Journal of Thermal Biology 129 (2025) 104127

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Automating thermal limits: continuous, objective, and high-throughput thermal data for small mobile ectotherms

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1. Introduction

Organisms vary in their physiological tolerance to temperature, and this will determine which species will move, adapt, or go extinct under climate change (Deutsch et al., 2008; Urban, 2015). Ectotherms, such as insects and reptiles, are especially sensitive to ambient temperature changes because they rely on external heat to regulate their body temperatures and sustain core biological processes (Guo et al., 2020; Clavijo-Baquet et al., 2022). Consequently, as temperature regimes change, ectotherm populations are at risk of range shifts and potential collapse, impairing the ecosystem services that they provide (Rumpf et al., 2019; McCain and Garfinkel, 2021; Wagner et al., 2021; Zattara and Aizen, 2021; Parr and Bishop, 2022). Subsequently, methods that generate objective, high-throughput, and precise thermal tolerance data are fundamental for predicting the behavioural and physiological limits of biodiversity in the future.

Currently, many studies use single-point estimates to quantify thermal tolerance and performance. Single-point estimates include survival times, lethal doses, and critical temperatures (CTs). Survival times represent the duration an organism can withstand a fixed temperature before succumbing to thermal stress, while lethal doses estimate the temperature at which a given percentage of the test population dies (i.e., LD50 estimates; (Vermeulen and Bijlsma, 2004; Terblanche et al., 2011; Scaccini et al., 2019; Jørgensen et al., 2021). For terrestrial invertebrates, CTs are the most common tolerance measure and indicate the highest (CT_{max}) and lowest (CT_{min}) temperatures at which an organism experiences a loss of righting response (Ali et al., 2019). CTs are very close to lethal estimates, as once the CT is reached, an organism is functionally dead and often unable to recover and escape from additional ecological or environmental pressures. This tolerance information has allowed ecologists to understand and predict the activity and distribution limits of organisms in many contexts (Oberg et al., 2012; Guo et al., 2020; Ion Scotta et al., 2021; Anderson et al., 2023). The methods used to collect CTs are simple, but also diverse, which can pose issues for replicability and objectivity (Terblanche et al., 2007; Nascimento et al., 2022). Whether in a dry bath, water bath, or on a hot plate, CTs are estimated by ramping the temperatures experienced by organisms up or down at a set rate until a loss of righting response is observed (Leong et al., 2022). Conventional dry bath methods are rarely automated and require frequent manual input from the observer to achieve the desired ramping rate, to identify the point at which an organism's righting response is reached, and to record CTs. Dry baths have been used to study the effects of temperature on the development and neuromuscular function of a broad range of small ectothermic insects, including ants, fruit flies, and grasshopper and mosquito eggs, for over 30 years (Huey et al., 1992; Chown et al., 2009; Kong et al., 2016; Roeder et al., 2021). Further, the throughput of CT assays is typically low. The number of individuals that can be simultaneously tested is limited by the capacity of the equipment (often ~15 individuals) and by the observer's ability to monitor numerous individuals reliably. Subsequently, these methods are low throughput, subjective and laborious. Further, CTs measure thermal performance as single endpoints, representing the most extreme temperatures an organism can endure. These extreme limits fail to capture sublethal variation – for example, progressive changes to physiological

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https://doi.org/10.1016/j.jtherbio.2025.104127
Received 18 November 2024; Received in revised form 22 April 2025; Accepted 24 April 2025
Available online 2 May 2025

stress – which occur across temperatures leading up to the CT. This distinction between lethal and sublethal effects potentially leads to underestimation of biotic responses to modified thermal regimes (Gangloff and Telemeco, 2018; Guo et al., 2020; Parratt et al., 2021).

An alternative to single-point estimates of thermal tolerance and performance is continuous estimates. These can be used to capture temperature-dependent behaviour across a range of temperatures and encompass both lethal and sub-lethal effects. Continuous estimates measure rate-based fitness traits, such as reproduction, growth, or activity over time. Measuring reproductive or growth rates, however, requires the meticulous management and monitoring of laboratory populations over time. Consequently, they are often too slow, expensive and laborious for many research projects and many organisms simply cannot be cultured in the lab (Brown et al., 1994; Penick et al., 2017). Continuous data can be used to estimate thermal performance curves (TPCs) and analyse sublethal temperature effects (Schulte et al., 2011). TPCs are unimodal, where performance increases with temperature to a performance optimum (T_{opt}), either side of which is CT_{min} and CT_{max} (Fig. 1; Angilletta et al., 2002; Angilletta, 2006; Schulte et al., 2011). Through comparison of TPC characteristics and CTs, it is possible to predict variations in activity and spatial distribution of different populations and species across different temperatures (Angilletta, 2006; Clusella-Trullas et al., 2011). We argue that a more accessible solution to collecting continuous thermal performance data is to focus on locomotor activity. Measuring locomotor activity can be associated with ecologically relevant changes to fitness such as the ability to forage efficiently and flee from unfavourable environments (Wilson, 1976; Powell and Franks, 2007; Kaspari et al., 2016). Compared to single point estimates, monitoring activity provides valuable insights into ectotherm responses to temperature and is a straightforward method for scaling up thermal phenotyping. Commercial and freely available video tracking software have previously been used to study movement of different insect life stages with temperature (Laursen et al., 2021; MacLean et al., 2022; Perez-Galvez et al., 2023; Vermaak et al., 2025). However, when tracking organisms with diverse phenotypes they are often difficult to distinguish from one another or from the background due to lack of a simple unifying feature, e.g., colour, shape, size. Further, challenges such as expensive videography setups, long experiments requiring extensive data storage, and complex data analyses limit the practicality of this approach (Cecchetto et al., 2020; Terlau et al., 2023).

Here, we test a new high-throughput approach to collecting locomotor activity data in the context of thermal ecology across continental scales. Instead of assessing righting response to capture CTs in dry baths, we propose the use of locomotor activity to capture conceptually comparable values for CTs. We aim to convey that accurately, efficiently, and objectively measuring a different temperature dependent trait can provide new insights into thermal responses, making the LAM a valuable tool alongside conventional thermal limit assay equipment (water or dry baths). We use a locomotor activity monitor (LAM; Trikinetics Inc., USA) housed in a programmable incubator to automate the collection of activity data across a broad range of temperatures and characterise these patterns as TPCs. The LAM relies on free and open-source software which outputs data in easily accessible text files (DAMSystem3 Software, Trikinetics Inc., USA). LAMs function by detecting the frequency at which infrared beams are broken by the movement of individual organisms housed in glass tubes. This removes the need for an observer to detect critical temperatures, offering the capacity to monitor many more individuals at once (Hawkins and DuRant, 2020; Terlau et al., 2023). LAMs can monitor movement of up to 32 individuals as frequently as every second, generating large amounts of continuous data and enhancing throughput compared to conventional methods.

Several studies have used infrared sensors to assess thermal responses in insects, including in the context of genetic variation, thermal performance curves, or CT_{min} and knock-down time assays for *Drosophila* (Rolandi et al., 2018; Kellermann et al., 2019; Awde et al., 2020). However, using LAMs specifically in the context of thermal ecology and behaviour, for example to use movement and temperature data to predict species distributions, remains largely unexplored and untested (Shik et al., 2019). We argue that the LAM could allow us to obtain macro-scale data (i.e. many taxa from many locations) more easily and objectively by boosting experimental throughput compared to conventional methods. It is unknown whether CT estimates generated from a LAM are comparable to CT estimates obtained from conventional dry bath methods. Here, we compare CT estimates obtained via conventional dry bath methods and the LAM-incubator method by conducting thermal performance assays on workers from six European and seven Australian ant species. Since the LAM depends on beam breaks to detect activity, it is not ideal for organisms with low activity levels (e.g., slugs, worms, pupae). We recommend using the LAM primarily for insects with mobile life stages or those capable of rapid movement. Ants

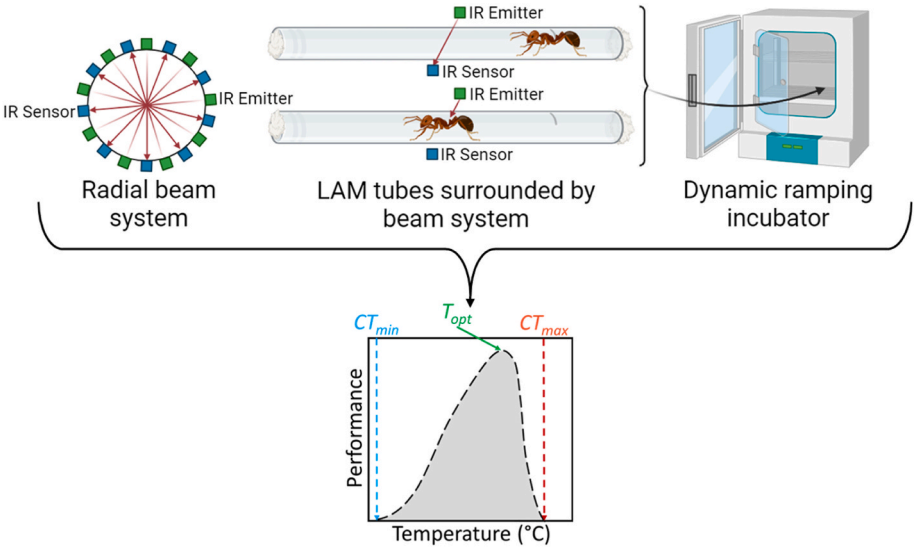

Fig. 1. Locomotor Activity Monitor (LAM) setup and Thermal Performance Curve. The LAM's radial beam configuration (top left) consists of nine infrared (IR) emitters and sensors per glass tube, each containing a single ant. Ant activity disrupts the IR beams, registering movement. The setup is placed in a dynamic ramping incubator (top right) to track activity across temperature. The thermal performance curve (bottom) shows performance versus temperature, with critical temperatures, CTmin (blue) and CTmax (red), and Topt (green) indicating optimal performance.

are an ideal study taxon due to their widespread distribution across the globe, their thermophilic nature, and their ease of collection and transport (Hölldobler and Wilson, 1990; Roeder et al., 2021; Nascimento et al., 2022). Specifically, we test whether CT estimates from dry bath and LAM approaches align and are similarly reliable.

2. Materials and methods

This work involved collection and processing of 13 ant species across Europe and Australia to assess their thermal tolerances. This required collaboration between European and Australian research groups, who conducted thermal assays in parallel. We tested whether thermal tolerance estimates obtained using two different methods—the dry bath and the LAM, each measuring a distinct thermal trait—could produce comparable and repeatable results. We aimed to showcase the LAM as a new tool as part of a wider toolkit in thermal ecology, which can contribute to understanding species' responses to temperature across different geographic regions.

2.1. Ant sampling

Six European species were processed at Cardiff University, Wales, and seven Australian species were processed at La Trobe University, Melbourne, Australia. For the European ant species, individual worker ants from six species were collected from the end of August 2023 to beginning of January 2024. These included four common species from South Wales: *Lasius niger, Lasius flavus, Myrmica rubra,* and *Formica rufa.* Individual workers of *Lasius* and *Myrmica* were each collected from urban parks in Cardiff using an aspirator and stored in 25 mL falcon tubes with air holes in the lids. *Formica* workers were collected from Wentwood Forest (latitude: 51.65, longitude −2.84) by hand with forceps into plastic tubs (15 cm × 10 cm x 5 cm) with a small amount of their nesting material and air holes pierced into the lids. We also collected *Temnothorax unifasciatus* and *Aphaenogaster senilis* in Switzerland and Spain, respectively. *Temnothorax* was collected from Lausanne (latitude: 46.52, longitude: 6.58) and found in hollow twigs. *Temnothorax* was collected in an identical way to *Lasius* and *Myrmica* using an aspirator, or by directly placing twigs housing ant nests into a falcon tube. *Aphaenogaster* workers were collected from Parque Nacional de Doñana (latitude: 36.99, longitude: 6.45) by hand with forceps into plastic tubs. The mainland European samples were sent by post to Cardiff University and arrived four to five days after collection. Samples were shipped with each unique colony isolated within separate 25 mL falcon tubes for *Temnothorax* or plastic tubs for *Aphaenogaster*. Falcon tubes and plastic tubs both had holes pierced into the lids, with moistened cotton wool firmly secured in each container to provide a water supply and scrunched tissue paper for the ants to hide in. Once in the laboratory at Cardiff University, ants were placed in plastic containers lined with Fluon® (Blades Biological, Kent, UK) to prevent escape. Ants were supplied with sugar solution and maintained at 20–25 °C in the laboratory.

For Australian samples, ant collection was conducted at the Nangak Tamboree Wildlife Sanctuary, La Trobe University Campus, Melbourne, Australia from October 2023 to April 2024. Ants were housed at 20 °C in plastic nest boxes with the top 10 cm of the inner rim lined with fluon and provided with water soaked in cotton wool balls.

2.2. Thermal tolerance assays

We used dynamic ramping assays with a 0.5 °C min^{-1} ramping rate across both dry bath and LAM methods, starting at 25 °C and 20 °C for European and Australian experiments, respectively. Most Welsh ants were assayed within the following three days of collection, while ants from mainland Europe (*Temnothorax* and *Aphaenogaster)* were assayed on day of arrival to the lab or, at most, three days later. Australian ants underwent thermal assays the day after collection. In total, the analysis included data from over 1500 individuals across the 13 species assayed (Table S2).

2.3. Locomotor activity monitor (LAM)- incubator assays

We used a LAM10H locomotor activity monitor (LAM; Trikinetics Inc., USA) to quantify the activity of individual ants across different temperatures. The LAM unit hosts 32 separate transparent glass tubes of 10 mm diameter surrounded at the midpoint by an array of nine radial infrared beams. These beams are broken as organisms pass across them during experiments and these "beam breaks" are recorded as counts every 10 s on the associated LAM software (DAMSystem3 Software, Trikinetics Inc., USA). We housed the LAM unit in a programmable incubator to manipulate the temperature experienced by the individual ants during experiments (Fig. 1). European species were incubated using the INCU-160C B0D Cooled Incubator (SciQuip, UK), while Australian species were incubated using PHCBI MIR-554-PE cooled incubator (PHCbi Group, PHC Corporation, Asia and Pacific).

In European experiments, individual ants were placed inside each tube of the LAM using soft forceps and each end of the tube was plugged with cotton wool. A type-K thermocouple was inserted within each tube to individually monitor the temperatures experienced by each ant and accounted for any non-uniform heat distribution within the incubator. We connected thermocouples to TC-08 Pico data loggers (Pico Technology Ltd., UK, advertised 0.025 °C resolution) and recorded temperature data using PicoLog Software 6.2.8. We also left three thermocouples outside of the LAM but inside the incubator to quantify differences between incubator temperature and within-tube temperature. In Australian experiments, the position of individual tubes per colony were randomised using a random number generator across the tubes and arrays. This was done to avoid systematic biases such as temperature gradients in the incubator, variation in tube quality or measurement bias of the LAM. One thermocouple was also placed in an empty tube blocked at the ends with foam.

The LAM-incubator method lasted approximately 3.5 h including set-up. Before introducing ants into the LAM in the European experiments, we preheated the incubator to 25 °C. At the start of the experiment, individuals are kept at 25 °C for 20 min before decreasing the temperature to 4 °C at a rate of 0.5 °C min^{-1}. Once the temperature reaches 4 °C, it is maintained for 10 min before returning to 25 °C in another 10 min. The incubator is held at 25 °C for another 20 min before being increased to 60 °C at a rate of 0.5 °C min^{-1}. We aimed to assay a minimum of 10 individuals per colony. For Australian experiments, loaded LAMs were placed in the incubator and ants were allowed to settle for 20 min at 20 °C. Temperature was then ramped down at a rate of 0.5 °C min^{-1} to 0 °C. Temperature was then ramped up to 20 °C over 15 min and ants were allowed to rest for a further 15 min at this temperature. Temperatures were then ramped up at the rate of 0.5 °C min^{-1} from 20 °C to 60 °C. Australian experiments aimed to trial 30 individuals from 1 to 3 colonies per species. All samples were weighed to identify average worker weight per colony and stored.

2.4. LAM manual observations

The dry bath method allows for direct testing of an individual's loss of righting response by tapping tubes to flip ants onto their backs and observing their ability to recover. However, the LAM does not allow for this same direct assessment of tapping the tubes. Subsequently, loss of righting response is more challenging to characterise in the LAM as individuals were unlikely to flip over. To evaluate the accuracy of the Pawar TPC model in predicting loss of righting response at CT_{min} or CT_{max} from the LAM data, we conducted a second set of experiments: visual LAM assays to manually record CTs. For this, we collected 5 fresh colonies each of *Lasius flavus, Lasius niger, Myrmica rubra,* and *Formica rufa* from South Wales using the same methods outlined previously. For visual LAM experiments, we observed activity of each individual in the

LAM through the incubator window and logged the temperature of the associated thermocouple when loss of righting response was identified. Loss of righting response was characterised by an individual's inability to maintain basic righting response and loss of other core motor function such as inability to move one or more legs, to climb and permanent cessation of movement. Visual LAM assays were conducted for the four South Wales species, each species with 5 colonies and 16 individuals per colony (n = 80). For the same species and colonies, 10 individuals per colony were assayed in the dry bath to compare CTs with the LAM (n = 50).

2.5. Dry bath assays

European dry bath assays used a PCH-3 Personal Benchtop Cooler/Heater (Grant Instruments Ltd., UK, advertised stability = ± 0.1 °C and resolution = ± 0.1 °C). Whereas Australian assays used custom digital dry bath with 20 individual wells set into an insulated aluminium block above a Peltier plate that was programmed to heat and cool at a set rate. Across regions, we assayed five ants per colony of each species for each assay type. This data was used to estimate CT_{max} and CT_{min} for each colony. Three thermocouples housed in cotton wool-plugged Eppendorf tubes were evenly distributed across the dry bath and connected to a data logger as above. Individual ants were placed in 1.5 mL Eppendorf tubes which were sealed with cotton wool to eliminate thermal refuge at the top of the tube (Oberg et al., 2012). In European assays, tubes were placed into the dry block which was preheated to 25 °C. Individuals were maintained at 25 °C for 10 min before the temperature was increased (CT_{max}) or decreased (CT_{min}) at 0.5 °C min^{-1} until all individuals lost their righting response (Chown et al., 2009). In European assays, different individuals from the same colonies were used for separate CT_{min} and CT_{max} ramps. A maximum of ten individual ants were assayed in a single experimental run and each run took approximately 1.5 h including set-up. In Australian assays, the same individuals were used to determine both CT_{min} and CT_{max}. The dry bath temperature ramped down, followed by a recovery period, and then ramped up, mimicking the LAM method. Upon reaching CT_{min} ants were removed from the experiment and left to acclimate back to room temperature. Once all Australian ants were removed, they were left for 15 min, after which they were checked for survival and normal movement. Ants that did not recover were replaced by a worker from the same colony for the CT_{max} assay. Ants were then acclimated for another 15 min at 20 °C in the thermal block before temperature was ramped up. To estimate the point at which righting responses were lost, Eppendorf tubes were briefly removed from the dry block and flicked to assess for loss of righting response. Upon the loss of a righting response, we recorded the average thermocouple temperature as the individual's CT_{max} or CT_{min}, rather than the advertised temperature of the dry bath unit.

From this point on, all data processing, construction of TPCs, and analysis were the same for Australian and European datasets. We combined European and Australian datasets to add broader taxonomic coverage and to explore whether the recorded metrics were generalisable across these different regions. Further, by testing the method in two independent research laboratory settings we can provide increased confidence in our comparative assessment of the validity of the LAM technique.

To justify the use of a combined ramp (CT_{min} and CT_{max}) for the LAM, compared to the dry bath with separate CT_{min} and CT_{max} assays, we conducted a smaller secondary experiment. This experiment aimed to determine if there was any difference in CT_{max} estimates generated by the combined ramp and a CT_{max} only ramp in the LAM. Combined and CT_{max} assays were run once each, both with 21 *Lasius niger* individuals taken from the same colony. Using a Welch Two Sample *t*-test, we found that CT_{max} estimates were not significantly different between ramp types (mean for Combined = 45.01, mean for CT_{max} = 45.65; difference = −0.64, 95 % CI [−2.22, 0.95], t(37.62) = −0.81, p = 0.422; Fig. S1).

2.6. Data processing

We aligned the LAM activity data to the thermocouple data per individual tube by matching the timestamps of each data type. We also identified the recovery period from the end of the CT_{min} ramp until the beginning of the CT_{max} ramp. For each ant, activity data was summed per minute and used to calculate the average activity per degree, both with and without considering the recovery period. We then continued our analysis using the activity data without the recovery period to represent our final raw LAM dataset. Some individuals are presumed to have died early in the experiment or were already unfit for testing, therefore we only included individuals where at least 20 % of the data is non-zero. The exclusion percentage is very low at only 2 % of individuals from the European sample population.

2.7. Thermal performance curve (TPC) fitting process

Using the LAM data, we tested four thermal performance models: Gaussian, Pawar, Quadratic and Weibull from the rTPC package (Padfield et al., 2021). We selected these thermal performance models based on their use in the existing literature and theoretical benefits (Angilletta, 2006). We calculated the mean activity per degree Celsius for each colony by averaging individual level data for a given colony. We then fitted each model to the colony-level data and assessed their Akaike information criterion (AIC) scores. We also assessed R-squared (R^2) for each model. For each model, we calculated predicted values based on the fitted model. Predicted values were used to fit a linear regression model between the actual and predicted values, from which we calculated the adjusted R-squared. Out of the four models tested, we selected Pawar which had the lowest AIC score and highest R^2 value (mean AIC = 76.753 and mean r^2 = 0.912) across all colonies (Fig. S2, Table S1).

Next, we fitted the Pawar model to individual ant data to produce individual TPCs. We used the default extraction parameters for CT_{min} and CT_{max} from the rTPC package, which would facilitate meaningful comparisons between the LAM and dry bath data (Padfield et al., 2021). For each individual, we also extracted the thermal optimum, T_{opt} (the temperature at which performance is maximised; (Huey and Bennett, 1987; Padfield et al., 2021).

Extreme outlier values were excluded from further analysis. This aimed to exclude individuals that died early due to stress factors, such as *Formica rufa* releasing formic acid, or those with suboptimal health that did not survive at temperatures broadly experienced within their ecological context (Hurlbert et al., 2008; Prather et al., 2018; Ślipiński et al., 2021; Corley et al., 2023). For dry bath data we excluded CT_{min} values above 16 °C and CT_{max} values below 36 °C based on histograms for each species. LAM observations had CT_{min} values above 18 °C and CT_{max} values below 35 °C excluded based on bar plot distributions. Based on histograms of LAM model estimates, we excluded CT_{min} values below −20 °C and above 30 °C. For CT_{max}, we excluded values below 30 °C and above 60 °C. For T_{opt}, we removed values below 20 °C. For that absolute number of samples analysed by species for each method see Table S2.

3. Data analysis

3.1. Comparing thermal metrics between methods

We analysed our data at an individual level, but recognise colonies as true biological replicates (Gotelli et al., 2011). We used the LMER function to compare CT_{min}, CT_{max} and T_{opt} metric estimates from dry bath and LAM assays. Temperature per individual was the dependent variable, while a combined factor (method and metric, i.e., LAM model CT_{min}, dry bath CT_{max}) and species identity and their interaction were used as fixed effects. Colony identity was included as a random effect. We used an ANOVA to test the significance of each model variable and calculated estimated marginal means (EMMs, or 'least-squares means')

to explore which species-method-metric combinations were significantly different.

3.2. Comparing coefficients of variation (CVs) of thermal metrics between methods

We calculated coefficients of variation (CVs) at a colony level as a measure of repeatability, or 'precision', of our different methods in detection of CT_{min}, CT_{max} and T_{opt}. For each metric, the CV represents the degree of closeness in temperature estimates between individuals of a colony. CVs were calculated per colony per species using the formula:

CV of Metric = (sd(Metric) / mean(Metric)) * 100

Lower CVs indicate higher repeatability, however there is no universal threshold that identifies a 'reliable' CV value. Our goal here is to test whether LAM estimates are less repeatable compared to the well-known dry bath method. We compared CVs using ANOVA with the dependent variable as the average CV value per colony and independent variables as species identity, metric, method type and their interactions. We used Post-hoc Tukey honest significant differences tests to determine if the different methods are equally reliable at predicting the same metrics for each species. Australian species were not included in this analysis due to insufficient colony replicates.

Table 1
Estimated marginal means comparisons of temperatures (°C) for species, metric, and method interactions. Combinations span thirteen species, three distinct metric types (CTmax, CTmin and Topt) and two methods (LAM model and Dry Bath observation), i.e., LAM model CTmax compared to Dry Bath CTmax.

Contrast	Species name	Estimate of difference	Standard error	p-value
Dry Bath CTmax - LAM model CTmax	*Chelaner cinctum*	−0.82	1.63	1.00
	Camponotus claripes	−2.60	2.26	1.00
	Lasius flavus	−1.86	0.93	1.00
	Crematogaster laeviceps	1.70	2.10	1.00
	Lasius niger	−3.54	0.98	0.23
	Camponotus nigroaeneus	−1.99	1.78	1.00
	Myrmica rubra	−2.77	1.03	0.93
	Formica rufa	**−4.37**	**0.98**	**0.01***
	Aphaenogaster senilis	−2.35	1.20	1.00
	Iridomyrmex septentrionalis	−1.16	1.99	1.00
	Papyrius sp.*A*	−0.52	2.05	1.00
	Temnothorax unifasciatus	−3.78	1.02	0.18
	Rhytidoponera victoriae	−2.30	1.63	1.00
Dry Bath CTmax - LAM model Topt	*Chelaner cinctum*	2.65	1.63	1.00
	Camponotus claripes	1.39	2.26	1.00
	Lasius flavus	2.39	0.92	0.97
	Crematogaster laeviceps	8.01	2.10	0.14
	Lasius niger	0.70	0.97	1.00
	Camponotus nigroaeneus	1.31	1.78	1.00
	Myrmica rubra	1.09	1.02	1.00
	Formica rufa	0.97	0.98	1.00
	Aphaenogaster senilis	1.42	1.20	1.00
	Iridomyrmex septentrionalis	2.76	1.99	1.00
	Papyrius sp.*A*	7.80	2.05	0.14
	Temnothorax unifasciatus	0.32	1.01	1.00
	Rhytidoponera victoriae	2.91	1.63	1.00
Dry Bath CTmin - LAM model CTmin	***Chelaner cinctum***	**−15.04**	**1.64**	**0.00***
	Camponotus claripes	**−11.55**	**2.07**	**0.00***
	Lasius flavus	**−9.46**	**0.92**	**0.00***
	Crematogaster laeviceps	−6.01	2.23	0.93
	Lasius niger	**−16.34**	**1.01**	**0.00***
	Camponotus nigroaeneus	0.64	1.81	1.00
	Myrmica rubra	**−12.94**	**1.02**	**0.00***
	Formica rufa	**−11.81**	**1.08**	**0.00***
	Aphaenogaster senilis	−1.79	1.29	1.00
	Iridomyrmex septentrionalis	−5.85	2.06	0.87
	Papyrius sp.*A*	−1.72	2.08	1.00
	Temnothorax unifasciatus	**−6.15**	**1.11**	**0.00***
	Rhytidoponera victoriae	**−8.13**	**1.64**	**0.00***

Table 2
Post-hoc Tukey HSD output for comparison of coefficients of variation (CVs) for species, metric, and method interactions. Combinations span six species, three distinct metric types (CTmax, CTmin and Topt) and two methods (LAM model and Dry Bath observation), i.e., LAM model CTmax compared to dry bath CTmax.

Contrast	Species name	Estimate of difference	Lower estimate of difference	Upper estimate of difference	p-value
LAM model CTmax – Dry Bath CTmax	*Lasius flavus*	3.89	−95.14	102.91	1.00
	Lasius niger	2.55	−98.10	103.20	1.00
	Myrmica rubra	3.20	−108.69	115.09	1.00
	Formica rufa	2.42	−102.87	107.71	1.00
	Aphaenogaster senilis	2.54	−129.15	134.24	1.00
	Temnothorax unifasciatus	2.30	−111.88	116.48	1.00
LAM model CTmin – Dry Bath CTmin	*Lasius flavus*	41.14	−57.88	140.17	1.00
	Lasius niger	2.53	−98.13	103.18	1.00
	Myrmica rubra	8.24	−103.65	120.13	1.00
	Formica rufa	25.24	−82.96	133.43	1.00
	Aphaenogaster senilis	**267.97**	**136.28**	**399.67**	**0.00***
	Temnothorax unifasciatus	89.54	−24.64	203.72	0.44
LAM model Topt – Dry Bath CTmax	*Lasius flavus*	7.15	−91.87	106.18	1.00
	Lasius niger	3.63	−97.02	104.28	1.00
	Myrmica rubra	4.77	−107.12	116.66	1.00
	Formica rufa	4.21	−101.08	109.50	1.00
	Aphaenogaster senilis	4.81	−126.88	136.50	1.00
	Temnothorax unifasciatus	3.88	−110.30	118.06	1.00

3.3. Comparing temperature observations with model estimates

We compared CTs generated by the LAM model and by visual assays (LAM visual observations and dry bath) using LME with identical model parameters as described above. Here, we included the additional method of LAM visual observations alongside our two pre-existing methods, dry bath and LAM model.

3.4. Identifying effect of mass on temperature estimates

A linear regression analysis was conducted to investigate the association between mean species mass and differences in temperature estimates between contrast types. For each species the estimated differences in temperature were derived from EMM comparisons of unique metric and method combinations (e.g., Dry bath CT_{max} vs. LAM model CT_{max}). This analysis was conducted on European and Australian data. All data manipulation and statistical analysis took place within the R version 4.3.2 (R Core Team, 2023).

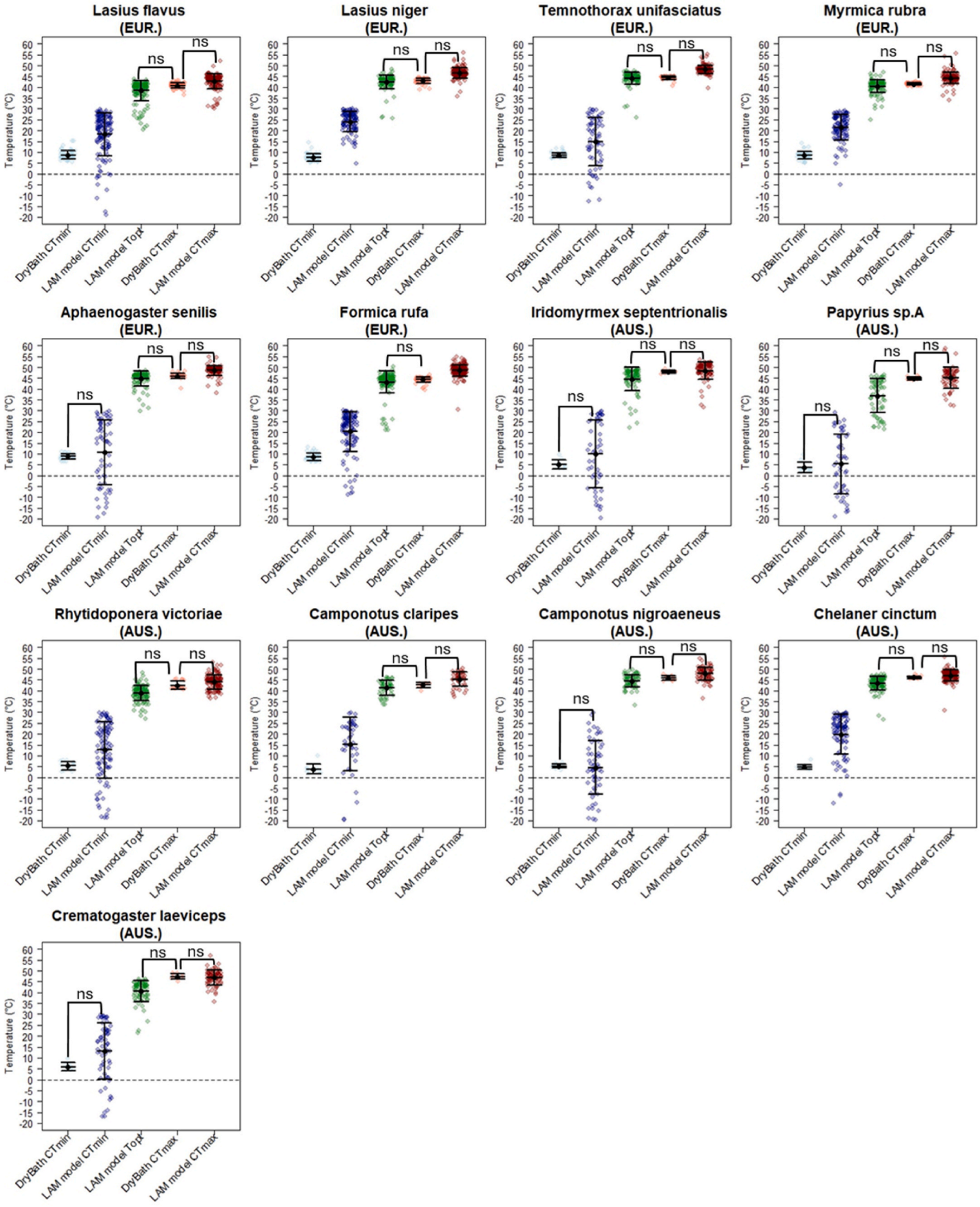

Fig. 2. Scatterplots of temperature estimates for thirteen species across five method-metric combinations. Plot titles indicate genus, species, and sampling origin (EUR or AUS). Each colored point represents an individual ant's temperature value. Metrics include CTmin, CTmax, and Topt, measured using two methods: DryBath and LAM. We statistically compared LAM CT_{min} vs dry bath CT_{min}, LAM CT_{max} vs dry bath CT_{max} and LAM T_{opt} vs dry bath CT_{max}. Comparisons with no significant difference in temperature estimates ($p > 0.05$) are indicated by 'ns.'

Table 3
Estimated marginal means comparisons of temperatures (°C) for species, metric and method interactions using individual level data. Combinations span four species, three distinct metric types (CTmax, CTmin and Topt) and three methods (LAM model, LAM visual and Dry Bath), i.e., LAM visual CTmax compared to dry bath CTmax.

Contrast	Species name	Estimate of difference	Standard error	p-value
Dry Bath CTmax - LAM model CTmax	***Lasius flavus***	**−3.17**	**0.60**	**0.00***
	Formica rufa	**−5.97**	**0.66**	**0.00***
	Lasius niger	**−3.59**	**0.60**	**0.00***
	Myrmica rubra	**−4.57**	**0.55**	**0.00***
Dry Bath CTmax - LAM model Topt	*Lasius flavus*	0.36	0.60	1.00
	Formica rufa	−0.37	0.66	1.00
	Lasius niger	1.05	0.60	0.99
	Myrmica rubra	0.30	0.55	1.00
Dry Bath CTmax -LAM visual CTmax	*Lasius flavus*	−1.59	0.60	0.63
	Formica rufa	**−3.91**	**0.66**	**0.00***
	Lasius niger	**−2.64**	**0.60**	**0.00***
	Myrmica rubra	**−2.88**	**0.55**	**0.00***
Dry Bath CTmin - LAM model CTmin	***Lasius flavus***	**−14.07**	**0.61**	**0.00***
	Formica rufa	**−16.52**	**0.78**	**0.00***
	Lasius niger	**−13.58**	**0.66**	**0.00***
	Myrmica rubra	**−18.35**	**0.60**	**0.00***
Dry Bath CTmin - LAM visual CTmin	*Lasius flavus*	1.04	0.60	1.00
	Formica rufa	0.37	0.59	1.00
	Lasius niger	**2.32**	**0.60**	**0.03***
	Myrmica rubra	0.17	0.60	1.00
LAM model CTmax - LAM visual CTmax	*Lasius flavus*	1.58	0.52	0.35
	Formica rufa	**2.06**	**0.54**	**0.03***
	Lasius niger	0.94	0.53	0.99
	Myrmica rubra	1.69	0.53	0.24
LAM model CTmin - LAM visual CTmin	***Lasius flavus***	**15.11**	**0.54**	**0.00***
	Formica rufa	**16.89**	**0.74**	**0.00***
	Lasius niger	**15.90**	**0.60**	**0.00***
	Myrmica rubra	**18.52**	**0.54**	**0.00***
LAM visual CTmax - LAM model Topt	***Lasius flavus***	**1.95**	**0.52**	**0.05***
	Formica rufa	**3.55**	**0.54**	**0.00***
	Lasius niger	**3.70**	**0.53**	**0.00***
	Myrmica rubra	**3.19**	**0.53**	**0.00***

4. Results

4.1. Comparing thermal metrics between methods

We found that the type of method, metric and species identity influenced estimates of thermal tolerance. Our analysis found significant effects of all model variables on temperature: the method-metric combined factor (ANOVA: df = 4, F = 5635.89, $p < 0.01$), species (ANOVA: df = 12, F = 6.94, $p < 0.01$) and their interaction (ANOVA: df = 48, F = 19.19, $p < 0.01$, Table S3). Similar analysis of visual data for four Welsh species showed the same significance patterns (Table S5).

Across 13 species, T_{opt} and CT_{max} were found to be similar between different methods and on average within 2.59 ± 1.50 °C of each other. However, CT_{min} estimates between different methods varied significantly across species. For all European and Australian species, post-hoc comparisons showed no significant difference in temperatures between T_{opt} and CT_{max} from the dry bath ($p > 0.05$, Table 1, Fig. 2). Visual data also indicated no significant difference between T_{opt} and dry bath CT_{max} temperature estimates for the four Welsh species ($p > 0.05$, Table 3, Fig. 3). In the dataset that included European and Australian species, only *Formica rufa* showed a significant difference in CT_{max} estimates between the LAM and dry bath methods ($p = 0.01$). However, this contrasted with our visual data, which showed that all four Welsh species had significantly higher CT_{max} estimates in the LAM model compared to the dry bath ($p < 0.01$, Table 3). Visual CT_{max} estimates differed significantly from T_{opt} for all species, from dry bath CT_{max} for three species, and from LAM model CT_{max} for one species ($p < 0.05$, Table 3). Upper thermal limits consistently increased in this order: dry bath CT_{max}, LAM T_{opt}, LAM visual CT_{max}, and LAM model CT_{max} (Fig. 2). Estimates of difference between T_{opt} and dry bath CT_{max} produced temperature estimates closer to zero than comparisons between CT_{max} estimates. This finding suggests that T_{opt} is a better predictor of dry bath CT_{max} than LAM model or visual CT_{max} e. 4, Table 1). Comparing CT_{min} between LAM and dry bath methods revealed significant differences in 9 out of 13 species, with larger differences than those seen in upper thermal limit comparisons ($p < 0.05$, Table 1, Figs. 2 and 3). Visual data from four Welsh species supported this trend, showing significant differences in CT_{min} between dry bath and LAM model methods. Visual LAM CT_{min} estimates were significantly lower than the LAM model and more closely matched dry bath CT_{min}. Only *Lasius niger* had a significant difference between visual and dry bath CT_{min} ($p = 0.03$, Table 3, Fig. 3). CT_{min} estimates consistently increased in temperature from dry bath to LAM visual, to LAM model (see Fig. 4).

4.2. Comparing coefficients of variation (CVs) between methods

Australian species were excluded from this analysis due to insufficient numbers of colony replicates, consequently this analysis includes the data for six European species only. Thermal metric, method (LAM versus dry bath) and their interaction had significantly different CVs (ANOVA: $p < 0.05$, Table S4). Compared to the LAM model and dry bath, the visual data also showed a significant difference in CVs between thermal metric, method and their interaction (ANOVA: $p < 0.05$, Table S6). Post-hoc Tukey honest significant differences tests identified that the TPC model of the LAM data is equally reliable at predicting T_{opt} and CT_{max} as the dry bath is at predicting CT_{max}. These findings are attributed to no significant differences in CV value for comparisons between LAM CT_{max} and dry bath CT_{max}, or T_{opt} and dry bath CT_{max} ($p > 0.05$, Table 2, Fig. 5). These results are mirrored in our visual data, with the addition of LAM visual CT_{max} estimates having no significant differences in CV value between dry bath CT_{max}, model CT_{max} or T_{opt} ($p > 0.05$, Table S4). However, for CT_{min} - CT_{min} comparisons across regional datasets, we find that the LAM model is very unreliable compared to the dry bath, where there were broad differences in CV value ($p < 0.05$, Table 2 and S7, Fig. 5 and S3).

4.3. Identifying effect of mass on temperature estimates

Average species body mass showed a weak negative relationship with differences in temperature estimates between contrasts, though this effect was not statistically significant. Linear regression of temperature estimates with average species mass and contrast type (e.g., Dry bath CT_{max} vs LAM model CT_{max}) showed that mass had no impact on estimates of difference across contrasts (estimate = −1.76, df = 43, $p > 0.05$; Table S8).

5. Discussion

We aimed to determine whether the LAM and dry bath methods produced similar physiological estimates when extracting comparable thermal performance metrics. To achieve this, we tested multiple

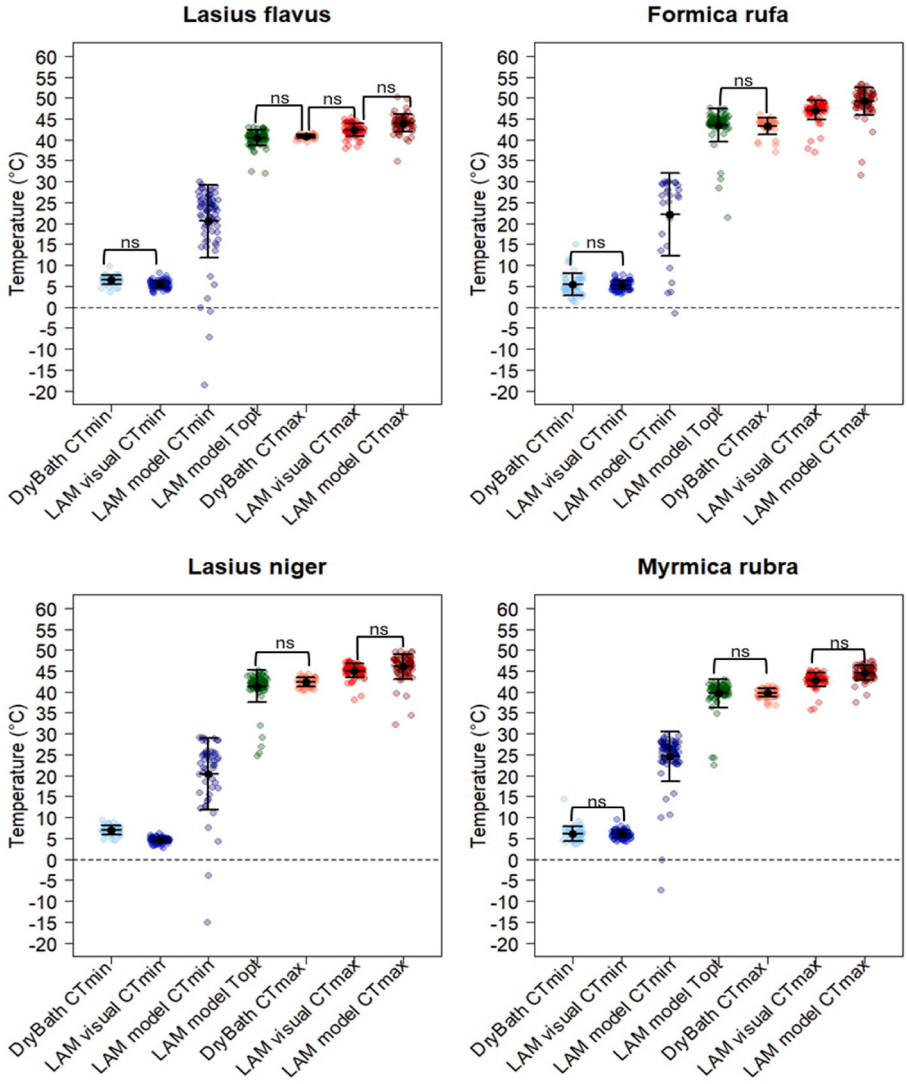

Fig. 3. Scatterplots showing mean and standard deviation bars of temperature (°C) estimates for four species across seven different method-metric combinations. Each plot title corresponds to the genus and species. Each colored point represents an individual ant's temperature value. Metrics include CTmin, CTmax, and Topt, measured using three methods: DryBath, LAM visual observation, and LAM model. We statistically compared LAM CT_{min} vs dry bath CT_{min}, LAM CT_{max} vs dry bath CT_{max} and LAM T_{opt} vs dry bath CT_{max}. Comparisons with no significant difference in temperature estimates ($p > 0.05$) are indicated by 'ns.'

replicated ant species using both methods in parallel across two geographically distinct laboratory environments (Europe and Australia). Additionally, we used visual LAM observations to assess the validity of CT estimates from the LAM model by detecting subtle behavioural and physiological changes not captured by infrared beams and TPC modelling. Our results show that we can accurately and reliably predict dry bath CT_{max} with LAM T_{opt}, but that LAM CT_{max} does not translate to an equivalent dry bath CT_{max}. From these findings, we suggest that T_{opt} reflects peak stress rather than optimal thermal performance. For cold thermal limits, the LAM method is considerably less reliable than the dry bath when quantifying CT_{min}. We show that the LAM method is highly effective for predicting upper thermal limits but not in the way we originally anticipated: LAM T_{opt} is equivalent to a traditional metric of CT_{max} (produced by a dry bath), indicating it could represent a stressful temperature rather than an optimal temperature for the ants. These findings highlight that the traits being assessed by each method are physiologically different and responding to temperature differently. However, we maintain that the LAM will be a valuable tool for quantifying the upper thermal limits of small ectotherms at speed. The LAM surpasses the dry bath in several ways, as it can generate continuous data in the form of TPCs from which we can reliably extract upper thermal limits (Fig. 6). Furthermore, compared to the dry bath, the LAM is objective, has higher throughput and lower labour costs due to automation. We acknowledge that the LAM and the study of different physiological traits should not replace existing methods or metrics, but rather complement our current toolkit. By doing so, they can enhance the efficiency, accuracy, and objectivity of thermal biology assessments, leading to new insights.

For the LAM method, our first key result is that T_{opt} reflects 'traditional' measures of CT_{max} and does this more reliably than TPC model estimates of CT_{max} (Figs. 2 and 3, Table 1). Across species and regions, our data follows a consistent pattern of lowest to highest temperatures in the following order: LAM T_{opt} = dry bath CT_{max} < LAM visual CT_{max} < LAM CT_{max} (Fig. 2). Between European and Australian datasets, there are differences in significance between dry bath CT_{max} and LAM CT_{max}. The Welsh red wood ant species, *Formica rufa,* is the only species with significant difference in temperature estimate of CT_{max} between methods (Table 1). Whereas, for the visual data of the Welsh species only, there are significant differences between dry bath CT_{max} and LAM CT_{max} estimates for all four species (Table 3). Differences in sample sizes may partially account for this discrepancy. The visual data included only the four Welsh species, each with only five colonies, which is about half

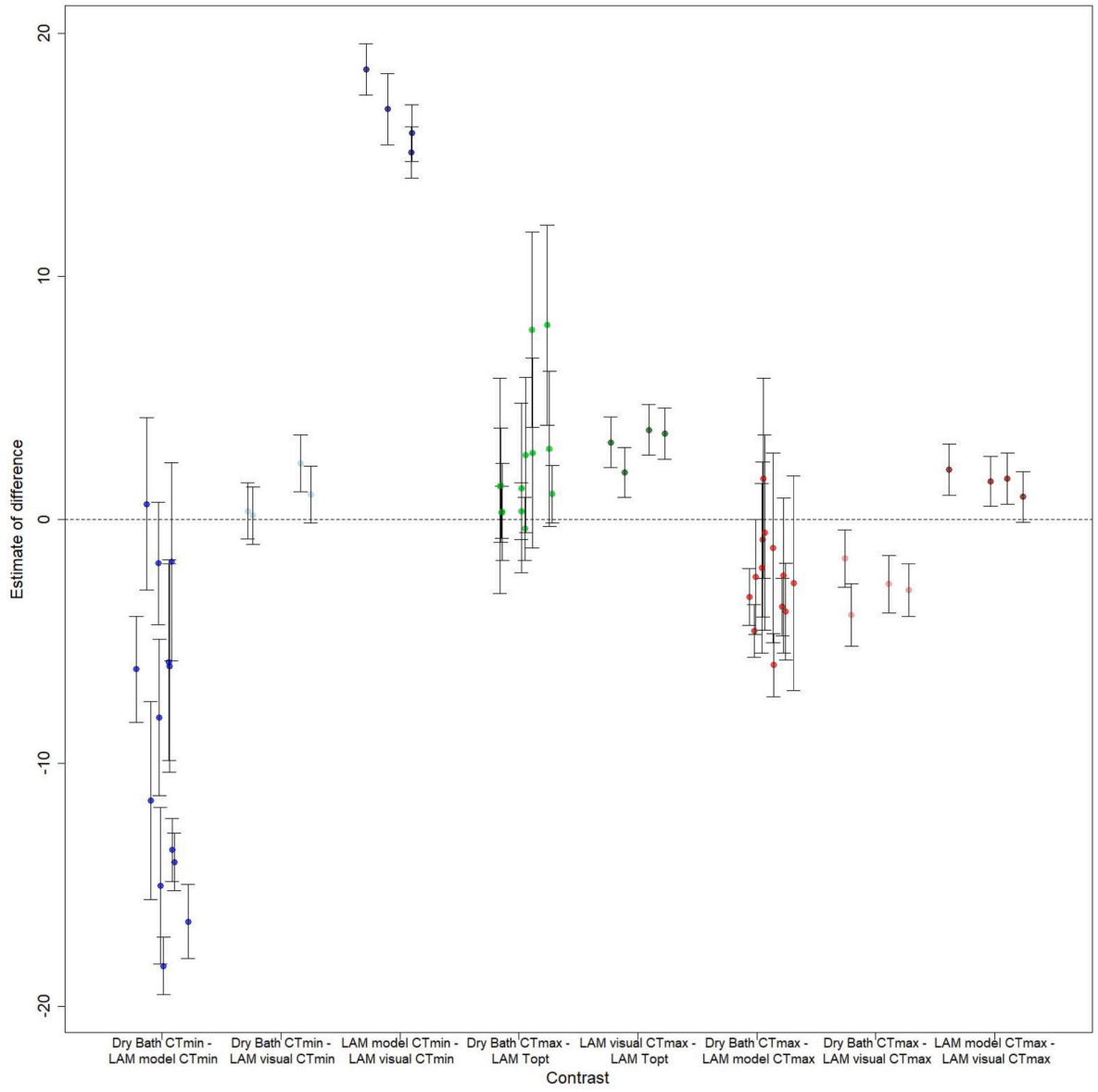

Fig. 4. Forest plots displaying the mean and standard deviation bars of estimate of difference by contrast type. Individual points represent species. Contrast types include Dry bath CT_{max} and CT_{min}, LAM visual CT_{max} and CT_{min}, and LAM model T_{opt}, CT_{max} and CT_{min}.

the number of colonies compared to the European species. Generally, a sample size greater than 10 is more representative when trying to detect broad-scale patterns of trait variation, especially in populations or species with large physiological and behavioural variation such as ants (Herrando-Pérez et al., 2019; Duffy et al., 2021). Many studies also document the positive influence of body size on thermal tolerance (Hurlbert et al., 2008; Johnson and Stahlschmidt, 2020). However, we identified that average species mass had no impact on the difference in temperature estimates between different metric and method comparisons (e.g., Dry bath CT_{max} vs LAM model CT_{max}) (estimate = −1.76, df = 43, $p > 0.05$; Table S8). Several papers document that the plasticity of upper thermal limits enables ectotherms to survive in fluctuating thermal conditions (Allen et al., 2016; Ben-Yosef et al., 2021; Turriago et al., 2023). An acclimation effect may be increasing the LAM CT_{max} results relative to the dry bath in European assays, where the same individuals were assessed for CT_{min} and CT_{max} in the LAM, but fresh individuals were used for each assay in the dry bath assays. Whereas, Australian dry bath assays ramped down and up on same individuals, where individuals that died on the CT_{min} ramp were excluded. In a study on neotropical tadpoles, acclimation to higher temperatures and thermal fluctuations resulted in higher CT_{max} values (Turriago et al., 2023). We consider that thermal fluctuation of our ramping down to CT_{min} and then up to CT_{max} may also be influencing higher CT_{max} values in the LAM than in the dry bath.

Our second key finding is that T_{opt} reflects 'peak' stress (σ_{peak}) or the fastest running speed before exhaustion, rather than 'optimal' thermal performance. We use the Greek letter σ to represent stress, as is commonly done in mechanics (Swallowe, 1999). This hypothesis is supported by our visual observations, which show that movement capacity varies across methods and among species. The tubes that individual ants are held in with the dry bath are smaller and constrain movement more compared to those used in the LAM. This practical constraint likely induces stress, which may cause T_{opt} and CT_{max} to be underestimated in the dry bath or, conversely, overestimated in the LAM. While there is limited literature on how restricted space affects physiological stress responses in laboratory experiments, it is widely accepted that larger organisms need more space and oxygen than smaller ones (Pörtner and Knust, 2007; Gangloff and Telemeco, 2018; Duncan et al., 2023). Therefore, larger organisms are likely to be more physically constrained and with lower relative oxygen availability in

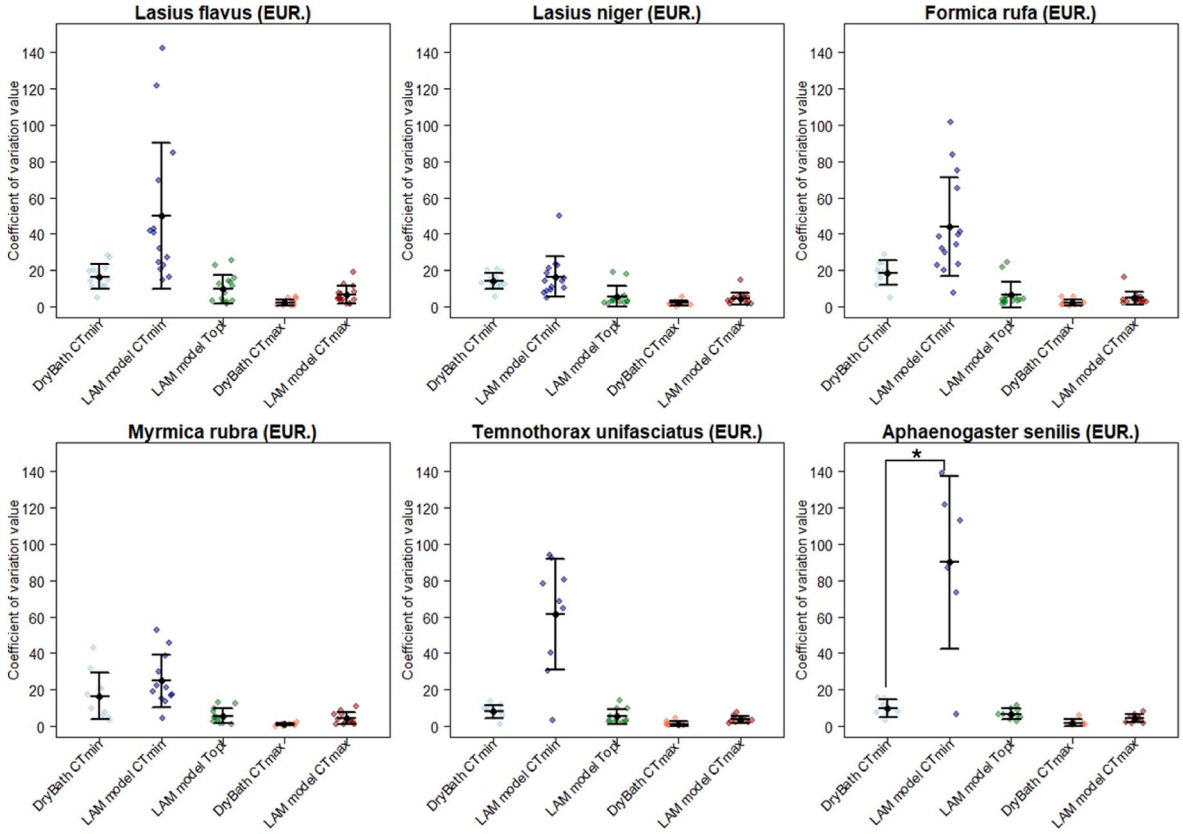

Fig. 5. Scatterplots showing mean and standard deviation bars for coefficient of variation (CVs) for six species across six different method-metric combinations. Each colored point represents a colony average CV for a specific method and metric. There are three unique metrics (CT_{min}, CT_{max}, and T_{opt}) and two unique methods (DryBath and LAM model). We statistically compared LAM CT_{min} vs dry bath CT_{min}, LAM CT_{max} vs dry bath CT_{max} and LAM T_{opt} vs dry bath CT_{max}. Comparisons with significant differences in coefficient of variation ($p < 0.05$) are indicated by '*.'

Eppendorf tubes compared to LAM tubes. LAM visual observations also show that individuals cross the infrared beam despite loss of righting response, potentially causing an overestimation of T_{opt} and CT_{max} in LAM model estimates compared to the dry bath. The hierarchal mechanisms of thermal limitation (HMTL) framework indicates that under extreme hypoxia (less than 10 kPa), active organisms experience T_{opt} and CT_{max} at lower temperatures than under normoxia, where there is no effect on the temperature performance curve (TPC) characteristics. Under moderate hypoxia (10–20 kPa), only T_{opt} is reduced (Gangloff and Telemeco, 2018). We suggest that limited space availability for movement in dry bath Eppendorfs may be shaping organism thermal performance through changes to oxygen availability. The dynamic interactions between stressful temperatures, oxygen limitation, body mass, and space availability contribute to early-onset stress responses. These factors together lead to underestimations in T_{opt} and CT_{max} in the dry bath compared to the LAM. Some research has linked thermal stress to increased escape behaviours. For example, beetles show an increase in clicking due to escape behaviour caused by subcritical temperatures (Riddell et al., 2023). In our experiment, the fastest running speed before exhaustion may represent an equivalent behavioural marker to clicking in the beetles, i.e., a marker of peak stress. However, there is limited information available on stress assays and their mechanistic underpinnings compared to TPCs to support this claim, making this a core target area for future research.

The third key finding is that Australian dry bath CT_{max} and LAM model CT_{max} are closer in temperature than for the same comparisons for European species (Figs. 2 and 3, Table 1). Slight differences in experimental design could be key to understanding the differences in CT_{max} estimates between regional datasets. Observer bias in detection of loss of righting response may lead to variation in identification of CT_{max} in dry bath experiments. This is a core issue of the dry bath method that is overcome with the LAM, where the LAM is automated and does not require manual adjustment of ramping protocol or loss of righting response detection. Subsequently the LAM is at lower risk of human error and subjectivity compared to the dry bath. Australian samples were also more 'field-fresh' at processing, as they were typically processed within one day of collection, compared to up to seven days for Welsh species and longer for non-Welsh European species, which were posted to the UK from Spain and Switzerland.

Our fourth major finding is that CT_{min} estimates from the LAM do not match those from the dry bath (Fig. 2, Table 1). The unreliability of LAM CT_{min} estimates arises from the system's inability to quantify low activity levels (Fig. 5). Our visual observations confirm that low activity often occurs at the ends of the tube outside the beam's range, in so-called 'dead space'. The inability to quantify low levels of activity in the current LAM system raise questions about how we can overcome this limitation. Video tracking could be used to quantify low levels of activity seen at CT_{min}, but it involves significant effort and cost to set up the appropriate videography system (Anderson et al., 2023; Terlau et al., 2023). This is especially true for capturing movement patterns of organisms like ants, which vary greatly in phenotype, affecting the quality of video tracking. To address these limitations, we suggest implementing a multi-beam LAM system. This enhanced design would feature several beams positioned at regular intervals along the length of the tubes. The primary advantage of this approach is reduction of 'dead space' where ants may not cross a single beam, enabling improved detection of low levels of activity and accuracy in quantifying lower thermal limits. This proposed system offers a more efficient and cost-effective solution for measuring low levels of activity than video tracking, particularly in organisms with diverse morphological characteristics.

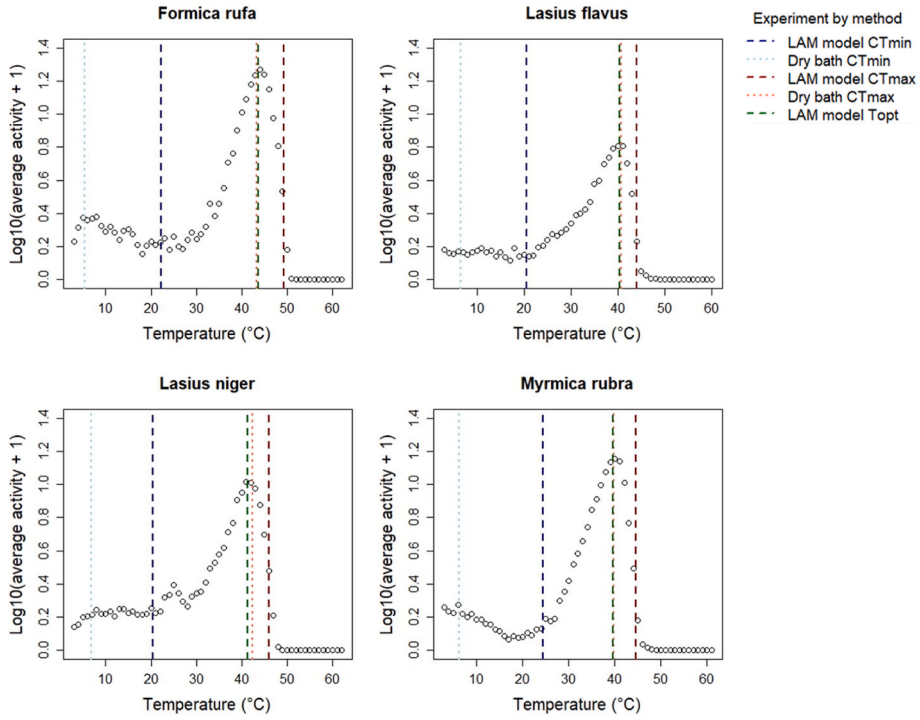

Fig. 6. Example thermal performance curves for four Welsh ant species with critical temperature estimates. Each panel illustrates the average locomotor activity of a species across a temperature gradient (°C), generating thermal performance curves (TPCs). Overlaid on each panel are the average critical thermal minimum (CT_{min}), critical thermal maximum (CT_{max}), and thermal optimum (T_{opt}) for each species. Thermal metrics were derived from two methods: the dry bath and the LAM model.

Additional caveats to the LAM method include a lack of incorporation of other stress factors associated with temperature, namely humidity, which could influence the reliability of CT estimates. Some studies argue that a loss of righting response occurs at lower temperatures under more ecologically relevant experimental conditions, for example with realistic humidity levels, suggesting that the thermal tolerance range is narrower than what we have predicted in our current LAM-incubator approach (Terblanche et al., 2007; Kovacevic et al., 2019). Our findings confirm that methodological approach can have a significant influence on CTs and their ecological relevance (Terblanche et al., 2007; Kovacevic et al., 2019). Fortunately, the LAM-incubator method has strong scope for incorporating humidity as an interacting variable, which cannot easily be manipulated in dry baths. In incubators, humidity can be altered manually by using different saturated salt solutions to absorb or release moisture to achieve a desired humidity (Eggert, 2022), or automatically with an incubator that has an inbuilt capacity to modify humidity.

Our findings underscore the method's capability to rapidly and accurately estimate upper thermal limits from locomotor activity. Compared to traditional dry bath methods, the LAM-incubator offers key advantages, including continuous, objective, and high-throughput thermal data generation via automation. Each LAM unit can assay 32 individuals every 3 h, generating large amounts of continuous data without the need for an observer. Additionally, multiple LAM units can be connected together to scale up data collection. In comparison, the dry bath method can only assay 15 individuals in 1.5 h, providing single-point data and requiring an observer. We have also generated thermal stress curves for woodlice and spiders using the LAM, highlighting its applicability to a suite of small mobile ectotherms (Fig. S4). By using the LAM-incubator method to scale up data collection, we will enable faster, higher quality comparisons of intra- and inter-species differences in thermal performance across broader biogeographic areas and time scales. We hope the LAM-incubator method will be a valuable tool for accurately forecasting whether species will move, adapt, or die as climate change intensifies (Nascimento et al., 2022).

CRediT authorship contribution statement

Sophie L. Mallett: Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Lily Leahy:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Ian P. Vaughan:** Writing – review & editing, Supervision. **Tristan Klaftenberger:** Resources. **Xim Cerdá:** Writing – review & editing, Resources. **Lucy J. Wheatley:** Resources, Methodology. **Kester Leyshon:** Resources, Methodology. **Shane King:** Resources, Methodology. **Will Dawson:** Resources, Methodology. **Kelsey Harrendence:** Resources, Methodology. **Icaro Wilker:** Resources. **Tom R. Bishop:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Data accessibility statement

All **data** are available from https://doi.org/10.6084/m9.figshare.27846969.

All **code** are available from https://doi.org/10.6084/m9.figshare.28668341.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the lead author used ChatGPT in order to make the text more concise. After using this tool, the authors reviewed and edited the content as needed and takes full responsibility for the content of the published article.

Funding sources

This work was supported by the School of Biosciences at Cardiff University and the Royal Society (RGS\R1\221207).

Declaration of competing interest

There are no competing interests to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2025.104127.

References

Ali, S., et al., 2019. Comparison of upper sublethal and lethal temperatures in three species of rice planthoppers. Sci. Rep. 9 (1), 16191. https://doi.org/10.1038/s41598-019-52034-7.

Allen, J.L., et al., 2016. Interactions between rates of temperature change and acclimation affect latitudinal patterns of warming tolerance. Conservat. Physiol. 4 (1), cow053. https://doi.org/10.1093/conphys/cow053.

Anderson, R.O., et al., 2023. Linking physiology and climate to infer species distributions in Australian skinks. J. Anim. Ecol. 92 (10), 2094–2108. https://doi.org/10.1111/1365-2656.14000.

Angilletta, M.J., 2006. Estimating and comparing thermal performance curves. J. Therm. Biol. 31 (7), 541–545. https://doi.org/10.1016/j.jtherbio.2006.06.002.

Angilletta, M.J., Niewiarowski, P.H., Navas, C.A., 2002. The evolution of thermal physiology in ectotherms. J. Therm. Biol. 27 (4), 249–268. https://doi.org/10.1016/S0306-4565(01)00094-8.

Awde, D.N., et al., 2020. High-throughput assays of critical thermal limits in insects. JoVE J. 160, e61186. https://doi.org/10.3791/61186.

Ben-Yosef, M., et al., 2021. Effects of thermal acclimation on the tolerance of bactrocera zonata (Diptera: tephritidae) to hydric stress. Front. Physiol. 12. https://doi.org/10.3389/fphys.2021.686424.

Brown, G.P., Bishop, C.A., Brooks, R.J., 1994. Growth rate, reproductive output, and temperature selection of snapping turtles in habitats of different productivities. J. Herpetol. 28 (4), 405–410. https://doi.org/10.2307/1564950.

Cecchetto, N.R., Medina, S.M., Ibargüengoytía, N.R., 2020. Running performance with emphasis on low temperatures in a Patagonian lizard, Liolaemus lineomaculatus. Sci. Rep. 10 (1), 14732. https://doi.org/10.1038/s41598-020-71617-3.

Chown, S.L., et al., 2009. Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. Funct. Ecol. 23 (1), 133–140. https://doi.org/10.1111/j.1365-2435.2008.01481.x.

Clavijo-Baquet, S., et al., 2022. How do ectotherms perform in cold environments? Physiological and life-history traits in an Andean viviparous lizard. Front. Ecol. Evol. 10. https://doi.org/10.3389/fevo.2022.974968.

Clusella-Trullas, S., Blackburn, T.M., Chown, S.L., 2011. Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. Am. Nat. 177 (6), 738–751. https://doi.org/10.1086/660021.

Corley, R.B., Dawson, W., Bishop, T.R., 2023. A simple method to account for thermal boundary layers during the estimation of CTmax in small ectotherms. J. Therm. Biol. 116, 103673. https://doi.org/10.1016/j.jtherbio.2023.103673.

Deutsch, C.A., et al., 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proc. Natl. Acad. Sci. 105 (18), 6668–6672. https://doi.org/10.1073/pnas.0709472105.

Duffy, G.A., et al., 2021. Adequate sample sizes for improved accuracy of thermal trait estimates. Funct. Ecol. 35 (12), 2647–2662. https://doi.org/10.1111/1365-2435.13928.

Duncan, M.I., et al., 2023. Oxygen availability and body mass modulate ectotherm responses to ocean warming. Nat. Commun. 14, 3811. https://doi.org/10.1038/s41467-023-39438-w.

Eggert, G., 2022. Saturated salt solutions in showcases: humidity control and pollutant absorption. Heritage Sci. 10 (1), 54. https://doi.org/10.1186/s40494-022-00689-3.

Gangloff, E.J., Telemeco, R.S., 2018. High temperature, oxygen, and performance: insights from reptiles and Amphibians. Integr. Comp. Biol. 58 (1), 9–24. https://doi.org/10.1093/icb/icy005.

Gotelli, N.J., et al., 2011. Counting Ants (Hymenoptera: Formicidae): Biodiversity Sampling and Statistical Analysis for Myrmecologists. College of Arts and Sciences Faculty Publications [Preprint]. https://scholarworks.uvm.edu/casfac/95.

Guo, F., et al., 2020. Activity niches outperform thermal physiological limits in predicting global ant distributions. J. Biogeogr. 47 (4), 829–842. https://doi.org/10.1111/jbi.13799.

Hawkins, W.D., DuRant, S.E., 2020. Applications of machine learning in behavioral ecology: quantifying avian incubation behavior and nest conditions in relation to environmental temperature. PLoS One 15 (8), e0236925. https://doi.org/10.1371/journal.pone.0236925.

Herrando-Pérez, S., et al., 2019. Intraspecific variation in lizard heat tolerance alters estimates of climate impact. J. Anim. Ecol. 88 (2), 247–257. https://doi.org/10.1111/1365-2656.12914.

Hölldobler, B., Wilson, E.O., 1990. The Ants. Belknap Press, Cambridge, MA.

Huey, R.B., et al., 1992. A method for rapid measurement of heat or cold resistance of small insects. Funct. Ecol. 6 (4), 489–494. https://doi.org/10.2307/2389288.

Huey, R.B., Bennett, A.F., 1987. Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. Evolution 41 (5), 1098–1115. https://doi.org/10.2307/2409194.

Hurlbert, A.H., Ballantyne Iv, F., Powell, S., 2008. Shaking a leg and hot to trot: the effects of body size and temperature on running speed in ants. Ecol. Entomol. 33 (1), 144–154. https://doi.org/10.1111/j.1365-2311.2007.00962.x.

Ion Scotta, M., et al., 2021. Genetic variability, population differentiation, and correlations for thermal tolerance indices in the minute wasp, Trichogramma cacoeciae. Insects 12 (11), 1013. https://doi.org/10.3390/insects12111013.

Johnson, D.J., Stahlschmidt, Z.R., 2020. City limits: heat tolerance is influenced by body size and hydration state in an urban ant community. Ecol. Evol. 10 (11), 4944–4955. https://doi.org/10.1002/ece3.6247.

Jørgensen, L.B., et al., 2021. A unifying model to estimate thermal tolerance limits in ectotherms across static, dynamic and fluctuating exposures to thermal stress. Sci. Rep. 11, 12840. https://doi.org/10.1038/s41598-021-92004-6.

Kaspari, M., et al., 2016. Thermal adaptation and phosphorus shape thermal performance in an assemblage of rainforest ants. Ecology 97 (4), 1038–1047.

Kellermann, V., et al., 2019. Comparing thermal performance curves across traits: how consistent are they? J. Exp. Biol. 222 (11), jeb193433. https://doi.org/10.1242/jeb.193433.

Kong, J.D., et al., 2016. Novel applications of thermocyclers for phenotyping invertebrate thermal responses. Methods Ecol. Evol. 7 (10), 1201–1208. https://doi.org/10.1111/2041-210X.12589.

Kovacevic, A., Latombe, G., Chown, S.L., 2019. Rate dynamics of ectotherm responses to thermal stress. Proc. Biol. Sci. 286, 20190174. https://doi.org/10.1098/rspb.2019.0174, 1902.

Laursen, S.F., et al., 2021. Contrasting manual and automated assessment of thermal stress responses and larval body size in black soldier flies and houseflies. Insects 12 (5), 380. https://doi.org/10.3390/insects12050380.

Leong, C.-M., Tsang, T.P.N., Guénard, B., 2022. Testing the reliability and ecological implications of ramping rates in the measurement of Critical Thermal maximum. PLoS One 17 (3), e0265361. https://doi.org/10.1371/journal.pone.0265361.

MacLean, H.J., Hjort Hansen, J., Sørensen, J.G., 2022. Validating the automation of different measures of high temperature tolerance of small terrestrial insects. J. Insect Physiol. 137, 104362. https://doi.org/10.1016/j.jinsphys.2022.104362.

McCain, C.M., Garfinkel, C.F., 2021. Climate change and elevational range shifts in insects. Curr. Opin. Insect Sci. 47, 111–118. https://doi.org/10.1016/j.cois.2021.06.003.

Nascimento, G., Câmara, T., Arnan, X., 2022. Critical thermal limits in ants and their implications under climate change. Biol. Rev. 97 (4), 1287–1305. https://doi.org/10.1111/brv.12843.

Oberg, E.W., Del Toro, I., Pelini, S.L., 2012. Characterization of the thermal tolerances of forest ants of New England. Insectes Sociaux 59 (2), 167–174. https://doi.org/10.1007/s00040-011-0201-y.

Padfield, D., O'Sullivan, H., Pawar, S., 2021. *rTPC* and *nls.multstart* : a new pipeline to fit thermal performance curves in r. Methods Ecol. Evol. 12 (6), 1138–1143. https://doi.org/10.1111/2041-210X.13585.

Parr, C.L., Bishop, T.R., 2022. The response of ants to climate change. Glob. Change Biol. 28 (10), 3188–3205. https://doi.org/10.1111/gcb.16140.

Parratt, S.R., et al., 2021. Temperatures that sterilize males better match global species distributions than lethal temperatures. Nat. Clim. Change 11 (6), 481–484. https://doi.org/10.1038/s41558-021-01047-0.

Penick, C.A., et al., 2017. Beyond thermal limits: comprehensive metrics of performance identify key axes of thermal adaptation in ants. Funct. Ecol. 31 (5), 1091–1100. https://doi.org/10.1111/1365-2435.12818.

Perez-Galvez, F.R., et al., 2023. Scoring thermal limits in small insects using open-source, computer-assisted motion detection. J. Exp. Biol. 226 (22), jeb246548. https://doi.org/10.1242/jeb.246548.

Pörtner, H.O., Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315 (5808), 95–97. https://doi.org/10.1126/science.1135471.

Powell, S., Franks, N.R., 2007. How a few help all: living pothole plugs speed prey delivery in the army ant Eciton burchellii. Anim. Behav. 73 (6), 1067–1076. https://doi.org/10.1016/j.anbehav.2006.11.005.

Prather, R.M., et al., 2018. Using metabolic and thermal ecology to predict temperature dependent ecosystem activity: a test with prairie ants. Ecology 99 (9), 2113–2121. https://doi.org/10.1002/ecy.2445.

R Core Team, 2023. R: a language and environment for statistical computing (No Title) [Preprint], version 4.3.2. https://cir.nii.ac.jp/crid/1370294721063650048. Accessed: 20 February 2024.

Riddell, E.A., Mutanen, M., Ghalambor, C.K., 2023. Hydric effects on thermal tolerances influence climate vulnerability in a high-latitude beetle. Glob. Change Biol. 29 (18), 5184–5198. https://doi.org/10.1111/gcb.16830.

Roeder, K.A., Roeder, D.V., Bujan, J., 2021. Ant thermal tolerance: a review of methods, hypotheses, and sources of variation. Ann. Entomol. Soc. Am. 114 (4), 459–469. https://doi.org/10.1093/aesa/saab018.

Rolandi, C., et al., 2018. Genetic variation for tolerance to high temperatures in a population of Drosophila melanogaster. Ecol. Evol. 8 (21), 10374–10383. https://doi.org/10.1002/ece3.4409.

Rumpf, S.B., et al., 2019. Elevational rear edges shifted at least as much as leading edges over the last century. Global Ecol. Biogeogr. 28 (4), 533–543. https://doi.org/10.1111/geb.12865.

Scaccini, D., Duso, C., Pozzebon, A., 2019. Lethal effects of high temperatures on Brown marmorated stink bug adults before and after overwintering. Insects 10 (10), 355. https://doi.org/10.3390/insects10100355.

Schulte, P.M., Healy, T.M., Fangue, N.A., 2011. Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. Integr. Comp. Biol. 51 (5), 691–702. https://doi.org/10.1093/icb/icr097.

Shik, J.Z., et al., 2019. Evidence for locally adaptive metabolic rates among ant populations along an elevational gradient. J. Anim. Ecol. 88 (8), 1240–1249. https://doi.org/10.1111/1365-2656.13007.

Ślipiński, P., et al., 2021. The influence of age and development temperature on the temperature-related foraging risk of Formica cinerea ants. Behav. Ecol. Sociobiol. 75 (7), 107. https://doi.org/10.1007/s00265-021-03029-w.

Swallowe, G.M., 1999. Stress and strain. In: Swallowe, G.M. (Ed.), Mechanical Properties and Testing of Polymers: an A–Z Reference. Springer Netherlands, Dordrecht, pp. 219–224. https://doi.org/10.1007/978-94-015-9231-4_48.

Terblanche, J.S., et al., 2007. Critical thermal limits depend on methodological context. Proc. Biol. Sci. 274 (1628), 2935–2943. https://doi.org/10.1098/rspb.2007.0985.

Terblanche, J.S., et al., 2011. Ecologically relevant measures of tolerance to potentially lethal temperatures. J. Exp. Biol. 214 (22), 3713–3725. https://doi.org/10.1242/jeb.061283.

Terlau, J.F., et al., 2023. Predicting movement speed of beetles from body size and temperature. Movement Ecol. 11 (1), 27. https://doi.org/10.1186/s40462-023-00389-y.

TriKinetics (no date). Available at: https://trikinetics.com/(Accessed: 26 October 2023). .

Turriago, J.L., et al., 2023. The time course of acclimation of critical thermal maxima is modulated by the magnitude of temperature change and thermal daily fluctuations. J. Therm. Biol. 114, 103545. https://doi.org/10.1016/j.jtherbio.2023.103545.

Urban, M.C., 2015. Accelerating extinction risk from climate change. Science 348 (6234), 571–573. https://doi.org/10.1126/science.aaa4984.

Vermaak, M., et al., 2025. Assessing the potential for predator-prey interactions in mesofaunal arthropod communities through temperature dependence of locomotion. J. Therm. Biol. 128, 104084. https://doi.org/10.1016/j.jtherbio.2025.104084.

Vermeulen, C.J., Bijlsma, R., 2004. Changes in mortality patterns and temperature dependence of lifespan in Drosophila melanogaster caused by inbreeding. Heredity 92 (4), 275–281. https://doi.org/10.1038/sj.hdy.6800412.

Wagner, D.L., et al., 2021. Insect decline in the Anthropocene: death by a thousand cuts. Proc. Natl. Acad. Sci. U. S. A 118 (2), e2023989118. https://doi.org/10.1073/pnas.2023989118.

Wilson, E.O., 1976. The organization of colony defense in the ant Pheidole dentata mayr (Hymenoptera: formicidae). Behav. Ecol. Sociobiol. 1 (1), 63–81. https://doi.org/10.1007/BF00299953.

Zattara, E.E., Aizen, M.A., 2021. Worldwide occurrence records suggest a global decline in bee species richness. One Earth 4 (1), 114–123. https://doi.org/10.1016/j.oneear.2020.12.005.