under the fluctuating

thermal regime could be associated with a decrease in population growth. Another important factor to account for in the case of insect vectors of infectious diseases is the effect temperature variations have on the parasites (Lambrechts et al., 2011; Paaijmans et al., 2010), and its interaction with the vector's biology (de O Rodrigues et al., 2016; Elliot et al., 2015; Raffel et al., 2012). Thus, future studies should take into account the interaction between relevant biological variables, fluctuations around higher temperatures in order to have a deeper understanding the effects of climate change in the biology of this insect, and hence the impact in Chagas disease.

5. Conclusion

In brief, the results presented in this study suggest that at a population and epidemiological level, the higher energetic costs of *R. prolixus* in a fluctuating thermal environment would imply an increase in nutrient consumption rate, biting frequency, and, consequently increasing disease transmission. However, the lower fecundity of mated females at DTF could be associated with a decrease in population growth. Thus, understanding the complex relations between environmental temperature variation and biological parameters requires more empirical work. This knowledge will not only provide basic information to the field of insect ecophysiology, but also could be useful background information to develop population and disease transmission models.

Funding

This work was supported in part by Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) /Argentina (PICT-2015–3491) and Consejo Nacional de Investigación Científica y Tecnológica (CONICET)/Argentina (PIP- 112–201301–00550). C.R. was also supported by a Post-doctoral fellowship from CONICET and P.E.S. by a researcher position at CONICET and Universidad de Buenos Aires (UBA)/Argentina.

Acknowledgements

The authors wish to thank Julian Mensch for constructive comments on an early version of the manuscript and the Laboratory of Insect Physiology (UBA-IBBEA-FCEN-DBBE) for sharing the rearing of insects and lively discussions. Authors also thank the editor Professor Dr. Hans-Otto Pörtner and an anonymous reviewer for helpful suggestions that improved the manuscript.

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