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Supplementary material

# Appendix 1

# Hummingbird torpor in context: duration, more than temperature, is the key to nighttime energy savings

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**Figure A1**: Equilibration time for the respirometry chamber, representing change in fractional oxygen values (baseline oxygen (.2095) – excurrent oxygen values ( $F_eO_2$ )). To approximate the equilibration time of our metabolic chambers using the negative pressure, "mask" design, we first baselined with atmospheric air, then added a constant flow of 99.99% nitrogen gas near the perch sufficient to generate an oxygen depression similar to that observed during hummingbird metabolic measurements. Air flow rate through the chambers was the same as that used during our metabolic trials. This figure shows an equilibration measurement for the system used in Ecuador (6L torpor chamber, flowrate = 1000 mL/min). We found that the flow rates we used were sufficient to equilibrate within ~8 minutes from start of nitrogen flow in the chamber.



**Figure A2**: Sample graph of energy expenditure (Joules) of a green-crowned brilliant over the course of a night (1930h – 0530h), colored by its metabolic state. This graph excludes baseline periods. At the beginning, the individual was active and perhaps flew around the chamber initially before settling down. Individuals were considered torpid (purple) when metabolic rate fell below resting normothermic values (a minimum of 0.4  $O_2$  mL/min change, and an average of 1.1 mL  $O_2$ /min in 30-90 minutes; Hiebert 1990, Powers et al. 2003). 'Rewarming' or arousal from torpor (red) began when VO<sub>2</sub> started to steadily increase and ended when VO<sub>2</sub> values peaked as the birds stopped actively increasing their metabolism (Bartholomew and Lighton 1986).



**Figure A3**: Site-wise measures of a. duration (hours) of torpor and b. nighttime energy expenditure (kJ). Boxplots show median (horizontal black central line), first and third quartiles (25<sup>th</sup> and 75<sup>th</sup> percentiles, at the ends of the box), and whiskers extending up to 1.5\*(inter-quartile range). Duration of torpor was positively related to night length and nighttime energy expenditure was strongly correlated with duration.

**Table A1**: Stepwise model DIC values for parameter combinations in the nighttime energy expenditure MCMCglmm models. All the independent variables in this model were modelled as continuous variables except for energy savings, which had a bimodal distribution and was transformed into an ordinal variable (in  $25^{th}$  quantiles) for this model. The best model was *Nighttime energy expenditure ~ Duration*. Other models, such as the 'duration + T<sub>c</sub> min', had similar DIC scores but were less parsimonious.

Model	DIC	a	Cetructuro	eta, pMCMC	eta, pMCMC	eta, pMCMC	$\beta$ , pMCMC	$\beta$ , pMCMC
Model	DIC	u	G-structure	Mass	Duration	T <sub>c</sub> _min	Savings	Rewarming
		-2.95 (-	40.32					
	213.2	15.40,	(3.49,	2.40 (0.60,	-	-	-	-
1. Mass	4	11.96)	100.7)	4.11), 0.014				
		15.75	44.46					
	192.8	(8.79,	(7.64,	-	-1.01 (-1.39, -	-	-	-
2. Duration	5	22.26)	109.4)		0.63), <2e-04			
3. T <sub>c</sub> min	216.2 2	13.01 (2.85, 23.81)	88.7 (16.19, 209.2)	-	-	0.004 (-0.26, 0.24), 0.96	-	-
4. Savings	207.5 4	16.27 (7.40, 24.07)	62.54 (10.62, 148.1)	-	-	-	-1.94 (-3.18, - 0.66), 0.0036	-
5. Duration + T <sub>c</sub> min	192.0 0	18.73 (11.22, 26.59)	41.53 (7.99, 100.4)	-	-1.09 (-1.46, -0.71), <2e-04	-0.16 (-0.36, 0.03), 0.10	-	-
	193.3	6.23 (-1.58,	8.93 (0,	1.92 (0.95,	-1.09 (-1.43, -0.72),	-0.21 (-0.39, -		
6. Mass + Duration + $T_c$ min	1	14.33)	25.66)	2.89), 0.0052	<2e-04	0.02), 0.03	-	-
7. Duration + T <sub>c</sub> min + savings	193.9 6	18.61 (10.99, 26.45)	41.76 (8.15, 99.78)	-	-1.17 (-1.73, -0.64), <2e-04	-0.17 (-0.36, 0.03), 0.093	0.27 (-1.21, 1.69), 0.69	-
8. Mass + Duration + T <sub>c</sub> min + savings	195.4 3	6.00 (-2.07, 14.35)	9.09 (0.0003, 27.03)	1.94 (0.95, 2.91), 0.0036	-1.14 (-1.63, -0.63), <2e-04	-0.21 (-0.40, - 0.04), 0.025	0.22 (-1.17, 1.70), 0.76	-

			8.78					-1.70 (-
9. Mass + Duration + $T_c$ min	194.7	4.33 (-4.02,	(0.0002,	2.15 (1.07,	-0.95 (-1.50, -0.39),	-0.21 (-0.40, -	-0.36 (-1.07,	4.04, 0.59),
+ savings + rewarming	8	13.06)	25.88)	3.07), 0.002	0.0008	0.04), 0.03	1.78), 0.62	0.15

M	DIC	MCMC		$\beta$ , pMCMC	$\beta$ , pMCMC Tc
Model	DIC	$\alpha$ , pmcmc	G-structure	mass	
		-0.63 (-2.48,	0.29 (0.0001,	0.20 (-0.003,	-
1. Mass	30.18	0.73), 0.24	1.06)	0.48), 0.012	
2. Mass + T <sub>c</sub> during		-0.55 (-3.60,	0.59 (0.0002,	0.25 (0.01,	-0.02 (-0.06,
rewarming	29.73	1.05), 0.63	2.45)	0.75), 0.004	0.01), 0.19

**Table A2**: Comparing MCMCglmm stepwise model results for the rewarming models Rewarming (kJ) ~ Mass (g) and Rewarming (kJ) ~ Mass (g) + chamber temperature ( $T_c$  in °C).



**Figure A4**: a. Torpor duration vs. minimum chamber temperature ( $T_c$  min) for the night. b. Torpor duration vs. average hourly energy savings in torpor relative to normothermy. Both  $T_c$  min and hourly energy savings were uncorrelated with torpor duration. *CYLA* (*Cynanthus latirostris*), *EUFU* (*Eugenes fulgens*), and *LACL* (*Lampornis clemenciae*) were in Arizona; *HEJA* (*Heliodoxa jacula*), *HERU* (*H. rubinoides*), *HEIM* (*H. imperatrix*), *PHSY* (*Phaethornis syrmatophorus*), and *FLME* (*Florisuga mellivora*) were Ecuadorian birds.



**Figure A5**: Average hourly torpid energy savings relative to normothermy for all individuals that used torpor across all sites. *CYLA (Cynanthus latirostris)* and *EUFU (Eugenes fulgens)* were in Arizona; *HEJA (Heliodoxa jacula), HERU (H. rubinoides), PHSY (Phaethornis syrmatophorus),* and *FLME (Florisuga mellivora)* were Ecuadorian birds. Numbers refer to the sample sizes of torpid individuals. *LACL (Lampornis clemenciae)* and *HEIM (H. imperatrix)* are not shown, as they did not enter torpor.



**Figure A6:** The probability of entering torpor is a negative binomial function of the mass of the individual. This is a graphical depiction of model 1 in Table 3 of the main paper.



**Figure A7**: Schematic diagram depicting the relationship between hourly energy savings (calculated as % energy saved/hour of torpor relative to normothermy), minimum  $T_b$ , and  $T_a$  for the broad-billed hummingbird under current and future temperatures at two Arizona sites (Harshaw and Sonoita). Overall, energy savings depend on how close  $T_a$  is to minimum  $T_b$ . Assuming a future increase of 3°C in nighttime temperatures, energy savings could decrease in Sonoita and increase in Harshaw under warming conditions. As per our calculations, these changes in hourly energy savings do not significantly impact nighttime energy expenditure but may have longer-term physiological relevance. Colour bars and temperature scales at the base of each plot represent temperature ranges at that time period and site. Minimum  $T_b$  for broad-billed hummingbirds (~15°C) is depicted by the bold vertical dashed lines. Lighter vertical dashed lines represent the range of ambient temperatures for that time period. The 'current' plots have light dotted shading; future portions of the plots have dense vertical shading; portions of the plots that overlap have chequered shading. The circle in the middle represents overall nighttime energy savings under that scenario- green is current and orange is future.

#### References

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- Hiebert, S. M. 1990. Energy costs and temporal organization of torpor in the rufous hummingbird (*Selasphorus rufus*). Physiological Zoology 63:1082–1097.
- Powers, D. R., A. R. Brown, and J. A. Van Hook. 2003. Influence of normal daytime fat deposition on laboratory measurements of torpor use in territorial versus nonterritorial hummingbirds. Physiological and Biochemical Zoology 76:389–97.

Metadata for

# Hummingbird torpor in context: duration, more than temperature, is

# key to nighttime energy savings

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## Introduction

This metadata describes and explains the data and methods that accompany the hummingbird torpor and nighttime energy expenditure study conducted June 2013 – August 2014. The objectives of the study were to measure the parameters of torpor use across either hummingbird species. This study was performed at three sites in the Patagonia and Chiricahua Mountains in south-eastern Arizona, as well as at two sites in the mid-elevation cloud forests of the Ecuadorian Andes.

#### Site table

These site codes are used in all other data tables.

Site code	Site	Latitude,	Altitude (m)	Dates sampled
		Longitude		
HC	Harshaw Creek	31.50, -110.68	1370 – 1635	Jun – Jul 2013
SC	Sonoita Creek	31.50, -110.86	1100 – 1180	Jun – Jul 2013
SWRS	Southwest Research Station	31.88, -109.21	1650 – 1720	May – Jul 2014
MQ	Maquipucuna ('Maqui')	0.12, -78.64	1275 – 1370	Jul – Aug 2014
SL	Santa Lucia	0.12, -78.61	1800 – 2100	Jul – Aug 2014

## Species table

These species codes are used in some of the data tables below

Species code	Species scientific name	Species common name	Site(s) studied
CYLA	Cynanthus latirostris	broad-billed hummingbird	HC, SC
EUFU	Eugenes fulgens	Rivoli's hummingbird	SWRS
LACL	Lampornis clemenciae	blue-throated hummingbird	SWRS
HEIM	Heliodoxa imperatrix	empress brilliant	SL
FLME	Florisuga mellivora	white-necked jacobin	MQ, SL
HEJA	Heliodoxa jacula	green-crowned brilliant	MQ, SL
PHSY	Phaethornis syrmatophorus	tawny-bellied hermit	MQ
HERU	Heliodoxa rubinoides	fawn-breasted brilliant	SL

# Summary of respirometry data

#### Dataset file

Identity: Torpor\_individual\_summaries.csv

Size: 41 records, not including header row, 12 kilobytes.

Format and storage mode: comma delimited

**Header information:** The first row of the file contains the variable names. See below for detailed descriptions of the column contents

#### Alphanumeric attributes: Mixed

**Special characters/fields:** If no information is available for a given record, or if a value is not appropriate, this is indicated by NA. O's indicate true zero.

Variable name	Variable definition	Storage type	Variable definitions
Site	Abbreviated site code where experiment was performed	Character	See Site table above
Temptrop	Code for whether the site is temperate or tropical	Character	Temperate = Arizona sites; Tropical = Ecuadorian sites
Species	Abbreviated species name	Character	See Species table above
Sp_indiv_no	Number of the individual within the species	Integer	NA
ID_AZ_BBLH	Individual identifier	Float	NA
Day	Day the experiment started	Integer	NA
Month	Month the experiment was performed	Integer	NA
Year	Year the experiment was performed	Integer	NA
Mass	Capture mass of the individual	Float	NA
Nectar_ consumption	Fed mass before start of experiment – end mass at the end of the night	Float	NA
Torpid_not	Binary character code indicating whether the individual used torpor or not	Character	T = torpid; N = normothermic
Tornor	Binary numeric code indicating whether the individual used torpor or not	Integer	1 = Used torpor; 0 = Did not use torpor
Time_of_entry	Hour in military time at which the bird entered torpor; NA if bird did not use torpor	Integer	NA
EntryTime_ numeric	Hour relative to start of night at which bird entered torpor;	Integer	NA

#### Variables

AOC duration minutes	mostly reliable for comparison	Float	ΝΑ
Reliable_rewarming _mmt	Binary character variable indicating whether the rewarming measurements were complete or not. Details in comments. Though some were missing the very end of rewarming the overall values are	Character	Y = Complete rewarming bout captured; N = Incomplete rewarming bout
Rewarming_O2_rate	Rate of change of O2 consumption over the rewarming bout	Float	NA
Rewarming_O2_change	Total difference in O2 consumption after rewarming minus before rewarming had started	Float	NA
Rewarming_stable_ end_VO2	VO2 after rewarming, at stable value	Float	NA
Rewarming_ duration_min	Duration in minutes of the total rewarming bout, from start to peak overshoot	Integer	NA
Rewarming_overshoot _end_time	Military hour at which the bird's oxygen consumption stopped increasing and started to stabilize after rewarming	Integer	NA
Rewarming_overshoot _max_VO2	Value of oxygen consumption at its peak during rewarming	Float	NA
Rewarming_ overshoot_ max_time	Military hour at which the bird's oxygen consumption peaked during rewarming	Integer	NA
Rewarmingbefore_ overshoot_time	Military time at which rewarming was ending, but before oxygen consumption overshot stable normothermic values.	Integer	NA
Rewarming_start_VO2	VO <sub>2</sub> at time when rewarming started.	Float	NA
Rewarming_ start_time	Military hour at which the bird started to rewarm after torpor, to	Integer	NA
Hours_normo	Number of hours spent normothermic, rounded to the nearest half hour	Float	NA
Hours_torpid	Number of hours spent torpid, rounded to the nearest half hour	Float	NA
	beginning of 1 <sup>st</sup> hour of night is 0, end of first hour is 1, and so on.		

AOC_mintopeak_O2ml_	Area under the curve of oxygen	Float	NA
Min	consumption in mL O2 consumed		
	per minute, from minimum end		
	torpor O2 consumption to peak O2		
	during rewarming		
Rewarming Tc	The average temperature of	Float	NA
	chamber during the rewarming		
	period		
kJ rewarming	The area under the oxygen	Float	NA
BeforeOvershoot	consumption curve through the		
	duration of rewarming after torpor,		
	converted to kiloJoules		
kJ RER071 rewarming	The area under the oxygen	Float	NA
BeforeOvershoot	consumption curve through the		
-	duration of rewarming after torpor.		
	converted to kiloJoules. We used		
	this value in the paper (with a		
	lower RER of 0.71 than the		
	previous column), because the		
	birds were likely burning fat at this		
	point		
Rate kJ	Rate of change of energy	Float	NA
_	expenditure in kJ from the		
	beginning to end of rewarming		
Total_hours	Night length in hours at that site	Integer	NA
Prop_hours	Proportion of the night spent	Float	NA
	torpid. Calculated as		
	Hours_torpid/Total_hours		
NEE_kJ_with_constRER	Total nighttime energy	Float	NA
	expenditure, in kiloJoules. These		
	calculations were made assuming a		
	constant RER through the night of		
	0.85.		
NEE_constRER_minus_	Total nighttime energy	Float	NA
rewarming_kJ	expenditure, minus rewarming		
	costs, in kiloJoules. These		
	calculations were made assuming a		
	constant RER through the night of		
	0.85.		
NEE_kJ_variableRER	Total nighttime energy	Float	NA
	expenditure, in kiloJoules. These		
	calculations were made allowing		
	RER to change through the night.		
	from 1 (carbohydrates) in the first		
	two hours, to 0.71 (fat) later in the		
	night.		

NEE_variableRER_minus _rewarming_kJ	Total nighttime energy expenditure in the previous column (variable RER), minus rewarming costs, in kiloJoules.	Float	NA
O2_ml_night	Total nighttime oxygen consumption in mL O <sub>2</sub> .	Float	NA
Tc_mean_C	Mean chamber temperature through the night	Float	NA
Tc_range_C	Range of chamber temperatures in Celsius		
Tc_min_C	Minimum chamber temperature for the night	Float	NA
Tc_max_C	Maximum chamber temperature for the night	Float	NA
Avg_EE_ hourly_torpid	Average energy expenditure over all torpid hours that night, in kiloJoules; NA if the individual did not use torpor. Accounts for variable RER.	Float	NA
Avg_EE_ hourly_normo	Average energy expenditure over all normothermic hours that night, in kiloJoules. Accounts for variable RER.	Float	NA
Percentage_avg	Percentage energy spent in torpor relative to normothermy. Uses constant RER value of 0.85 all night. Calculated as (Avg_EE_hourly_torpid/ Avg_EE_hourly_normo)*100	Float	NA
Percentage_avg_varRER	Percentage energy spent in torpor relative to normothermy. Uses varying RER values as presented in paper. Calculated as (Avg_EE_hourly_torpid/ Avg_EE_hourly_normo)*100	Float	NA
Comments	Comments	Character	NA

# Summary of ambient temperatures for all sites

On nights that torpor measurements were collected.

#### Dataset file

Identity: Ta\_AllSites\_summ.csv Size: 57 records, not including header row, 2 kilobytes. Format and storage mode: comma delimited Header information: The first row of the file contains the variable names. See below for detailed descriptions of the column contents Alphanumeric attributes: Mixed Special characters/fields: If no information is available for a given record, or if a value is not appropriate, this is indicated by NA. 0's indicate true zero.

#### Variables

Variable name	Variable definition	Storage type	Variable definitions
Row	Row number, excluding header	Integer	NA
Site	Abbreviated site code where	Character	See Site table above
	experiment was performed		
Hour2	Military time (only hour, not minutes),	Integer	e.g. 19 = 7pm, 1 = 1am
	over which temperature was averaged.		
Mean_Ta	Mean ambient temperature over that hour	Float	NA
Min_Ta	Minimum ambient temperature over that hour	Float	NA
Max_Ta	Maximum ambient temperature over that hour	Float	NA

## Summary of chamber temperatures for all individuals

#### Dataset file

Identity: Tc\_AllSites\_summ.csv Size: 57 records, not including header row, 2 kilobytes. Format and storage mode: comma delimited Header information: The first row of the file contains the variable names. See below for detailed descriptions of the column contents Alphanumeric attributes: Mixed Special characters/fields: If no information is available for a given record, or if a value is

**Special characters/fields:** If no information is available for a given record, or if a value is not appropriate, this is indicated by NA. O's indicate true zero.

#### Variables

Variable name	Variable definition	Storage type	Variable definitions
Row	Row number, excluding header	Integer	NA
Site	Abbreviated site code where	Character	See Site table above
	experiment was performed		
Hour2	Military time (only hour, not minutes),	Integer	e.g. 19 = 7pm, 1 = 1am
	over which temperature was averaged.		
Mean_Tc	Mean chamber temperature over that	Float	NA
	hour		
Min_Tc	Minimum chamber temperature over	Float	NA
	that hour		
Max_Tc	Maximum chamber temperature over	Float	NA
	that hour		

### Scholander-Irving curve measurements with broad-bill hummingbirds

<u>Controlled conditions</u>: Metabolic rate measurements on eight *Cynanthus latirostris* individuals at 5°C temperature steps under basal conditions, in Harshaw Creek.

#### Dataset file

Identity: Broadbill.csv

Size: 39 records, not including header row, 2 kilobytes.

Format and storage mode: comma delimited

**Header information:** The first row of the file contains the variable names. See below for detailed descriptions of the column contents

Alphanumeric attributes: Mixed

**Special characters/fields:** If no information is available for a given record, or if a value is not appropriate, this is indicated by NA. O's indicate true zero.

#### Variables

Variable name	Variable definition	Storage type	Variable definitions
Row	Row number, excluding header	Integer	NA
ID	Individual ID	Integer	NA
Temp_C	Temperature of the controlled	Float	NA
	chamber in degrees Celsius		
N_T	Character code denoting whether	Character	N = Normothermic;
	the measurement was on a		T = Torpid
	normothermic or torpid individual		
VO2_all	Average VO2 or oxygen	Float	NA
	consumption, with one value per		
	row for both normothermic and		
	torpid measurements		
VO2_Normothermic	Average VO2 for just normothermic	Float	NA
	measurements		

<u>Field conditions</u>: Oxygen consumption measurements, in mL O<sub>2</sub>/min, from all 15 *Cynanthus latirostris* individuals in the main study, under natural temperature cycles and photoperiods, in both Harshaw Creek and Sonoita Creek.

#### Dataset file

Identity: BBLH\_ VO2\_field.csv
Size: 572 records, not including header row, 24 kilobytes.
Format and storage mode: comma delimited
Header information: The first row of the file contains the variable names. See below for detailed descriptions of the column contents
Alphanumeric attributes: Mixed

Special characters/fields: If no information is available for a given record, or if a value is not appropriate, this is indicated by NA. O's indicate true zero. Variables

Variable name	Variable definition	Storage type	Variable definitions
Site	Abbreviated site code where	Character	See Site table
	experiment was performed		
Bird_no	Individual ID, as links to other	Character	NA
	datasets		
Bird_numeric	Just the numeric component of	Integer	NA
	Bird_no		
Time	Time at which file was saved and	DateTime	NA
	VO2 measured, since last file save		
	(usually ~ every 15 minutes)		
V02	Average VO2 or oxygen	Float	NA
	consumption (in mL/min) over		
	measurement period		
Time2	Hour of measurement	Integer	NA
Temperature	Temperature of the chamber in	Float	NA
	degrees Celsius, averaged over the		
	time of the measurement		
Torpid_not	Category of measurement: whether	Character	NA = measurement
	normothermic, torpid, entering		was excluded from
	torpor, or in the rewarming phase		plotting and
			analyses;
			Normo = bird was
			Formula Pird was
			entering terner so
			value was not a
			stable one.
			Torpid = bird was in
			torpor:
			Rewarm = bird was
			rewarming, so value
			was not a stable one.