





Usefulness and limitations of thermal performance curves in predicting ectotherm development under climatic variability

Rassim Khelifa^{1,2}  | Wolf U. Blanckenhorn¹  | Jeannine Roy¹ | Patrick T. Rohner¹  | Hayat Mahdjoub¹ 

¹Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland

²Department of Botany, University of British Columbia, Vancouver, BC, Canada

Correspondence

Rassim Khelifa
Email: erassimkhelifa@gmail.com

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Abstract

1. Thermal performance curves (TPCs) have been estimated in multiple ectotherm species to understand their thermal plasticity and adaptation and to predict the effect of global warming. However, TPCs are typically assessed under constant temperature regimes, so their reliability for predicting thermal responses in the wild where temperature fluctuates diurnally and seasonally remains poorly documented.
2. Here, we use distant latitudinal populations of five species of sepsid flies (Diptera: Sepsidae) from the temperate region (Europe, North Africa, North America) to compare estimates derived from constant TPCs with observed development rate under fluctuating temperatures in laboratory and field conditions.
3. TPCs changed across gradients in that flies originating from higher latitudes showed accelerated development at higher temperatures, an adaptive response. TPCs were then used to predict development rates observed under fluctuating temperatures; these predictions were relatively accurate in the laboratory but not the field. Interestingly, the precision of TPC predictions depended not only on the resolution of temperature data, with daily and overall temperature summing performing better than hourly temperature summing, but also on the frequency of temperatures falling below the estimated critical minimum temperature. Hourly temperature resolution most strongly underestimated actual development rates, because flies apparently either did not stop growing when temperatures dropped below this threshold, or they sped up their growth when the temperature rose again, thus most severely reflecting this error.
4. We conclude that when flies do not encounter cold temperatures, TPC predictions based on constant temperatures can accurately reflect performance under fluctuating temperatures if adequately adjusted for nonlinearities, but when encountering cold temperatures, this method is more error-prone.
5. Our study emphasizes the importance of the resolution of temperature data and cold temperatures in shaping thermal reaction norms.

KEYWORDS

Altitude, chilling, climate change, latitude, Sepsidae, temperature variation, thermal adaptation

1 | INTRODUCTION

Changes in world temperature, not only on average but also in how temperature varies daily and seasonally, are a fundamental factor influencing global biodiversity via species' distribution, physiology and ecology (Andrew et al., 2013; Hughes, 2000; Nooten, Andrew, & Hughes, 2014; Parmesan & Yohe, 2003). Ectotherms, the most diverse organisms on earth (Wilson, 1992), are particularly susceptible to changes in environmental conditions since all their physiological and biological functions depend on temperature (Jarošík, Honěk, & Dixon, 2002; Jarošík, Kratochvíl, Honěk, & Dixon, 2004; Paaïjmans et al., 2013). The biological and ecological responses of organisms to increasing temperature have already been demonstrated in various taxa showing plasticity (Charmantier et al., 2008; Parmesan & Yohe, 2003; Seebacher, White, & Franklin, 2015) and/or adaptation to the new environmental conditions (Hoffmann, Sørensen, & Loeschcke, 2003). As the average temperature across the globe is expected to rise by 1–4°C in the next 100 years (Intergovernmental Panel on Climate Change, 2014), and temperature variation is predicted to increase as well (Alexander et al., 2006; Donat & Alexander, 2012), understanding the thermal responses of species to this change is crucial to predicting their fate in the future and to inform conservation practice (Bozinovic, Bastías, et al., 2011; Bozinovic, Calosi, & Spicer, 2011; Clavijo-Baquet et al., 2014; Vazquez, Gianoli, Morris, & Bozinovic, 2017).

To reliably predict the response of organisms to future temperature changes, a good understanding of their current thermal adaptation and the relevant thermal parameters to which species respond is needed (Bozinovic, Catalan, Estay, & Sabat, 2013; Bozinovic, Medina, Alruiz, Cavieres, & Sabat, 2016a; Paaïjmans et al., 2013). Previous studies on warming typically focused on the effects of average temperatures for simplicity, but more recently, temperature fluctuations also have been shown to be crucial for accurately estimating the spatiotemporal thermal responses of temperate species (Bernhardt, Sunday, Thompson, & O'Connor, 2018; Bozinovic, Bastías, et al., 2011; Ragland & Kingsolver, 2008; Thompson, Beardall, Beringer, Grace, & Sardina, 2013; Vasseur et al., 2014). Although there is a great body of literature that experimentally estimated the response of species to constant temperatures (Scheiner, 2002), the reliability of these estimates to predict responses to fluctuating temperatures (Estay, Lima, & Bozinovic, 2014; Kingsolver, Higgins, & Augustine, 2015), which clearly better reflect natural conditions at least in temperate regions, has yet to be tested.

The thermal performance curve (TPC) is an important tool to understand thermal adaptation and the physiology of organisms (Angilletta, 2009; Bozinovic, Sabat, Rezende, & Canals, 2016b). TPCs are usually estimated across a range of relevant constant temperatures and has a shape of a negatively skewed normal distribution, with zero performance beyond the critical minimum (CT_{min}) and maximum (CT_{max}) temperatures and a peak at the optimal temperature (T_{opt}), which typically is closer to CT_{max} (Deutsch et al., 2008). From CT_{min} to T_{opt} , the performance increase is quasi-exponential. This part of the curve is very important because it

includes the temperature range that organisms are most often exposed to in their natural habitat (Bernhardt et al., 2018). Due to the nonlinearity of TPCs and to the fact that temperature in the wild varies daily and seasonally (Colinet, Sinclair, Vernon, & Renault, 2015), the average performance under fluctuating temperatures is not equal to the performance at the corresponding average temperature (Ruel & Ayres, 1999; Vasseur et al., 2014; Williams et al., 2012), a mathematical property known as Jensen's inequality. Although TPCs derived from constant temperatures have been regularly used to determine the responses of organisms to fluctuating temperatures (Thompson et al., 2013; Vasseur et al., 2014), assuming or suggesting that the overall response is the product of the additive responses to temperatures (Liu, Zhang, & Zhu, 1995), experimental studies are needed to confirm this under laboratory and especially field conditions.

Thermal performance curves vary across geographic gradients in ectotherms (Sinclair et al., 2016). Higher latitude and higher elevation sites have shorter warm periods during the year, shifting the TPC of species horizontally towards a lower temperature range implying faster development at lower temperatures or vertically towards overall higher performance implying faster development at higher latitudes and elevations at any given temperature (countergradient variation: Yamahira, Kawajiri, Takeshi, and Irie (2007). Thus, a species' temperature sensitivity may evolve in response to environmental conditions. For instance, Kingsolver, Massie, Ragland, and Smith (2007) showed that the TPC of cabbage white butterflies (*Pieris rapae*) evolved after range expansion in North America. However, evolutionary adaptation can involve various traits (Angilletta, 2009), such that the absence of differences between geographically distant populations for a certain trait may hide adaptive compensatory processes in other traits (Blanckenhorn & Demont, 2004). Common garden experiments are useful tools to reveal such geographic adaptations (Thompson et al., 2013).

The general relevance of TPCs in predicting the response of species to climate change in the wild nonetheless remains questionable. One reason is that TPCs are typically estimated under controlled laboratory conditions, which do not reflect the actual natural conditions where a large spectrum of factors will affect the involved physiological responses (Cook, Wolkovich, & Parmesan, 2012). For instance, it has been widely shown in plants that there are clear carry-over effects of winter temperatures on spring development rate and phenology (Cook et al., 2012; Fu et al., 2015; Porter & Gawith, 1999) in that spring development is usually fast after cold winters (chilling fulfilment) but relatively slow after warm winters (chilling loss). Although this topic has so far received little attention in animals, there is evidence that post-winter development is faster after longer winters (accumulating more chilling) (Bosch & Kemp, 2003; Stålhandske, Gotthard, & Leimar, 2017; Stålhandske, Gotthard, Posledovich, & Leimar, 2014). If this pattern is general among insects, it is reasonable to suggest that TPCs for development rate estimated under controlled laboratory conditions would not reliably estimate the phenology under natural conditions featuring cold temperatures. Here, we test this hypothesis with a field

experiment comparing observed and TPC-predicted development time.

Investigating five species of closely related black scavenger flies (Diptera: Sepsidae), we here asked whether and how development rate estimated under constant temperatures differs from that estimated under fluctuating temperatures in the laboratory, and to which extent TPCs established using constant temperatures can reliably predict future responses of natural populations to climate change, especially when temperatures fall under the critical minimum. We first estimated the thermal tolerance range of each species (TPC) by raising the flies under various constant temperatures (12° to 35°C). We then quantified the effect of Jensen's inequality by subjecting the flies to two diurnally fluctuating temperatures ($18 \pm 3^\circ\text{C}$ and $18 \pm 7^\circ\text{C}$) plus one constant temperature ($18 \pm 0^\circ\text{C}$) with the same average in the laboratory. Finally, to assess the predictive reliability of laboratory TPCs for the development observed in the field, we exposed the flies to natural field temperatures towards the end of the season. We focused on development rate as a measure of performance because of its strong life-history implications (Gillooly, Charnov, West, Savage, & Brown, 2002) and its tight relationship to fitness (Dmitriew, 2011) and adult phenology (Tauber, Tauber, & Masaki, 1986).

2 | MATERIALS AND METHODS

2.1 | Study organisms

Sepsid flies are widespread acalyptrates that use decaying organic matter as breeding substrate. Larvae of the genus *Sepsis* are coprophagous and dwell preferentially in vertebrate dung (often cattle), whereas adults feed on nectar of flowering plants and dung (Figure S1a). They represent good model organisms for studies of thermal adaptations (e.g. Berger, Postma, Blanckenhorn, & Walters, 2013; Berger, Walters, & Blanckenhorn, 2014) as they are easy to maintain in the laboratory and their geographic distribution is wide across both latitude and elevation (Pont & Meier, 2002; Roy, Blanckenhorn, & Rohner, 2018). Egg-to-adult development time varies among species from 1–6 weeks under field conditions (Pont & Meier, 2002).

We performed field and laboratory experiments with five of the most common temperate *Sepsis* species that regularly coexist on pastures in Europe: *S. cynipsea* (Linnaeus, 1758), *S. thoracica* (Robineau-Desvoidy, 1830), *S. punctum* (Fabricius, 1794), *S. neocynipsea* (Melander & Spuler, 1917), and *S. fulgens* (Meigen, 1826). Populations used in the experiment originated from Europe, North Africa and North America (Table S1). For each species, we used two populations except for *S. punctum*, for which we used four because this species is differentiated in Europe versus North America (Puniamorthy, Schäfer, & Blanckenhorn, 2012). To additionally assess potentially systematic geographic patterns of intraspecific differentiation, the population pairs were selected from latitudinal (*S. cynipsea*, *S. thoracica*, and *S. punctum*), elevational (*S. punctum*, *S. neocynipsea*, and *S. fulgens*), or distant transcontinental locations (*S. neocynipsea*).

2.2 | Sample preparation

Flies were collected in the field and brought to the laboratory at various times. Single females were isolated into plastic containers with a mesh lid and provided with dung as oviposition substrate. Offspring emerged were subsequently kept for multiple generations as isofemale lines under constant conditions (temperature 18°C, 60% humidity and 16L: 8D photoperiod) in a climate chamber, with sugar and water ad libitum as food sources and fresh cattle dung for breeding.

To perform our experiments, we outcrossed 5 isofemale lines for each population and species to avoid inbreeding effects, increase the genetic variation and reduce maternal carry-over effects by randomly mixing 40 offspring individuals (20 males and 20 females) from each isofemale line for two generations in plastic containers ($20 \times 5 \times 5$ cm) at the above conditions.

2.3 | Experiments

2.3.1 | Experiment I – Estimation of thermal performance curves for development rate at constant temperatures

Thermal performance of individuals from each population and species was estimated at seven constant temperatures (12°, 17°, 22°, 27°, 32°, 34° and 35°C) and constant photoperiod (16L:8D) in separate climate chambers. Temperatures were chosen using prior knowledge about *Sepsis* thermal adaptation (Berger et al., 2013; Blanckenhorn, 1999) and the theoretical shape of the thermal performance curve. The interval between the first five temperatures (12°–32°C) is 5°C because these temperatures fall within the gradually increasing range of the curve, with 12°C being close to the putative lower thermal threshold; however, the thermal interval was reduced to 2° and 1°C beyond 32°C, where performance was expected to decrease abruptly (Figure S1b).

For each species and population, we supplied a group of 20–30 flies of similar age in a given replicate container with food and fresh dung in a dish for oviposition at our standard holding temperature of 18°C (cf. below). After 24 hr, the dung dish (containing eggs) was removed, transferred to a cylindrical glass vial (10 × 3 cm) and checked daily for emergence of adult offspring. The position within the climate chamber of replicates, populations and species was randomized. In total, we had 9 replicates for each species ($n = 5$), population ($n = 2$, except for *S. punctum*: $n = 4$) and temperature treatment ($n = 3$) combination. Development time was estimated as the number of days from egg laying to the first adult emergence; development rate was then calculated as the inverse of development time.

2.3.2 | Experiment II – Assessment of development rate at fluctuating temperatures

We exposed flies of each species and population in separate climate chambers to three treatments with the same average temperature, 60% humidity and 16L:8D photoperiod. We set one treatment at constant 18°C, adding two fluctuating treatments

with a mean of 18°C and an amplitude of 15–21°C (18 ± 3°C) or 11–25°C (18 ± 7°C) (Figure S1c). The choice of our standard holding/rearing temperature of 18°C in our experiment was based on (a) the natural phenology of our flies, 18°C being a temperature that is regularly encountered in the wild by all species during the reproductive season, and (b) Jensen's inequality, as 18°C is located at a point of the TPC where temperature fluctuation induces a physiological response that is different from that of the mean. Apart from using non-constant temperatures here, the methodology of the previous experiment to assess development rate was applied, with 10 replicates for each species ($n = 5$), population ($n = 2$, except for *S. punctum*: $n = 4$) and temperature treatment ($n = 3$) combination.

2.3.3 | Experiment III – Assessment of development rate under field conditions at the end of the season

A field experiment assessing development rate of the same five species was conducted on the Irchel campus of the University of Zurich towards the end of the season 2016. For this experiment, we used flies collected from two local populations in Switzerland (*S. neocynipsea* from Sörenberg, all others from Zürich). Starting in mid-August (12.08.2016) until late November, wild-caught (parental) flies were kept in 3-L containers with sugar, water and dung ad libitum. Four replicate containers per species were placed outside in randomized order in a largely shaded location to avoid the risk of overheating due to direct sunlight. Fresh cow dung was supplied (i.e. replaced) twice a week in 100-ml plastic cups for oviposition. When eggs were detected in the dung, the cups were isolated into a separate container at the same field site and checked daily for emergence of F1 offspring adults to estimate development time under the ever-decreasing field temperatures in autumn (Figure S2). We obtained corresponding hourly and daily mean air temperature data from a nearby meteorological station (4 km from the field site) from IDAweb (<https://gate.meteoswiss.ch/idaweb/>) to assess the effect of fluctuating field temperatures on development rate.

2.4 | Statistical analyses

Statistical analyses were performed with R 3.2.2 (R Development Core Team, 2019). Regular (normally distributed) and phylogenetically corrected mixed-effects models were applied with the R packages LME4 (Bates, Mächler, Bolker, & Walker, 2015) and MCMCGLMM (Hadfield, 2010). The phylogenetic relationship among species was accounted for in all MCMCglmm models based on the phylogeny reported by Zhao, Annie, Amrita, Yi, and Rudolf (2013). The phylogenetic signal λ was calculated by dividing the phylogenetic variance by the total variance (phylogenetic, random effects and residual variance) (Currie & Meade, 2014). To check for model convergence, diagnostic plots were produced with the scapeMCMC package (Magnusson, Stewart, & Magnusson, 2009). Values are mean ± SD (unless indicated otherwise).

2.4.1 | Experiment I

A two-way ANOVA was used to test for the differences among species and viable temperatures (12–32°C) on development rate (log-transformed) and the potential effect of species-by-temperature interaction which reflects interspecific difference in reaction norms. We further investigated the inter-population differences in development rate and thermal reaction norms by using a phylogenetically corrected mixed-effects regression which includes temperature, latitude, elevation and the three-way-interactions as fixed effects and species as a random effect. Since three-way interaction did not significantly improve the fit (based on AIC), only two-way interactions were kept in the model. In this analysis, the temperature treatments of 34° and 35°C were excluded because no flies emerged.

To estimate the TPC at constant laboratory conditions for each population and species, temperature was used as a continuous variable and all seven temperature treatments were included. Since all individuals died at 34° and 35°C, development rate was set to zero. We fitted the O'Neill function (Krenek, Berendonk, & Petzoldt, 2011). This function estimates three of the four key TPC parameters of the response to temperature (T_{opt} , CT_{max} , and Q_{10}) (Equation 1). The model assumes that growth increases with temperature with a coefficient Q_{10} prior to the optimal temperature (T_{opt}), where development is maximal (r_{max}), and decreases abruptly afterwards towards the critical maximum temperature (CT_{max}), where development is no longer possible.

$$\Phi(T) = r_{max} \left(\frac{CT_{max} - T}{CT_{max} - T_{opt}} \right)^p \exp \left(\frac{p(T - T_{opt})}{T_{max} - T_{opt}} \right), \quad (1)$$

where T is the ambient temperature, $p = \frac{1}{400} J^2 \left(1 + \sqrt{1 + \frac{40}{J}} \right)^2$, $J = (Q_{10} - 1)(T - T_{opt})$, and $\varphi(T)$ is the respective mean development rate estimated from experimental data.

2.4.2 | Experiment II

We again used both regular and phylogenetically corrected linear mixed-effects regression models to investigate development rate as function of the temperature fluctuation treatment (18 ± 0°, 18 ± 3°, 18 ± 7°C), species, plus their interaction, and latitude/elevation (i.e. population) as covariate. To assess the usefulness of the TPC estimated with constant temperatures (in experiment I) in predicting the actually observed performance under fluctuating temperatures, we derived estimates based on hourly temperature readings in the climate chambers to predict the development rate observed in our laboratory experiment II. We estimated the hourly development rate by dividing the TPC-estimated development rate by 24 hr (since the unit of TPC is day). Then, we summed all hourly development rates to obtain the predicted daily development rate. Because the same frequency distribution of temperatures is used every day in the laboratory experiment, the mean development rate for the entire development time is theoretically identical to that for any given day. To directly compare the observed and TPC-predicted development

rate, reduced major axis regressions (observed development rate being the response) assessing deviations from unity were conducted for each species using the lmodel2 R package (Legendre, 2014).

2.4.3 | Experiment III

For each species, the relationship between TPC-predicted and field-observed development rate was assessed at three different resolutions of temperature measurements with reduced major axis regression (observed development rate being the response), as above for potential deviations from unity in experiment II. The three temperature scales were hourly average, daily average and overall average temperature (i.e. average of daily temperatures across the entire development).

To test for the potential relationship between the precision of the estimated development time and the frequency of cold conditions (i.e. temperatures below the estimated CT_{min} ; see Supporting information), we fitted a linear mixed-effects model with the ratio of observed-to-predicted development time as the response variable, the number of days below CT_{min} as fixed effect, and species as random effect. The slope of the relationship between field-observed and TPC-estimated development time was additionally estimated separately for flies that did not encounter cold conditions (no temperatures below CT_{min}) and those that experienced cold conditions (number of days below CT_{min}).

3 | RESULTS

3.1 | Effects of constant laboratory temperatures on development and TPC

There was a significant effect of species (ANOVA: $F_{4,477} = 284.2$, $p < .0001$), temperature (ANOVA: $F_{4,477} = 5,569.2$, $p < .0001$), and species-by-temperature interaction (ANOVA: $F_{16,477} = 11.3$, $p < .0001$) on

development rate (log-transformed) (Figure 1). The fastest developing species were *S. cynipsea* and *S. thoracica*, whereas the slowest were *S. neocynipsea* and *S. fulgens* (Figure S3). When controlling for phylogeny, species effect is no longer significant and the variance in development rate was mostly explained by the positive effect of temperature and the interaction between species and temperature (Table S2), which reveal interspecific differences in the thermal reaction norm. The linear mixed-effects model corrected for phylogeny testing for the effect of latitude and elevation on development rate showed a significant effect of latitude and temperature-by-latitude interaction but not of elevation. Interestingly, development rate declined with latitude in cold temperatures (12–17°C), but increased with latitude in warm temperatures (22–32°C) (Table S3). Flies of all species did not survive to the adult life stage at 34° and 35°C; we thus took the former as the critical maximum temperature. There was a significant positive relationship between r_{max} and T_{opt} (linear model: slope = 0.03, SE = 0.007, $R^2 = 0.62$, $p = .002$), showing that species that have higher optimal temperature T_{opt} tend to have faster development rate (Figure S4).

3.2 | Relationship between observed and predicted development rate at fluctuating temperatures in the laboratory

All species responded similarly to temperature fluctuations: the greater the fluctuation, the faster the development rate (Figure 2, Table S5). On average, moderately ($18 \pm 3^\circ\text{C}$) and highly fluctuating ($18 \pm 7^\circ\text{C}$) temperatures increased development rate by 4.3% and 12.9%, respectively, relative to constant 18°C. However, development rate did not vary with latitude or elevation (Table S5).

When pooling all species, populations and treatments, the slope of the relationship between experimentally observed and TPC-based predicted development rate was slightly but not significantly lower

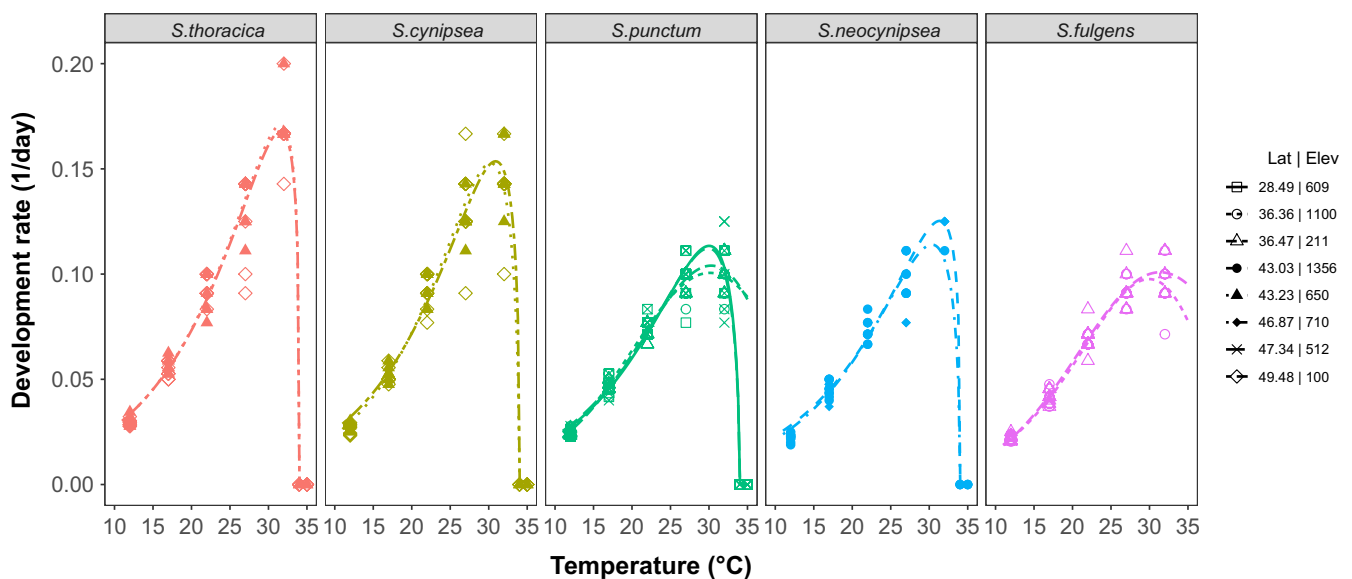


FIGURE 1 Thermal performance curves (TPC) for development rate of five species of *Sepsis* at seven temperatures (12°, 17°, 22°, 27°, 32°, 34°, 35°C) as fitted by the O'Neil function. Parameter estimates for each species are shown in Table S4. The legend refers to the latitude (°) and elevation (m a.s.l) for each population studied

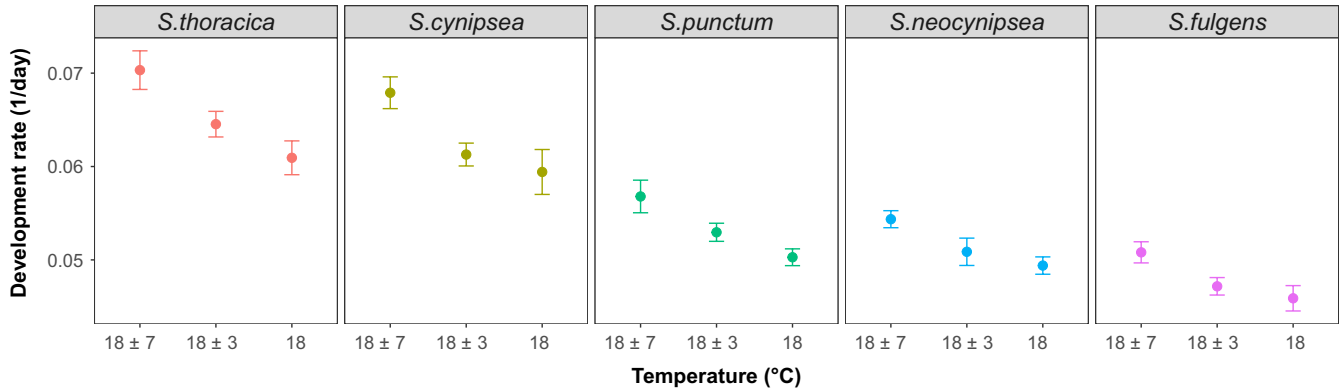


FIGURE 2 Effect of diurnal temperature fluctuation on the development rate of five *Sepsis* species. The three temperature regimes were a constant ($18 \pm 0^\circ\text{C}$), a moderately variable ($18 \pm 3^\circ\text{C}$) and a highly variable temperature ($18 \pm 7^\circ\text{C}$) treatment with an average of 18°C . Error bars are 95% confidence intervals

than unity (slope = 0.97 [95% CI: 0.92, 1.03], $R^2 = 0.97$, $N = 36$), revealing that estimates derived from constant TPCs are close to those observed even when temperature fluctuates (Figure 3). Separate analyses for each species also showed no significant deviations from unity in *S. cynipsea* (slope = 0.86 [95% CI: 0.59, 1.24], $R^2 = 0.93$, $n = 6$), *S. punctum* (slope = 0.98 [95% CI: 0.77, 1.22], $R^2 = 0.90$, $n = 12$), *S. fulgens* (slope = 0.77 [95% CI: 0.57, 1.03], $R^2 = 0.96$, $n = 6$), or *S. thoracica* (slope = 0.86 [95% CI: 0.44, 1.53], $R^2 = 0.85$, $n = 6$); only in *S. neocynipsea* a significant deviation was observed (slope = 0.69 [95% CI: 0.50, 0.94], $R^2 = 0.95$, $n = 6$). The same analysis for each temperature fluctuation treatment equally showed no deviations from unity: 18°C (slope = 1.01 [95% CI: 0.95, 1.06], $R^2 = 0.99$, $n = 12$), $18 \pm 3^\circ\text{C}$ (slope = 1.04 [95% CI: 0.96, 1.13], $R^2 = 0.98$, $n = 12$), and $18 \pm 7^\circ\text{C}$ (slope = 0.99 [95% CI: 0.87, 1.12], $R^2 = 0.97$, $n = 12$). Of course, the power to detect deviations was low in these latter, separate analyses.

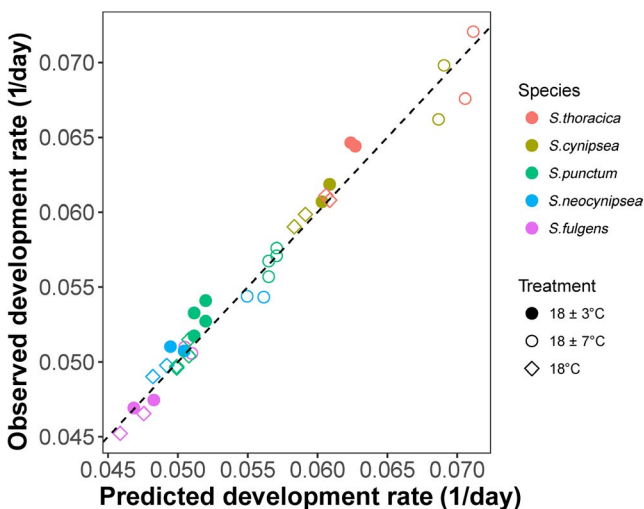


FIGURE 3 Relationship between predicted and observed development rate estimated under fluctuating conditions in the laboratory. Predicted development rates were estimated from the thermal performance curve using hourly temperature measurements. The hatched diagonal line has a slope of 1

3.3 | Relationship between observed and predicted development rates in the field towards the end of the season

Mean \pm SD development times observed in the field towards the end of the season varied within and across species: 26.86 ± 12.06 d ($n = 84$) for *S. cynipsea*, 32.66 ± 17.15 d ($n = 84$) for *S. thoracica*, 34.68 ± 13.27 d ($n = 84$) for *S. punctum*, 35.38 ± 17.44 d ($n = 84$) for *S. fulgens*, and 36.35 ± 17.70 d ($n = 63$) for *S. neocynipsea*. We estimated the development rate from the (constant) TPC for field temperatures at three different resolutions: the overall average temperature, daily average, and hourly average. Although the relationship between the field-observed and TPC-based predicted development rate differed significantly from unity at all scales (Figure S5), daily and overall average temperature yielded slopes closer to 1 (0.95 [95% CI: 0.93, 0.97] and 0.94 [0.92, 0.96], respectively) than hourly temperature (1.20 [1.16, 1.25]). This suggests that, under field conditions, laboratory-estimated TPCs tend to underestimate development rate when using hourly temperatures, whereas estimation became more accurate at lower resolutions.

We appraised the prediction precision as the ratio of observed over predicted development rate (ratio = 1 indicating perfect prediction) for the hourly, daily and overall mean temperature to find that, across species, this ratio was 1.09 [95% CI: 1.08, 1.11] for the hourly, 1.13 [95% CI: 1.11, 1.14] for the daily, and 1.02 [95% CI: 1.01, 1.03] for the overall average temperature, revealing that the crude latter resolution provides the best prediction (Table S6). However, prediction precision was negatively related to the number of chilling days (the number of days below CT_{\min}) at daily and overall average temperature resolutions for all three CT_{\min} estimations (Figure 4), unsurprisingly showing highest prediction reliability when the number of chilling days was zero (Table S7: intercept \approx 1): as chilling days became more frequent towards the end of the season, the predicted development rate became slower than observed (observed/predicted ratio $>$ 1).

When subdividing individuals into two categories – those that encountered temperatures that fell below CT_{\min} ($CT_{\min 1}$: chilled individuals) and those that did not (non-chilled individuals) – we were able

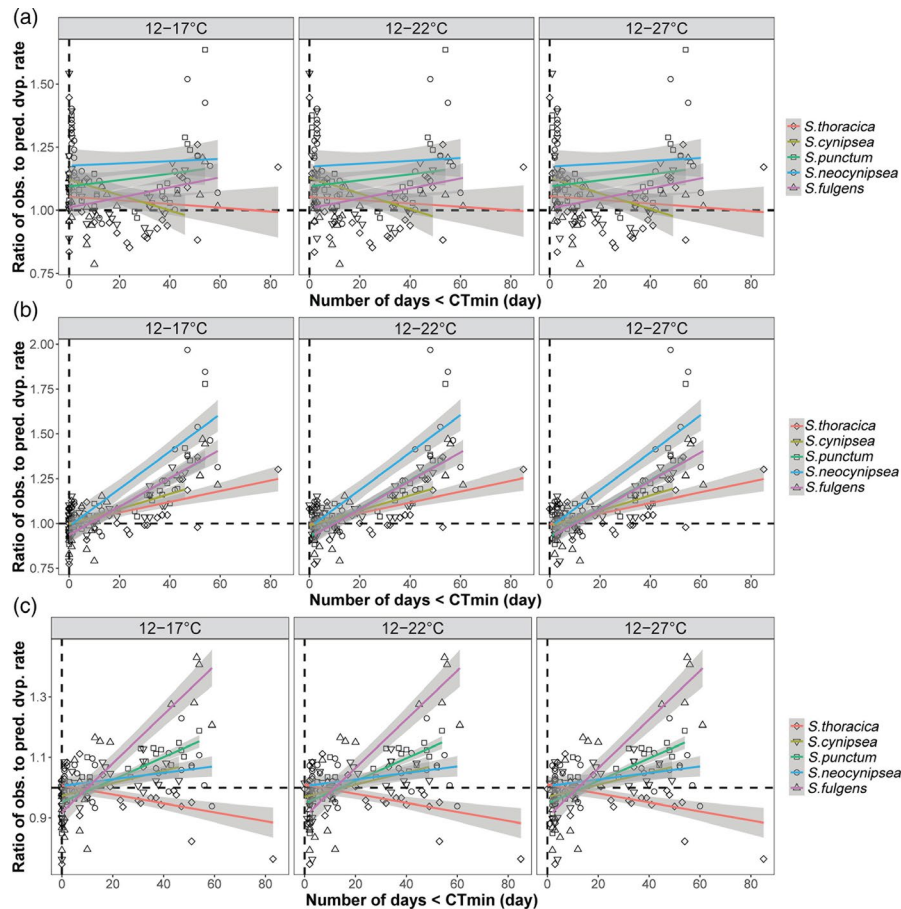


FIGURE 4 Relationship between the ratio of observed-to-predicted development rate and the number of days below the estimated critical minimum temperature CT_{min} (see Table S7) for hourly (a), daily (b), and overall mean temperature (c) summing. The three panels represent three estimates of CT_{min} (see Material and Methods). When the ratio > 1 , the observed values are underestimated by the (constant) thermal performance curve, whereas when the ratio < 1 , the observed values are overestimated. Fitted lines are simple linear regressions and grey bands are standard errors

to reveal that for non-chilled individuals, daily average temperature performed better at predicting the observed development rate than hourly or overall average temperature, showing a slope of 0.98 [0.97, 1.00] compared to 1.10 [1.08, 1.12] and 0.94 [0.93, 0.95], respectively. The inverse was true for chilled individuals, for which the overall average temperature performed better (1.04 [1.03, 1.06]) than the hourly (1.09 [1.07, 1.11]) or daily average temperature (1.15 [1.13, 1.18]).

4 | DISCUSSION

Whether thermal performance curves (TPC) of ectotherms estimated with constant temperatures can be used to predict performance at naturally fluctuating temperatures is a fundamental and timely question (Angilletta, 2009; Kingsolver et al., 2015; Kingsolver & Woods, 2016; Sinclair et al., 2016). Combining laboratory and field experiments on five temperate *Sepsis* fly species, we here investigated the plastic responses of developmental rate. This trait is of particular interest due to its critical role in shaping phenology and its direct impact on fitness (Dmitriew, 2011; Gillooly et al., 2002). TPCs

covering the full viable temperature range were typical for ectotherms, but differed significantly between species and populations within species. As flies were exposed to identical environmental conditions in a common garden, this variation is genetic (i.e. evolved) and demonstrates local adaptation to climate variation with latitude. We found that TPCs derived from constant temperatures yield reliable estimates of development time and rate under fluctuating laboratory temperatures when properly corrected. However, this technique has limitations for predicting development under natural conditions when winter approaches. This is because fluctuating temperatures below the minimum temperature threshold CT_{min} positively affect development rate. In this regard, our data further demonstrate that in order to gain adequate predictions, it is crucial to select an appropriate thermal resolution for the organism under scrutiny.

4.1 | Predicting development rate using TPCs

Theoretically, temperature fluctuations below the optimal temperature (T_{opt}), where the TPC is convex and increases exponentially, should speed up development relative to the corresponding constant

temperature, and this effect should become stronger as fluctuations increase, as found here (compare $18 \pm 7^\circ\text{C}$ versus $18 \pm 3^\circ\text{C}$ in Figure 2; Ruel & Ayres, 1999). Similar results were, for example observed in the mosquito *Anopheles stephensi* (Paaijmans et al., 2013) and the green alga *Tetraselmis tetrahele* (Bernhardt et al., 2018). According to Jensen's inequality (see Introduction), opposite effects result when the fluctuations extend to the concave section of the TPC to the right of T_{opt} , as observed, for example in the cold-adapted yellow dung fly (Kjærsgaard, Pertoldi, Loeschcke, & Blanckenhorn, 2013). These results imply that the phenology of adults may shift more strongly than expected based on mean temperatures in regions where warming increases thermal extremes.

Our main objective was to evaluate the predictive power of TPCs estimated from constant temperatures to data generated at fluctuating temperatures. To test this, we compared development rates predicted from constant TPC with the actually observed development rates generated under (a) controlled laboratory conditions with specific diurnally fluctuating temperatures, and (b) erratically fluctuating field conditions towards the end of the season. We found that TPC predicts the observed development rate relatively well when the above-mentioned nonlinear effect (Jensen's inequality) is properly adjusted for by rate summation (Liu et al., 1995). While the estimated slopes were around 1, relatively large confidence intervals resulted due to the relatively small number of data points (five species, three treatments, 2–4 populations); increasing the number of treatments and populations should add power to the analysis and likely strengthen the predictions at the species and population levels. Else, when assuming constant as opposed to fluctuating temperatures, development times and rates may be considerably under- or overestimated (Figure 2). When applied to field conditions, this rate-summing method can be used to project the effect of climate warming in the future (Vasseur et al., 2014). However, our field experiments showed that the accuracy of TPC-based predictions of development rate was limited and quite complex. As the accuracy of rate summing must depend on the resolution of the temperature data, we applied three temperature resolutions including hourly, daily and overall average temperatures to test which gives most reliable estimates of pre-winter development. Interestingly and unexpectedly, and in contrast to our laboratory study, daily and gross average temperatures yielded more accurate predictions, whereas hourly resolution generally most severely underestimated actual development rates (Figures S5 and Figure 4). Not surprisingly perhaps, the predictability of field development rates towards the end of the season here depended on the frequency of cold temperatures below the critical minimum temperature CT_{min} . Estimates of the latter here hovered around $6\text{--}7^\circ\text{C}$ for all species (Table S4), and more cold days or hours encountered in the field led to greater underestimation (i.e. individuals emerged earlier than expected; Figure 4). When excluding individuals that encountered temperatures below their critical minimum, predictions were more reliable, showing that not accounting for cold conditions (chilling) underestimates pre-winter development, potentially leading to

erroneous predictions and misleading conclusions. The use of meteorological station data 4km away instead of in situ data loggers might have added some noise to the results, but likely no systematic bias, as other factors such as humidity and day length might account for additional variation in development rate in the field. Nevertheless, there is evidence in other insects indicating that the number of chilling days positively affects (i.e. advances) the day of spring emergence (Bosch & Kemp, 2003; Stålhandske et al., 2014). Hence, cold temperatures seem to trigger an increase in development rate, ultimately leading to earlier emergence before winter frost might kill the juveniles (adaptive explanation). Alternatively, the standard method for estimating the lower temperature threshold (Blanckenhorn, 1999) may generally overestimate CT_{min} , meaning that flies do *not* stop growing after all when temperatures fall below this threshold, thus generally leading to underestimated growth and development rates during winter (non-adaptive explanation). Hourly temperature resolution most strongly underestimated actual development rates here. This is because temperatures fall under the putative lower threshold only for some hours during any given day while daily means remain above the threshold. Hourly measurements, therefore, most severely (i.e. accurately) reflect this error. Since we think that this pattern of underestimated pre-winter (and perhaps also post-winter) growth at cold temperatures might be general among ectotherms, it is essential that future attempts to predict climate change effects on phenology should consider the potential effect of cold temperatures (ranging below critical minimum) on the thermal responses of species, before or after entering diapause (Stålhandske et al., 2017, 2014). Although our study shows that temperature fluctuations augmented the plastic response by accelerating the development of species, an increase in both the variance and the average might in fact decrease development rate and potentially increase mortality (Bozinovic, Bastías, et al., 2011). It is still uncertain how species will respond to future climate change given the potential changes in thermal sensitivity through adaptive processes (Araújo et al., 2013; Hoffmann & Sgrò, 2011). A recent study on bacteria found that species adapt to temperature fluctuations by lowering their sensitivity to temperature variations (Saarinen, Laakso, Lindström, & Ketola, 2018). We highlight a research opportunity for experimental evolutionary studies of adaptation to fluctuating temperatures in order to unravel the temporal dynamics of thermal sensitivity in ectotherms.

4.2 | Intra- and interspecific variation in TPCs

We observed significant differences among species, with *S. cynipsea* and *S. thoracica*, the most common species in north-central and southern Europe (respectively), showing fastest development especially at warm temperatures ($22\text{--}32^\circ\text{C}$), and *S. neocynipsea* and *S. fulgens* showing the slowest development. These interspecific differences at least partly relate to these flies' distribution range, with the faster-developing species being warm-loving (and common in the southern temperate region), and slower developing species

being cold-loving or widespread (*S. punctum*, *S. fulgens* and *S. neocynipsea*) (Pont & Meier, 2002; Rohner et al., 2015). In all five species, we documented an optimal temperature (T_{opt}) for development rate around 29–32°C that was positively correlated to their maximum development rate, suggesting that fast-developing species are more warm-adapted. However, because of the typically steep decline of the TPC towards the critical maximum beyond T_{opt} (Figure 1), further detailed studies scrutinizing potential interspecific differences at temperatures between 32° and 34°C are needed to reveal possible adaptations of the upper thermal extreme (CT_{max}) at the species and population levels that could not be resolved here.

In addition to these interspecific differences, populations of most species varied geographically in their responses to constant laboratory temperatures along latitude. Extending Berger et al.'s (2014) study of *S. punctum*, flies consistently developed faster at higher latitudes when exposed to high temperatures, revealing adaptation to cold climates across latitude. Faster development in cooler regions with short seasons is adaptive because flies can complete their life cycle faster at any given (cool) temperature, thus likely explaining the observed systematic changes in thermal reaction norms at higher latitudes found here and elsewhere (Homeny & Juliano, 2007; Kipyatkov & Lopatina, 2010; Mikolajewski, De Block, & Stoks, 2015; Yamahira et al., 2007). However, such countergradient variation was not found in elevation. Studies on dung fly species *Scathophaga stercoraria* and *S. cynipsea* have shown low levels of altitudinal genetic differentiation in quantitative life-history and morphological traits, suggesting high gene flow between low and high elevation populations (Blanckenhorn, 1997a, 1997b, 1998; Kraushaar, Goudet, & Blanckenhorn, 2002). Furthermore, following another study on *S. fulgens* showing no latitudinal cline for development time across Europe (Roy et al., 2018), we found no elevational differentiation in Algerian *S. fulgens*. This study confirms that the geographic cline of life-history traits in sepsids are complex, and the underlying ecological, behavioural and physiological mechanisms need further investigation.

5 | CONCLUSIONS

In recent years, increasing attention has been paid to the importance of temperature fluctuations for more accurately predicting the thermal and phenological responses of temperate ectotherms (Cavieres, Bogdanovich, Toledo, & Bozinovic, 2018; Vasseur et al., 2014). It is by now clear that organisms are well adapted to fluctuating temperatures (Saarinen et al., 2018); hence, using constant temperatures in laboratory experiments may obscure patterns such that, in the extreme, we may reach wrong conclusions, or at least quantitatively inaccurate predictions. Our study adds a new layer of information regarding the usefulness and limitation of TPCs for predicting organisms' responses to climate change by combining theoretical, laboratory and field data. We confirmed that fluctuating temperatures alter development rate also in sepsid flies, mandating that future studies of thermal responses

of life-history traits should consider naturally variable temperatures to obtain more accurate estimates better reflecting those observed in the wild (Cavieres, Bogdanovich, & Bozinovic, 2016). We also confirmed that TPCs derived from constant temperatures can be used to reasonably predict thermal responses to fluctuating temperatures under controlled laboratory conditions if properly adjusted. However, when applying the same approach to field data, predictions derived from constant TPCs can be less reliable, notably when temperatures fall below the critical lower temperature threshold (i.e. at the cold end of the TPC). We have shown that predictions and estimations further depend on the resolution of temperature data (hourly, daily, or gross average) and that considering different thermal scales relevant to the study organisms is essential. As seasonal and diurnal temperature fluctuations in the temperate region often span the left-hand region of TPC, crossing the critical minimum temperature, climate change is likely to alter the thermal sensitivity of species, which makes long-term projections of the future phenology of ectotherms using TPCs difficult.

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AUTHORS' CONTRIBUTIONS

R.K., W.U.B. and H.M. conceived the ideas and designed the experiment; H.M., P.T.R. and J.R. collected the data; R.K. and H.M. analysed the data; R.K., H.M., P.T.R. and W.U.B. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data are deposited in Dryad Digital Repository: <https://doi.org/10.5061/dryad.fc75292> (Khelifa et al., 2019).

ORCID

Rassim Khelifa  <https://orcid.org/0000-0001-6632-8787>

Wolf U. Blanckenhorn  <http://orcid.org/0000-0002-0713-3944>

Patrick T. Rohner  <http://orcid.org/0000-0002-9840-1050>

Hayat Mahdjoub  <https://orcid.org/0000-0002-8972-9628>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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