Department of Ecology & Evolutionary Biology

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How I Wrote My Prospectus

My prospectus started in the fall of my second year from a list of half-formed ideas and several stressful meetings with my advisor – whom I feared would find that list the silliest, least thought-provoking, impractical thing he'd ever read. While those fears were, on the whole, unfounded, spending a few hours talking over where my areas of interest overlapped with notable gaps in the literature and the skillset of the lab was crucial for laying out the plan moving forward. Together, we worked out which area I would likely have the most time to flesh out some preliminary results for by the spring semester, which ideas overlapped well with upcoming grants, and what parts of the research I was most interested in completing. I then spent a month or so on my own writing out a short version of the prospectus, which I passed back and forth with my advisor for edits before the pre-prospectus meeting with my full committee in February. That meeting provided some valuable insights on what needed to be expanded or changed from the original document, plus a sizable reading list of key literature that might be relevant to include moving forward. The next few months were spent generating initial results, going over the reading list to incorporate those ideas into my thinking and into the document, and fleshing out my research plan before sending the committee the final document in May.

Advice for Prospectus Writers

1. Collaborate with your advisor on ideas. They know the literature and the skillset of the lab much better than you, and have most likely been on enough committees to offer good advice about which of your project ideas work best together and for the prospectus.
2. Seek feedback from the other students in the department. Passing written prospectuses between members of my cohort was a great sanity check for all of us. Presenting my prospectus slides in speakeasy was so helpful for building confidence, and several people whose advisors were on my committee were able to give me very detailed and practical advice about their expectations.
3. Don't panic about not having too much preliminary research. Our department is not set up to require that; given my teaching schedule and how new my ideas were when I started writing, most of the preliminary data I did have was more a proof of concept than anything concrete. This was not an issue for the committee, all of whom were much more focused on making sure ideas had a firm basis and projects asked interesting questions.
4. Meet with your other committee members individually beforehand if you can. I did not do this, and it would have been extremely helpful for me to understand what they were looking for from my final prospectus and to know what they wanted me to get out of the readings they assigned me.
5. Try to keep some perspective about what this is. The goal of the prospectus is to help you come up with a good plan, get some expert feedback on it, and demonstrate some key

knowledge in the field. All of those things will help you moving forward, even if your dissertation plan ends up evolving a lot from what you end up presenting pre-candidacy – mine has changed a ton, but I still refer back to the document and my notes from the committee meeting frequently for citations and ideas!

Heat Stress & Population Forecasting

Incorporating mechanistic death rates, population dynamics, and the variability of thermal performance into the risk assessments of the Anthropocene

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Prospectus – Written Document
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May 22, 2023

SUMMARY: As ecosystems around the world face increasingly warm and variable climates, predictions about the impacts of changing temperatures on the biota experiencing them remain uncertain. My dissertation aims to improve these forecasting efforts by combining mechanistic ecological modeling with established biological metrics. In Chapter 1, I incorporate the different physiological causes of death into mechanistic thermal performance curves and dynamic population models to extend our predictive capacity into the range of stressful temperatures. Chapter 2 then explores the population-level costs of different thermal acclimations and adaptations to determine optimal strategies and their feasibility in changing thermal landscapes. In the remaining chapters, I consider the added complications of deviations from predicted thermal performance due to confounding ecological factors (Chapter 3) and spatiotemporal variation (Chapter 4). By informing comparisons with established models, predictions about thermal risks, and hypotheses about the eco-evolutionary theory of thermal performance curves, these modeling efforts will contribute crucial advancements to translating our rapidly growing empirical understanding of thermal performance into the theory required to meet the urgent conservation needs of today's changing climate.

INTRODUCTION & BACKGROUND

Forecasting the impacts of climate change on organisms around the world is a critical goal of current ecology. However, many of our best predictive models rely on faulty extrapolations and simplifications about the impacts of temperature—particularly its hottest extremes—on individuals, populations, and communities (e.g., Deutsch et al., 2008; Pinsky et al., 2019; Vasseur et al., 2014; Woods et al., 2018). Heat waves, one of the most prominent drivers of acute extinction events (Grant et al., 2017; Harley and Paine, 2009; Jørgensen et al., 2022; McKechnie and Wolf, 2010; Parmesan et al., 2000; Pörtner et al., 2022) (e.g., Harvell et al., 2019; Rasmont and Iserbyt, 2012; Thomsen et al., 2019; Welbergen et al., 2008), are projected to globally increase in intensity, frequency, and duration over the next century (Ganguly et al., 2009; Meehl and Tebaldi, 2004; Pörtner et al., 2022; Rahmstorf and Coumou, 2011). Given the disproportionate impact these hot temperatures will have on population fitness and subsequent extinction and extirpation risks, better incorporation of their consequences into our forecasting models is imperative.

The aim of this project is to improve our ability to incorporate those consequences into predictions made with thermal performance curves. Thermal performance curves, or TPCs, show the relationship between temperature and performance (Angilletta, 2009; Huey and Stevenson, 1979). Generally, TPCs are measured exclusively in ectotherms and are unimodal and left-skewed, with x-intercepts at the critical thermal minimum and maximum (CT_{min} and CT_{max}, respectively) enclosing the organism's thermal breadth (T_{breadth}) and a peak at the thermal optimum (T_{opt}) (Angilletta, 2009) (Fig 1). The independent variable of a TPC is either an organism's external experienced temperature or internal body temperature; these temperatures are often assumed equal in ectotherms, but in practice are decoupled by temperature variation and thermoregulation (e.g., Huey et al., 2012; Kearney et al., 2009; Sears et al., 2016). The dependent variable of a TPC is either:

a) **performance**: a metric of organism functionality which changes, usually rapidly and reversibly, with respect to temperature, such as sprint speed. Most performance metrics are feasible to collect experimentally, bounded from below by zero, and of great interest in evolutionary biology (Angilletta, 2009).
b) or **fitness**: a metric of population success which changes with respect to temperature, defined in this context as *Malthusian fitness*, or the intrinsic population growth rate (r) equivalent to the instantaneous birth rate (b) minus the instantaneous death rate (d), absent of density dependent factors (Amarasekare and Savage, 2012; this contrasts with defining fitness as lifetime reproductive output, which is less appropriate in the models used herein). Fitness metrics are typically difficult to collect experimentally, not bounded below by zero, and of great interest in population ecology.

These metrics of performance and fitness are sometimes naively equated, but that conversion is usually nontrivial due to disparate assumptions and bounds, as well as the uncorrelated, non-linear thermal dependences of birth and death rates (Angilletta, 2009; Irschick and Higham, 2016; Sinclair et al., 2016).

In the context of forecasting, it is critical to understand the theoretical connection between experimentally measured TPCs and their broader ecological relevance: how do we arrive at the relationship between temperature and fitness, and what should we understand that relationship to mean? Direct connections between performance metrics and fitness can be tenuous, but their

association has a strong theoretical backbone. The ubiquitous unimodal left-skew of TPCs arises not only from curve-fitting experimental datapoints, but also from summing realistic birth and death curves (Amarasekare, 2015; Amarasekare and Savage, 2012; Vinton and Vasseur, 2022). The characteristic temperature dependence of birth across many taxa is symmetric and unimodal (e.g., Carrière and Boivin, 1997; Dannon et al., 2010; Dreyer and Baumgärtner, 1996; Hou and Weng, 2010; Morgan et al., 2001), while the characteristic temperature dependence of death tends to exponentially increase according to a Boltzmann-Arrhenius relationship (e.g., Gillooly et al., 2002; Savage et al., 2004). By the definition of Malthusian fitness *r*, subtracting the characteristic death rate from the characteristic birth rate thus yields a TPC of fitness that increases gradually towards a peak near the optimum of birth, then drops off quickly into negative values as death rates accelerate, with slopes and curve skew determined by species-specific scaling rates (Fig 1).

The parallel between performance- and fitness-derived TPCs, as well as the intuitive practical connection between the two, suggests a clear relationship between the preexisting experimental data on biological performance and the ecological fitness metrics required to forecast extinction risks. Currently, making such predictions with temperature-dependent performance data relies on integrating an organism's TPC with climate data to compare its average observed fitness under past temperatures with its average projected fitness under future temperatures (e.g., Deutsch et al., 2008; Vasseur et al., 2014). However, in order to use the experimental data we have to make the predictions we need, several key theoretical gaps in this framework must be addressed. Resolving those gaps through improved modeling efforts is the first step towards increasing the accuracy and confidence of population forecasting in a warming world.

Chapter 1: Extending mechanistic TPCs to stressful temperatures

A fundamental issue with using TPCs predictively is their inaccuracy with respect to extremely hot temperatures. Most published models rely on extrapolating organismal TPCs measured exclusively under permissive temperatures—those temperatures under which organisms can successfully grow and reproduce—into the range of those hotter, stressful temperatures. Because physiological insights show that death rates accelerate rapidly under heat wave conditions (Baker and Geider, 2021; Hollingsworth, 1969; Stroustrup et al., 2016; Jørgenson et al. 2022), this naive extrapolation is likely resulting in erroneously optimistic assessments of impending extinction risks. However, gathering sufficient data under these extremes is prohibitively difficult. Avoiding this inaccuracy thus requires a better theoretical understanding of the mechanisms underlying death rates as temperatures increase.

The aim of this chapter is to mechanistically determine what TPCs should look like at stressful temperatures. Increasing thermal means and variation around the globe are leading to higher likelihoods that organisms will experience longer, more frequent exposures to heat wave conditions. Because current predictions use TPCs that do not include dynamic modeling or the acceleration of death rates under heat stress, they miss the quick and devastating impacts of the extreme temperature events that will shape extinction and extirpation risks over the next century.

1.1 Motivation and Background

Typically, the TPCs used to determine population fitness r in classic projection papers like Deutsch et al. (2008) are derived by fitting a 'TPC-shaped' function to several values of r; these in turn are calculated from empirical measurements of vital rates at a few temperatures. The standard definition of Malthusian fitness offers a more mechanistic method for deriving this relationship from life history parameters; as shown in Amarasekare & Savage's landmark 2012 derivation, the characteristic TPC shape also arises from the difference between a normal distribution of birth rates and an Arrhenius distribution of death rates with respect to temperature. While this framework offers limited support to the phenomenological Deutsch method, common approaches still incorporate several issues that I would like to highlight.

a) Fitting to Limited Empirical Data: TPC curves are typically fit to empirical studies that only provide 4-8 datapoints, with only 1-2 of those measured at temperatures higher than T_{opt} (see any of the 38 datasets used in Deutsch et al., 2008). Notably, the prominent method of curve fitting TPCs to this data introduced by Deutsch et al. (and subsequently used by Vasseur et al., 2014 and Duffy et al., 2022, among others; see Table 2) includes a breakpoint, such that increasing performance up to T_{opt} is described by fitting a Gaussian function to data gathered at $T \leq T_{opt}$, while decreasing performance past T_{opt} is described by fitting a quadratic function to data gathered at $T > T_{opt}$. In practice, this method means that the predominant description of death rates at stressful temperatures arises from the extrapolation of a curve fit to only two datapoints. These fitting functions may be surprisingly close to correct, but as there is no mechanistic theory underlying them, their accuracy is not rigorous, repeatable, or well understood.

b) Accelerating Death Rates: Once a TPC intersects the x-axis on the right ($r = 0$, $T > T_{opt}$), its value is either (1) assumed equal to 0 (e.g., Deutsch et al., 2008), despite knowledge that $r < 0$ simply indicates the very biologically plausible scenario of a shrinking population (see Vasseur, 2020), or (2) extrapolated from the existing fitness curve (e.g., Vasseur et al., 2014), despite empirical evidence that death rates are controlled by different mechanisms at stressful temperatures (Somero et al., 2017) and, consequently, sharply accelerate (Baker and Geider, 2021; Hollingsworth, 1969; Jørgensen et al., 2022; Stroustrup et al., 2016).

c) Limits of Metabolic Theory: The theory underlying the shape of mechanistic TPCs ($r = b - d$) relies on a death rate controlled by respiratory costs, as explained by metabolic theory. However, metabolic theory itself relies on the assumption that proteins are functioning as they should (Brown et al., 2004; Savage et al., 2004); stressful temperatures, by disrupting protein function, break this assumption and incur a different mechanism for death which we should not expect to be able to model with the same equation (Knapp and Huang, 2022; Ratkowsky et al., 2005; Santra et al., 2019; Somero et al., 2017).

The crux of these three points is that death rates at permissive temperatures do not explain death rates at stressful temperatures – and we should not expect them to. The physiological evidence clearly shows that while proteins work, the leading cause of degradation and death is the slow ramping up of metabolic costs, giving us the well-known death function underlying classic TPCs; however, when proteins degrade, that metabolic mechanism is immediately overtaken by the disastrous impacts of a crumbling proteome.

If we wish to use TPCs predictively at the stressful temperatures where they have not been (and frequently cannot be) measured, we must understand the underlying mechanisms. By using birth and death rates to calculate fitness instead of estimating it phenomenologically, I will avoid issue (a) by fitting the death function to all available datapoints instead of extrapolating fitness at stressful temperatures from only a couple of them; (b) by relying on a mechanistic explanation of underlying reaction rates instead of an assumed relationship with temperature; and (c) by incorporating appropriate, nuanced assumptions about the different sources of death and their relationships with temperature.

1.2 Proposed Methods

Let the temperature-dependent fitness function $r(T) = b(T) - d(T)$.

Temperature-Dependence of Birth Rates: Let $b(T)$, the birth rate with respect to temperature, be unimodal and fit to data compiled on the organism in question (e.g., parabolic distribution for phytoplankton in Baker and Geider, 2021; Weibull distribution for pea aphids in Xia et al., 1999). This general relationship is well established in the literature (Dannon et al., 2010; Dreyer and Baumgärtner, 1996; Medeiros et al., 2003, 2000; Morgan et al., 2001; e.g., Reznick, 1985; Southwood, 1988) and arises theoretically (a) in a biomass framework through the symmetric relationship of ingestion rate around an optimal temperature (Englund et al., 2011; Uiterwaal and DeLong, 2020; Vinton and Vasseur, 2022) and (b) in a strict birth-death framework from the temperature limitations on the composite processes involved in one cohort of adults producing another (Amarasekare and Savage, 2012).

Temperature-Dependence of Death Rates: Let $d(T)$, the death rate with respect to temperature, be a Boltzmann-Arrhenius function of the form $d(T) = k_0\, Exp[\frac{E_A}{kT}]$, where k_0 is the intercept value, E_A is the activation energy, and k is the Boltzmann constant (~8.61×10^{-5} eV/K). This follows the established practice of assuming an Arrhenius relationship between death rate and temperature due to gradually increasing metabolic costs (Amarasekare and Savage, 2012; Cossins and Bowler, 1987; Jørgensen et al., 2022). In general, the Boltzmann-Arrhenius function indicates the effect of temperature on the rate of a reaction by mapping the increasing proportion of molecules with the sufficient kinetic energy (E_A) for that reaction to occur (Brown et al., 2004).

Classical empirical estimates of activation energies for most biological rates, including death rate under permissive temperatures, consistently fall within 0.6-0.7eV – the very same narrow, observed range of activation energies of the biochemical reactions underlying metabolism (Brown et al., 2004; Gillooly et al., 2002). A recent meta-analysis found a slightly wider range of 0.5-0.8eV across 1,351 vital rates in 314 ectothermic species (Jørgensen et al., 2022). These consistent findings correspond to the low temperature sensitivity of death rates under permissive temperatures, and these temperature-insensitive death rates are the ones that are utilized in the classic fitness TPC framework.

Currently, these same temperature-insensitive death rates are being extrapolated into stressful temperatures. However, death rates are *extremely* temperature sensitive in the stressful range (Baker and Geider, 2021; Hollingsworth, 1969; Jørgensen et al., 2022; Stroustrup et al., 2016). Consequently, instead of modeling the death rate as a single Arrhenius function, we now shift to modeling the death rate as the sum of two Arrhenius functions. The first, $d_m(T)$, describes death

due to metabolic costs and dominates death rates under permissive temperatures. The second, $d_p(T)$, describes death due to declining protein function and dominates death rates under stressful temperatures. Since the processes controlling metabolic cost and declining proteostasis are different, we parameterize $d_p(T)$ differently than $d_m(T)$, and we now write the temperature dependence of death as $d(T) = d_m + d_p = k_{0m}\, Exp\left[\frac{E_{Am}}{k\,T}\right] + k_{0p}\, Exp\left[\frac{E_{Ap}}{k\,T}\right]$. With this unified model of death, we now have a set of mechanistic assumptions for the birth and death processes under both permissive and stressful temperatures.

For d_m, the activation energy E_{Am} should fall within the 0.5-0.8eV range detailed above. For d_p, however, the activation energy E_{Ap} should be much greater, reflecting the extreme temperature sensitivity of death in the stressful range. Jørgensen et al. (2022) gathered data on the heat failure rates of 112 species in this range and found a median activation energy of 6.12eV. To demonstrate the magnitude of this discrepancy, they explain that for each single degree of warming, the median increase in vital rates under permissive temperatures (E_A = 0.5eV) is 7%; under stressful temperatures (E_A = 6.12eV), it is 110%.

Data availability: While strict birth and death or growth and decline data are unfortunately difficult to find in the literature or to collect empirically, these values have been reported in a few notable cases (e.g., Baker and Geider, 2021) and can be extracted from some reported life tables (e.g., Liu and Tsai, 2000) utilizing the methodology of Birch (1948) (see Fig 2 for examples).

Generating model results: With the framework of the mechanistic TPCs in place and appropriate datasets located, what remains unclear is the value at which declining proteostasis overwhelms increasing metabolic costs as the leading cause of death. Call the temperature at which this occurs the breakpoint T_{break}. Brown et al. (2004) proposed that death rates should be explained fully by metabolic costs anywhere within the "normal activity temperatures" of 0 and 40^{o}C; this assumption is, in essence, the null model that former work has relied upon. Because it assumes T_{break} is hot enough that most organisms are unlikely to ever experience temperatures above it, declining proteostasis would never be an important cause of death and preexisting TPCs should already describe death rates well. This null model, however, clearly does not fit with the empirical evidence of a breakpoint in death rates or the experimental work on protein denaturation temperatures, and we are thus free to reject it from the start.

Savage et al. (2004) described 'biologically relevant' temperatures more conservatively as only those lower than T_{opt}, the peak of the fitness curve. Jørgensen et al. (2022) describe a critical temperature separating the permissive and stressful ranges roughly equal to CT_{max}, the temperature "at which biological processes dictating the 'rate of death' become dominant over those determining the 'rate of life.'" More research is needed to carefully assess these possibilities by asking when the assumption of normal protein function is actually broken. The closer T_{break} is to T_{opt}, the riskier stressful temperatures become; consequently, the lower the T_{break} value, the smaller the thermal safety margin of the organism. We start with three possible biologically relevant values of T_{break}, listed in order of decreasing severity: T_{opt}, CT_{max}, and $b = 0$.

Within a modeling framework, the first question to answer is the *impact* of T_{break}'s relative location. To assess the impacts of T_{break} as a free parameter on temperature-dependent fitness, we turn to dynamical population modeling. The mechanistic $r(T)$ is plugged into a logistic growth

framework and paired with a simulated temperature regime to compute the differential 'riskiness' of various T_{break} values. This population dynamical approach is an important missing component of current models because it captures the impact of sequential population sizes on each other; they thus are able to capture the rapid, severe impacts of sequential and transient temperature extremes, which previous approaches based on non-linear averaging (e.g., Deutsch et al., 2008; Vasseur et al., 2014) explicitly cannot quantify. Only one study has thus far broadly integrated temperature-dependent population dynamics into extinction risk estimates (see Duffy et al., 2022, Methods: Population dynamical modeling), but further incorporation of dynamical approaches is critical to assessing extinction risks in variable thermal environments.

The first, simplest version of this method (1) incorporates $r(T)$ into the r-α population growth model, a logistic growth model which replaces the carrying capacity K with the density-dependence parameter α (where $K = r/\alpha$) and (2) generates sequential temperatures T using a Stochastic Differential Equation framework (in this case, an Ornstein-Uhlenbeck process (see Mangel, 2006)) modeled using the Stratonovich Process function in Wolfram Mathematica V12.0.0.0, then (3) calculates sequential population sizes using the r-α model at each temperature to examine the population dynamics over time. This simulation is then (4) assigned an arbitrary extinction threshold, (5) repeatedly run for a set amount of time, and (6) made to output the proportion of simulations in which the population 'goes extinct' by crossing the assigned extinction threshold. This output is then (7) calculated for a range of T_{break} values to (8) generate a relationship between ultimate extinction risk and the breakpoint temperature.

Possible extensions of this model may include (1) using the classic logistic growth model instead of the r-α model, which may be useful if the carrying capacity K and its relationship to r can be better constrained, (2) using more realistic, data-based, or autocorrelated temperature regimes, though this will be more useful for specific predictions about particular species in set locations than for generating the broad relationships sought here, and (3) incorporating a more informed extinction threshold, such as one generated through a relationship between the carrying capacity K and the threshold under which Allee effects are relevant. Each of these avenues may have utility depending on the specific questions we end up asking.

1.3 Model Predictions & Outlook

From the preliminary TPCs examined so far, fitness curves calculated by the Deutsch method tend to be more left-skewed than those calculated with the birth-death framework, with variability in which curve crosses the x-intercept first but generally steeper fitness curves beyond $r = 0$ from the latter. Inclusion of the population dynamical framework consistently increases the overall variability of population size and risk of extinction above non-linear averaging approaches. The combination of this dynamic framework with realistic death rates suggests that simulations run with higher thermal variability are thus extremely likely to find higher extinction risks (as predicted by Slein et al., 2023; Vasseur et al., 2014), with lower T_{break} values corresponding to smaller safety margins.

Despite the number of assumptions incorporated into this preliminary modeling scheme, it will be able to clearly show the consequences of the existence and location of a breakpoint in death rates. However, these results ultimately do not meet the hope for a mechanistic, temperature-dependent model of population fitness because the value of T_{break} will be determined only by

available empirical evidence – its location and movement have not been tied to any underlying processes. Even if higher T_{break} values show sharply diminishing returns in extinction reductions, there would be no point in any organism evolving low T_{break} values; this could only serve to increase potential death rates, and, so far, we have not considered any costs of doing such a thing. This will be the subject of Chapter 2.

Goal Outcomes: *Mathematical model and accompanying paper assessing the importance of and available data for mechanistic fitness TPCs, particularly in relation to the breakpoint in death rates and shape of curves beyond that point*

Chapter 2: What thermal regimes are organisms optimally adapted for?

With a mechanistic explanation of temperature-dependent death rates in hand, the next question is why T_{break} is where it is. Presumably, organisms ought to push the breakpoint to as high a temperature as possible to avoid any risks of slipping into the regime of stressful temperatures and their aggressively accelerating death rates. However, moving T_{break} to hotter temperatures does not come without cost; it requires keeping one's internal proteome intact for as long as possible by adapting more stable protein states or rapidly producing new, defensive proteins to mitigate the damage stressful temperatures cause. Both mechanisms can be extremely energetically costly and, as such, it only makes sense for organisms to adapt as much as they need to: each species should seek the optimal balance between the energetic cost of having high T_{break} values and the risk of actually needing them. Given a certain temperature regime, there will thus be some optimal breakpoint value that maximizes population success and minimizes extinction risks. That optimization – and where, given past, current, and projected temperature regimes, it falls in relation to observed breakpoints – is the subject of this chapter.

2.1 Motivation & Background

The primary risk posed to organisms by heat stress is the denaturation of proteins – the loss of their native, functional structure – which can, in turn, lead to cytotoxic protein aggregations (Stefani and Dobson, 2003) and a cascade of cellular damage (Santra et al., 2019). There are two ways to avoid this damage: *adaptation* or *mitigation*.

Adaptation is the long-term strategy employed under consistent levels of thermal stress. As such, the intrinsic thermal stability of proteins is not equal across organisms; there is an inherent structural trade-off between cold-adapted organisms, whose more flexible proteins tend can function more quickly, and warm-adapted organisms, whose more rigid proteins are less susceptible to thermal denaturation (Gu and Hilser, 2009). This trade-off maintains the consistent amount of fluidity required for protein functionality across organisms that have evolved at different temperatures (Somero et al., 2017). The temperature-dependent denaturation of proteins thus depends on the adaptations of the organism in question.

Mitigation is the short-term strategy employed during acute events of thermal stress. Through adaptation, organisms evolve proteins that are well-adapted for the permissive temperature environments they have evolved in. Mitigation tactics, collectively known as the Heat-Shock Response (HSR), are the fail-safe for any forays out of the adaptive, permissive regime and into the stressful one. This HSR mediates the decline in protein function that dominates death rates

under stressful temperatures in most taxa (Feder and Hofmann, 1999; Richter et al., 2010). However, its efficacy depends on the duration, intensity, and abruptness of the experienced thermal stress (Boopathy et al., 2022).

On the cellular level, heat stress induces the HSR by causing the dissociation of a transcription factor (HSF1) from a multichaperone protein complex. The freed chaperone proteins from that complex (collectively known as Heat Shock Proteins, or HSPs) bind to denatured proteins to initiate refolding or degradation, while HSF1 moves to the nucleus to transcribe more HSPs. Once no denatured proteins remain, HSPs instead re-bind to HSF1, reforming the multichaperone complex and turning the HSR 'off' (Somero et al., 2017; Sørensen et al., 2003). The induction temperature of the HSR again depends on the acclimation history of the organism and alters the inflection point of death rates under heat stress (Vasseur et al., unpublished).

A key component to consider about the HSR is its energetic expense. Handling heat-induced damage incurs high ATP costs, as demonstrated by the synchronized uptick in metabolic response genes with molecular chaperone genes in response to acute heat stress in intertidal invertebrates (Connor and Gracey, 2011; Gracey et al., 2008; Han et al., 2013; Somero et al., 2017) and *Drosophila* (Feder et al., 1996; Krebs and Loeschcke, 1994; Welte et al., 1993). These metabolic costs and the consequent metabolic depression (see Hoekstra and Montooth, 2013) are known to result in reductions to growth rate (DiDomenico et al., 1982; Feder et al., 1992; Gomez-Pastor et al., 2018; Krebs and Loeschcke, 1994; López-Maury et al., 2008), fecundity (Krebs and Loeschcke, 1994; Silbermann and Tatar, 2000), and lifespan (Krebs and Feder, 1998, 1997) proportional to the frequency and severity of heat stress. Consequently, it is impossible to accurately incorporate the population-level effects of experiencing heat stress without explicitly considering temporal dynamics within a mechanistic model inclusive of both metabolic costs and proteostasis loss. Our model is thus uniquely suited to the task.

2.2 Proposed Methods

In the model described in Chapter 1, T_{break} was a free parameter whose location relied on hypothesized mechanisms. In this framework, we instead incorporate those mechanisms organisms use to shift T_{break} (adaptation and mitigation), as well as the energetic costs of doing so. This method requires determining both what the metabolic cost *is* and what that cost is *to*. There is consensus in the literature that the metabolic cost of the HSR is high; the single empirical study measuring that cost found an increase of 35% and noted a positive correlation with the gene copy number of heat shock protein *Hsp70* in *Drosophila melanogaster* (Hoekstra and Montooth, 2013). As a first approach, we can use these correlations (ideally in the same genus or species, for which there is significant thermal performance data) to estimate the cost of HSR activation, alongside the preliminary unified death rate model of Vasseur et al. (unpublished) to calculate the quantity of HSP production given different heat shocks. Because our model incorporates three mechanistic vital rates (birth, death due to metabolic costs, and death due to proteostasis loss), we can then compare the favorability of initiating the HSR at different temperatures by running simulations where the HSR ameliorates proteostasis loss while incurring short- and long-term metabolic costs. We can then use this trade-off to determine the optimal HSR initiation temperature under different thermal regimes. Next steps include carefully determining if and how (1) this approach can be applied to other organisms and (2) to incorporate the costs separately into fecundity or growth rate.

Once we have established a model that determines the optimum temperature for HSR initiation (and, consequently, the optimum T_{break} value), there are two interesting applications to explore. The first is incorporating *adaptation* as well as *mitigation*; the initial model only includes the ability to respond to stressful temperatures in the short-term, but *trends* of increasing temperature may also induce adaption of more stable proteomes and, consequently, warmer-adapted organisms in the long-term. Functionally, this likely means increasing CT_{max} of the actual thermal performance curve by shifting the birth curve *b(T)* to the right in conjuncture with some metabolic cost of adaptation, but I have yet to work out the underlying mechanisms for this piece of the model.

The second application approaches the question of optimal adaptation for the actual thermal environment. Given thermal regimes simulated to look like the past, current, and projected temperature regimes of a specific organism in a specific location (examples of potential methods for doing so may be found in Deutsch et al., 2008, Methods: Climate Data; Duffy et al., 2022, Methods: CMIP6 simulations & Statistical analyses of climate data; Jørgensen et al., 2022, Fig 3a and Extended Data Fig 1; Vasseur et al., 2014, (b) Empirical data and climate change scenarios), we should be able to determine the optimal thermal strategies for each. The extent of the mismatch between actual observed adaptations and the optimal simulated adaptations for the different regimes will offer a glimpse into how fast the organism is adapted to changing temperatures (comparison between past and present), how well-adapted they are to current temperatures (comparison between empirical and optimal), and how quickly they need to adapt to survive under future temperatures (comparison between present and future, with respect to adaptation speed).

Goal Outcomes: *Mathematical model and accompanying paper assessing the optimal T_{break} value given the costs of HSR; comparisons between actual value and past/future temperature projections to assess riskiness and adaptation speed*

Chapter 3: TPCs are not static

In the first two chapters of this project, the goal was the creation and implementation of a mechanistic TPC of population fitness to use for forecasting extinction and extirpation risks. The mechanistic nature of that model eliminated some of the least verified assumptions underlying predictive TPCs. While many assumptions necessarily remain, building a mechanistic framework for fitness TPCs offers a new avenue to deal with others. Most notably, TPCs are typically assumed to be both static over time and uniform across populations, both of which have been experimentally disproven (Sinclair et al., 2016). Important differences between measured TPCs in the lab and realized TPCs in the field may arise from acclimation, thermoregulation, life history, interacting stressors, thermodynamics, and ecological interactions (Dell et al., 2014; Fey et al., 2015; Fey and Vasseur, 2016; Kearney et al., 2013; Muñoz and Bodensteiner, 2019; Sinclair et al., 2016; Vinton and Vasseur, 2022). Addressing the discrepancies between the fundamental TPCs derived in these initial chapters and the realized TPCs that accurately describe dynamics in the field requires a clearer understanding of how these processes alter the underlying mechanisms. While I continue to gather sources hinting at which complicating factors can be

better addressed by this new framework, three potential avenues to explore dynamic TPCs are examined below.

3.1 Motivation & Potential Methods

Interacting stressors: Temperature is rarely, if ever, the only fluctuating factor in an organism's environment. Varying levels of humidity, water, nutrients, salinity, or contaminants add new dimensions to the fitness reaction norms expected of a population. Our focus on temperature in this project stems from a particular interest in understanding the impacts of changing thermal regimes. While some empirical work has been done measuring two or three axes of this landscape of stress (e.g., Cross et al., 2015; Galindo et al., 2018; Lee and Roh, 2010; Loureiro et al., 2015; Thomas et al., 2017), empirically testing the thousands of combinations of each different level of each different factor is impossible. However, it may be easier to understand the component relationships of stressful factors within the underlying processes of birth and death. For example, theory and experimental work show that TPCs are compressed by limitations on food availability (Brett et al., 1969; Huey and Kingsolver, 2019; Vinton and Vasseur, 2022). Because food limitation has known impacts on metabolic and birth rates, the compression of the TPC can be understood as an amplification of the death function under permissive temperatures (through higher respiratory costs incurred by resource limitation) and/or a contraction of the birth function (through decreased biomass availability incurred by resource limitation).

Life history: It is well established that many organisms have different thermal tolerances during different stages of their lives. For example, Kingsolver et al. (2011) found substantial differences in the TPC shape of sphinx moths during their egg, larval, and pupal stages. This increased vulnerability during certain life stages could be extremely important for forecasting; if juveniles are more thermally vulnerable than adults, but TPCs only reflect adult tolerance, there could be an unforeseen bottleneck where the population cannot replace itself due to unsuccessful maturation, despite reasonable survivorship among mature individuals. Some empirical data on these differences is already available in the life table datasets used in Chapter 1, as those parameters were frequently gathered for each of several stages in an age-structured population. To incorporate life history into the model, a possible method would thus be creating an r_x for each age class x, where the birth function b_x incorporated into each r_x is the rate of new individuals joining that age class and the death function d_x is the sum of the death rate of that cohort added to the rate of individuals leaving that cohort. If such specific data proves too difficult to locate or parse, an alternative method would be assuming some stable age distribution and basing each age class's fitness TPC on empirical data. Then the change in population size would equal the proportional sum of changes to the population size of each age class.

Thermoregulation: In variable thermal environments, most organisms are capable of physiological or behavioral thermoregulation. This thermoregulation essentially acts as a filter between the environmental temperature the organism exists in and the body temperature it experiences. Our current model set-up includes a paired set of equations (one to calculate temperature T and one to calculate changes in population size based on $r(T)$); to add thermoregulatory mediation to that model would require adding a third equation which transforms the environmental temperature T to the body temperature T_b based on thermoregulatory accuracy. Changes to population size would then be calculated using $r(T_b)$. Some simple examples of this method could include (1) physiological thermoregulation of

endotherms, which transforms any environmental temperature within their thermoneutral zone to a constant body temperature (see Levesque and Marshall, 2021) or (2) behavioral thermoregulation of ectotherms, which could assume some percentage effectiveness of thermoregulation with higher efficacy under less extreme temperatures or in more variable environments (based on theory and data explored by Kearney et al., 2009).

Chapter 4: Spatiotemporal Variation and the Eco-Evolutionary Dynamics of TPCs

A final unexplored assumption in using TPCs for population forecasting is their ignorance of thermal variation. This assumption is particularly important because changes in thermal variation are likely to be even more important to impending population persistence than changes to the thermal mean (Dillon et al., 2016; Vasseur et al., 2014). The spatiotemporal scale of thermal data rarely matches the spatiotemporal scale of experienced thermal variation (Collins et al., 2018; Dillon et al., 2016; Sears et al., 2011) and how decoupled these scales are may critically impact the predictive power of TPCs. Some progress can be made on this problem by better understanding how behavioral thermoregulation alters both TPCs (see Chapter 3) and experienced temperature (see Sears et al., 2016), but more thought is needed on the broader theory of connecting an organism's TPC with the environment in which that TPC evolved.

Without the data to thoroughly resolve this issue, we can instead turn to emerging correlations between experienced temperature variation and evolved thermal tolerances (e.g., Magni et al., 2018). For example, experimental work shows positive correlations between capacity for cold hardening and living in more predictable seasonal environments (compared to more random ones) in tropical lizards, implying that greater acclimation ability over the long term might increase hardening capacity over the short term (Phillips et al., 2016). Field studies have also shown higher quantities of HSP expression correlating with higher variation in thermal history based on the microsites of intertidal limpets across a wide range of latitudes (Lima et al., 2016; Moreira et al., 2021). Because the modeling framework developed throughout earlier chapters is built explicitly to incorporate various temperature regimes and dynamic thermal strategies, it is well-suited to examine the potential for evolutionary rescue given histories of thermal variation that provide different tools for future adaptation. This may illuminate gaps in our understanding of how spatiotemporal variation actually impacts the eco-evolutionary dynamics of TPCs and, critically, where the hard limits on the short-term acclimation and long-term evolvability of thermal tolerance might be (see Donoghue, 2008), with key implications for the prediction and management of global risks and species distributions in a warming world.

Proposed Timeline:

Ch 1 – preliminary research and modeling complete; finish and present initial modeling and results, Summer 2023 (ESA); write-up Fall 2023 (start of year 3)
Ch 2 – preliminary research in progress; model and write-up Winter-Spring 2024 (end of year 3)
Ch 3 – begin work Summer-Fall 2024, completed Fall-Winter 2024 (start of year 4)
Ch 4 – begin work Winter-Spring 2025 (end of year 4), completed Fall 2025 (start of year 5)

FIGURES & TABLES

VARIABLE	MEANING
α	Density-dependence parameter ($K = r/\alpha$)
b	Instantaneous birth rate
CT_{max}	Critical thermal maximum
CT_{min}	Critical thermal minimum
d	Instantaneous death rate
d_m	Instantaneous death rate due to metabolic cost
d_p	Instantaneous death rate due to proteostasis loss
E_A	Activation energy of the Boltzmann-Arrhenius function
K	Carrying capacity
k	Boltzmann constant; ~8.61×10^{-5} eV/K
k_0	Intercept of the Boltzmann-Arrhenius function
N	Population size
r	Malthusian fitness
t	Time
T	Environmental temperature
T_b	Body temperature
$T_{breadth}$	Range of temperature with positive fitness
T_{break}	Breakpoint between permissive and stressful temperatures
T_{opt}	Thermal optimum; peak of fitness TPC

Table 1: Compiled variables and their definitions.

EQUATION	NAME
$d(T) = k_0\, Exp\left(\frac{E_A}{kT}\right)$	Boltzmann-Arrhenius function
$r(t) = \begin{cases} Exp\left\{-\left(\frac{T - T_{opt}}{2\sigma_p}\right)^2\right\}, & T \le T_{opt} \\ 1 - \left(\frac{T - T_{opt}}{T_{opt} - CT_{max}}\right)^2, & T > T_{opt} \end{cases}$	Deutsch et al. (2008)'s phenomenological fitness function, where $CT_{min} = T_{opt} - 4\sigma_p$
$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right)$	Logistic growth equation
$\frac{dN}{dt} = N(r - \alpha N)$	r-α model
$r(T) = b(T) - d(T)$	Temperature-dependent fitness function
$d(T) = d_m + d_p = k_{0m}\, Exp\left[\frac{E_{Am}}{k\,T}\right] + k_{0p}\, Exp\left[\frac{E_{Ap}}{k\,T}\right]$	Unified death model

Table 2: List of mentioned equations.

ABBREVIATION	MEANING
ATP	Adenosine triphosphate; cellular energy units
HSF1	Heat shock factor 1
HSP	Heat Shock Protein
Hsp70	Heat shock protein 70
HSR	Heat Shock Response
TPC	Thermal Performance Curve

Table 3: Compiled abbreviations and their definitions.

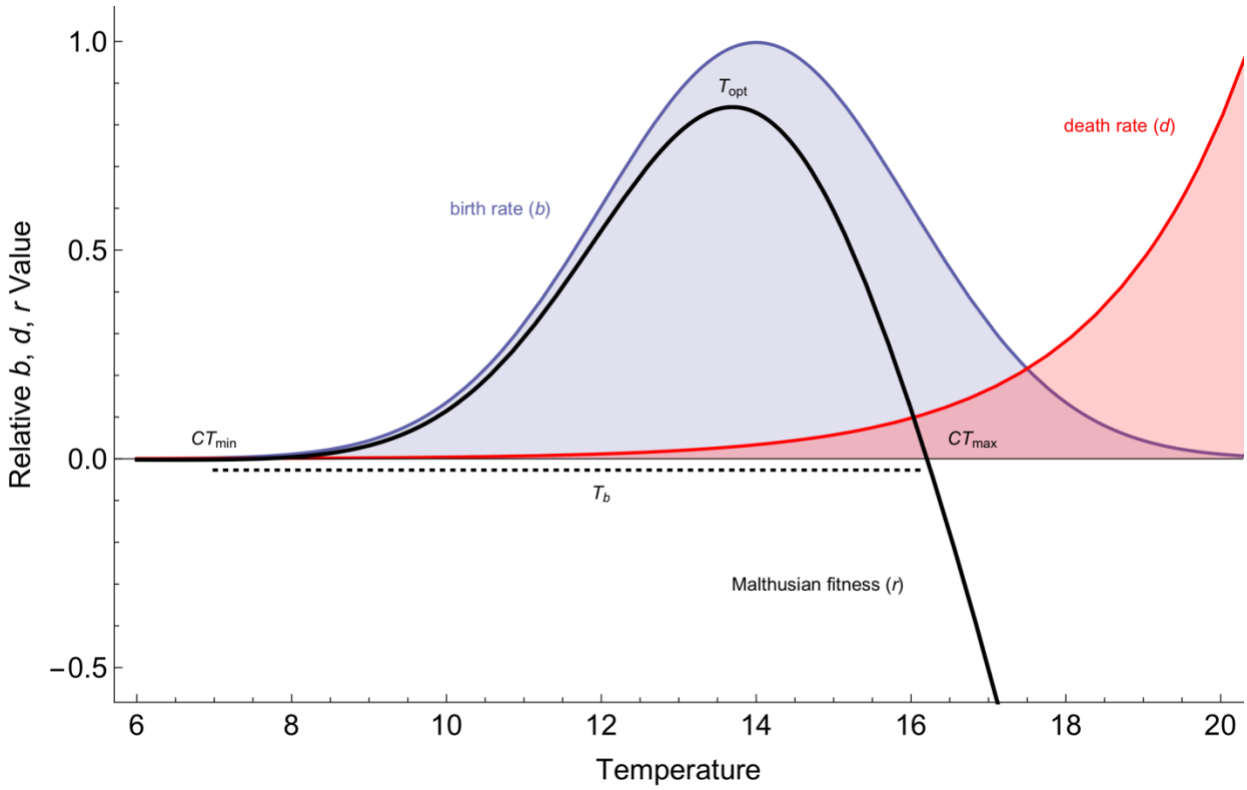

Fig 1: In black, an example TPC of Malthusian fitness (r) with peak fitness at the thermal optimum (T_{opt}), positive fitness within the thermal breadth ($T_{breadth}$), and fitness ≤ 0 outside of the critical thermal minimum and maximum (CT_{min} and CT_{max}, respectively). TPCs can be obtained phenomenologically by fitting the black curve to experimentally gathered data or theoretically by subtracting the exponential death rate (d) in red from the unimodal birth rate (b) in blue; the latter method is used here.

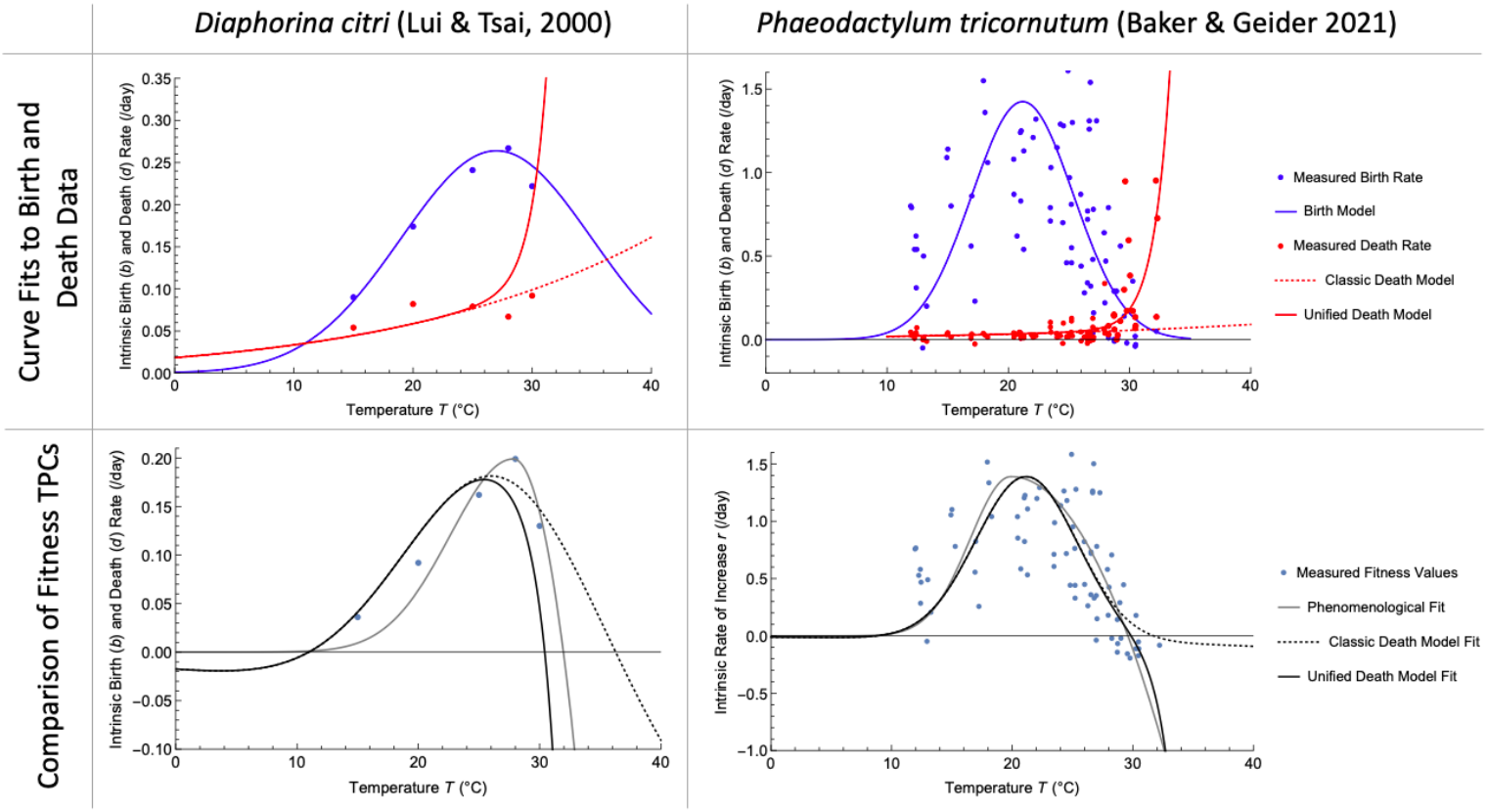

Fig 2: Example data and resulting TPCs from the insect *Diaphorina citri* from Liu and Tsai (2000) (left) – a typical dataset from the 38-species compilation used by Deutsch et al. (2008), Vasseur et al. (2014), and Duffy et al. (2022) – and the phytoplankton *Phaedactylum tricornutum* from Baker and Geider (2021) (right). The birth and death rates for the former (*D. citri*) were calculated from a reported life table (see Birch, 1948) and illustrate (1) the small size of many used datasets, including only one data point measured beyond T_{opt}, and (2) the potential of phenomenological fits to overestimate fitness under stressful temperatures. The death rate and fitness values for the latter (*P. tricornutum*) were directly reported (instantaneous birth rate from straightforward calculation) and illustrate a much closer match between phenomenological and mechanistic TPCs.

Top: Birth rates (blue dots) were fitted with a normal distribution (blue lines) while death rates (red dots) were fitted first, with a single Boltzmann-Arrhenius function for death rate resulting only from metabolic cost (E_A = 0.4) (dashed red line) and second, with the sum of two Boltzmann-Arrhenius functions for death rate resulting from both metabolic cost (E_A = 0.4) and proteostasis loss (E_A = 6) (solid red line).

Bottom: Fitness TPCs were calculated from the measured fitness data (dots) using the phenomenological method of Deutsch et al. (2008) (gray line) and the mechanistic method of subtracting death rate from birth rate (black lines; dotted show result using only metabolic death rates, while solid lines show result using the sum of metabolic cost and proteostasis loss).

BIBLIOGRAPHY

Amarasekare, P., 2015. Effects of temperature on consumer–resource interactions. Journal of Animal Ecology 84, 665–679. https://doi.org/10.1111/1365-2656.12320

Amarasekare, P., Savage, V., 2012. A Framework for Elucidating the Temperature Dependence of Fitness. The American Naturalist 179, 178–191. https://doi.org/10.1086/663677

Angilletta, M.J., 2009. Thermal Adaptation: A Theoretical and Empirical Synthesis. OUP Oxford.

Baker, K.G., Geider, R.J., 2021. Phytoplankton mortality in a changing thermal seascape. Global Change Biology 27, 5253–5261.

Birch, L., 1948. The intrinsic rate of natural increase of an insect population. The Journal of Animal Ecology 15–26.

Boopathy, L.R.A., Jacob-Tomas, S., Alecki, C., Vera, M., 2022. Mechanisms tailoring the expression of heat shock proteins to proteostasis challenges. Journal of Biological Chemistry 298. https://doi.org/10.1016/j.jbc.2022.101796

Brett, J.R., Shelbourn, J.E., Shoop, C.T., 1969. Growth Rate and Body Composition of Fingerling Sockeye Salmon, Oncorhynchus nerka, in relation to Temperature and Ration Size. J. Fish. Res. Bd. Can. 26, 2363–2394. https://doi.org/10.1139/f69-230

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a Metabolic Theory of Ecology. Ecology 85, 1771–1789. https://doi.org/10.1890/03-9000

Carrière, Y., Boivin, G., 1997. Evolution of thermal sensitivity of parasitization capacity in egg parasitoids. Evolution 2028–2032.

Collins, S.L., Avolio, M.L., Gries, C., Hallett, L.M., Koerner, S.E., La Pierre, K.J., Rypel, A.L., Sokol, E.R., Fey, S.B., Flynn, D.F.B., Jones, S.K., Ladwig, L.M., Ripplinger, J., Jones, M.B., 2018. Temporal heterogeneity increases with spatial heterogeneity in ecological communities. Ecology 99, 858–865. https://doi.org/10.1002/ecy.2154

Connor, K.M., Gracey, A.Y., 2011. Circadian cycles are the dominant transcriptional rhythm in the intertidal mussel Mytilus californianus. Proceedings of the National Academy of Sciences 108, 16110–16115. https://doi.org/10.1073/pnas.1111076108

Cossins, A.R., Bowler, K., 1987. Temperature Biology of Animals. Springer Dordrecht.

Cross, W.F., Hood, J.M., Benstead, J.P., Huryn, A.D., Nelson, D., 2015. Interactions between temperature and nutrients across levels of ecological organization. Global Change Biology 21, 1025–1040. https://doi.org/10.1111/gcb.12809

Dannon, E.A., Tamò, M., van Huis, A., Dicke, M., 2010. Functional response and life history parameters of Apanteles taragamae, a larval parasitoid of Maruca vitrata. BioControl 55, 363–378. https://doi.org/10.1007/s10526-009-9263-4

Dell, A.I., Pawar, S., Savage, V.M., 2014. Temperature dependence of trophic interactions are driven by asymmetry of species responses and foraging strategy. Journal of Animal Ecology 83, 70–84.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., Martin, P.R., 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proc. Natl. Acad. Sci. U.S.A. 105, 6668–6672. https://doi.org/10.1073/pnas.0709472105

DiDomenico, B.J., Bugaisky, G.E., Lindquist, S., 1982. Heat shock and recovery are mediated by different translational mechanisms. Proceedings of the National Academy of Sciences 79, 6181–6185.

Dillon, M.E., Woods, H.A., Wang, G., Fey, S.B., Vasseur, D.A., Telemeco, R.S., Marshall, K., Pincebourde, S., 2016. Life in the Frequency Domain: the Biological Impacts of Changes

in Climate Variability at Multiple Time Scales. Integrative and Comparative Biology 56, 14–30. https://doi.org/10.1093/icb/icw024
Donoghue, M.J., 2008. A phylogenetic perspective on the distribution of plant diversity. Proc. Natl. Acad. Sci. U.S.A. 105, 11549–11555. https://doi.org/10.1073/pnas.0801962105
Dreyer, H., Baumgärtner, J., 1996. Temperature influence on cohort parameters and demographic characteristics of the two cowpea coreids Clavigralla tomentosicollis and C. shadabi. Entomologia Experimentalis et Applicata 78, 201–213. https://doi.org/10.1111/j.1570-7458.1996.tb00783.x
Duffy, K., Gouhier, T.C., Ganguly, A.R., 2022. Climate-mediated shifts in temperature fluctuations promote extinction risk. Nat. Clim. Chang. 12, 1037–1044. https://doi.org/10.1038/s41558-022-01490-7
Englund, G., Öhlund, G., Hein, C.L., Diehl, S., 2011. Temperature dependence of the functional response. Ecology Letters 14, 914–921. https://doi.org/10.1111/j.1461-0248.2011.01661.x
Feder, J.H., Rossi, J.M., Solomon, J., Solomon, N., Lindquist, S., 1992. The consequences of expressing hsp70 in Drosophila cells at normal temperatures. Genes Dev. 6, 1402–1413. https://doi.org/10.1101/gad.6.8.1402
Feder, M.E., Cartaño, N.V., Milos, L., Krebs, R.A., Lindquist, S.L., 1996. Effect of Engineering Hsp70 Copy Number on Hsp70 Expression and Tolerance of Ecologically Relevant Heat Shock in Larvae and Pupae of Drosophila Melanogaster. Journal of Experimental Biology 199, 1837–1844. https://doi.org/10.1242/jeb.199.8.1837
Feder, M.E., Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annual review of physiology 61, 243–282.
Fey, S.B., Siepielski, A.M., Nusslé, S., Cervantes-Yoshida, K., Hwan, J.L., Huber, E.R., Fey, M.J., Catenazzi, A., Carlson, S.M., 2015. Recent shifts in the occurrence, cause, and magnitude of animal mass mortality events. Proceedings of the National Academy of Sciences 112, 1083–1088. https://doi.org/10.1073/pnas.1414894112
Fey, S.B., Vasseur, D.A., 2016. Thermal variability alters the impact of climate warming on consumer–resource systems. Ecology 97, 1690–1699. https://doi.org/10.1890/15-1838.1
Galindo, C.A., Cruz, E.X., Bernal, M.H., 2018. Evaluation of the combined temperature and relative humidity preferences of the Colombian terrestrial salamander Bolitoglossa ramosi (Amphibia: Plethodontidae). Can. J. Zool. 96, 1230–1235. https://doi.org/10.1139/cjz-2017-0330
Ganguly, A.R., Steinhaeuser, K., Erickson, D.J., Branstetter, M., Parish, E.S., Singh, N., Drake, J.B., Buja, L., 2009. Higher trends but larger uncertainty and geographic variability in 21st century temperature and heat waves. Proceedings of the National Academy of Sciences 106, 15555–15559. https://doi.org/10.1073/pnas.0904495106
Gillooly, J.F., Charnov, E.L., West, G.B., Savage, V.M., Brown, J.H., 2002. Effects of size and temperature on developmental time. Nature 417, 70–73. https://doi.org/10.1038/417070a
Gomez-Pastor, R., Burchfiel, E.T., Thiele, D.J., 2018. Regulation of heat shock transcription factors and their roles in physiology and disease. Nat Rev Mol Cell Biol 19, 4–19. https://doi.org/10.1038/nrm.2017.73
Gracey, A.Y., Chaney, M.L., Boomhower, J.P., Tyburczy, W.R., Connor, K., Somero, G.N., 2008. Rhythms of Gene Expression in a Fluctuating Intertidal Environment. Current Biology 18, 1501–1507. https://doi.org/10.1016/j.cub.2008.08.049

Grant, P.R., Grant, B.R., Huey, R.B., Johnson, M.T.J., Knoll, A.H., Schmitt, J., 2017. Evolution caused by extreme events. Philosophical Transactions of the Royal Society B: Biological Sciences 372, 20160146. https://doi.org/10.1098/rstb.2016.0146

Gu, J., Hilser, V.J., 2009. Sequence-Based Analysis of Protein Energy Landscapes Reveals Nonuniform Thermal Adaptation within the Proteome. Molecular Biology and Evolution 26, 2217–2227. https://doi.org/10.1093/molbev/msp140

Han, G., Zhang, S., Marshall, D.J., Ke, C., Dong, Y., 2013. Metabolic energy sensors (AMPK and SIRT1), protein carbonylation and cardiac failure as biomarkers of thermal stress in an intertidal limpet: linking energetic allocation with environmental temperature during aerial emersion. Journal of Experimental Biology 216, 3273–3282. https://doi.org/10.1242/jeb.084269

Harley, C.D.G., Paine, R.T., 2009. Contingencies and compounded rare perturbations dictate sudden distributional shifts during periods of gradual climate change. Proceedings of the National Academy of Sciences 106, 11172–11176. https://doi.org/10.1073/pnas.0904946106

Harvell, C.D., Montecino-Latorre, D., Caldwell, J.M., Burt, J.M., Bosley, K., Keller, A., Heron, S.F., Salomon, A.K., Lee, L., Pontier, O., Pattengill-Semmens, C., Gaydos, J.K., 2019. Disease epidemic and a marine heat wave are associated with the continental-scale collapse of a pivotal predator (Pycnopodia helianthoides). Science Advances 5, eaau7042. https://doi.org/10.1126/sciadv.aau7042

Hoekstra, L.A., Montooth, K.L., 2013. Inducing extra copies of the Hsp70 gene in Drosophila melanogaster increases energetic demand. BMC Evolutionary Biology 13, 68. https://doi.org/10.1186/1471-2148-13-68

Hollingsworth, M.J., 1969. Temperature and length of life in Drosophila. Experimental Gerontology 4, 49–55. https://doi.org/10.1016/0531-5565(69)90026-6

Hou, Y., Weng, Z., 2010. Temperature-Dependent Development and Life Table Parameters of Octodonta nipae (Coleoptera: Chrysomelidae). Environmental Entomology 39, 1676–1684. https://doi.org/10.1603/EN10015

Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A., Jess, M., Williams, S.E., 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. Philosophical Transactions of the Royal Society B: Biological Sciences 367, 1665–1679.

Huey, R.B., Kingsolver, J.G., 2019. Climate Warming, Resource Availability, and the Metabolic Meltdown of Ectotherms. The American Naturalist 194, E140–E150. https://doi.org/10.1086/705679

Huey, R.B., Stevenson, R.D., 1979. Integrating Thermal Physiology and Ecology of Ectotherms: A Discussion of Approaches. American Zoologist 19, 357–366. https://doi.org/10.1093/icb/19.1.357

Irschick, D.J., Higham, T.E., 2016. Animal athletes: an ecological and evolutionary approach. Oxford University Press.

Jørgensen, L.B., Ørsted, M., Malte, H., Wang, T., Overgaard, J., 2022. Extreme escalation of heat failure rates in ectotherms with global warming. Nature 611, 93–98. https://doi.org/10.1038/s41586-022-05334-4

Kearney, M., Shine, R., Porter, W.P., 2009. The potential for behavioral thermoregulation to buffer “cold-blooded” animals against climate warming. Proceedings of the National Academy of Sciences 106, 3835–3840.

Kearney, M.R., Simpson, S.J., Raubenheimer, D., Kooijman, S.A.L.M., 2013. Balancing heat, water and nutrients under environmental change: a thermodynamic niche framework. Functional Ecology 27, 950–966. https://doi.org/10.1111/1365-2435.12020
Kingsolver, J.G., Arthur Woods, H., Buckley, L.B., Potter, K.A., MacLean, H.J., Higgins, J.K., 2011. Complex Life Cycles and the Responses of Insects to Climate Change. Integrative and Comparative Biology 51, 719–732. https://doi.org/10.1093/icb/icr015
Knapp, B.D., Huang, K.C., 2022. The Effects of Temperature on Cellular Physiology. Annual Review of Biophysics 51, 499–526. https://doi.org/10.1146/annurev-biophys-112221-074832
Krebs, R.A., Feder, M.E., 1998. Experimental manipulation of the cost of thermal acclimation in Drosophila melanogaster. Biological Journal of the Linnean Society 63, 593–601.
Krebs, R.A., Feder, M.E., 1997. Deleterious consequences of Hsp70 overexpression in Drosphilla melanogaster larvae. Cell stress & chaperones 2, 60.
Krebs, R.A., Loeschcke, V., 1994. Costs and benefits of activation of the heat-shock response in Drosophila melanogaster. Functional Ecology 8, 730–737.
Lee, K.P., Roh, C., 2010. Temperature-by-nutrient interactions affecting growth rate in an insect ectotherm. Entomologia Experimentalis et Applicata 136, 151–163. https://doi.org/10.1111/j.1570-7458.2010.01018.x
Levesque, D.L., Marshall, K.E., 2021. Do endotherms have thermal performance curves? Journal of Experimental Biology 224, jeb141309.
Lima, F.P., Gomes, F., Seabra, R., Wethey, D.S., Seabra, M.I., Cruz, T., Santos, A.M., Hilbish, T.J., 2016. Loss of thermal refugia near equatorial range limits. Global Change Biology 22, 254–263. https://doi.org/10.1111/gcb.13115
Liu, Y.H., Tsai, J.H., 2000. Effects of temperature on biology and life table parameters of the Asian citrus psyllid, Diaphorina citri Kuwayama (Homoptera: Psyllidae). Annals of Applied Biology 137, 201–206. https://doi.org/10.1111/j.1744-7348.2000.tb00060.x
López-Maury, L., Marguerat, S., Bähler, J., 2008. Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation. Nature Reviews Genetics 9, 583–593.
Loureiro, C., Cuco, A.P., Claro, M.T., Santos, J.I., Pedrosa, M.A., Gonçalves, F., Castro, B.B., 2015. Progressive acclimation alters interaction between salinity and temperature in experimental Daphnia populations. Chemosphere 139, 126–132. https://doi.org/10.1016/j.chemosphere.2015.05.081
Magni, S., Succurro, A., Skupin, A., Ebenhöh, O., 2018. Data-driven dynamical model indicates that the heat shock response in Chlamydomonas reinhardtii is tailored to handle natural temperature variation. Journal of the Royal Society Interface 15, 20170965.
Mangel, M., 2006. The Theoretical Biologist's Toolbox. Cambridge University Press.
McKechnie, A.E., Wolf, B.O., 2010. Climate change increases the likelihood of catastrophic avian mortality events during extreme heat waves. Biology Letters 6, 253–256. https://doi.org/10.1098/rsbl.2009.0702
Medeiros, R.S., Ramalho, F.S., Lemos, W.P., Zanuncio, J.C., 2000. Age-dependent fecundity and life-fertility tables for Podisus nigrispinus (Dallas) (Het., Pentatomidae). Journal of Applied Entomology 124, 319–324. https://doi.org/10.1046/j.1439-0418.2000.00482.x
Medeiros, R.S., Ramalho, F.S., Zanuncio, J.C., Serrão, J.E., 2003. Effect of temperature on life table parameters of Podisus nigrispinus (Het., Pentatomidae) fed with Alabama argillacea

(Lep., Noctuidae) larvae. Journal of Applied Entomology 127, 209–213. https://doi.org/10.1046/j.1439-0418.2003.00728.x
Meehl, G.A., Tebaldi, C., 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. Science 305, 994–997.
Moreira, C., Stillman, J.H., Lima, F.P., Xavier, R., Seabra, R., Gomes, F., Veríssimo, A., Silva, S.M., 2021. Transcriptomic response of the intertidal limpet Patella vulgata to temperature extremes. Journal of Thermal Biology 101, 103096. https://doi.org/10.1016/j.jtherbio.2021.103096
Morgan, D., Walters, K.F.A., Aegerter, J.N., 2001. Effect of temperature and cultivar on pea aphid, Acyrthosiphon pisum (Hemiptera: Aphididae) life history. Bulletin of Entomological Research 91, 47–52. https://doi.org/10.1079/BER200062
Muñoz, M.M., Bodensteiner, B., 2019. Janzen's hypothesis meets the Bogert effect: connecting climate variation, thermoregulatory behavior, and rates of physiological evolution. Integrative Organismal Biology 1, oby002.
Parmesan, C., Root, T.L., Willig, M.R., 2000. Impacts of Extreme Weather and Climate on Terrestrial Biota. Bulletin of the American Meteorological Society 81, 443–450. https://doi.org/10.1175/1520-0477(2000)081<0443:IOEWAC>2.3.CO;2
Phillips, B.L., Munoz, M.M., Hatcher, A., Macdonald, S.L., Llewelyn, J., Lucy, V., Moritz, C., 2016. Heat hardening in a tropical lizard: geographic variation explained by the predictability and variance in environmental temperatures. Functional Ecology 30, 1161–1168.
Pinsky, M.L., Eikeset, A.M., McCauley, D.J., Payne, J.L., Sunday, J.M., 2019. Greater vulnerability to warming of marine versus terrestrial ectotherms. Nature 569, 108–111. https://doi.org/10.1038/s41586-019-1132-4
Pörtner, H.-O., Roberts, D.C., Tignor, M.M.B., Poloczanska, E.S., Mintenbeck, K., Alegría, A., Craig, M., Langsdorf, S., Löschke, S., Möller, V., Okem, A., Rama, B. (Eds.), 2022. Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change.
Rahmstorf, S., Coumou, D., 2011. Increase of extreme events in a warming world. Proceedings of the National Academy of Sciences 108, 17905–17909. https://doi.org/10.1073/pnas.1101766108
Rasmont, P., Iserbyt, S., 2012. The Bumblebees Scarcity Syndrome: Are heat waves leading to local extinctions of bumblebees (Hymenoptera: Apidae: *Bombus*)? Annales de la Société entomologique de France (N.S.) 48, 275–280. https://doi.org/10.1080/00379271.2012.10697776
Ratkowsky, D.A., Olley, J., Ross, T., 2005. Unifying temperature effects on the growth rate of bacteria and the stability of globular proteins. Journal of Theoretical Biology 233, 351–362. https://doi.org/10.1016/j.jtbi.2004.10.016
Reznick, D., 1985. Costs of Reproduction: An Evaluation of the Empirical Evidence. Oikos 44, 257–267. https://doi.org/10.2307/3544698
Richter, K., Haslbeck, M., Buchner, J., 2010. The Heat Shock Response: Life on the Verge of Death. Molecular Cell 40, 253–266. https://doi.org/10.1016/j.molcel.2010.10.006
Santra, M., Dill, K.A., De Graff, A.M., 2019. Proteostasis collapse is a driver of cell aging and death. Proceedings of the National Academy of Sciences 116, 22173–22178.

Savage, V.M., Gillooly, J.F., Brown, J.H., West, G.B., Charnov, E.L., 2004. Effects of Body Size and Temperature on Population Growth. The American Naturalist 163, 429–441. https://doi.org/10.1086/381872
Sears, M.W., Angilletta, M.J., Schuler, M.S., Borchert, J., Dilliplane, K.F., Stegman, M., Rusch, T.W., Mitchell, W.A., 2016. Configuration of the thermal landscape determines thermoregulatory performance of ectotherms. Proceedings of the National Academy of Sciences 113, 10595–10600.
Sears, M.W., Raskin, E., Angilletta Jr, M.J., 2011. The world is not flat: defining relevant thermal landscapes in the context of climate change. Integrative and Comparative Biology 51, 666–675.
Silbermann, R., Tatar, M., 2000. Reproductive costs of heat shock protein in transgenic Drosophila melanogaster. Evolution 54, 2038–2045.
Sinclair, B.J., Marshall, K.E., Sewell, M.A., Levesque, D.L., Willett, C.S., Slotsbo, S., Dong, Y., Harley, C.D., Marshall, D.J., Helmuth, B.S., 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? Ecology Letters 19, 1372–1385.
Slein, M.A., Bernhardt, J.R., O'Connor, M.I., Fey, S.B., 2023. Effects of thermal fluctuations on biological processes: a meta-analysis of experiments manipulating thermal variability. Proceedings of the Royal Society B: Biological Sciences 290, 20222225. https://doi.org/10.1098/rspb.2022.2225
Somero, G.N., Lockwood, B.L., Tomanek, L., 2017. Biochemical adaptation: response to environmental challenges from life's origins to the anthropocene. (No Title).
Sørensen, J.G., Kristensen, T.N., Loeschcke, V., 2003. The evolutionary and ecological role of heat shock proteins. Ecology letters 6, 1025–1037.
Southwood, T.R.E., 1988. Tactics, Strategies and Templets. Oikos 52, 3–18. https://doi.org/10.2307/3565974
Stefani, M., Dobson, C.M., 2003. Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. J Mol Med 81, 678–699. https://doi.org/10.1007/s00109-003-0464-5
Stroustrup, N., Anthony, W.E., Nash, Z.M., Gowda, V., Gomez, A., López-Moyado, I.F., Apfeld, J., Fontana, W., 2016. The temporal scaling of Caenorhabditis elegans ageing. Nature 530, 103–107. https://doi.org/10.1038/nature16550
Thomas, M.K., Aranguren-Gassis, M., Kremer, C.T., Gould, M.R., Anderson, K., Klausmeier, C.A., Litchman, E., 2017. Temperature–nutrient interactions exacerbate sensitivity to warming in phytoplankton. Global Change Biology 23, 3269–3280. https://doi.org/10.1111/gcb.13641
Thomsen, M.S., Mondardini, L., Alestra, T., Gerrity, S., Tait, L., South, P.M., Lilley, S.A., Schiel, D.R., 2019. Local Extinction of Bull Kelp (Durvillaea spp.) Due to a Marine Heatwave. Frontiers in Marine Science 6.
Uiterwaal, S.F., DeLong, J.P., 2020. Functional responses are maximized at intermediate temperatures. Ecology 101, e02975. https://doi.org/10.1002/ecy.2975
Vasseur, D.A., 2020. The impact of temperature on population and community dynamics, in: Theoretical Ecology, Concepts and Applications. Oxford University Press.
Vasseur, D.A., DeLong, J.P., Gilbert, B., Greig, H.S., Harley, C.D., McCann, K.S., Savage, V., Tunney, T.D., O'Connor, M.I., 2014. Increased temperature variation poses a greater risk

to species than climate warming. Proceedings of the Royal Society B: Biological Sciences 281, 20132612.
Vasseur, D.A., Robey, A.J., Bernhardt, J., unpublished. A unified approach for studying the ecological effects of permissive and stressful temperatures on ectotherm populations.
Vinton, A.C., Vasseur, D.A., 2022. Resource limitation determines realized thermal performance of consumers in trophodynamic models. Ecology Letters.
Welbergen, J.A., Klose, S.M., Markus, N., Eby, P., 2008. Climate change and the effects of temperature extremes on Australian flying-foxes. Proceedings of the Royal Society B: Biological Sciences 275, 419–425. https://doi.org/10.1098/rspb.2007.1385
Welte, M.A., Tetrault, J.M., Dellavalle, R.P., Lindquist, S.L., 1993. A new method for manipulating transgenes: engineering heat tolerance in a complex, multicellular organism. Current Biology 3, 842–853. https://doi.org/10.1016/0960-9822(93)90218-D
Woods, H.A., Kingsolver, J.G., Fey, S.B., Vasseur, D.A., 2018. Uncertainty in geographical estimates of performance and fitness. Methods in Ecology and Evolution 9, 1996–2008. https://doi.org/10.1111/2041-210X.13035
Xia, J.Y., Van, W., der Werf, Rabbinge, R., 1999. Influence of temperature on bionomics of cotton aphid, Aphis gossypii, on cotton. Entomologia Experimentalis et Applicata 90, 25–35. https://doi.org/10.1046/j.1570-7458.1999.00420.x