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 new R package (torpor) enabling distinction of torpid versus euthermic *M* measured in stable environmental conditions. Functions are based on the variation in *M* measured along varying ambient temperatures (T_a). This model determines physiological state membership using a binary mixture model, and further allows prediction of *M* along T_a. Secondly, we challenge this method by applying it to previously published data (*N*=28) on the *M* of various mammals covering a diversity of metabolic and thermal strategies.

3. The fractions of values for which the original assignment matched that of the model ranged between 0.78 and 1 (median=0.99). Most of the conflicts concerned *M* measured at temperatures close to the thermoneutral zone. Parameters describing torpid *M* could not always be identified accurately with some smaller data sets.

4. The package “torpor” provides an objective method to assign measurements of *M* to euthermia and/or torpor, and to predict *M* values at any given T_a below the thermoneutral zone. Necessary considerations for a lucid application of this method are further discussed. Ultimately, the use of this package should improve the standardization of respirometry analyses in heterotherms.

Table 1: Abbreviations used in the present paper and descriptions of the functions of the package “torpor”.	
M	metabolic rate
BMR	basal metabolic rate
TMR	minimal metabolic rate in torpor
T_a	ambient temperature
T_b	body temperature
T_{lc}	lower critical temperature (i.e., lower limit of the thermoneutral zone)
T_m	extrapolated modelled temperature where the conforming torpor function reaches *BMR*
T_t	threshold ambient temperature below which thermoregulation occurs in torpor
tor_fit ()	fits a binomial mixture model using Bayesian inference.
tor_plot ()	provides a plot of the *M* values over the respective T_a. Raw data and predictions are presented in different colors depending on the metabolic state. Predicted values are represented by continuous and stripped lines for the estimates’ median and 95% credible interval bounds of the posterior distribution, respectively. The function is a wrapper function around the tor_fit () and tor_predict (). For more flexibility, output of tor_fit () can be directly included as argument.
tor_predict ()	provides the predicted *M* and 95% credible interval bounds at a given T_a, for the normothermic and torpid states.
tor_summary()	provides a comprehensive summary of the output returned by tor_fit (). Reported are the mean, 95% credible interval bounds, median and Brooks–Gelman–Rubin criterion (i.e., chain convergence

	check) of parameters posterior distributions. Additionally, prior posterior overlap values for different parameters are generated. Finally, the mean *M* value used to standardize the *M* variable is reported.
tor_classify ()	returns the raw data with the related predicted state values (between 1 and 2), which leads to the state classification (torpor or euthermy). Additionally, it also provides the predicted *M* at the given T_a.

Introduction

Torpor is a state of controlled reduction of *M* and T_b (Ruf & Geiser, 2015) that underlies most of the heterothermia observed in endotherms (Geiser & Ruf, 1995; Lovegrove, 2012). This physiological state 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). Hence, reports on its occurrence, frequency and depth are highly welcome.

Assigning steady-state measurements of *M* or T_b to torpor or euthermia can be challenging, especially in species that are thermolabile (e.g., Geiser, Coburn, Körtner, & Law, 1996) or that defend an only slightly lowered minimal T_b during torpor (e.g., Mzilikazi & Lovegrove, 2002). Barclay, Lausen, and 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 (e.g., Brice, Grigg, Beard, & Donovan, 2002), the vast majority of studies have 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), or 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, 1988). 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). It can also be predicted by an equation on the basis of body mass and T_a (Willis, 2007). All available techniques have their limitations and are at least partly arbitrary (Barclay et al., 2001), despite the efforts made to render them more objective.

Here, we present a new package “torpor” running under R (R Development Core Team, 2012) allowing an objective assignment of *M* values measured in stable environmental conditions to torpor or euthermia. Furthermore, “torpor” can also be useful to estimate T_t and any *M* values along a range of *Ta,* including *TMR*. The package comprises several useful functions listed in table 1, that will improve standardization of the analyses of steady-state metabolic measures.

This novel approach is based on the variation observed among measurements of *M* made in steady-state conditions. We assume that *M* follows the "standard" relationship with T_a modelled by Humphries, Thomas, and Speakman (2002) and Speakman and Thomas (2003) for bats. We additionally consider the possibility for a metabolic inhibition during torpor to occur (Geiser, 2004; Withers, Cooper, Maloney, Bozinovic, & Cruz-Neto, 2016). This model describes fairly well the vast majority of patterns observed among endotherms entering daily torpor or hibernation. We further explore the advantages and limitations of this new method by applying it to previously published data on the *M* of various mammals.

Material and Methods

The Model

The package “torpor” is based on a binary mixture model using Bayesian inference. It determines the physiological state membership (euthermia vs torpor) of each *M* measurement. The package is constructed around the standard model for bats (Humphries, Thomas, & Speakman, 2002; Speakman & Thomas, 2003) with the consideration of metabolic inhibition during torpor (Geiser, 2004; Withers, Cooper, Maloney, Bozinovic, & Cruz-Neto, 2016; Fig. 1; more details in the package documentation). In addition to measured values of *M* along T_a, the method only requires two parameters from researchers (i.e., *BMR* and T_{lc}).

Data collection and model testing

We tested the model on 28 published sets of metabolic data obtained by respirometry on 26 mammal species (supplementary table). In each set, *M* measured at T_a's below the thermoneutral zone were assigned by the authors to either of two states, hereafter referred to as euthermia and torpor (details in supplementary material). Chosen studies reported *BMR* and T_{lc} estimates, although the latter had to be extracted from graphs in two cases. We calculated a corroboration index as the fraction of values for which the authors’ assignment matched the model's one.

Results

Corroboration index ranged between 0.78 and 1 (median = 0.99, N = 28, supplementary material). It was equal to 1 in 12 data sets (e.g., Fig. 2A and 2D). Corroboration indexes lower than 0.95 were restricted to heterotherms for which a limited number of values (N < 50) were available (e.g., Fig. 2B). Most of the conflicts (27 out of 41 cases) concerned M measured less than 6°C below T_{lc}. In 12 data sets for which T_t was estimated both by the original authors and by the model, the difference between the two estimates amounted 1.47 ± 0.83°C (range 0.05°C – 2.9°C, supplementary material).

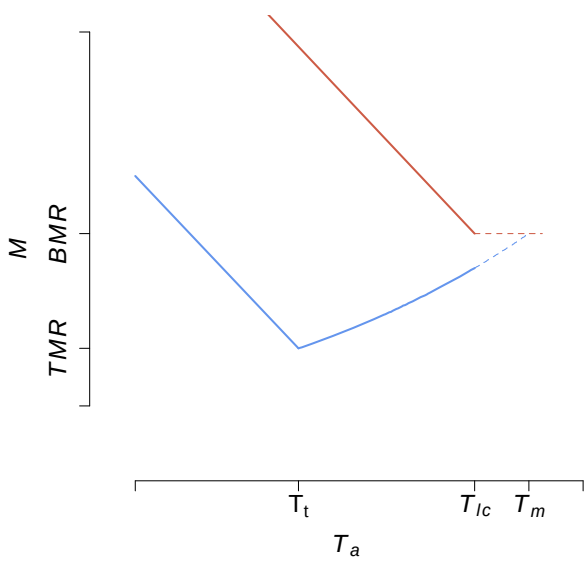

Figure 1: Representation of the relation between M and T_a following the standard model for bats (Humphries, Thomas, & Speakman, 2002; Speakman & Thomas, 2003) with the consideration of metabolic inhibition during torpor (Geiser, 2004; Withers, Cooper, Maloney, Bozinovic, & Cruz-Neto, 2016). Torpor is indicated in blue and euthermia in red. Lines are continuous within the temperature range considered by the model.

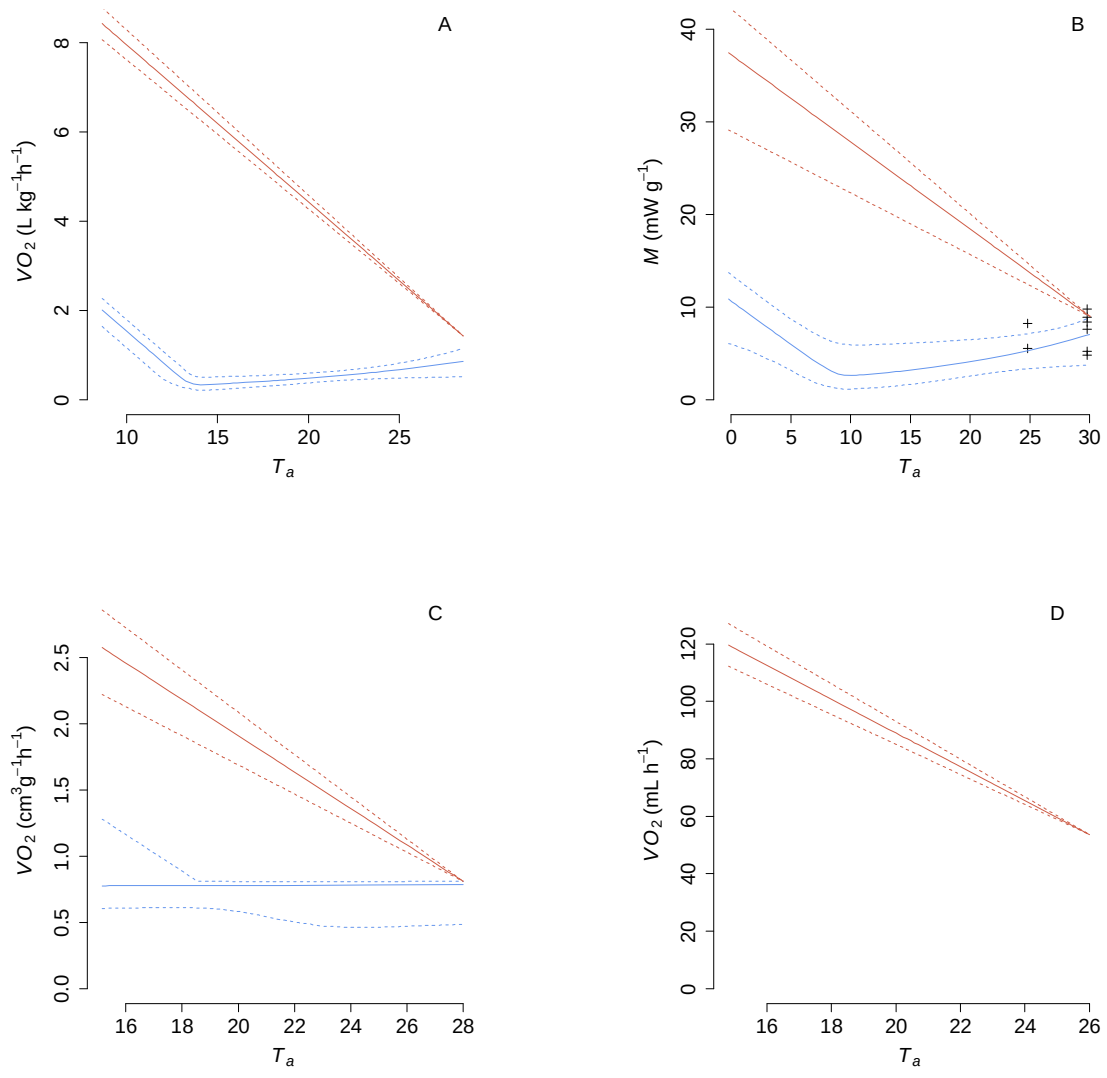

Figure 2: Four sets of M measured at different T_a below thermal neutrality. Values were assigned to torpor (blue) or euthermia (red) by the binary mixture model. Predicted values: median and 95% credible intervals are represented by continuous and dotted curves, respectively. Mismatches between authors and model assignments are highlighted with a cross. A/ Perfect corroboration between model and authors assignments: *Planigale gilesi* (Geiser & Baudinette, 1988). B/ Some mismatches in the assignments: *Lasiurus cinereus* (Cryan & Wolf, 2003). C/ Perfect corroboration between model and authors assignments, but insufficient number of torpor values to identify torpor function parameters: *Melonycteris melanops* (Bonaccorso & McNab, 1997). D/ Perfect corroboration between model and authors assignments in a strict homeotherm: *Rattus niobe* (Genoud, 2014).

Discussion

The new package “torpor” provides an objective method to assign measurements of *M* to euthermia and/or torpor. Additionally, it enables prediction of *M* at any T_a (including *TMR*), as well as of T_t. The estimation of T_t further enables the objective assignment of *M* values to regulated versus conforming torpor.

The assignments made by the model and by the original authors agreed fairly well as illustrated by a corroboration index that equalled or approached one. Most conflicting assignments were observed close to the thermoneutral zone (e.g., Fig. 2B). At these temperatures, the two states are associated with only slightly different *M*. Small sample size and consequently, a poor description of the *M* in either torpor or euthermia is surely one cause for these conflicts. Some of them also occurred in species exhibiting a large intra-state variability in *M* or a minimum T_b in torpor that is relatively close to the euthermic T_b (Geiser, Coburn, Körtner, & Law, 1996, Mzilikazi & Lovegrove, 2002).

For a lucid application of torpor, some considerations should be kept in mind. Firstly, the standard model on which our method is based (Humphries et al., 2002, Speakman & Thomas 2003) may not describe the thermal biology of all heterothermic species. Torpid *M* may sometimes depend more strongly on body mass than on T_a (Kelm & von Helversen, 2007). In addition, T_b and/or thermal conductance may sometimes vary with T_a below thermal neutrality (McNab, 1980) in such a way that the relationship between euthermic *M* and T_a is no longer linear. Secondly, the exclusive use of stable *M* meeting steady-state conditions during respirometry runs is an important requirement of the proposed method. In particular, transitions between euthermia 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 (Lyman, 1982) should be excluded. Thirdly, the proposed method requires that values of *BMR* and T_{lc} for the species under investigation are known. Because *M* in euthermia is assumed to converge at a point of coordinates [T_{lc}, *BMR*], these two parameters affect the assignment of the data points below the thermoneutral zone. Ultimately and with consideration of the above-mentioned points, the new R-package "torpor" should ease and standardize the analysis of respirometry data in heterotherms.

Authors contribution

NJF designed methodology; MG collected data; CV wrapped the package; All authors gave final approval for publication.

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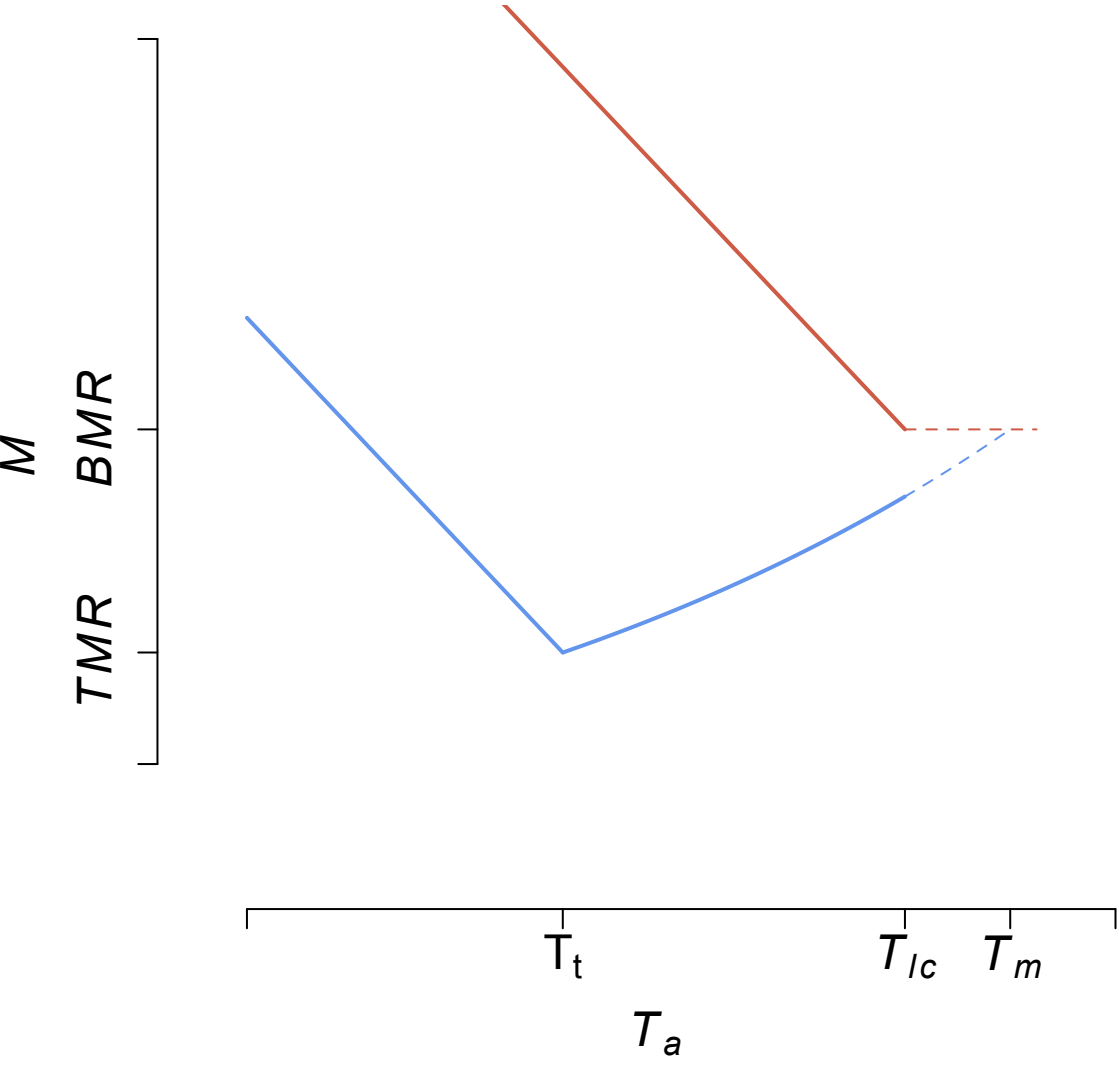
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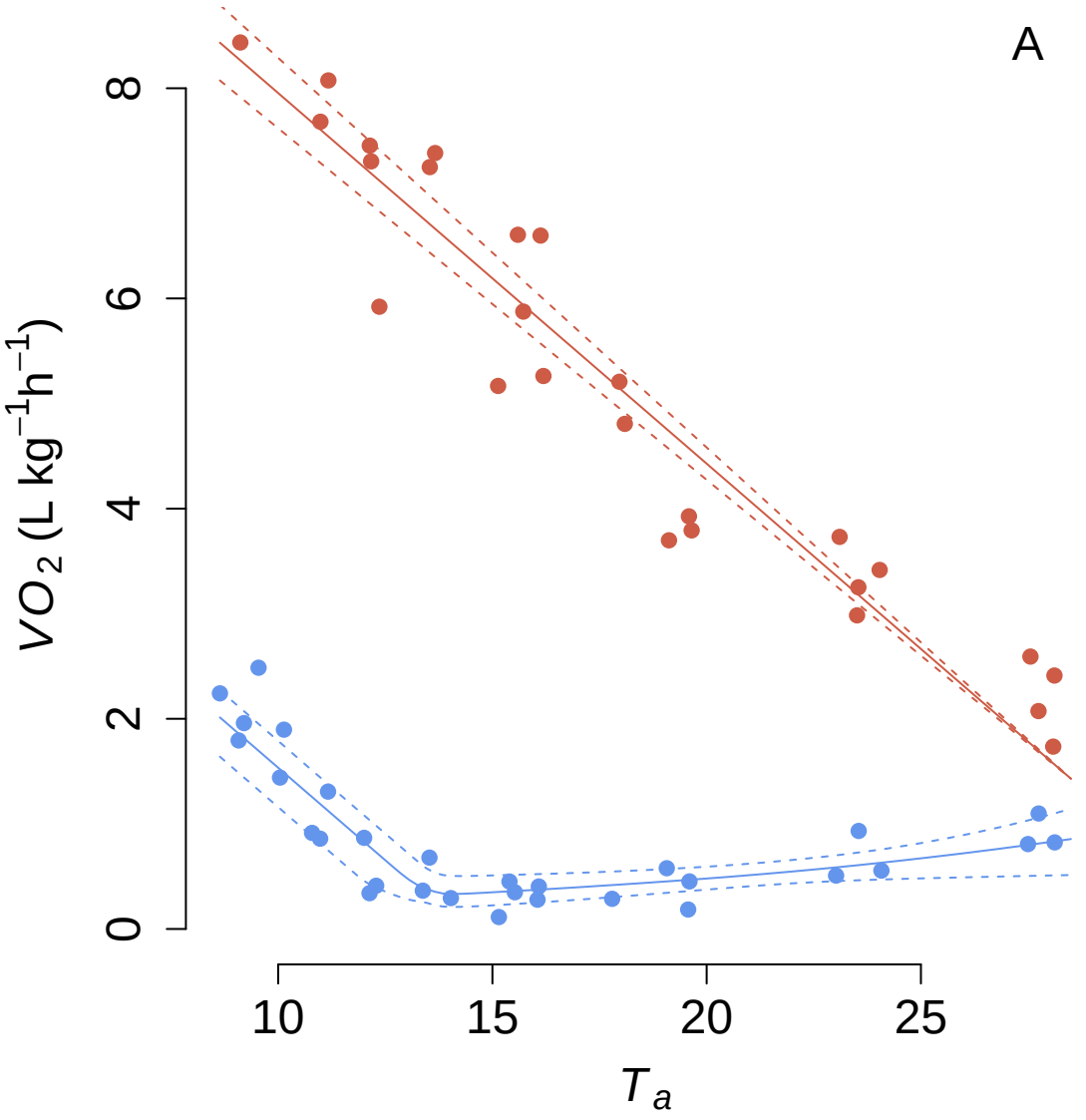
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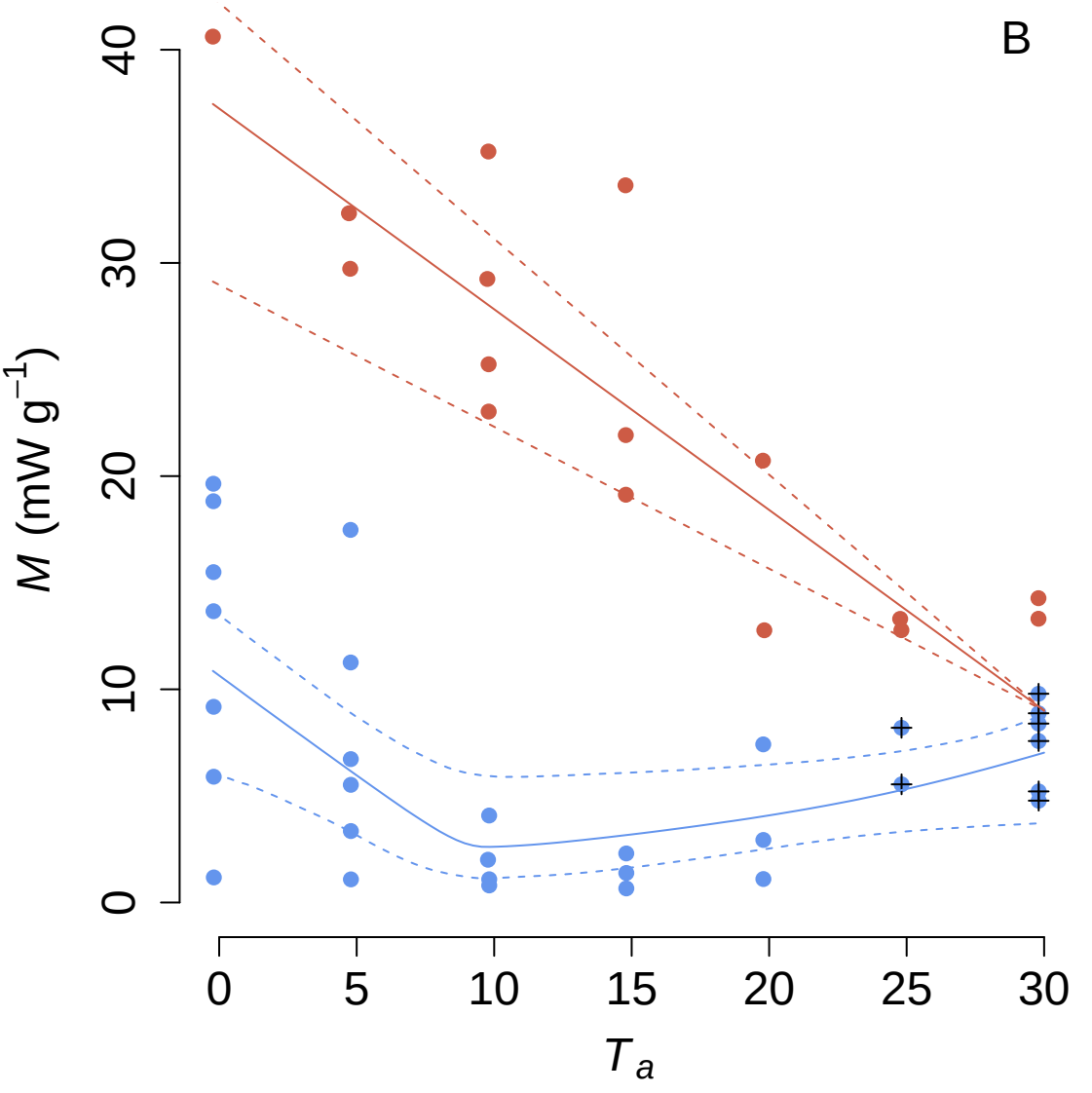
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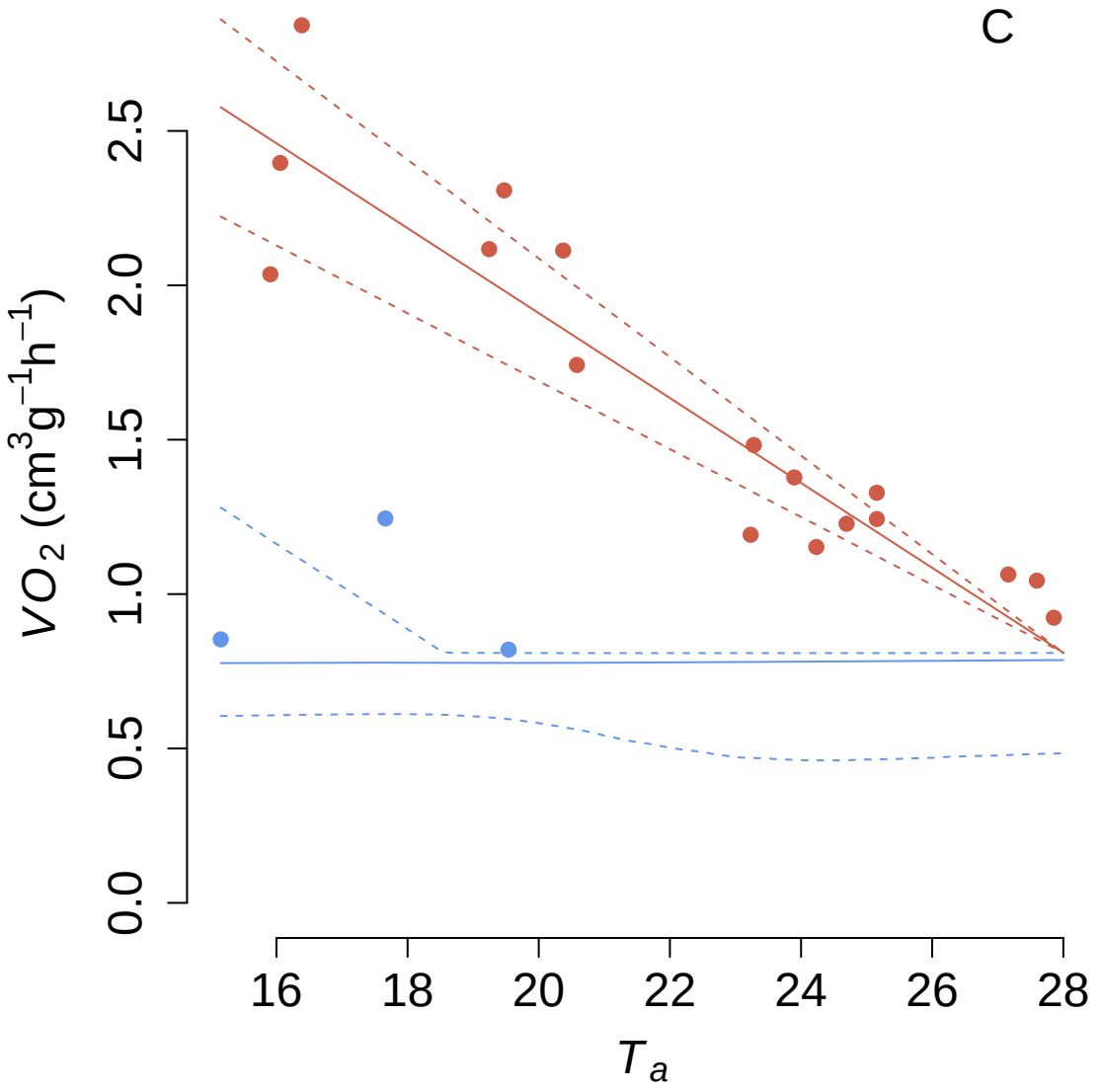
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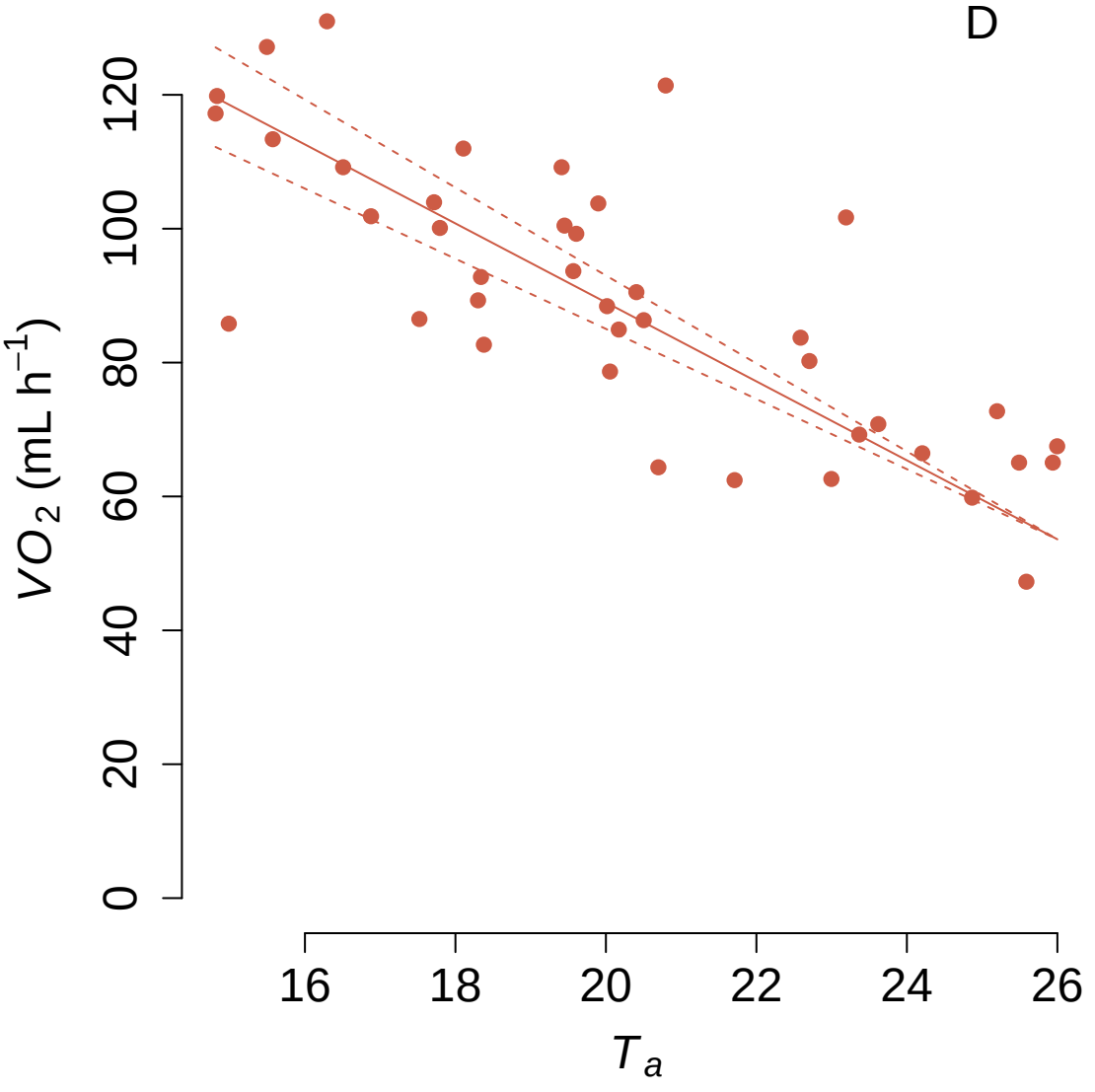
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