

1 **Diurnal temperature fluctuations outweigh population origin**
 2 **in shaping thermal performance and range predictions**
 3 **in European *Aedes albopictus***
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Abstract

Predicting invasive ectotherm responses to climate change requires understanding how population-level variation and diurnal temperature fluctuations shape thermal performance, and whether constant-temperature measurements accurately capture performance under fluctuating conditions. Yet many models, including those used to predict mosquito distribution and disease transmission, still rely on constant-temperature measurements. We addressed both questions in the invasive mosquito species *Aedes albopictus* by rearing first filial offspring (F1) from three European populations spanning a 1,200 km latitudinal gradient (Frankfurt–Rhine-Main, Germany; Trento and Palermo, Italy). We tested F1 performance under five mean temperatures (13–33°C) exposing them to constant and fluctuating ($\pm 5^\circ\text{C}$) thermal regimes, and quantifying larval survival, larva-to-adult development time, wing length, and adult longevity. The thermal regime dominated all immature traits: fluctuating temperatures reduced survival, depressed the survival optimum from $\sim 30^\circ\text{C}$ to $\sim 24^\circ\text{C}$, and extended development by $\sim 71\%$ compared to equivalent constant means. A competing-risks discrete-time hazard analysis demonstrated that thermal variability simultaneously increased pre-adult mortality and decreased adult emergence. Population origin effect was significant in only a subset of traits, with divergence concentrated at the adult life-stage and thermal extremes rather than across the whole temperature range. Constant-temperature thermal performance curves overestimated fluctuating-temperature development rates by 42–60% at 23–28°C. When mapped across Europe, this inflated both predicted development rates and the geographic extent of viable development. Incorporating realistic thermal variability into experimental parameterisation is therefore progressively essential, as reliance on constant-temperature measurements can substantially bias predictions of *Ae. albopictus* development and spread, with potentially substantial consequences for downstream epidemiological modelling.

Keywords: adaptation; invasive species; life-history traits; mosquito; plasticity

52 1. Introduction

53 Temperature governs virtually all aspects of ectotherm biology, from developmental rate
 54 and survival to reproductive output and senescence^{1–3}. For disease vectors, this
 55 dependence extends beyond individual life-history performance as temperature also
 56 modulates transmission risk directly, so accurate thermal performance estimates are a
 57 prerequisite for predicting vector-borne disease dynamics under current and future
 58 climates^{1,4}. Climate change is expected to raise both mean temperatures and the
 59 variability around those means, alongside more frequent and intense transient extremes⁵
 60 ^{–7}. Insects are already responding to these shifting thermal regimes, with well-documented
 61 changes in their geographic distributions and seasonal phenology^{8–11}.

62 The invasive mosquito vector *Aedes albopictus* (Skuse, 1894) has become one of the
 63 most widespread mosquito species globally, having spread from its native range in
 64 Southeast Asia to establish self-sustaining populations across Europe, the Americas,
 65 Africa and Oceania during the past four decades^{12,13}. Its European invasion history
 66 involved multiple independent introductions, originally from Asia and North America,
 67 detected in Albania and Italy^{12,14}. Subsequent expansion into central Europe has been
 68 characterised again by independent introductions and by ongoing dispersal along major
 69 highway networks from southern to northern Europe^{15,16}. This establishment has
 70 substantially increased the risk of local arbovirus transmission, with the overall average
 71 risk of dengue outbreaks estimated to have increased by 297% across Europe in the last
 72 decade compared to 1981–2010¹⁷.

73 Given its rapid expansion across Europe, it is important to determine whether thermal
 74 performance varies among populations across the invasive range, as such variation may
 75 influence temperature-dependent model predictions. Although differences have been
 76 reported between temperate and tropical *Ae. albopictus* populations¹⁸, population-level
 77 variation along climatic gradients within Europe remains insufficiently characterized. Such
 78 variation integrates genetic, environmental and developmental components, and
 79 disentangling them requires experimental approaches that decouple environmental
 80 influences from genetic variation, such as common garden designs along climatic
 81 gradients that serve as spatial proxies for climate change^{19,20}.

82 A separate question concerns whether differences in thermal performance among
 83 populations, or their absence, are even estimable from the constant-temperature
 84 experiments on which thermal biology has largely relied. Organisms in the field
 85 experience continuous thermal variability, and because thermal performance curves
 86 (TPCs) are non-linear, performance at a constant temperature does not equal mean
 87 performance under fluctuating temperatures. Jensen's inequality²¹ predicts the direction
 88 of this discrepancy from the local curvature of the response function: variability depresses
 89 mean performance on the concave, decelerating approach to the thermal optimum, and
 90 elevates it on the convex, accelerating phases near the lower and upper thermal limits^{22,23}.

91 Empirical support is consistent across mosquito systems: daily fluctuating temperatures
 92 alter larval development, survival and parasite transmission in *Anopheles* and *Aedes*

93 *aegypti* in the directions predicted by Jensen's inequality, and thermal performance
 94 parameters estimated from constant-temperature experiments systematically mispredict
 95 performance under ecologically more realistic conditions^{24–26}. For *Ae. albopictus*
 96 specifically, daily fluctuating regimes substantially reduce pupation and emergence rates
 97 relative to equivalent constant temperatures²⁷, yet whether life-history traits measured at
 98 constant-temperature accurately capture the thermal performance of this species across
 99 its European invasive range, and whether any such bias is consistent across populations
 100 or temperature-dependent in its magnitude, remains unresolved.

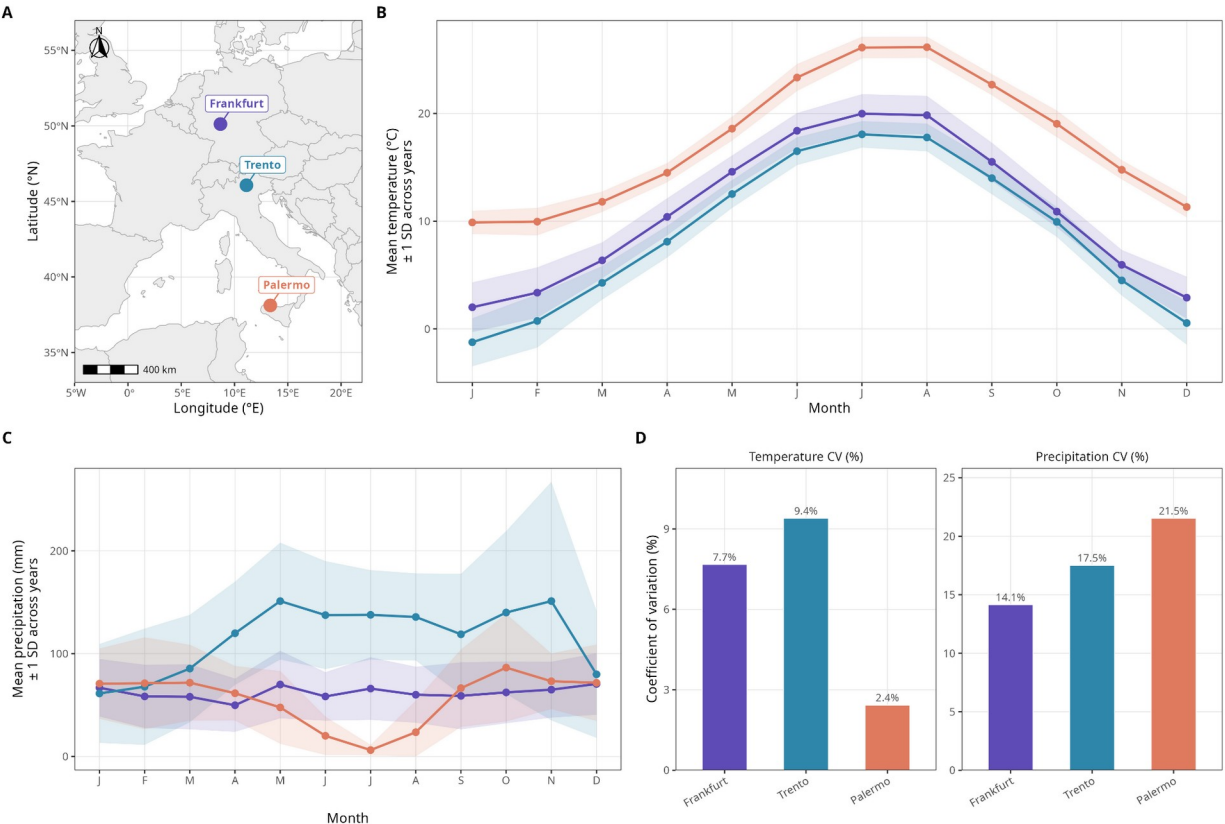
101 Here we focus on four questions that therefore remain unaddressed for *Ae. albopictus* in
 102 Europe: (1) whether thermal life-history traits performance varies among populations
 103 established along a European climatic gradient; (2) whether traits measured under a
 104 constant-temperature regime match the thermal performance of the species under
 105 fluctuating conditions; (3) whether the discrepancy between constant and fluctuating
 106 temperature regimes is uniform across populations or varies in direction and magnitude
 107 between them; and (4) what the downstream consequences of this parameterisation
 108 choice are for population dynamics models, which have not been quantified for the most
 109 abundant and epidemiologically relevant mosquito species in Europe^{28,29}.

110 We address these questions using *Ae. albopictus*, the primary invasive arboviral vector
 111 in temperate Europe, as an empirical model system. We reared first filial offspring (F1) of
 112 field-collected individuals from three populations spanning approximately 1,200 km along
 113 a latitudinal gradient (38.1°N–50.1°N) in the European invasive range (Frankfurt-Rhine-
 114 Main metropolitan region, Germany; Trento, Italy; Palermo, Italy) under five mean
 115 temperatures (13–33°C) held either constant or fluctuating diurnally by $\pm 5^\circ\text{C}$, and
 116 measured larval survival, larval-to-adult development rate, wing length and adult
 117 longevity. We then tested for population-level differences in thermal performance and
 118 their interaction with temperature regime, fitted TPCs to both regimes, used rate
 119 summation to quantify the bias introduced by constant-temperature parameterisation, and
 120 propagated the fitted traits into a population growth model and a Europe-wide map of
 121 predicted developmental rate.

122 2. Methods

123 2.1 Study site selection and long-term climate characterisation

124 Three established invasive *Ae. albopictus* populations were selected to represent a
 125 latitudinal gradient spanning the geographical distribution range in Europe (Figure 1): the
 126 Frankfurt–Rhine-Main metropolitan region (Hesse, Germany, approximately 50 km south
 127 of Frankfurt city centre, 50.1°N), Trento (Autonomous Province of Trento, northern Italy,
 128 46.1°N), and Palermo (Sicily, southern Italy, 38.1°N). *Ae. albopictus* was first detected in
 129 northern Italy in 1990³⁰ and rapidly expanded its distribution range throughout the country
 130 over the following decades³¹. In the Autonomous Province of Trento, the species was first
 131 recorded in the southern part of the province in the late 1990s and has since colonised
 132 all major lowland valleys³². In Palermo, *Ae. albopictus* was first documented in autumn
 133 2004, with the first city-wide surveillance conducted in 2005 using 300 ovitraps across 28
 134 sites³³. In Germany, the species was first detected in 2007 at the A5 motorway corridor
 135 connecting Italy to Germany via Switzerland³⁴. The Frankfurt–Rhine-Main metropolitan
 136 region was reached by *Ae. albopictus* in 2018³⁵, making it among the most recently
 137 established of the three study populations^{15,34}. Together these sites span approximately
 138 1,200 km of latitudinal gradient. To quantify the climatic differences across the gradient,
 139 long-term climate at each sampling location was characterised using monthly mean 2-m
 140 air temperature and total precipitation from the ERA5 single-level monthly means
 141 reanalysis product (ECMWF; ~0.25° resolution) for the period 1995–2025, with annual
 142 means and interannual coefficients of variation computed for each site³⁶ (Figure 1A-D).
 143 Full details of the climate extraction and processing pipeline are provided in the SM 1.1.



144

145 **Figure 1.** Climatic context of the study populations and experimental thermal design. (A)
146 Sampling locations of the three *Aedes albopictus* populations along the European invasive range,
147 spanning approximately 12 degrees of latitude (Frankfurt, Germany; Trento, Italy; Palermo, Italy).
148 (B) Mean seasonal temperature cycle at each location, calculated from ERA5 reanalysis monthly
149 means (1995–2025); shaded ribbons show ± 1 SD across years. (C) Mean seasonal precipitation
150 cycle at each location over the same period, with shaded ribbons showing ± 1 SD across years.
151 (D) Interannual coefficients of variation (CV) in annual mean temperature and annual total
152 precipitation for each population, calculated across the 1995–2025 period.

153 2.2 Study populations and colony establishment

154 Field populations of *Ae. albopictus* were collected from June to August 2025 from the
 155 three locations. For the Italian populations (Trento and Palermo), a continuously
 156 supplemented colony approach was adopted for each site to obtain F1-offspring whilst
 157 maintaining genetic diversity representative of each field population, introducing ~500
 158 wild collected eggs from five ovitraps for each location during the entomological
 159 surveillance activities to a location-specific colony throughout the summer season
 160 (sampled at the end of June, mid July and early August; Trento coordinates 46.19179,
 161 11.13615; Palermo coordinates 38.10347, 13.33611; EPSG: 4326). For the German
 162 population, ~700 mosquito eggs originating from the metropolitan area around Frankfurt
 163 (Büstadt; coordinates: 49.64344, 8.46069) sampled in July 2025 were hatched from five
 164 ovitraps. The Italian and German populations were reared in two different laboratories -
 165 University of Frankfurt, Germany, and Edmund Mach Foundation, Italy - following
 166 standard established rearing protocols for both laboratories and harmonised experimental
 167 setups whenever logistically possible. Differences in lab procedures are explicitly
 168 mentioned in the methods section.

169 Eggs were hatched at 25 °C, and larvae were reared until the pupal stage and fed *ad*
 170 *libitum* with finely ground cat food (Adult Fit 32™, Royal Canin®, Aimargues, France).
 171 Pupae were separated into 2 ml tubes, and species identity was verified morphologically
 172 at the adult stage. Adult mosquitoes were then transferred to cages for egg production,
 173 where they were provided with sugar solution and coffee filter paper as oviposition
 174 substrate³⁷. For egg production, F0-females were offered multiple blood meals presented
 175 via an artificial membrane-feeding system consisting of a plastic Petri dish heated to 36
 176 °C with a temperature-controlled heating pad and covered with a Parafilm membrane to
 177 simulate host skin. The population originating from Germany was reared at the University
 178 of Frankfurt and was offered human blood meals, the populations reared at the Edmund
 179 Mach Foundation (Trento and Palermo populations) were offered cow blood. When F1-
 180 eggs were present on the coffee filters, the coffee filters were removed from the cage to
 181 prevent interbreeding between wild-caught F0-females and their F1-progeny and placed
 182 in a 1 L vessel under controlled conditions at 25 °C and 80% humidity. F1-eggs were
 183 subsequently used for life-cycle experiments. This approach ensured that each
 184 population colony retained the genetic pool of the contemporary field population whilst
 185 producing F1-offspring under standardised laboratory conditions, thereby removing the
 186 direct influence of the field environment on individual phenotype.

187 2.3 Thermal regime treatments

188 Individuals of the three populations were reared under five mean temperatures (13, 18,
 189 23, 28 and 33°C) under a constant (CT) and a daily fluctuating (FT) regime per mean
 190 temperature. Under the constant regime, temperature was maintained at the target mean
 191 for the full 24-hour cycle. Under the FT, a stepped daily cycle approximating natural
 192 diurnal temperature variation was applied using the same five mean temperatures, with a
 193 fixed diurnal temperature range of 10°C structured as follows: eight hours at the mean
 194 plus 5°C (daytime peak), four hours at the mean (morning and evening transitions), and
 195 eight hours at the mean minus 5°C (nocturnal trough; Fig. 1 E). This schedule produces
 196 a daily mean equivalent to the corresponding constant treatment, enabling direct
 197 comparison of trait expression at equivalent mean temperatures under contrasting
 198 temperature variability. Due to logistical constraints, namely equipment failure and the
 199 need to run experiments sequentially rather than in parallel, juvenile life-history traits
 200 could not be measured at 18°C under the FT regime; for the same reasons, adult longevity
 201 could not be assessed under the FT regime. Climatic chambers were maintained at 75–
 202 80% relative humidity (RH) and a 16hL:8hD photoperiod with one-hour simulated dawn
 203 and dusk transitions to mimic natural light conditions during the mosquito active season.
 204 Technical details are available in SM 1.2.

205 2.4 Rearing protocol

206 For each population, eight replicate containers were established per temperature and
 207 treatment combination, following previously published rearing protocols^{43,44}. Each
 208 replicate consisted of 10 first-instar larvae less than 24 hours old, hatched at 25°C and
 209 80% RH, and placed in a standardised plastic container (Picnic-style cups) filled with 100
 210 ml of dechlorinated water, maintaining a density of 0.1 larvae ml⁻¹. Larvae were isolated
 211 individually from hatching containers using small pipettes and were fed daily with finely
 212 ground cat food (Adult Fit 32™, Royal Canin®, Aimargues, France) at stage-specific
 213 rates: 0.1 mg larva⁻¹ day⁻¹ for first-instar larvae and 0.2 mg larva⁻¹ day⁻¹ from second
 214 instar onwards³⁸. Every two days, two-thirds of the water in each replicate container was
 215 replaced with fresh dechlorinated water and food was replenished. Daily inspections were
 216 conducted for all replicates, at which the number of live individuals, the day of pupation
 217 and the sex of the emerged individuals were recorded.

218 Upon pupation, individual pupae were transferred to pupal containers. Under the FT
 219 regime, pupae were transferred to 50 ml Falcon tubes containing 20 ml of dechlorinated
 220 water, sealed with a mesh lid to allow adult eclosion under the same thermal regime
 221 without escape or desiccation. Day of adult eclosion was recorded for each individual and
 222 newly emerged mosquitoes were killed by freezing. Under the CT regime, pupae were
 223 transferred to 5 ml press-on lid glasses filled to one quarter with dechlorinated water²⁰.
 224 Following adult eclosion, under the CT regime the water was removed by syringe leaving
 225 only a small residual volume to maintain humidity inside the 5 ml glass³⁹ and allow
 226 measuring adult longevity.

227 2.5 Life-history traits measured

Larvae-to-adult survival was calculated as the proportion of larvae introduced into each replicate that successfully emerged as adults. Development time was recorded in two components: larva-to-pupal development time (days from larval introduction to pupation), and pupal-to-adult development time (days from pupation to adult eclosion). Adult longevity was recorded for individuals that emerged under CT conditions only (individuals reared under FT were not retained for adult longevity measurement due to logistical issues). Adult longevity was defined as the number of days from eclosion to natural death, during which mosquitoes were maintained under starvation conditions within 5 mL press-on lid glass vial. Sex ratio was determined at adult eclosion for each replicate.

Wing length was measured for a subset of adults emerging from the life-cycle experiments. Measurements were obtained for 4-7 females and 4-7 males per population × temperature combination. The exact number of adult individuals with wing length measurements for each combination of population, thermal regime and sex is reported in Table SM 1.3.

Wing length was measured following a standardised protocol across the two laboratories with a calibrated microscope (SM 1.3). One wing of each individual was excised at the base using entomological forceps after thawing at room temperature, and placed on a microscope slide under a coverslip, and measured along the Radius 1 (R1) vein using a Leica S8APO stereomicroscope at 5× magnification with the 'Segment line' tool in the Leica Application Suite (LAS, version 4.9). The length of the R1 wing vein was used as a standardised measure of adult body size, following established morphometric approaches in mosquitoes. Moreover, wing length is positively associated with female fecundity^{40–43}.

2.6 Statistical analysis

2.6.1 Generalized Linear Mixed Models

Each life-history trait was analysed using a linear or generalized mixed-effects model fitted with the *glmmTMB* package⁴⁴. For each trait, a set of candidate models was compared via the Akaike Information Criterion (AIC) and likelihood ratio tests (LRTs). Model selection was based on parsimony; the model with the lowest AIC was retained for inference, unless a simpler model competed closely ($\Delta\text{AIC} < 2$). For the full initial model (Eq. 1) of each life-history trait, we considered as fixed effects population and thermal treatment regime (categorical variables), temperature (linear and quadratic terms; continuous variables), and their interactions. Replicate cup ID was included as a random effect in every model to account for non-independence of individuals reared in the same container.

$$\text{Life-history trait} \sim (\text{Temperature} + \text{Temperature}^2) \times \text{Thermal Regime} + \text{Population} \times (\text{Temperature} + \text{Temperature}^2) + (1 \mid \text{Replicate}) \quad (\text{Eq. 1})$$

Moreover, for adult life-history traits, namely wing length and longevity, the sex of the individuals was added as additional fixed effects variables described above. After the model selection, the fixed-effects of each best model varied between life-history traits and the best selected models for each trait is reported in the Results section. Model adequacy was also evaluated through simulation-based residual diagnostics implemented in the *DHARMA* package version 0.4.7⁴⁵. All hypothesis tests used a two-tailed significance threshold of $\alpha = 0.05$. All analyses were performed in R version 4.5.2⁴⁶.

2.6.2 Additional analyses

In addition to these trait-specific models, we conducted complementary analyses to identify the processes underlying temperature-dependent larva-to-adult performance and to determine how individual life-history responses translate into broader thermal and demographic patterns. Specifically, we (1) used a discrete-time competing-risks models to distinguish whether temperature, thermal regime, and population origin affected successful adult emergence through daily mortality, delayed emergence, or both; (2) fitted TPCs to characterise the nonlinear temperature dependence of development rate and estimate its thermal limits and optimum; (3) used rate summation to test whether the performance observed under fluctuating temperatures could be predicted from the constant-temperature response after accounting for nonlinear averaging; (4) mapped the resulting development-rate predictions to assess how constant- and fluctuating-temperature parameterisations differ geographically; (5) and integrated the temperature-dependent life-history traits into a population growth model to estimate how these responses combine to determine net reproductive output across the thermal gradient. All analyses were performed in R version 4.5.2⁴⁶.

Larva-to-adult survival proportion. The response variable was specified as the number of individuals reaching adulthood out of the initial number of larvae and modelled using a GLMM with binomial error. Additionally, for the constant-temperature experiments, a Kaplan–Meier analysis was performed using the complete lifespan of each individual. Survival time was calculated as the number of days from hatching to death, incorporating adult longevity for individuals that successfully emerged. Kaplan–Meier curves and pairwise log-rank tests evaluating population differences³⁹ within each temperature were computed using the survival package⁴³ (version 3.8-3) and visualised using the survminer package⁴⁷ (version 0.4.9) in R.

Development time. Larva-to-adult development time, i.e., the number of days from experimental onset (L1 larvae younger than 12 hours; day 0) to adult emergence, was analysed for individuals that reached adulthood ($n = 846$). Because development times are strictly positive and right-skewed, a Gamma GLMM with a log link was fitted. Marginal

means at each tested temperature were estimated from the fitted model and back-transformed to the response scale using the *emmeans* package (v1.10.6)⁴⁸.

Wing length. Wing length was analysed for $n = 317$ adults collected under both CT and FT thermal regimes using a Gaussian linear mixed-effects model (LMM) and involving the fixed and random effects described above.

Adult longevity. Adult longevity was modelled using the fixed and random effects described above under constant-temperature conditions only ($n = 592$) with a Poisson GLMM with a log link.

Cause-specific discrete-time hazard analysis. To resolve the daily mechanisms governing larval fates beyond aggregate survival rates, we modeled death and adult emergence as competing daily outcomes. This framework allowed us to evaluate, day by day, how temperature, thermal regime, and population origin jointly shape whether an individual is more likely to die or to emerge on a given day, and whether these drivers act differently on each outcome. Each individual faced exactly two possible fates: dying as a larva or pupa (event = 1) or successfully emerging as an adult (event = 2). On any given day that an individual remained alive and not yet emerged, it could experience either of these two outcomes. We modelled this explicitly as a competing-risks problem, where each day, the individual has a certain probability of dying and a separate probability of emerging, with the two probabilities summing to less than one (the remainder being the probability of surviving without emerging and remaining in the risk set the following day). Eventually, one of the two outcomes occurs, and at that point the individual leaves the risk set permanently.

We expanded the individual-level dataset to individual-period format (one row per individual per day at risk, up to 150 days), producing 45,556 individual-day rows, 1,519 of which ended in death and 846 in adult emergence. On each day, the daily hazard of dying was estimated in one model and the daily hazard of emerging in a separate model, with the competing event treated as censored on its event day, meaning an individual that emerges is no longer at risk of dying as a larva, and vice versa. Both daily hazards were modelled using binomial GLMMs with a complementary log-log link⁴⁹, which is the appropriate discrete-time formulation when the outcome is a binary event occurring at most once per individual⁴⁵. The two models shared the same fixed-effect structure (Eq. 2), which included the baseline hazard shape, thermal covariates, population identity, and their interactions:

$$\text{Event} \sim \log(\text{day}) + (\text{Temperature} + \text{Temperature}^2) \times \text{Thermal Regime} + \text{Population} \times (\text{Temperature} + \text{Temperature}^2) + \log(\text{day}) \times \text{Temperature} + (1 \mid \text{Replicate}) \quad (\text{Eq. 2})$$

Likelihood-ratio tests assessed the contribution of each term; hazard ratios (HR) and 95% confidence intervals were obtained by exponentiating model coefficients and their Wald intervals.

The same discrete-time hazard framework was subsequently extended to adult mortality, treating death as the sole event of interest after adult emergence. All individuals that reached adulthood ($n = 592$) eventually died during the experiment. As for juvenile mortality, daily adult mortality was modelled using a binomial GLMM with a complementary log-log link (Eq. 3). Because adult longevity was recorded only under constant-temperature conditions, thermal regime was not included as a covariate. Population and sex were retained as fixed effects based on the corresponding trait-level analyses:

$$\text{Adult death} \sim \log(\text{day}) + \text{Population} + (\text{Temperature} + \text{Temperature}^2) + \text{Sex} + \text{Population} \times \text{Temperature} + \log(\text{day}) \times \text{Temperature} + (1 \mid \text{Replicate}) \text{ (Eq. 3)}$$

Thermal performance curves and rate summation bias. Mechanistic models of vector-borne disease risk commonly parameterise the temperature dependence of mosquito life-history traits using TPCs. These mathematical functions describe how a trait varies continuously across the full biologically relevant temperature range. For development rate, the TPC framework is preferred over linear degree-day models because insect development is inherently nonlinear, as it accelerates from zero at a lower thermal limit ($T_{\min}$), reaches a maximum at an intermediate thermal optimum (T_{opt}), and collapses back to zero above an upper thermal limit ($T_{\max}$)^{1,50}. Fitting a TPC to development rate, therefore, simultaneously estimates these three biologically interpretable parameters, allows the thermal niche to be characterised on a species scale across populations and treatments, and provides the functional form required for downstream rate-summation analysis and population modelling. To this end, we fitted Brière-1 (Eq. 4) nonlinear curves to mean development rates (1/days) for each treatment.

$$r(T) = a \cdot T \cdot (T - T_{\min}) \cdot \sqrt{(T_{\max} - T)} \text{ (Eq. 4)}$$

Since only one population marginally ($n = 1$ for CT treatment; $n = 6$ for FT treatment) completed larval development at 13 °C, zero-rate anchors were added at that temperature to constrain the estimated lower thermal limit. Curves were fitted by weighted nonlinear least squares (*minpack.lm*). Uncertainty was quantified by non-parametric bootstrap (1,000 replicates, resampling individuals within each treatment $\times$ temperature cell).

TPCs are non-linear, so the rate predicted by evaluating the curve at a cycle's mean temperature is not the same as the mean of the rates actually experienced across the

temperatures within that cycle (Jensen's inequality^{21,51,52}). Near the thermal optimum, where the curve is concave (curving downward as it approaches the peak), temperature variability lowers the average rate relative to the mean-temperature prediction; near the lower thermal limit, where the curve is convex (curving upward from near zero), variability can instead raise it. To correct for this, we applied rate summation (RS), therefore, rather than evaluating the constant-temperature Brière-1 curve at a single mean temperature, we evaluated it separately at each plateau temperature of the stepped daily cycle imposed in the fluctuating chambers, then averaged the resulting rate predictions. This RS-predicted rate gives a Jensen-corrected estimate of expected performance under fluctuating conditions, which we compared against the rate actually observed under the fluctuating treatment (Eq. 5):

$$\text{Bias} = (\text{RS_predicted_rate} - \text{observed_FT_rate}) / \text{observed_FT_rate} \times 100 \text{ (Eq. 5)}$$

with bias tested against zero using one-sample t-tests at each temperature, to assess whether RS adequately predicts fluctuating-temperature performance from constant-temperature data alone, or whether additional factors beyond simple non-linear averaging are involved.

Spatial mapping of development rate estimates and constant- versus fluctuating-temperature bias. To assess how the choice of constant- versus fluctuating-temperature-fitted TPCs propagates into geographic predictions of larva-to-adult development, we mapped the Brière-1-predicted daily development rate under each parameter set across the species' current European range. Daily mean 2 m air temperature for July from 2016 to 2025 was obtained from the ERA5 single-level hourly reanalysis product (2 m temperature, parameter ID 167; 0.25° × 0.25° spatial resolution), produced by the European Centre for Medium-Range Weather Forecasts (ECMWF) and accessed via the Copernicus Climate Change Service Climate Data Store³⁶. For each grid cell and day, development rate was predicted from the daily mean temperature using the constant-fitted and fluctuating-fitted Brière-1 curves independently, with predictions set to zero outside each curve's respective $[T_{\min}, T_{\max}]$ range. Daily rate rasters were aggregated to a single July mean per cell by arithmetic averaging, consistent with the additive nature of daily development-rate accumulation. This produced two independent raster surfaces representing the constant- and fluctuating-predicted mean development rate for the month of July. To visualise where the two parameterisations diverge, we computed their percentage difference relative to the fluctuating parameters:

$$\% \text{ difference} = 100 \times (\text{constant} - \text{fluctuating}) / \text{fluctuating} \text{ (Eq. 6)}$$

at each grid cell and mapped it on a diverging colour scale centred on zero, with the display range set to the 85th percentile of absolute differences to prevent a small number of extreme cells from compressing the visual range. All spatial analyses were performed using the *terra* and *tidyterra* packages, with country boundaries from Natural Earth (*rnaturalearth*).

Population growth model. Individual life-history traits were integrated into a temperature-dependent demographic model. Temperature-dependent larval development time, $d(T)$, was estimated from the fitted Brière-1 TPC, larva-to-adult survival probability, $l_s(T)$, from a binomial generalized linear model, adult longevity from a Poisson generalized linear model, and female wing length, $WL(T)$, from a linear model. Adult mortality was calculated as the inverse of predicted longevity,

$$\mu(T) = 1 / longevity(T) \text{ (Eq. 7)}$$

Female wing length estimates were converted to per-reproductive-cycle fecundity⁴⁰ using the allometric regression of Blackmore & Lord⁵³:

$$\log_{10}(F(T)) = 0.68 + 1.35 \times \log_{10}(WL) \text{ (Eq. 8)}$$

where $F(T)$ is the expected number of eggs produced per gonotrophic cycle.

These empirical components were supplemented with the temperature-dependent egg-hatching rate $h(T)$, obtained from the experimental work of Marini et al.⁵⁴. The duration of the gonotrophic cycle was assumed to vary with temperature as:

$$GC(T) = \max(2, 15 - 0.3 \times T) \text{ (Eq. 9)}$$

producing the expected number of reproductive cycles during an adult female's lifetime,

$$n_{cycles}(T) = longevity(T) / GC(T) \text{ (Eq. 10)}$$

The net reproductive rate was then calculated as:

$$R_0(T) = h(T) \times l_s(T) \times 0.5 \times F(T) \times n_{cycles}(T) \text{ (Eq. 11)}$$

where the factor 0.5 is the sex ratio. Thus, $R_0(T)$ represents the expected number of female offspring produced per female over her lifetime: when $R_0 > 1$, each female contributes more than one female to the next generation and the population grows, whereas $R_0 < 1$ indicates demographic decline.

464

465 This integrative approach was applied to constant-temperature data only, as adult
 466 longevity was not measured under the fluctuating regime, precluding estimation of $\mu(T)$ for
 467 that treatment. Uncertainty was propagated through a non-parametric bootstrap (500
 468 replicates) in which individuals were resampled within each temperature cell and all four
 469 trait models were simultaneously refitted; 95% confidence intervals correspond to the
 470 2.5th and 97.5th percentiles of the resulting distributions.

471 3. Results

472 Across the three *Ae. albopictus* populations, thermal conditions emerged as the main
 473 determinant of life-history performance. Mean rearing temperature produced significant
 474 nonlinear effects on larva-to-adult survival and development time, and thermal regime
 475 strongly affected performance across all traits (Table 1, SM 2.1, SM 2.3, and SM 2.5).
 476 Relative to constant temperatures, fluctuating temperatures reduced larva-to-adult
 477 survival, prolonged development, and resulted in smaller adult wing length.
 478 Population identity did not improve models fit and was excluded during model selection
 479 ($\Delta AIC < 2$), therefore it was not included in the final models for larva-to-adult survival
 480 (Table SM 2.1), development time (Table SM 2.3), and wing length (Table SM 2.5). The
 481 following results are therefore reported pooled across the three populations, unless
 482 otherwise stated. Population identity was, however, retained in the model of adult
 483 longevity, and population-level patterns in adult mortality and at the thermal extremes are
 484 presented separately in section 3.2.

485 3.1 Juvenile thermal performance is largely conserved across 486 populations but strongly affected by thermal regime

487 A total of 2,252 individuals with complete terminal event records were included in the
 488 survival analysis (846 reaching adulthood, 1,401 dying as larvae or pupae). Larva-to-
 489 adult survival was modelled with a binomial GLMM (Table SM 2.1; Figure 2A), with the
 490 proportion of individuals reaching adulthood as the response and replicate batch as a
 491 random intercept

492

$$493 \quad Hatched / Total \sim (Temperature + Temperature^2) \times Thermal Regime + (1 | Replicate)$$

494

495 Fluctuating temperatures both reduced overall larval-to-adult survival and narrowed the
 496 thermal breadth over which survival was possible. The thermal optimum for survival was
 497 estimated at approximately 30°C under the constant regime and approximately 24°C
 498 under the fluctuating regime, indicating a downward and inward shift of the survival curve
 499 under temperature variability (Table SM 2.2).

500

44

45

Among the individuals that survived to adulthood, thermal regime also affected how long development took. Of the 846 individuals reaching adulthood, larva-to-adult development time ranged from 7 to 111 days across all temperature and treatment combinations (Figure SM 3.1). A Poisson GLMM with a log link (Table SM 2.3, Figure 2B) was fitted with mean temperature, its quadratic term, thermal regime, and their interactions as fixed effects, and replicate batch as a random intercept:

$$\text{Development Time} \sim (\text{Temperature} + \text{Temperature}^2) \times \text{Thermal Regime} + (1 \mid \text{Replicate})$$

Mean temperature had a strong nonlinear effect on larva-to-adult development time, with both the linear and quadratic components of the orthogonal polynomial being highly significant (both $p < 0.001$). The temperature regime also had a strong effect: the estimated coefficient for the fluctuating-temperature regime was 0.504 on the log scale, corresponding to a 1.66-fold increase in expected development time relative to the constant-temperature regime. At the grand-mean rearing temperature of 23°C, model-based predictions indicated mean development times of 11.8 days under constant temperatures and 20.2 days under fluctuating temperatures, a 8.4-day (70.8%) increase. Neither the linear nor quadratic temperature $\times$ regime interaction was significant ($p = 0.102$ and $p = 0.738$, respectively), indicating that the effect of variability did not change systematically across the tested temperature gradient.

The thermal regime also shaped the size of the adults that did emerge. Wing length was analysed for $n = 278$ adults using a Gaussian linear mixed model with sex $\times$ temperature and sex $\times$ regime terms and replicate batch as a random intercept:

$$\text{Wing Length} \sim \text{Sex} \times \text{Temperature} + \text{Sex} \times \text{Thermal Regime} + (1 \mid \text{Replicate})$$

Wing length decreased significantly with increasing mean temperature, with the magnitude of the decline differing between sexes (Table SM 2.5, Figure 2C). For females, wing length decreased by an estimated 0.035 mm per 1°C increase in temperature ($p < 0.001$), whereas the corresponding decline in males was 0.019 mm per 1°C ($-0.035 + 0.016$), indicating a significantly shallower thermal decline in males (sex $\times$ temperature: $p < 0.001$). Females were significantly larger than males, with a 0.55 mm difference at the 23°C. The thermal regime also had a strong effect: relative to constant temperatures, fluctuating temperatures reduced predicted wing length by 0.492 mm in females ($p < 0.001$). This reduction was significantly smaller in males, at approximately 0.344 mm ($-0.492 + 0.148$), as indicated by the significant sex $\times$ treatment interaction ($p < 0.001$). Predicted female wing lengths derived from the model were used to estimate per-reproductive-cycle fecundity via the allometric

regression of Blackmore and Lord⁵³, and are reported in the population growth model section below.

The trait-level models above quantify how performance differs between the two thermal regimes at each tested temperature. To characterise the continuous thermal response and to test whether constant-temperature data can predict fluctuating-temperature performance, we fitted Brière-1 TPCs and applied rate summation. Brière-1 TPCs fitted to constant-temperature mean development rates (Figure 2D) estimated the lower thermal limit at 8.8°C, the thermal optimum at 31.2°C, and the upper thermal limit at 37.7°C. Rate summation (RS) predictions, derived by applying this constant-temperature curve to the stepped fluctuating daily cycle and averaging by phase duration, systematically exceeded the observed fluctuating-temperature development rates and differed from the empirical fluctuating-temperature curve in the location of the thermal limits. Within the imposed 5-40°C evaluation range, the RS curve remained positive from the lower boundary of 5.0°C, approximately 5.8°C below the empirically fitted fluctuating-temperature limit of 10.8°C, to the upper boundary of 40.0°C, approximately 1.7°C above the empirical limit of 38.3°C. A discontinuity in the RS-predicted curve near 33°C arises from the stepped structure of the daily cycle, and it corresponds to the mean temperature at which one plateau phase crosses a thermal limit of the constant-temperature curve, abruptly removing one third of the day's contribution to the daily mean rate.

One-sample t-tests against a null of zero bias indicated significant overestimation of observed fluctuating-temperature rates at 23°C (+60.3%, $p < 0.05$) and 28°C (+41.9%, $p < 0.05$). At 33°C, the bias declined to a small, non-significant value (-2.6%). The result at 13°C ($n = 7$ Trento survivors only) could not be reliably quantified, as the empirical fluctuating-temperature mean is dominated by sampling noise, and is therefore excluded from the quantitative bias summary.

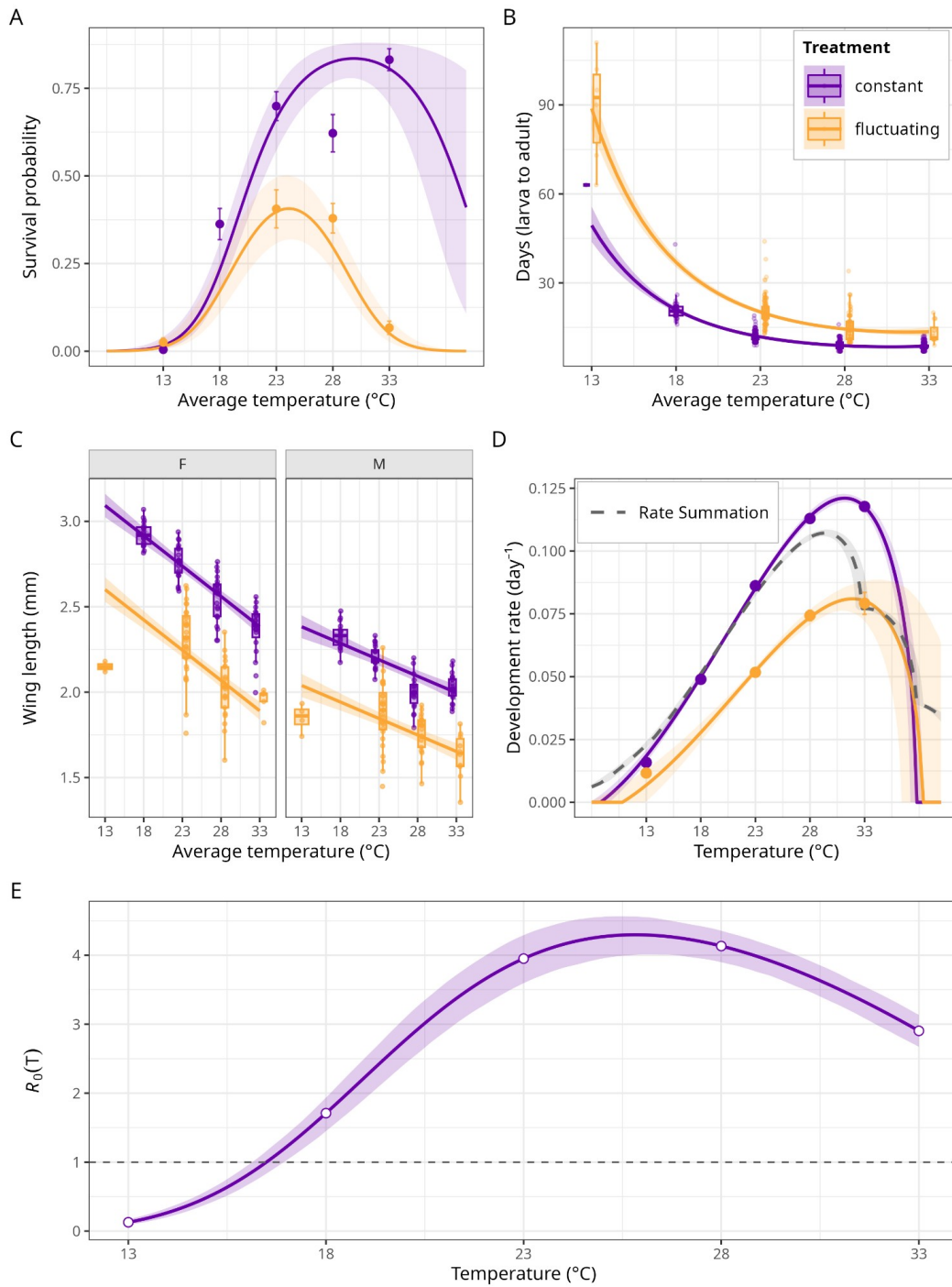
Integrating these trait functions gives a single demographic summary of thermal performance. Given the absence of population-level differences in survival, development time and wing length, the temperature-dependent trait functions were integrated into a single population growth framework pooled across the three populations. The net reproductive rate $R_0(T)$ (Figure 2E) was unimodal, exceeding the replacement threshold ($R_0 = 1$) between 16.5°C and 37.6°C and peaking at 25.8°C with $R_0 = 4.3$. At the five tested temperatures R_0 was 0.128 (13°C), 1.71 (18°C), 3.95 (23°C), 4.13 (28°C) and 2.90 (33°C), so only 13°C fell below replacement. Below 16.5°C, low larval survival and prolonged development jointly suppressed output to sub-replacement levels; above 37.6°C, accelerated adult mortality, reduced larval survival and smaller female body size drove R_0 below replacement.

579

580 The survival and development-time models above treat each outcome in isolation;
581 survival counts only whether an individual reached adulthood, and development time is
582 calculated only for those that did. To separate these processes, we additionally modelled
583 larval/pupal death and adult emergence as competing daily outcomes in a discrete-time
584 hazard framework (see Methods section). For the daily probability of emerging as an adult

(Figure 3A), population identity did not significantly improve model fit (LRT $\chi^2 = 3.53$, $df = 6$, $p = 0.740$) and was excluded from the final model, while the non-proportional hazards term $\log(\text{day}) \times \text{temperature}$ was retained (HR = 1.11, 95% CI 1.06–1.16, $p < 0.001$; Table SM 2.7), indicating that temperature's effect on the daily emergence probability intensified as individuals aged. Mean temperature strongly raised the daily probability of emerging (HR = 5.52, 95% CI 3.52–8.66, $p < 0.001$), with a significant negative quadratic term (HR = 0.969, 95% CI 0.961–0.976, $p < 0.001$). The thermal regime main effect was non-significant in isolation, but combined with the regime $\times$ temperature interaction, fluctuating temperatures produced a net strong suppression of the daily emergence probability across all tested mean temperatures. None of the thermal regime terms reached significance individually (main effect $p = 0.762$; linear interaction $p = 0.957$; quadratic interaction $p = 0.403$), but their combined predicted effect was a net suppression of the daily emergence probability under fluctuating conditions across all tested mean temperatures (Figure 3A).

In the companion model for larval and pupal death, thermal regime acted in the opposite direction. Fluctuating temperatures altered the daily hazard of larval/pupal death (Figure 3B, Table SM 2.6) in a temperature-dependent manner, reflected in significant linear (HR = 0.729, 95% CI: 0.595–0.894, $p = 0.002$) and quadratic (HR = 1.01, 95% CI: 1.00–1.01, $p < 0.001$) interactions between thermal regime and mean temperature. Together these terms indicate that fluctuating temperatures elevated mortality risk most strongly at cool mean temperatures, with this excess narrowing progressively as mean temperature increased. Temperature's effect on the daily hazard of death also intensified as individuals aged, reflected in a significant non-proportional hazards term ($\log(\text{day}) \times \text{temperature}$; HR = 1.01, 95% CI 1.00–1.03, $p = 0.031$), mirroring the pattern in the emergence model. Taken together with the emergence results above, fluctuating temperatures simultaneously increased the daily probability of larval/pupal death and decreased the daily probability of successful adult emergence, so that thermal variability acted on both competing outcomes in a mutually reinforcing direction.



613 **Figure 2** Observed and modelled temperature-dependent life-history traits of *Aedes albopictus*
 614 under constant and fluctuating thermal regimes. Pooled observations from all three populations
 615 are depicted. (A) Larva-to-adult survival probability across average temperatures. (B) Larva-to-
 616 adult development time (days) as a function of average temperature. (C) Adult wing length (mm)
 617 across temperatures for females (F) and males (M). (D) Constant-temperature and fluctuating-

618 temperature thermal performance curves (TPC) and rate-summation (RS) prediction. (E) Net
 619 reproductive rate $R_0(T)$ as a function of temperature.

620 3.2 Population-level differences are concentrated in adult traits and 621 thermal extremes

622 While population origin did not predict any of the pooled juvenile traits described above,
 623 nor the cause-specific hazards of larval/pupal death (LRT $\chi^2 = 8.84$, $df = 6$, $p = 0.183$) or
 624 adult emergence (LRT $\chi^2 = 3.53$, $df = 6$, $p = 0.740$), it was retained in the model of adult
 625 longevity and emerged in the analysis of full-lifespan adult mortality. Population-level
 626 structure in this system is therefore confined to the adult stage.

627

628 In the adult mortality hazard model (Table SM 2.8, Figure 3C), Palermo adults had
 629 a significantly lower daily mortality hazard than Frankfurt ($\beta = -1.515$, $p = 0.035$;
 630 HR = 0.22), whereas Trento did not differ significantly ($\beta = -0.425$, $p = 0.555$). Males
 631 had more than twice the daily mortality hazard of females ($\beta = 0.764$; HR = 2.15).
 632 Temperature had a strong nonlinear effect, with mortality increasing but
 633 decelerating at higher temperatures, and this effect varied with age ($\log(\text{day}) \times$
 634 temperature, $p = 0.025$). Population $\times$ temperature interactions were not significant
 635 (Trento $p = 0.801$; Palermo $p = 0.125$). Pairwise comparisons at each tested
 636 temperature localised the effect: Frankfurt and Palermo differed significantly at
 637 13°C ($p = 0.028$), 18°C ($p = 0.010$), 23°C ($p = 0.002$) and 28°C ($p = 0.031$), but not at
 638 33°C ($p = 0.713$); Frankfurt and Trento did not differ significantly at any
 639 temperature.

640

641 Modelling longevity directly, rather than as a daily hazard, resolved a population signal
 642 that the hazard model did not detect. Adult longevity was analysed for individuals reared
 643 under CT conditions only ($n = 592$). The selected model was a Poisson GLMM including
 644 population, average temperature, sex, the population $\times$ temperature interaction, and
 645 replicate batch as a random intercept (Table 1, Figure 3D):

646

647 *AdultLongevity ~ Population $\times$ Temperature + Sex + (1 | Replicate)*

648

649 In the Frankfurt reference population, each 1°C increase in temperature was
 650 associated with a 5.1% reduction in expected longevity ($\beta = -0.052$). This
 651 temperature dependence was significantly stronger in Trento, as indicated by the
 652 negative population $\times$ temperature interaction ($\beta = -0.023$, $p = 0.006$), therefore the
 653 estimated temperature slope for Trento was -0.075 on the log scale, corresponding

62

63

to an approximately 7.2% reduction in expected longevity per 1°C increase. Trento therefore exhibited greater expected longevity than Frankfurt at cooler temperatures, but this difference progressively diminished with increasing temperature because of the steeper decline in Trento. Palermo did not differ from Frankfurt neither in its main effect ($p = 0.126$) nor in its interaction with temperature ($p = 0.435$). Further, males had significantly lower expected longevity than females, by approximately 7.7% ($\beta = -0.080$, $p = 0.015$).

Consistent with the temperature-dependence of these model-based population effects, non-parametric survival analysis localised population differences to the ends of the tested thermal range. Kaplan–Meier survival analyses (Figure SM 3.2) showed population-specific differences in survival only at the thermal extremes. At 13°C, the Trento population exhibited significantly higher survival than the Frankfurt population, while the Palermo population did not differ significantly from either population. At 28°C, survival was highest in the Palermo population, intermediate in the Trento population, and lowest in the Frankfurt population. In contrast, no significant differences among populations were detected at 33°C.

The clearest single instance of population divergence occurred at the lower thermal limit. No larvae completed development at 13°C in the Frankfurt or Palermo populations (Figure 2A; Table SM 2.1): only Trento recorded adult emergence at 13°C ($n = 7$ of 155 individuals; one adult emerged under the constant temperature regime and six under the fluctuating regime), whereas no individuals from Frankfurt or Palermo reached adulthood at this temperature.

Table 1. Results of a Poisson GLMM with a log-link function testing the effects of population origin (city), average temperature (avg_T), their interaction (city $\times$ avg_T), and sex on adult longevity ($n = 592$ individuals). Replicate batch (replic_uid) was included as a random intercept (variance = 0.0073, SD = 0.085). Parameter estimates are reported on the log scale, together with standard errors (SE), z-values, and P-values. Model fit statistics were AIC = 2782.1, BIC = 2817.2, and log-likelihood = -1383.0.

Term	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	3.195	0.149	21.401	<0.001
Trento	0.628	0.214	2.937	0.003
Palermo	0.332	0.217	1.528	0.126
avg_T	-0.052	0.006	-8.965	<0.001
sexM	-0.080	0.033	-2.423	0.015

Term	Estimate	Std. Error	z value	Pr(> z)
Trento:avg_T	-0.023	0.008	-2.735	0.006
Palermo:avg_T	-0.007	0.008	-0.780	0.435

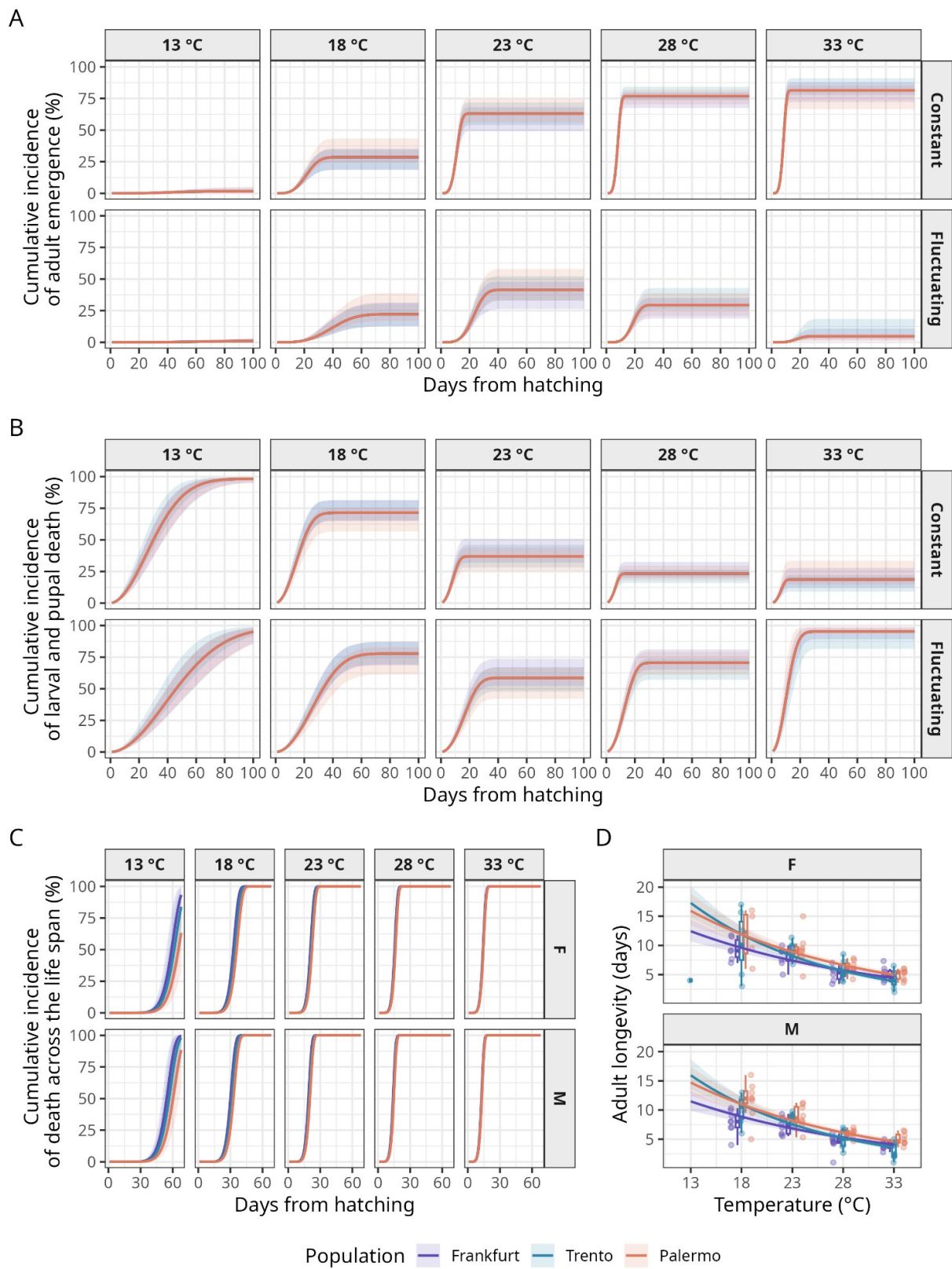


Figure 3 Cause-specific cumulative incidence of adult emergence and larval/pupal death, and full life-span mortality and longevity, across temperatures and populations. Predicted cause-specific cumulative incidence curves from discrete-time hazard GLMMs (binomial family, complementary log-log link) fitted separately to adult emergence (A) and larval/pupal death (B), under both constant and fluctuating thermal regimes, and to full life-span mortality (C), under constant temperature only. Sex could not be assigned during the immature stages captured in A and B, so those panels are pooled across sexes; C and D are faceted by sex instead. Each panel shows the predicted probability of the event having occurred by day t from hatching, for one population at one mean temperature. Cumulative incidence functions were computed as $CI(t) = \sum h(s) \cdot S(s-1)$, where $h(s)$ is the cause-specific daily hazard and $S(s-1)$ is the probability of having experienced neither event by the start of day s . The final plateau of each curve represents the total proportion of individuals expected to experience that event under the given conditions. (D) Adult longevity as a function of mean temperature under constant temperature, by population and sex, from a Poisson GLMM; points show mean longevity per replicate vial, boxplots summarise the distribution at each temperature, and lines with shaded ribbons show model-predicted longevity with 95% confidence intervals.

3.3 Bias from constant-temperature parameterisation is amplified in spatial predictions

Whether the constant-temperature bias documented above is a laboratory curiosity or a practically consequential source of error depends on how it propagates when TPCs are extrapolated to real-world temperature data at a continental scale. Mapping the average 2016-2025 Brière-1-predicted larva-to-adult development rate for the month of July across Europe under CT and FT parameters (Figure 4) revealed a consistent directional bias across the mapped domain: the CT-fitted curve predicted higher daily development rates than the FT-fitted curve everywhere in the climatically suitable range, corresponding to systematically shorter predicted development times under the CT parameterisation. While the difference between the two treatments was relatively stable across most of continental Europe (~25-50%), the largest gap is visible toward the cooler margins of the continental thermal gradient, specifically in the Alpine and Arctic areas. Because development rate is clamped to zero outside each curve's fitted thermal limits, and the CT-fitted lower limit ($T_{\min} = 8.79^{\circ}\text{C}$) was lower than the FT-fitted limit ($T_{\min} = 10.8^{\circ}\text{C}$), the CT parameterisation also predicted non-zero development across a geographically larger area, extending the apparent northern and altitudinal boundary of viable development. Using CT-derived thermal parameters therefore does not just overestimate development rate within the region where both parameterisations predict viable development; it also overestimates the total geographic extent over which development is predicted to occur at all.

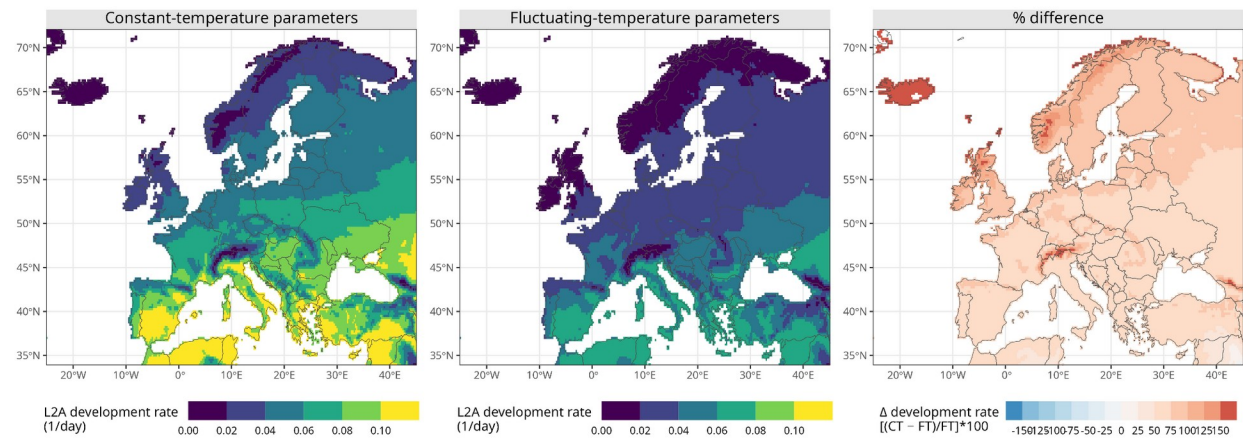


Figure 4 Spatial variation in the predicted *Aedes albopictus* larva-to-adult development rate across Europe in July from 2016 to 2025 under parameterisations derived from constant-temperature (CT) and fluctuating-temperature (FT) laboratory experiments. Left and middle panels show the monthly mean development rate predicted using the CT and FT thermal performance curves, respectively. The right panel shows the relative difference between parameterisations, expressed as $[(CT-FT)/FT]\times100$. Positive values indicate higher development rates predicted by the CT parameterisation, whereas negative values indicate higher rates under the FT parameterisation. The color scale for the difference map was truncated at the 85th percentile of the absolute percentage differences to improve visualisation of spatial patterns; values beyond these limits are saturated.

4. Discussion

Our results show that temperature-dependent life-history traits of *Ae. albopictus* in the European invasive range are primarily determined by mean temperature and by whether laboratory temperatures conditions are held constant or diurnally fluctuate. Fluctuations of $\pm 5^{\circ}\text{C}$ reduced survival and shifted its optimum by almost 6°C (Table SM 2.2), prolonged development by 71% (Table SM 2.3) and raised the daily hazard of larval and pupal death (Table SM 2.6). Constant-temperature parameters, when projected across Europe, inflated both predicted development rates and the extent of viable habitat. Population origin, by contrast, explained no detectable variation in any juvenile trait, and population differences emerged only in the adult stage.

Where population differences did emerge they were confined to the thermal extreme and the adult life stage: Palermo adults sustained a lower mortality hazard than Frankfurt across $13\text{--}28^{\circ}\text{C}$, with the difference disappearing at 33°C , and Trento adults were longer-lived than Frankfurt at cool temperatures. The RS bias, by contrast, was uniform across populations, identifying it as a species-level property of the TPC. When applied to ERA5 temperature data across Europe, the CT-derived Brière-1 parameters predicted higher larval development rates than the FT-derived parameters throughout the climatically suitable range, and additionally extended the predicted northern and altitudinal boundary of viable development beyond what the FT parameterisation supports, suggesting that current distribution models relying on CT parameterisation overestimate both the development rate and the poleward extent of suitable habitat.

While temperate and tropical *Ae. albopictus* populations have been shown to differ in thermal performance¹⁸, previous work reported phenotypic and genomic differentiation along a broad latitudinal gradient in the species' native range, with phenotypic divergence being most evident at thermal extremes, including cold tolerance, diapause incidence and wing-size clines⁵³. Our results are broadly consistent with this pattern: divergence across the European invasive range was limited overall, and the few detectable differences clustered near the thermal limits, suggesting that population differences might become apparent only at the edges of thermal performance, although some differences were evident at 28°C but not at 33°C , possibly because severe thermal stress overrides population-level variation⁵⁵. Whether comparable differences arise among populations within the same climatic zone along continuous environmental gradients is less clear¹⁸, and the available evidence points in different directions. In the mosquito *Culex pipiens*, substantial among-population variation was found in thermal response curves of larval survival, adult survival and development rate varied by up to 45, 79 and 84% respectively across four populations, yet this variation was not correlated with local temperatures and therefore did not support local thermal adaptation⁵⁶. Invasive North American *Ae. albopictus* populations show minimal divergence in non-diapause larval growth traits despite clear divergence in diapause-associated cold tolerance^{57,58}, and in *Ae. sierrensis* the upper thermal limits varied by less than 3°C across a 1,200 km latitudinal gradient, with phenotypic differences attributed to plasticity rather than genetic variation^{59,60}. Kramer et al.²⁰ similarly found full life-cycle performance across a Nepalese altitudinal gradient to be largely uniform at $18\text{--}28^{\circ}\text{C}$ in both *Ae. aegypti* and *Ae. albopictus*,

776 with divergence confined to cold tolerance after sub-zero exposure. This is consistent
 777 with the characterisation of *Ae. albopictus* as a species whose invasive success depends
 778 critically on phenotypic plasticity^{19,37,61}.

779 At the same time, even relatively small population-level differences may have important
 780 biological consequences, as Dennington et al.^{62,63} showed that apparently modest
 781 horizontal shifts in *Ae. aegypti* TPCs can produce disproportionately large fitness
 782 differences at the thermal extremes. In the European *Ae. albopictus* populations
 783 examined here, population-level variation is detectable in subsets of analyses and at
 784 particular temperatures, but is consistently overshadowed in magnitude and generality by
 785 mean temperature and thermal variability, and may reflect genetic divergence, residual
 786 field carry-over, or stochastic developmental variation⁵⁵.

787 These limited phenotypic differences do not exclude adaptive differentiation among
 788 European *Ae. albopictus* populations, particularly because genomic differentiation has
 789 already been documented across the invasive range. Multiple independent introductions
 790 and subsequent admixture across the European range^{13–15} provide standing genetic
 791 variation, but the short time since establishment, under three decades in most areas north
 792 of the Alps^{14,20}, constrains the extent of adaptive differentiation on active-season
 793 performance⁶⁴. Adaptation could be instead concentrated on a distinct axis: temperate
 794 invasive populations have evolved enhanced cold tolerance of diapausing eggs relative
 795 to tropical populations, partly through epigenetic mechanisms^{37,65}, and North American
 796 populations show rapid divergence in diapause incidence and duration while non-
 797 diapause larval growth traits remain uniform⁶⁶. Diapause is a threshold trait under acute
 798 directional winter selection, and its divergence is among the clearest documented cases
 799 of rapid evolution in an invasive insect²⁰.

800 Fluctuating temperatures consistently reduced both larval survival and development rate
 801 relative to constant temperatures with equivalent mean, uniformly across all three
 802 populations. This result is consistent with the predictions of Jensen's inequality when
 803 mean temperatures fall in the concave, decelerating phase of the TPC above the thermal
 804 optimum: temperature variance reduces mean performance because the cost of
 805 supraoptimal excursions outweighs the benefit of equivalent suboptimal excursions given
 806 the asymmetry of the Brière curve²¹ (Figure 2D). Models of *Ae. albopictus* population
 807 dynamics parameterised from constant temperature experiments are therefore expected
 808 to overestimate larval development rate systematically under field conditions. Across the
 809 European active season temperature range of 18 to 33°C, we found this overestimation
 810 to be substantial and temperature-dependent: RS overestimated observed FT-rates by
 811 approximately 60% at 23°C and 42% at 28°C, with the bias declining to non-significant
 812 levels at 33°C as the Brière curve approaches its near-linear upper portion. Crucially, this
 813 bias is a species-level property rather than a population-specific one, which simplifies the
 814 modelling problem: a single set of fluctuating-temperature Brière parameters might be
 815 sufficient to represent the species across the European invasion gradient, though the
 816 magnitude of residual bias will depend on how closely the experimental fluctuation
 817 amplitude matches the specific field conditions being modelled. The same pattern holds
 818 in the mosquito *Culex pipiens*⁶⁷ and fruit fly *Drosophila suzukii*²³, where constant-
 819 temperature TPCs also overestimated development and viability under fluctuating

820 conditions, most strongly near the thermal optimum. Its consistency across taxonomically
821 distant ectotherms indicates a general property of non-linear TPCs.

822 Beyond the magnitude of the CT-to-FT bias documented above, the rate-summation
823 analysis also failed qualitatively. Applied to the constant-temperature curve, it placed the
824 thermal limits at 3.8°C and 42.7°C, whereas the curve fitted directly to fluctuating-
825 temperature data bounded development between 10.8°C and 38.3°C. This widening is
826 inherent to applying RS to any curve truncated at zero: as soon as one phase of the daily
827 cycle enters the positive range, the daily average rises above zero, producing a tail where
828 there should be a limit. The same artefact was recently described for malaria transmission
829 in *Anopheles stephensi*, where constant-temperature models predicted thermal limits
830 more accurately than RS despite both failing to capture the full effect of variability⁶⁹. The
831 RS curve (Figure 2D) shows a discontinuity around 33°C corresponding to the
832 temperature at which the warm phase of the fluctuating cycle crosses the CT-derived
833 temperature maximum. This is a geometric consequence of the stepped daily cycle used
834 in the experiment and shows how RS fails near thermal limits: at a mean of 33°C larvae
835 spend a full 8 h at 38°C, above the larval thermal limit, and presumably accumulate
836 damage during that phase⁵⁵.

837 The antagonistic effects we documented, fluctuating temperatures simultaneously raising
838 the daily hazard of larval death (Figure 3B) and suppressing adult emergence (Figure
839 3A), echo the stage-dependent trade-offs reported for the mosquito *Anopheles coluzzii*⁶⁸,
840 as it was found that the direction of the fluctuation effect reversed between larvae and
841 adults, underscoring that single-stage thermal performance summaries can be
842 misleading about whole-organism fitness under variable conditions. Jacob et al.⁶⁴ likewise
843 found that constant-temperature models poorly captured both the magnitude and the
844 direction of variability effects across malaria and dengue vectors.

845 Our results extend this conclusion to an arboviral vector and to a life-history endpoint,
846 reinforcing that empirical measurement under fluctuating regimes, not non-linear
847 averaging of constant-temperature data, is required where thermal limits matter.
848 Hagstrum & Milliken⁷⁰ flagged the same RS overestimation for stored-product insects
849 three decades ago; the present study confirms that it persists in a disease vector system,
850 at a magnitude relevant to macroecological modelling. Overall, these results suggest that
851 the choice of thermal regime in laboratory parameterisation is a systematic source of bias
852 with measurable geographic consequences.

853 Limitations

854 Experimental protocols were harmonised across institutions where local constraints
855 allowed, but procedural differences remained: Italian populations were sampled three
856 times across the summer against a single sampling event for the German population, the
857 experiments carried over in Italy required sequential evaluation in two climatic chambers
858 whereas the German facility ran five temperatures simultaneously, and blood-feeding
859 sources differed across institutions, though since fecundity and egg viability were not
860 directly measured here, this difference is not expected to affect the trait comparisons
861 reported. All sampling nonetheless fell within the same photoperiodic window, after the

summer solstice and before the critical photoperiod for diapause induction^{37,71}, and the core findings were consistent across populations. Our fluctuating regime used a single amplitude ($\pm 5^{\circ}\text{C}$) and stepped profile, providing a controlled test of how diurnal variability alters thermal performance. Because the constant-to-fluctuating discrepancy depends on both, extrapolation to other field regimes nevertheless requires caution. Only a single laboratory generation (F1) was assessed, which limits rapid laboratory adaptation and preserves field-derived phenotypes but cannot exclude maternal or other transgenerational effects; multi-generation common-garden rearing would help determine whether the observed lack of population-level differences persists after such carry-over effects are reduced^{19,72}. Although our study focused only on three source populations spanning a broad latitudinal gradient, the limited differentiation observed here is consistent with studies that sampled substantially more populations^{59,62,73}.

5. Conclusion

Across a 1,200 km European climatic gradient, invasive *Ae. albopictus* populations share a common juvenile thermal phenotype. Population-level differences were limited and concentrated near thermal extremes, with survival at 28°C following Palermo > Trento > Frankfurt and adult emergence at 13°C occurring only in Trento. The thermal regime under which larvae are reared, by contrast, has large and consistent effects: fluctuating temperatures reduce survival, prolong development, depress adult body size, and simultaneously elevate pre-adult mortality while suppressing emergence. Constant-temperature thermal performance curves systematically overestimate fluctuating-temperature development and misplace thermal limits, and the standard rate-summation correction does not recover empirical performance, with substantial consequences for spatial predictions of *Ae. albopictus* across Europe. For population dynamics models of *Ae. albopictus* in Europe, these results suggest that population-specific parameterisation is largely unnecessary, whereas incorporating realistic thermal variability is essential. Accurate forecasting of this vector's establishment, spread and pathogen transmission potential under current and future climates may depend less on resolving fine-scale population structure, especially for larval thermal performance, than on measuring performance under the variable thermal conditions organisms actually experience in the field, while population-specific differences in egg^{37,65,66,73} and adult traits^{18,62,63} and near thermal extremes remain important to consider.

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 901 Commission.

902 Declaration of generative AI use

903 During the preparation of this work, D.D.R. used Claude Sonnet 5.0 to check the grammatical
 904 consistency and flow of the text of the first draft, and M.B. used Claude Sonnet 4.6 to assist with
 905 restructuring and standardising analysis scripts for reproducibility. After using these tools, all
 906 authors reviewed and edited the content as needed and take full responsibility for the content of
 907 the published article.

908 Data availability

909 All the R scripts in which the analyses were performed are available at
 910 https://github.com/margoblaha/aedes_thermal_performance_modeling, as well as permanently
 911 archived, together with the original experimental observations, in Zenodo upon acceptance for
 912 publication.

914 CRediT author statement

915 *Conceptualisation*: D.D.R. and R.R. conceived the initial idea for the study, which was further
 916 conceptualized and developed jointly with G.M. and I.M.K.

917 *Methodology*: D.D.R., D.A., F.R., G.M., and I.M.K. jointly discussed, refined, and agreed upon
 918 the experimental protocol.

919 *Investigation*:

- 920 - Sampling in Trento: D.D.R., D.A., C.D.S.
- 921 - Sampling in Palermo: M.L.D.P, F.L.R.
- 922 - Sampling in Frankfurt: A.J.

923 *Lab work*:

- 924 - D.D.R., D.A., C.D.S., M.B., G.M. performed fluctuating temperature experiments and
 925 measurements
- 926 - A.G.M-S., Y.E.G., M.M., I.M.K, performed constant temperature experiments and
 927 measurements

928 *Formal analysis*: D.D.R. and M.B. performed the data analyses, with substantial input from
 929 I.M.K., G.M., and R.R. regarding the analytical approach and interpretation of the results.

930 *Writing – original draft*: D.D.R. and M.B. led the writing of the first manuscript draft.

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Supplementary Materials of: Diurnal temperature fluctuations outweigh population origin in shaping thermal performance and range predictions in European *Aedes albopictus*

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Supplementary Materials 1

Additional methodological details

SM 1.1 Climatic characterisation of the three populations

Long-term climate at each origin was characterised using monthly mean air temperature and total precipitation from the ERA5 reanalysis dataset produced by the European Centre for Medium-Range Weather Forecasts. Data were obtained from the ERA5 single-level monthly means product at a spatial resolution of approximately 0.25° (~28 km) for the period 1995–2025. Monthly values were extracted from the grid cell nearest to each sampling location (Frankfurt: 50.110°N, 8.682°E; Trento: 46.067°N, 11.121°E; Palermo: 38.115°N, 13.361°E).

Monthly mean 2-m air temperature was converted from Kelvin to degrees Celsius. Monthly precipitation totals were derived from ERA5 total precipitation fields and expressed in millimetres. Annual climate summaries were calculated for each year by averaging monthly temperatures and summing monthly precipitation totals. To quantify interannual climatic variability, the coefficient of variation (CV) was calculated across annual values for both mean temperature and total precipitation over the 1995–2025 period. These metrics were used to compare the long-term climatic conditions and variability experienced by the three source populations (Figure 1 in the main text).

SM 1.2 Experimental thermal regimes

Temperatures were monitored with loggers (HOBO Logger MX2301 Bourne USA) at 15 minutes intervals and the experimental temperatures deviated by maximum $\pm 0.71^{\circ}\text{C}$ from target temperatures in all treatments. The experiments performed under FT were instead executed using two climatic chambers (PROCLIMATIC Model Lt 900 ACLLI 4000). Temperature was monitored through both the integrated control system of the chamber, which guarantees a temperature deviation within $\pm 1^{\circ}\text{C}$, and was additionally monitored with an iButton Hygrochron Temperature/Humidity Logger (model DS1923) measuring a deviation within $\pm 0.45^{\circ}\text{C}$ in all treatments.

Table SM1.2. Climatic chambers used for constant-temperature (CT) and fluctuating-temperature (FT) life-cycle experiments, by institution.

Target T (°C)	Regime	Institution	Population	Chamber (model, manufacturer)
13	CT	University of Frankfurt	Frankfurt, Trento, Palermo	Reptile Egg Incubator, 25 L, VEVOR
13	FT	FEM	Frankfurt, Trento, Palermo	PROCLIMATIC LT 900 ACLLI 4000
18	CT	University of Frankfurt	Frankfurt, Trento, Palermo	FKU 1800, Liebherr (Bulle, Switzerland)

23	CT	University of Frankfurt	Frankfurt, Trento, Palermo	Climate chamber
23	FT	FEM	Frankfurt, Trento, Palermo	PROCLIMATIC LT 900 ACLLI 4000
28	CT	University of Frankfurt	Frankfurt, Trento, Palermo	FKU 1800, Liebherr (Bulle, Switzerland)
28	FT	FEM	Frankfurt, Trento, Palermo	PROCLIMATIC LT 900 ACLLI 4000
33	CT	University of Frankfurt	Frankfurt, Trento, Palermo	FK 3640, Liebherr (Bulle, Switzerland)
33	FT	FEM	Frankfurt, Trento, Palermo	PROCLIMATIC LT 900 ACLLI 4000

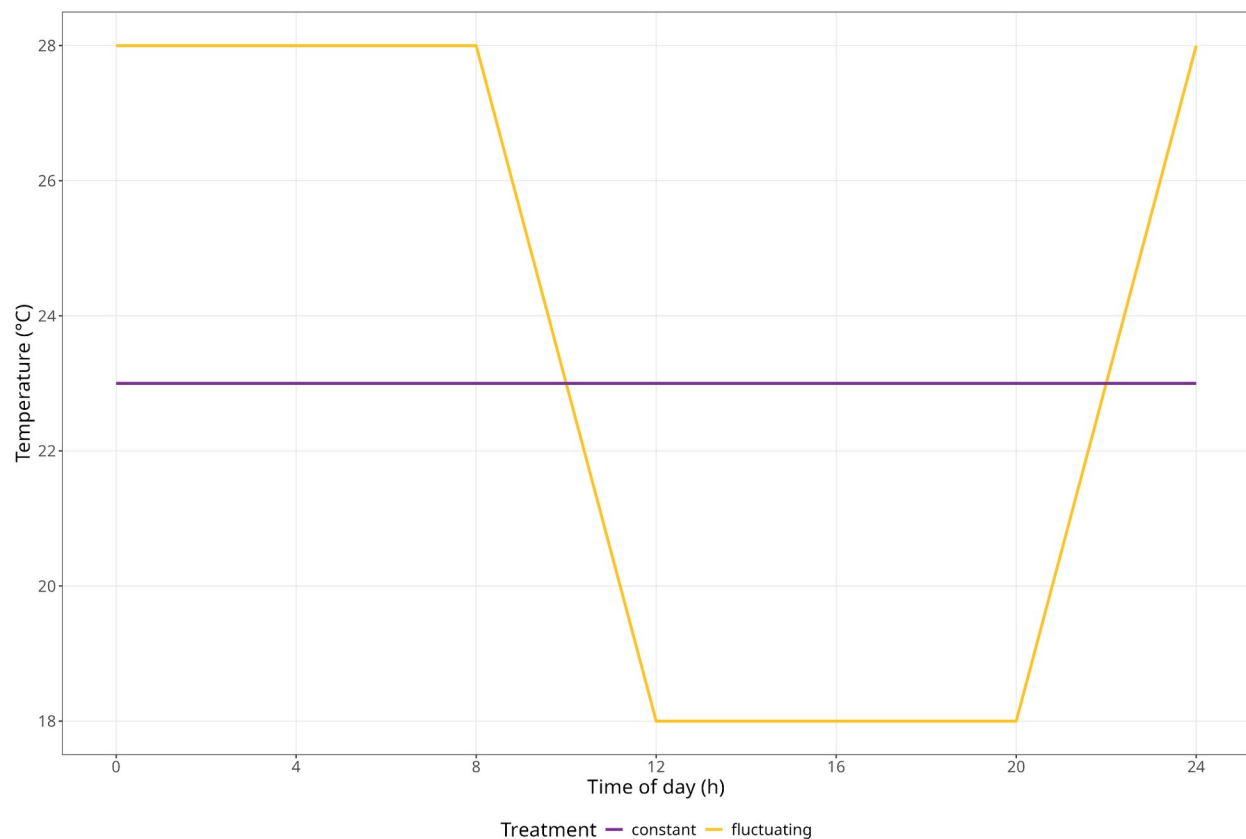


Figure SM 1 Schematic of the constant and fluctuating experimental thermal regimes used in the experiment, illustrated for a representative mean temperature of 23°C. The fluctuating regime followed a four phase diel cycle with an amplitude of $\pm 5^{\circ}\text{C}$ around the mean (8 h at the

upper plateau, 4 h linear transition, 8 h at the lower plateau, 4 h linear transition), applied identically for each population across all five mean temperatures tested (13, 18, 23, 28 and 33°C).

SM 1.3 Wing length measurements methodology

Wing length was measured for adults emerging from the life-cycle experiments conducted under both constant and fluctuating temperature regimes. Individuals from the adult heat experiment were not measured because all mosquitoes were reared under identical thermal conditions (23°C) before heat exposure, and no treatment-related differences in body size were expected.

A subset of 4–7 females and 4–7 males was measured for each population × temperature combination. Variation in sample size resulted from wings that were damaged during dissection and could not be measured reliably.

Adults were stored individually at –20°C until processing. After thawing at room temperature, one wing was removed at the wing base using fine entomological forceps under a stereomicroscope. The wing was mounted on a microscope slide beneath a coverslip and photographed using a Leica S8 APO stereomicroscope (5× magnification). Wing length was measured using the *Segment Line* tool in Leica Application Suite (LAS, version 4.9) as the distance from the projected alula notch to the distal end of the R vein (R1), following standard protocols commonly used for *Aedes* mosquitoes^{1–4}. When a wing exceeded the field of view, overlapping images were acquired using a fixed anatomical landmark to ensure continuous measurement.

Table SM1.3. Sample sizes for wing length measurements by population (Frankfurt, Trento and Palermo), thermal regime (Constant vs. Fluctuating) and sex (F = female; M = male).

	Constant regime		Fluctuating regime	
	F	M	F	M
Frankfurt	25	24	20	16
Trento	29	27	17	18
Palermo	28	35	20	29

1. Armbruster, P. & Hutchinson, R. A. Pupal Mass and Wing Length as Indicators of Fecundity in *Aedes albopictus* and *Aedes geniculatus* (Diptera: Culicidae). *J. Med. Entomol.* **39**, 699–704 (2002).

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4. Oliver, J. & Howard, J. J. Fecundity of Naturally Blood-Fed *Ochlerotatus japonicus*. *J. Med. Entomol.* **42**, 254–259 (2005).

Supplementary Materials 2

Statistical Model outputs

Fixed-effect estimates from generalised linear mixed models (glmmTMB) fitted to *Aedes albopictus* life-history data. Estimates are on the link scale of each model; S.E., z-value and p-value refer to Wald tests against zero.

Table SM 2.1 Fixed-effect estimates from a binomial GLMM (logit link) fitted to larva-to-adult survival data pooled across three *Ae. albopictus* populations (Frankfurt, Trento, Palermo). The response variable is the number of adults emerging out of the total number of larvae introduced per replicate cup (n = 228 cups). Population was not retained in the final model. Estimates are on the logit scale; S.E., z-value and Wald p-values are reported for each term. Temperature was modelled as a quadratic.

Family: binomial (logit)

Formula: cbind(n_survived, n_failed) ~ avg_T + l(avg_T²) + treat + avg_T:treat + l(avg_T²):treat + (1 | replic_uid)

Fit: AIC = 803.3 | BIC = 827.3 | logLik = -394.6 | n = 228 cups

Random effects: replic_uid: Var = 1.23, SD = 1.109

Term	Estimate	S.E.	z value	Pr(> z)
(Intercept)	-16.233	2.299	-7.060	<0.001
avg_T	1.196	0.197	6.079	<0.001
l(avg_T ²)	-0.020	0.004	-4.985	<0.001
FT_treat	-2.846	3.178	-0.896	0.371
avg_T:FT_treat	0.355	0.271	1.310	0.190
l(avg_T ²):FT_treat	-0.012	0.006	-2.174	0.030

Table SM 2.2 Estimated lower thermal limits (Tmin), thermal optima (Topt) and upper thermal limits (Tmax), in degrees Celsius, derived from the binomial GLMM of larva-to-adult survival (Table SM 2.1) for each thermal regime. Limits were obtained by solving the fitted quadratic temperature response for the roots and the optimum of the predicted survival curve. Because population was not retained in the survival model, limits are pooled across the three populations.

Treatment	Tmin (°C)	Topt (°C)	Tmax (°C)
Constant	20.9	29.8	38.8
Fluctuating	15.3	24.1	32.9

Table SM 2.3 Fixed-effect estimates from a Poisson GLMM (log link) fitted to larva-to-adult development time for individuals that successfully reached adulthood (n = 846 individuals nested in replicate cups). Population was not retained in the final model. Temperature was modelled as a second-order orthogonal polynomial to capture the non-linear relationship between mean temperature and development rate. Estimates are on the log scale; S.E., z-value and Wald p-values are reported for each term.

Family: Poisson (log)

Formula: days_larva_adult ~ poly(avg_T, 2) * treat + (1 | replic_uid)

Fit: AIC = 4119.5 | BIC = 4152.7 | logLik = -2052.7 | n = 846 individuals

Random effects: replic_uid: Var = 2.15×10^{-10} , SD = 1.47×10^{-5}

Term	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	2.380	0.013	184.45	<0.001
poly(avg_T, 2) ₁	-7.560	0.314	-24.05	<0.001
poly(avg_T, 2) ₂	4.273	0.342	12.50	<0.001
FT_treat	0.504	0.022	22.41	<0.001
poly(avg_T, 2) ₁ :FT_treat	-1.211	0.742	-1.63	0.102
poly(avg_T, 2) ₂ :FT_treat	0.158	0.472	0.33	0.738

Table SM 2.4 Fitted parameters of the Brière-1 thermal performance curve for larva-to-adult development rate, reported separately for each of the two treatment combinations. Values are mean $\pm$ bootstrap SE from 1,000 non-parametric resampling replicates. Fitting was performed by weighted nonlinear least squares on mean development rates (1/days) per temperature level.

Treatment	Tmin (°C)	Topt (°C)	Tmax (°C)
Constant	8.78 [7.82–9.61]	31.2 [30.8–31.6]	37.7 [37.2–38.3]
Fluctuating	10.8 [7.49–12.9]	31.9 [30.6–34.5]	38.2 [36.3–41.9]

Table SM 2.5 Fixed-effect estimates from a Gaussian LMM (identity link) fitted to adult wing length (mm) for individuals from both thermal regimes with valid measurements (n = 317 adults). Population was not retained in the final model. The model includes a sex $\times$ temperature interaction and a sex $\times$ treatment interaction, both of which were statistically significant. Estimates are on the response (mm) scale; S.E., z-value and Wald p-values are reported for each term.

Family: Gaussian (identity)

Formula: wing_mm ~ sex * avg_T + sex * treat + (1 | replic_uid)

Fit: AIC = -272.7 | BIC = -243.6 | logLik = 144.3 | n = 278 adults

Random effects: replic_uid: Var = 0.000147, SD = 0.012 | Residual SD = 0.143

Term	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	3.554	0.066	54.03	<0.001
sexM	-0.920	0.090	-10.21	<0.001
avg_T	-0.035	0.003	-14.13	<0.001
FT_treat	-0.492	0.025	-19.71	<0.001
sexM:avg_T	0.016	0.003	4.76	<0.001
sexM:FT_treat	0.148	0.033	4.53	<0.001

Table SM 2.6 Fixed-effect estimates from a binomial GLMM (complementary log-log link) fitted to the daily probability of larval or pupal death in a competing-risks person-period framework. In this framework, each individual-day row represents one individual on one day at risk; an individual exits the risk set permanently on the day of either death or adult emergence, whichever occurs first. Hazard ratios (HRs) are obtained by exponentiating the estimates; a positive estimate indicates that the covariate increases the daily probability of dying. Reference levels: FK (population), CT treatment. S.E., z-values and Wald p-values are reported for each term.

Family: binomial (cloglog)

Formula: died_pp ~ log(day) + avg_T + I(avg_T²) + treat + avg_T:treat + I(avg_T²):treat + log(day):avg_T + (1 | replic_uid)

Fit: AIC = 11405.0 | BIC = 11483.0 | logLik = -5693.5 | n = 42,755 person-days

Random effects: replic_uid: Var = 0.325, SD = 0.570

Term	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	-10.135	1.212	-8.364	<0.001
log(day)	0.794	0.143	5.534	<0.001
avg_T	0.383	0.107	3.559	<0.001
I(avg_T ²)	-0.008	0.002	-3.437	<0.001
FT_treat	1.828	1.090	1.677	0.094
avg_T:FT_treat	-0.316	0.104	-3.040	0.002
I(avg_T ²):FT_treat	0.009	0.002	3.728	<0.001
log(day):avg_T	0.014	0.007	2.154	0.031

Population was excluded after likelihood-ratio testing (LRT $\chi^2 = 8.84$, $df = 6$, $p = 0.183$).

Table SM 2.7 Fixed-effect estimates from a binomial GLMM (complementary log-log link) fitted to the daily probability of adult emergence in a competing-risks person-period framework. Estimates are on the complementary log-log scale; a positive estimate indicates that the covariate increases the daily probability of emerging. Reference level: CT treatment. S.E., z-values and Wald p-values are reported for each term.

Family: binomial (cloglog)

Formula: $\text{eclosed_pp} \sim \log(\text{day}) + \text{avg_T} + \text{l}(\text{avg_T}^2) + \text{treat} + \text{avg_T}:\text{treat} + \text{l}(\text{avg_T}^2):\text{treat} + \log(\text{day}):\text{avg_T} + (1 \mid \text{replic_uid})$

Fit: AIC = 5196.6 | BIC = 5274.5 | logLik = -2589.3 | n = 42,755 person-days

Random effects: replic_uid: Var = 1.143, SD = 1.069

Term	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	-32.561	3.426	-9.504	<0.001
log(day)	1.089	0.608	1.790	<0.001
avg_T	1.709	0.229	7.451	<0.001
l(avg_T ²)	-0.032	0.004	-8.116	<0.001
FT_treat	-0.913	3.016	-0.303	0.353
avg_T:FT_treat	-0.013	0.251	-0.053	0.746
l(avg_T ²):FT_treat	-0.004	0.005	-0.836	0.220
log(day):avg_T	0.103	0.024	4.339	0.033

Population was excluded after likelihood-ratio testing ($LRT \chi^2 = 3.53$, $df = 6$, $p = 0.740$).

Table SM 2.8 Fixed-effect estimates from a binomial GLMM (complementary log-log link) fitted to the daily probability of adult death in a person-period framework using constant-temperature adults only. Each individual-day row represents one adult on one day alive; an individual exits the risk set on the day of death. Population, quadratic temperature, sex, and non-proportional log(day) × temperature and population × temperature interaction terms are included. Reference levels: FK (population), female (sex).

Family: binomial (cloglog)

Formula: died_pp ~ log(day) + population + avg_T + I(avg_T²) + sex + log(day):avg_T + population:avg_T + (1 | replic_uid)

Fit: AIC = 2845.9 | BIC = 2926.5 | logLik = −1412.0 | n = 11,204 person-days

Random effects: replic_uid: Var = 0.136, SD = 0.369

Term	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	-64.313	6.313	-10.187	<0.001
log(day)	9.890	1.290	7.669	<0.001
TN	-0.532	0.704	-0.756	0.450
PA	-1.526	0.719	-2.122	0.034
avg_T	2.543	0.325	7.815	<0.001
I(avg_T ²)	-0.035	0.004	-8.967	<0.001
sex_M	0.769	0.094	8.186	<0.001
log(day):avg_T	-0.102	0.046	-2.229	0.026
TN:avg_T	0.010	0.026	0.396	0.692
PA:avg_T	0.041	0.026	1.544	0.123

Supplementary Materials 3

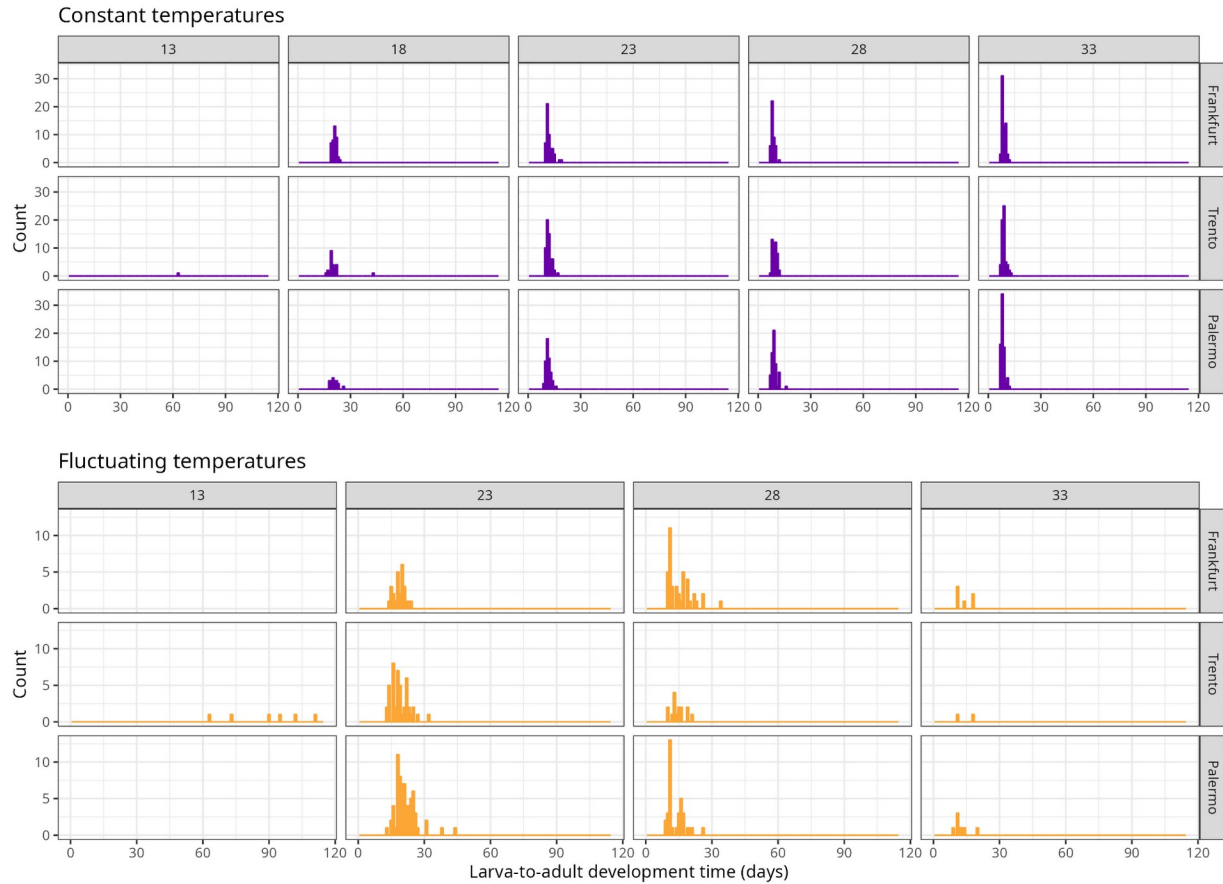


Figure SM 3.1 Observed distributions of larva-to-adult development time in *Ae. albopictus*, by population, mean rearing temperature and thermal regime. Histograms of the number of days from hatching to adult emergence for all individuals that completed development ($n = 846$). Upper block: constant thermal regime, at mean temperatures of 13, 18, 23, 28 and 33°C. Lower block: fluctuating regime ($\pm 5^\circ\text{C}$ stepped diel cycle about the same means), at 13, 23, 28 and 33°C. Panels with no or few bars indicate treatment combinations in which few or no individuals reached adulthood: no adults emerged at 13°C in Frankfurt or Palermo under either regime, and only 7 Trento individuals emerged at 13°C (1 constant, 6 fluctuating). Development time ranged from 7 to 111 days across all combinations.

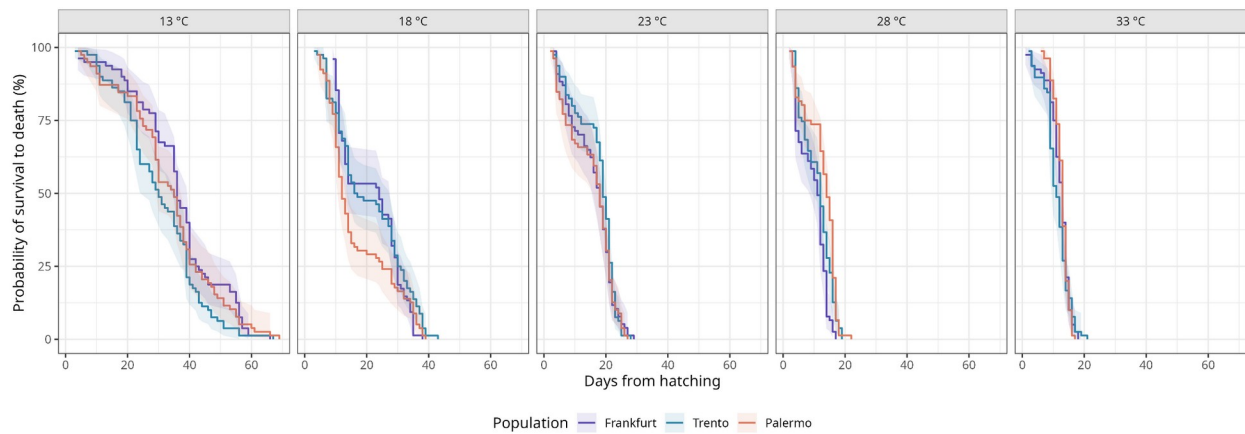


Figure SM 3.2. Non-parametric Kaplan–Meier survival curves by population and mean rearing temperature for the constant thermal regime. Survival time was measured in days from hatching. Curves are faceted by mean rearing temperature (columns: 13, 18, 23, 28, 33°C) and population (rows: Frankfurt, Trento, Palermo). shaded bands show 95% confidence intervals. Population differences within each temperature were assessed with pairwise log-rank tests and were detected only at the thermal extremes: at 13°C Trento survived significantly longer than Frankfurt, with Palermo intermediate and not significantly different from either; at 28°C survival was highest in Palermo, intermediate in Trento and lowest in Frankfurt; no significant differences among populations were detected at 33°C. Sample sizes were 78–80 individuals per population per constant-temperature treatment.