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

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Abstract

1. Torpor is a state of controlled reduction of metabolic rate (*M*) and body temperature in endotherms. Assigning measurements of *M* to torpor or euthermia can be challenging. All current techniques available to distinguish between those two states have their limitations and are at least partly arbitrary.

2. Here, we firstly propose a novel method for distinguishing torpor from euthermia. We use the variation in *M* measured during euthermic rest and torpor at different ambient temperatures (T_a) to estimate the lower critical temperature (T_{lc}) of the thermoneutral zone (TNZ) and determine physiological state membership using a mixture model. In addition, this method allows the prediction of *M* during euthermic rest and torpor along T_a, including resting *M* within the TNZ. Secondly, we test this method on previously published datasets (*N*=28), covering a diversity of metabolic and thermal strategies.

3. The method's results match the original assignment with very high accuracy (mean=0.95). Most of the conflicts concerned *M* measured around T_{lc}.

4. Our method provides an objective approach to estimate T_{lc} and assign measurements of *M* to euthermia or torpor. Our method further enables the prediction of *M* during euthermic rest and torpor at any given T_a below the upper limit of the TNZ. The present method is applicable to species conforming to the "Scholander-Irving model" and its later extensions to include torpor. This model has proven to be an appropriate description of the variation of *M* along T_a in most endotherms studied to date.

5. Our method aims ultimately at improving the standardization of respirometry analyses in heterotherms. Finally, the development of the associated R-package (torpor), should spread and ease the use of this method.

Introduction

Torpor is a state of controlled reduction of metabolic rate and body temperature observed in numerous mammals and birds (Ruf & Geiser, 2015). This physiological state tends to be either shallow and short (< 24h, daily torpor) or deep and long-lasting (> 24h, hibernation) (Schleucher & Withers, 2001; McKechnie & Lovegrove, 2002; Ruf & Geiser, 2015). However, a continuity between these types of torpor as well as between torpor and euthermic rest has been suggested (McKechnie & Lovegrove, 2002; Boyles et al., 2013; van Breukelen & Martin, 2015). Torpor has profound implications on energy use and allocation (Lyman, Willis, Malan, & Wang, 1982) and potentially affects many biological functions (Geiser & Brigham, 2012; Nowack, Stawski, & Geiser, 2017). Accordingly, a vast literature describes the occurrence of torpor and its associated energy savings (Geiser & Ruf, 1995; Lovegrove, 2012; Ruf & Geiser, 2015; Nowack, Levesque, Reher, & Dausmann, 2020).

Assigning measurements of metabolic rate (M) or body temperature (T_b) to torpor or euthermic rest can be challenging. The distinction between the two states is usually straightforward in typical hibernators, which undergo deep torpor bouts at low ambient temperature (T_a). But even in such species, that distinction can become problematic at ambient temperatures slightly below the thermoneutral zone (TNZ), i.e. the range of T_a where temperature regulation can be achieved without regulatory changes in metabolic heat production or evaporative heat loss (IUPS Thermal Commission, 2003). Indeed, at T_a slightly below the TNZ, the differences in state-specific average M and T_b are small compared to the state-specific variability in each of these variables. For the same reason additional difficulties can be encountered in species whose minimum T_b in torpor lies only slightly below the

euthermic T_b (McNab, 1980a; Genoud, Bonaccorso, & Anends, 1990; Bonaccorso & McNab, 1997; Bartels, Law, & Geiser, 1998) and in species exhibiting a large variability in metabolic rate and body temperature at rest (Geiser, Coburn, Kourtner, & Law, 1996; Coburn & Geiser, 1998).

Barclay, Lausen, & Hollis (2001) reviewed the variety of criteria that have been chosen in the past to distinguish torpor from euthermia. Apart from a minority of reports that identified torpor on the basis of behavioural features (Geiser & Kenagy, 1988; Geiser & Masters, 1994; Brice, Grigg, Beard, & Donovan, 2002), the vast majority of studies used patterns of variation in body (or skin) temperature and/or rate of metabolism to separate the two states in the field or in the laboratory. Animals were usually deemed to be in torpor below a threshold T_b or M (Geiser et al., 1996; Coburn & Geiser, 1998; Hosken & Withers, 1999; Mzilikazi & Lovegrove, 2002; Kelm & von Helversen, 2007; Levesque, 2008), below some threshold temperature differential between the body and air (Levesque & Lovegrove, 2014), or below a threshold percentage of the euthermic rate of metabolism (Hudson & Scott, 1979; Geiser, 1988a). The 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). The diagnostic threshold can also be predicted on the basis of body mass and T_a (Willis, 2007). Despite the efforts made to render them more objective, all available techniques have their limitations and are at least partly arbitrary (Barclay et al., 2001; Boyles, Smit, & McKechnie, 2011).

With this paper, we firstly propose an automatized method for distinguishing torpor from euthermic rest in species undergoing daily or seasonal torpor, hereafter referred to as

heterotherms following the terminology of Geiser & Ruf (1995) or Lovegrove (2012). Assignment to an either euthermic or torpid state is based on the variation observed among measurements of *M*. We assume that the relationship between *M* of resting animals and T_a 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 (Hainsworth & Wolf, 1970; Humphries, Thomas, & Speakman, 2002; Speakman & Thomas, 2003; Geiser, 2011, Fig. 1). That model predicts the vast majority of patterns observed among endotherms entering daily torpor or hibernation (but see discussion). We explicitly consider that metabolic inhibition may occur during torpor (Geiser, 1988b, 2004; Geiser & Kenagy, 1988; Withers, Cooper, Maloney, Bozinovic, & Cruz-Neto, 2016), by allowing the curve of torpor *M* to reach the lower limit of the thermoneutral zone (or lower critical temperature, T_{lc}) at a level equal to – or lower than the resting metabolic rate within the TNZ (M_{TNZ}). Our method is specifically intended to facilitate the discrimination between torpor and euthermia in laboratory experiments using respirometry, although it can also be useful to model T_{lc}, M_{TNZ}, the minimum rate of metabolism in torpor (*TMR*), as well as T_t, the threshold T_a below which a minimum T_b in torpor is maintained by varying *M*. Secondly, we assess the performance of our method, by applying it to previously published data on the metabolic rate of various mammals. Finally, we provide a new package running under R (R Development Core Team, 2012) allowing researchers to apply the present method to their own data. This package ("torpor") comprises several useful functions that will improve standardization of the analyses of metabolic measures for thermal biology.

Material and Methods

The three-steps Method

Our present method is based on three steps and requires measurements of the metabolic rate (M) of resting or torpid animals at ambient temperatures (T_a) ranging from below – to within the TNZ. Animals are assumed to be in one of three "states": euthermic rest below the TNZ, torpor below the TNZ or rest within the 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 consider that resting M measured within the TNZ is independent of T_a. This rate is hereafter referred to as M_{TNZ}, although it corresponds to the basal metabolic rate (*BMR*) provided that the specific criteria for the *BMR* are met (see discussion). Below T_{lc}, the M of euthermic animals (M_e) increases linearly with decreasing T_a (Fig. 1):

$$M_e \sim \alpha_e + \beta_e * T_a \mid T_a < T_{lc}$$

The model assumes that M_e equals M_{TNZ} at T_{lc} and therefore:

$$\alpha_e = M_{TNZ} - \beta_e * T_{lc}$$

Where:

$$\beta_e = \frac{M_{TNZ}}{T_{lc} - T_{be}}$$

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

Below some threshold ambient temperature (T_t), the metabolic rate in torpor (M_t) increases linearly with decreasing T_a to maintain a minimal body temperature (Fig. 1). This state is usually referred to as "regulated torpor":

$$M_t \sim \alpha_r + \beta_r * T_a \mid T_a < T_t$$

Where:

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

and

$$\alpha_r = -T_{bt} * \beta_r$$

TMR represents the minimal M_t 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 provided that thermal conductance and body temperature in regulated torpor do not vary with T_a below T_t. Between T_t and T_{lc}, M_t follows an exponential curve (Fig. 1). In this T_a range, torpor is referred to as "conforming torpor".

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

Where:

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

and

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

M_r represents M_t at T_{lc}. In accordance with the general trend observed (Geiser, 2004), we assume that

$$\beta_r = \beta_e$$

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 T_{lc_low} as the highest possible T_a within the data set that still underestimates T_{lc}. 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 & 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

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

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

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

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

In the third step, only M values situated between the predicted M_e and M_t are assigned. Measurements' states that are automatically assigned during the second step are provided 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. T_{be} prior distribution 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 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). Those conditions are verified by fixing the upper range of the prior distribution of T_{bt} as:

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

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 & 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 data set 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 & 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) can enable the evaluation of the modelled some parameters' relevance. 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, Trautmann, Steuer, & Bornkamp, 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 step: Estimation of M_{TNZ} and T_{lc}), as the value at T_a of the T_{lc} cumulative posterior distribution function, or 1 minus that function respectively. A threshold proportion is then selected to consider as invalid any assignment with a significantly lower assignment confidence. That hypothesis is tested with a binomial test, function: “binom.test”, with the alternative hypothesis set as “greater”. The significance level for the binomial test is set at 0.05. For the present study, assignments with a confidence higher than 0.8 are considered as valid.

Data collection and method evaluation

We evaluate our method by applying it on 28 published sets of metabolic data obtained by respirometry on 26 species of small or medium-sized mammals at rest or in torpor (Table 1). This assemblage of species covers a diversity of metabolic and thermal strategies along the continuum from permanent homeothermy to heterothermy including shallow, daily torpor and deep, long-term hibernation. For each published set, we consider all metabolic data provided, except those made above the upper critical temperature defined by the authors (see “the three-steps Method” above). In 20 species (here considered as "heterotherms", Table 1), rates of metabolism measured at T_a's below the described thermoneutral zone were originally assigned to either of two states, which were usually referred to as euthermia (or normothermia) and torpor. In *Cynopterus brachyotis*, values corresponding to particularly low T_b were distinguished but not explicitly referred to as torpor (McNab, 1989). In *Lasiurus seminolus*, some of the values measured during torpor were originally distinguished as the bats displayed "irregular fluctuations" in rate of metabolism (Genoud, 1993). For simplicity, we hereafter treat these values as if the authors assigned them to torpor. The remaining six

species are hereafter referred to as "homeotherms" (Table 1), as none of the values in the corresponding data sets were assigned to torpor by the original authors. Chosen studies reported M_{TNZ} (mostly referred to as *BMR*) and T_{lc} estimates, although the latter had to be extracted from a graph in two cases and calculated as the intercept between the euthermic line below thermal neutrality and M_{TNZ} provided by the authors in another case (Table 1). They all provided a sufficiently precise graph of metabolic rate values as a function of T_a and several of them also estimated T_t. Plotted data were digitized using the software Plot Digitizer (Huwaldt & Steinhorst, 2015). Sample sizes of the digitized data range between 27 and 204 (Table 1). We test the correlation between the M_{TNZ}, T_{lc} and T_t values modelled with the present method and those provided by the authors with Pearson's paired-samples correlation test, function "cor.test". Cases in which the estimation of T_t falls outside the range of measured T_a are excluded from this analysis as T_t is considered unidentifiable. Then, we examine whether the assignment of the metabolic values to torpor or euthermia made by the authors and provided by the method coincides, and we calculate a corroboration index as the fraction of the matched assignments. Only measures with valid assignments are considered for the correlation tests and the corroboration index calculations. Finally, in order to define a PPO range highlighting an identifiable parameter, the PPOs of *TMR* from studies where *M* measurements are assigned to torpor are compared with those from studies where no *M* measurement is assigned to torpor and *TMR* is consequently unidentifiable.

Results

In studies where no *M* is assigned to torpor (i.e. "homeotherms"), PPOs of *TMR* range from 76.80 to 95.60%. In identified heterotherms, PPOs of TMR values range from 4.60 to 64.60% (Table 1).

Values of T_{lc}, M_{TNZ} and T_t modelled by the model and values obtained from the original studies are significantly correlated (T_{lc}: N=28, Pearson's coefficient: 0.65, $p<0.001$, M_{TNZ}: N=28, Pearson's coefficient: 1.00, $p<0.001$, T_t: N=14, Pearson's coefficient: 0.97, $p<0.001$).

Over all data sets, the proportions of valid assignments (i.e. assignment confidence > 0.8) range from 0.63 to 1.00 (median=0.87, Table 1). The corroboration indexes range between 0.66 and 1.00 (median=1.00, Table 1). Complete matches between the method assignments and the authors' descriptions are found for 15 data sets (e.g. *Nyctophilus geoffroyi* (Hosken & Withers, 1999), Fig. 2A). Mismatches mostly occur close to T_{lc} but can also be found as far as 41.0°C from T_{lc} (Fig. 3). Among homeotherms, no *M* measurement is assigned to torpor (e.g. *Sorex minutus* (Sparti & Genoud, 1989), Fig. 2B).

Discussion

The present method aims at standardizing the assignment of *M* measurements to physiological states and at estimating the parameters' functions which link *M* to T_a. The use of published data sets enable us to assess our method's performance, although it is not our goal to challenge the original analyses. Authors of the published data sets may have benefited from additional information (e.g. body temperature or behaviour) to assign *M* to the various physiological states, while our method is based on statistics exploiting only T_a and *M*.

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. Considering only the PPO of *TMR*, we can define that PPO higher than 75% clearly reveals that the parameter is not identifiable.

The parameters estimated by our method correlate significantly with those extracted from the original studies; T_{lc} values modelled with our method differ appreciably from those provided originally, while remaining within an acceptable range (Table 1). Our estimation of T_{lc} requires numerous *M* values of euthermic rest to precisely model the T_a at which M_e reaches M_{TNZ}. It is possible that the amount of M_e values is sometimes not sufficient to model T_{lc} adequately. Additionally, a statistical estimation of T_{lc} (e.g. Song, Körtner, & Geiser, 1995, 1997; Bonaccorso & McNab, 1997; Genoud, Martin, & Glaser, 1997; Hosken & Withers, 1999; Willis, Lane, Liknes, Swanson, & Brigham, 2005; Genoud, 2014) was hitherto often lacking,

leaving potentially space for some misleading subjectivity on the part of the original authors. Finally, an overestimation of T_{lc} by our method remains possible if an appreciable metabolic inhibition occurs during torpor (see below). The difference in the estimations of T_t is consistently smaller than that of the estimations of T_{lc} (Table 1). This could be linked to the smaller variation in *M* exhibited during steady conforming torpor in comparison to that measured during euthermic rest, hence to the higher precision in the estimation of T_t. Finally, it is not surprising that the M_{TNZ} obtained by our method and by the original authors are close to identical (Table 1), since both are largely based on the same *M* values.

The assignments made by our method and by the original authors agree well, as illustrated by the corroboration index ($\geq$ 0.9 for 22 out of 28 data sets, Table 1). Many of the conflicting assignments in the region of T_{lc} are a mere consequence of the difference between the estimated T_{lc} and that of authors (Table 1, Figs. 2C & 3). In particular, differences in modelled T_{lc} explain all homeotherm species mismatches, as no *M* value is assigned to torpor for these species. The remaining mismatches can generally be explained by intra-state frequency distributions of *M* that do not segregate clearly. This condition is often found close to the thermoneutral zone, but also occurred in species where T_t is relatively close to T_{lc} (e.g. McNab, 1980a, 1989). Such discrepancies illustrate the difficulty of the assignment process when torpor and euthermia have to be distinguished solely on the basis of measurements of *M*. In this study, we consider assignments with a confidence lower than 0.8 as invalid. Obviously, an increase of this threshold value would lead to fewer assignments' mismatches. 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 and T_b control (e.g. Daniels, 1984; McNab, 1988; Heldmaier, Ortmann, & Elvert, 2004).

The present method furthermore aims at providing the parameters of the functions relating M in resting and torpid animals to T_a. These functions enable the estimation of *TMR* and T_t. Predicting M at any T_a is crucial to model energy costs related to torpor expression (e.g. Boyles, Johnson, Blomberg, & Lilley, 2020). Another central aspect lies in that our method allows the M function for torpor to be lower than M_{TNZ} at T_{lc}. We thus explicitly admit that metabolic inhibition could occur in some species during torpor (Geiser, 2004; Withers et al., 2016). Caution should be kept in the interpretation of a difference between the estimated M_r and M_{TNZ}, as the latter will often overestimate metabolic inhibition in torpor. Indeed, insufficient amount of M_t values corresponding to conforming torpor leads to the unidentifiability of M_r (e.g. *Melonycteris melanops* (Bonaccorso & McNab, 1997), Fig. 2D). Consequently, metabolic inhibition should only be considered when sufficient M_t values corresponding to the conforming torpor are provided and M_r PPO indicates adequate parameter's identifiability.

Considering only identifiable parameters (i.e. *Mr* PPO < 75%), we quantify metabolic inhibition as M_r over M_{TNZ}. Values range between 0.27 and 0.93 (N=18, median=0.77, Table 1). By extrapolation, we should consider the possibility that a hypometabolic state might occur within the TNZ as well (e.g. Grimpo, Legler, Heldmaier, & Exner, 2013; Reher, Ehlers, Rabarison, & Dausmann, 2018). With our method, we have not implemented an assignment to a state of torpor within the thermoneutral zone, hence M_{TNZ} corresponds to the average of all values from the TNZ. Our method might underestimate the eumetabolic M_{TNZ} if animals occasionally enter a hypometabolic state in the TNZ, and might consequently overestimate

T_{lc}. A predicted M_t at T_{lc} much lower than M_{TNZ} should be viewed as an indication for a strong metabolic inhibition occurring during torpor and should invite for a careful consideration of the estimated M_{TNZ} and T_{lc}.

While the performance of our method appears to be excellent with the selected data sets, its main limitations correspond to those of the model on which it is based; the Scholander-Irving model (Scholander et al., 1950) and its latter extensions (Hainsworth & Wolf, 1970; Humphries et al., 2002; Speakman & Thomas, 2003; Geiser, 2011). Although extremely fruitful, this model cannot be considered as more than a descriptive simplification of the standard energetics of mammals and birds (McNab, 2002). It surely does not describe the thermal biology of all heterothermic species. Causes for significant divergence from the hereby applied model (Fig. 1) include 1) a non-linear relationship between M_e and T_a (e.g. *Cercartetus nanus*: Song, Körtner, & Geiser, 1997) that might be due to a variation of thermal conductance and/or T_b as a function of T_a below thermal neutrality (McNab, 1980b); 2) a strong difference in the slopes of the regressions of M vs T_a in regulated torpor and euthermic rest (Geiser, 2004); and 3) a strong dependence of M_t on factors other than T_a, such as body mass (Kelm & von Helversen, 2007) and duration since the last activity period or since last meal (Grigg, Augee, & Beard, 1992; Morris, Curtin, & Thompson, 1994). This model also does not describe adequately circadian (e.g. the shallow "rest-phase hypothermia" of many small birds (McKechnie & Lovegrove, 2002) or ultradian (Heldmaier, Steinlechner, Ruf, Wiesinger, & Klingenspor, 1989) variations in M. Finally, it must be recalled that our method constrains the minimal rate of metabolism in torpor (*TMR*) between 0 and 80% of M_{TNZ} (Table 1). This constraint would exclude heterotherms undergoing extremely shallow torpor with a *TMR* above 80% of M_{TNZ}. Nevertheless, it is noteworthy that all heterotherms that were reviewed by Ruf & Geiser

(2015) had a lower (and usually much lower) relative *TMR* (4.4 – 66% in birds; 1 - 67% in mammals). The above limitations should be kept in mind for a lucid application.

In the selected data sets, the metabolic values reported usually correspond to minimal and stable rates for a given physiological state. Similarly, our method is designed for an analysis of sets of stable *M* values. Transitions between activity and rest or between euthermic rest and torpor (i.e. entrance into and arousal from torpor) as well as incomplete torpor bouts during which *M* never reaches a stable, minimal level (e.g. "test drops" (Hudson & Scott, 1979); see also (Lyman, 1982; Genoud, 1993)) should be excluded. It should also be recalled that the duration of the runs can affect estimations of *M* (Cooper & Withers, 2009), and that long runs may be necessary for some heterothermic animals to achieve a state of steady torpor (e.g. Heldmaier, Ortmann, & Elvert, 2004). Our method is able to reveal the presence or the absence of distinct groups of values (euthermic rest vs torpor) within a given data set. It is clearly not intended to judge whether any of the data points entered correspond to stable rates and/or to minimal values. Furthermore, throughout this paper, resting metabolic rate within the TNZ is referred to as M_{TNZ}, rather than to the basal rate of metabolism (*BMR*). Estimating the *BMR* requires several specific criteria: animals should be post-absorptive, adult, non-reproductive, and resting during a major inactive phase of the daily cycle (McNab, 1997). If these criteria are not met, the method still remains applicable, but the resting *M* estimated within the TNZ (i.e. M_{TNZ}) will correspond to a minimal *RMR* (resting metabolic rate) rather than to the *BMR*.

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 euthermia

in a complex manner. Indeed, *BMR* (thus also M_{TNZ}) and thermal conductance depend on body mass in an allometric manner at an intraspecific level (Nespolo, Arim, & Bozinovic, 2003; Glazier, 2005; Genoud, 2014; Sassi & Novillo, 2015), as they do at an interspecific level (e.g. reviewed by Schleucher & Withers, 2001; Genoud, Isler, & Martin, 2018). Moreover, T_t and *TMR* might also be affected by body mass within species, as they too are known to scale to body mass at an interspecific level (Ruf & Geiser, 2015). However, there would be no particular difficulty to apply our method to single individuals, other than having enough measurements for each of them. The method could also be applied repeatedly on single individuals, in order to account for intra-individual variation.

Despite its limitations, 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 euthermia. Rather than being assigned on the basis of a particular threshold value, the method calculates for each data point the probability of belonging to one state or another. Data points that might not be assignable are clearly identified. Thus, the partly arbitrary nature of the assignment process is removed. Furthermore, the method also estimates the parameters' functions which link *M* to T_a and several parameters describing the standard energetics of the animal(s) under study, including M_{TNZ}, *TMR*, T_{lc} and T_t. It can be applied to study intraspecific as well as interspecific variation. Parameters of standard energetics 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.

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Table 1: Model parameters and corroboration index for 28 data sets 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
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
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
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
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
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
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
Setifer setosus	Heterotherm	530	0.231	$mlO_2\ g^{-1}\ h^{-1}$	28	–	McNab 1980a	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
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
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
Tolypeutes matacus	Homeotherm	1160	0.181	$mlO_2\ g^{-1}\ h^{-1}$	28	–	McNab 1980a	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
Zaedyus pichiy	Heterotherm	1740	0.226	$mlO_2\ g^{-1}\ h^{-1}$	28	–	McNab 1980a	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.

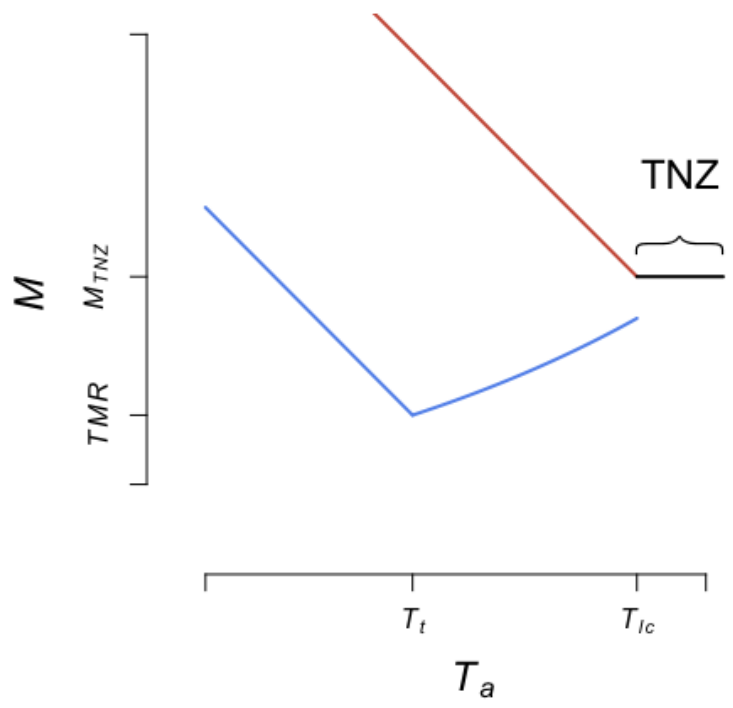

Figure 1: Relation between *M* during rest and torpor and T_a on which the present method is based. Torpor is indicated in blue, euthermic rest below the TNZ in red and rest within the TNZ in black. *TMR*: minimal metabolic rate in torpor, M_{TNZ}: resting metabolic rate within the TNZ, T_t: threshold T_a below which a minimum T_b in torpor is regulated by varying heat production (regulated torpor), T_{lc}: lower critical temperature of TNZ.

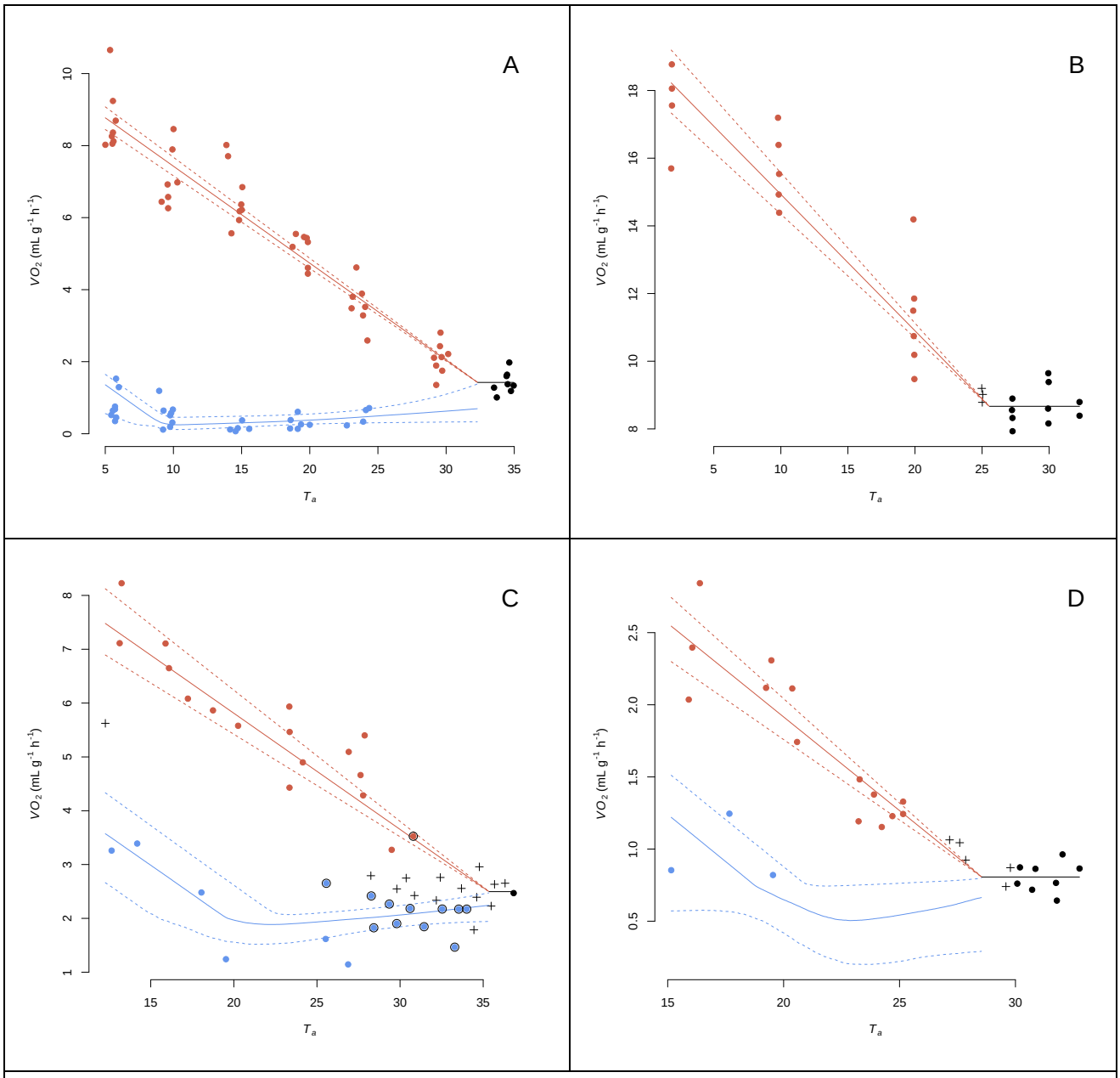

Figure 2: Four data sets of *M* measured at different ambient temperature (T_a). Values were assigned to torpor (blue), euthermia (red) and M_{tnz} (black) using the presented three-step method. Predicted values: median and 95% credible intervals are represented by continuous and segmented lines, respectively. 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 & Withers, 1999). B/ Absence of mismatched assignment between model and authors assignments with some invalid assignments in a homeotherm: *Sorex minutus* (Sparti & 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).

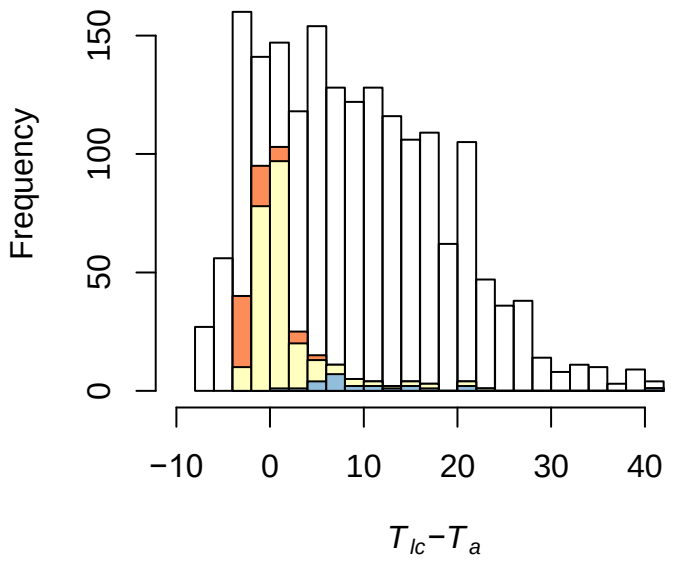

Figure 3: Frequency distribution of all the measurements from the 28 considered studies in relation to the difference between the method's estimated T_{lc} and the experimental T_a (N=1898). Invalid assignments (i.e. assignment confidence < 0.8) are represented in yellow and assignments mismatches in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

670