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

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Abstract: 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. 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. 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. 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. Our study emphasizes the importance of the resolution of temperature data and cold temperatures in shaping thermal reaction norms.

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**Usefulness and limitations of thermal performance curves in predicting
ecotherm development under global change**

Running title: Thermal performance curve performance

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Abstract (292 words)

Thermal performance curves (TPCs) have been estimated in multiple temperate ectotherm species and used 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. 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. TPCs changed across gradients in that flies originating from higher latitudes or altitudes showed accelerated development, 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 in the field. Interestingly, the precision of TPC-predictions depended not only on the resolution of temperature data, with diurnal 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 speed up their growth when the temperature rises again, thus most severely reflecting this error. 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 non-linearities, but when they encounter cold temperatures this method is more error-prone. Our study emphasizes the importance of the resolution of temperature data and cold temperatures in shaping thermal reaction norms, thus improving predictions of the responses of ectotherms to climate change in the age of big data and citizen science.

Keywords: Altitude, chilling, climate change, latitude, temperature variation, thermal adaptation, Sepsidae.

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 (Hughes 2000, Parmesan and Yohe 2003, Andrew et al. 2013, Nooten et al. 2014). 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 et al. 2002, Jarošík et al. 2004, Paaijmans et al. 2013). The biological and ecological responses of organisms to increasing temperature have already been demonstrated in various taxa showing either plasticity (Charmantier et al. 2008, Seebacher et al. 2015), shifting phenology (Parmesan and Yohe 2003, Root et al. 2003, Thomas et al. 2004), or adaptation to the new environmental conditions (Hoffmann et al. 2003). As the average temperature across the globe is expected to rise by 1°–4°C in the next 100 years (IPCC 2014), and temperature variation is predicted to increase as well, understanding the thermal responses of species to this change is crucial to predict their fate in the future and to inform conservation practice.

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. 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 (Bozinovic et al. 2011, Thompson et al. 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, which clearly better reflect natural conditions at least in temperate climes, 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). 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. Due to the nonlinearity of TPCs and to the fact that temperature in the wild varies daily and seasonally, the average performance under fluctuating temperatures is not equal to the performance at the corresponding average temperature (Ruel and Ayres 1999, Vasseur et al. 2014), 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, assuming or suggesting that the overall response is the product of the additive responses to temperatures (Liu et al. 1995), experimental studies are needed to confirm this under laboratory and especially field conditions.

TPCs 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 range of temperatures (e.g. implying faster development at lower temperatures), or vertically towards higher rates, implying faster development at higher latitudes and elevations at any given temperature (countergradient variation; Yamahira et al. 2007). Thus a species' temperature sensitivity may evolve in response to environmental conditions. For instance, Kingsolver et al. (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 and Demont 2004). Common garden experiments are useful tools to reveal such geographic adaptations (Angilletta 2009).

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 et al. 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 (Porter and Gawith 1999, Cook et al. 2012, Fu et al. 2015) in that spring development is usually fast after cold winters (chilling fulfillment) 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 and Kemp 2003, Stålhandske et al. 2014, 2017). If this pattern is general among insects, it is reasonable to suggest that TPCs for development rate estimated under controlled laboratory conditions would not yield reliable estimates of the phenology under natural conditions featuring cold temperatures. Here we test this hypothesis with a field experiment comparing observed and TPC-predicted development time.

Using five species of black scavenger fly (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 estimated the quantitative effect of Jensen's inequality by subjecting the flies to two diurnally fluctuating temperatures (15-21°C and 11-25°C) and one constant temperature (18°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 et al. 2002) and its tight relationship to fitness (Dmitriew 2011) and adult phenology (Tauber et al. 1986).

Materials and Methods

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. They represent good model organisms for studies of thermal adaptations (e.g. Berger et al. 2013, 2014) as they are easy to maintain in the lab and their geographic distribution is wide across both latitude and elevation (Pont and Meier 2002; Roy et al. 2018). Egg-to-adult development time varies among species from 1-6 weeks under field conditions (Pont and Meier 2002).

We performed field and laboratory experiments with five of the most common temperate *Sepsis* species: *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 vs. North America (Puniamoorthy et al. 2012). Each pair of populations was selected from latitudinal

(*S. cynipsea*, *S. thoracica*, *S. punctum*), elevational (*S. punctum*, *S. neocynipsea*, *S. fulgens*), or distant transcontinental locations (*S. neocynipsea*).

Sample preparation

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

To perform our experiments, we out-crossed 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 (20×5×5 cm) at the above conditions.

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°, 35°C) and constant photoperiod (16L:8D) in separate climate chambers. Temperatures were chosen using prior knowledge about *Sepsis* thermal adaptation (Blanckenhorn 1999; Berger et al. 2013) 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.

For each species and population, we supplied a group of 20-30 flies in a given replicate container with food and fresh dung in a dish for oviposition. After 24 h, the dung dish (containing eggs) was removed, transferred to a round 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 population, species and temperature treatment combination. Development time was estimated as the number of days from egg laying to the first adult emergence. We measured the effect of temperature and latitude on development rate, calculated as the inverse of development time.

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, plus two fluctuating treatments with a mean of 18°C and an amplitude of 15-21°C ($\pm 3^\circ\text{C}$) or 11-25°C ($\pm 7^\circ\text{C}$). Apart from the non-constant temperatures, the same methodology used in the previous experiment was applied, again assessing development rate. Here we had 10 replicates for each population, species and treatment.

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 wild 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 3L-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 a 100-ml plastic cup 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. We obtained corresponding hourly and daily average air temperature data of 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.

Statistical analyses

Statistical analyses were performed with R 3.2.2 (R Development Core Team 2017). All mixed-effects models were fitted with the R package *lme4* (Bates et al. 2015). Values are mean $\pm$ SD (unless indicated otherwise).

Experiment I

To assess the effect of temperature and latitude on development rate (i.e. the inverse of development time), we used generalized mixed-effects Poisson regression with temperature and latitude as fixed effects and species as random effect. 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 (Spain 1982). This function estimates three of the four components of the response to temperature (T_{opt} , CT_{max} , 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 afterward towards the critical maximum temperature (CT_{max}) where development is no longer possible.

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

223 where T is the ambient temperature, $p = \frac{1}{400} J^2 \left(1 + \sqrt{1 + \frac{40}{J}} \right)^2$, $J = (Q_{10} - 1) (T - T_{opt})$,

224 and $\phi(T)$ is the respective mean development rate estimated from experimental data.

225 *Experiment II*

226 Linear mixed-effects Poisson regressions were again performed for each species to assess the
227 effect of temperature fluctuation treatment (18° , $18\pm3^\circ$, $18\pm7^\circ\text{C}$) and geographic location
228 (low vs. high latitude or elevation) on development rate. To assess the usefulness of the TPC
229 estimated with constant temperatures (as in experiment I) in predicting the actually observed
230 performance under fluctuating temperature, we derived estimates based on hourly temperature
231 readings in the climate chambers to predict development rate obtained from our laboratory
232 experiment II. We estimated the hourly development rate by dividing the TPC-estimated
233 development rate by 24 h (since the unit of TPC is day). Then we summed all hourly
234 development rates to obtain the predicted daily development rate. Because the same frequency
235 distribution of temperatures is used every day in the laboratory experiment, the mean
236 development rate for the entire development time is theoretically identical to that for any
237 given day. To directly compare the observed and TPC-predicted development rate, reduced
238 major axis regressions (observed development rate being the response) assessing deviations
239 from unity were conducted for each species using the lmodel2 R package (Legendre 2014).

240 *Experiment III*

241 For each species, the relationship between TPC-predicted and field-observed development
242 rate was assessed at three different resolutions of temperature measurements with reduced
243 major axis regression (observed development rate being the response), as above for potential
244 deviations from unity in experiment II. The three temperature scales were hourly average,
245 daily average, and overall average temperature (average of daily temperatures across the
246 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 CTmin; see Supplementary material), 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 CTmin 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 CTmin) and those that experienced cold conditions (number of days below CTmin).

Results

Effects of constant laboratory temperatures on development and TPC

Development time differed among species (Figure S1a). From fastest to slowest, respective mean $\pm$ SD development time across all viable temperatures (from 12° to 32°C) and populations were: *S. cynipsea* (19.57 $\pm$ 12.00 d, n=206), *S. thoracica* (20.81 $\pm$ 11.98 d, n=274), *S. punctum* (23.59 $\pm$ 12.29 d, n=569), *S. neocynipsea* (25.61 $\pm$ 14.54 d, n=198), and *S. fulgens* (23.83 $\pm$ 13.09 d, n=311) (Table S2). Development time was significantly negatively related to temperature (Figure S1a; Table S3). The fastest change in development time was recorded between 12° and 17°C with an average decline (across species) of 0.60 [95%CI: 0.57-0.62] d°C⁻¹. In all species, flies did not reach the adult life stage at 34° and 35°C, thus taking the former as the critical maximum temperature. Interestingly, development time was affected by elevation similarly at all temperatures (shorter when higher; Figure S1b; Table S3). In contrast, development time decreased with latitude only for the three highest temperature treatments 22°, 27° and 32°C.

Development rate was estimated as the inverse of development time, and the thermal performance curve was then fitted by the O'Neil function using seven temperatures: 12°, 17°, 22°, 27°, 32°, 34° and 35°C.

22°, 27°, 32°, 34° and 35°C (Figure 1). Our model showed that T_{opt} , r_{max} and Q_{10} varied across species (Table S4). T_{opt} varied between 29.9°C in *S. punctum* and 31.5°C in *S. thoracica*; r_{max} (maximal development rate) ranged between 0.10 d⁻¹ in *S. fulgens* and 0.17 d⁻¹ in *S. thoracica*; and Q_{10} varied between 1.7 in *S. punctum* and 1.82 in *S. cynipsea*. 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 = 0.002), showing that species that have larger optimal temperature T_{opt} tend to have faster development rate.

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 estimated critical minimum CT_{min} consistently increased with increasing temperature range, yielding an average across species of 6.49°C [95%CI: 6.32-6.67] for 12-17°C, 7.64°C [95%CI: 7.36-7.91] for 12-22°C, and 7.85°C [95%CI: 7.50-8.19] for 12-27°C. CT_{min} estimated with 12-22°C was positively correlated with that estimated with 12-27°C (Spearman's rank correlation: r = 0.86, P = 0.0005), while the other pairwise correlations were lower and not significant (range: r =0.30-0.55; P > 0.05).

Relationship between observed and predicted development rate 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, low ($18\pm 3^\circ\text{C}$) and high fluctuating ($18\pm 7^\circ\text{C}$) temperature increased development rate by 4.3% and 12.9%, respectively. However, in this experiment neither latitude nor elevation affected development rate (Table S5).

Figure 2 Mean $\pm$ SD effect of diurnal temperature fluctuation on the development rate of five *Sepsis* species. The three temperature regimes were a constant (18°C), a relatively variable ($15\text{--}21^\circ\text{C}$), and a highly variable temperature ($11\text{--}25^\circ\text{C}$) treatment with an average of 18°C .

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 than unity (slope = 0.97 [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). A separate analysis for each species also showed no significant deviations from unity in four species: *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], $R^2 = 0.96$, $n = 6$), *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±3°C (slope = 1.04 [95%CI: 0.96– 1.13], $R^2 = 0.98$, $n = 12$) and 18±7°C (slope = 0.99 [95%CI: 0.87– 1.12], $R^2 = 0.97$, $n = 12$). Note that the power to detect deviation was low in these latter, separate analyses.

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

Relationship between observed and predicted development rates in the field

Mean ± SD development times observed in the field experiment 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 and 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 S2), diurnal and overall average temperature showed slopes closer to 1 (0.95 [95%CI: 0.93 - 0.97] and 0.94 [0.92 - 0.96], respectively) than hourly temperature (1.18 [1.14 - 1.23]). 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.

While the performance of daily and overall average temperature in predicting the observed development rate was similar, they showed some differences for two species (Table S6). Daily average temperature yielded better prediction for *S. cynipsea* with a slope of 0.98 [95%CI: 0.93-1.04] compared to 0.94 [0.9-0.99], whereas overall average temperature gave a better prediction for *S. neocynipsea* with a slope of 0.98 [0.95-1.01] compared to 0.92 [0.88-0.96].

We appraised the prediction precision as the ratio of observed over predicted development rate (ratio = 1 indicating perfect prediction) for the hourly, daily average and overall temperature to find that, across species, the ratio was 0.93 [95%CI: 0.92 – 0.94] for the hourly average temperature, 0.92 [95%CI: 0.90 – 0.93] for the daily average temperature, and 1 [0.99 – 1.00] for the overall average temperature (during development time), revealing that the latter (crude) resolution provides the best prediction. Nevertheless, prediction precision was negatively related to the number of chilling days (the number of days below CTmin) at daily and overall average temperature resolutions for all three CTmin estimations (Figure 4), unsurprisingly showing highest prediction reliability when the number of chilling days was zero (Table S7: intercept ≈ 1): as chilling days became more frequent towards the

352 end of the season, the predicted development time became longer than observed
353 (observed/predicted ratio < 1).

354 When dividing individuals into two categories – those that encountered temperatures
355 that fell below CT_{min} (CT_{min}1; chilled individuals) and those that did not (non-chilled
356 individuals) – we were able to reveal that for non-chilled individuals, daily average
357 temperature performed better than the hourly and overall average temperature at predicting
358 the observed development rate, showing a slope of 1.02 [1.00 – 1.03] compared to 0.92 [0.91-
359 0.93] and 1.07 [1.05-1.08], respectively. The inverse was true for chilled individuals, for
360 which the overall average temperature performed better (0.98 [0.97-0.99]) than the hourly
361 (0.93 [0.92-0.95]) and daily average temperature (0.89 [0.87-0.91]).

Figure 4. Relationship between the ratio of predicted-to-observed development rate and the number of days below the estimated critical minimum temperature CT_{min} (see Table S4) for hourly average temperature (a), daily temperature (b), and for the overall average temperature (c). The three panels represent three estimates of CT_{min} (see 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.

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, 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 (Gillooly et al. 2002, Dmitriew 2011). 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, this variation is genetic (i.e. evolved) and demonstrates local adaptation to climate variation with latitude and altitude. We found that TPCs derived from constant temperatures yield reliable estimates of development time and rate under laboratory conditions 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 respect, our data also demonstrate that in order to gain adequate predictions, it is crucial to select an appropriate thermal resolution for the organism under scrutiny.

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 $18 \pm 7^\circ\text{C}$ vs. $18 \pm 3^\circ\text{C}$ in Figure 2; Ruel and Ayres 1999). Similar results were e.g. 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 e.g. in the cold-adapted yellow dung fly (Kjaersgaard et al. 2013). The latter 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 quantify the predictive power of TPCs estimated from constant temperatures to data generated at fluctuating temperatures, a common situation. To test this, we compared development rates predicted from constant TPC with the actually observed development rates generated under (1) controlled laboratory conditions with specific diurnally fluctuating temperatures, and (2) 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 non-linear effect (Jensen's inequality) is properly adjusted for by rate summation (Liu et al. 1995). Else, when assuming constant as opposed to fluctuating temperatures, development times and rates can be considerably under- or overestimated (Figure 2). When applied to field conditions, this rate-summing method may 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 experiments, daily and gross average temperatures yielded more accurate predictions, whereas hourly resolution generally most severely underestimated actual development rates (Figure S2; 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 – 7°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. Although factors such as humidity or day length in the field might account for some variation in development rate, there is evidence in other insects indicating that the number of chilling days positively affects (i.e. advances) the day of spring emergence (Bosch and 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 (e.g. Blanckenhorn 1999; see Methods) 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 rates (non-adaptive explanation). Hourly temperature resolution most strongly underestimates actual development rates. 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 must 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. 2014, 2017).

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 at warm temperatures (22-32°C), 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*, *S. neocynipsea*) (Pont and 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, even though we could not detect any significant interspecific differences in their critical maxima. 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.

In addition to these interspecific differences, populations of most species varied geographically in their responses to constant laboratory temperatures along elevation and latitude. Extending Berger et al.'s (2014) study of *S. punctum*, flies consistently developed faster at higher elevations across the entire range of temperatures (12-32°C), whereas they developed faster at higher latitudes only at the warmer temperatures used here (22-32°C), revealing adaptation to cold climates across both gradients. 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 of thermal reaction norms at higher latitudes and elevations found here (Homeny and Juliano 2007,

Yamahira et al. 2007, Kipyatkov and Lopatina 2010, Mikolajewski et al. 2015). However, such countergradient variation was not recovered in all species here. Following another study on *Sepsis fulgens* showing no latitudinal cline for development time across Europe (Roy et al. 2018), we found no elevational differentiation in Algerian *S. fulgens*.

Conclusions

In recent years, increasing attention has been paid to the importance of temperature fluctuations for predicting the thermal and phenological responses of temperate ectotherms (Vasseur et al. 2014). It is by now clear that organisms are well adapted to fluctuating temperatures; 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. 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. The next challenge to urgently tackle would be understanding the relationship between cold conditions and thermal sensitivity of development in the age of big data and citizen science (Stålhandske et al. 2017).

Authors' contributions

- RK, WB & HM conceived the ideas and designed the experiment;
- HM, PR and JR collected the data;
- RK & HM analyzed the data;
- RK, HM, PR, and WB led the writing of the manuscript.

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Conflict of interest

The authors have no conflict of interest

All authors contributed critically to the drafts and gave final approval for publication.

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Supplementary information

**Usefulness and limitations of thermal performance curves in predicting
ecotherm development under global change**

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1. Supplementary Methods

Estimating critical minimum temperature

The critical minimum temperature CT_{min} was estimated as *-intercept//slope* of the linear regression of development rate across three temperature ranges in the quasi-linear left-hand part of the TPC (Blanckenhorn 1999): 12-17°C, 12-22°C and 12-27°C. We chose different temperature ranges because the quasi-linear left-hand part of the TPC is actually exponential and thus the slope changes somewhat with an increase in temperature range. By pooling data from different species and populations, we tested for relationships between the various CT_{min} estimated for 12-17°C, 12-22°C and 12-27°C with Spearman correlation tests.

2. Supplementary Figures

Figure S1 (a) Development time of five *Sepsis* species at five viable temperature treatments (12°, 17°, 22°, 27°, 32°C). Error bars are 95% confidence intervals. (b) The effect of latitude (standardized), elevation (standardized) and temperature on development rate. Values on contours are predictions of standardized development time from generalized mixed-effects Poisson models. The smaller the value (the lighter the color), the earlier the adult emergence.

Figure S2 Relationship between predicted and observed development rate estimated in the field. Predicted development rate was estimated using hourly average temperature (a), daily average temperature (b), or overall average temperature (c). The diagonal line has a slope of 1, and the fitted line is a simple linear regression.

3. Supplementary Table

Table S1. Geographic coordinates and elevation of study sites.

Species	Country	Site	Latitude (°)	Longitude (°)	Elevation (m)
<i>S. thoracica</i>	Germany	Ludwigshafen	49.48	8.42	100
<i>S. thoracica</i>	Italy	Petroia	43.23	12.56	650
<i>S. punctum</i>	Switzerland	Zurich	47.34	8.54	512
<i>S. punctum</i>	Spain	La Laguna (Tenerife)	28.49	-16.31	609
<i>S. cynipsea</i>	Germany	Ludwigshafen	49.48	8.42	100
<i>S. cynipsea</i>	Italy	Petroia	43.23	12.56	650
<i>S. punctum</i>	Algeria	Maouna	36.36	7.41	1100
<i>S. punctum</i>	Algeria	Old Bridge	36.47	7.37	211
<i>S. fulgens</i>	Algeria	Maouna	36.36	7.41	1100
<i>S. fulgens</i>	Algeria	Old Bridge	36.47	7.37	211
<i>S. neocynipsea</i>	Switzerland	Sörenberg	46.87	8.27	710
<i>S. neocynipsea</i>	USA	Fort Hall (ID)	43.03	-112.44	1356

Table S2. Development time averaged across all temperature treatments.

Species	Population	Mean	n	sd	LCI	UCI
<i>S. cynipsea</i>	Ludwigshafen	21.45	104	12.59	19.03	23.87
<i>S. cynipsea</i>	Petroia	17.40	90	10.94	15.13	19.66
<i>S. fulgens</i>	Maouna	24.16	149	13.71	21.96	26.37
<i>S. fulgens</i>	Old Bridge	23.48	150	12.48	21.48	25.48
<i>S. neocynipsea</i>	Fort Hall	24.19	87	13.88	21.27	27.11
<i>S. neocynipsea</i>	Sorenberg	26.84	99	15.05	23.88	29.81
<i>S. punctum</i>	La Laguna	24.71	138	13.22	22.51	26.92
<i>S. punctum</i>	Maouna	21.99	121	11.90	19.87	24.11
<i>S. punctum</i>	Old Bridge	24.07	155	12.19	22.15	25.99
<i>S. punctum</i>	Zurich	23.32	131	11.70	21.32	25.33
<i>S. thoracica</i>	Ludwigshafen	21.60	155	12.06	19.70	23.49
<i>S. thoracica</i>	Padula	19.66	107	11.81	17.42	21.90

Table S3. Summary statistics of the generalized mixed-effects Poisson regression testing for the effect of temperature, elevation and latitude on development rate of five *Sepsis* species (N = 1486). The intercept is temperature treatment 12°C.

Fixed effects	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	3.740	0.052	71.380	< 2e-16
Temp [17]	-0.600	0.013	-44.940	< 2e-16
Temp [22]	-1.045	0.016	-66.890	< 2e-16
Temp [27]	-1.382	0.022	-63.950	< 2e-16
Temp [32]	-1.555	0.031	-49.440	< 2e-16
z.Latitude	0.004	0.010	0.450	0.653
z.Elevation	-0.014	0.007	-2.130	0.033
Temp [17]:z.Latitude	-0.001	0.013	-0.060	0.949
Temp [22]:z.Latitude	-0.036	0.016	-2.280	0.022
Temp [27]:z.Latitude	-0.058	0.022	-2.610	0.009
Temp [32]:z.Latitude	-0.096	0.036	-2.660	0.008

Table S4. Parameter estimates for the thermal performance curves of five species of *Sepsis*. $r_{\max}$ (maximum development rate), T_{opt} (optimal temperature), $T_{\max}$ (maximum critical temperature) and Q_{10} were estimated with the O'Neil function, whereas $T_{\min}$ was estimated with a linear model (see Material and methods).

Species	Geographic location	$r_{\max}$	T_{opt}	$CT_{\max}$	Q_{10}	$CT_{\min}$
<i>S. fulgens</i>	High elevation	0.10	30.02	34.00	1.75	6.23
	Low elevation	0.11	30.35	34.00	1.73	6.36
<i>S. cynipsea</i>	High latitude	0.15	30.26	34.00	1.82	6.45
	Low latitude	0.15	30.90	34.00	1.76	6.73
<i>S. thoracica</i>	High latitude	0.17	31.57	34.00	1.74	6.41
	Low latitude	0.17	31.57	34.00	1.75	6.35
<i>S. neocynipsea</i>	High latitude	0.11	30.35	34.00	1.74	7.21
	Low latitude	0.13	31.36	34.00	1.71	6.74
<i>S. punctum</i>	High latitude	0.11	29.92	34.00	1.73	6.01
	Low latitude	0.11	30.02	34.00	1.74	6.71
	High elevation	0.11	29.97	34.00	1.70	6.37
	Low elevation	0.11	30.13	34.00	1.72	6.38

Table S5. Summary statistics of the linear mixed-effects regression testing for the effects of temperature fluctuation and geographic location on development rate of five *Sepsis* species (N = 313).

Fixed effects	Estimate	Std. Error	df	t	Pr(> t)
				value	
Intercept [18°C]	0.053	0.003	4.030	15.691	< 2e-16
Temperature [18 ± 3°C]	0.002	0.000	304.60	4.965	< 2e-16
Temperature [18 ± 7°C]	0.007	0.000	304.60	14.26	< 2e-16
z.Latitude	0.000	0.000	306.50	0.623	0.534
z.Elevation	0.000	0.000	305.70	1.281	0.201

Table S6. Slope estimation of the relationship between field-observed and TPC-based predicted development time using reduced major axis regression. Bold slopes differ significantly from 1.

Scale of temperature	Species	Slope	95% CI Slope
Hourly average	<i>S. cynipsea</i>	1.31	1.18-1.45
	<i>S. fulgens</i>	1.22	1.12-1.34
	<i>S. punctum</i>	1.19	1.11-1.27
	<i>S. thoracica</i>	1.20	1.11-1.29
	<i>S. neocynipsea</i>	1.25	1.17-1.35
Diurnal average	<i>S. cynipsea</i>	0.98	0.93-1.04
	<i>S. fulgens</i>	0.90	0.84-0.96
	<i>S. punctum</i>	0.87	0.84-0.91
	<i>S. thoracica</i>	1.01	0.97-1.06
	<i>S. neocynipsea</i>	0.92	0.88-0.96
Average across development time	<i>S. cynipsea</i>	0.94	0.9-0.99
	<i>S. fulgens</i>	0.90	0.83-0.96
	<i>S. punctum</i>	0.87	0.84-0.9
	<i>S. thoracica</i>	1.01	0.97-1.05
	<i>S. neocynipsea</i>	0.98	0.95-1.01

785 **Table S7.** Summary results of the linear mixed-effects model testing for the effect of cold
786 conditions on the ratio of observed-to-predicted development rate of five *Sepsis* species using
787 hourly average temperature (a) daily average temperature (b) or overall average temperature
788 (c) and three CT_{min} estimates (N= 267). CT_{min1}, CT_{min2} and CT_{min3} were calculated using linear
789 regression across the following temperature ranges: 12-17°C, 12-22°C and 12-27°C, respectively (see
790 Methods).

CT _{min}	Resolution		Estimate	Std. Error	df	t value	Pr(> t)
CT _{min1}	(a) Hourly	(Intercept)	0.9303	0.0241	4.8600	38.6790	<0.0001
		No. days < CTmin	-0.0001	0.0003	262.0000	-0.2720	0.7860
	(b) Daily	(Intercept)	1.0210	0.0179	4.9600	57.1700	<0.0001
		No. days < CTmin	-0.0048	0.0003	262.1000	-19.260	< 2e-16
	(c) Overall	(Intercept)	1.0280	0.0167	5.4300	61.4570	<0.0001
		No. days < CTmin	-0.0019	0.0003	262.6000	-7.0610	<0.0001
CT _{min2}	(a) Hourly	(Intercept)	0.9307	0.0242	4.9800	38.4640	< 2e-16
		No. days < CTmin	-0.0001	0.0003	261.8000	-0.3150	0.7530
	(b) Daily	(Intercept)	1.0270	0.0188	4.9700	54.5900	< 2e-16
		No. days < CTmin	-0.0048	0.0003	261.8000	-19.260	< 2e-16
	(c) Overall	(Intercept)	1.0294	0.0168	5.6500	61.1510	< 2e-16
		No. days < CTmin	-0.0019	0.0003	262.2200	-6.9190	< 2e-16
CT _{min3}	(a) Hourly	(Intercept)	0.9305	0.0242	5.0000	38.3790	< 2e-16
		No. days < CTmin	-0.0001	0.0003	261.8000	-0.2750	0.7840
	(b) Daily	(Intercept)	1.0280	0.0193	4.9500	53.280	< 2e-16
		No. days < CTmin	-0.0048	0.0003	261.8000	-19.160	< 2e-16
	(c) Overall	(Intercept)	1.0300	0.0171	5.6400	60.2260	< 2e-16
		No. days < CTmin	-0.0019	0.0003	262.2000	-6.8650	< 2e-16

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1. Supplementary Methods

Estimating critical minimum temperature

The critical minimum temperature CTmin was estimated as *intercept/slope* of the linear regression of development rate across three temperature ranges in the quasi-linear left-hand part of the TPC (Blanckenhorn 1999): 12-17°C, 12-22°C, or 12-27°C. We chose different temperature ranges because the quasi-linear left-hand part of the TPC is actually exponential and thus the slope changes somewhat with an increase in temperature range. By pooling data from different species and populations, we tested for relationships between the various CTmin estimated for the three temperature ranges using Spearman correlation tests.

The estimated critical minimum CTmin consistently increased with increasing temperature range, yielding an average across species of 6.49°C [95%CI: 6.32-6.67] for 12-17°C, 7.64°C [95%CI: 7.36-7.91] for 12-22°C, and 7.85°C [95%CI: 7.50-8.19] for 12-27°C. CTmin estimated with 12-22°C was positively correlated with that estimated with 12-27°C (Spearman's rank correlation: $r = 0.86$, $P = 0.0005$), while the other pairwise correlations were lower and not significant (range: $r = 0.30-0.55$; $P > 0.05$).

2. Supporting Figures

A

5 species

Sepsis cynipsea (2 pop)
Sepsis thoracica (2 pop)
Sepsis punctum (4 pop)
Sepsis neocynipsea (2 pop)
Sepsis fulgens (2 pop)

B

C

Figure S1. Experimental design for experiments I and II. (a) Five species of *Sepsis* flies were used in the experiment with two populations per species, except for *S. punctum*, where four populations were used. (b) Design of experiment I to estimate the thermal performance curve for development rate as the measure of performance using 7 constant temperature treatments.. (c) Design of experiment 2 to assess the effect of daily temperature fluctuations on development rate using three treatments with the same average: 18° , $18\pm3^\circ$, and $18\pm7^\circ\text{C}$. The frequency of temperatures used for each treatment is shown in the figure.

Figure S2. Average hourly temperatures during the field experiment. Vertical red-dashed lines indicate the dates at the start (when eggs were laid in the field) and the end (when the last fly emerged) of the experiment. Temperature data were provided by a meteorological station 4 km away from the experimental site.

Figure S3. Development times of five *Sepsis* species at five viable temperatures (12°, 17°, 22°, 27°, 32°C). Error bars are 95% confidence intervals.

Figure S4. Relationship between predicted and observed development rate in the field towards the end of the season. Predicted development rate was estimated using hourly (a), daily (b), or overall (c) average temperature summing. The diagonal line has a slope of 1, and the fitted line is a simple linear regression.

3. Supporting Tables

Table S1. Geographic coordinates and elevation of all sampled populations.

Species	Country	Site	Latitude (°)	Longitude (°)	Elevation (m)
<i>S. thoracica</i>	Germany	Ludwigshafen	49.48	8.42	100
<i>S. thoracica</i>	Italy	Petroia	43.23	12.56	650
<i>S. punctum</i>	Switzerland	Zurich	47.34	8.54	512
<i>S. punctum</i>	Spain	La Laguna (Tenerife)	28.49	-16.31	609
<i>S. cynipsea</i>	Germany	Ludwigshafen	49.48	8.42	100
<i>S. cynipsea</i>	Italy	Petroia	43.23	12.56	650
<i>S. punctum</i>	Algeria	Maouna	36.36	7.41	1100
<i>S. punctum</i>	Algeria	Old Bridge	36.47	7.37	211
<i>S. fulgens</i>	Algeria	Maouna	36.36	7.41	1100
<i>S. fulgens</i>	Algeria	Old Bridge	36.47	7.37	211
<i>S. neocynipsea</i>	Switzerland	Sörenberg	46.87	8.27	710
<i>S. neocynipsea</i>	USA	Fort Hall (ID)	43.03	-112.44	1356

Table S2. Mean, sample size (n), standard deviation (SD), and standard error (se) of the estimated development time (averaged across all temperature treatments) for all studied *Sepsis* species.

Species	Mean	n	sd	se
<i>S.cynipsea</i>	19.6	194	12.0	0.862
<i>S.fulgens</i>	23.8	299	13.1	0.757
<i>S.neocynipsea</i>	25.6	186	14.5	1.07
<i>S.punctum</i>	23.6	545	12.3	0.527
<i>S.thoracica</i>	20.8	262	12.0	0.740

Table S3. Pairwise comparison of the development times of all five species. The table presents the P-values of TukeyHSD, performed with the multcomp R package (Hothorn, Bretz, & Westfall 2008), of the generalized linear Poisson model (without overdispersion) including temperature, species as fixed effects.

	<i>S.fulgens</i>	<i>S.neocynipsea</i>	<i>S.punctum</i>	<i>S.thoracica</i>
<i>S.cynipsea</i>	<0.0001	<0.0001	<0.0001	0.35
<i>S.fulgens</i>		0.31	<0.0001	<0.0001
<i>S.neocynipsea</i>			0.71	<0.0001
<i>S.punctum</i>				<0.0001

Table S4. Phylogenetically corrected generalized mixed-effects Poisson regression model results testing for the effect of temperature, elevation and latitude on development rate of five *Sepsis* species (N = 1486). Temperatures 33 and 34°C were excluded because all flies died. The analysis was performed with MCMCglmm package (Hadfield 2010) using 500000 MCMC iterations, accounting for phylogeny and overdispersion (= observation level) as random effects. The phylogenetic signal λ was 0.96 [95%CI: 0.88-1]. Eff.samp: effective sample size, Post.mean: posterior mean.

	Post.mean	Lower 95% CI	Upper 95% CI	Eff.samp	pMCMC
Intercept[S.cynipsea]	4.233	-5.097	18.655	5913	0.134
Temperature[S.cynipsea]	0.799	-18.524	15.986	6075	0.970
Species[S.fulgens]	1.261	-17.992	14.999	4700	0.899
Species[S.neocynipsea]	0.656	-16.519	15.158	4929	0.944
Species[S.punctum]	-0.788	-17.021	13.737	4396	0.977
Species[S.thoracica]	-0.092	-0.102	-0.081	1415	<0.0001
zLatitude	0.002	-0.037	0.042	2426	0.924
zElevation	0.006	-0.029	0.039	2160	0.714
Temperature:Species[S.fulgens]	0.009	-0.005	0.023	1688	0.238
Temperature:Species[S.neocynipsea]	-0.004	-0.019	0.013	1511	0.606
Temperature:Species[S.punctum]	0.013	0.000	0.025	1458	0.054
Temperature:Species[S.thoracica]	-0.001	-0.016	0.013	1336	0.858

Table S5. Parameter estimates for the thermal performance curves of five species of *Sepsis*. $r_{\max}$ (maximum development rate), T_{opt} (optimal temperature), $T_{\max}$ (maximum critical temperature) and Q_{10} were estimated with the O'Neil function, whereas $T_{\min}$ was estimated with a linear model (see Material and methods).

Species	Geographic location	$r_{\max}$	T_{opt}	$CT_{\max}$	Q_{10}	$CT_{\min}$
<i>S. fulgens</i>	High elevation	0.10	30.02	34.00	1.75	6.23
	Low elevation	0.11	30.35	34.00	1.73	6.36
<i>S. cynipsea</i>	High latitude	0.15	30.26	34.00	1.82	6.45
	Low latitude	0.15	30.90	34.00	1.76	6.73
<i>S. thoracica</i>	High latitude	0.17	31.57	34.00	1.74	6.41
	Low latitude	0.17	31.57	34.00	1.75	6.35
<i>S. neocynipsea</i>	High latitude	0.11	30.35	34.00	1.74	7.21
	Low latitude	0.13	31.36	34.00	1.71	6.74
<i>S. punctum</i>	High latitude	0.11	29.92	34.00	1.73	6.01
	Low latitude	0.11	30.02	34.00	1.74	6.71
	High elevation	0.11	29.97	34.00	1.70	6.37
	Low elevation	0.11	30.13	34.00	1.72	6.38

Table S6. Phylogenetically corrected (??) generalized Poisson mixed-effects regression model results testing for the effects of temperature fluctuation, latitude and elevation on development time of five *Sepsis* species (N = 313). The analysis was performed with MCMCglmm package (Hadfield 2010) using 500000 MCMC iterations, accounting for phylogeny and overdispersion (= observation level) as random effects. The phylogenetic signal λ : 0.89 [95%CI: 0.72-0.98]. Eff.samp: effective sample size, Post.mean: posterior mean.

	Estimate	Lower 95% CI	Upper 95% CI	eff.samp	pMCMC
Intercept [18°C]	2.933	2.399	3.466	49700	< 0.0001
Temperature [18 ± 3°C]	-0.042	-0.123	0.037	27125	0.298
Temperature [18 ± 7°C]	-0.119	-0.204	-0.035	24292	0.006
z.Latitude	-0.004	-0.056	0.046	26793	0.875
z.Elevation	-0.004	-0.046	0.038	28044	0.848

Table S7. Slope estimation of the relationship between field-observed and TPC-based predicted development time using reduced major axis regression. Bold slopes differ significantly from 1.

Resolution of temperature	Species	Slope	95% CI Slope
Hourly average	<i>S. cynipsea</i>	1.31	1.18-1.45
	<i>S. fulgens</i>	1.22	1.12-1.34
	<i>S. punctum</i>	1.19	1.11-1.27
	<i>S. thoracica</i>	1.20	1.11-1.29
	<i>S. neocynipsea</i>	1.25	1.17-1.35
Diurnal average	<i>S. cynipsea</i>	0.98	0.93-1.04
	<i>S. fulgens</i>	0.90	0.84-0.96
	<i>S. punctum</i>	0.87	0.84-0.91
	<i>S. thoracica</i>	1.01	0.97-1.06
	<i>S. neocynipsea</i>	0.92	0.88-0.96
Average across development time	<i>S. cynipsea</i>	0.94	0.9-0.99
	<i>S. fulgens</i>	0.90	0.83-0.96
	<i>S. punctum</i>	0.87	0.84-0.9
	<i>S. thoracica</i>	1.01	0.97-1.05
	<i>S. neocynipsea</i>	0.98	0.95-1.01

Table S8. Summary results of the linear mixed-effects model testing for the effect of cold conditions on the ratio of observed-to-predicted development rate of five *Sepsis* species using hourly average temperature (a), daily average temperature (b), or overall average temperature (c) and three CT_{min} estimates (N= 267). CT_{min1}, CT_{min2} and CT_{min3} were calculated using linear regression across 12-17°C, 12-22°C and 12-27°C, respectively (see Material and Methods). The analysis was performed with MCMCglmm package (Hadfield 2010) using 50000 MCMC iterations, corrected for phylogeny and using species as random effects.

CT _{min}	Resolution		post.mean	Lower 95% CI	Upper 95% CI	eff.samp	pMCMC
CT _{min1}	(a) Hourly	Intercept	0.930	0.837	1.030	4700	0.0004
		No. days < CTmin	0.000	-0.001	0.001	4700	0.810
	(b) Daily	Intercept	1.021	0.933	1.112	4700	<0.0001
		No. days < CTmin	-0.005	-0.005	-0.004	4700	<0.0001
	(c) Overall	Intercept	1.027	0.944	1.115	4700	<0.0001
		No. days < CTmin	-0.002	-0.002	-0.001	4700	<0.0001
CT _{min2}	(a) Hourly	Intercept	0.932	0.839	1.043	4449	<0.0001
		No. days < CTmin	0.000	-0.001	0.001	4428	0.783
	(b) Daily	Intercept	1.028	0.938	1.115	4700	<0.0001
		No. days < CTmin	-0.005	-0.005	-0.004	4700	<0.0001
	(c) Overall	Intercept	1.029	0.943	1.113	4700	<0.0001
		No. days < CTmin	-0.002	-0.002	-0.001	4700	<0.0001
CT _{min3}	(a) Hourly	Intercept	0.932	0.830	1.032	4700	<0.0001
		No. days < CTmin	0.000	-0.001	0.001	4391	0.818
	(b) Daily	Intercept	1.028	0.938	1.119	4307	<0.0001
		No. days < CTmin	-0.005	-0.005	-0.004	4700	<0.0001
	(c) Overall	Intercept	1.029	0.941	1.116	4700	<0.0001
		No. days < CTmin	-0.002	-0.002	-0.001	4700	<0.0001

987 **References**

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