The Physical and Chemical Basis for Temperature Effects on Metabolic Rate and Biological Processes – A Brief History

By Mark Ritchie (corresponding author, Department of Biology, Syracuse University, Syracuse NY 13244 USA, meritchi@syr.edu

José Ignacio Arroyo, Santa Fe Institute, Santa Fe, New Mexico USA

One of the most salient features of metabolic theory is its reliance on predictions derived from principles of physics and chemistry (Brown *et al.* 2004). It is what have been called an "efficient theory"; a theory that from a few principles/assumptions is able to make many predictions (Marquet *et al.* 2014). It is therefore useful to know the origin of the principles that derive temperature-dependence of metabolism to understand how they drive translation of rates from biochemistry to whole-organism or even ecosystem metabolism and to predict the key features of thermal performance curves (TPCs), such as the optimum, maximum, and range temperature. There have been two primary paths forward in generating hypotheses for biological temperature dependence, or BTD: one rooted in physics, chemistry, and development of the Second Law of thermodynamics (e.g., Boltzmann, Clausius, Gibbs, Arrhenius, Van't Hoff, Arrhenius, Eyring) and one rooted in biology and physiology and the detailed mechanisms of gas exchange in model organisms (Boyle, Lavoisier, Krogh, Wu). The dominant paradigms for BTD in biology versus chemistry and physics remained largely separated through the 19th and 20th Century, and only in the last 20 years have there been concerted efforts to unify them.

The formal scientific study of biological temperature dependence began, perhaps ironically, simultaneously with that of early ideas about the scaling of circulatory systems, as highlighted by the work of early 18th Century Dutch physician and chemist Herman Boerhaave (Cook 2007; Lindemann 2013). He promoted several prescient ideas: 1) the human body is a mechanical, hydrological structure, 2) components vibrate more frequently at higher temperature and 3) organisms maintain themselves near what we would now describe as "steady-state." Boerhaave viewed illness as an indicator of the body being out of steady-state and was apparently one of the first to use a thermometer to measure the magnitude of fever.

From Boerhaave's concepts, another 200 years would pass before biologists would begin to formally study biological temperature dependence. In the meantime, the first measurements of gas exchange in animals by Robert Boyle and John Mayow and the various discoveries of chemist Anton Lavoisier in the later 18th Century set the stage for thinking about organisms as systems exchanging energy with their environment. However, it is at the turn of the 18th century that the chains of advances in understanding temperature's role in biology and physiology versus chemistry and physics began to diverge.

Advances in understanding the relationships between materials, energy and temperature proceeded apace in the 19th Century, unencumbered as physicists and chemists were by the need to measure gas exchange in live organisms. Spurred by Avogadro's discovery of chemical "particles," or molecules in 1811, theory and concepts related to "macroscopic" physics emerged by the 1830's. This approach applies statistical descriptions to understand the collective behavior of very large numbers of particles. Advances featured the formulation of the Ideal Gas Law by Benoit Paul Emile Clapeyron, conceptualization and definition of work and heat by Sadi Carnot, the measurement of heat-work equivalence by James Joule and the concept of kinetic energy available for work by Josiah Gibbs. Further developments included the formulation of entropy and the Second Law of Thermodynamics by Rudolf Clausius and Ludwig Boltzmann.

Here we describe the history of the modeling of temperature dependence in physical-chemistry and biology, focusing on the origins of the Arrhenius equation, their extensions and use (e.g.

metabolic theory), then we go to the development of the standard thermodynamic theory to finally briefly mention recent developments based on thermodynamics.

An exploration of Arrhenius kinetics

These breakthroughs in physics, all based on descriptions of moving particles and the probability of reactants colliding or combining as a function of their kinetic energy, fueled the development of physical chemistry in the 1880's (see Figure 2). The first equation attempted to describe the response of reaction rate was proposed by Ludwig Wilhelmy in 1850 (Laidler 1984). However, the first, significant contribution to modeling the temperature dependence was made by Jacobus Van't Hoff, the first winner of the Nobel Prize for Chemistry in 1901, and the French chemist Henry LeChatelier in the mid 1870's formulated the relationship between the energy required for chemical conversions and equilibrium (when the conversion of a reactant to a product is balanced by the reverse conversion of product to reactant). Van't Hoff's (1884) theory recognized that reacting compounds, and reactants and catalysts in particular, form an intermediate "transition state" during the path from reactants to products. The equation proposed by Van't Hoff for the temperature dependence of the catalyzed reaction rate under constant pressure P was

$$\left(\frac{\partial \ln(k)}{\partial T}\right)_P = \frac{E_a}{RT^2} \quad (1)$$

Where k is rate constant, T is absolute temperature in degrees Kelvin, R is the gas constant (0.00831 kJ mol^{-1} $^oK^{-1}$), and E_a a constant which subsequently was called "activation energy" (see below).

Around the end of 19th and beginning of the 20th centuries other models were proposed (see Laidler 1984) but there was no consensus of which of the models proposed was universal. In this sense, Arrhenius compiled data from many previous studies on the temperature response of chemical reactions and fit different models, and found that Vant Hoff's model fit the data better. This form, currently used, is an exponentially increasing function of temperature that is obtained from the direct integration of Eq. (1) (Fig. 2A)

$$k = Ae^{-E_a/RT} \quad (2)$$

where *A* is a "pre-factor" containing information about the reaction not related to temperature dependence, *e* is the natural base and E_a is the "activation" energy for the reaction, *T*.Besides demonstrating the convergence of the above equation for the data on reaction rates available at that time, Svante Arrhenius, also a Novel Prize winner (in 1903), further developed the concept of "activation energy" (E_a) and a fuller description of the molecular kinetics of chemical reactions during 1889-1901.

The determination of a reaction rate constant *k* from physical principles has dominated the fields of physical chemistry and biochemistry for the past 120 years and the mechanism is summarized in Box 1. In addition, the fundamental thermodynamics involved is critical to understanding both the past and current state of the field.

In summary, what today we know as the Arrhenius equation was originally proposed by Vant Hoff, and is an empirical (not derived from first-principles) function, but that later was contextualized and interpreted in further developments in thermodynamics (in the 20th century,. In this vein, another empirical model was suggested as an extension of the Arrhenius equation and that proposes a power-law to account for deviations from the exponential phase, the Kooij (1893) equation

$$k = Ae^{-E_a/RT}T^C \quad (3)$$

Where C is a constant. This early extension paradoxically did not become popular to explain model deviations from the exponential form in current biological data, perhaps because of the lack of an interpretation, or principles-based explanation of the C parameter.

Further development of the Arrhenius equation occurred in 1935. The quantum-mechanical details and thermodynamic properties of transition states inferred in the work of Van't Hoff, LeChatelier, Arrhenius, and Gibbs were explored simultaneously in more detail by the USA team of Eyring and his student W.F.K. Wynne-Jones and the UK team of Meredith Gwynne Evans and Michael Polanyi (Eyring 1935, Evans and Wynne-Jones 1935, Evans and Polanyi 1935). Their equivalent theories describe the reaction constant *k* being driven by the heat required for large numbers of rotating, vibrating molecules to collide and the change in entropy resulting from collapse of the transition state to product

$$k = \frac{\kappa k_B}{h}Te^{-\Delta G^{\ddagger}/k_BT} \quad (4),$$

Where $\Delta G^{\ddagger}$ is the Gibbs energy or the activation energy, k_B is Boltzmann constant, h is Planck's constant, and κ is a constant (transmission coefficient, often assumed to be 1).

Given that $\Delta G^{\ddagger} = \Delta H^{\ddagger} + T\Delta S^{\ddagger}$, Eq. (4) can be rewritten as

$$k = \frac{\kappa k_B}{h}Te^{(-\Delta H^{\ddagger}+\Delta S^{\ddagger}T)/k_BT} = \frac{\kappa k_B}{h}Te^{\Delta S^{\ddagger}/k_B}e^{-\Delta H^{\ddagger}/k_BT} \quad (5),$$

The coefficient *A* was now defined by the constants κ, a transfer coefficient referring to the proportion of reactant-enzyme complexes that are at or higher than $\Delta G^{\ddagger}$, h, Planck's constant, and k_B, the Boltzmann constant. It is worth noting that if we compare the Arrhenius empirical model with the Eyring first-principles derivation we have that, $E_a = \Delta H + RT$.

Box 1 Enthalpy and entropy and chemical reaction rates This approach considers work done by heat added to a collection of reactant molecules. At a given initial temperature, reactants have a bond energy, called *enthalpy*, H_r or the amount of energy released if a mole of reactant molecules is broken into its component atoms. Likewise, reactions proceed by the formation of one or more intermediate compounds, called *transition states*. Typically, the transition states for a particular reaction have a higher enthalpy, or potential energy in their bonds, $H^{\ddagger}$, than the reactants. Therefore, an additional energy, called the *activation energy*, is required to form this higher potential energy transition state (Fig.1A).

This activation energy has two components – the energy captured in the formation of the bonds of the transition state, or $\Delta H^{\ddagger}$ (which is the difference between the enthalpies of the transition state and the reactants, $H^{\ddagger} - H_r$), and the energy spent on changing the position of molecules and forming a new type of molecule, the transition state. Boltzmann had previously shown that these changes in the number of "microstates" - position, type, and potential energy of molecules can be quantified as a change in the *entropy of activation* $\Delta S^{\ddagger}$. Gibbs extended this idea to understand that this additional component of free energy was equal to $\Delta S^{\ddagger}$ multiplied by temperature. Thus, the activation energy, E_a, can be written as the quantity known as the "free energy of activation," $\Delta G^{\ddagger}$

$$\Delta G^{\ddagger} = H^{\ddagger} - H_r + \Delta S^{\ddagger} T \;=\; \Delta H^{\ddagger} + \Delta S^{\ddagger} T$$

In this case, $\Delta G^{\ddagger}$ is positive because energy must be added and entropy increased for the reaction to proceed.

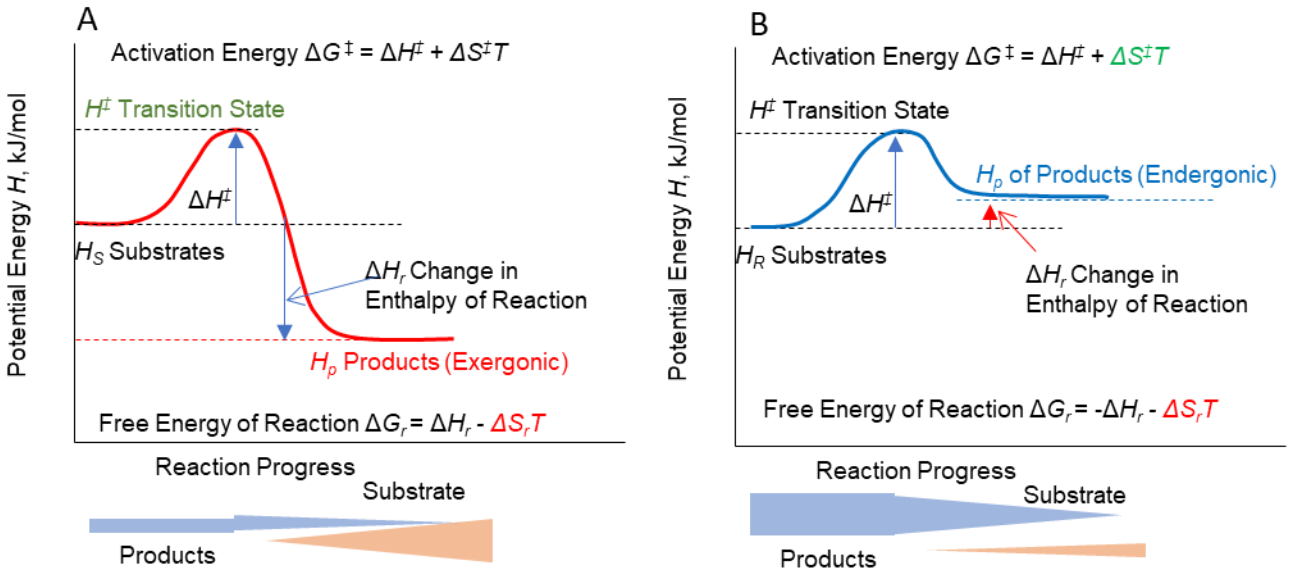

Figure 1. Enthalpy versus reaction progress. The plots show the effect of an enzyme in decreasing the needed energy for a reaction to occur.

An attractive feature of equations (2) and (3) are that they can be transformed into a linear relationship between the logarithm of reaction rate and the inverse of temperature (Fig. 2B)

$$\ln(k) = \ln(A) - (\Delta G^{\ddagger}/\mathrm{R})(1/T) \quad (6)$$

in which the x-axis (the inverse of temperature) reflects a shift from hot (towards the origin) to cool (to the right) temperatures, and the y-axis is the natural logarithm of the reaction constant. The slope of the line estimates (since we already know the gas constant *R*) the activation energy (E_a/R) of a reaction and the intercept is ln(*A*). This linearization proposed by both Van't Hoff and Arrhenius stimulated a century of exploring temperature sensitivity of biochemical reactions by (1) plotting the logarithm of rates measured at different temperatures against the inverse of those temperatures and estimating the slope (Gillooly *et al.* 2001) and (2) comparing activation energies and entropies for different reactions and catalysts (Piskulich *et al.* 2019).

The Ratio Q_{10}

In contrast to developments in physics and chemistry and their applications to biology, progress in understanding the role of temperature to metabolic rate in biological research lagged during the 19th Century. Physiologists did not develop instruments that could precisely measure exchanges of particular gases (oxygen versus carbon dioxide versus dinitrogen) in live organisms until very early in the 20th Century. Biology as a science in the 19th Century also was heavily influenced by Carl Linnaeus (Carl von Linne'), Alfred Wallace, and Charles Darwin to focus on classifying and comparing attributes of the many forms of life. Relatively few scientists, with most of those the groups working on environmental influences on plant gas exchange, had interest in connecting physical and chemical "first principles" to biological measurements. Finally, most practicing 19th Century physiologists were physicians and largely focused on practical methods of diagnosis and response rather than fundamental physical and chemical theories.

As the 20th Century arrived, physiologists had the opportunity to link gas exchange measurements with the late 19th century developments in physical chemistry. One important outcome of the early work on thermodynamics of chemical reactions was the derivation of a temperature coefficient Q_{10} for a reaction at equilibrium derived from Van't Hoff's equation, which compares two rates, k_1 and k_2 at temperatures 10 degrees apart (Gillooly *et al.* 2001).

$$Q_{10} = \frac{k_1}{k_2} = Ae^{\left(\frac{-\Delta G\ddagger}{R}\right)\left(\frac{1}{T+10}-\frac{1}{T}\right)} = Ae^{\left(\frac{\Delta G\ddagger}{R}\right)\left(\frac{10}{T^2+10T}\right)} \quad (7),$$

which implies that the temperature coefficient is not a constant over the range of temperatures *T* to *T*+10, since the change in enthalpy, or difference in potential energy of the chemical bonds in the transition state molecule(s) compared to the reactant molecule(s), $\Delta H^{\ddagger}$, is assumed to be constant.

Early physiologists recognized that the temperature coefficient was not a constant with temperature, and the first measurements of metabolic rate – temperature relationships by a trio of

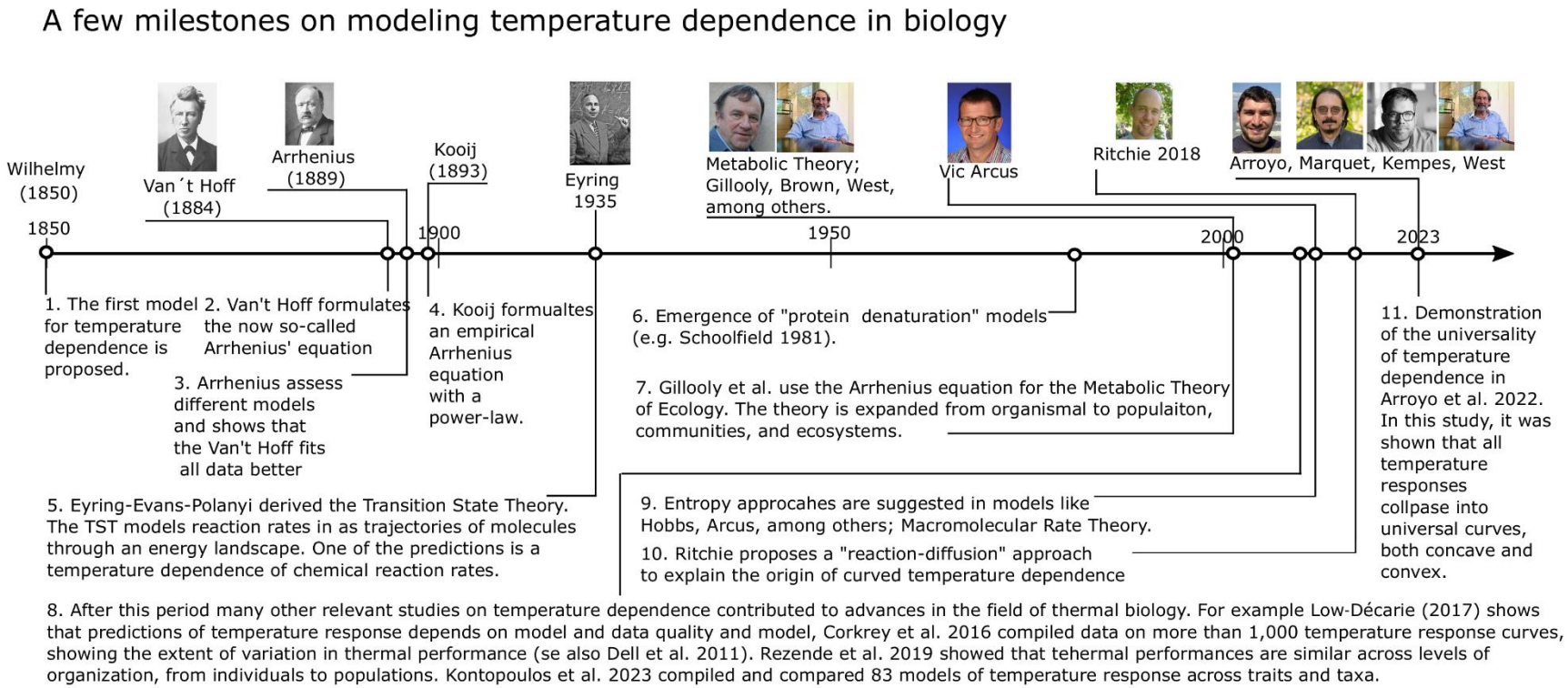

Figure. 2. Timeline of some milestones in the history of the study of temperature dependence modeling. Some relevant findings in the understanding and modeling of temperature dependence in biology are depicted. A few of them include the proposal of the Arrhenius equation, their application in developing the Metabolic Theory of Ecology, and some of their subsequent extensions to account for the whole curvature of temperature response. These extensions include the development of "protein denaturation" models and "heat capacity models". After the 2010s several major empirical findings are summarized in point 11, which include an exhaustive compilation of data on traits and thermal responses at different levels of organization. This empirical data together with other recent theoretical developments (e.g. Arroyo et al. 2022) indicate that temperature dependence is a broad pattern in biology, from enzymes to ecosystems. Not just there have been a huge accumulation of data but also models, as almost 100 different models there exist to explain temperature dependence (Kontopoulus et al. 2023), but not unified theory that could explain all the different fundamental aspects (denaturation, entropy, etc.) of the temperature dependence of biological quantities across levels of organization.

German scientists were interpreted in terms of the Arrhenius' function (Snyder 1908; Putter 1914; Kanitz 1915). In the 2016 book *The Respiratory Exchange of Animals and Man* by the 1920 Nobel Prize winner for Physiology or Medicine, August Krogh, along with his wife and collaborator Marie, described the response of metabolic rate to temperature as the ratio of two rates measured at temperatures ten degrees apart, or Q_{10}. Krogh pointed out that Q_{10} would not be constant across different temperature ranges, but rather be lower at higher temperatures and showed that the Arrhenius expression for metabolic rate provided a superior fit to data than a constant Q_{10}.

Despite these early cautions and linkages to thermodynamics, estimation of Q_{10} as a ratio of rates measured over comparable temperature ranges, perhaps taking a cue from biochemists' tendency to compare reaction characteristics at different temperatures relative to a standard 25^{o}C, became the convention for measuring temperature sensitivity in physiological and growth measurements. The ratio was used as a diagnostic for acclimation or adaptation to extreme temperatures (lower Q_{10} in better adapted or acclimated organisms) and the basis for consensus of an "average" Q_{10} of 2 – 3 for various physiological rates. These outcomes led to a general understanding of the magnitude of response of physiological and other rates to temperature and Q_{10} values were routinely compared among organism taxa and environments (Schulte 2015).

The inadequacies of the Q_{10} framework to explore BTD at temperature extremes emerged in the 1990's as researchers' interest in the potential effects of climate change pushed analyses into larger temperature intervals with higher maximum T and lower Q_{10}. In addition, without any direct physical or chemical explanations for a constant Q_{10} (Mahecha *et al.* 2010) it has been difficult to explain increasingly observed organism responses to temperature that changed with other environmental factors, such as elevated CO_2, nitrogen deposition, and altered thermal environments.

The problem of declining rates at higher temperatures.

Whether oriented around the Arrhenius or Q_{10} interpretations, the substantial bulk of research on BTD has focused on the range of temperatures where metabolic rate for ectotherms increases with temperature. This focus largely ignored observed physiological rates that declined at higher temperatures, which implied an optimal T_{opt} associated with maximum rates and maximum temperature T_{max} for life. Observation of these limits occurred even prior to Arrhenius' work, such as Boerhaave's recognition of fever as a signal of the body being "out of equilibrium." Krogh's 1916 monograph mentioned evidence of temperatures at which gas exchange rates of ectotherms decline with increasing temperature but cited a lack of data and did not discuss the issue further.

Copious data collected since 1916 indicate that biochemical, gas exchange and other physiological rates in organisms exhibit an "optimal" temperature, T_{opt}, in the range of 20-40^{o}C, above which rates decline. In addition, a massive number of measurements of "temperature performance curves," or TPCs indicate an additional limit called critical maximum temperature, or CT_{max}, at which organism rates reduce dramatically or death occurs. These limits to either rate

(at T_{opt}) or the temperature range of life have been extensively studied since the 1970's. However, the theoretical basis for them, and thus the ability to predict how they might change for different rate processes, organisms or environments remains unresolved.

Declining rates at $T > T_{opt}$ have largely been interpreted through the critical role of enzymes as catalysts of biochemical reactions and the *enzyme degradation hypothesis* (Fig. 2) the idea that reactions decline at higher temperatures because enzymes unfold or denature and thus lose their catalytic capacity. Ironically, enzymes were discovered and their role as catalysts hypothesized in the 1830's by Jon Jakob Berzelius, well before the key developments in thermodynamics and physical chemistry. However, their catalytic function was not experimentally proven for another 60 years and the enzymes themselves not purified until the 1920's. Thus, the enzyme degradation hypothesis arose at about the time that metabolic rate measurements became routine.

Without a clear mechanism from statistical mechanics or thermodynamics to explain T_{opt} and/or T_{max}, biochemists and physiologists turned to empirical observations to infer mechanisms that might explain these phenomena in BTD. Hsien Wu, an obscure contemporary of Eyring, Wynne-Jones, Evans, and Polanyi, was a Chinese biochemist who used the methods of isolating enzymes newly available in the 1920's to explore the causes of protein denaturation. In a series of 13 papers published over the period 1924-1931, such as (Wu & Yen 1924), Wu proposed a theory that environmental factors, including temperature, break the polar (driven by electrical charge) bonds that hold enzymes together.

Following subsequent replication of Wu's experiments, the enzyme degradation hypothesis has become the largely unchallenged paradigm for interpreting T_{opt} and declining rates for the past 90 years. Organisms adapted to cooler temperatures and showing lower T_{opt} in the same physiological rates were assumed to have evolved different isozymes (enzymes that catalyze the same reaction but have different amino acid sequences). Improvements in protein isolation and molecular analysis developed during the 1980's and 1990's fostered analysis of the kinetic properties of a vast array of enzymes from many different organisms (Ritchie 2018).

Simultaneously with the measurements of reaction kinetics and thermodynamics, many thermal performance curves, or TPCs, have been measured for a variety of physiological rates in a large range of ectothermic organisms from microbes to invertebrates (Deutsch *et al.* 2008; Knapp & Huang 2022). These TPCs estimate T_{opt} and CT_{max} along with other parameters that define the range of temperatures at which performance occurs and increases exponentially. Since the 1970's, understanding "thermal performance curves" or TPCs has become important for assessing the likely impacts of climate change (Deutsch *et al.* 2008; Kontopoulos *et al.* 2020; Bennett *et al.* 2021; Montagnes *et al.* 2022). Generally, the data from these experiments has been fitted to the Sharpe-Schoolfield model (Schoolfield *et al.* 1981) or similar models (Box 2). These models accounts for temperature declines by assuming that enzymes become "deactivated," presumably through degradation of enzyme structure under the enzyme degradation hypothesis, as temperature increases above T_{opt}.

Near the boundaries of an organism's thermal niche where temperatures are high or low enough to reversibly inactivate key metabolic enzymes, metabolic performance steeply drops toward

zero, resulting in a unimodal thermal performance. These dynamics are captured by models such as a unimodal extension of the Arrhenius model commonly called the Sharpe-Schoolfield model (Sharpe & DeMichele 1977; Schoolfield *et al.* 1981). A simplified version of the model that ignores deactivation of enzymes at low temperatures is

$$k(T) = \frac{k_0 e^{\frac{-\Delta G}{R}\left(\frac{1}{T} - \frac{1}{T_0}\right)}}{1 + +e^{\frac{E_H}{R}\left(\frac{1}{T_H} - \frac{1}{T}\right)}} \quad (8),$$

where k_0 is a reference rate at temperature T_0, T is the temperature of interest, ΔG is the activation energy estimated for the exponentially increasing portion of the curve (Fig. 3), E_H is the "de-activation energy that is fit to a slope of exponential decline above a peak temperature, and T_H is the temperature at which the rate reaches half of the maximum rate in the declining rate phase. This equation produces a shape that, when ΔG, E_H, and T_H are free parameters, often fits temperature performance data.

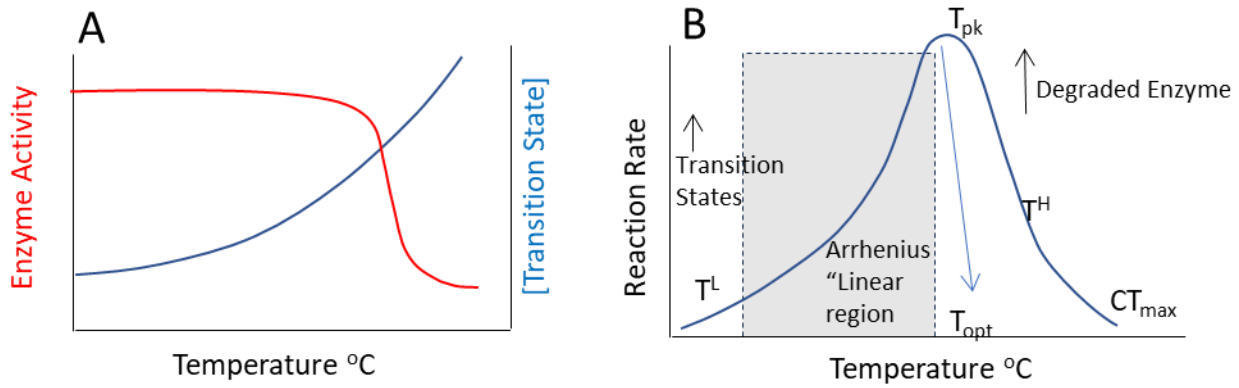

Figure 3. Illustration of the ***enzyme degradation hypothesis****. A. Enzyme activity (red curve) remains approximately constant up to a certain temperature, above which the enzyme begins to change conformation (degrade), thus causing a rapid decline in substrate affinity for the enzyme and thus the enzyme's catalytic capacity. Meanwhile the concentration of transition states (enzyme-substrate and enzyme-product complexes increases exponentially with the expected rate of molecular collisions (kinetics). B. Net reaction rate curve that increases exponentially across the lower range of* T *up to a temperature where enzyme degradation begins to occur, causing a decline in reaction rate above an optimal temperature* T_{opt}*. The resulting unimodal reaction rate showing* T_{opt}*, as well as the parameters of the Sharpe-Schoolfield Model: peak temperature* T_{pk} *(equivalent to* T_{opt}*), temperature at ½ decline from peak temperature* T_H *and maximum critical temperature* CT_{max}*. The shaded area demonstrates the temperature region where exponential or a linear Arrhenius relationship occurs, a region of "temperature scaling."*

However, there is no accompanying fundamental thermodynamic mechanism, such as a change in energy state, to explain the value of the deactivation energy E_H or T_H. Rather, the value is inferred from experimental data under the assumption that a decline could only be caused by conformational changes to enzymes, defined here as "degradation." Ultimately, the various formulae used provide mathematical descriptions that are fit to data under the assumption of enzyme degradation at temperatures above T_{opt}.

In addition to the Sharpe-Schoolfield model, there are diverse models that account for the curved temperature response. These models often include extensions of the Arrhenius equation or extensions of the Eyring equation, then there are empirical-theoretical and theoretical approaches to explain the declining portion of the TPC (DeLong *et al.* 2017; Grimaud *et al.* 2017; Low-Décarie *et al.* 2017). All of these models are complex, i.e. they have many parameters that are fit to data and have no physical or chemical underlying explanation. Confidence in these models is often lacking as a result, and this has motivated recent attempts to formulate a simple theory grounded in fundamental thermodynamics of catalytic reactions.

"Re-discovery" of physical mechanisms for biological temperature dependence.

As the 21st Century arrived, BTD of physiological rates was largely understood through the lens of Q_{10} and the enzyme degradation hypothesis. Variation among organisms and environments in the response to temperature was viewed as idiosyncratic and reflective of differences in evolutionary history. Variation in T_{opt} was attributed to evolved variation in enzyme isozymes with different thermal tolerances and plastic adaptation to exposure to particular thermal regimes, or acclimation. The success of physical first principles in deriving the network model for explaining body size scaling of metabolism (West *et al.* 1997) as well as thermal heat exchange (Gates 1980) set the stage unifying physical and chemical first principles to other important relationships in metabolism.

A first step in such a reconciliation, mostly absent since the early 19th Century, was the work of Jamie Gillooly, James H. Brown, Geoffrey West and others (Gillooly *et al.* 2001) who re-analyzed a large dataset of mass-specific metabolic rates for various vertebrate taxa and plants as a function of temperature below T_{opt}. In a result that would not have surprised the physiologists of the very early 20th Century, Gillooly and colleagues found that the Arrhenius function fit the data very well for all the taxa, with some variation in the estimated activation energy among taxa (Fig. 1). This result yielded two important outcomes: 1) gas exchange measurements were once again connected to the thermodynamics of biochemical reactions, and 2) the concept of activation energy, nominally one applied to a single reaction and its transition state(s), was introduced as an alternative metric of thermal sensitivity.

Alternative models for the curved temperature response

The Gillooly et al. (2001) meta-analysis rejuvenated interest since 2001 in understanding the theoretical basis for rate declines above T_{opt}. Simultaneously, a wealth of enzyme kinetic and thermodynamic data generated by 2010 suggested that the enzyme degradation hypothesis,

despite its paradigm status, is unlikely to explain the limits of metabolic rate under increasing temperature (Ritchie 2018). Denaturation temperatures of virtually all important enzymes involved in metabolism maintain activity above 50 ^{o}C and denature above 55 ^{o}C, a temperature well above most estimates of CT_{max} and T_{opt} , which largely lie below this range at 20-46 ^{o}C, (Kontopoulos *et al.* 2020; Montagnes *et al.* 2022). One review offered this quote:

The textbook explanation for reduced enzyme activity at high temperatures is protein denaturation or unfolding; however, for many enzymes, this explanation cannot account for experimental observations (Arcus & Mulholland 2020).

These results suggest that alternative explanations for declining metabolic rates at temperatures above T_{opt} need to be explained from physical and chemical principles that are important at relevant temperature ranges. For example, one theoretical approach considers an additional transition in the reaction description between active and inactive enzymes and noting that the equilibrium ratio of these two "states," respectively, declines with increasing temperature (Daniel & Danson 2010).

Re-examination of the thermodynamics of reaction rates has led to other alternative models for rate declines above T_{opt}. These models explore the consequences of the change in entropy that occurs during the chemical transitions during enzyme-catalyzed reactions. This entropy change was first recognized by Van't Hof and Gibbs but was re-derived from quantum mechanics in the Eyring – Polanyi equation from 1935 (equation (3)). These models track changes in entropy as a change in heat capacity, C_P (J/^{o}K) among transition states, $\Delta C_P^{\ddagger}$, and that $\Delta C_P^{\ddagger} < 0$ (Hobbs *et al.* 2013; Arcus *et al.* 2016; Arroyo *et al.* 2022). This means that, as temperature increases, less of the energy added to the system is incorporated into bond energy, and thus activation, of transition states. This results in a lower rate of enzyme-substrate binding and thus a slower reaction.

One approach is that of Arroyo et al. (2022). They hypothesized that a critical point and subsequent decrease of rates given by temperature was related to the change in the entropy of activation $\Delta S^{\ddagger}$ and temperature, similar. They described the relationship between entropy change and the difference in heat capacity with increasing temperature from a reference temperature T_0 to a new temperature T, as

$$\Delta S^{\ddagger} = \int_{T_0}^{T} \frac{{C_P}^{\ddagger}}{T}\, dT = \Delta {S_0}^{\ddagger} + \Delta {C_P}^{\ddagger} ln\left(\frac{T}{T_0}\right) \quad (6),$$

where T_0 is a reference temperature (usually 25 ^{o}C or 298 ^{o}K) and $\Delta S_0^{\ddagger}$ is the molar entropy change at the reference temperature, a quantity measured at 25^{o}C for many common reactions.

The Eyring equation with the change in entropy defined as in equation (6) becomes

$$k = B_0 \left(\frac{1}{T}\right)^{-\frac{\Delta {C_P}^{\ddagger}}{R} - \alpha} e^{-\Delta H^{\ddagger}/RT}$$

(7)

where B_0 is a parameter defined as

$$B_0 = \frac{k_B e^{\Delta S_0/R} T_0^{\frac{-\Delta C_P^{\ddagger}}{R}}}{h}$$

with variables defined as in equation (3). This model was conceived to be applied to both the molecular and macroscopic level, where for the macroscopic level h is removed from the equation, and $\alpha = 1$ at the molecular level or $\alpha = 1$ otherwise. Because the change in heat capacity between transition states is negative, the exponent of the (1/*T*) term in equation (7) is actually positive and generates a negative influence of temperature on the reaction constant *k*, thus producing a T_{opt} and a T_{max}.

Another model, named "Macromolecular Rate Theory," also begins with the Eyring-Polanyi equation but invokes Kirchoff's Law to argue that changes in heat capacity of transition states also affect the heat portion of the free energy of activation, not just the entropy component (e.g., Hobbs et al 2013, Arcus et al 2016).

Although both the MMRT and Arroyo et al. model include change in heat capacity in their formulation, the underlying philosophy and mechanisms of these models are different. In Arroyo et al.'s model, the minimal mechanism that could generate a critical transition from increasing to decreasing rate is used, and yields a model that is relatively easy to fit to data. In contrast MMRT applies the known influences of a change in heat capacity on the thermodynamics of rates, with a correspondingly more complicated model that is harder to fit to data and difficult to assess whether the entropy versus enthalpy changes associated with $\Delta C_P^{\ddagger}$ are more important.

These models, which can be grouped as new versions of traditional "transition state theory," generate unimodal relationships of reaction rate versus temperature and provide a first principles explanation other than enzyme degradation for declining rates with temperatures above T_{opt}. They focus attention on changes in entropy, rather than just kinetic energy, associated with increased temperature, and thus suggest new questions and mechanisms to explore. However, as with the enzyme degradation models, e.g., these state-transition models are typically fitted to reaction or metabolic rate data to estimate parameters and the risk is therefore high that model predictions cannot be discriminated easily between models. Few studies have compared different models and their accompanying assumptions and/or estimated parameters independently, though some have compared a mechanistic model with a phenomenological or statistical model (Liang *et al.* 2017). A relatively recent study compared different temperature dependence models and concluded that model performance is "contingent on model choice and data quality" (Low-Décarie et al. 2017). This is conclusion makes sense as models with more parameters can fit the data better, and data with less noise can fit better to the model.

Transport and diffusion

Traditionally, the effect of temperature on biochemical reactions, and by extension metabolic rate, has focused on the conversion of substrates to products. This focus ignores supply of substrates to and dissipation of products from enzyme-dense reaction sites or simply assumes

that such processes do not limit reaction rate. In addition, living things exist as "open systems" that must be maintained at or near steady-state by the influx and outflux of materials and thus their metabolic rate might be better described as a reaction-displacement system.

Responses to temperature in a reaction-displacement system may differ from those captured by an analysis of reaction progress. A recent meta-analysis revealed that diffusion and transport rates are much less temperature-sensitive than substrate to product conversion, exhibiting slopes in Arrhenius plots equivalent to 25-35 kJ/mol as compared to average Arrhenius slopes (true activation energies) for common hydrolysis reactions and metabolic rate of 60-70 kJ/mol (Ritchie 2018). This difference arises because the additional energy required to move a molecule between other molecules is typically much less than that required to attain the bond energy to form enzyme-substrate complex molecules (Benesi 1986; Herrero & Rodrigo 2005). This seemingly innocuous outcome introduces several new complications into the theory of BTD.

First, asymmetry in temperature-sensitivity means that temperature affects the ratio of product to substrate when the reaction is maintained at steady-state (Niven 2009; England 2013). More specifically, dissipation of products away from reaction sites may not keep up with product formation at higher temperatures, thereby resulting in what chemists refer to as product inhibition due a greater reverse reaction rate and lower net overall reaction rate. Thus, it is conceivable that asymmetric temperature effects on diffusion and transport, which may be strongly limited in crowded cells (Roosen-Runge *et al.* 2011; Kekenes-Huskey *et al.* 2016) might drive the decline in reaction rates.

A second consequence of asymmetry in temperature sensitivity of diffusion/transport versus transition state formation is that T_{opt} may be sensitive to the thermodynamic favorability of the reaction. Many synthesis reactions are endergonic, requiring additional energy to form products in addition to the activation energy (Box 1). Thus, such reactions require much higher concentrations of substrate than product, or K_{eq}, in order to generate a net forward reaction, and any limits to product dissipation may favor the reverse reaction. If so, T_{opt} for unfavorable or synthesis reactions in organisms, such as those critical for cell replication, growth and development, may be cooler than for typical metabolic reactions that often feature highly favorable hydrolysis or oxidation reactions (Ritchie 2018). Such dependence of T_{opt} on K_{eq} is *not* predicted by the enzyme degradation hypothesis (Sharpe & DeMichele 1977; Schoolfield *et al.* 1981).

A third consequence is that a reaction-displacement framework allows consideration of *entropy production*, or the rate at which entropy is increased *outside* reaction sites by dissipation of heat and products (Niven 2009; Ritchie 2018). This contrasts with the *internal* entropy changes quantified in state transition theory and its various models. If indeed diffusion transport limits in crowded cytoplasm limits product dissipation and reduces reaction rate at T far below enzyme denaturation temperatures, then the reaction-displacement framework may lead to a more general theory for BTD based on entropy changes both inside and outside reaction sites, cells, or entire organisms.

Conclusion

After two centuries of largely separated research efforts, one based on physical chemistry and the study of biochemical reactions and the other based on whole-organism measurements of gas exchange and additional physiological rates, a reconciliation and synthesis of approaches to understand the effect of temperature on metabolic rate now seems possible. Advances in just the last two decades have emphasized the possibility of linking whole organism thermal responses to chemical and physical first principles. In particular, the field is beginning to connect to advances over this same recent time period in the thermodynamics of far from equilibrium systems and the role of entropy in limiting biological activity and metabolic rate in particular. Rather than entrenchment in the paradigms of Q_{10} and the enzyme degradation hypothesis, new models have emerged to explore the entire thermal performance relationship for metabolic rates to include T_{opt} and maximum T. These new models, as will be discussed in much more detail in ensuing chapters, provide the potential for experimental testing of new predictions among different models and that link thermal performance to environmental and thermodynamic influences and constraints well beyond issues of enzyme thermal stability. These models also are expected to have further extensions that could explain many other observed relationships in thermal biology, such as the relationships between the thermal traits of the thermal performance curve. Such potential growth in the field should render physiologists much better able to assess potential impacts of climate change.

1. Arcus, V.L. & Mulholland, A.J. (2020). Temperature, dynamics, and enzyme-catalyzed reaction rates. *Annual Review of Biophysics and Bioengineering*, 49, 163-180.

2. Arcus, V.L., Prentice, E.J., Hobbs, J.K., Mulholland, A.J., Van der Kamp, M.W., Pudney, C.R. *et al.* (2016). On the temperature dependence of enzyme-catalyzed rates. *Biochemistry*, 55, 1681-1688.

3. Arroyo, J.I., Diez, B., Kempes, C.P. & Marquet, P.A. (2022). A general theory for temperature dependence in biology. *Proceedings of the National Academy of Sciences*, 112, e2119872119.

4. Benesi, A.J. (1986). Diffusion in potentials - a method for solving the Smoluchowski equation. *Journal of Chemical Physics*, 85, 374-376.

5. Bennett, J.M., Sunday, J., Calosi, P., Villalobos, F., Martínez, B., Molina-Venegas, R. *et al.* (2021). The evolution of critical thermal limits of life on Earth. *Nature Communications*, 12, 1198.

6. Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G. (2004). Toward a metabolic theory of ecology. *Ecology*, 1771-1789.

7. Cook, H. (2007). *Commerce, Medicine, and Science in the Dutch Golden Age*. Yale University, New . Haven.

8. Daniel, R.M. & Danson, M.J. (2010). A new understanding of how temperature affects the catalytic activity of enzymes. *Trends in Biochemical Sciences*, 35, 584-591.

9. DeLong, J.P., Gibert, J.P., Luhring, T.M., Bachman, G., Reed, B., Neyer, A. *et al.* (2017). The combined effects of reactant kinetics and enzyme stability explain the temperature dependence of metabolic rates. *Ecology and Evolution*, 7, 3940-3950.

10. Deutsch, C.A., Tewksbury, J.J., Huey, R.B. & Martin, P.R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences*, 105 6668-6672.

11. England, J. (2013). Statistical physics of self-replication. *Journal of Chemical Physics*, 139, 121923.

12. Gates, D.M. (1980). *Biophysical ecology*. University of Chicago, Chicago, USA.

13. Gillooly, J.F., Brown, J.F., West, G.B., Savage, V.M. & Charnov, E.L. (2001). Effects of size and temperature on metabolic rate. *Science*, 293 2248-2251.

14. Low-Décarie, E., Boatman, T. G., Bennett, N., Passfield, W., Gavalás-Olea, A., Siegel, P., & Geider, R. J. (2017). Predictions of response to temperature are contingent on model choice and data quality. *Ecology and evolution*, *7*(23), 10467-10481.

15. Corkrey, R., McMeekin, T. A., Bowman, J. P., Ratkowsky, D. A., Olley, J., & Ross, T. (2016). The biokinetic spectrum for temperature. *PLoS One*, *11*(4), e0153343.

16. Dell, A. I., Pawar, S., & Savage, V. M. (2011). Systematic variation in the temperature dependence of physiological and ecological traits. *Proceedings of the National Academy of Sciences*, *108*(26), 10591-10596.

17. Kontopoulos, D. G., Sentis, A., Daufresne, M., Glazman, N., Dell, A. I., & Pawar, S. (2023). No model to rule them all: a systematic comparison of 83 thermal performance curve models across traits and taxonomic groups. *bioRxiv*, 2023-09.

18. Rezende, E. L., & Bozinovic, F. (2019). Thermal performance across levels of biological organization. *Philosophical Transactions of the Royal Society B*, *374*(1778), 20180549.

19. Grimaud, G.M., Mairet, F., Sciandra, A. & Bernard, O. (2017). Modeling the temperature effect on the specific growth rate of phytoplankton: a review. *Reviews in Environmental Science and Biotechnology*, hal-01576871.

20. Herrero, M.A. & Rodrigo, M. (2005). A note on Smoluchowski's equations with diffusion. *Applied Mathematics Letters*, 18, 969-975.

21. Hobbs, J.K., Jiao, W., Easter, A.D., Parker, E.J., Schipper, L.A. & Arcus, V.L. (2013). Change in heat capacity for enzyme catalysis determines temperature dependence of enzyme catalyzed rates. *ACS Chemical Biology*, 8, 2388–2393.

22. Kanitz, A. (1915). *Temperature und Levensforgange*, Berlin.

23. Kekenes-Huskey, P.M., Scott, C.E. & Atalay, S. (2016). Quantifying the influence of the crowded cytoplasm on small molecule diffusion. *Journal of Physical Chemistry B*, 120, 8696-8706.

24. Knapp, B.D. & Huang, K.C. (2022). The effects of temperature on cellular physiology. *Annual Review of Biophysics*, 51, 499-526.

528 25. Kontopoulos, D.G., van Sebille, E., Lange, M., Yvon-Durocher, G., Barraclough, T.G. &
529 Pawar, S. (2020). Phytoplankton thermal responses adapt in the absence of hard
530 thermodynamic constraints. *Evolution*, 74, 775-790.

531 26. Laidler, K.J. (1984). The development of the Arrhenius equation. *Journal of Chemical*
532 *Education*, 61, 494.

533 27. Liang, L.L., Arcus, V.L., Heskel, M.A., O'Sullivan, O.S., Weerasinghe, L.K., Creek, D. *et al.*
534 (2017). Macromolecular Rate Theory (MMRT) provides a thermodynamics rationale to
535 underpin the convergent temperature response in plant leaf respiration *Global Change*
536 *Biology*, doi: 10.1111/gcb.13936.

537 28. Lindemann, M. (2013). *Medicine and Society in Early Modern Europe* 2nd edn. Cambridge
538 University Press. , Cambridge.

539 29. Low-Décarie, E., Boatman, T.G., Bennett, N., Passfield, W., Gavalás-Olea, A., Siegel, P. *et*
540 *al.* (2017). Predictions of response to temperature are contingent on model choice and
541 data quality. *Ecology and Evolution*, 7, 10467-10481.

542 30. Mahecha, M.D., Reichstein, M., Carvalhais, N., Lasslop, G., Lange, H., Seneviratne, S.I. *et*
543 *al.* (2010). Global Convergence in the Temperature Sensitivity of Respiration at
544 Ecosystem Level. *Science*, 329, 838-840.

545 31. Marquet, P.A., Allen, A.P., Brown, J.H., Dunne, J.A., Enquist, B.J., Gillooly, J.F. *et al.*
546 (2014). On Theory in Ecology. *BioScience*, 64, 701-710.

547 32. Montagnes, D.J.S., Wang, Q., Lyu, Z. & Shao, C. (2022). Evaluating thermal performance of
548 closely related taxa: Support for hotter is not better, but for unexpected reasons.
549 *Ecological Monographs*, 92.

550 33. Niven, R.K. (2009). Steady state of a dissipative flow-controlled system and the maximum
551 entropy production principle. *Physical Review E*, 80, 0211131-02111315.

552 34. Piskulich, Z.A., Mesele, O.O. & Thompson, W.H. (2019). Activation Energies and Beyond.
553 *The Journal of Physical Chemistry A*, 123, 7185-7194.

554 35. Putter, A. (1914). Temperaturkoeffizienten. *Zeitschrift fur Allgemeine Physiologik*, 16, 574-
555 627.

556 36. Ritchie, M.E. (2018). Reaction-diffusion thermodynamics explains optimal temperatures of
557 biochemical reactions. *Scientific Reports*, 8.

558 37. Roosen-Runge, F., Henniga, M., Zhang, F., Jacobs, R.M.J., Sztucki, M., Schober, H. *et al.*
559 (2011). Protein self-diffusion in crowded solutions. *Proceedings of the National Academy*
560 *of Sciences*, 108, 11815–11820.

561 38. Schoolfield, R.M., Sharpe, P.J.H. & Magnuson, C.E. (1981). Non-linear regression of
562 biological temperature-dependent rate models based on absolute reaction-rate theory.
563 *Journal of Theoretical Biology*, 88, 719-731.

564 39. Schulte, P.M. (2015). The effects of temperature on aerobic metabolism: towards a
565 mechanistic understanding of the responses of ectotherms to a changing environment.
566 *Journal of Experimental Biology*, 218, 1856-1866.

567 40. Sharpe, P.J.H. & DeMichele, D.W. (1977). Reaction kinetics of poikilotherm development.
568 *Journal of Theoretical Biology*, 64, 649-670.

569 41. Snyder, C.D. (1908). A comparative study of the temperature coefficients of various
570 physiological actions. *American Journal of Physiology*, 22, 309-334.

571 42. West, G.B., Brown, J.H. & Enquist, B.J. (1997). A general model for the origin of allometric
572 scaling laws in biology. *Science*, 276, 122-126.

573 43. Wu, H. & Yen, D. (1924). Studies of denaturation of proteins I. Some new observations
574 concerning the effects of acids and alkalies on proteins. *Proceedings of the Society of*
575 *Experimental Biology and Medicine*, 1924, 345-384.

576 44. Wilhelmy, L. (1850). The law by which the action of acids on cane sugar occurs. *Annalen*
577 *Physik Chemie*, *81*, 413-433.