





RESEARCH ARTICLE

Long-term forecast of thermal mortality with climate warming in riverine amphipods

Wilco C. E. P. Verberk¹  | K. Natan Hoefnagel¹ | Ignacio Peralta-Maraver^{2,3}  |
Mathieu Floury⁴  | Enrico L. Rezende⁵ 

¹Department of Animal Ecology and Physiology, Institute for Water and Wetland Research, Radboud University, Nijmegen, The Netherlands

²Departamento de Ecología e Instituto del Agua, Facultad de Ciencias, Universidad de Granada, Granada, Spain

³Research Unit Modeling Nature (MNat), Universidad de Granada, Granada, Spain

⁴Univ Lyon, Université Claude Bernard Lyon 1, CNRS, ENTPE, UMR 5023 LEHNA, Villeurbanne, France

⁵Departamento de Ecología, Facultad de Ciencias Biológicas, Center for Applied Ecology and Sustainability (CAPES), Pontificia Universidad Católica de Chile, Santiago, Chile

Correspondence

Wilco C.E.P. Verberk, Department of Animal Ecology and Physiology, Institute for Water and Wetland Research, Radboud University, PO Box 9010, 6500 GL Heyendaalseweg 135, 6525 AJ, Nijmegen, The Netherlands.
Email: w.verberk@science.ru.nl

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Abstract

Forecasting long-term consequences of global warming requires knowledge on thermal mortality and how heat stress interacts with other environmental stressors on different timescales. Here, we describe a flexible analytical framework to forecast mortality risks by combining laboratory measurements on tolerance and field temperature records. Our framework incorporates physiological acclimation effects, temporal scale differences and the ecological reality of fluctuations in temperature, and other factors such as oxygen. As a proof of concept, we investigated the heat tolerance of amphipods *Dikerogammarus villosus* and *Echinogammarus trichiatus* in the river Waal, the Netherlands. These organisms were acclimated to different temperatures and oxygen levels. By integrating experimental data with high-resolution field data, we derived the daily heat mortality probabilities for each species under different oxygen levels, considering current temperatures as well as 1 and 2°C warming scenarios. By expressing heat stress as a mortality probability rather than a upper critical temperature, these can be used to calculate cumulative annual mortality, allowing the scaling up from individuals to populations. Our findings indicate a substantial increase in annual mortality over the coming decades, driven by projected increases in summer temperatures. Thermal acclimation and adequate oxygenation improved heat tolerance and their effects were magnified on longer timescales. Consequently, acclimation effects appear to be more effective than previously recognized and crucial for persistence under current temperatures. However, even in the best-case scenario, mortality of *D. villosus* is expected to approach 100% by 2100, while *E. trichiatus* appears to be less vulnerable with mortality increasing to 60%. Similarly, mortality risks vary spatially: In southern, warmer rivers, riverine animals will need to shift from the main channel toward the cooler head waters to avoid thermal mortality. Overall, this framework generates high-resolution forecasts on how rising temperatures, in combination with other environmental stressors such as hypoxia, impact ecological communities.

Wilco C.E.P. Verberk and Enrico L. Rezende have contributed equally to this work.

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KEYWORDS

climate vulnerability, heat tolerance, oxygen limitation, thermal biology, thermal safety margin, water quality

1 | INTRODUCTION

Global warming threatens the survival of many species into the future (Thomas et al., 2004). Ectothermic organisms which constitute approximately 99.9% of the total animal biodiversity on the planet (Atkinson & Sibly, 1997) are especially at risk as their fitness is largely determined by environmental temperatures (Amarasekare & Savage, 2012; Angilletta Jr, 2009). The threat of global warming has stimulated a multitude of studies on the thermal biology of ectotherms as this holds the promise to predict how they will fare in a warming world (Sinclair et al., 2016; Sunday et al., 2012). Many data have been collected on heat tolerance (Bennett et al., 2018), especially the critical thermal maxima (CT_{max}). CT_{max} is often compared against the habitat temperature to gauge vulnerability to warming (Deutsch et al., 2008). Not surprisingly, CT_{max} tends to exceed the temperature species experience in their habitat, resulting in positive and generally high warming tolerance estimates, especially in temperate regions (Calosi et al., 2008; Deutsch et al., 2008; Gunderson & Stillman, 2015; Hoffmann et al., 2013; Rezende et al., 2011, 2020; Stillman, 2003; Verberk, Leuven, et al., 2018). However, critical endpoints for heat tolerance do not fully quantify thermal tolerance (Rezende et al., 2020) and therefore deserve further scrutiny.

In many if not most physiological studies, the endpoints imply ~50% mortality within minutes or hours, something which in an ecological context equals a selection bottleneck that should result in local extinction in a matter of a few days. Clearly, the limits of heat tolerance will be lower over longer timescales, which are more relevant from an ecological perspective. Tolerance limits not only vary as a function of time, but the relationship with time differs across species (Leiva et al., 2019; Peralta-Maraver & Rezende, 2021). It is therefore challenging to forecast how species will respond to heat stress. This challenge is further complicated because in their natural habitats, animals may experience multiple bouts of heat stress of different intensity and duration. Repeated exposures may enhance heat mortality but may also trigger acclimation responses and adaptive evolution that ameliorate the negative impact of warming, and such responses will also have a specific time frame (Calosi et al., 2008; Rezende et al., 2011; Stillman, 2003; Verberk, Calosi, et al., 2018). Most ectotherms exhibit an increase in CT_{max} after exposure to warmer conditions, but such plasticity is argued to be insufficient to buffer ectotherms from the effects of global warming (Gunderson & Stillman, 2015; Pottier et al., 2022).

Finally, environmental factors other than temperature, such as food or oxygen availability, may act in synergy with heat stress and increase vulnerability to environmental warming (Huey & Kingsolver, 2019; Rubalcaba et al., 2020; Verberk, Durance, et al., 2016). An interaction between heat stress and hypoxia is of particular significance in the case of aquatic ectotherms, for which

obtaining oxygen is more challenging than for their terrestrial counterparts (Dejours, 1981; Verberk & Bilton, 2013). A mismatch between oxygen supply and demand has been proposed to explain thermal limits; as water temperatures rise, metabolic demand for oxygen increases, eventually exceeding the capacity of an organism to supply its tissues with sufficient oxygen (Pörtner, 2010). Whether or not oxygen limits heat tolerance under normoxia is debated (Jutfelt et al., 2018; Pörtner et al., 2017; Verberk, Overgaard, et al., 2016), but hypoxia is widely acknowledged to amplify the detrimental effects of warming (Woods et al., 2022). This includes reductions in heat tolerance under hypoxia which have been reported for a range of aquatic ectotherms including crustaceans, insects, mollusks, and fish (Ern et al., 2016; Healy & Schulte, 2012; Koopman et al., 2016; Rutledge & Beiting, 1989; Verberk & Bilton, 2013; Verberk, Leuven, et al., 2018). Therefore, the combined effect of heat stress and low oxygen levels can negatively impact the fitness and geographical distribution of aquatic ectotherms (Deutsch et al., 2008; Ern, 2019; Verberk, Overgaard, et al., 2016). This is especially alarming, given the widespread deoxygenation of aquatic ecosystems and its interactions with global warming and eutrophication (Breitburg et al., 2018; Jane et al., 2021; Woods et al., 2022).

Resolving the fundamental question of how heat tolerance quantified in the laboratory translates to field conditions is thus complicated by physiological acclimation effects, and by the effects of temporal scale when measuring heat tolerance using standardized protocols which rarely reflect the ecological reality of fluctuations in temperature, and other factors such as oxygen. The advancements in sensor technology and remote sensing methods have facilitated the acquisition of environmental data of high spatial and temporal resolution, including temperature and oxygen. Therefore, integrating information from laboratory heat tolerance trials and field observations would represent a powerful tool to explain and predict how ectotherms will fare in the warmer, deoxygenated waters of the future.

Here, we present a way forward by using the framework of 3D “thermal tolerance landscapes” (whereby mortality probability, time, and temperature form the three dimensions, see Rezende et al., 2020) to calculate mortality probabilities as a function of time, acclimation history, and environmental factors such as temperature and oxygen. We constructed this dynamic model (i.e., temperature varies in time) of mortality probabilities using data on heat tolerance measurements on riverine amphipods that were exposed to a range of conditions either weeks prior to testing (acclimation effects) or during testing (acute effects). We then apply our dynamic model to high-resolution temperature data from the river in which these amphipods live to calculate both daily mortality risk and annual mortality risk, bridging the physiological unit of investigation (i.e., the individual) and the ecological unit of investigation (i.e., the population). We ask how acclimation effects and acute effects modulate individual survival and population

persistence. Finally, we ask how well annual mortality can be estimated from coarser temperature data with a logistic model to allow forecasting heat mortality across larger spatial and temporal scales, illustrated by projecting mortality ~50 years into the future and across Europe. The analytical framework developed in here has significant implications for improving our understanding of thermal responses of riverine ectotherms, as well as for forecasting future impacts of global warming.

2 | METHODS

2.1 | Animal collection and maintenance

We collected amphipods from the river Waal in Nijmegen (N51°51'22", E5°52'55"), the Netherlands, in six batches between spring 2015 and spring 2016. Amphipods were highly abundant in the submerged rocky embankment of the river, and after lifting stones, amphipods were collected with kitchen sieves (mesh size 2 mm). Water was also collected from the site and used to maintain the animals in the lab.

Directly following collection, animals were exposed to one of two temperatures (10 and 20°C) and one of two oxygen levels (10 and 20 kPa) during the acclimation period. These acclimation temperatures reflect non-extreme temperatures that the amphipods experience in the field during either spring and autumn (10°C) or during early and late summer (20°C). Amphipods were maintained in a continuous flow system with two header tanks, with either hypoxic or normoxic water. Water oxygen content was achieved by aerating the water with a mixture of oxygen and nitrogen gas using gas flow controllers (M+W Instruments, Mass-Stream D-6341-DR). Water flowed from the header tanks into eight separate glass aquaria (2 temperatures × 2 oxygen contents × 2 size classes) by gravity. These aquaria were fitted with an overflow feeding into a common collection basin at the bottom of the setup and from there, water was pumped back up again to the header tanks. Water was filtered with a biological filter to maintain water quality. The temperature was regulated by placing the aquariums in heated or cooled trays, connected to heating baths (Grant Industries TXF200). The amphipods were separated into two size classes in the laboratory to prevent cannibalism and were fed twice a week with fish food (Sera Vipan) and live mosquito larvae (Chironomidae).

Temperature and oxygen content of each aquarium were monitored daily, and we used the realized measured values in our analysis rather than nominal values. Realized values for the low- and high-temperature acclimation were 10.3°C (±0.49 SD) and 20.0°C (±0.16 SD), respectively, and for normoxia and hypoxia acclimation, they were 19.6 kPa (±1.20 SD) and 9.5 kPa (±0.83 SD), respectively. Due to logistic constraints, the acclimation period varied between 7 days and 9 weeks as we ran sets of trials for each batch of collected amphipods until we ran out of amphipods. Thus, amphipods within each trial had been acclimated for the same amount of time. Most physiological changes take place in the initial phase of acclimation, with diminishing effects later during the acclimation period. This was also confirmed

in preliminary analyses that showed that acclimation time modulated the effect of acclimation temperature but adding acclimation time resulted in only very small improvements in model performance. To contextualize, in these preliminary analyses, the model estimate for acclimation temperature differed between 0.25 (1 week) and 0.27 (9 weeks), so well within the values given by the model presented in the manuscript (Table S3: $0.24 \pm 0.07SE$).

2.2 | Heat tolerance trials

We measured how long amphipods survived in heat stress of varying intensities for 1040 individuals. All heat trials and pretreatments were performed in Dutch standard water (0.2 g/L $CaCl_2$, 0.18 g/L $MgSO_4$, 0.10 g/L $NaHCO_3$, and 0.02 g/L $KHCO_3$). Before each heat tolerance trial, animals were transferred to 25°C for 10 min to prevent exposure to large temperature increments. Subsequently, they were either immediately transferred to the heat trials or exposed to a heat shock of 30°C for 5 min and left to recover at their acclimation temperature for 120 min. After the recovery and prior to the heat trials, the heat shocked animals were again transferred to 25°C for 10 min to ensure similar starting conditions. In each trial, 10 amphipods were monitored while being exposed to one of five stressful temperatures (30, 31, 32, 33.5, or 35°C). Water in the setup was temperature controlled using a water bath (Grant Instruments GP200) and continuously filtered through a UV filter (Sera UV-C-System 5W). Water was aerated with a gas mixture of oxygen and nitrogen to achieve one of three oxygen levels during the heat trials (5, 20, or 60 kPa, hereafter referred to as hypoxia, normoxia, and hyperoxia).

During a heat tolerance trial, amphipods were placed under small, upside-down petri dish lids (3 cm diameter) that prevented them from escaping, and these were made of glass so they could be easily observed from above. The petri dishes themselves were placed on a nylon mesh (0.3 mm mesh) which ensured exchange of oxygen with the surrounding water. Short trials at the highest temperatures allowed for live observation until the endpoint was reached. Longer trials were video recorded (ThorLabs Inc. 1.4 megapixel CCD camera with ThorLabs APT ThorCam software version 2.6.7064) for later analysis. Recordings were made for 2 min at 10-min intervals using Tiny Task software (Tiny Task for Windows 1.45 Vista-software).

The amphipods in the study showed a specific repeatable pattern, leading to death during the heat trials (see also Verberk, Leuven, et al., 2018). First, they moved less and used their pereopods to grapple the mesh provided. Next, pleopod movement halted, although amphipods continued to move their antennae and pereopods. Just prior to death, the body stretches which was used as the endpoint for all heat trials, although some twitches of appendages could be observed after this point. All animals were preserved in 70% ethanol for later identification, and determination of sex and wet mass. Wet mass was determined by blotting the preserved animal on tissue paper, adding it to a preweighed cup of water on a balance (Mettler-Toledo XA105, Switzerland), and noting the mass to the nearest 0.1 mg.

2.3 | Analysis of heat tolerance data

Amphipods were identified postmortem and were found to belong to one of three species (70% *D. villosus* (Sowinsky, 1894), 29% *E. trichiatum* (Martinov, 1932), and 1% *E. ischnus* (Stebbing, 1899)). Too few datapoints were obtained for *E. ischnus* and we therefore excluded individuals of this species as well as data from those amphipods we could not identify to species level. Diagnostics on the remaining data revealed abnormally low survival times for trials performed between 7 March 2017 and 11 April 2017 (Figure S1). These trials concerned almost all amphipods from Batch 5. As a cautionary measure, we decided to remove these data and focus our results on the remaining data ($n=808$). This resulting dataset had survival time measurements for most of the 24 treatment combinations but lacked complete representation of measurements under hyperoxia and from warm-acclimated amphipods (Table S1). To address this issue, the models were constrained and only included two-way interactions with test temperature in single-species models, and three-way interactions with test temperature and species in the models for both species. Six mixed models were run for each species to analyze the effect of test temperature on (log10 transformed) survival time (Table S2). The first model considered only the effect of test temperature (i.e., the null model), followed by models incorporating test oxygen conditions (model 2), heat shock (model 3), acclimation temperature (model 4), acclimation oxygen (model 5), and all four treatment conditions (model 6). Thus, the order in which we added complexity to our simplest model (model 1) reflects the timescale at which effects exert an effect, ranging from immediate (test oxygen), to hours (the heat shock response), to weeks (acclimation effects). The collection batch was included as a random intercept in all models. We used a similar model structure for the analyses combining the data on the two species but allowed the responses for all the conditions to differ between the two species by including interactions with the species factor. The models were ranked based on their performance using AICc and Akaike's weights. To specifically test whether heat tolerance differed between hyperoxia and normoxia, we used a likelihood ratio test to compare the best-performing model with an identical model except for pooling the normoxia and hyperoxia treatments (i.e., oxygen was included instead as a two-level categorical variable differentiating between hypoxia treatments on the one hand and on the other hand both the normoxia and hyperoxia treatments). Although body mass of the amphipods and river temperature (i.e., the water temperature at the time amphipods were collected) were explored as potential factors, they only showed minor improvements and were not included in the final models. The complex experimental design and random effects prevented us from obtaining reliable standard errors for the 24 different treatment combinations (2 acclimation temperatures, 2 acclimation oxygen levels, 3 test oxygen levels, and 2 heat shock conditions). To overcome this, the predicted response curve was calculated for each of the 24 unique treatment combinations and then averaged for each of the treatment contrasts. These response curves are also referred to as thermal death time curves as they separate between life (below the curve) and death (above the curve). Linear mixed models

were fitted using the function *lmer* within the R-packages *lme4* (Bates et al., 2016; R-Core Team, 2022). Data and code are available online (Verberk et al., 2023).

2.4 | River temperature

Data on river temperature were obtained from Rijkswaterstaat (www.rijkswaterstaatdata.nl accessed on 2020-03-17). We initially obtained river temperatures for two sites: Lobith (located upstream of the site where amphipods were collected) and Vuren (located downstream of the collection site), but since there was little difference between these two sites, we focused our analysis on Lobith which was closest to the collection site and here the measuring frequency was highest (typically once every 10 min). We calculated the rate of change using a 2-h moving window and found that the 95th percentile of rates of change was 0.102°C per minute, while the 5th percentile of rates of change was -0.069°C per minute. On average, the rate of change was much lower (0.0165°C per minute), enabling us to obtain river temperatures by interpolation on a 1-min interval resolution for each Julian day in each year.

2.5 | Constructing a dynamic model of the thermal tolerance landscape

We used our experimental data on heat tolerance to fit the dynamic model developed by Rezende et al. (2020), which generalizes the thermal tolerance landscape approach for variable temperatures, for each of the two amphipod species. Briefly, the analytical protocol combines survival times obtained at different constant temperatures to obtain the probability of survival as a function of heat stress intensity (i.e., the constant temperature) and heat stress duration (i.e., the survival time). It then assesses how well this probability function predicts the original estimates. To fit the model, we first selected the thermal death time curve of interest, representing a treatment combination, and centered the remaining 23 thermal death time curves onto it. We then fed this composite thermal death time curve into the model. Diagnostic regressions showed a strong fit to the data for both species ($R^2 > .9$ in all analyses), indicating that a single survival probability function described the mortality observed across treatments well. Subsequently, we coupled the survival probability function obtained for each treatment with "instantaneous" changes in ambient temperature (i.e., 1-min bins in the numerical approach) to obtain the predicted survival under a variable thermal regime. Thermal tolerance landscapes were fitted with a 0.1°C resolution and used to estimate daily survival probability given temperature data with a 1-min time resolution (i.e., 1440 temperature records for each Julian day in a given year; see above). These analyses were accomplished using the *tolerance.landscape* and the *dynamic.landscape* functions in R, which were developed specifically for this purpose by Rezende et al. (2020).

2.6 | Validation of the dynamic model

We validated model predictions against published estimates of CT_{max} from Verberk, Leuven, et al. (2018) that have been obtained empirically for amphipods under incremental warming (0.25°C/min starting at 10°C). Predicted and observed values were strongly correlated (Pearson $r=0.95$, $p=0.008$) and replicated the impact of oxygen levels and differences between species on CT_{max} (Figure S2). Predicted values are on average $1.37 \pm 0.82^\circ\text{C}$ ($\pm\text{SD}$) lower than empirical measurements, which imply that experimental animals are succumbing to heat stress roughly 5 min after predicted by our model. The model also accurately predicted mortality probability for *D. villosus* maintained for 15 days at eight temperatures from 0 to 30°C (Maazouzi et al., 2011; Pearson $r=0.994$, $p=6.4 \times 10^{-7}$), demonstrating that this dynamic model can also accurately predict mortality for longer periods (Figure S3).

2.7 | Applying the dynamic model to field data to estimate mortality

We used the dynamic model to estimate daily mortality probability based on river temperature data collected over 7 years and interpolated to a minute resolution. Daily mortalities were accumulated to annual mortality by multiplying daily survivorship probabilities. Cumulative, annual mortality was calculated for each of the 7 years and then averaged. The study assumed no recovery at night, because daily fluctuations in water temperatures in the rivers were small (on average, a temperature range of $0.57 \pm 0.68^\circ\text{C}$ per day), contrasting with the study of Rezende et al. (2020) for fruit flies on land where ambient temperatures dropped during night time and likely provide opportunity to recover from heat stress. Mortality probabilities were calculated for four different combinations of experimental treatments (2 temperature \times 2 oxygen): We contrasted cold acclimation versus warm acclimation and normoxia versus hypoxia (acclimated to 10 kPa and tested under 5 kPa). Note that this hypoxia scenario is not the most extreme condition as acclimation to 10 kPa improved heat tolerance, while testing under 5 kPa led to a (greater) reduction in heat tolerance (see results). With this approach, we first disentangled the impact of thermal acclimation and oxygen availability on mortality under current temperatures (treatment combinations \times 7 years = 28), and then repeated the process for two warming scenarios by 1 or 2°C to the temperature records.

2.8 | Constructing a logistic model for annual mortality

We estimated the relationship between annual mortality and summer temperatures (mean temperature of the hottest month, i.e., August). This approach allows us to predict annual thermal mortality from coarse indicators in climate forecast models and project how mortality is expected to increase in the future and change across thermal gradients. We fitted logistic regressions for annual mortality predicted for each of the four treatment combinations, for each

of the 7 years, and under current and 1 and 2°C warming temperatures ($n=4 \times 7 \times 3=84$) and obtained high goodness of fit ($R^2 > .78$).

Knowing how summer temperatures will increase in the future, we can project the annual mortality in time. With this purpose, we relied on climate forecasts and assumed that a mean summer temperature of 22.3°C in our dataset corresponds to 2015 and that by 2075, it will reach 25°C. In other words, rivers were projected to warm at a rate of 0.045°C/year, which in the river studied is caused by both climate change and the discharge of cooling water (Reeze et al., 2017). Using the information on predicted future summer temperatures, we projected the annual mortality $\pm 95\%$ confidence intervals into the future. We constructed four projections of annual mortality into the future, corresponding to the four treatment combinations that we employed for the dynamic model (see above), which together encapsulate the variability in long-term forecasts of rising temperature impacts on field communities.

Annual mortality was also projected in space by using a global map of freshwater temperature. This map of how maximum weekly water temperature (averaged over 1976–2005) varies spatially across surface waters is constructed by coupling a dynamic water temperature model to a global hydrological model with meteorological input from five climate models and the map accounts for topographic differences in river width, depth, and slope (Barbarossa et al., 2021 and references therein). These modeled global freshwater temperatures were compared to water temperatures measured in running freshwaters in France, leading to the derivation of a correction factor (Figure S4; raw data publicly available at <https://naiades.eaufrance.fr>; processed datasets are provided in Verberk et al., 2023). These corrected modeled temperatures were then used in the spatial projection of annual mortality across Europe for current temperatures and for 1 and 2°C warming scenarios, for the best-case scenario (i.e., normoxia, warm-acclimated amphipods).

3 | RESULTS

3.1 | Heat tolerance experiment

Across treatments, survival of heat stress was strongly affected by test temperature in both amphipod species (Figure 1; Figures S5 and S6; Tables S2 and S3), with survival time logically declining when heat stress became more intense. Both acute and acclimation effects modulated the effect of test temperature and differences in heat tolerance across treatments were generally more prominent at lower test temperatures (representing chronic, mild heat stress) whereas at higher test temperatures (representing acute heat stress), heat tolerance was more similar across treatments (Figures S5 and S6). Both warm acclimation (see Figure 1b) and heat shock increased heat tolerance at low test temperatures, with acclimation effects being substantially larger and confirming that thermal history can result in beneficial acclimation. For oxygen levels, acclimation and acute effects were in opposite directions (Figure 1c): reduced oxygen availability during measurements greatly decreased heat tolerance, whereas hypoxia acclimation improved

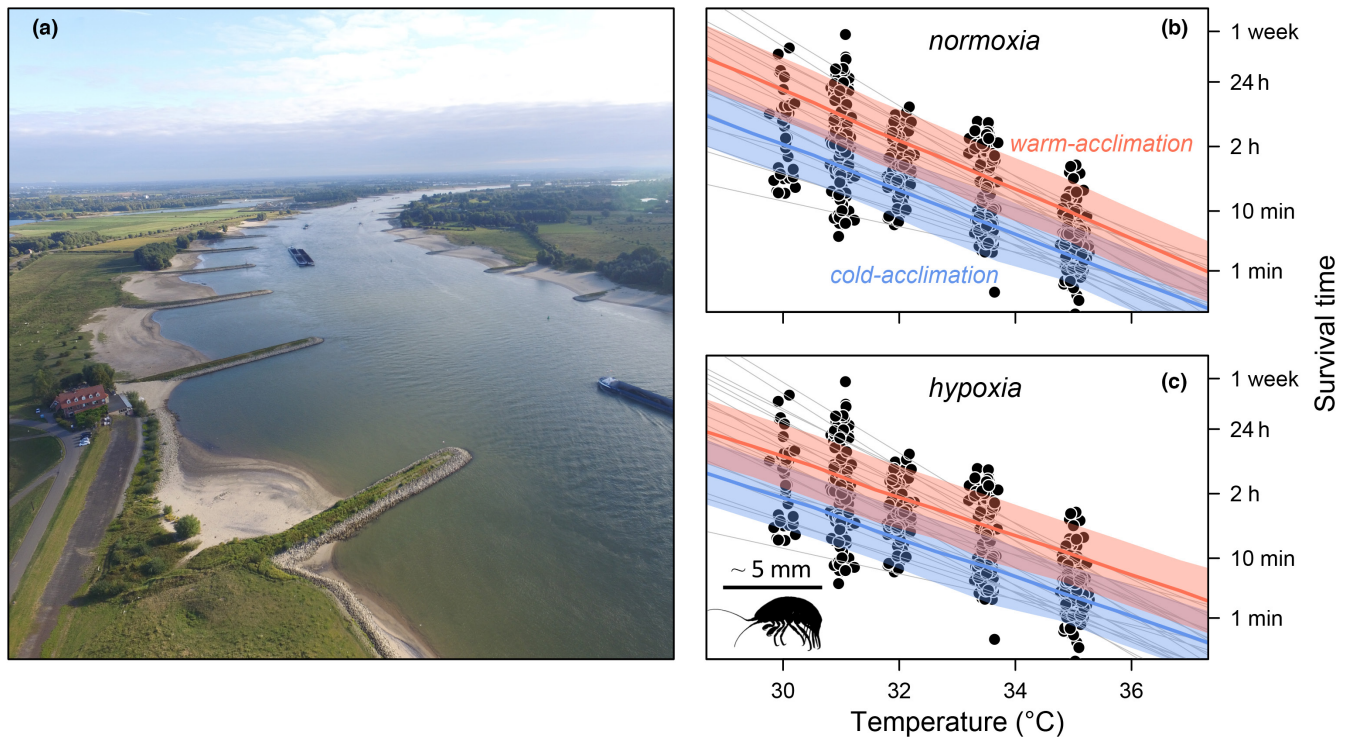


FIGURE 1 Heat tolerance in amphipods from river Waal. (a) We studied the amphipods *D. villosus* and *E. trichiatus*, both invasive species currently found across rivers in West Europe, including the Waal, the Netherlands (pictured; Photo credit: Frank Collas). The collection site was at 0.98 km from this spot (N51° 51' 22", E5° 52' 55"). (b, c) Thermal death time curves, showing the survival time of *D. villosus* at different temperatures from a cross-factorial experiment. Empirical measurements are shown in black, individual regressions of the 24 different combinations of acclimation and measurement conditions in gray, and mean survival for cold- and warm-acclimated animals, respectively, in blue and red, and separately for (b) normoxia ($PO_2 = 20$ kPa) and (c) hypoxia ($PO_2 \leq 10$ kPa). Note that survival time was \log_{10} -transformed.

heat tolerance, partially compensating for the acute effects of hypoxia (Figures S5 and S6). Hyperoxia, that is, increased oxygen availability during the measurements, resulted in a modest improvement in heat tolerance of both amphipods (Figures S5 and S6; a competing model which did not differentiate between normoxia and hyperoxia treatments (i.e., hypoxia vs. non-hypoxia) received less support; $\Delta AIC = 43.4$; likelihood ratio test: $p < 0.001$).

3.2 | Dynamic model of thermal tolerance landscape

We used the experimental data on heat tolerance to fit dynamic models that calculate survival probabilities for a given day (i.e., a 24-h thermal regime). As input for the thermal regime, we used field data on river temperature interpolated to a 1-min resolution (i.e., 1440 temperature records for each Julian day for each of the 7 years). Heat mortality for *D. villosus* was predicted to occur only during a 4-month window around summer between June and September. Projected cumulative, annual mortality differed across the four different temperature and oxygen treatment combinations. According to a two-way ANOVA, predicted annual mortality ($n = 7$ years \times 4 treatments = 28) under current conditions is 79.2% higher in cold-acclimated than warm-acclimated individuals ($F_{1,25} = 183.9$, one-tailed $p < 2.5 \times 10^{-13}$)

and 15.2% higher in hypoxia than normoxia ($F_{1,25} = 6.8$, one-tailed $p = 0.007$). With 1°C and 2°C warming, the annual mortality increases, eventually reaching 100% in all groups except warm-acclimated animals in normoxia, with a predicted average mortality of 49.5% across years (Figure 2). In this warming scenario, it is clear that amphipod populations of *D. villosus* could only remain viable in this warming scenario in oxygenated waters, and that a reduction in oxygen availability would result in local extinction. Results were similar for the amphipod *E. trichiatus*, except that survival was considerably higher following warm acclimation and this species seems to be more resilient to warming temperatures of 1°C or 2°C (Figure S7).

3.3 | Logistic model to project annual mortality across space and time

Annual mortality of *D. villosus* could be quite well predicted from summer temperatures, quantified here as the mean temperature of the hottest month in the analysis (i.e., August) via logistic models (odds ratio $OR = 4.58$, $z = 3.430$, and $p = 0.0006$; Figure 3). The effect of summer temperature on annual mortality was further modulated by the effects of warm acclimation ($OR = 0.008$, $z = -3.964$ and $p < 7.4 \times 10^{-5}$) and oxygen ($OR = 0.038$, $z = -3.367$ and $p = 0.0007$). Comparing the summer temperature at which annual mortality equals 50%, the effects of warm

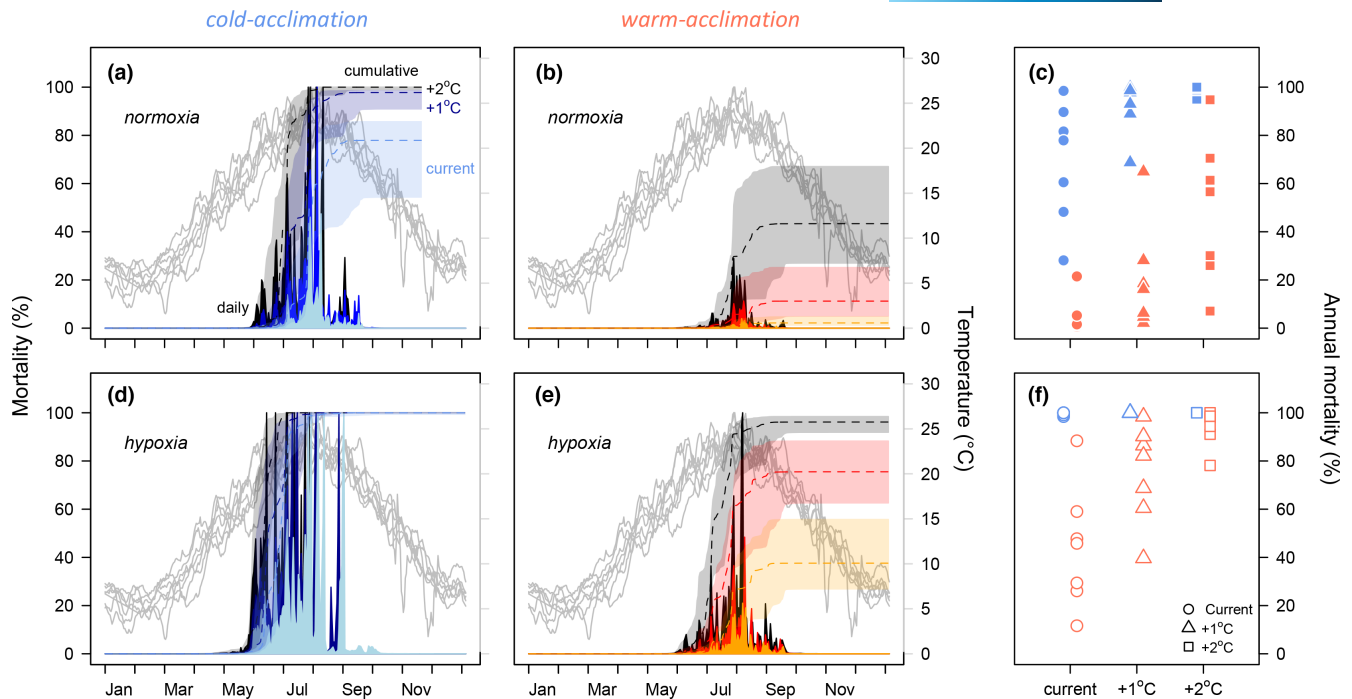


FIGURE 2 Daily and cumulative thermal mortality for *D. villosus*. Daily and cumulative mortality across seven different years for amphipods measured under (a) normoxia with cold and (b) warm acclimation, and (d) hypoxia with cold and (e) warm acclimation. By combining the tolerance landscapes with hourly temperature records, we calculated daily mortality (vertical spikes) under three different scenarios: current temperatures, and 1°C and 2°C warming. We obtained water temperature records for 7 years, shown in gray, and added 1°C and 2°C for the warming scenarios. Cumulative, annual mortality was calculated for each of the 7 years and then averaged. The spikes represent the daily mortality, the dotted lines the cumulative mortality averaged across 7 years (shaded areas indicate the 25% and 75% percentiles). We estimated the annual mortality for (c) normoxia ($PO_2 = 20$ kPa) and (f) hypoxia ($PO_2 \leq 10$ kPa) as the cumulative mortality at the end of each year, showing estimates for cold-acclimated amphipods in blue and estimates for warm-acclimated amphipods in red. Daily and cumulative thermal mortality for *E. trichiatus* is available as [Figure S7](#).

acclimation and normoxia translated, respectively, into a 3.2 and 2.1°C wider thermal window of mortality, adding up to 5.3°C and illustrating the crucial role played by both phenotypic plasticity and adequate oxygenation to reduce the impact of warming waters ([Figure 3](#)).

This logistic model, which translates summer temperature estimates into annual mortality, can be used to predict how mortality will increase with warming through time and space. According to these analyses, even under optimal conditions of oxygenation and warm acclimation, thermal mortality in *D. villosus* is expected to increase and approach 100% by 2100 ([Figure 3](#)). This effect is amplified under hypoxia, where the model predicts mean annual mortalities >80% by 2040. Similar analyses for *E. trichiatus* suggest that this species is more resilient to warmer waters and should be able to cope with the scenarios studied here ([Figure S8](#)), with the caveats discussed below.

The spatial projections show a clear pattern of increased mortality risk for *D. villosus* toward southern, warmer rivers ([Figure 4](#)), which roughly corresponds to the known distribution range ([Figures S9](#) and [S10](#)). In addition, under a warming scenario, mortality risks increase, with clear increases in predicted mortality, for example, the Loire, Oder, and Volga catchments ([Figure 4](#); [Figure S11](#) for the location of the named catchment basins). On a regional scale (south-eastern France), thermal mortality is predicted to be higher for *D. villosus* in the main river compared to the cooler upstream headwaters. Thus,

avoiding heat stress by thermal niche tracking would require an upstream shift in distribution at the catchment scale and a shift in distribution ranges northward and upward on a continental scale. Similar analyses for *E. trichiatus* show a higher resilience to warmer waters, similar to the temporal projections ([Figures S12](#) and [S13](#)).

4 | DISCUSSION

Our stepwise analytical framework can be used to predict heat mortality on a range of spatial and temporal scales, using laboratory measurements on thermal tolerance and water temperatures (measured or modeled). In addition, the framework can accommodate multiple stressors aside from temperature (e.g., hypoxia), including physiological acclimation to these stressors. The analytical steps involve: (i) estimating the thermal landscapes for different species or conditions from laboratory measurements, (ii) predicting thermal mortality from high-resolution temperature records for multiple years and under different warming scenarios (+1 and +2°C here), (iii) estimating the association between a coarser descriptor of temperature (e.g., summer temperature) and cumulative annual mortality, and (iv) determining how this coarser descriptor is expected to change in the future to forecast temporal trends and spatial patterns in annual mortality ([Figures 3](#) and [4](#)).

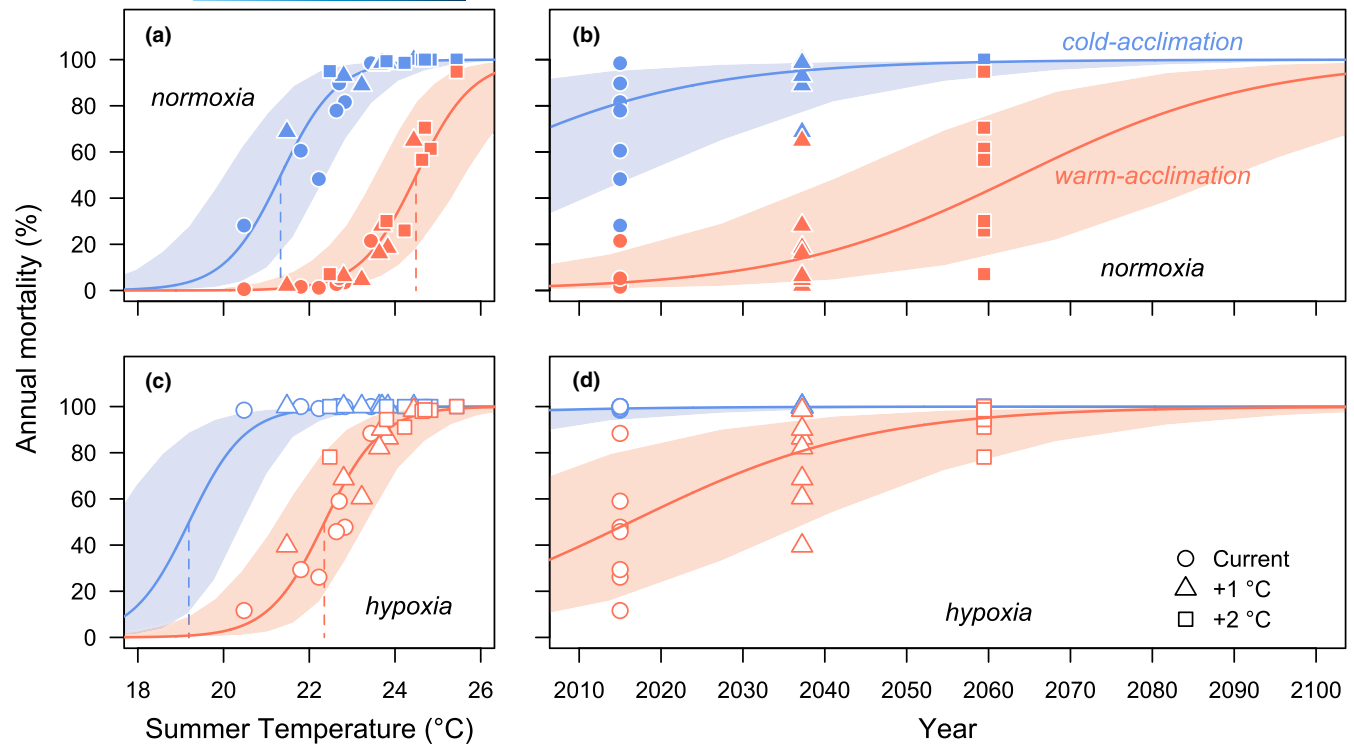


FIGURE 3 Logistic regression and long-term mortality projection. (a) Annual mortality for *D. villosus* predicted from summer temperatures under current conditions, 1°C and 2°C warming in normoxia ($PO_2 = 20$ kPa). (b) We then combine this logistic model with forecasts of summer temperatures to predict how annual mortality will change in time, given projected increases in river temperatures. (c) Under hypoxia ($PO_2 \leq 10$ kPa), the logistic curves are displaced toward lower temperatures, reflecting the hypoxia-induced reduction in heat tolerance, increasing the vulnerability of amphipod populations to warming conditions. (d) As a result, future annual mortalities are projected to increase. Solid lines represent the logistic model plotted against summer temperature or remapped onto years, assuming a linear warming from 22.3°C in 2015 to 25°C in 2075, and the shaded areas show the 95% confidence intervals. The dotted lines indicate the temperatures where annual mortalities are 50%. At a rate of 0.045°C/year, the 1°C and 2°C warming scenarios correspond to 2037 and 2059. Logistic models and long-term mortality projections for *E. trichiatus* are presented in Figure S8.

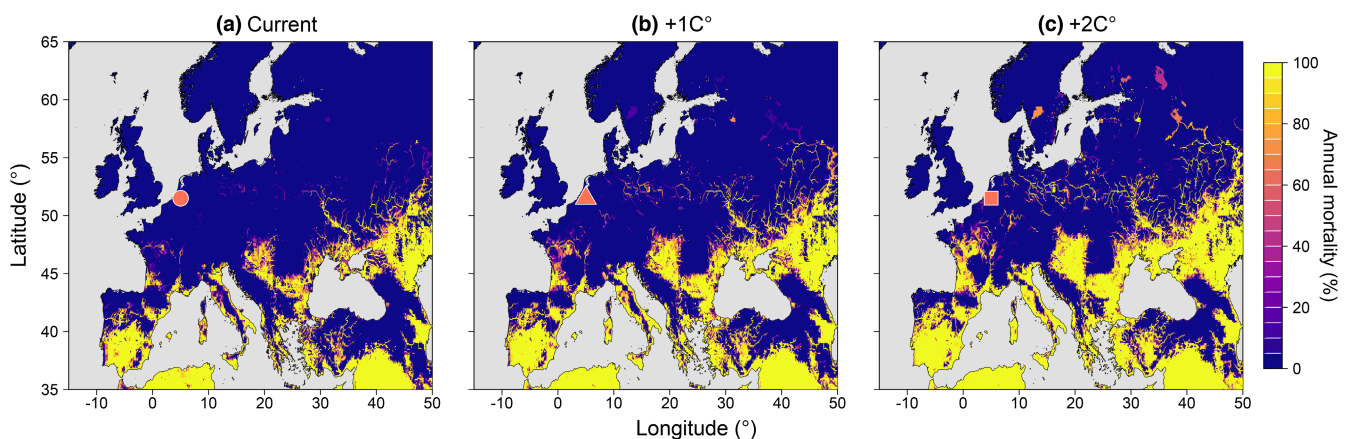


FIGURE 4 Spatial forecast of annual mortality in *D. villosus*. We combined our logistic model with the global map of freshwater temperatures (after applying a correction factor derived from water temperatures measured in running freshwaters in France) to predict how annual mortality will vary spatially across Europe. Projections were derived for current temperatures (a), and for the 1°C and 2°C warming scenarios (b, c). Location of the study area is shown with a symbol.

Importantly, these analytical steps involve several caveats and caution is crucial to obtain reliable simulations and interpret the outcomes appropriately. It goes without saying that good predictions require good data; hence, experiments must be appropriately designed and

ideally involve large sample sizes. In addition, the current implementation of the dynamic model does not include potential evolutionary responses to thermal selection. Furthermore, the model makes implicit assumptions about recovery, which are neglected here because water

temperature remains generally constant throughout the day. In contrast, daily recovery was included in estimations of thermal mortality in the fruit fly *Drosophila subobscura* because thermal fluctuations on land are large (Rezende et al., 2020), and future analyses may include physiological recovery explicitly within the model. Also, developmental conditions can considerably alter sensitivity to heat stress (Truebano et al., 2018), which will have an important impact on predictions. And finally, simulations of warming scenarios (+1 and +2°C) assume that thermal variation remains unchanged, which will not always be the case. Fortunately, the approach is quite flexible and other scenarios with higher variation and thermal extremes can be simulated as required (e.g., multiplying current temperatures by a factor).

Our experimental results on heat tolerance in these species is mechanistically related to insufficient oxygen supply (see Portner & Knust, 2007; Verberk et al., 2011): Both hypoxia acclimation and acute hyperoxia improve oxygen supply to meet increased oxygen demand at high temperatures (Verberk, Calosi, et al., 2018; Verberk, Leuven, et al., 2018). Importantly, these oxygen effects became stronger at longer timescales associated with mild heat stress as has been recently reported in another amphipod (Semsar-kazerouni et al., 2020) and suggested for aquatic ectotherms in general (Leiva et al., 2019). Thus, rather than there being a threshold effect of demand surpassing supply capacity, animals gradually become more and more functionally hypoxic, having to divert more and more energy away from basic physiological processes to fuel survival (Harrison et al., 2018), explaining why the observed effects of oxygen limitation are more pronounced at the longer timescales associated with mild heat stress. Coupled field data on species distributions, water oxygenation, and water temperature likewise suggested that adequate oxygenation can offset the impact of warming in aquatic mayflies (Verberk, Durance, et al., 2016).

In general, plasticity in thermal tolerance following acclimation has been reported to be insufficient in buffering ectotherms from global warming (Gunderson & Stillman, 2015; Pottier et al., 2022). Here, we report that, similar to the effects of oxygen, the beneficial effects of thermal history appear to be greater at longer timescales, corroborating previous results on another amphipod species (Semsar-kazerouni & Verberk, 2018). In fact, annual mortality can approach 100% (for cold-acclimated amphipods under hypoxia) or be negligible (for warm-acclimated amphipods under normoxia) (Figure 2), widening the thermal window of mortality by up to 5.3°C (Figure 3). The temporal scale employed in heat tolerance trials may reconcile these different findings, as most studies measure heat tolerance as CT_{max} and employ short, rapid ramping assays, where the effects of acclimation may be relatively minor. In addition, our four treatment combinations used for the simulations do not include the most extreme conditions. Including the hardening effects of a heat shock would further improve heat tolerance, at least in *D. villosus*, while exposure to hypoxia of normoxia-acclimated animals will reduce heat tolerance. Thus, plasticity in heat tolerance due to thermal history and adequate oxygenation can reduce the impact of global warming to a much greater extent than previously thought.

Previous measurements suggested that invasive *D. villosus* is more sensitive to warm temperatures and reduced oxygenation than other amphipods (Verberk, Leuven, et al., 2018). To evaluate vulnerability

to warming, the critical thermal maxima is often compared against the habitat temperature (Deutsch et al., 2008). For *D. villosus*, this approach would translate to a comfortable warming tolerance of 10°C (given a CT_{max} of 32.3°C and a mean summer temperature of 22.3°C). Yet, our modeling results show that an amphipod species from a temperate river at 51.8° N, with relatively cool summer temperatures of 22.3°C, will struggle to persist by 2100 in the best-case scenario (i.e., normoxia, warm-acclimated). Again, the timescale employed may be important to consider here. Obviously, for the persistence of a population, detrimental effects of warming occur at temperatures lower than those at which animals may survive heat stress when exposed for a few hours. In our approach, we first estimated mortality over a 24-h period which is already considerably longer than the typical durations of ramping trials to measure heat tolerance. Moreover, while ramping trials typically focus on the lethal temperature at which 50% of the animals die, in our model even daily mortality risks well below 50% could result in substantial cumulative mortality over many days. For example, a daily mortality of only 5% will accumulate to 50% mortality after a fortnight. Thus, while the metric of warming tolerance may be useful in a comparative study (Comte & Olden, 2017; Pinsky et al., 2019), ranking species from more to less vulnerable, a more sophisticated methodology is necessary to link physiological heat tolerance directly to in situ river conditions (regarding oxygenation and temperature) and derive predictions for mortalities due to heat.

Elevated mortalities due to heat are not necessarily synonymous with local extinction (though they certainly portray higher environmental pressure on the population). The likelihood of extinction will also depend on the population's intrinsic rate of increase, which varies with temperature and across different organisms, on their life-history strategies and the connectivity between different populations. Consequently, additional knowledge specific to each lineage is required to understand how mortality rates relate to overall population vulnerability to climate change. Having said that and assuming appropriate empirical data, this approach opens the venue to forecast in unprecedented detail the impact of thermal mortality on ecological communities, diagnose which lineages are more vulnerable, quantify how other stressors affect their vulnerability and design appropriate mitigation strategies and conservation timelines. Indeed, the framework is very flexible and readily applicable to a variety of terrestrial and aquatic ectotherms, especially small invertebrates and vertebrates (Rezende et al., 2014, 2020). Its widespread implementation could provide a rapid assessment on the impact of current temperatures on natural populations, with emphasis in regions with high biodiversity such as tropical forests, coral reefs, and freshwater habitats in general (Strayer & Dudgeon, 2010).

AUTHOR CONTRIBUTIONS

Wilco C.E.P. Verberk, K. Natan Hoefnagel, and Enrico L. Rezende were designed the study; K. Natan Hoefnagel was performed the research; Wilco C.E.P. Verberk, Enrico L. Rezende, Ignacio Peralta-Maraver, and Mathieu Floury were analyzed the data; Ignacio Peralta-Maraver, Enrico L. Rezende, Wilco C.E.P. Verberk, and Mathieu Floury were involved in visualization; Wilco C.E.P. Verberk, Enrico L. Rezende, and

Ignacio Peralta-Maraver wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data supporting the results are archived in a public repository: Verberk et al. (2023) doi: <https://doi.org/10.5281/zenodo.7640253>.

ORCID

Wilco C. E. P. Verberk  <https://orcid.org/0000-0002-0691-583X>
 Ignacio Peralta-Maraver  <https://orcid.org/0000-0002-3467-7664>
 Mathieu Floury  <https://orcid.org/0000-0002-4952-5807>
 Enrico L. Rezende  <https://orcid.org/0000-0002-6245-9605>

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SUPPORTING INFORMATION

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