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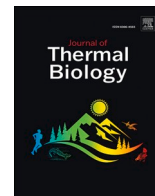
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Modelling thermal reaction norms for development and viability in *Drosophila suzukii* under constant, fluctuating and field conditions

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ABSTRACT

Phenological models for insect pests often rely on knowledge of thermal reaction norms. These may differ in shape depending on developmental thermal conditions (e.g. constant vs. fluctuating) and other factors such as life-stages. Here, we conducted an extensive comparative study of the thermal reaction norms for development and viability in the invasive fly, *Drosophila suzukii*, under constant and fluctuating thermal regimes. Flies, were submitted to 15 different constant temperatures (CT) ranging from 8 to 35 °C. We compared responses under CT with patterns observed under 15 different fluctuating temperature (FT) regimes. We tested several equations for thermal performance curves and compared various models to obtain thermal limits and degree-day estimations. To validate the model's predictions, the phenology was monitored in two artificial field-like conditions and two natural conditions in outdoor cages during spring and winter. Thermal reaction norm for viability from egg to pupa was broader than that from egg to adult. FT conditions yielded a broader thermal breadth for viability than CT, with a performance extended towards the colder side, consistent with our field observations in winter. Models resulting from both CT and FT conditions made accurate predictions of degree-day as long as the temperature remained within the linear part of the developmental rate curve. Under cold artificial and natural winter conditions, a model based on FT data made more accurate predictions. Model based on CT failed to predict adult's emergence in winter. We also document the first record of development and adult emergence throughout winter in *D. suzukii*. Population dynamics models in *D. suzukii* are all based on summer phenotype and CT. Accounting for variations between seasonal phenotypes, stages, and thermal conditions (CT vs. FT) could improve the predictive power of the models.

1. Introduction

In ectotherms, the relationship between development and temperature is typically curvilinear at low and high temperatures and linear in between (Beck, 1983). The lowest and highest temperatures at which development occurs correspond to the lower and upper developmental thresholds (T_0 and T_{max}). If the upper threshold typically occurs near or at the upper lethal temperature (i.e., CT_{max}), lower lethal temperature (CT_{min}) is a different notion accounting for a critical temperature affecting performance. Temperature-dependent development in insects is often represented using the inverse of development time, that is, the development rate. Several models have been used to describe both the development and survival rate of insects in response to temperature

(Damos and Savopoulou-Soultani, 2012; Rebaudo et al., 2017). Yet, the output is sensitive to the choice of the equation as well as uncertainties in model parameters. For example, the linear model can estimate the lower developmental threshold (i.e., extrapolated x-intercept) but cannot calculate the T_{max} . Additionally, it fails to describe developmental rates close to, or above, the optimal developmental temperature (Shi et al., 2011). Despite this, the linear Campbell equation (Campbell et al., 1974) remains the most widely used model. However, this model raises questions about the capacity to predict the dynamics of natural populations that may experience temperatures below or above the lower or upper thermal thresholds. Moreover, insects typically experience fluctuating temperatures (FT) in the field, while most developmental thresholds and degree-day accumulations are experimentally

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determined using constant temperatures (CT). The ecological relevance of using CT to predict insect life cycle in the field has therefore been extensively questioned (Colinet et al., 2015; Kingsolver et al., 2015).

Numerous studies comparing development rate under CT vs. FT found, at low temperatures, a significantly faster development rate under FT compared to development rate at the corresponding average temperature under CT. The contrary was found at high temperatures (Bayu et al., 2017; Hagstrum and Milliken, 1991; Vangansbeke et al., 2015). These differences can be at least partly explained mathematically by Jensen's inequality (Colinet et al., 2015; Ruel and Ayres, 1999) which states that, except under rare circumstances, the response of a system to average conditions is different from the system's average response to variable conditions. The average performance rate does not represent the nominal performance rate especially under sub/supra-optimal thermal conditions. Because of the nonlinear shape of performance curves, under FT conditions we should expect performances to be lower than CT in the upper range (concave part of the curve) and higher in the lower range (convex part of the curve) (Denny, 2017; Foray et al., 2014). Some data suggest that development rate can be relatively well predicted with models parameterized under CT (von Schmalensee et al., 2021). This may not be true for all performance traits such as viability/survival (Colinet et al., 2015). Indeed, FT during development may enable survival at low or high thermal conditions that may be lethal if continually applied (Petavy et al., 2001). It is known that accumulation and repair of thermal injuries differ substantially under FT vs. CT (Colinet et al., 2006; Enriquez et al., 2020; Grumiaux et al., 2019; Košťál et al., 2016). At low temperature, physiological repair mechanisms are possible when temperature fluctuates (Colinet et al., 2018). Obtaining accurate developmental and lethal thresholds is critical for modelling population dynamics and phenology, but this poses problems near upper and lower thresholds because of high mortality and inability of insects to complete critical developmental steps (i.e., inability to hatch or molt) (Régnière et al., 2012). Régnière et al. (2012) showed that problems associated with measuring development and viability near thresholds can be circumvented by including temperatures alternating between extreme and optimum ranges in experimental designs with a sufficient proportion of the development taking place at the extreme temperature.

The spotted wing drosophila, *Drosophila suzukii*, is a fruit pest endemic to Asia that has successfully colonized Europe and North America and is now found almost worldwide (Tait et al., 2021). In contrast to other frugivorous *Drosophila*, females lay their eggs in healthy ripening fruits. This feature means that fruits are attacked while maturing on plant, making them improper for consumption and commercialization. In addition, larval feeding inside the fruit triggers bacterial infections and favors the entry of plant pathogens (Dreves et al., 2009). Depending on the invaded area and climatic conditions, *D. suzukii* can cause up to 80% yield loss (Bolda et al., 2010). Integrated pest management (IPM) against *D. suzukii* has been widely explored over the last decade and population control measures require precise phenological models.

Understanding the ecological factors influencing development, survival and reproduction of pests are key issues in developing control strategies (Chuine and Régnière, 2017). Indeed, accurate predictions of population dynamics can reduce the number and the cost of control interventions. Decision making tools thus rely on reliable phenological models, which are mainly based on parameters such as development, survival and reproduction to estimate population size independently regardless of the actual growth of the crop (Tonngang et al., 2022). Various distribution and population dynamic models exist for *D. suzukii*, but to our knowledge there have been no practical applications of these (Asplen et al., 2015; Langille et al., 2016, 2017; Leach et al., 2019; Ørsted et al., 2021; Tochen et al., 2016; Wiman et al., 2016; Winkler et al., 2020). A common feature of insect phenology is the environmental control of their developmental rate (Chuine and Régnière, 2017). Insect population dynamics is indeed influenced by many abiotic and biotic factors, but temperature and humidity are often the main drivers

of their abundance and distribution (Stange and Ayres, 2010). As with other *Drosophila*, there is a broad consensus that temperature is the main driver of *D. suzukii* population distribution, dynamics and seasonal phenology (de la Vega and Corley, 2019; Dos Santos et al., 2017; Gutierrez et al., 2016; Hamby et al., 2016; Langille et al., 2016). Yet, there are still many knowledge gaps that currently hamper the accurate evaluation and prediction of *D. suzukii* field population dynamics (Asplen et al., 2015). For instance, natural complexity is overlooked (e.g. FT) and degree-day models are often based on empirical constant or average temperatures (Leach et al., 2019; Wiman et al., 2016).

Development of *D. suzukii* has mostly been studied under CT (de la Vega and Corley, 2019; Tochen et al., 2014; Wiman et al., 2016, 2016, 2016; Winkler et al., 2021), apart from Sánchez-Ramos et al. (2019) who tested three FT regimes. Here we examined *D. suzukii* developmental and viability rates under 15 CT and 15 FT conditions spanning the species thermal range. Based on previous observations (Enriquez et al., 2020), we expected i) viability to differ in flies developing under FT vs. CT, especially at temperatures near lower and upper thresholds. Based on Jensen's inequality (Ruel and Ayres, 1999), we assumed that ii) viability would be improved and development time accelerated under FT near CT_{min}/T_0 and the opposite pattern for temperatures near CT_{max} . To model our data, we tested a range of equations (linear and nonlinear) and we assumed that iii) asymmetrical and unimodal models would best fit the data and provide more accurate parameters. Here, we also validated thermal responses *in natura* in spring and winter and we expected that iv) model parametrized using FT would provide more accurate predictions than model parametrized using CT, especially when natural temperatures approach lower thermal limits.

2. Material and methods

2.1. Fly origin and rearing

A wild line was established from a sampling of *D. suzukii* infected fruits in a raspberry crop in Thorigné fouillard (France: 48.14, -1.57) in October 2020. One female was kept from a single infected fruit to create an isofemale line and about 190 lines in total were created. These isofemale lines were then mixed in a population cage to create a mass-bred population. The stock population was then maintained in 100 mL bottles containing 20 mL of standard food media composed of 15 g of agar, 50 g of sugar, 30 g of brewer yeast, 20 g of corn meal, 50 g of carrot powder and 16 mL of Nigapin for 1 L of water. Bottles were placed in incubators (MIR-154-PE, Panasonic) at 25 °C, 65% of relative humidity and a 12/12 LD photoperiod. A minimum of 20 bottles was kept every generation. In order to maintain genetic diversity, individuals were shuffled at each generation.

2.2. Developmental temperatures

Under CT regimes, we tested 15 different temperatures spanning from 8 to 35 °C (see Fig. 1). Three temperatures proved to be lethal (i.e., no development): 8, 33 and 35 °C. Under FT conditions, we used 15 different regimes with two temperatures alternating daily with a 12h–12h cycle. The mean of the two alternating temperatures, referred to as the mid-temperature, ranged from 11.5 to 32 °C (see Fig. 1). In all treatments the photoperiod was a light-dark cycle of 12h–12h.

2.3. Assessing viability and development rate

In the evening, we placed petri-dishes filled with egg-laying medium, composed of grape agar (Genesee Scientific), in rearing cages with mature adults at 25 °C for a maximum of 12 h. In early morning, eggs were collected under a binocular with a small spatula. Ten eggs were placed in 2 mL eppendorfs filled with 1 mL of standard media. This process enabled larval density to be fully controlled, as it may affect both development and viability (Henry et al., 2020). Eppendorfs were closed

CT conditions	FT conditions	
	Range	Mean
8 °C	8 °C-15 °C	11.5 °C
9 °C	8 °C-20 °C	14 °C
10 °C	8 °C- 25 °C	16.5 °C
13 °C	15 °C-20 °C	17.5 °C
16 °C	15 °C-25 °C	20 °C
17 °C	15 °C-30 °C	22.5 °C
19 °C	15 °C-35 °C	25 °C
22 °C	20 °C- 30°C	25 °C
25 °C	23 °C-31 °C	27 °C
28 °C	25 °C-31 °C	28 °C
29 °C	25 °C-33 °C	29 °C
30 °C	26 °C-32 °C	29 °C
31 °C	28 °C-32 °C	30 °C
33 °C	27 °C-33 °C	30 °C
35 °C	30 °C-34 °C	32 °C

Fig. 1. Experimental thermal conditions tested under constant (CT) and fluctuating conditions (FT). For FT, the two temperatures alternated on a daily 12h–12h cycle and the range as well as the average temperature are shown (i.e. mid temperature). The color code is the same in figures.

with a piece of cotton wool to allow air circulation. Groups of eppendorfs, each containing 10 eggs, were then randomly transferred to incubators regulated at a specific thermal regime (see Fig. 1). Eppendorfs were placed within a plastic box containing humid pieces of cotton wool to maintain saturated relative humidity. We dedicated a total 20 eppendorfs/replicates (i.e., N total of 200 eggs) to each developmental temperature: a group of 10 eppendorfs ($n = 10 \times 10$ eggs) was used to assess egg-to-pupa development and viability and another group of 10 eppendorfs ($n = 10 \times 10$ eggs) was used to assess egg-to-adult development and viability. In such way, tubes dedicated to monitor adult's traits were undisturbed and remained continuously in incubators. Eppendorfs with developing flies were checked daily until the end of emergence. Egg-to-pupa and egg-to-adult development were obtained by recording the time spent by every newly formed pupa or emerged adult in each tube individually. Egg-to-pupa and egg-to-adult viability were determined for each individual tube as the proportion (expressed as percentage) of formed pupae and enclosed adults respectively, from the 10 eggs deposited in each tube. At emergence, the sex was also determined which allowed sex ratio to be determined.

2.4. Modelling thermal reaction norms

To estimate the parameters of the thermal reaction norms for viability, we used `rTPC` and `nls.multstart` packages in R (Padfield et al., 2021). Among the 24 models proposed in `rTPC`, we selected the best nonlinear equation according to several criteria: the lowest AIC, the accuracy of the estimated parameters relative to current knowledge and observations, and a good fit of data. The best model that met all these criteria was the model “kamykowski_1985” (Kamykowski, 1987):

$$Rate(T) = a \times (1 - \exp(-b(T - t_{min}))) \times (1 - \exp(-c(t_{max} - T)))$$

In this equation, the rate (i.e., survival rate) is modeled according to temperature T and other parameters in the function. Parameter a estimates critical thermal maximum (CT_{max}), b estimates the temperature for optimal performance (T_{opt}), c estimates the thermal performance breadth and t_{min}/t_{max} are the low/high temperatures at which the rate becomes negative (Kamykowski, 1987).

To estimate parameters of the thermal reaction norms for develop-

ment (i.e., phenological model), we use the `devRate` package in R (Rebaudo et al., 2018). Among the 30 equations proposed in the package, we selected the best based on the lowest RMSE value, the accuracy of the estimated parameters relative to current knowledge and observations, and a good fit of data. In this case, the best predictive model was the Performance model “per2_11” (Shi et al., 2011):

$$Development\ rate(T) = cc \times (T - T_1) \times (1 - \exp(k(T - T_2)))$$

The performance model was built on how rising temperatures affect enzyme activity and denaturation in ectotherms. In this equation, the developmental rate is modeled according to temperature T and other parameters in the function. T_1 estimates the lower developmental threshold (T_0) and T_2 estimates the upper developmental threshold (CT_{max}) whereas cc and k are constants. For FT, the mid temperature was used as T (see Fig. 1). For both traits (i.e., development and viability), the estimated parameters resulting from the modeled reaction norms were retrieved after the models had converged and 95% confidence intervals of the estimated values were obtained using bootstrapping methods.

2.5. Model's validation

The data on development in two different stages and two thermal regimes allowed using the Performance model's equation (Shi et al., 2011) with four different combinations of entry parameters: CT in egg-to-pupa, CT in egg-to-adult, FT in egg-to-pupa and FT in egg-to-adult. The accuracy of the resulting estimated parameters was tested by confronting estimated vs. observed emergence data. To do so, we assessed the development and viability (as described before) i) in two fluctuating lab conditions and ii) in natural conditions at two seasons. First we designed two field-mimicking thermoperiods in incubators with multiple steps changing every 2 h. The first cycle mimicked a typical hot summer day in Brittany (“summer-like”: daily mean of 25 °C, minimum 20 °C) with a daily 2 h heat stress at 33 °C, and the second regime was a typical winter day in Brittany (“winter-like”: daily mean of 13 °C, maximum of 20 °C) with a daily 2 h cold stress at 5 °C (see Figs. 5A and 6A). In addition, we also assessed the development and viability of *D. suzukii* in uncontrolled natural conditions using tubes with eggs on food placed within cages in open wood structures situated in a small wood located on University Campus in Rennes (Eastern France, 48.6°N 1.40°W) (see Fig. A.1). A net was fixed on each structure to avoid direct exposure to sunrays. The experiment was launched twice: in spring (starting in April 2022, see Fig. 7A) and in winter (starting in November 2022; see Fig. 8A). We recorded microhabitat developmental temperatures of larvae by putting a thermocouple (type K) connected to a Testo 175T3 temperature data logger (Testo SE & Co., Germany) directly inside a tube containing eggs and food. We did not collect any phenotypical response data from this tube. As for the other experiments, for each of these validation experiments (in lab or field), a group of 10 eppendorfs/replicate ($n = 10 \times 10$ eggs) was dedicated to assess egg-to-pupa development time and viability, and another group of 10 eppendorfs/replicate to assess egg-to-adult development time and viability. To calculate model's rate prediction of field-mimicking lab experiments and field conditions, we used Performance model's equation with the appropriate entry parameters (depending on development stage) and calculated cumulated rate by hour as a function of microhabitat temperatures. By the end of the day, the accumulation of the hourly rate was reduced to a daily rate and cumulated with the following daily rates (i.e., degree-days).

2.6. Statistical analyses

All analyzes were performed with R (R Core Team, 2022) and some graphs were also performed using GraphPad Prism (Version 8.0.1). Data from viability and sex ratio were analyzed using binomial generalized linear model (GLM) with a logit link function for proportional outcome

(i.e., dead vs. alive or male vs. female). Development (rate = 1/development time) was analyzed using generalized linear models (GLM) with an inverse Gamma function as data were always non-null and strictly positive. In models, main effects of temperature, thermal regimes and sex were tested, as well as interactions, and the statistical significance of each variable was determined by the analysis of deviance via the ‘Anova’ function implemented in the car package.

3. Results

3.1. Sex ratio

The sex ratio at emergence was rather balanced (i.e., around 0.5) and not affected by any factors (Fig. A.2). All statistical outputs are listed in Table A.1.

3.2. Viability

The egg-to-adult viability increased with temperature until it reached an optimum range and after which it decreased (Fig. 2). Viability was generally greater under FT than under CT conditions at the coldest temperatures. Under CT regimes, the lowest viability was reached at 9 °C for egg-to-adult with 20% viability, while at the upper extreme temperature, constant 30 °C led to 33%. CT conditions below and above these temperatures were lethal (e.g. 8, 31, 33, 35 °C). Under FT regimes, the lowest viability was reached at 8–15 °C (mid 11.5 °C) with 62% while at the upper extreme temperature, 28–32 °C (mid 30 °C) led to lowest values with 31% viability. FT conditions above these values were lethal (e.g. 15–35 °C, 27–33 °C and 30–34 °C). An important point to note is that under FT conditions, with a 12 h temperature cycle, *D. sukukii* managed to develop and survive even when exposed daily to temperatures that would normally be lethal if applied continuously (e.g. 8, 31, 33 °C). It resulted that temperature ($\chi^2 = 1074.47, p < 2.2e-16$) and the thermal regime ($\chi^2 = 90.59, p < 2.2e-16$) had a strong influence on egg-to-adult viability. Similar patterns were observed for egg-to-pupa

viability, as illustrated in Figure A.3 with all statistics listed in Table A.2.

Based on the Kamykowski nonlinear equation, we could fit reaction norms for viability according to temperatures (CT egg-to-pupa: RSS = 2.53, convergence <0.001; FT egg-to-pupa: RSS = 3.33, convergence <0.001; CT egg-to-adult: RSS = 2.41, convergence <0.001; FT egg-to-adult: RSS = 5.64, convergence <0.001). From these models, we could then estimate key parameters for viability such as CT_{min}, CT_{max}, T_{opt}, T_{breadth} and thermal safety margin that are summarized in Table 1. We could note from these values that the main difference between CT and FT was in CT_{min}, with up to 7 °C difference, while CT_{max} values were much less variable. This resulted in broader thermal breadth under FT than CT for viability of pupae and adults (see Table 1).

Table 1

Predicted parameters [$\pm 95\%$ CI] derived from Kamykowski (1987) equation describing the thermal reaction norms for viability from egg-to-pupa and egg-to-adult, under CT and FT conditions. CT_{min} and CT_{max} are respectively the lower and the upper lethal limits, T_{opt} is the optimal temperature for viability, thermal breadth is the estimated range of tolerable temperatures, and thermal safety margin represents the estimated buffer zone before reaching upper lethal limit (CT_{max} - T_{opt}). The Akaike Information Criterion (AIC) of the models are also shown for each condition.

	Egg-to-pupa		Egg-to-adult	
	CT condition	FT condition	CT condition	FT condition
CT _{min} (°C)	7.9 [7.6–8.0]	1.2 [1.2–6.6]	8.0 [7.8–8.1]	2.9 [1.2–6.5]
T _{opt} (°C)	22.9	22.4	23.9	18.9
	[21.4–24.1]	[21.5–23.4]	[22.6–25.3]	[17.8–20.2]
CT _{max} (°C)	33.0	31.9	31.0	31.5
	[32.9–33.0]	[31.8–32.0]	[30.9–31.0]	[31.1–31.8]
Thermal breadth (°C)	25.05	30.8	23.0	28.6
	[24.9–25.3]	[25.5–30.9]	[22.8–23.2]	[25.0–30.6]
Thermal safety margin (°C)	10.04	9.6	7.04	12.6
	[8.8–11.5]	[8.6–10.5]	[5.8–8.4]	[11.3–13.9]
AIC	-152.2	-113.8	-137.5	-40.24

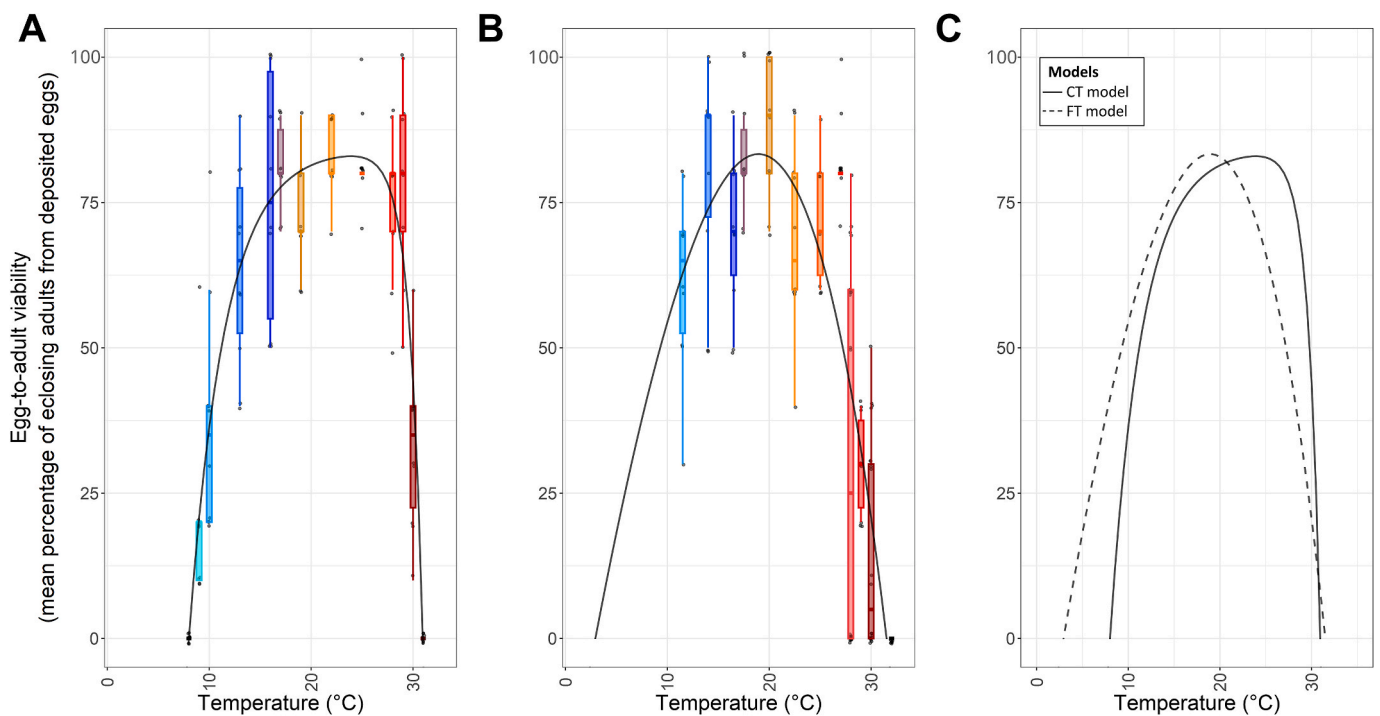


Fig. 2. Egg-to-adult viability (%) according to developmental temperatures under (A) CT and (B) FT conditions. Boxplots are shown with the color codes as in Fig. 1. Bottom and top edges of the boxes indicate the 25th and 75th percentiles. The lines result from the modelling with Kamykowski equation (Kamykowski, 1987). (C) Comparison of the model’s prediction curves under CT vs. FT.

3.3. Development rate

The comparison of development rate between CT and FT is shown in Fig. 3 for egg-to-pupa and in Fig. 4 for egg-to-adult. Under CT regimes, the minimum temperature that allowed development was 9 °C and development took 42.9 d for egg-to-pupa and 74.5 d for egg-to-adult. The temperature that allowed maximum development rate of pupae and adults was 29 and 28 °C, with 5 and 8.8 d respectively. The upper extreme temperatures for development were 31 °C with 5.3 d for egg-to-pupa and 30 °C with 9.1 d for egg-to-adult (Figs. 3 and 4). Under FT, the longest development was for the regime 8–15 °C (mid 11.5 °C) with 23.3 d for egg-to-pupa and 43.3 d for egg-to-adult. The maximum rate of development was under 25–31 °C (mid 28 °C) with 5.6 d for egg-to-pupa and 9 d for egg-to-adult. The upper extreme conditions for development were under FT regime 27–33 °C (mid 30 °C) with 6.9 d for egg-to-pupa and 28–32 °C (mid 30 °C) with 10.1 d for egg-to-adult (Figs. 3 and 4).

Development rate was strongly affected by rearing temperature (egg-to-pupa: $\chi^2 = 47,173$, $p < 2.2e-16$; egg-to-adult: $\chi^2 = 5701.2$, $< 2.2e-16$; Table A.3). Development rate of pupae was also affected by thermal regime and was on average faster under FT conditions (egg-to-pupa: $\chi^2 = 583$, $p < 2.2e-16$). Similar pattern was found in egg-to-adult development rate, yet no main overall effect of thermal regime was detected ($\chi^2 = 0.5$, $p = 0.4995$). Moreover, there was no sex-specific influence in egg-to-adult development rate (Figure A.4 and Table A.3).

Based on the Performance equation, we could fit well the data (CT egg-to-pupa: RSS = 0.0001, convergence <0.001; FT egg-to-pupa: RSS = 0.0004, convergence <0.001; CT egg-to-adult: RSS = $2.57e^{-5}$, convergence <0.001; FT egg-to-adult: RSS = $4.18e^{-5}$, convergence <0.001). From these models, we could then estimate the key parameters for development (T_0 , CT_{max} , r_{max} and thermal breadth) which are summarized in Table 2. The estimated maximal rate temperature (r_{max}) ranged between 28.7 °C and 29 °C (see Table 2, Figs. 3 and 4). The estimated thresholds T_0 were generally lower under FT than under CT, with about 2 °C difference in both egg-to-pupa and egg-to-adult (Table 2). Changes in thermal limits (T_0 and CT_{max}) also induced

changes in thermal breadth which were larger under FT than under CT in egg-to-adult, mainly due to a shift to the left (towards lower temperatures) (Fig. 4).

3.4. Testing predictive model's accuracy

For the validation, we first compared the estimated development times from models based on CT and FT data with the observations from a lab experiment conducted under summer-like conditions, involving repeated heat stress at 33 °C (Fig. 5). Under these conditions, observations showed that pupal development peaked after 7.45 days [95% CI: 7.3–7.6] and adult emergence peaked after 12 days [95% CI: 11.8–12.1]. Despite daily stress at 33 °C, we observed 86% viability in egg-to-pupa and 80% in egg-to-adult. For egg-to-pupa development, the performance model with FT entry parameters estimated 8 days [95% CI: 7–9] of pupal development, and with CT entry parameters, 6 days [95% CI: 6–7] of pupal development. For egg-to-adult development, the performance model with FT entry parameters estimated 20 days [95% CI: 20–21] for adult emergence, and with CT entry parameters, 14 days [95% CI: 13–14]. Hence, in this summer-like lab conditions, models based on FT data better performed in estimating pupal development than that based on CT data, whereas models based on CT data better estimated time to adult emergence than that based on FT.

Next, we compared estimated development times, from CT and FT data, with observations from a lab experiment conducted under winter-like condition with repeated cold stress at 5 °C (Fig. 6). Under these conditions, observations showed that pupal development peaked after 22.45 days [95% CI: 22.1–22.8] and adult emergence peaked after 39.7 days [95% CI: 39.3–40.9]. Here, we observed 69% viability in egg-to-pupa and 64% in egg-to-adult. For egg-to-pupa development, the performance model with FT entry parameters estimated 21 days [95% CI: 19–23] of pupal development, and with CT entry parameters, 25 days [95% CI: 23–26] of pupal development. For egg-to-adult development, the performance model with FT entry parameters estimated 42 days [95% CI: 40–44] for adult emergence, and with CT entry parameters, 52

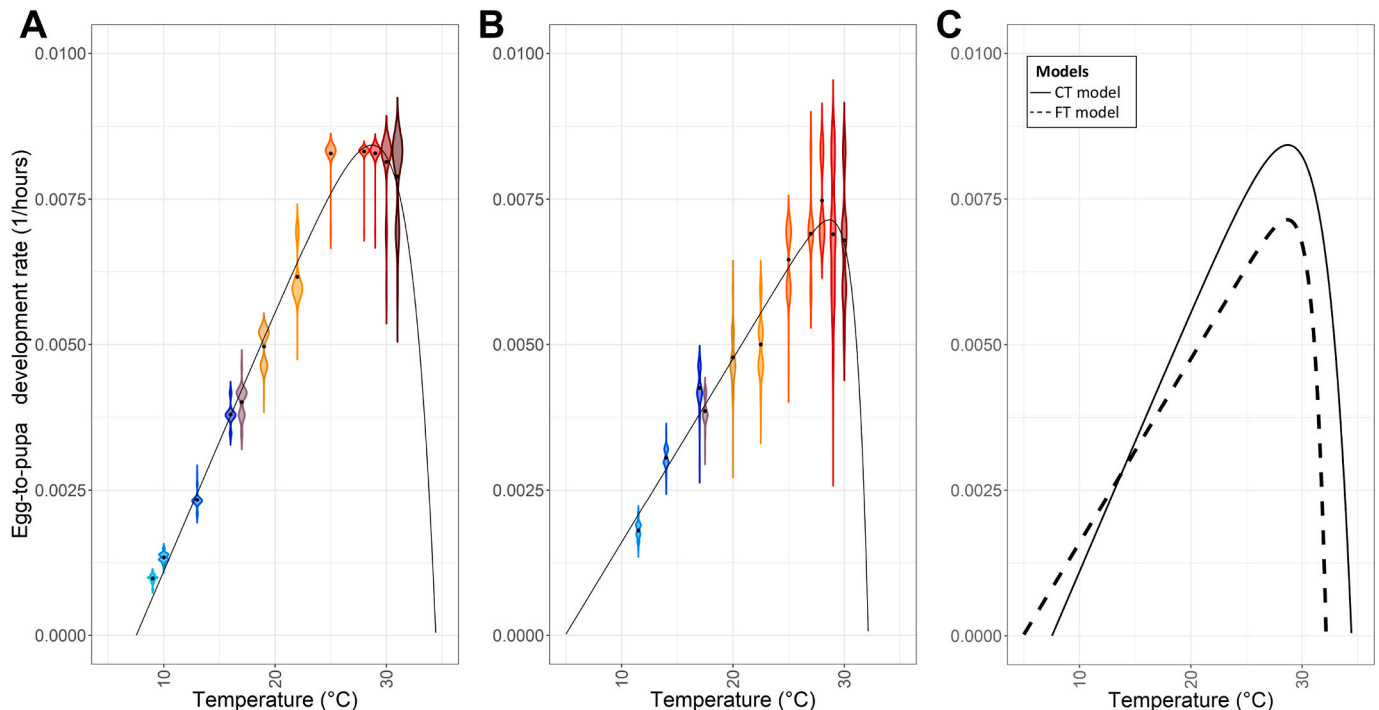


Fig. 3. Egg-to-pupa development rate according to developmental temperatures under (A) CT and (B) FT conditions. Violin plots are shown with the color codes as in Fig. 1. Tails of violin plots trim to the range of the data. The lines result from the modelling with Performance equation (Shi et al., 2011). (C) Comparison of the model's prediction curves under CT vs. FT.

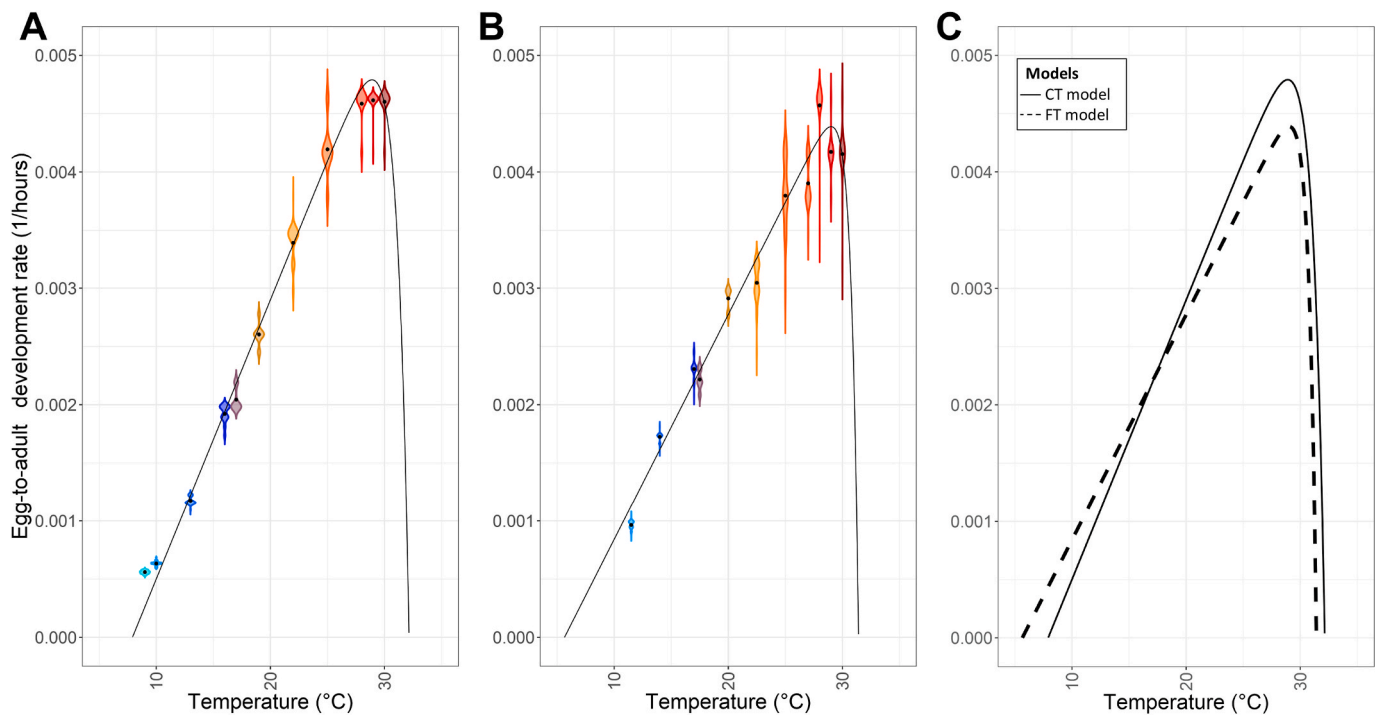


Fig. 4. Egg-to-adult development rate according to developmental temperatures under (A) CT and (B) FT conditions. Violin plots are shown with the color codes as in Fig. 1. Tails of violin plots trim to the range of the data. The lines result from the modelling with Performance equation (Shi et al., 2011). (C) Comparison of the model's prediction curves under CT vs. FT.

Table 2

Predicted parameters [$\pm 95\%$ CI] derived from Performance model (Shi et al., 2011) describing thermal reaction norms for development rate from egg-to-pupa and egg-to-adult under CT and FT conditions. T_0 is the lower developmental threshold, CT_{max} the upper limit for development, r_{max} is the temperature allowing the maximal development rate, thermal breadth is the estimated range of temperatures allowing development, and RMSE is the root mean squared error of each model.

	Egg-to-pupa		Egg-to-adult	
	CT condition	FT condition	CT condition	FT condition
T_0 (°C)	7.5 [7.3–7.7]	4.9 [4.3–5.5]	7.9 [7.8–8.1]	5.6 [5.4–5.9]
CT_{max} (°C)	34.5 [34.1–34.8]	32.2 [31.6–33.0]	32.2 [31.8–32.6]	31.4 [30.9–31.4]
r_{max} (°C)	28.7 [28.7–28.7]	28.7 [28.7–28.7]	28.9 [28.9–28.9]	29 [28.8–29.1]
Thermal breadth (°C)	27 [26.4–27.5]	27.3 [26.1–28.7]	24.3 [23.7–24.8]	25.9 [25–26]
RMSE	0.0004	0.0007	0.0002	0.002

days [95% CI: 49–53]. Hence, in this winter-like lab conditions, models based on FT data better performed in estimating pupal development than that based CT data, and models based on FT data distinctly better estimated time to adult emergence than model based on CT.

Then, we compared estimated development times, from CT and FT data, with observations from a field experiment conducted in the Spring (April–May 2022) (Fig. 7). Observations showed that pupal development peaked after 15.5 days [95% CI: 15.4–15.6] and adult emergence peaked after 28.1 days [95% CI: 28.0–28.2]. Here, we observed 89% viability in egg-to-pupa and 76% in egg-to-adult. For egg-to-pupa development, the performance model with both FT and CT entry parameters estimated 15 days [95% CI: 14–16] of pupal development. For egg-to-adult development, the performance model with FT and CT entry parameters both estimated 30 days [FT: 95% CI: 29–32; CT: 95% CI: 30–32] for adult emergence. Hence, in this spring field conditions, all equations estimated rather accurately development times.

Finally, we compared estimated development times from CT and FT data with observations from a field experiment conducted during the winter (November 2022 to February 2023) (Fig. 8). During that period, temperatures went below 0 °C several times, and the lowest daily mean microhabitat temperature was -1.4 °C. In these rather cold winter conditions, observations showed that pupal development peaked after 46.55 days [95% CI: 45.4–47.7], and adult emergence peaked after 83.9 days [95% CI: 81.6–86.2]. Here, we unexpectedly observed 34% viability in egg-to-pupa and 31% in egg-to-adult despite rather harsh winter conditions endured during development. For egg-to-pupa development, the performance model with FT entry parameters estimated 44 days [95% CI: 40–47] of development, and with CT entry parameters, 50 days [95% CI: 48–51] of development. For egg-to-adult development, the performance model with FT entry parameters estimated 86 days [95% CI: 80–94] for adult emergence. With CT entry parameters, the temperature data during 100 days of recording only permitted the model to estimate 77% of the total development. Indeed, with the CT parameters (e.g., T_0 of 7.9 °C instead of 5.6 °C with FT) and the temperatures provided from November 2022 to February 2023, only 77% of development could be estimated under CT at the end of this 100-d experiment, compared to 100% with parameters from the FT model. In these cold winter conditions, the model based on FT data thus gave a better estimation of development for pupae than CT. For adult emergence, only the equation with FT parameters managed to estimate a complete development time with rather good accuracy.

4. Discussion

Understanding and modelling how ectotherms respond to future climates requires an understanding of thermal performances and sensitivity (Angilletta, 2009). Life stages often co-occur in different seasons and live in different microhabitats, and stages may also differ in thermal performances and sensitivity (Bowler and Terblanche, 2008). Incorporating variations among life stages in phenological models would enhance their predictive accuracy and contribute to a more

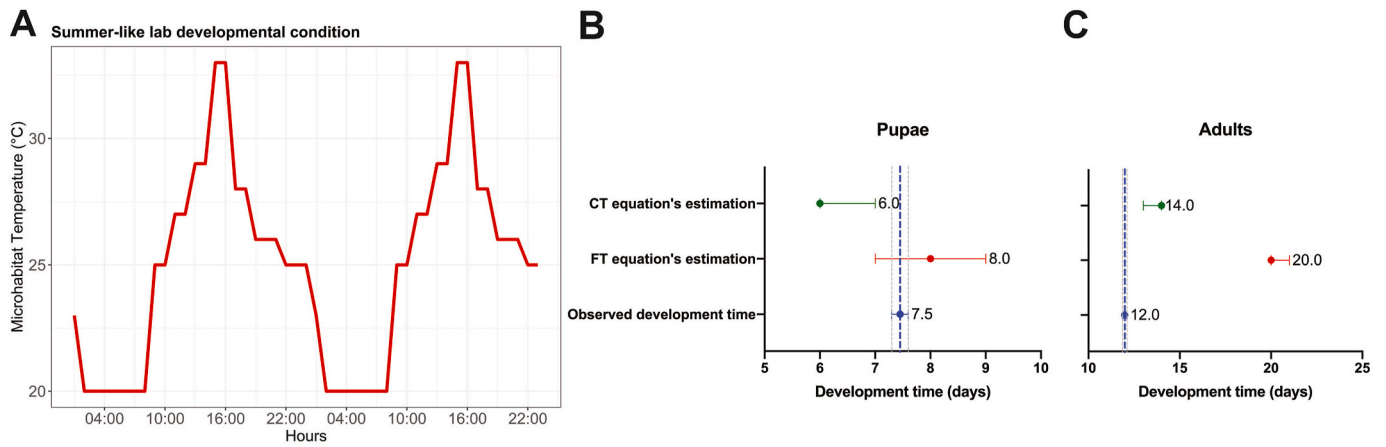


Fig. 5. (A) Temperature setting of the “Summer-like” thermal conditions in incubators; (B) egg-to-pupa development time effectively observed (in blue) and estimated with FT model (in red) and with CT model (in green); (C) egg-to-adult development time effectively observed (in blue) and estimated with FT model (in red) and with CT model (in green). Error bars all represent 95% CI.

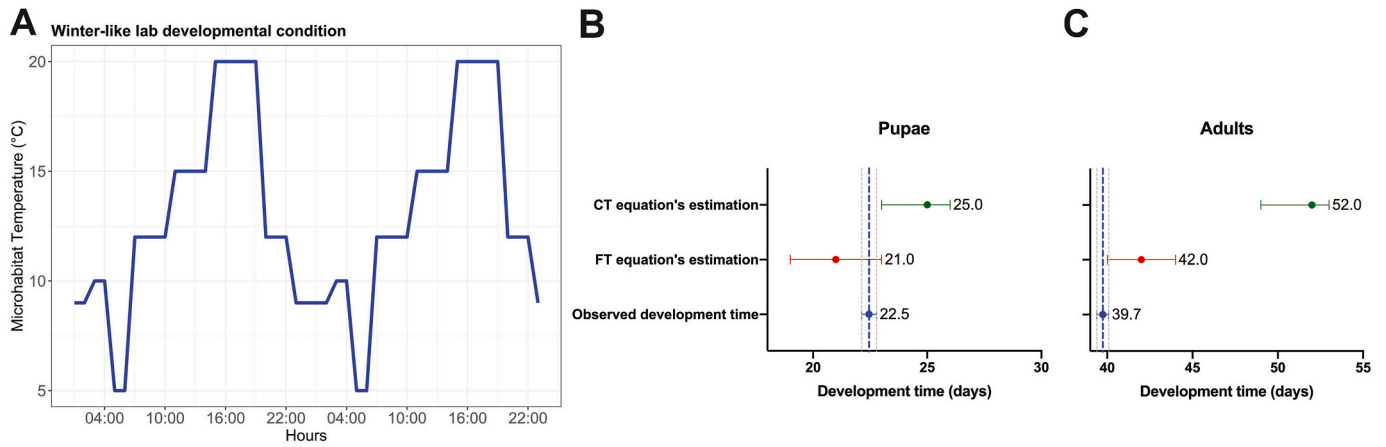


Fig. 6. (A) Temperature setting of the “Winter-like” thermal conditions in incubators; (B) egg-to-pupa development time effectively observed (in blue) and estimated with FT model (in red) and with CT model (in green); (C) egg-to-adult development time effectively observed (in blue) and estimated with FT model (in red) and with CT model (in green). Error bars all represent 95% CI.

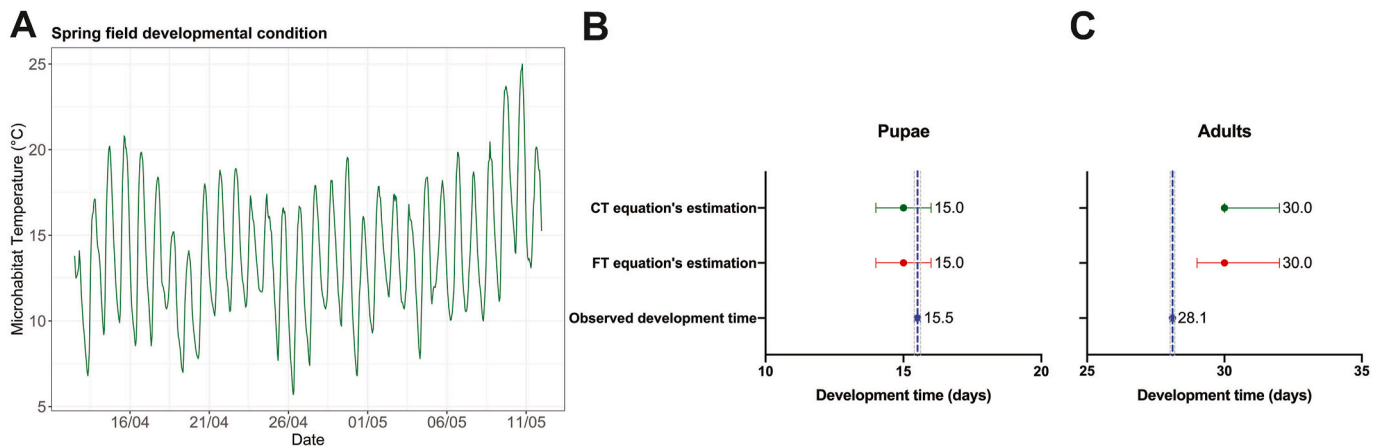


Fig. 7. (A) Microhabitat temperatures recorded during the “Spring experiment” in cages (from April to May, 2022); (B) egg-to-pupa development time effectively observed (in blue) and estimated with FT model (in red) and with CT model (in green); (C) egg-to-adult development time effectively observed (in blue) and estimated with FT model (in red) and with CT model (in green). Error bars all represent 95% CI.

comprehensive understanding of pest population dynamics in changing environments (Rebolledo et al., 2020). In addition, gathering data across a large range of temperatures is crucial for accurate model predictions

(de Jong and Van der Have, 2009). However, measuring traits near lethal limits can be difficult due to high mortality (Régnière et al., 2012). Yet, this problem can be circumvented by including alternating

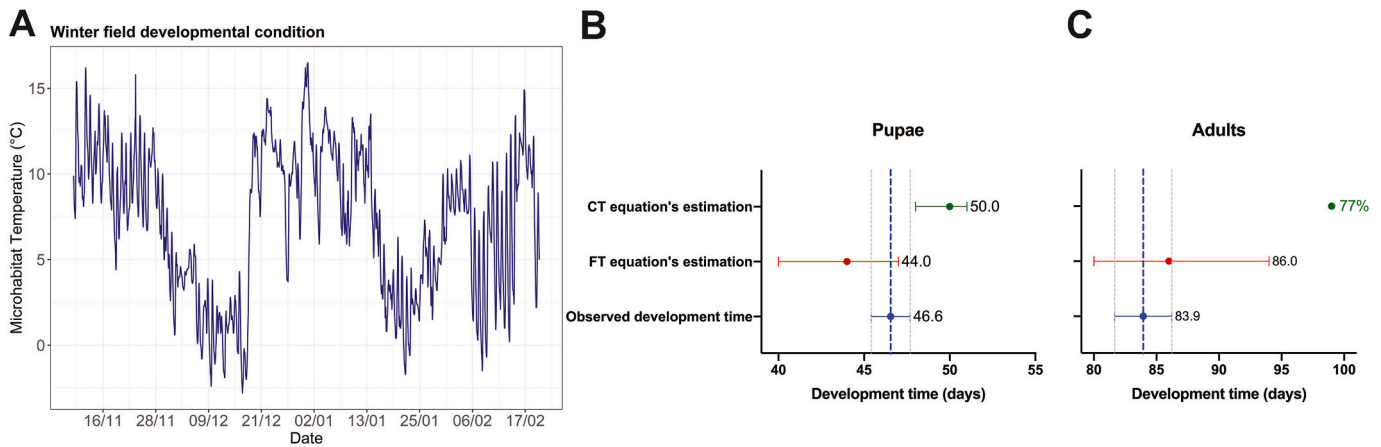


Fig. 8. (A) Microhabitat temperatures recorded during the “Winter experiment” in cages (from Nov 2022 to Feb 2023); (B) egg-to-pupa development time effectively observed (in blue) and estimated with FT model (in red) and with CT model (in green); (C) egg-to-adult development time effectively observed (in blue) and estimated with FT model (in red) and with CT model (in green). Error bars all represent 95% CI. With CT entry parameters, the temperature data during 100 days of recording only permitted the model to estimate 77% of the total development (as indicated in green).

temperatures (Régnière et al., 2012).

Our study revealed strong discrepancies between CT and FT regimes for both viability and development rate. We observed that FT regimes extended the survival limits, particularly towards the colder side, primarily due to reduced CT_{min} , while CT_{max} remained relatively stable (Fig. 2 and A3). Beyond the upper limit, the heat survival might be physiologically constrained due to denaturation and destabilization of macromolecules and macrostructures (e.g., DNA, proteins, membranes), causing irreversible damages (Lubawy et al., 2019; González-Tokman et al., 2020). Under FT, deleterious effects of repeated heat exposure were observed. For instance, constant 30 °C allowed a high pupal viability (>85%, Fig. A.3) while viability was halved in the corresponding FT regimes with an equivalent average temperature of 30 °C (i.e., 27–33 °C and 28–32 °C). Our observations thus align with Jensen’s Inequality, as the percentage of viability under FT did not correspond to that predicted under an equivalent CT regime. This discrepancy was particularly notable in the ascending part of the curve as well as in the descending part (Colinet et al., 2015). If we move beyond simply comparing the effects of equivalent average temperatures between FT and CT, an important point must be noted. Near the lower and upper extremes, FT conditions allowed organisms to survive and develop at stressful temperatures (both heat and cold) which should normally be lethal under CT conditions. For instance, 8 °C was lethal under CT, but flies managed to sustain FT regimes of 8–15 °C, 8–20 °C, and 8–25 °C with about 60–80% survival (Fig. 2 & Fig. A3). Likewise, temperatures of 31 °C or above were fully lethal under CT, but flies managed to survive with FT regimes of 26–32 °C, 28–32 °C, 25–33 °C and even 27–33 °C. Hence FT regimes allow daily thermal stress of 12 h to be tolerable when alternating with permissive temperatures. It is commonly accepted that during FT regimes, thermal injuries accumulate during stress phases but insects manage to quickly recover during non-stressful phases (Colinet et al., 2015; Grumiaux et al., 2019; Enriquez et al., 2020).

The model validations revealed a lack of precision in the phenological models when they were calibrated with CT compared with models calibrated with FT, and this was particularly noticeable when development took place at low temperature (Figs. 6 and 8). Indeed, we noted marked variations in lower developmental thresholds (T_0) between thermal regimes with a 2.3 °C shift in T_0 values between CT and FT (7.9 and 5.6 °C respectively; Table 2). This led to a 10-d difference in the prediction of adult emergence under winter-like lab conditions (52 days vs. 42 days with CT vs. FT, respectively) (Fig. 6). Our observations revealed that using the T_0 derived from FT regimes for degree-days predictions was accurate in simulated cold conditions, while using T_0

derived from CT regimes poorly predicted adult emergence. The estimated T_0 values reported in the literature for *D. sukii* are all derived from CT protocols and are generally in the range (or higher) of the values we found here under CT (i.e., 7.9 °C): 7.2 °C (Tochen et al., 2014), 8.1 °C (Ryan et al., 2016), 11.6 °C (Tonina et al., 2016); 7.4–8.2 °C (Sánchez-Ramos et al., 2019), 9.6 °C (Winkler et al., 2021). Yet, all these T_0 values derived from CT regimes are some degrees higher than the values we found under FT regimes (i.e., 5.6 °C). Overestimating the lower threshold value will result, under cold winter conditions, in not accounting for a portion of the heat accumulation in degree-days calculation, leading to a delay in the estimation of the expected emergence dates. In winter, temperature may repeatedly fall below T_0 , elevating the likelihood of errors in calculating accumulated degree-days if the T_0 value is inaccurate. Temperatures measured in our validation trials *in natura* throughout winter repeatedly dropped below both the developmental and the lethal thresholds estimated under CT (T_0 and CT_{min} in Tables 1 and 2). Indeed, environmental temperatures in that period remained below 8 °C for several consecutive days or even a week or more. Therefore, the predictive model that used estimated T_0 from a CT regime managed to cumulate heat units to complete only 77% of the total development at the end of 100 days of test in winter. On the other hand, the predictive model that used estimated T_0 from an FT regime was much more successful at predicting adult emergence after 86 days on average, against 84 days effectively observed (see Fig. 8). This highlights again how the use of FT can be useful and relevant for parameterizing phenology models, as already emphasized (Régnière et al., 2012).

Under warm conditions, survival and development are constrained by CT_{max} . In summer-like conditions (with daily stress at 33 °C), FT proved inefficient for estimating development time of adults. Since CT_{max} was lower under FT compared to CT for pupae and adults (Table 2), when temperatures exceeded this threshold in summer-like conditions, the degree-days accumulated during these periods were not taken into account. This may have led to a delay in the accurate estimation of development. Our data thus illustrate how a few degrees of difference between thresholds can influence development predictions, especially during winter when development is extended. In practice, using incorrect thresholds in models is not an issue as long as daily temperatures remain in between. However, when field temperatures meet and cross these limits it may lead to significant errors in the calculated cumulative degree days. Our results suggest relying on thresholds obtained through FT regimes to better estimate phenology during/after winter.

In addition, we found that pre-imaginal stages (e.g., larval/pupal

stages) were not as cold sensitive as the literature suggests in *D. suzukii* (e.g., Kaçar et al., 2016). Indeed, during the field trials in winter, environmental temperatures dropped below 5 °C for several consecutive days and we also recorded multiple frost events (Fig. 8). Despite that, about a third of the eggs deposited in tubes in November managed to develop into adults throughout winter 2022 in Rennes and emergence took about 84 days on average (start-end range: 72–99 days). This finding is significant as it suggests that *D. suzukii* may have the ability to overwinter not only as reproductively-dormant adult females, as repeatedly reported in the literature (e.g., Stephens et al., 2015; Stockton et al., 2019; Winkler et al., 2020; Sario et al., 2023), but also as developing larvae/pupae. Just as adults, immature stages (larvae or pupae) have likely developed effective acclimation mechanisms throughout the winter to withstand cold conditions (e.g., Enriquez and Colinet 2019). In nature, larvae might be adapted to feed on available winter resources such as rotting apples on the ground (Deconninck et al., 2024) and profit from microhabitats that offer protection from harsh conditions, for instance under the leaf litter (Wallingford et al., 2018). This finding is noteworthy because, so far, it was largely assumed that overwintering temperatures are lethal to immature stages of *D. suzukii* (e.g., Dalton et al., 2011; Kaçar et al., 2016; Stockton et al., 2018). Our results suggest that in regions with mild winters, as in Brittany (France), *D. suzukii* might employ a mixed overwintering strategy involving dormant adults (in reproductive quiescence) as well as developing immature stages. This mixed opportunistic strategy would allow flies to capitalize on the benefits of both states. Such flexibility may allow *D. suzukii* to adjust to variations and stochasticity in climate and successfully emerge/reproduce once conditions become favorable. By employing several strategies, *D. suzukii* may thus minimize the risks associated with unpredictable or variable environmental conditions (i. e., bet-hedging).

The course of the field trials during winter 2022 witnessed an adult emergence period that lasted for about one month, even though all tubes had been loaded with synchronized eggs in a density-controlled protocol. Emergence started after 72 days and ended 99 days after egg deposition. To explain such a high inter-individual variability in development time, a hypothesis might be again the bet-hedging strategy, also called risk spreading. It implies that a single genotype can stochastically generate a distribution of phenotypes, guaranteeing that at least some individuals will be well suited to any environmental condition (Hopper, 1999; Levy et al., 2012; Simons, 2011). If development were more synchronized, there would be a risk of all individuals emerging during certain periods, which could potentially coincide with lethal conditions during winter. Such an evolutionary strategy, which maximizes the geometric mean of fitness rather than arithmetic across time and environmental conditions, has already been studied in *D. melanogaster* (Kain et al., 2015) but was not yet described in *D. suzukii*.

To conclude, here we employed a basic FT design with only two alternating temperatures (i.e., cold during the scotophase and warmer during the photophase). Despite this simplistic approach, which still deviates from the daily fluctuations in nature, we were able to better describe and predict thermal and overwintering responses than with CT. This study brings new perspectives for developing phenological models and novel data for building a robust population dynamic model (under construction). We show that phenological models parametrized using CT data are not sufficiently accurate to predict long development periods. Furthermore, we underscore that certain temperatures deemed lethal under CT may still permit viability when experienced under FT regime. Finally, we demonstrate here for the first time that *D. suzukii* may employ various strategies for overwintering: adults in reproductive dormancy, but also developing immature stages from which acclimated adults may gradually emerge from protected microhabitats during/after winter.

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CRediT authorship contribution statement

Bréa Raynaud-Berton: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Patricia Gibert:** Writing – review & editing, Supervision. **Christelle Suppo:** Writing – review & editing, Formal analysis, Data curation. **Sylvain Pincebourde:** Writing – review & editing, Formal analysis, Data curation. **Hervé Colinet:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2024.103891>.

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