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3   **The mathematics of thermal sub-optimality: Nonlinear regression**

4   **characterization of thermal performance of reptile metabolic rates.**

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20   The thermal performance of ectotherms is characterised as unimodal and asymmetrical.
21   Although still debated, it has been assumed that the peak of a performance curve indicates
22   the optimal temperature for an organism. The temperature at this peak rarely coincides with
23   preferred body temperatures, suggesting that the assumption is unsound. Any mathematical
24   formulation hoping to characterise the thermal performance of ectotherms must
25   accommodate the disagreement between the peak of the performance function and the
26   preferred temperature of the target organism(s). The mathematical function examined and
27   advocated here provides the most intuitive equation to describe thermal performance by
28   ectotherms. It also quantifies several physiologically informative parameters, one of which
29   seems congruent with preferred body temperatures.

30

Abstract

Although several approaches have been suggested, there is no broadly accepted single approach for quantitative characterization of thermal performance in ectotherms. I sought to identify the most appropriate non-linear function with which to represent thermal performance of ectothermic metabolic rate, and to interrogate the biological relevance of the thermal parameters of this function. I used published data for exercise-induced metabolic rates of nine species of reptile from a broad phylogenetic base and global distribution. Using an Akaike Information Criterion, I compared 12 different models proposed to characterize thermal performance adapted from a broad range of disciplines, finding that a beta-distribution model described the reptile metabolic rate data most parsimoniously. Using the beta-distribution model, unique functions were parameterized for each species. Four parameters were extracted from each species-specific fit: the temperature coincident with the peak of the thermal performance curve, T_{opt}; the point at which the function intersected the x-axis, CT_{max}; and two points indicative of thermal breadth, $T_{d(lower)}$ and $T_{d(upper)}$. There was a positive relationship between the species' preferred body temperatures (T_{pref}) reported in the scientific literature and both T_{opt} and $T_{d(lower)}$ extracted from the species-specific beta functions. While $T_{d(lower)}$ estimates were not different to published T_{pref} values, T_{opt} estimates were statistically higher than T_{pref}. This is consistent with previous observations that the point of peak performance does not match T_{pref}. The predicted CT_{max} also correlated well with published values. The model in its current form was not able to estimate CT_{min}, and this parameter was not explored here, but should be in future research. By providing a quantitative description of the thermal performance, the beta-distribution function offers a new theoretical basis for thermal optimality. I contend that T_{pref} aligns with the mathematical threshold $T_{d(lower)}$, where metabolic rate is at its maximum prior to thermal inhibition.

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Introduction

Temperature is one of the most ubiquitous abiotic factors encountered by organisms (Withers, 1992), and the influence that it has upon their performance is a critical determinant of fitness (Somero, 2005). Since thermal performance is critical in both driving and constraining many aspects of ecology and evolution (Somero, 2005; Dowd *et al.*, 2015), attempts to quantify its optimum have been incorporated into many contributions to conservation, environmental management, and pure biological research (e.g. Buckley (2008); Kearney *et al.* (2009); Evans *et al.* (2015); Tuff *et al.* (2016)). The basis of all of these applications is that there should be an optimal temperature at which performance is maximized while accruing minimal deleterious effects such as oxidative damage. Beyond this threshold performance declines rapidly (Angilletta, 2006). This thermal optimum, T_{opt}, might be intuitively expected to occur at the peak of the thermal performance curve (Huey & Kingsolver, 1989; Martin & Huey, 2008; Dowd *et al.*, 2015). However, accumulating evidence indicates that preferred body temperatures, T_{pref}, of many ectotherms do not and should not coincide with the hypothesized T_{opt} (Martin & Huey, 2008).

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The diverse array of potential performance metrics includes, among a great many others, sprint speed, juvenile development rates (both in invertebrates and vertebrates), and respiration patterns (Angilletta, 2009). Underpinning many of these measures is the constraint imposed by the effects of temperature on enzymatic reactions (Huey & Kingsolver, 1989), or oxygen limitation on mitochondrial activity (Pörtner, 2010). There is a long heritage of seeking phylogenetic and ecological signals in the average metabolic rates of species as well (Andrews & Pough, 1985; Lighton & Fielden, 1995; Chown *et al.*, 2007). Furthermore, the involvement of metabolic rates in thermal performance has gained increasing recognition in the last decade (Angilletta, 2009; Pörtner, 2010). Yet, while there is much empirical evidence for the characteristic asymmetrical, unimodal curve describing a number of performance metrics (Angilletta (2006); Figure 1) from sprint speed to fecundity to metabolic rates, there has been no broadly accepted analysis that provides consistent interpretation of these curves between species (Dowd *et al.*, 2015). Here I have investigated various potential models of

92   thermal performance using exercise-induced peak metabolic rates of reptiles, and following

93   the assumption that patterns in metabolic rates should be broadly indicative of the patterns

94   of most performance metrics (Gillooly *et al.*, 2001; Gillooly *et al.*, 2002) at least insofar as they

95   are measured for whole animals, rather than isolated enzyme-catalysed reactions (Huey,

96   1982).

97   Understanding thermal performance of metabolic rates in ectotherms has long been of

98   interest to comparative physiologists and ecophysiologists. However, unlike the study of

99   endotherms for which Scholander *et al.* (1953) provides a unifying theoretical construct, a

100   consistent theoretical model for ectotherms has been lacking (Angilletta, 2006), despite many

101   attempts in the last half century (Thornton & Lessem, 1978; Wollkind *et al.*, 1978; Hertz *et al.*,

102   1983; Crowley, 1985; Kovac *et al.*, 2007; Tomlinson & Menz, 2015; Tomlinson & Phillips,

103   2015). The lack of a broadly agreed arithmetic model describing thermal performance in

104   ectotherms (analogous to the Scholander-Irving model) has generated a great deal of debate

105   concerning the ecological and evolutionary bases to thermal sensitivity in ectotherms (Huey

106   & Kingsolver, 1989; Gilchrist, 1995; Angilletta *et al.*, 2003; Angilletta, 2009). From this debate,

107   two consistent elements have emerged that require synthesis: (a) there is expected to be a

108   continuous, non-linear increase in performance related to increased chemical kinetics (Huey

109   & Stevenson, 1979; Huey & Kingsolver, 1989; Angilletta, 2009) to (b) a point where thermal

110   limitation is either imposed by increased mitochondrial inefficiency (Pörtner *et al.*, 2006), by

111   enzyme denaturation (Somero, 2002) or several other potential physiological constraints

112   (Somero, 2002). This schema establishes how thermal increase and inhibition accelerate

113   chemical reaction rates, leading to the Arrhenius model of thermal performance (Sharpe &

114   DeMichele, 1977), but offers little insight into an appropriate mathematical function to

115   characterize the physiological performance more broadly, including any intrinsic interspecific

116   differences in the performance traits themselves.

117   Additional to the thermokinetic aspects above, I contend that there must be both minimum

118   and maximum asymptotes to the function. The minimum asymptote must be zero or more

119   because performance of any type, including metabolic rate, cannot be negative. The existence

120   of the upper asymptote is less well-articulated in ectotherms, although it is a common

121   concept for endotherms (Tomlinson, 2016). My reasoning for its existence in relation to

122   ectothermic metabolic rate is that the supply networks required to fuel metabolic activity

123   must impose some structural limitations to maximum performance. These could be anything

124   from oxygen supply limitation leading to mitochondrial inefficiency (*sensu* (Pörtner, 2010)),

125   limitations in the production and transport of ATP within the cell (Suarez, 1996), or any

126   number of other biophysical mechanisms. Pörtner (2001) presents relatively strong evidence

127   for aquatic ectotherms and there has been some tentative suggestion for terrestrial species

128   (Pörtner, 2001; Tomlinson *et al.*, 2017) that, when an ectotherm approaches thermal maxima,

129   as its energetic expenditure peaks, it may no longer have any capacity to supply the oxygen

130   requirements for activity (Pörtner, 2001; Pörtner, 2010). Regardless of the specific

131   mechanism(s) at play, their ultimate outcome would be an upper limit where metabolic rate

132   is not thermally constrained, but is operating at the peak structural capacity of the animal.

133   Represented graphically (Figure 1), these four elements imply that below the critical

134   temperature where thermal inhibition takes place, the thermal performance of metabolic

135   rate is S-shaped. This might traditionally imply that thermal performance is a logistic function,

136   characterized by a lower asymptote (r_{min}), an upper asymptote (r_{max}), a nonlinear "slope", or

137   exponential scaling factor b, and a midpoint (T_i) , representing 50% of r_{max}, around which the

138   thermal function is symmetrical (Ritz & Streibig, 2008). Finally, the rapid, non-linear decline

139   in performance above a critical threshold (Angilletta, 2006), while an essential element of the

140   model, can be captured arithmetically in a number of ways. Models encapsulating these

141   theoretical constraints have previously been proposed to describe thermal performance by

142   ectotherms (Hertz, 1983; Crowley, 1985; Kovac *et al.*, 2007); however, there are other,

143   structurally simpler thermal performance models in the broader literature that serve similar

144   purposes (Yan & Hunt, 1999; Arnall *et al.*, 2014; Tomlinson *et al.*, 2015; Tomlinson & Phillips,

145   2015).

146

147   Fitting non-linear functions to thermal performance provides the mathematical basis to such

148   a theory, where the majority of parameters represent physiological responses of the organism

149   and interspecific differences can then be interpreted consistently. Traditionally, where

150   thermal performance of metabolic rates has been statistically modelled at all, it has been

151   interrogated by linearizing the data through mathematical transformation and fitting

152   segmented regressions to it (e.g. Bennett and Dawson (1972); Snyder and Weathers (1976);

153   Tomlinson and Phillips (2012); Mason *et al.* (2013)). Transformations of this type simplify the

154   data, reducing the number of parameters required to describe any patterns, but they also

155   alter the variance structure (Hayes & Shonkwiler, 2006; Gurka & Edwards, 2011), generally

156   compressing it and constraining the interpretation of potentially adaptive traits. More

157   realistically, these relationships should be analyzed by the application of nonlinear functions

158   in order to maintain as many physiologically informative factors as possible. My objective here

159   was to identify the most appropriate, broadly applicable theoretical model of thermal

160   performance of metabolic rate in ectotherms by resolving the most appropriate of the

161   proposed non-linear functions, using a meta-data set of reptile metabolic rates. I also aimed

162   to see whether the most appropriate model of thermal performance was capable of

163   estimating preferred body temperatures, T_{pref}, by correlating known preferred body

164   temperatures of my nine study species with the modelled temperature thresholds derived

165   from the species-specific functions.

166

167   **Methods**

168   *Study taxa*

169   This study required a solid database of standardized metabolic rates of ectothermic taxa to

170   be coupled with reliable estimates of their preferred body temperatures (T_{pref}). While

171   metabolic rates have been studied in a great diversity of ectotherms, there are several

172   decades of studies specifically focusing on reptiles, and their thermoregulation and thermal

173   preferences (Martin & Huey, 2008). Few of these studies, however, have managed to capture

174   a breadth of thermal performance suitable for fitting the non-linear functions that I explored

175   here. Thus, the data that I have garnered from the literature represent the small number of

176   papers where the exercise-induced maximal metabolic rates (MMR) of active squamate

177   reptiles increase with T_b and show a decrease beyond their peak capacity. This data set

178   encompasses only eight species of reptiles from three families, including the Agamidae

179   (Wilson, 1974), Iguanidae (Moberly, 1968; Bennett, 1972; Bennett & Dawson, 1972; Bennett

180   *et al.*, 1975), and Scincidae (Wilson, 1974) (Table 1).

Data handling

Raw data of individual responses were extracted by calculating Cartesian co-ordinates of the published figures and converting these to independent data points. For the purposes of comparison, all metabolic data were allometrically standardized to $mLO_2.g^{-0.80}.h^{-1}$ (Andrews & Pough, 1985). Given that individual masses were rarely available, all mass corrections were undertaken using the published mean values for the species. Since the data used for the analyses herein do not perfectly represent the values published for each species, the individual data are included in Appendix 1.

Statistical analysis

(Huey, 1982) characterised models of thermal performance as falling into three categories: empirical, descriptive, and deductive, noting that, while performance data would ideally be fitted to a theoretical function derived from an underlying set of physical principles, the only deductive curves available were based upon chemical reactions, and were far removed from whole-animal physiology. In a similar manner, three of the functions that I identified for my candidate library have been derived from chemical kinetics. The first model was a multi-exponential model, first developed by Sharpe and DeMichele (1977) to characterize the relationship between temperature and the development rates of insects based upon Arrhenius laws of chemical kinetics. This has been reformulated for application to metabolic rates (Kooijman, 2010), and used to characterize the thermal performance of at least one reptile (Arnall *et al.*, 2014) in the form:

$$\dot{V}O_2 = \frac{exp^{(\frac{T_A}{T_R} - \frac{T_A}{T_b})}}{1 + exp^{(\frac{T_{AL}}{T_b} - \frac{T_{AL}}{T_L})} + exp^{(\frac{T_{AH}}{T_H} - \frac{T_{AH}}{T_b})}} \tag{1}$$

where T_R represents a reference temperature, T_L and T_H represent the lower and upper thermal tolerance limits respectively. Distinct from earlier iterations constructed around activation energy and enthalpy of specific reactions (Sharpe & DeMichele, 1977; Schoolfield *et al.*, 1981), the reformulation makes use of "Arrhenius temperatures", described as "the slope of the relationship between the logarithm of a physiological rate an d the inverse

209 absolute temperature" (Kooijman, 2010). While these parameters have the dimension of

210 temperature (K), this is presumably an energetic equivalent value to standardize the units of

211 the function. I this context, Kooijman (2010) characterizes T_A to represent the Arrhenius

212 temperature of the reaction within the tolerance thresholds, and T_{AL} and T_{AH} are the Arrhenius

213 temperatures for the rate of decrease beyond these thresholds.

214 A second function derived from chemical kinetics was proposed by Johnson *et al.* (1954) on

215 the basis of enzyme activation:

216
$$\dot{V}O_2 = \frac{c \times T_b \times exp^{-\frac{\Delta H^{\ddagger}}{R \times T_b}}}{1 + exp^{-\frac{\Delta H_1}{R \times T_b}} \times exp^{\frac{\Delta S_1}{R}}} \tag{2}$$

217 Where $\Delta H^{\ddagger}$ represents the heat activation for transition state intermediates ΔH_1 represents

218 the heat activation for the reaction (in this case respiration), ΔS_1 represents the entropy of

219 the reaction (in this case respiration), R represents the gas law constant (~8.31) and c

220 represents a constant. Consistent with this interest in chemical kinetics mediated by enzyme

221 thermal tolerance, Ratkowsky *et al.* (2005) proposed the third function in my candidate set:

222
$$\dot{V}O_2 = \frac{c_T \times exp^{\frac{-\Delta H^{\ddagger}}{R \times T_b}}}{1 + exp^{\frac{-n \times (\Delta H_1 - 18.1 \times T_b + \Delta C_P) \times ((T_b - 373.6) - T_b \times \ln(\frac{T_b}{385.2}))}{R \times T_b}}} \tag{3}$$

223   Where additional parameters included are the heat capacity change between the native and

224 denatured states of the controlling enzyme (ΔC_P), the enthalpy change at the convergence

225 temperature for enthalpy (ΔH_1), and the number of amino acid residues in the protein (n).

226

227 The functions described above notably don't parameterize variables that are informative to

228 whole animal physiology (Huey, 1982), namely minimum performance, maximum

229 performance, the temperature of peak performance (the canonical T_{opt}), nor any suggestion

230 of T_{pref}. Functions of that do describe these parameters fall into the empirical and descriptive

231 categories of functions identified by Huey (1982), which were distinguished on the basis of

232 their mathematical origin (empirical) and their use of specific physiological parameters

233 (descriptive). I draw no distinction between the two categories here, but included in my

234 candidate library eight published functions which specifically incorporate species-specific

235   performance traits such as the upper asymptotes (parameterized here as r_{max}) and/or lower

236   asymptotes (r_{min}), and physiologically informative temperature thresholds such as T_{opt}, CT_{max}

237   (the point where performance equals zero), T_l (the lower critical temperatures), T_u (the upper

238   critical temperature) into the equations. These functions also typically include some form of

239   reaction rate multiplier, k. For purposes of comparison, I have re-parameterized these from

240   their original forms such that all the equivalent parameters are represented by consistent

241   cyphers here.

242   The first of these physiologically informed functions I re-parameterized from a skewed normal

243   function (Lehman *et al.*, 1975) that originally incorporated temperature thresholds and a

244   constant, *c*, that defined the breadth of the curve (originally enumerated as -2.3; Lehman *et*

245   *al.* (1975)), but not performance asymptotes. In order to translate this to represent metabolic

246   rates, the upper asymptote r_{max} had to be introduced, and the "sign" function (sgn) employed

247   to mathematically articulate the piece-wise nature of the original algorithm:

248
$$\dot{V}O_2 = r_{max} \times exp^{\, c \times (\frac{T_b - T_{opt}}{T_l - T_{opt}} \times \frac{T_b - T_{opt}}{T_u - T_{opt}})} \tag{4}$$

249

250   The fifth of my candidate thermal performance function was a special Poisson-density

251   function (O'Neill *et al.*, 1972):

252
$$\dot{V}O_2 = r_{max} \times (\frac{CT_{max} - T_b}{CT_{max} - T_{opt}})^{\frac{(\ln(Q_{10}) \times (CT_{max} - T_{opt}))^2 \times \left(1 + \sqrt{1 + \frac{40}{(\ln(Q_{10}) \times (CT_{max} - T_{opt} + 2))}}\right)^2}{400}} \times$$

253
$$exp^{\frac{(\ln(Q_{10}) \times (CT_{max} - T_{opt}))^2 \times \left(1 + \sqrt{1 + \frac{40}{(\ln(Q_{10}) \times (CT_{max} - T_{opt} + 2))}}\right)^2}{400} \times (1 - \frac{CT_{max} - T_b}{CT_{max} - T_{opt}})} \tag{5}$$

254   Where Q_{10} represents the ratio of the metabolic rate at two temperatures separated by 10 °C

255   (IUPS Thermal Commission, 2003). Equation 6 of my candidate set was an exponential x power

256   function (Lassiter, 1975):

257
$$\dot{V}O_2 = r_{min} \times exp^{a \times (T_b - T_{opt})} \times (\frac{T_l - T_b}{T_l - T_{opt}})^{a \times (T_l - T_{opt})} \tag{6}$$

258   Where *a* is a constant. Equation 7 was a logistic x logistic function (Thornton & Lessem, 1978):

259
$$\dot{VO}_2 = \frac{k_l \times \exp^{\left(\frac{1}{T_{opt}-T_b}\times\ln\left(\frac{0.98\times(1-k_l)}{0.2\times k_l}\right)\right)\times(T_b-T_l)}}{1+k_l \times \left(\exp^{\left(\frac{1}{T_{opt}-T_b}\times\ln\left(\frac{0.98\times(1-k_l)}{0.2\times k_l}\right)\right)\times(T_b-T_l)}-1\right)} \times$$

260
$$\frac{k_u \times \exp^{\left(\frac{1}{T_{opt}-T_b}\times\ln\left(\frac{0.98\times(1-k_u)}{0.2\times k_u}\right)\right)\times(T_b-T_u)}}{1+k_u \times \left(\exp^{\left(\frac{1}{T_{opt}-T_b}\times\ln\left(\frac{0.98\times(1-k_u)}{0.2\times k_u}\right)\right)\times(T_b-T_u)}-1\right)} \tag{7}$$

261 Where k_l represents the reaction rate multiplier near the lower critical temperature and k_u

262 represents the reaction rate multiplier near the upper critical temperature. Equation 8 was a

263 two-way negative exponential function derived by (Huey & Stevenson, 1979):

264
$$\dot{VO}_2 = r_{max} \times \left(1 - exp^{-k_l\times(T_b-T_l)}\right) \times \left(1 - exp^{-k_u\times(T_b-T_u)}\right) \tag{8}$$

265 The ninth model in the candidate set was a bi-exponential model, developed independently

266 in two publications (Logan et al., 1976; Tomlinson et al., 2015):

267
$$\dot{VO}_2 = r_{min} + e^{k_l\times T_b} - e^{T_b-T_d} \tag{9}$$

268 where T_d is the temperature of deviation from the pure exponential function.

269 The tenth model was a six-parameter, exponentially modified logistic function, broadly

270 consistent with those previously published (Hertz, 1983; Crowley, 1985; Kovac et al., 2007):

271
$$\dot{VO}_2 = r_{min} + \frac{r_{max}-r_{min}}{1+e^{k_l\times(T_b-T_i)}} - \left(e^{k_u\times(T_b-T_d)}\right) \tag{10}$$

272 Here, T_d is again the temperature of deviation from the pure logistic function. There is also a

273 parameter around which the logistic base function is symmetrical, T_i.

274 In recognition of the complexity of this model, containing six parameters, I also tested two
275 simpler models. The first of these simpler models was a modified cubic function (Knies et al.,
276 2006; Knies et al., 2009);

277
$$\dot{VO}_2 = r_{min} + k \times \left(T_b^3 - T_b^2 - T_b\right) \times k \times (T_b - T_{opt}) \tag{11}$$

278

279 Alternatively, a model proposed to describe the thermal performance of plant physiology (Yan

280 & Hunt, 1999) was used as the fourth candidate model, characterized as:

281
$$\dot{VO}_2 = r_{max} \times \left(\frac{T_{max}-T_b}{T_{max}-T_{opt}}\right) \times \left(\frac{T_b}{T_{opt}}\right)^{\frac{T_{opt}}{T_{max}-T_{opt}}} \tag{12}$$

282   Here, r_{max} indicates the maximal capacity of the organism, or the upper asymptote of the

283   function. T_{max} indicates the upper thermal tolerance limit, where performance intersects the

284   horizontal axis, (the traditional CT_{max}), and T_{opt} represents the temperature at which the

285   performance function peaks (the traditional interpretation of T_{opt}). The beta-distribution

286   model formulated by Yan and Hunt (1999) does not provide a parameter analogous to T_d. As

287   such, I propose that similar thresholds can be estimated from half the breadth between T_{opt}

288   and T_{max} thus:

289    $$T_{d(upper)} = T_{opt} + \left(\frac{T_{max} - T_{opt}}{2} \right)$$ (12.1)

290    $$T_{d(lower)} = T_{opt} - \left(\frac{T_{max} - T_{opt}}{2} \right)$$ (12.2)

291   The four candidate non-linear models were fitted to the combined data for all species, and

292   were compared using an information-criterion approach (Burnham & Anderson, 2002) using

293   the 'thermperf' package (Bruneaux, 2017) in the *R* statistical environment version 3.2.1 (R

294   Core Team, 2016). The most parsimonious model, with the lowest resulting Akaike

295   Information Criterion (AIC), was inferred to capture the maximal complexity of the data with

296   the smallest reduction in likelihood of fit.

297

298   Once the best model form was identified in this manner, unique fits were resolved for each

299   species of reptile. Each of these unique fits resulted in a humped relationship, the maximum

300   of which is consistent with a classical interpretation of optimal temperature, T_{opt} (Huey &

301   Kingsolver, 1989). All nonlinear modelling was undertaken using the '*nls2*' package

302   (Grothendieck, 2013) in the *R* statistical environment version 3.2.1 (R Core Team, 2016). All

303   data are presented as means, as generated from published data.

304

305   **Results**

306   *Selection of an appropriate thermal performance function*

307   The complete data set extracted from the literature showed the distinctive, humped

308   distribution characteristic of thermal performance data (Figure 2), which eight of the

309   candidate models were capable of mimicking. The functions constructed on the basis of

310   enzyme kinetics (Johnson *et al.*, 1954; Ratkowsky *et al.*, 2005) were not able to be fitted to

311   the data describing the metabolic rates of whole animals that I collected here. Of the

312   candidate performance functions, the beta-distribution function (Yan & Hunt, 1999) best

313   accounted for the variation in the data, followed by the exponentially-penalised logistic

314   function (Kovac *et al.*, 2007), and finally the Arrhenius function (Sharpe & DeMichele, 1977)

315   (beta-distribution AICc = 513.7, AIC weight = 0.35, ΔAIC = 0.00; exponentially penalized logistic

316   AICc = 514.1, AIC weight = 0.29, ΔAIC = 0.35; Arrhenius AICc = 515.1, AIC weight = 0.18, ΔAIC

317   = 1.31). All other candidate models were more than two AIC units distant from the best model

318   (Table 2). The residuals resulting from the beta-distribution function had the narrowest range

319   (-0.89 to 5.51 mL $O_2.g^{-0.8}.h^{-1}$), followed by the exponentially penalized logistic (-0.85 to 5.55

320   mL $O_2.g^{-0.8}.h^{-1}$), and finally the Arrhenius function (-0.91 to 5.53 mL $O_2.g^{-0.8}.h^{-1}$). Of the three,

321   however, the beta-distribution function best indicated the decline in metabolic rate that is

322   expected beyond an upper thermal threshold, projected at 48.1 °C, and at 57.0 °C for the

323   exponentially penalized logistic function. The Arrhenius function did not identify a peak for

324   this data set. The temperature range between the peak of the function and CT_{max} was 23.0 °C

325   for the beta-distribution function, compared to 6.6 °C for the exponentially-penalized logistic.

326   The Arrhenius function cannot be resolved in such a manner as to estimate CT_{max}, because

327   the function never intersects the horizontal axis, so mathematically the tolerance breadth of

328   this function is infinite. On these bases I sought ways of parameterizing the thermal optima

329   of the individual reptile species by applying the beta-distribution function, as the best model

330   with the most plausible physiological interpretations.

331

332   *Parameterizing thermal optima*

333   The beta-distribution function could be grouped into unique parameters for each family

334   (Figure 3) and for each of the nine study species (Table 3). Each species-specific function

335   facilitated the estimation of an optimum at the peak of the thermal performance function,

336   T_{opt}. A lower threshold, $T_{d(lower)}$ could also be estimated for each species from the breadth

337   between the parameterized T_{opt} and T_{max}. T_{opt} ranged between 34.0 °C and 41.3 °C, while

338      $T_{d(lower)}$ ranged from 29.7 °C to 38.5 °C (Table 1). There was a strong correlation between T_{pref}

339   and T_d across all species (r = 0.72) and also between T_{pref} and T_{opt} across all species (r = 0.78)

340   However, the T_d estimates were much closer to equivalence with T_{pref} than were the T_{opt}

341 estimates (Figure 4). This was supported statistically, where there were no differences

342 between paired T_{pref} and $T_{d(lower)}$ estimates (t_8 = 0.274, p = 0.791), but T_{opt} estimates were

343 higher than the observed T_{pref} of the species (t_8 = 6.77, p = 1.40×10^{-4}). The T_{max} estimates

344 resulting from beta-distribution functions fitted for each species were also not significantly

345 different to the published CT_{max} values (t_5 = 1.50, p = 0.192).

346

347 **Discussion**

348 The initial aim of this study was to identify the most appropriate of the diverse candidate set

349 of non-linear functions proposed to represent thermal performance by ectotherms. Of these,

350 three functions were statistically indistinguishable in describing the pattern of thermal

351 performance in metabolic rates of reptiles, including the beta-distribution function (Yan &

352 Hunt, 1999), the exponentially-penalized logistic function (Kovac et al., 2007) and the

353 Arrhenius function (Sharpe & DeMichele, 1977). However, there are a number of

354 idiosyncrasies associated with adapting the Arrhenius function to model metabolic rates.

355 Firstly, it is dependent upon a reference temperature (the T_R parameter) which I fitted on the

356 basis of the data, but which previously appears to have been selected somewhat arbitrarily

357 (Arnall et al., 2014). The lack of a parameterized r_{min} also assumes that the minimum

358 metabolic rate is zero, which is a relic of its original application to insect development rates

359 (Sharpe & DeMichele, 1977). This is captured by an implicit step change in rates between k_L

360 and k_R (Eqn. 3.2), but it does not parameterize an estimate of the standard metabolic rate

361 (SMR), which is the minimum maintenance cost of a living animal. Secondly, the resulting

362 function does not resolve CT_{max} in the manner of the other candidate models tested (i.e. it

363 does not ever intersect the temperature axis). Finally, it applies no theoretical upper limit to

364 metabolic rates (or a peak metabolic rate; PMR), assuming that uninhibited reactions will

365 increase in speed, which makes it increasingly complicated to apply to data sets where

366 seasonal acclimation or diverse phylogeny can imply interactions between temperature and

367 PMR. There is an emerging literature discussing the disconnect between statistical viability

368 and biological plausibility (Martinez-Abrain, 2008; Halsey et al., 2015), and I contend that the

369 Arrhenius function, while being statistically indistinguishable from the beta-distribution and

370 exponentially-penalised logistic functions in its ability to characterize ectothermic thermal

371 performance, its interpretive value is limited because of the five shortcomings in biological

372 relevance described above.

373 A biologically relevant function to describe thermal performance by ectotherms must have

374 some further components beyond the simple concepts that performance declines beyond an

375 upper and lower threshold, but increases within those bounds. The assumptions discussed in

376 the introduction, that 1) there must be both a lower and upper horizon to performance, 2)

377 the increase from minimum to maximum must be continuous and non-linear as a result of the

378 thermal kinetics of chemical reactions (Angilletta, 2009), and 3) there must be an upper

379 tolerance threshold, establish at least four parameters integral to the more physiologically

380 appropriate models, r_{min}, r_{max}, T_{opt}, and $T_{d(lower)}$. Hence, the most statistically robust *and*

381 physiologically relevant of the four candidate functions was the beta-distribution model,

382 which was originally developed to describe plant thermal physiology (Yan & Hunt, 1999). As

383 with the Arrhenius model, this function assumes that r_{min} is zero, as a relic of its development

384 in the context of growth rates, but it is capable of estimating r_{max} and two critical thermal

385 tolerance thresholds. As further data are collected, these estimated values may provide a

386 more objective comparative approach to understanding ecological and evolutionary patterns

387 in the energetic requirements of ectotherms. This task remains complicated by the question

388 of the most appropriate temperature at which to make comparative measurements (Huey &

389 Stevenson, 1979; Atkinson, 1994).

390 The beta-distribution function provides estimates of two thresholds of thermal tolerance, T_{opt}

391 representing the classical interpretation of thermal optimum at the peak of the performance

392 function (Huey & Kingsolver, 1989, 1993), and an upper threshold, T_{max}, which closely

393 approximates CT_{max}. Using these two parameters in concert, however, it is possible to

394 calculate two further threshold points, the upper and lower T_d. The lower appears to

395 approximate T_{pref} in ectothermic reptiles, and I venture that the upper may represent the

396 body temperature at which the animal actively seeks refuge, as is used to parameterize some

397 mechanistic niche envelope models (e.g. Kearney and Porter (2016)). While there are

398 established techniques to identify the performance breadth based upon proportions of

399 maximal performance (usually 80% or 95% thresholds) as has often been applied to

400 locomotor performance (e.g. van Berkum (1988); Huey *et al.* (1989); Navas (1996); Angilletta

401 *et al.* (2002)), these thresholds are somewhat arbitrary and difficult to compare between

402 studies. It is notable that the scope between metabolic rates objectively estimated at $T_{d(lower)}$

403 and T_{opt} in all cases is approximately 90% (Table 1), regardless of the phylogenetic heritage or

404 ecological niche and biome in which the species originated (van Berkum, 1988).

405

406 *Statistical considerations*

407 Non-linear regression is an approach to studying a number of physiological processes,

408 including dose responses (Ritz *et al.*, 2015), water relations in plants (Lewandrowski *et al.*,

409 2016), and thermal performance in both endotherms (Tomlinson, 2016) and ectotherms

410 (Hertz, 1983; Crowley, 1985; Kovac *et al.*, 2007; Tomlinson & Menz, 2015; Tomlinson &

411 Phillips, 2015). There are a number of packages in the *R* statistical environment that can

412 automate this process, including the '*drc*' package (Ritz & Streibig, 2012; Ritz *et al.*, 2015). I

413 caution, however, that it is critical to understand both the parameters and structures of the

414 functions used, and their physiological analogues. The beta-distribution function applied

415 here, for example, is only made appropriate if temperature is converted from degrees Celsius

416 to Kelvin, because in this format there are no hard limits in the independent variable (i.e. T_a =

417 0 °C is just another point along the continuum). If the function is fitted in degrees Celsius it

418 become undefined at temperatures below zero, and clearly loses relevance to the real world.

419 This is easily remedied by the addition of 273 to all temperature components of the function,

420 meaning that all parameters are still fitted in degrees Celsius, thus:

421 $$\dot{V}O_2 = W_f \times \left(\frac{(T_{max}+273) - (T_a + 273)}{(T_{max} + 273) - (T_{opt}+273)} \right) \times \left(\frac{(T_a+273)}{(T_{opt}+273)} \right)^{\frac{(T_{opt}+273)}{(T_{max}+273) - (T_{opt}+273)}} \quad (12.3)$$

422

423 It is also critically important to understand the structure of the data required to fit these

424 functions. Although a non-linear regression approach is the most appropriate to interrogate

425 thermal biology, it requires a specific experimental design to accommodate the analysis

426 appropriately, and this needs to be considered prior to the collection of data. Experimental

427 design was a substantial constraint on the selection of appropriate data for this study; the

428 thermal performance data for only the nine species selected had been collected over a wide

429 enough temperature range to capture the decline in metabolic rate beyond the upper

430   tolerance threshold, despite the fact that there were numerous other species available in the

431   literature where performance had been measured over a narrower range (see Appendices).

432   In the event that further data are to be collected to test these functions, it is critical that

433   measurements are made as far beyond this threshold as is ethically defensible. Furthermore,

434   studies should increase the number of intervals in the key parts of the range to increase the

435   sensitivity of the fitted function to critical turning points, consistent with discussion by Knies

436   and Kingsolver (2010) concerning T_b near T_{opt}.

437   Nonlinear regression using the '*nls*' protocol in the *R* statistical environment assumes a

438   normal distribution of errors (Ritz & Streibig, 2008; Baty *et al.*, 2015). Studies such as this fall

439   prey to the quality of data that can be extracted from the published literature. Ideally, had I

440   experimentally collected all these data myself I would have access to all the individual stat

441   required to ensure independence of the data, such as specific temperatures, body mass and

442   individual identifications for the purposes of identifying and accounting for deviations from a

443   normal distribution of errors. Failing the ability to incorporate these into a repeated measures

444   design, or as a series of random factors within my analyses, I'm restricted to the next best

445   thing, which is to acknowledge this uncontrolled effect. The sample sizes in my data are not

446   equal, and some of my data will bear a flattening of the variance structure resulting from the

447   application of average body masses into my calculations. Given that these effects should be

448   equivalent across all the models that I applied here, however, I feel that this is at least

449   consistent across my analyses.

450

451   *Why "suboptimal" is optimal*

452   Several discussions have identified a lack of congruence between the optimal performance

453   temperature (T_{opt}) at the peak of the performance curve, and the preferred body temperature

454   (T_{pref}) of many reptiles (Martin & Huey, 2008; Dowd *et al.*, 2015). My question was

455   parameterized thus: if T_{opt} is not indicative of thermal preferences and thermoregulatory

456   behavior, does an appropriate non-linear function parameterize more accurate ways to

457   estimate them? The preferred body temperatures published for the reptiles studied here

458   were most closely approximated by $T_{d(lower)}$, rather than T_{opt} (Figure 4), estimated from the

459   beta-distribution function. Where T_{opt} is the mathematical representation of the thermal

460 optimum at the peak of the performance function, t-testing indicated that the fitted T_{opt}

461 values were higher than the published T_{pref}. The lower temperature threshold that I

462 calculated at $T_{d(lower)}$ was statistically indistinguishable from T_{pref} of the nine species studied.

463 One theory underpinning the apparent paradox that mathematically suboptimal

464 temperatures are in fact biologically optimal rests on the asymmetry of thermal performance

465 curves, where performance (or apparent fitness) declines much more rapidly when T_b exceeds

466 T_{opt} than when T_b is below T_{opt} (Huey & Stevenson, 1979; Gilchrist, 1995; Izem & Kingsolver,

467 2005; Martin & Huey, 2008). In this context, as well as avoiding the risk of rapid declines in

468 fitness when T_b exceeds T_{opt}, fitness should also be optimized across the thermal performance

469 function. This results in a relationship between the distance between Tpref and Topt and the

470 degree of asymmetry of the thermal performance function (Martin & Huey, 2008). When the

471 thermal performance functions that I resolved here for each species were assessed for

472 asymmetry in this manner, however, the relationship appeared to be the reverse of that

473 expected. Although performance optima are typically characterized using only a single

474 performance metric, such as locomotor capacity, fecundity, or metabolic rate, there is an

475 implicit assumption that they are all proxy indicators for the thermal effects on enzyme-

476 catalyzed reactions (Sharpe & DeMichele, 1977; Huey & Kingsolver, 1989). Regardless of

477 whether that assumption is justified, there should be strong selection to maximize lifetime

478 fitness by centering T_{pref} below the T_{opt} (Martin & Huey, 2008), because performance declines

479 so rapidly beyond this tolerance threshold regardless of underlying mechanisms. As such, I

480 remain unconvinced that the small number of species that I have investigated here suitably

481 replicate the data set reported by Martin & Huey (2008) for sprint speed to provide much

482 support in comparing the patterns of performance asymmetry and thermal preference

483 breadth between the two.

484

485 Alternative to Martin and Huey (2008),the oxygen- and capacity-limited thermal tolerance

486 (OCLTT) theory (eg. Pörtner (2010)) suggests that maintaining a buffer of aerobic scope could

487 be the mechanistic basis to the observed patterns, whereby ectotherms maintain T_{pref} below

488 the putative performance maximum. The theory poses that critical temperature thresholds

489 represent the transition points from aerobic to anaerobic mitochondrial metabolism (Pörtner,

490 2010). Furthermore, Pörtner (2010) concludes that oxygen limitation sets in prior to

functional failure. Bearing in mind that aerobic scope best represented as the maximum amount of oxygen available for any aerobic activity above routine for any specific temperature, maintaining T_b at a point lower than T_{opt} should allow the organism to maintain some scope for increased metabolic output prior to catastrophic oxygen limitation. Essentially, one way of interpreting these theories is in terms of an "engineering failsafe": maintaining T_b at levels below the temperature where the performance curve peaks allows some leeway for metabolic rates to increase in response to environmental stimuli or increased exertion. Assuming that the metabolic rates of the fitted performance functions for each species genuinely reflect the maximum aerobic capacity of the reptiles studied, maintaining body temperatures close to the estimated T_d values should allow the reptiles in question to consistently maintain an aerobic scope of about 10% of their maximal capacity (Table 1).This is advantageous compared to maintaining a higher T_b close to T_{opt} because, consistent with Pörtner (2010), maintaining T_b at levels that appear suboptimal allows the animals to remain responsive to their environment, with some capacity for increased O_2 consumption required for increased activity associated with foraging, escaping predation and other behaviors.

The species included in this analysis represent a broad spectrum of ecological niches and phylogenetic spread. Both of these elements undoubtedly contributed some variation to my findings, some of which can be speculated at but some remain too data-deficient to interpret, and I have therefore chosen not to speculate upon them. Generally, a study with the phylogenetic breadth represented here should include phylogenetic corrections (Feder, 1987; Garland & Adolph, 1994). The data requirement that the temperatures over which performance was measured were broad enough to indicate a decline in metabolic rate constrained my sample size, limiting scope for accommodating phylogenetic corrections.

Furthermore, there is a disconnect between the physiological heritage of the term "optimal temperature" which was initially coined in a mathematical context as the point where performance was maximal, and evolutionary contexts where it implies lifetime fitness gains (Martin & Huey, 2008). Obviously lifetime fitness is the culmination of a wide array of traits and pressures, not all of which will have the same thermal requirements, and some of which may trade off against each other. The final caveat on the generality of the beta-distribution model's ecological and evolutionary interpretations is that although this function appears

competent to describe the thermal performance of metabolic rate for the reptiles described here, it may not apply to all ectotherms (Dowd *et al.*, 2015) or all life history stages (Kingsolver *et al.*, 2011), nor to all possible performance traits (Huey, 1982). The ecological and evolutionary interpretations of this modelling approach, therefore, remain tentative until further data can contribute to these models. Nevertheless, having a broadly applicable model of thermal performance should facilitate these kinds of comparisons, and should be rigorously tested.

Conclusions

Thermal performance by ectotherms is a non-linear interaction between physiological processes (including the regulated body temperature) and ambient temperature. Although any performance metric should conform to the same relationship, the metabolic rates of ectotherms are best related to T_a by a beta-distribution function. This function provides the capacity to calculate the parameter T_d, which approximates the preferred body temperatures of the animal, at least in the nine species of reptiles that I investigated here. This threshold, while not consistent with a classical paradigm of thermal optimality in ectotherms, is consistent with existing alternative hypotheses.

Acknowledgements

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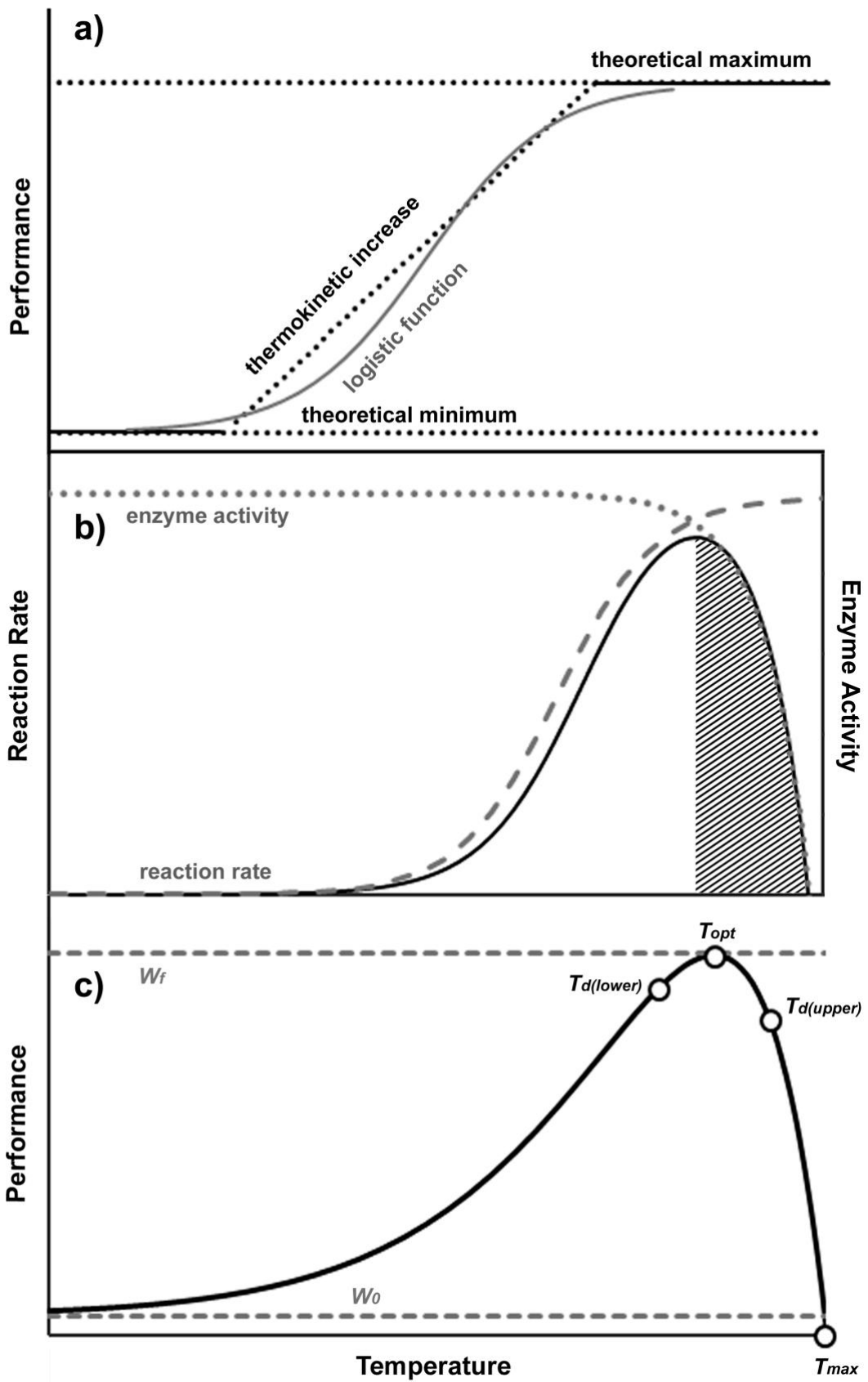766 tropical iguanas. *Herpetologica*, **14**, 161-164.
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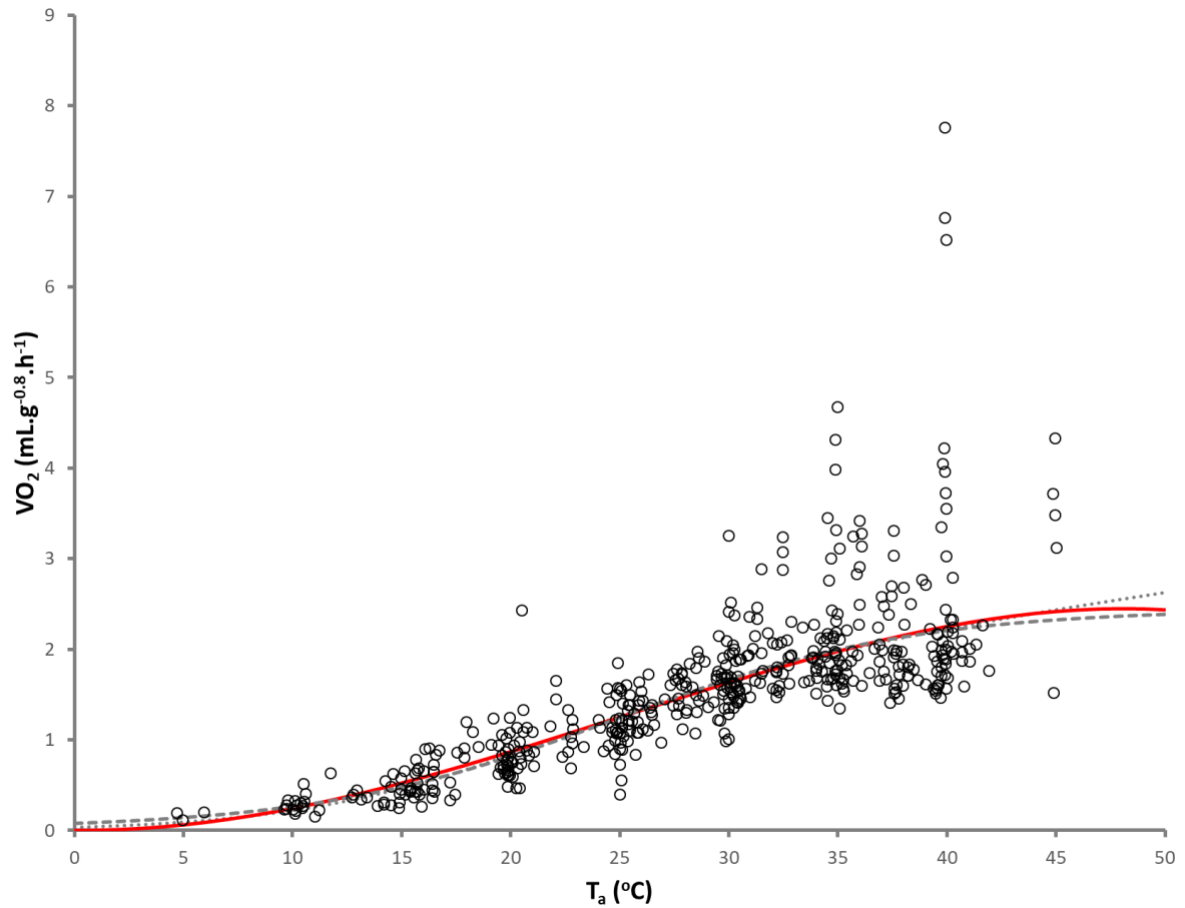770 Wollkind, D.J., Logan, J.A. & Berryman, A.A. (1978) Asymptotic methods for modeling biological
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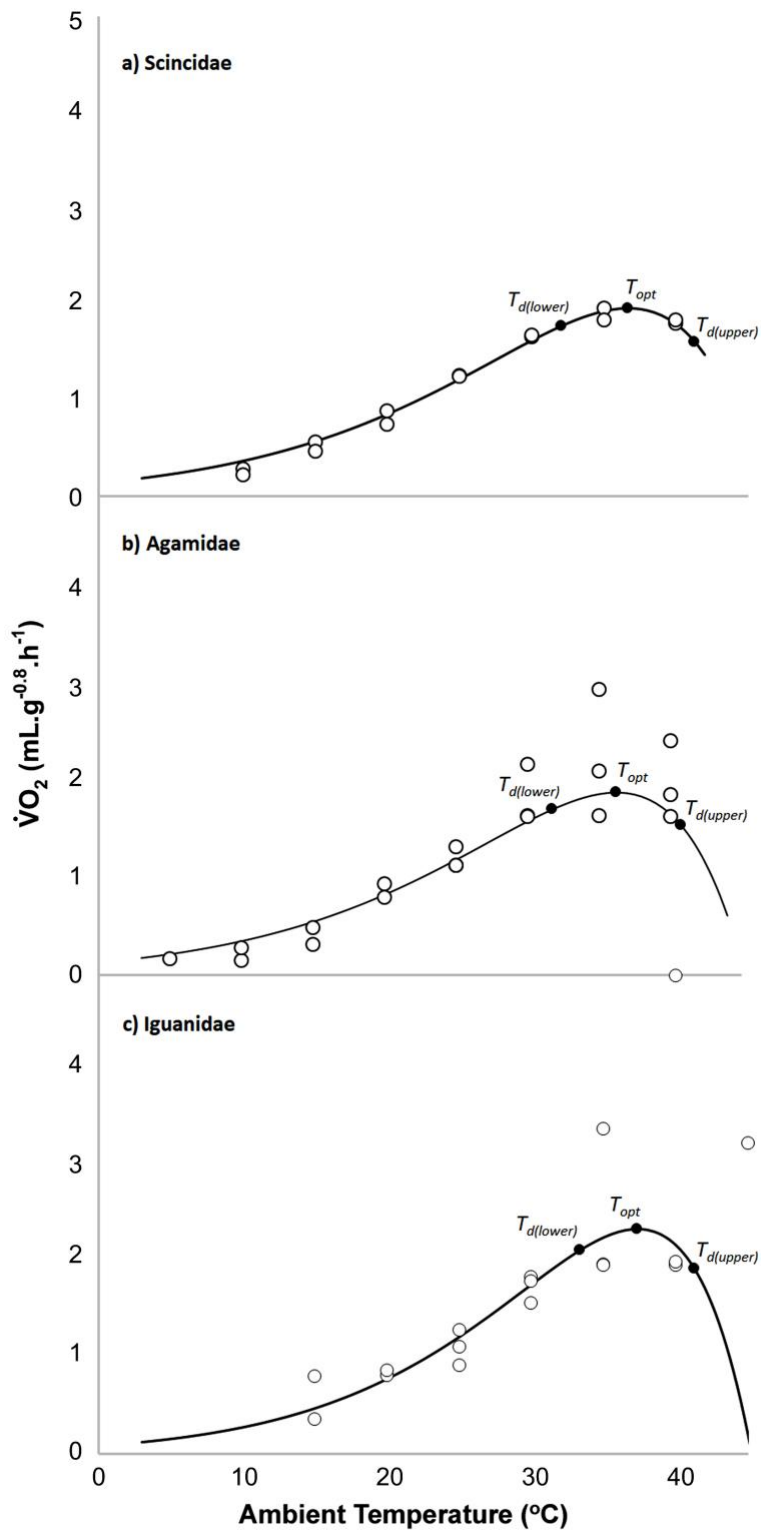
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a)
theoretical maximum
Performance
thermokinetic increase
logistic function
theoretical minimum

b)
enzyme activity
Reaction Rate
Enzyme Activity
reaction rate

c)
W_f
T_{opt}
$T_{d(lower)}$
$T_{d(upper)}$
Performance
W_0
Temperature
T_{max}

Figure 1: **a)** A schematic representation on the elements of thermal performance in ectotherms, within the bounds of the thermal tolerance limits. There must be a theoretical minimum to performance, and it must also increase with increasing temperature due to increased thermal kinetics of reaction rates. There should also be a theoretical upper limit to performance imposed by structural limitations. While these could be approached as a piece-wise series of linear fits, a more accurate representation is made by a non-linear approach such as the logistic function shown **b)** When converted to a non-linear paradigm, increased performance mimics the "s" curve of increasing reaction rates with increasing temperature (dashed grey line). The addition of thermal inhibition of performance implies that, while reaction rates increase, some aspect of physiological constraint is imposed (cross-hatched area), here indicated by enzyme denaturation (dotted line). The interaction between reaction kinetics and physiological constraint results in the characteristic unimodal, asymmetrical performance curve. **c)** The proposed thermal performance functions examined here are all characterized by some or all parameters that relate to physiological traits. These include a lower limit, r_{min}, and an upper performance asymptote r_{max}. Critical to the question that I explore here, three temperature thresholds can be resolved using these functions: T_{max} occurs at the only temperature where the function is equal to zero, T_{opt} occurs at the peak of the function and two T_d thresholds can be developed in unique ways from each function.
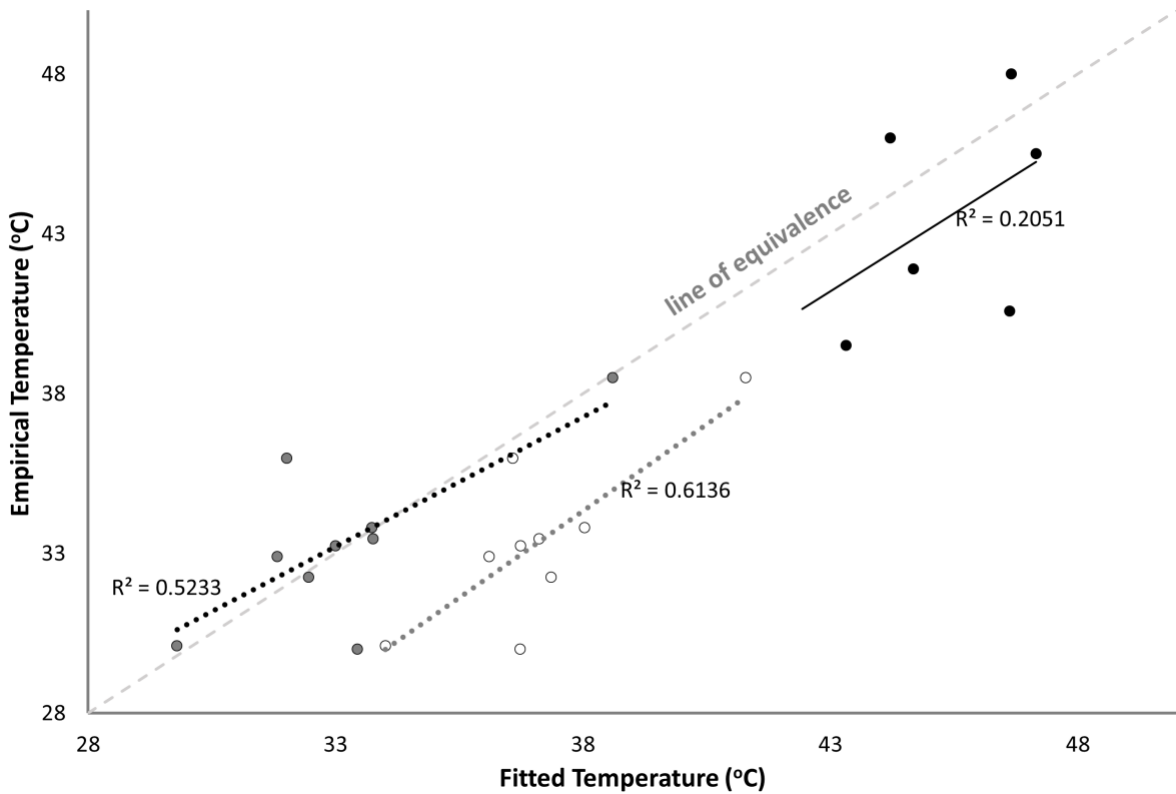
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Figure 2: Fitted estimates of the four candidate functions, the Arrhenius curve (grey dotted line), the exponentially penalized logistic curve (grey dashed line), and the beta-distribution curve (red solid line), describing the thermal performance of exercise induced peak metabolic rates in reptiles. The beta-distribution function resulted in the most parsimonious fit (AICc = 513.8), however the exponentially penalized logistic (AICc = 514.1), and the Arrhenius function (AICc = 515.1) were statistical indistinguishable from it, with a ΔAICc less than 2.0 (Raftery, 1995). All the other functions tested feel beyond this limit.

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Figure 3: Beta-distribution performance functions of allometrically corrected maximum metabolic rates for the nine reptile species examined here from **a)** the Scincidae, **b)** the Agamidae and **c)** the Iguanidae. The solid, black line represents the average beta-distribution function for each family. T_{max} is parameterized by the function directly, while T_d is parameterized by the first derivative of the function. The points are the average published metabolic rates measured for each species at each temperature.

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Figure 4:   Relationships between T_{opt} (○) and $T_{d(lower)}$ (◐) values interpolated from the species-specific

beta-distribution fits and published T_{pref} for each species, and between T_{max} (●) parameters resulting

from the beta-distribution fits and published CT_{max} for each species. The lines represent simple

correlations to indicate trends, however they serve to show that $T_{d(lower)}$ predictions fall much more

closely upon the line of equivalence than T_{opt} predictions resulting from the beta-distribution function,

while the T_{max} parameter is relatively consistent with published CT_{max} values.

816   **Table 1:**   Mass and thermal characteristics of the nine species of reptile used to investigate the thermal performance of metabolic rates. Mass, T_{pref} and
817   CT_{max} values were extracted from published literature sources, while T_{opt} and T_d were interpolated from fitted exponentially penalized logistic thermal
818   performance functions. The number of data points contributing to each species is indicated in the column headed n. Breadth is calculated here as the
819   difference between T_d and T_{opt}. Scope indicates the percentage of metabolic rates predicted at T_d of those predicted at T_{opt}, assuming that T_{opt} indicates the
820   absolute maximal metabolic capacity of the species.

Family	Species	n	Mass (g)	T_{pref} (°C)	CT_{max} (°C)	T_d (°C)	T_{opt} (°C)	T_{max} (°C)	Breadth (°C)	Scope (%)	Sources
Scincidae	*Tiliqua rugosa*	87	421	32.2	45.5	32.4	37.3	47.1	4.90	90.6	Warburg (1965); Wilson (1974); Bennett and John-Alder (1986)
	Egernia cunninghamii	105	257	32.9	41.9	31.8	36.1	44.7	4.284	90.7	Spellerberg (1972); Wilson (1974); Bennett and John-Alder (1986)
Agamidae	*Pogona barbata*	37	239	33.2	46.0	33.0	36.7	44.2	3.74	90.7	Licht (1965); Warburg (1965); Schäuble and Grigg (1998)
	Physignathus leseuri	71	549	30.1	-	29.8	34.0	42.4	4.21	90.7	Wilson (1974)
Iguanidae	*Sauromalus hispidus*	35	574	35.9	-	32.0	36.6	45.7	4.56	90.7	Bennett (1972)
	Amblyrhynchus cristatus	23	489	33.4	-	33.8	37.1	43.8	3.35	90.7	Wilhoft (1958); Bartholomew (1966); White (1973)
	Iguana iguana	42	794.5	33.8	40.6	33.7	38.0	46.6	4.29	90.7	Moberly (1968); Brusch *et al.* (2016)
	Dipsosaurus dorsalis	29	35.2	38.5	48	38.6	41.3	46.7	2.68	90.8	Licht (1964); DeWitt (1967); Bennett and Dawson (1972)

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823   **Table 2:** Akaike Information Criterion (AIC) comparison of the fitted models characterized according to a candidate set of ten published thermal
824   performance functions. The function with the lowest AIC score, and hence a ΔAIC of zero, is deemed to be the most parsimonious model of the candidate
825   set; in this case the beta-distribution function. Canonically, models within two AIC distances are considered statistically indistinguishable from the most
826   parsimonious model (Raftery, 1995), in this case capturing three functions.

Performance Function		K	ΔAIC	Log Likelihood
Beta-distribution	(Yan & Hunt, 1999)	4	0.00	-252.84
Exponentially-penalized logistic	(Kovac et al., 2007)	6	0.24	-250.96
Arrhenius	(Sharpe & DeMichele, 1977)	7	1.31	-250.41
Skewed normal	(Lehman et al., 1975)	5	3.20	-253.42
Exponential power	(Lassiter, 1975)	5	4.61	-254.12
two-way negative exponential	(Huey & Stevenson, 1979)	4	7.74	-254.66
Modified cubic	(Knies et al., 2006; Knies et al., 2009)	4	10.03	-257.85
Biexponential	(Logan et al., 1976; Tomlinson et al., 2015)	6	26.42	-266.05
Logistic x logistic	(Thornton & Lessem, 1978)	5	596.62	-549.15
Special Poisson	(O'Neill et al., 1972)	6	5423.69	-2963.66

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Table 3:   Beta functions of thermal performance of maximum metabolic rates for the nine species
of reptile investigated.

Family	Species	Performance Function
Scincidae	*Tiliqua rugosa*	$1.94 \times \left(\dfrac{47.1 - T_b}{47.1 - 37.3}\right) \times \left(\dfrac{T_b}{37.3}\right)^{\frac{37.3}{47.1 - 37.3}}$
	Egernia cunninghamii	$1.99 \times \left(\dfrac{44.7 - T_b}{44.7 - 36.1}\right) \times \left(\dfrac{T_b}{36.1}\right)^{\frac{36.1}{44.7 - 36.1}}$
Agamidae	*Pogona barbata*	$2.19 \times \left(\dfrac{44.2 - T_b}{44.2 - 36.7}\right) \times \left(\dfrac{T_b}{36.7}\right)^{\frac{36.7}{44.2 - 36.7}}$
	Physignathus leseuri	$1.79 \times \left(\dfrac{42.4 - T_b}{42.4 - 34.0}\right) \times \left(\dfrac{T_b}{34.0}\right)^{\frac{34.0}{42.4 - 34.0}}$
Iguanidae	*Sauromalus hispidus*	$2.10 \times \left(\dfrac{45.7 - T_b}{45.7 - 36.6}\right) \times \left(\dfrac{T_b}{36.6}\right)^{\frac{36.6}{45.7 - 36.6}}$
	Amblyrhynchus cristatus	$2.89 \times \left(\dfrac{41.3 - T_b}{41.3 - 37.1}\right) \times \left(\dfrac{T_b}{37.1}\right)^{\frac{37.1}{41.3 - 37.1}}$
	Iguana iguana	$2.10 \times \left(\dfrac{46.6 - T_b}{46.6 - 38.0}\right) \times \left(\dfrac{T_b}{38.0}\right)^{\frac{38.0}{46.6 - 38.0}}$
	Dipsosaurus dorsalis	$5.12 \times \left(\dfrac{46.7 - T_b}{46.7 - 41.3}\right) \times \left(\dfrac{T_b}{41.3}\right)^{\frac{41.3}{46.7 - 41.3}}$

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