Assigning metabolic rate measurements to torpor and euthermy in heterothermic endotherms: “torpor", a new package for R.

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Summary statement: The presented method and its associated R-package (torpor) enable the assignment of metabolic rate measurements to torpor or euthermy, ultimately improving the standardization of respirometry analyses in heterotherms.

Abstract

Torpor is a state of controlled reduction of metabolic rate (*M*) in endotherms. Assigning measurements of *M* to torpor or euthermy can be challenging, especially when the difference between euthermic *M* and torpid *M* is small, in species defending a high minimal body temperature in torpor, in thermolabile species, and slightly below the thermoneutral zone (*TNZ*). Here, we propose a novel method for distinguishing torpor from euthermy. We use the variation in *M* measured during euthermic rest and torpor at varying ambient temperatures (T_a) to objectively estimate the lower critical temperature (T_{lc}) of the *TNZ* and to assign measurements to torpor, euthermic rest or rest within TNZ. In addition, this method allows the prediction of *M* during euthermic rest and torpor at varying T_a, including resting *M* within the *TNZ*. The present method has shown highly satisfactory results using 28 published sets of metabolic data obtained by respirometry on 26 species of mammals. Ultimately, this novel method aims to facilitate analysis of respirometry data in heterothermic endotherms. Finally, the development of the associated R-package (torpor) will enable widespread use of the method amongst biologists.

List of main abbreviations:

BMR: Basal metabolic rate.

M: Resting rate of metabolism, expressed in W, $mlCO_2\ h^{-1}$ or $mlO_2\ h^{-1}$. Often expressed as a mass-specific rate (e.g. in $W\ g^{-1}$).

M_{TNZ}: Resting rate of metabolism within the *TNZ.*

M_e: Euthermic *M*.

M_r: predicted M_t at T_{lc}. M_r may be lower than M_{TNZ} if additional metabolic inhibition is involved, other than the abolishment or reduction of thermogenesis necessary to maintain euthermy.

M_t: Torpid *M*.

PPO: prior and posterior distribution overlap.

T_a: Ambient temperature.

T_b: Body temperature.

T_{lc}: lower critical T_a or lower limit of the *TNZ*.

TMR: minimal metabolic rate in torpor, measured at T_t.

TNZ: Themoneutral zone. The range of T_a where *M* is independent of T_a.

T_t: Threshold T_a separating regulated from conforming torpor.

Introduction

Torpor is a state of controlled reduction of metabolic rate (*M*) and body temperature (T_b) observed in numerous mammals and birds (Ruf and Geiser, 2015). This physiological state can occur over short periods (i.e. < 24h), often referred to as 'daily torpor', or it can last up to many days, often referred to as 'hibernation', which typically involves multiday torpor bouts separated by short spontaneous arousals (McKechnie and Lovegrove, 2002; Ruf and Geiser, 2015; Schleucher and Withers, 2001). The drop in T_b may be considerable in hibernators, which often allow T_b to reach values close to 0°C, but it can also be much shallower, as is often the case in species entering daily torpor (Ruf and Geiser, 2015). It has been suggested that a continuum may exist between these types of torpor as well as between torpor and euthermic rest (Boyles et al., 2013; McKechnie and Lovegrove, 2002; van Breukelen and Martin, 2015). Torpor use has profound implications on energy expenditure and allocation (Lyman et al., 1982) and affects many biological functions (Geiser and Brigham, 2012; Nowack et al., 2017). Accordingly, a vast amount of literature describes the occurrence of torpor and its associated energy savings in a multitude of species (Geiser and Ruf, 1995; Lovegrove, 2012; Nowack et al., 2020; Ruf and Geiser, 2015).

Determining whether measurements of *M* can be assigned to torpor or euthermy can however be challenging. The distinction between these two states is usually straightforward in typical hibernators, due to the more than 90% reduction in *M* common in these species when torpid at their usually hibernation ambient temperature (T_a) (Geiser, 2004). Yet this distinction can become problematic when the difference between euthermic *M* (M_e) and torpid *M* (M_t) is small, which often occurs slightly below the thermoneutral zone (TNZ) (Geiser, 2011;

Hainsworth and Wolf, 1970; Humphries et al., 2002; Speakman and Selman, 2003). Additional difficulties are encountered with species that either enter daily torpor, referred to as 'daily heterotherms', whose minimum T_b in torpor often lies only slightly below the euthermic T_b (Bartels et al., 1998; Bonaccorso and McNab, 1997; Genoud et al., 1990; McNab, 1980b), or who exhibit a large variability in M and T_b at rest (Coburn and Geiser, 1998; Geiser et al., 1996).

Barclay, Lausen, & Hollis (2001) reviewed the variety of criteria that have been chosen in the past to distinguish torpor from euthermy. Apart from a minority of reports that identified torpor on the basis of behavioural features (Brice et al., 2002; Geiser and Kenagy, 1988; Geiser and Masters, 1994), the vast majority of studies have used patterns of variation in T_b (or skin temperature) and/or M to separate the two states in the field or in the laboratory. Animals have been deemed to be in torpor below a threshold T_b or M (Coburn and Geiser, 1998; Geiser et al., 1996; Hosken and Withers, 1999; Kelm and von Helversen, 2007; Levesque, 2008; Mzilikazi and Lovegrove, 2002), below some threshold temperature differential between the body and air (Levesque and Lovegrove, 2014), or below a threshold percentage of the euthermic rate of metabolism (Geiser, 1988a; Hudson and Scott, 1979). This diagnostic threshold value was sometimes calculated on the basis of the parameter's variation in the euthermic state (e.g. Lovegrove & Raman, 1998; McKechnie, Ashdown, Christian, & Brigham, 2007), but has also been predicted by an equation based on body mass and T_a (Willis, 2007). All available techniques have their limitations and are at least partly arbitrary (Barclay et al., 2001; Boyles et al., 2011), despite the efforts made to render them more objective.

With this paper, we propose an automated method for distinguishing torpor from euthermic rest in species that enter torpor, hereafter referred to as heterotherms following the

109 terminology of Geiser & Ruf (1995) or Lovegrove (2012). Here, assignment to an either
110 euthermic or torpid state is based on a probabilistic approach using the variation observed
111 among measurements of *M* at varying T_a. We assume that the relationship between *M* of
112 resting animals and T_a follows the classical "Scholander-Irving model" (Scholander, Hock,
113 Walters, Johnson, & Irving, 1950; for a discussion see McNab, 2002), which was later extended
114 to include torpor (Hainsworth & Wolf, 1970; Humphries, Thomas, & Speakman, 2002;
115 Speakman & Thomas, 2003; Geiser, 2011). This model (Fig. 1) predicts the vast majority of
116 patterns observed among endotherms entering daily torpor or hibernation. We explicitly
117 consider that additional metabolic inhibition involving mechanisms other than the
118 abolishment or reduction of thermogenesis necessary to maintain euthermy may occur during
119 torpor (Geiser, 1988b; Geiser, 2004; Geiser and Kenagy, 1988; Guppy and Withers, 1999;
120 Withers et al., 2016), by allowing the curve of torpor *M* to reach T_{lc} at a level equal to – or
121 lower than the resting metabolic rate within the *TNZ*. Thus, over all aforementioned
122 techniques, our novel method provides major improvements, as it assigns measurements
123 under an explicit framework (i.e. the extended Sholander-Irving model) and does so with a
124 probabilistic approach based on observed variation.
125
126 Our method is specifically intended to facilitate the discrimination between torpor and
127 euthermy in laboratory experiments using respirometry. Additionally, it can also be useful to
128 predict *M* at varying T_a, by modelling the different parameters of the thermoregulatory curves.
129 Further, we have assessed the performance of our method, by applying it to previously
130 published data on the *M* of various mammals, including both heterotherms and species not
131 undergoing torpor (hereafter referred to as homeotherms). Finally, we have provided a new
132 package running within R (R Development Core Team, 2012) to allow researchers to apply our

method to their own data. This package (“torpor”) comprises several useful functions that will improve standardization of the analyses of metabolic measurements for thermal biology.

136 ## Results

137 Over all datasets, the proportion of assignments, that could be validly assigned (i.e.
138 assignment confidence > 0.80, cf. "Assignment confidence" in section "Material and
139 Methods") ranged from 0.63 to 1.00 (median=0.87, Table 1). The corroboration index, which
140 assesses the similarity between valid assignments made by our method and by the authors of
141 the original datasets, ranged between 0.66 and 1.00 (median=1.00, Table 1). Complete
142 matches between the method valid assignments and the authors' descriptions were found for
143 15 out 28 datasets (e.g. *Nyctophilus geoffroyi* (Hosken and Withers, 1999), Fig. 2A).
144 Mismatches mostly occurred close to T_{lc} (e.g. *Peropteryx macrotis* (Genoud et al., 1990), Fig.
145 2C) but could also be found as far as 41.0°C below T_{lc} (Fig. 3). Among homeotherms, no *M*
146 measurement was assigned to torpor (e.g. *Sorex minutus* (Sparti & Genoud, 1989), Fig. 2B).
147
148 In studies of homeotherms, where no *M* was originally assigned to torpor, prior and posterior
149 distribution overlaps (*PPOs*) of *TMR* ranged from 76.80 to 95.60%. In identified heterotherms,
150 *PPOs* of *TMR* values range from 4.60 to 64.60% (Table 1).
151
152 Modelled values of T_{lc}, M_{TNZ} and T_t and extracted values from the original studies were
153 significantly correlated (T_{lc}: N=28, Pearson's coefficient: 0.65, $p<0.001$, M_{TNZ}: N=28, Pearson's
154 coefficient: 1.00, $p<0.001$, T_t: N=14, Pearson's coefficient: 0.97, $p<0.001$).
155
156

Discussion

Our method aims to facilitate the discrimination between torpor and euthermy using the variation of *M* measured at varying T_a. We tested it using 28 published sets of metabolic data obtained by respirometry on 26 species of small or medium-sized mammals. Selected species displayed a diversity of metabolic and thermal strategies ranging from permanent homeothermy to heterothermy including shallow, daily torpor and deep, long-term hibernation. The efficiency of our method proved satisfactory. Indeed, the corroboration index was generally high. Most conflicting assignments were a mere consequence of the difference between the estimated T_{lc} and that defined by the authors (Table 1, Figs. 2C & 3). In particular, all mismatches between the model's and the authors' assignments concerning M_e and M_{TNZ} measurements in homeotherm species were explained by differences in estimated T_{lc}. The remaining mismatches could generally be explained by intra-state frequency distributions of *M* that did not segregate clearly. This condition was often found close to *TNZ*, but also occurred in species where T_t was relatively close to T_{lc} (e.g. McNab, 1980a, 1989). Such discrepancies illustrate the difficulty of the assignment process when torpor and euthermy have to be distinguished solely on the basis of measurements of *M*. While the use of a statistical method enables an objective assignment, it is nevertheless worthwhile to recall that some authors also used patterns of T_b to assign their data (e.g. McNab, 1980a, 1989). These specific cases should deserve further attention as they can highlight mechanisms decoupling *M* from the control of T_b (e.g. Daniels, 1984; McNab, 1988; Heldmaier, Ortmann, & Elvert, 2004). One advantage of our method is to allow researchers to identify statistically data points that are difficult to assign. In this study, we considered assignments with a confidence lower than 0.8 as invalid. Obviously, an increase of this threshold value would lead to fewer assignment mismatches.

Our method also models the relationship between M and T_a specific to each state, as well as several parameters describing the standard energetics of the animal(s) under study, including M_{TNZ}, TMR, T_{lc} and T_t. Predicting M at any T_a is crucial to model energy costs (e.g. Boyles, Johnson, Blomberg, & Lilley, 2020). It should however be recalled that our Bayesian inference-based method models all parameters whatever the available data. It remains therefore crucial to consider the parameters' identifiability. The prior and posterior distribution overlaps (*PPO*) of T_{bt} and T_{lc} are not reliable for that purpose, because the prior distribution of those parameters largely depends on the data provided. From the *PPOs* of *TMR* obtained in studies where no M was assigned to torpor (i.e. "homeotherms") and those obtained in identified heterotherms, we define a *PPO* higher than 75% as indicative of a parameter that is not identifiable.

The parameters estimated by our method correlated significantly with those extracted from the original studies. The number of M_e values provided was probably not always sufficient to model T_{lc} adequately. On one hand, a statistical estimation of T_{lc} (e.g. in Song, Körtner, & Geiser, 1995, 1997; Bonaccorso & McNab, 1997; Genoud, Martin, & Glaser, 1997; Hosken & Withers, 1999; Willis, Lane, Liknes, Swanson, & Brigham, 2005; Genoud, 2014) was often lacking, leaving space for some potential misleading subjectivity by the authors of the original studies. On the other hand, an overestimation of T_{lc} by our method remains possible if appreciable additional metabolic inhibition occurs during torpor (see below). The estimated T_t was always close to that of the original studies. This higher precision in the estimation of T_t in comparison to that of T_{lc} is probably due to the lower variation of M exhibited during conforming torpor in comparison to that measured during euthermic rest. It is also not

205 surprising that the M_{TNZ} obtained by our method and by the authors of the original studies
206 were close to identical, since both were calculated as the mean of nearly the same *M* values
207 (i.e. all values above T_{lc})
208
209 An important aspect incorporated in our method is that we allow function for M_t to be lower
210 than M_{TNZ} at T_{lc}. We thus explicitly allow for additional metabolic inhibition to occur in some
211 species during torpor, other than the cessation or reduction of the thermogenesis necessary
212 to maintain euthermy (Geiser, 2004; Guppy and Withers, 1999; Withers et al., 2016). Caution
213 should be kept in M_r interpretation as an insufficient number of M_t values corresponding to
214 conforming torpor leads to the unidentifiability of this parameter (e.g. *Melonycteris melanops*
215 (Bonaccorso & McNab, 1997), Fig. 2D). Consequently, the mentioned additional metabolic
216 inhibition should only be considered when sufficient M_t values corresponding to conforming
217 torpor are provided and M_r *PPO* indicates adequate parameter's identifiability. Considering
218 only identifiable parameters (i.e. *Mr PPO* < 75%), we quantified additional metabolic inhibition
219 as the fraction M_r / M_{TNZ}. Values ranged between 0.27 and 0.93 (N=18, median=0.77, Table 1).
220 By extrapolation, we should consider the possibility that a hypometabolic state might occur
221 within the *TNZ* as well (e.g. Grimpo, Legler, Heldmaier, & Exner, 2013; Reher, Ehlers,
222 Rabarison, & Dausmann, 2018). With our method, we did not implement an assignment to
223 such a hypometabolic state within the TNZ, hence M_{TNZ} corresponds to the mean of all values
224 from the *TNZ*. Our method might therefore underestimate the eumetabolic $M_{TNZ,}$ and might
225 consequently overestimate T_{lc}, if animals occasionally enter a hypometabolic state in the *TNZ*,.
226 A predicted M_t at T_{lc} much lower than M_{TNZ} should be viewed as an indication for a strong
227 additional metabolic inhibition occurring during torpor and should invite for a careful
228 consideration of the estimated M_{TNZ} and T_{lc}.

229

230 Throughout this paper, resting metabolic rate within the *TNZ* is referred to as M_{TNZ} rather than
231 to the basal rate of metabolism (*BMR*). Estimating the *BMR* requires several specific criteria:
232 animals should be post-absorptive, adult, non-reproductive, and resting during a major
233 inactive phase of the daily cycle (McNab, 1997). If these criteria are not met, the method still
234 remains applicable, but the resting *M* estimated within the *TNZ* (i.e. M_{TNZ}) will correspond to
235 a minimal resting metabolic rate rather than to the *BMR*.

236

237 While the performance of our method appeared to be excellent with the selected datasets,
238 its main limitations correspond to those of the model on which it is based. Indeed, although
239 extremely fruitful, the Scholander-Irving model (Scholander et al., 1950) and its latter
240 extensions (Geiser, 2011; Hainsworth and Wolf, 1970; Humphries et al., 2002; Speakman and
241 Thomas, 2003) does not describe the thermal biology of all heterothermic species. Causes for
242 significant divergence from the hereby model include a non-linear relationship between M_e
243 and T_a (e.g. *Cercartetus nanus*: Song, Körtner, & Geiser, 1997), a difference in the slopes of
244 the regressions of *M* versus T_a in regulated torpor and euthermic rest (Geiser, 2004) and a
245 strong dependence of M_t on factors other than T_a, such as body mass (Kelm and von
246 Helversen, 2007) and duration since the last activity period or since last meal (Grigg et al.,
247 1992; Morris et al., 1994). Furthermore, this model also does not describe adequately
248 circadian (e.g. the shallow "rest-phase hypothermy" of many small birds (McKechnie and
249 Lovegrove, 2002) or ultradian (Heldmaier et al., 1989) variations in *M*.

250

251 Our method is able to reveal the presence or the absence of distinct groups of values
252 (euthermic rest vs torpor), but is not intended to judge whether any of the data points entered

correspond to stable rates and/or to minimal values. Transitions between activity and rest or between euthermic rest and torpor as well as incomplete torpor bouts during which *M* never reaches a stable, minimal level (e.g. "test drops" (Hudson and Scott, 1979); see also (Genoud, 1993; Lyman, 1982)) should be excluded. It should also be recalled that long runs may be necessary for some heterothermic animals to achieve a state of steady torpor (e.g. Heldmaier, Ortmann, & Elvert, 2004). The above limitations should be kept in mind for a lucid application.

Then, individual variation is not explicitly considered in our method. Especially, body mass variation within a studied population might affect the assignment of *M* to torpor or euthermy in a complex manner (Genoud, 2014; Genoud et al., 2018; Glazier, 2005; Nespolo et al., 2003; Sassi and Novillo, 2015; Schleucher and Withers, 2001). However, there would be no particular difficulty to apply our method to single individuals, or repeatedly on single individuals.

Our method and the associated R-package (torpor) provide a way to standardize the analysis of respirometry data in relation to T_a. Its major strength is that it uses a probabilistic approach to assign metabolic values to torpor or euthermy, rather than assigning them on the basis of a particular threshold value. Thus, the partly arbitrary nature of the assignment process is removed. Further, it can be applied to study intraspecific as well as interspecific variation in energetics. Parameters might now be extracted from the literature for comparative analysis, largely avoiding causes of variation due to the diversity of past assignment techniques. Ultimately, this method and the associated R-package (torpor) will ease intra- and inter-specific comparative analyses of endotherm energetics.

Material and Methods

The three-steps Method

Our present method is based on three steps and requires measurements of *M* of resting or torpid animals at T_a ranging from below – to within the *TNZ*. Animals are assumed to be in one of three "states": euthermic rest below the *TNZ* (M_e), torpor below the *TNZ* (M_t) or rest within the *TNZ* (M_{TNZ}). We do not consider here the relation between *M* and T_a above the *TNZ*. Consequently, metabolic rates measured at T_a higher than the upper limit of the *TNZ* should be excluded. During the different steps, a mixture model based on Bayesian inference is run under varying conditions.

The model

The Scholander-Irving model and its extensions (Fig. 1) consider that resting *M* measured within the *TNZ* is independent of T_a. This rate is hereafter referred to as M_{TNZ}. Below T_{lc}, the *M* of euthermic animals (M_e) increases linearly with decreasing T_a :

$$M_e \sim \alpha_e + \beta_e * T_a \mid T_a < T_{lc} \quad (1)$$

The model assumes that M_e equals M_{TNZ} at T_{lc}, which enabled calculation of α_e, the intercept of the line predicting M_e at varying T_a:

$$\alpha_e = M_{TNZ} - \beta_e * T_{lc} \quad (2)$$

and of the slope of that line (β_e):

$$\beta_e = \frac{M_{TNZ}}{T_{lc} - T_{be}} \quad (3)$$

T_{be} represents the hypothetical T_a where M_e equals 0, which correspond to the euthermic body temperature, provided that thermal conductance and body temperature do not vary with T_a below the *TNZ* (McNab, 1980a).

Below the threshold T_a separating regulated and conforming torpor (T_t), the metabolic rate in torpor (M_t) increases linearly with decreasing T_a to defend the animal's setpoint T_b in torpor. That state, corresponding to M_t measured at T_a lower than T_t, is usually referred to as "regulated torpor":

$$M_t \sim \alpha_r + \beta_r * T_a \mid T_a < T_t \quad (4)$$

Where :

$$T_t = \frac{(TMR - \alpha_r)}{\beta_r} \quad (5)$$

The intercept of the line for regulated torpor (α_r) is obtained as:

$$\alpha_r = -T_{bt} * \beta_r \quad (6)$$

TMR represents the minimal M_t measured at T_t. T_{bt} is the hypothetical T_a at which M_t of the regulated torpor would equal 0. T_{bt} would correspond to the minimal body temperature in torpor (i.e. setpoint T_b in torpor) provided that thermal conductance and body temperature during regulated torpor do not vary with T_a below T_t. Between T_t and T_{lc}, torpor is referred to as "conforming torpor" and M_t follows an exponential curve:

$$M_t \sim \alpha_c * \exp(\beta_c * T_a) \mid T_t \leq T_a \leq T_{lc} \quad (7)$$

Where the coefficient of the exponent (β_c) is calculated as:

$$\beta_c = \frac{\log(M_r) - \log(TMR)}{T_{lc} - T_t} \quad (8)$$

and the intercept for the exponential curve (α_c) is calculated as:

$$\alpha_c = \frac{M_r}{\exp(\beta_c * T_{lc})} \quad (9)$$

M_r represents M_t measured at T_{lc}. In accordance with the general trend observed (Geiser, 2004), we assume that the slopes of the lines linking T_a to M_e (β_e) and to M_t of the regulated torpor,(β_r) are similar:

$$\beta_r = \beta_e \quad (10)$$

The following parameters are modelled: the fractions of measurements belonging to each state, which provides the value specific state membership probabilities for each measurement (see Second step: Metabolic rate (*M*) measurements pre-assignment, below), T_{be}, T_{bt}, *TMR,* and the standard deviations for M_{TNZ} and regulated/conforming metabolic rates (SD_{TNZ}, SD_r *and* SD_c).

First step: Estimation of M_{TNZ} and T_{lc}

The method initially defines the highest possible T_a within the dataset that still underestimates T_{lc} (T_{lc_low}). Above T_{lc_low}, a linear regression between *M* and T_a should neither result in a significant negative slope nor be affected by heteroscedasticity. Beginning with the ten *M* values measured at the highest T_a, linear regressions are performed on sets of *M* values progressively including values at lower T_a. The inclusion of additional *M* values obtained below T_{lc} eventually leads to a significantly negative slope in the case of animals not entering torpor and/or to heteroscedasticity in the case of heterotherms. A significantly positive slope would reveal an absence or insufficient number of values for euthermic rest below the *TNZ*, hence it automatically stops the analysis as T_{lc_low} is undefinable. Heteroscedasticity is assessed with a Breusch-Pagan test with the function “bptest” from package “lmtest” (Zeileis and Hothorn, 2002). Significance level for the Breusch-Pagan test is set at 0.05. The significance of the negative regression is assessed with a linear regression, function “lm” and significance level is set at 0.01.

Then, in order to get T_{lc}, the model described in the second step (see Second step: Metabolic rate (M) measurements pre-assignment) is first run without data points measured above

368 T_{lc_low}. Moreover, within this step, the parameter T_{lc} is also modelled. M_{TNZ} provided in that
369 analysis is the mean of the M measured at T_a higher than T_{lc_low}. Once T_{lc} has been estimated,
370 M_{TNZ} is recalculated as the mean of the M measured at T_a higher than T_{lc}.
371

372 ## Second step: Metabolic rate (*M*) measurements pre-assignment

373

374 The assignment of M measurements to one of the three physiological states is based on a
375 probabilistic process. Below T_{lc}, a posterior categorical distribution is generated for each
376 coupled data (T_a and M), providing the probabilities to belong either to torpor or to euthermic
377 rest (i.e. value specific state membership probabilities). Independent of the pre-assignment,
378 M values higher than the predicted M_e or lower than the predicted M_t are automatically
379 assigned to M_e and M_t, respectively. This latter automatic procedure is, however, only
380 performed if at least one M measurement has been assigned to torpor during that second
381 step. As defined previously, M values located above T_{lc} are automatically assigned to M_{TNZ}.
382 Automatically assigned values get a state membership probability of one.
383

384 ## Third step: Metabolic rate (*M*) measurements final assignment and estimation
385 ## of the functions parameters

386

387 In the third step, only M values situated between the predicted M_e and M_t are assigned.
388 Measurements' states that are automatically assigned during the second step are provided
389 along with the coupled M and T_a values. During that final step, the parameters of the functions

relating M to T_a below the T_{lc}, as well as the standard deviation for the different physiological states are modelled.

Bayesian parametrization

Prior distributions are defined either uninformatively or with biologically relevant limits. Except when specified, all prior distributions are Gaussian with a mean of 0 and a precision (i.e. $1/SD^2$) of 0.001 and are truncated based on the specified limits. Specifically, the prior distribution of T_{lc} during the first step is constrained between T_{lc_low} and the maximal T_a recorded within the dataset. The prior distribution of T_{be} is constrained between T_{lc} (T_{lc_low} for the first step) and 50°C. The upper limit of the T_{bt} prior distribution satisfies two conditions. First, T_t should be inferior to the lower limit of the 95% credible interval (CI) of T_{lc}. Second, the ratio of conforming M_t values corresponding to body temperatures differing by 10°C (Q_{10}) should not exceed a value of 5 (Geiser, 1988b). These conditions are verified by fixing the upper range of the prior distribution of T_{bt} as:

$$\min\left(\frac{\text{lower 95CI } T_{lc}*\beta_t - TMR}{\beta_t}, T_{be} - \frac{M_{TNZ}*2}{TMR}\right) \quad (11)$$

The lower limit of the T_{bt} prior distribution is set at -5°C (Barnes, 1989). The upper limit of the prior distribution of TMR is defined as 80% of the M_{TNZ} (Ruf and Geiser, 2015), while its lower one is fixed at 0. For M_r, the prior distribution ranges from TMR to M_{TNZ}. An uninformative Dirichlet distribution (i.e. two concentration values of 1) is used for the priors of the dataset specific state membership fractions, which provide the value specific state membership

probabilities of each measurement (see Second step: Metabolic rate (*M*) measurements pre-assignment, above). Finally, the prior distributions of the standard deviations for M_{TNZ} and regulated/conforming metabolic rates (SD_{TNZ}, SD_r *and* SD_c) are uniform, with that of SD_r constrained between 0 and 3, that of SD_c constrained between one fifth of the value of SD_r and SD_r and that of SD_{TNZ} constrained between half the value of SD_r and SD_r.

Three different Markov chains are run during 50'000 iterations starting at initial values within the range of parameter space. The initial convergence phase is excluded by dropping the first 30'000 iterations. Markov chains are thinned by a factor of 10 and the Brooks–Gelman–Rubin criterion $\hat{R}$ (Brooks and Gelman, 1998) is used to assess the convergence of chains (< 1.1). To ease parameters' estimation, metabolic rate measurements are divided by their mean.

Identifiability of the parameters

The prior and posterior distribution overlap (*PPO*) enables the evaluation of the relevance of some modelled parameters. Overlap values are obtained with the function "MCMCtrace" from package "MCMCvis" (Youngflesh, 2018). Truncated Gaussian prior distributions are provided with the function "rtruncnorm" from package "truncnorm" (Mersmann et al., 2018).

Assignment confidence

The *M* value is assigned to the physiological state having the highest specific state membership probability. The assignment confidence represents the product of the highest specific state membership probability and of the probability that T_a is above (for M_{TNZ}) or below (for M_t and M_e) T_{lc}. That latter probability is calculated from the variation in the estimation of T_{lc} (cf. First

437 step: Estimation of M_{TNZ} and T_{lc}), as the value at T_a of the T_{lc} cumulative posterior distribution
438 function, or 1 minus that function respectively. A threshold proportion is then selected to
439 consider any assignment with a significantly lower assignment confidence as invalid. That
440 hypothesis is tested with a binomial test, function: “binom.test”, with the alternative
441 hypothesis set as “greater”. The significance level for the binomial test is set at 0.05. For the
442 present study, assignments with a confidence higher than 0.8 were considered as valid.
443

444 Data collection and method evaluation

445

446 We evaluated our method by applying it to 28 published sets of *M* data obtained by
447 respirometry. Mainly, we aimed at highlighting strengths and possible issues linked to the
448 application of our method to typical sets of measurements. Thus, we were specifically
449 interested in divergences between assignments made by our method and by the authors. The
450 26 species of small or medium-sized mammals investigated illustrate a diversity of metabolic
451 and thermal strategies ranging from permanent homeothermy to heterothermy including
452 shallow, daily torpor and deep, long-term hibernation. The selected datasets also differ in size
453 (Table 1). For each published set, we considered all metabolic data provided, except those
454 made above the upper critical temperature defined by the authors (see “the three-steps
455 Method” above). In 20 species (here considered "heterotherms", Table 1), rates of
456 metabolism measured at T_a's below the described thermoneutral zone were originally
457 assigned to either of two states, which were usually referred to as euthermy (or
458 normothermy) and torpor. In *Cynopterus brachyotis*, values corresponding to particularly low
459 T_b were identified but not explicitly referred to as torpor (McNab, 1989). Some of the values
460 obtained from torpid *Lasiurus seminolus* by Genoud (1993) were originally characterised by

461 "irregular fluctuations" in *M* (Genoud, 1993). For simplicity, we treated these values as if the
462 authors assigned them to torpor. The remaining six species are hereafter referred to as
463 homeotherms (Table 1), as none of the values in the corresponding datasets were assigned to
464 torpor in the original studies. Chosen studies reported M_{TNZ} (mostly referred to as *BMR*) and
465 T_{lc} estimates, although the latter had to be extracted from a graph in two cases, or calculated
466 as the intercept between the euthermic line below thermal neutrality and M_{TNZ} provided by
467 the authors in another case (Table 1). In all dataset the authors provided a sufficiently precise
468 graph of metabolic rate values as a function of T_a and several of them also estimated T_t.
469 Plotted data were digitized using the software Plot Digitizer (Huwaldt and Steinhorst, 2015)
470 except for the *Pipistrellus pipistrellus* dataset, which was provided by MG. Sample sizes of the
471 digitized data ranged between 27 and 204 (Table 1). We tested the correlation between the
472 M_{TNZ}, T_{lc} and T_t values modelled with the present method and those provided by the authors
473 with Pearson's paired-samples correlation test, function "cor.test". Cases in which the
474 estimation of T_t fell outside the range of measured T_a were excluded from this analysis as T_t
475 was considered unidentifiable. Then, we examined whether the assignment of the metabolic
476 values to torpor or euthermy made by the authors and provided by the method coincided,
477 and we calculated a corroboration index as the fraction of the matched assignments. Only
478 measures with valid assignments (i.e. assignment confidence > 0.80, cf. "Assignment
479 confidence" in previous section) were considered for the correlation tests and the
480 corroboration index calculations. Finally, in order to define a *PPO* range highlighting an
481 identifiable parameter, the *PPOs* of *TMR* from studies where part of the *M* measurements
482 were assigned to torpor were compared with those from studies where no *M* measurement
483 was assigned to torpor. In those later studies *TMR* was considered unidentifiable.
484

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Competing interests

No competing interests declared

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Table 1: Model parameters and corroboration index for 28 datasets on small or medium-sized mammals described as either heterotherms or homeotherms.

Species	Type	m	Autors M_{TNZ}	M units	Authors T_{lc}	Authors T_t	Authors	N	T_{lc}	M_{TNZ}	TMR	T_t	β_t	α_e	β_c	α_c	α_r	MR inhibit	T_{be}	T_{bt}	PPO TMR	PPO M_r	Valid assignments	Corroboration	M assigned to torpor?
Unit	-	g	M units	-	°C	°C	-	-	°C	M units	M units	°C	M units/ °C	M units	log(M unit)/ °C	M units	M units	%	°C	°C	%	%	-	-	-
Callithrix pygmaea	Homeotherm	153	98	$mlO_2\ h^{-1}$	27.5 [8]	–	Genoud et al. 1997	27	29.15	102.97	NI	NI	-9.78	388.45	NI	NI	NI	NI	39.77	NI	89.80	86.80	0.63	1.00	No
Marmosa robinsoni	Heterotherm	122	0.8	$mlO_2\ g^{-1}\ h^{-1}$	26.5	–	McNab 1978	38	28.73	0.77	NI	NI	-0.10	3.49	NI	NI	NI	NI	36.41	NI	77.70	92.10	0.68	1.00	No
Phenacomys intermedius	Homeotherm	21.5	3.13	$mlO_2\ g^{-1}\ h^{-1}$	25.1 [6]	–	McNab 1992	75	25.48	3.09	NI	NI	-0.25	9.36	NI	NI	NI	NI	37.78	NI	80.00	94.60	0.85	1.00	No
Rattus niobe	Homeotherm	42.3	53.6	$mlO_2\ h^{-1}$	26	–	Genoud 2014	62	27.56	56.18	NI	NI	-4.56	181.97	NI	NI	NI	NI	39.97	NI	84.90	89.00	0.76	0.98	No
Sorex coronatus	Homeotherm	9.1	5.7	$mlO_2\ g^{-1}\ h^{-1}$	27.5	–	Sparti & Genoud 1989	33	22.61	5.94	NI	NI	-0.24	11.37	NI	NI	NI	NI	47.56	NI	95.20	97.20	0.97	0.81	No
Sorex minutus	Homeotherm	3.3	8.6	$mlO_2\ g^{-1}\ h^{-1}$	27.5	–	Sparti & Genoud 1989	28	25.36	8.67	NI	NI	-0.40	18.98	NI	NI	NI	NI	46.99	NI	95.60	95.00	0.89	1.00	No
Tolypeutes matacus	Homeotherm	1160	0.181	$mlO_2\ g^{-1}\ h^{-1}$	28	–	McNab 1980b	66	29.57	0.19	NI	NI	-0.04	1.24	NI	NI	NI	NI	33.94	NI	76.80	95.30	0.85	1.00	No
Cercartetus lepidus	Heterotherm	12.6	1.49	$mlO_2\ g^{-1}\ h^{-1}$	28.8	5.3	Geiser 1987	57	29.01	1.54	0.11	4.16	-0.20	7.47	0.08	0.08	0.95	0.49	36.72	4.68	4.60	33.50	0.98	1.00	Yes
Cercartetus nanus	Heterotherm	36	0.66	$mlO_2\ g^{-1}\ h^{-1}$	28.7	4.8	Song et al. 1997	171	26.15	0.64	0.06	2.86	-0.14	4.29	0.05	0.05	0.46	0.27	30.55	3.26	8.10	27.10	0.95	0.85	Yes
Cynopterus brachyotis	Heterotherm	37.4	1.27	$mlO_2\ g^{-1}\ h^{-1}$	29.7 [6]	–	McNab 1989	51	29.08	1.25	0.74	25.23	-0.21	7.18	NI	NI	NI	NI	34.38	28.76	44.80	94.30	0.76	0.92	Yes
Dasyuroides byrnei [1]	Heterotherm	118.2	0.7	$mlO_2\ g^{-1}\ h^{-1}$	28.3 [7]	15	Geiser & Baudinette 1987	75	26.13	0.64	0.38	12.97	-0.12	3.66	0.01	0.34	1.89	0.67	31.51	16.24	21.50	29.90	0.89	0.87	Yes
Eptesicus fuscus	Heterotherm	15	16.98	$mlO_2\ h^{-1}$	26.7	0	Willis et al. 2005	29	27.46	6.69	0.61	NI	-2.81	83.39	NI	NI	NI	NI	29.66	NI	13.60	80.50	0.93	1.00	Yes
Lasiurus cinereus [2]	Heterotherm	23.5 [4]	9	$mW\ g^{-1}$	30	–	Cryan & Wolf 2003	48	26.87	8.95	1.67	8.68	-1.09	38.23	NI	NI	NI	NI	35.15	10.22	24.70	78.00	0.90	0.81	Yes
Lasiurus seminolus	Heterotherm	9.4 [5]	1.26 [5]	$mlO_2\ g^{-1}\ h^{-1}$	32	5	Genoud 1993	204	30.69	1.43	0.12	6.27	-0.42	14.22	0.08	0.08	2.74	0.60	34.03	6.57	7.60	23.20	0.95	0.99	Yes
Macroglossus minimus	Heterotherm	16.3	1.29	$mlO_2\ g^{-1}\ h^{-1}$	30.9	20.4	Bartels et al. 1998	46	30.54	0.90	0.47	20.38	-0.16	5.89	0.06	0.16	3.78	0.93	36.32	23.35	27.50	26.30	0.65	0.97	Yes
Macroglossus minimus [3]	Heterotherm	16.4	0.88	$mlO_2\ g^{-1}\ h^{-1}$	31	21	Bonaccorso & McNab 1997	47	30.05	1.23	0.78	18.79	-0.22	7.75	NI	NI	NI	NI	35.78	22.39	30.50	79.50	0.91	1.00	Yes
Melonycteris melanops	Heterotherm	53.3	0.81	$mlO_2\ g^{-1}\ h^{-1}$	28	–	Bonaccorso & McNab 1997	30	28.84	0.81	0.44	17.05	-0.13	4.47	NI	NI	NI	NI	35.00	20.53	64.60	87.30	0.83	1.00	Yes
Natalus tumidirostris	Heterotherm	5.4	1.54	$mlO_2\ g^{-1}\ h^{-1}$	28	20	Genoud et al. 1990	30	27.22	1.46	0.82	19.00	-0.58	17.07	NI	NI	NI	NI	29.54	20.42	58.70	76.70	0.83	1.00	Yes
Nyctophilus geoffroyi	Heterotherm	8	1.42	$mlO_2\ g^{-1}\ h^{-1}$	34	–	Hosken & Withers 1999	84	32.32	1.43	0.24	8.62	-0.27	10.12	0.05	0.18	2.56	0.52	37.60	9.53	23.70	65.60	1.00	1.00	Yes
Peropteryx macrotis	Heterotherm	5.1	2.31	$mlO_2\ g^{-1}\ h^{-1}$	30.5	20	Genoud et al. 1990	49	35.01	2.50	1.80	20.36	-0.22	10.14	0.02	1.35	6.20	0.90	46.99	28.70	22.30	60.40	0.71	0.66	Yes
Pipistrellus pipistrellus	Heterotherm	4.9	7.6	$mlO_2\ h^{-1}$	33.2	0	Genoud & Christe 2011	95	32.79	7.62	0.54	2.22	-3.01	106.17	0.08	0.46	7.20	0.80	35.32	2.40	6.40	19.60	1.00	1.00	Yes
Planigale gilesi	Heterotherm	8.3	1.43	$mlO_2\ g^{-1}\ h^{-1}$	28.5 [8]	14	Geiser & Baudinette 1988	62	27.76	1.53	0.30	13.41	-0.37	11.65	0.07	0.13	5.19	0.55	31.93	14.22	19.90	34.40	0.89	1.00	Yes
Setifer setosus	Heterotherm	530	0.231	$mlO_2\ g^{-1}\ h^{-1}$	28	–	McNab 1980b	56	29.37	0.23	0.14	NI	-0.03	1.18	0.01	0.14	NI	0.88	36.20	NI	34.70	56.60	0.77	0.95	Yes
Sminthopsis macroura	Heterotherm	24.8	0.89	$mlO_2\ g^{-1}\ h^{-1}$	31.3	14	Song et al. 1995	155	30.80	0.84	0.18	16.39	-0.19	6.63	0.09	0.05	3.27	0.77	35.24	17.36	16.50	30.60	0.97	1.00	Yes
Steatomys pratensis	Heterotherm	27.5	1.315	$mlO_2\ g^{-1}\ h^{-1}$	28	20	Ellison 1995	39	26.73	1.24	0.26	16.38	-0.18	6.02	0.07	0.13	3.18	0.42	33.88	17.85	21.60	45.40	0.77	1.00	Yes
Syconycteris australis	Heterotherm	17.8	1.44	$mlO_2\ g^{-1}\ h^{-1}$	29.5	18	Geiser et al. 1996	91	32.13	1.27	0.49	15.11	-0.14	5.78	0.05	0.24	2.61	0.90	41.31	18.62	21.60	28.60	0.88	0.95	Yes
Syconycteris australis [1]	Heterotherm	18	1.38	$mlO_2\ g^{-1}\ h^{-1}$	30.2	18	Coburn & Geiser 1998	93	27.53	1.49	0.61	16.37	-0.17	6.00	0.07	0.23	3.33	0.86	36.22	20.07	32.00	43.10	0.85	0.97	Yes
Zaedyus pichiy	Heterotherm	1740	0.226	$mlO_2\ g^{-1}\ h^{-1}$	28	–	McNab 1980b	57	31.82	0.23	0.15	22.49	-0.02	0.94	0.02	0.11	0.65	0.82	42.25	29.47	29.30	71.70	0.70	0.75	Yes

Subset of published data considered: [1]=summer group; [2]=males; [3]=lowland population.

m=average body mass; Autor's M_{TNZ}=author's estimation of metabolic rate within TNZ; M units=units for metabolic rate; Authors' T_{lc} and T_t=author's estimation of lower critical temperature and threshold ambient temperature for torpor, respectively. When m, M_{TNZ} or T_{lc} were not explicitly provided, they were: [4]=back-calculated from M_{TNZ} in % of expected from body mass; [5]=calculated as a weighted average of two seasonal groups; [6]=extracted from a graph; [7]=calculated as the intercept of the euthermic line and M_{TNZ} given by the authors; [8]=obtained as the

mean of a given range; *N*=number of digitized data points (sample size); Model parameters are defined in text. NI=Parameter considered as not identifiable.

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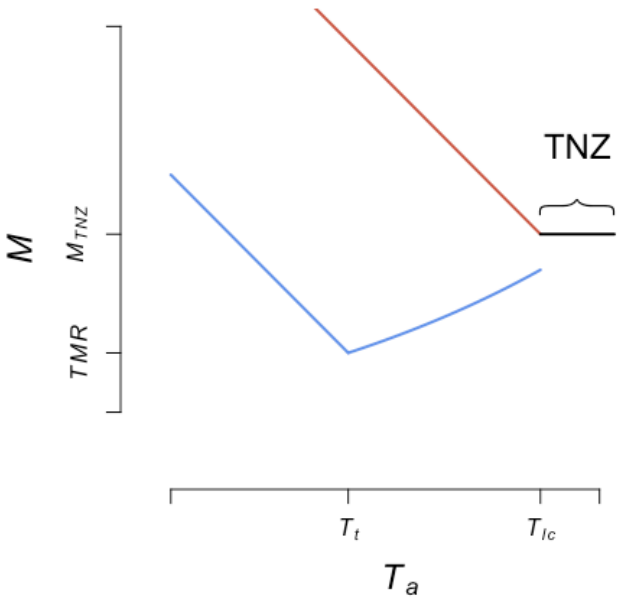

Figure 1: Representation of the relation between *M* during rest and torpor and T_a on which the present method is based. Torpor is indicated in blue, euthermic rest below the *TNZ* in red and rest within the *TNZ* in black. The relation follows the classical Scholander-Irving model (Scholander, Hock, Walters, Johnson, & Irving, 1950; for a discussion see McNab, 2002), which was later extended to include torpor (Geiser, 2011; Hainsworth and Wolf, 1970; Humphries et al., 2002; Speakman and Thomas, 2003). We further consider the possible occurrence of additional metabolic inhibition (Geiser, 2004; Geiser and Kenagy, 1988; Withers et al., 2016), hence allow the torpor curve to reach T_{lc} at a level equal to – or below M_{TNZ}.

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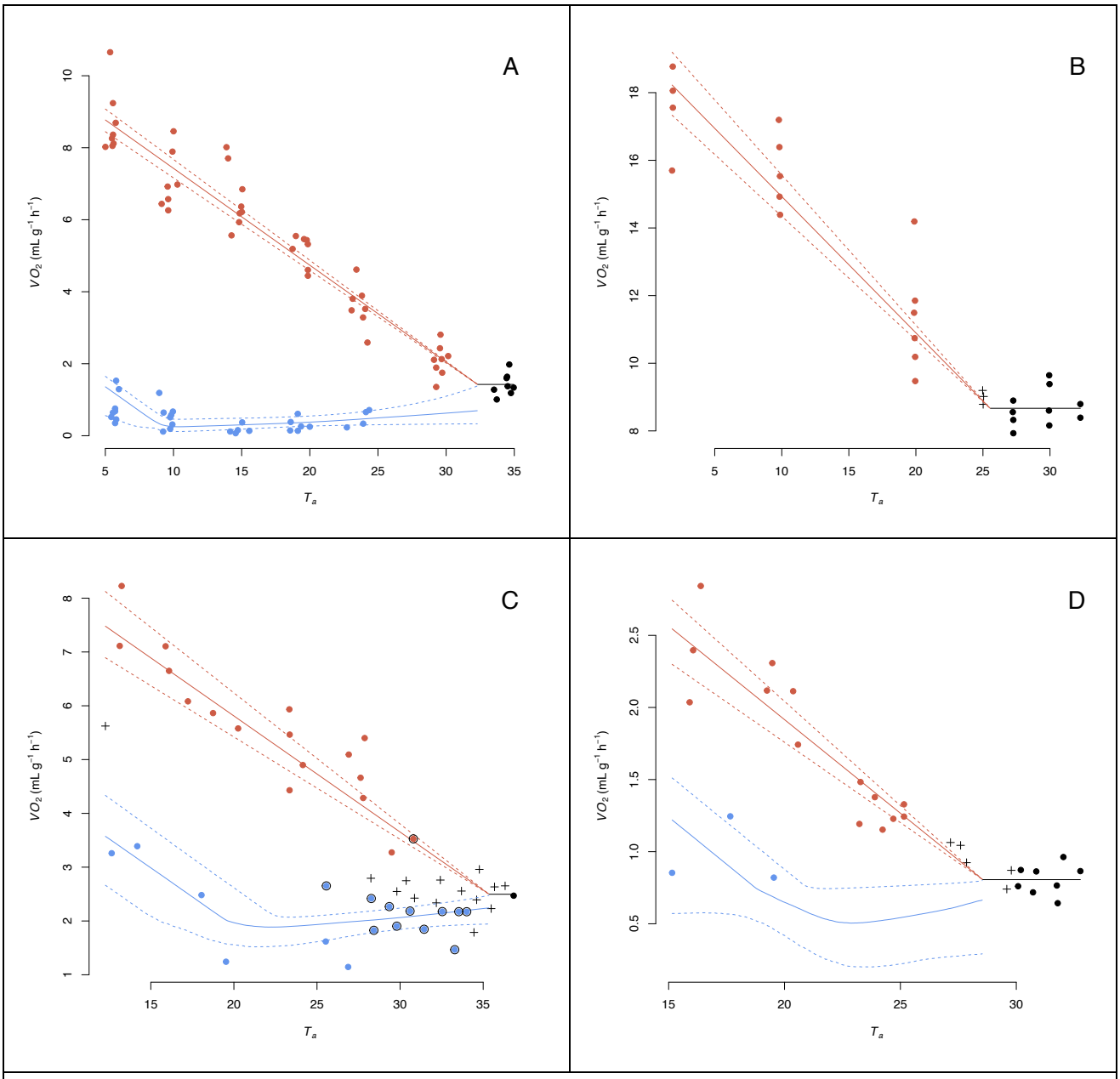

Figure 2: Four datasets of *M* measured at different ambient temperature (T_a). Values were assigned to torpor (blue), euthermy (red) and M_{tnz} (black) using the presented three-step method. Predicted values: median and 95% credible intervals are represented by continuous and segmented lines, respectively. Invalid assignments are highlighted with a cross and mismatches between authors and model assignments are surrounded with black circle. A/ Perfect corroboration between model and authors assignments in a heterotherm: *Nyctophilus geoffroyi* (Hosken and Withers, 1999). B/ Absence of mismatched assignment between model and authors assignments with some invalid assignments in a homeotherm: *Sorex minutus* (Sparti and Genoud, 1989). C/ Presence of

mismatched or invalid assignments: *Peropteryx macrotis* (Genoud et al., 1990). D/ Absence of mismatched assignment between model and authors assignments with some invalid assignments, but insufficient number of torpor values to identify some torpor function parameters: *Melonycteris melanops* (Bonaccorso & McNab, 1997).

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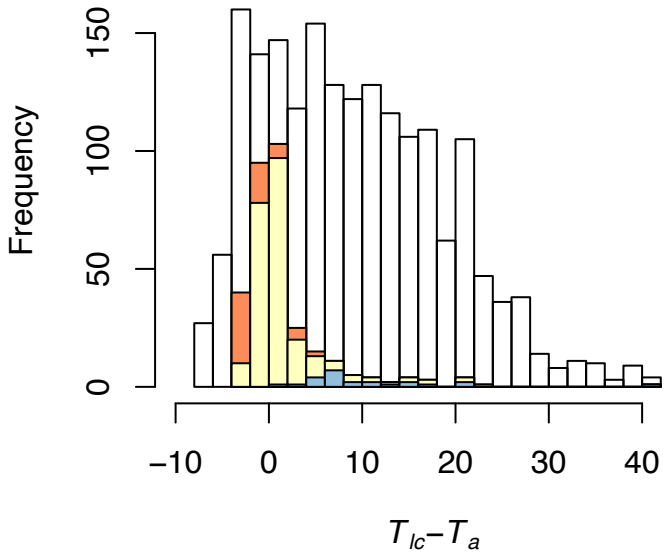

Figure 3: Frequency distribution of all the measurements from the 28 considered studies in relation to the difference between the method's estimated T_{lc} and the experimental T_a (N=1898). Invalid assignments (i.e. assignment confidence < 0.8) are represented in yellow and assignments mismatches in orange and blue. Mismatches caused by the difference between the model's estimated T_{lc} and that of the authors are highlighted in orange and the remaining mismatches in blue.

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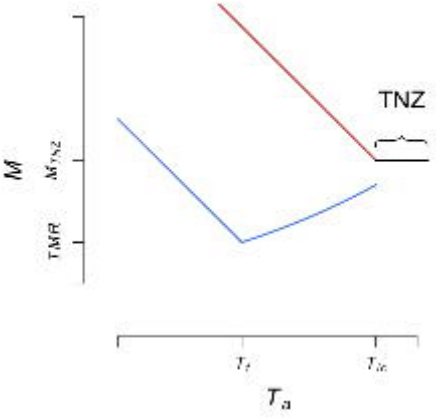
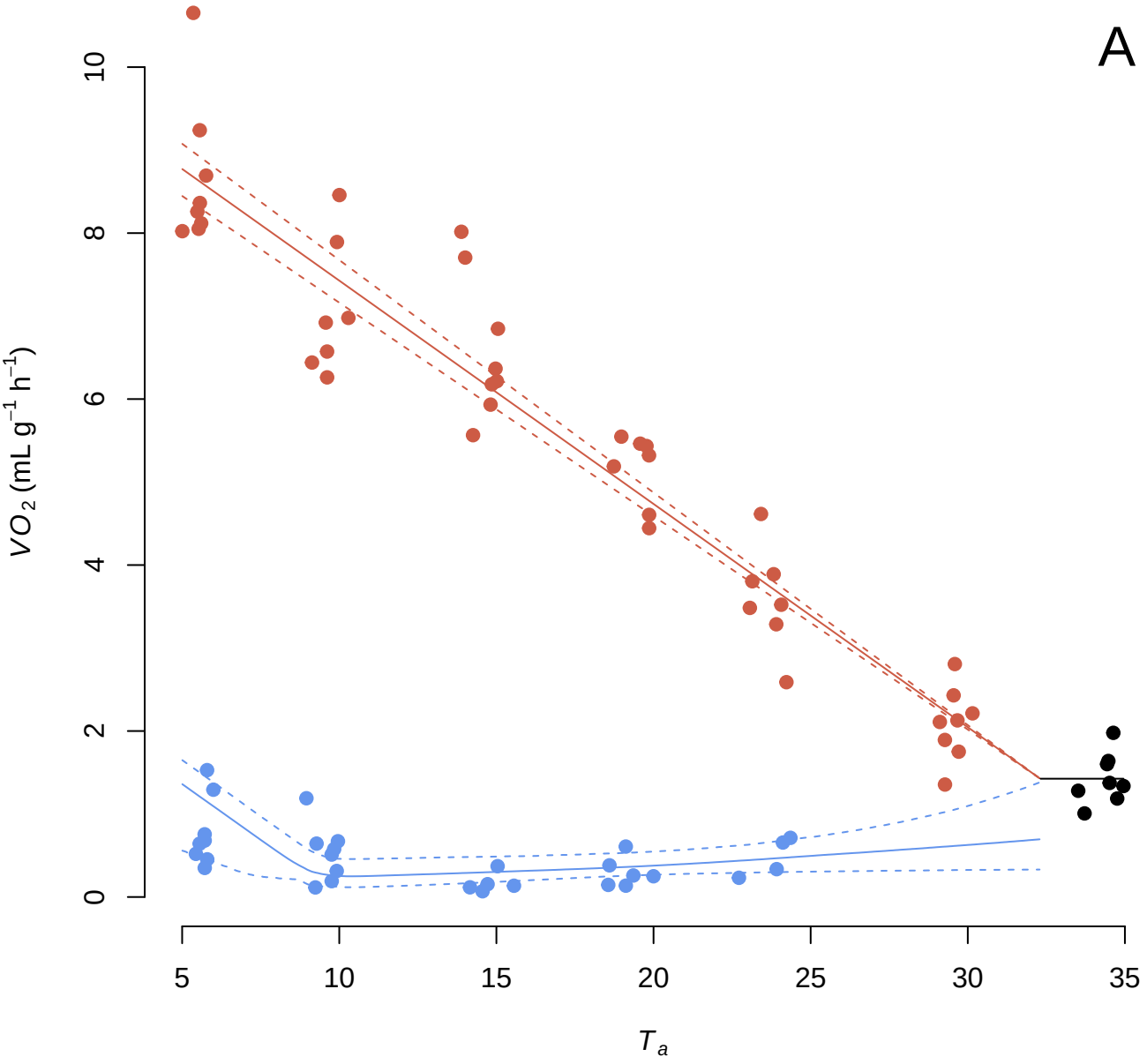
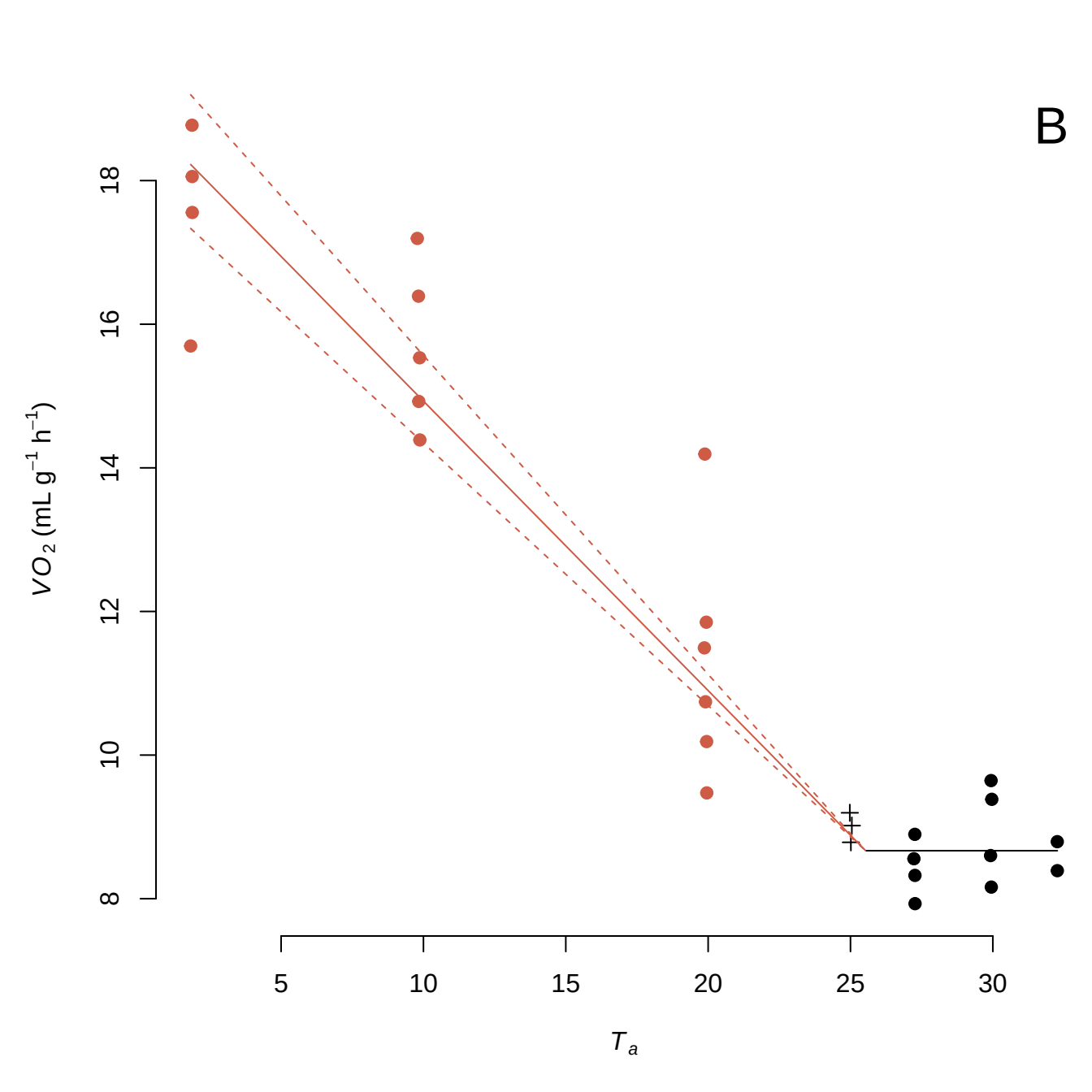
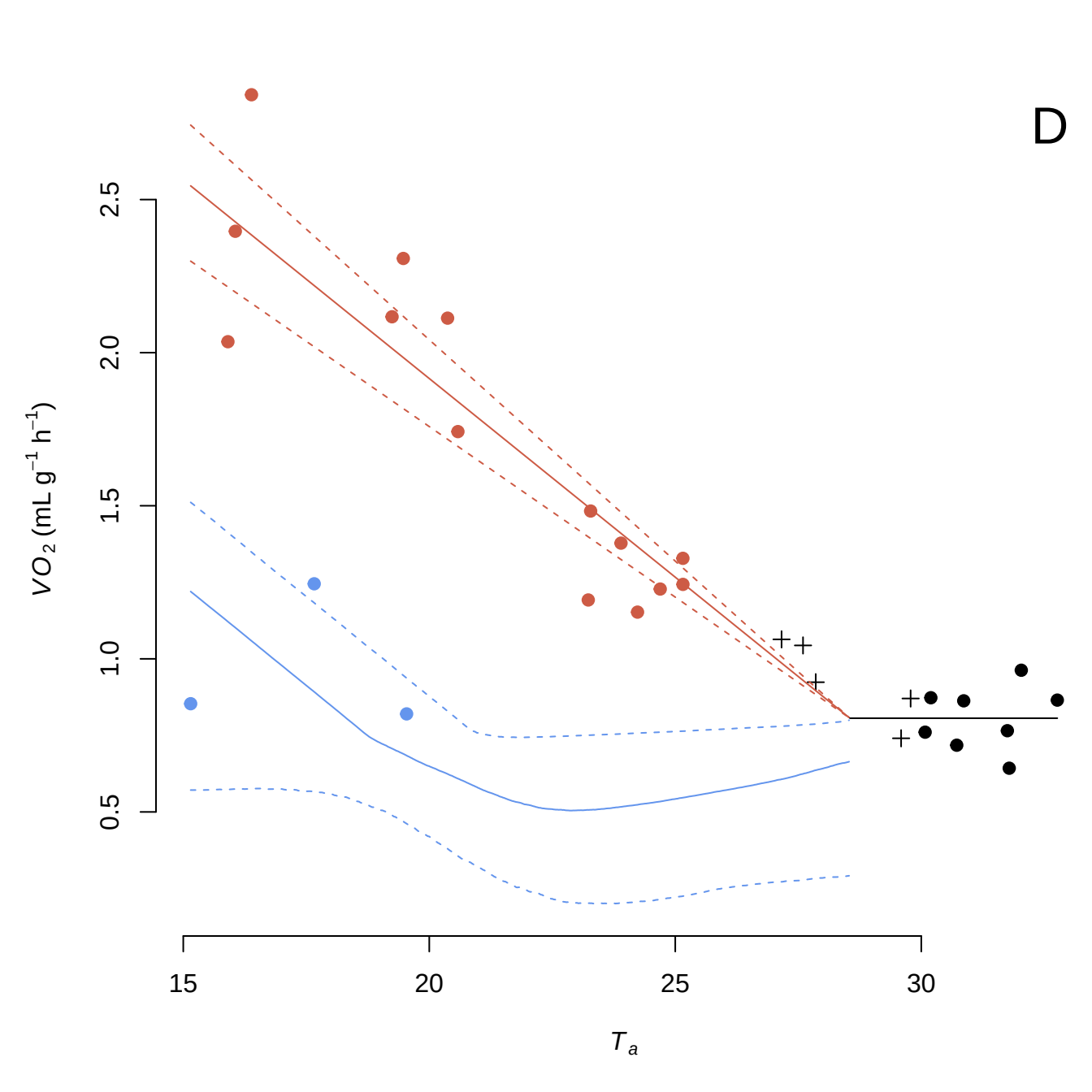
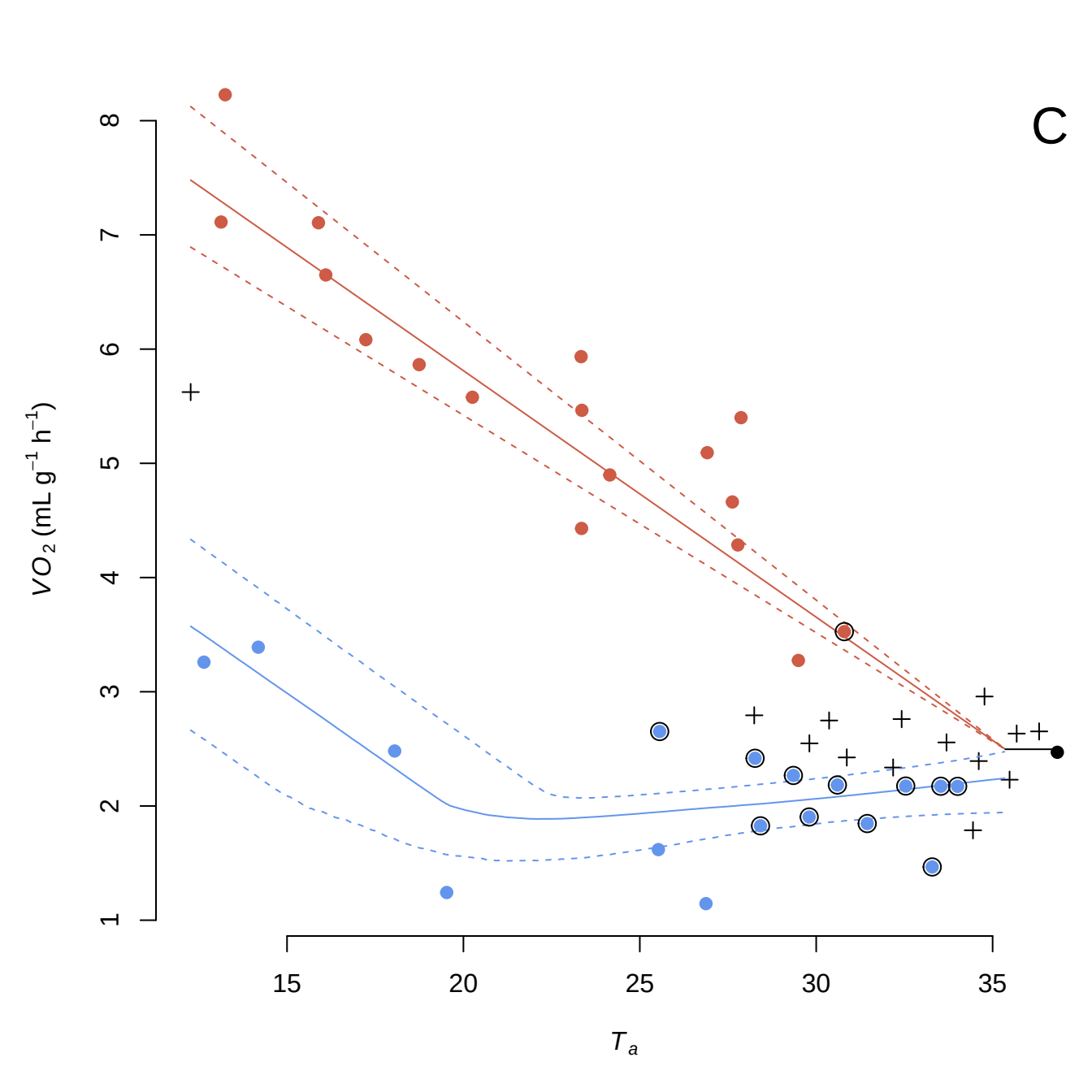
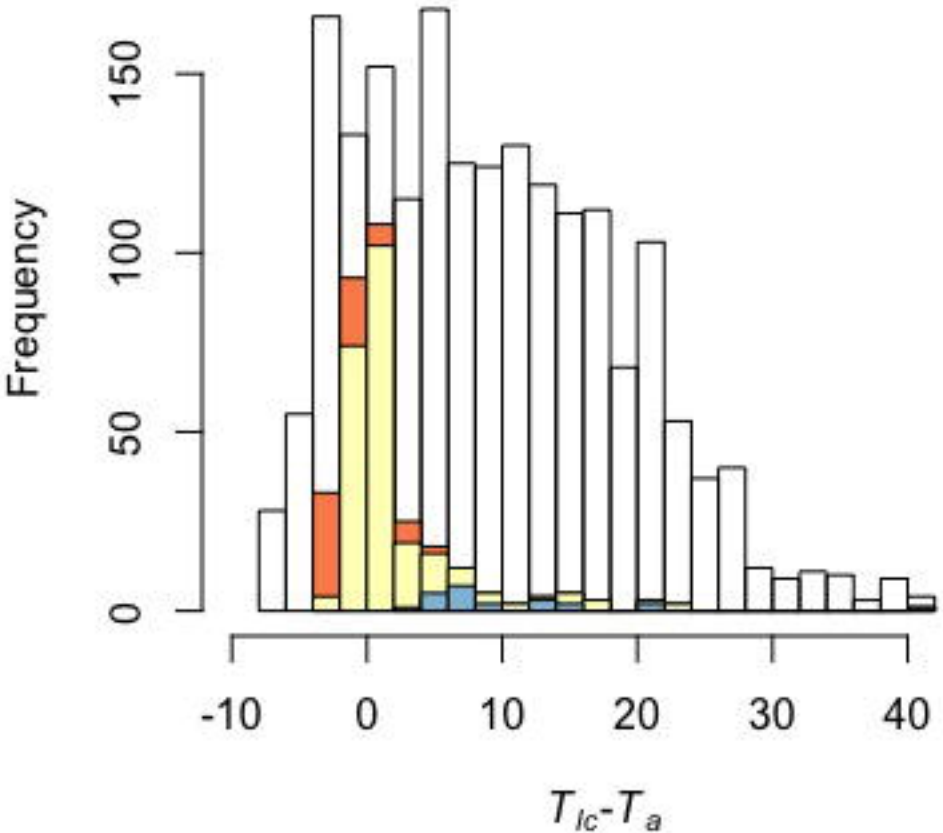

TNZ
M
TMR
T_a


A
10
8
6
4
2
0
VO_2 (mL g^{-1} h^{-1})
5
10
15
20
25
30
35
T_a


B
18
16
14
12
10
8
VO_2 (mL g^{-1} h^{-1})
5
10
15
20
25
30
T_a


D
2.5
2.0
1.5
1.0
0.5
VO_2 (mL g^{-1} h^{-1})
15
20
25
30
T_a


C
VO_2 (mL g^{-1} h^{-1})
1
2
3
4
5
6
7
8
15
20
25
30
35
T_a


Frequency
0
50
100
150
-10
0
10
20
30
40
T_{lc}-T_a