BIOLOGY OF *MYOTIS THYSANODES* AND *M. LUCIFUGUS* (CHIROPTERA: VESPERTILIONIDAE)—III. METABOLISM, HEART RATE, BREATHING RATE, EVAPORATIVE WATER LOSS AND GENERAL ENERGETICS

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Abstract—1. Oxygen consumption (OC), weight specific oxygen consumption (VO), heart rate (HR), breathing rate (RR) and evaporative water loss (EWL) differ in adult female *Myotis thysanodes* and *M. lucifugus* and are significantly related to thermoregulatory performance (regulating or conforming) and to reproductive condition but not to body composition, spleen or adrenal weights or age class.

2. Multiple regression equation models of these physiological parameters as possible linear or exponential functions of ambient temperature (T_a), body temperature (T_b), T_b to T_a differential, weight, and each other are presented.

3. The relations of the physiological variables to T_a is curvilinear with OC, VO, RR and oxygen pulse (cm³ of oxygen consumed/heart beat) reaching minimal levels at similar optimal T_a's which exceed normally controlled T_b. HR becomes minimal at T_a's approximating normally controlled T_b and EWL reaches minimal values at T_a's lower than controlled T_b. Evaporative heat loss equals metabolic heat production at T_a's which exceed normally controlled T_b.

4. Roosting energy budgets differ in the two species and are functions of reproductive status and thermoregulatory performance. The ability of regulating individuals to conform at low T_a's conserves a large portion of the roosting energy budget.

INTRODUCTION

The relationship of metabolic rate, as estimated by oxygen consumption, to ambient temperature has been investigated in a wide variety of animals. In 1974 in this journal alone there appeared twelve articles which depict this relationship in sixteen different animal species. Such studies are of extreme value since ambient temperature is probably the single environment factor which most profoundly affects metabolism and maintenance metabolism is the most important factor contributing to total energy budgets in animals. Additionally, however, many intrinsic factors, e.g. reproductive condition, hormone levels, fat and other stored energy levels, etc. as well as other extrinsic variables, e.g. light levels and cycles, water vapor pressures, etc., may also exhibit significant relationships to metabolic rate. As a continuation of previous studies of physiological and ecological aspects of the biology of two bat species (Studier & O'Farrell, 1972; Studier *et al.*, 1973; O'Farrell & Studier, 1973, 1975, 1976), we have investigated a wide variety of these variables to determine if they relate singly or in combination to oxygen consumption. Other physiological parameters such as heart rate, breathing rate, evaporative water loss and their interrelationships were also studied in these two species of vespertilionid bats, *Myotis thysanodes* and *M. lucifugus*.

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MATERIALS AND METHODS

Although other samples were sporadically taken, six adult female bats of each species were routinely collected in the early morning in weekly intervals from a maternity colony from mid-April–late September, 1970. During the lactation periods, small numbers of neonatal bats were also collected. Bats had recently returned from nightly feeding flights just prior to capture. Bats were quickly returned to the laboratory where they were weighed, aged, sexed and placed in a metabolism chamber which was designed to simulate their natural roosting areas. After an equilibrium period at an exposure temperature of 16°C, oxygen consumption (OC), body temperature (T_b), heart rate (HR), breathing rate (RR) and evaporative water loss (EWL) were determined. Ambient temperature was raised in 4°C intervals so that the laboratory exposure temperatures matched natural roost temperatures. Each physiological parameter was determined at each temperature interval. Additional details of the methods used to collect these data are given in Studier & O'Farrell (1972).

After final exposure to a T_a of 40°C, bats were reweighed and sacrificed. Embryos were removed from pregnant females, weighed, several measurements taken and carcasses were dried to constant weight for use in other studies (Studier *et al.*, 1973; O'Farrell & Studier, 1973, 1975, 1976). Spleen and adrenals from adults and neonates were removed and weighed. These bats were then dried to constant weight and fats extracted by the methods of Ewing *et al.* (1970). Fat-free residues were then ashed at 620°C for 12 hr. Fat indices (g fat/g lean dry wt), water index (g water/g lean dry wt) as well as percentage composition (fat, water, non-fat organic, mineral) were then calculated for each individual. Additional details of these methods are presented by O'Farrell & Studier (1973).

Two parameters involving interrelationships were analyzed as functions of T_a. Oxygen pulse (OP) is equivalent to the amount of oxygen consumed per heart beat and

is derived as OC/HR. Evaporative heat loss (EHL) is defined as the fraction of metabolic heat production which is accounted for by EWL. Thusly, EWL converted to calories/hr divided by OC converted to calories/hr yields this derived variable.

Polynomial and multiple regression and correlation analysis as well as analysis of variance were performed primarily through the University of Michigan–Flint computer facility using MIDAS (Michigan Interactive Data Analysis System).

RESULTS

When total data for any of the measured physiological parameters are plotted against ambient temperature (Figs. 1 and 2 are examples), the resultant graph shows extraordinarily great variability. In our attempts to explain or model the relationships between the measured physiological variables and other parameters, it became apparent that several "class" or categorical variables exerted a significant effect. Before multiple regression analysis could be completed, it was necessary to first analyze the species separately, then segregate all individual data points into regulator (endothermic) or conformer (ectothermic) and, finally, categorize the reproductive status of the bat as pregnant, lactating or post-lactating (see Studier & O'Farrell, 1972). Twelve multiple regression models were consequently generated for each of the physiological variables measured. In no case did spleen weight; left, right or total adrenal weights; fat index; water index; percentage fat, water, non-fat organic or mineral composition; or adult age category exhibit a significant relationship to OC, T, HR, RR or EWL.

Multiple regression models

Table 1 gives the multiple regression equations and ANOVA results for the four measured physiological variables (OC, HR, RR, EWL) for both species after individuals had been categorized as indicated above.
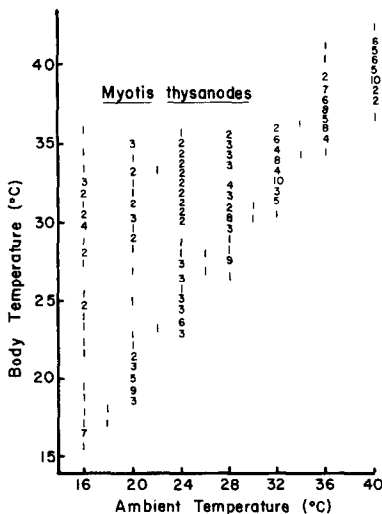

Fig. 1. Body temperatures of *Myotis thysanodes* at various ambient temperatures. Numbers shown in the figure represent the number of total data points at that approximate site. Points include adult females of both thermoregulatory types and all reproductive conditions.
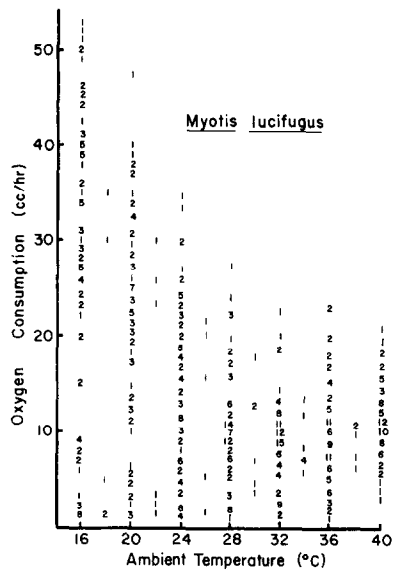

Fig. 2. Oxygen consumption of *Myotis lucifugus* at various ambient temperatures. See legend for Fig. 1 for further explanation.

Independent variables in each equation are listed in decreasing order of significance. All independent variables with $P < 0.1$ are included. No cause–effect inferences are implied by the relationships shown to exist. In most cases, the modelled physiological parameter is significantly related to several variables, often including other physiological parameters.

In most cases, particularly for regulating bats, the multiple regression models explain a large proportion of the data variability, based on the r^2 values. In nearly all cases more than half of the data variability is explained by the independent variables included in the equation. However, since the r^2 values seldom exceed 0.8, variables not measured may also relate significantly to the measured physiological variables.

In essentially all the multiple regression models, T, shows an extremely strong linear or curvilinear relationship to the modeled parameter directly or indirectly through T, to T, differential or through OC which is primarily a function of T. It is somewhat surprising that total body weight or physiological body weight ($wt^{0.75}$) is seldom significant as an independent variable in the models. This may be explained in large part by the observation that for either species, the total observed weight ranges never exceeded 4 g.

Relations to ambient temperature

Since T_a is frequently the single most important independent variable in the multiple regression models and since the relationship of T, to many physiological parameters is often presented in the literature, first and second order ANOVA, correlation and regression analyses were performed for OC, HR, RR, EWL and weight specific oxygen consumption (VO) as a function of T_a. Results of these analyses are presented in Table 2. Since, in many cases the r^2 value exceeds 0.5, T_a accounts for most of the variability in many of the physiological parameters examined.

DISCUSSION

At the initiation of this study, we thought that by measuring a large number of variables which might be reasonably expected to affect or relate to the physiological performance of the bats investigated, we could provide an extraordinarily clear picture of the interrelationships of intrinsic and extrinsic factors to these physiological parameters. Our success in this endeavor, however, has been sporadic.

Since fat, for instance, is classically considered to provide body energy stores and significantly change integumentary insulation properties (Bartholomew, 1972), one might reasonably expect fat index or percentage body fat to show a significant relationship to energetic parameters. In these two species, however, the amount of body fat bears no relationship to the tendency to regulate or conform (Studier & O'Farrell, 1972), bears no relationship to level of controlled T in regulators and, finally, bears no relationship to the rate of metabolic heat production as estimated by VO or OC. The seasonal accumulation of fat in these species, then (Ewing *et al.*, 1970), provides no immediate modification of daily energetics in these bats, but rather provides only energy stores for future use for the individuals.

Table 1. Multiple regression equations for measured physiological variables in regulating and conforming *Myotis thysanodes* and *M. lucifugus* during various reproductive states. Abbreviations and units used are DV = dependent variable; IV = independent variable; RC = reproductive condition; PR = pregnant; LA = lactating; PL = post-lactating; HR = heart rate (beats/min); OC = oxygen consumption (cc/hr); RR = respiratory rate (breaths/min); EWL = evaporative water loss (mg/hr); T_a = ambient temperature (°C); T = body temperature (°C); DIF = $T_b - T_a$ differential (°C); wt = live body wt (g). Figures in parentheses are S.E.M. Probability for all equations is equal to or less than 0.0001 except where indicated

RC	r^2	F	df	DV IV$_1$	IV$_2$	IV$_3$	IV$_4$		
				Regulating Myotis lucifugus					
PR	0.725	123.8	3,141	OC=1.53 DIF (0.42)	4.58 Ta (1.36)	-48.6 Ta$^{0.5}$ (15.7)	136. (47.)		
PR	0.688	35.3	4,64	HR=0.967 RR (0.141)	124. DIF$^{0.5}$ (32.)	-39.7 OC$^{0.5}$ (13.5)	0.212 Ta^2 (0.085)	-20.0 (132.8)	
PR	0.635	23.3	5,67	RR=12.3 DIF (2.7)	0.863 EWL (0.354)	0.0708 OC^2 (0.0303)	29.2 wt$^{0.75}$ (14.6)	-30.4 OC$^{0.5}$ (17.7)	27.8 (90.7)
PR	0.544	11.1	7,65	EWL=0.267 Ta^2 (0.091)	11.9 wt$^{0.75}$ (4.2)	31.0 OC$^{0.5}$ (12.7)	-13.5 Ta (5.9)	-0.182 DIF^2 (0.090)	0.0633 RR (0.0343)
				-2.49 OC (1.41)	76.5 (99.2)				
LA	0.780	44.3	4,50	OC=-6.03 Ta (0.84)	0.0879 Ta^2 (0.0150)	648. wt$^{0.75}$ (191.)	$-275.$ wt (81.)	$-777.$ (258.)	
LA	0.711	12.7	6,31	HR=127. OC$^{0.5}$ (31.)	-0.199 OC^2 (0.056)	123. wt$^{0.75}$ (36.)	1.30 DIF^2 (0.54)	-74.4 Ta (40.2)	$-834.$ Ta$^{0.5}$ (455.)
				2970. (1290.)					
LA				RR= too few data for analysis					
LA	0.635	28.9	3,50	EWL=0.0929 Ta^2 (0.0112)	54.1 OC$^{0.5}$ (18.6)	-4.03 OC (1.99)	$-152.$ (45.)		
PL	0.668	38.3	4,76	OC=5.98 Ta (1.48)	8.06 wt$^{0.75}$ (2.01)	-62.6 Ta$^{0.5}$ (16.2)	0.991 DIF (0.332)	126. (45.)	
PL	0.758	12.6	5,20	HR=350. OC$^{0.5}$ (68.)	-31.8 OC (7.3)	1.66 DIF^2 (0.40)	-40.5 DIF (10.5)	-2.02 EWL (0.64)	34.6 (103.3)
PL	0.635	9.6	4,22	RR=-0.802 OC^2 (0.253)	0.365 DIF^2 (0.130)	65.0 OC (25.0)	$-255.$ OC$^{0.5}$ (124.)	424. (165.)	
PL	0.862	34.4	4,22	EWL=-36.0 DIF (3.4)	8.28 OC (1.38)	-0.125 OC^2 (0.031)	-50.1 wt$^{0.75}$ (20.8)	388. (110.)	
				Regulating Myotis thysanodes					
PR	0.708	43.1	5,89	OC=-4.91 Ta (1.00)	0.0710 Ta^2 (0.0168)	0.0300 DIF^2 (0.0149)	-66.4 wt (33.6)	148. wt$^{0.75}$ (75.1)	-72.2 (90.0)
PR	0.830	19.5	7,28	HR=4.01 EWL (0.76)	-0.00298 RR^2 (0.00085)	1.78 RR (0.49)	83.1 OC (23.7)	$-470.$ OC$^{0.5}$ (141.)	-0.576 OC^2 (0.178)
				0.543 DIF^2 (0.212)	585. (224.)				
PR	0.536	21.4	2,37	RR=0.552 DIF^2 (0.107)	39.0 wt$^{0.75}$ (18.9)	-32.3 (88.6)			
PR	0.644	9.9	6,33	EWL=18.7 Ta (4.8)	32.6 OC$^{0.5}$ (8.9)	$-162.$ Ta$^{0.5}$ (49.)	16.4 DIF$^{0.5}$ (6.4)	-3.25 DIF (1.36)	-2.21 OC (0.95)
				288 (123.)					
LA	0.725	30.7	3,35	OC=0.127 DIF^2 (0.015)	242. wt (113.)	$-542.$ wt$^{0.75}$ (257.)	649. (321.)		

Table 1.

RC	r^2	F	df	DV	IV$_1$	IV$_2$	IV$_3$	IV$_4$		

Regulating _Myotis thysanodes_

RC	r^2	F	df	DV	IV$_1$	IV$_2$	IV$_3$	IV$_4$		
LA	0.532	15.3	2,27	HR=60.6 DIF^2 (13.7)	1.39 EWL (0.38)	172. (46.)				
LA				RR= too few data for analysis						
LA	0.492	10.0	3,31	EWL=61.2 wt$^{0.75}$ 15.4)	0.210 Ta^2 (0.064)	-99.7 Ta$^{0.5}$ (36.8)	131. (162.)			
PL	0.675	43.6	2,42	OC=-84.0 Ta$^{0.5}$ 23.5)	6.90 Ta (2.31)	265. (59.)				
PL	0.633	12.9	4,30	HR=218. DIF$^{0.5}$ (49.)	-8420. Ta$^{0.5}$ (2450.)	1260. Ta (366.)	-7.56 Ta^2 (2.32)	15300. (4560.)		
PL	0.630	6.4*	4,15	RR=0.0674 OC^2 0.0260)	6.19 Ta^2 (3.24)	-916. Ta (485.)	5940. Ta$^{0.5}$ (3190.)	-10500. (5870.)		
PL	0.858	22.6	4,15	EWL=-20.3 DIF^2 (3.8)	-31.0 wt$^{0.75}$ (6.4)	4.24 OC (1.02)	-0.0440 OC^2 (0.0159)	226. (36.)		

Conforming _Myotis lucifugus_

RC	r^2	F	df	DV	IV$_1$	IV$_2$	IV$_3$	IV$_4$		
PR	0.358	12.9	4,93	OC=8.61 Tb (3.26)	-83.1 Tb$^{0.5}$ (35.8)	-0.0646 Ta^2 (0.0293)	3.33 Ta (1.85)	163. (73.)		
PR	0.719	11.1	6,26	HR=-0.759 Ta^2 (0.211) 190. (518.)	2.42 Tb^2 (0.83)	229. RR$^{0.5}$ (94.)	-7.67 RR (3.53)	-101. Tb (47.)	-5.53 OC (2.86)	
PR	0.325	5.1$	3,32	RR=156. Tb$^{0.5}$ (67.)	-123. Ta$^{0.5}$ (65.)	0.548 EWL (0.304)	-86.3 (140.)			
PR	0.749	17.9	5,30	EWL=0.400 Ta^2 (0.117)	-21.0 Ta (7.4)	1.71 OC (0.70)	14.7 wt$^{0.75}$ (6.4)	0.000338 RR^2+216. (0.000182) (120.)		
LA	0.546	32,5	2,54	OC=16.2 Tb$^{0.5}$ (3.9)	-11.3 Ta$^{0.5}$ (3.7)	-18.9 (3.6)				
LA	0.824	24.6	4.21	HR=152. wt$^{0.75}$ (29.)	45.4 Ta (13.7)	-0.587 Ta^2 (0.227)	39.7 OC$^{0.5}$ (18.4)	-1430. (265.)		
LA				RR= too few data for analysis						
LA	0.675	35.4	3,51	EWL=0.310 Tb^2 (0.056)	-7.05 Ta (2.87)	-66.6 Tb$^{0.5}$ (37.1)	338. (131.)			
PL	0.470	23.4	2,53	OC=0.556 Tb (0.103)	1.21 wt (0.70)	-21.2 (5.9)				
PL	0.567	10.2	4,31	HR=-0.380 Ta^2 (0.186)	10.3 Tb^2 (5.7)	13600. Tb$^{0.5}$ (7600.)	-1830. Tb (1040.)	-28100. (15500.)		
PL				RR= too few data for analysis						
PL	0.787	36.2	5,49	EWL=0.798 Ta^2 (0.186)	3.62 OC (0.90)	-0.682 Tb^2 (0.210)	-37.2 Ta (11.7)	35.3 Tb (13.3)	6.94 (87.4)	

Conforming _Myotis thysanodes_

RC	r^2	F	df	DV	IV$_1$	IV$_2$	IV$_3$	IV$_4$		
PR	0.354	9.4	6,103	OC=0.767 Tb^2 (0.252) -346. (205.)	-135. Tb (46.)	975. Tb$^{0.5}$ (334.)	-0.660 Ta^2 (0.229)	115. Ta (41.)	-814. Ta$^{0.5}$ (293.)	
PR	0.578	71.3	1,52	HR=16.3 Tb (1.9)	-208. (59.)					
PR	0.474	11.7	2,26	RR=201. Tb$^{0.5}$ (56.)	-164. Ta$^{0.5}$ (55.)	-102. (53.)				
PR	0.731	66.6	4,98	EWL=2.98 OC (0.37)	13.2 Ta (4.4)	-116. Ta$^{0.5}$ (46.)	8.12 wt$^{0.75}$ (3.59)	238. (121.)		
LA	0.757	35.0	4,45	OC=-11.9 Ta$^{0.5}$ (4.0)	1.85 wt (0.63)	0.0142 Tb^2 (0.0054)	9.36 Tb$^{0.5}$ (4.58)	-8.06 (13.8)		
LA	0.770	53.6	2,32	HR=10.2 Tb (2.9)	1,42 EWL (0.40)	-133. (70.)				
LA				RR= too few data for analysis						
LA	0.692	31.4	3,42	EWL=49.8 wt$^{0.75}$ (16.9)	21.5 Ta (8.8)	-177. Ta$^{0.5}$ (92.)	147. (252.)			
PL	0.138	5.8$	1,36	OC=0.313 Tb (0.130)	-2.40 (4.36)					
PL	0.698	13.1	3,17	HR=25.8 Tb (4.2)	-3.01 EWL (0.78)	0.454 OC^2 (0.250)	-272. (113.)			
PL				RR= too few data for analysis						
PL	0.707	15.4	5,32	EWL=0.435 OC^2 (0.124)	0.202 Ta^2 (0.081)	-4.87 OC (2.55)	-8.62 Ta (4.64)	-20.5 wt$^{0.75}$ (11.5)	247. (80.)	

* = probability equal to or less than 0.0005; # = probability equal to or less than 0.005; $ = probability equal to or less than 0.05

Table 2. Best regression equations for the measured parameters. Values given are coefficients for lines of the form $y = ax^2 + bx + c$ for second order equations and $y = bx + c$ for linear equations. The independent variable is always ambient temperature (°C). Probability for all equations is equal to or less than 0.0001 except where indicated. Abbreviations and symbols used are defined in Table 1 except for VO = weight specific oxygen consumption (cc/gm per hr). Figures in parentheses are S.E.M.

REGULATORS

DV	RC	Myotis thysanodes a	b	c	r^2	F	df	Myotis lucifugus a	b	c	r^2	F	df
VO	PR	0.00827 (0.00109)	-0.620 (0.063)	13.0 (0.8)	0.662	212.0	2,216	0.00993 (0.00102)	-0.681 (0.057)	12.8 (0.7)	0.648	229.7	2,250
VO	LA	0.00733 (0.00180)	-0.496 (0.103)	9.64 (1.40)	0.550	27.4	2,45	0.00808 (0.00130)	-0.567 (0.075)	11.1 (1.0)	0.746	94.1	2,64
VO	PL	0.00779 (0.00182)	-0.579 (0.103)	11.7 (1.4)	0.677	75.5	2,72	0.00858 (0.00085)	-0.564 (0.048)	10.2 (0.6)	0.645	154.4	2,170
OC	PR	0.0720 (0.0085)	-5.34 (0.49)	109. (7.)	0.693	244.1	2,216	0.0829 (0.0079)	-5.69 (0.44)	108. (6.)	0.682	267.9	2,250
OC	LA	0.0634 (0.0171)	-4.33 (0.98)	84.6 (13.3)	0.527	25.0	2,45	0.0820 (0.0136)	-5.76 (0.78)	112. (10.)	0.735	88.7	2,64
OC	PL	0.0662 (0.0142)	-4.93 (0.80)	99.9 (10.6)	0.717	91.3	2,72	0.0766 (0.0081)	-5.06 (0.46)	91.8 (6.0)	0.627	142.7	2,170

DV	RC	a	b	c	r^2	F	dF	a	b	c	r^2	F	dF
HR	PR	0.950 (0.273)	-60.3 (15.1)	1310. (197.)	0.297	15.2	2,72	0.802 (0.194)	-51.0 (10.7)	1240. (138.)	0.260	23.0	2,131
HR	LA	too few data for analyses							-7.92 (1.88)	737. (55.)	0.303	17.8	1,41
HR	PL	0.766 (0.323)	-49.2 (18.0)	1210. (236.)	0.287	7.4[#]	2,37	0.570 (0.226)	-38.2 (12.6)	1090. (165.)	0.250	12.2	2,73
RR	PR	0.393 (0.193)	-28.9 (10.7)	658. (141.)	0.412	16.1	2,46	0.658 (0.155)	-43.4 (8.4)	873. (106.)	0.514	39.6	2,75
RR	LA	too few data for analysis						0.496 (0.374)	-37.2 (21.0)	922. (278.)	0.538	7.6	2,13
RR	PL	0.480 (0.400)	-32.4 (20.6)	706. (248.)	0.445	7.2[#]	2,18	0.690 (0.210)	-9.00 (11.7)	432. (151.)	0.334	6.5[#]	2,26
EWL	PR	0.188 (0.033)	-9.27 (1.86)	168. (25.)	0.241	32.8	2,207	0.320 (0.036)	-15.7 (2.0)	231. (26.)	0.389	75.4	2,237
EWL	LA	0.264 (0.101)	-13.1 (5.8)	233. (80.)	0.273	7.3[#]	2,39	0.238 (0.074)	-10.7 (4.3)	174. (57.)	0.404	21.4	2,63
EWL	PL	too few data for analysis						0.358 (0.420)	-16.6 (2.4)	238. (31.)	0.563	108.1	2,168

CONFORMERS

DV	RC	a	b	c	r^2	F	dF	a	b	c	r^2	F	dF
VO	PR		0.0435 (0.0057)	-0.208 (0.176)	0.198	57.8	1,234		0.0481 (0.0062)	-0.466 (0.199)	0.237	59.8	1,193
VO	LA		0.0604 (0.0069)	-0.837 (0.215)	0.613	76.1	1,48		0.0370 (0.0067)	-0.158 (0.218)	0.354	30.1	1,55
VO	PL		0.0269 (0.0121)	0.017 (0.401)	0.098	4.9	1,45		0.0522 (0.0062)	-0.772 (0.210)	0.399	70.4	1,106

DV	RC	a	b	c	r^2	F	dF	a	b	c	r^2	F	dF
OC	PR		0.364 (0.042)	-2.37 (1.30)	0.241	74.4	1,234		0.409 (0.050)	-4.00 (1.59)	0.260	67.9	1,193
OC	LA		0.504 (0.061)	-7.06 (1.89)	0.588	68.5	1,48		0.371 (0.061)	-2.03 (1.98)	0.401	36.8	1,55
OC	PL		0.217 (0.101)	0.616 (3.33)	0.093	4.6$	1,45		0.471 (0.056)	-7.13 (1.88)	0.401	70.9	1,106
HR	PR		15.5 (1.8)	-173. (55.)	0.551	71.0	1,58		15.3 (2.1)	-101. (70.)	0.392	51.0	1,79
HR	LA		17.0 (2.1)	-222. (66.)	0.657	63.1	1,33	-0.587 (0.296)	49.6 (17.2)	-583. (239.)	0.699	29.1	2,25
HR	PL		12.2 (3.7)	-1.79 (119.)	0.364	10.9[#]	1,19		12.8 (3.1)	-10.4 (105.)	0.319	17.3[*]	1,37

Table 2.

		Myotis thysanodes						*Myotis lucifugus*					
DV	RC	a	b	c	r^2	F	df	a	b	c	r^2	F	df
RR	PR		2.19 (1.02)	54.2 (31.4)	0.125	4.68	1,32		4.28 (1.73)	17.9 (57.3)	0.149	6.1§	1,35
RR	LA		3.32 (0.39)	5.14 (11.95)	0.878	72.0	1,10		7.30 (1.24)	-46.8 (36.9)	0.729	34.9	1,13
RR	PL		too few data for analysis						6.26 (1.91)	-42.4 (68.2)	0.518	10.7§	1,10
EWL	PR	0.127 (0.029)	-3.84 (1.66)	50.4 (22.3)	0.556	141.3	2,226	0.276 (0.043)	-11.6 (2.5)	138. (35.)	0.550	114.3	2,187
EWL	LA	0.169 (0.088)	-4.61 (5.04)	49.6 (67.6)	0.626	36.0	2,43	0.242 (0.084)	-9.74 (4.86)	132. (66.)	0.530	29.3	2,52
EWL	PL		2.80 (0.75)	-7.51 (24.75)	0.233	14.0*	1,46	0.367 (0.069)	-15.9 (4.2)	198. (61.)	0.600	78.8	2,105

The spleen functions partially in erythrocyte storage and its weight markedly decreases during arousal in bats as erythrocytes are added to the circulating blood (Lidicker & Davis, 1955). This modifies the oxygen carrying capacity of the blood and, perhaps, might modify such related physiological parameters as OC, VO, HR or T_i. Again, however, no relationship exists. Adrenal weights may be indicative of hormonal levels and secondarily to rates of energy liberation. Such is not the case in these species. Aging has been linked to changing metabolic rate and other energetic parameters (Guyton, 1971); however, again no relationships were found in these species. Although energy demands imposed by flight are presumably relatively constant and independent of age, roost resting metabolism might relate to age category. These age categories are, however, somewhat arbitrary and their accuracy has often been questioned.

It is, of course, not surprising that there exist significant differences in the physiological responses of the two species since their genetic composition varies. After our earlier reported findings concerning T_i (Studier & O'Farrell, 1972), it was expected that significant differences should occur in the physiological response of regulators and conformers. Since reproductive status of females relates significantly to their tendency to regulate as well as the level of controlled T_i in regulators (Studier & O'Farrell, 1972), the finding that reproductive condition significantly affects other physiological parameters related to energetics is expected.

The multiple regression equations depict many varied interrelationships among the measured variables; however, a few patterns emerge. For regulating individuals of both species, OC relates best to T_a in 2/3 of the instances and to T_i to T_a differential (DIF) in the other 1/3. OC is, therefore, best explained by a linear relationship to T_a or DIF with curvilinear functions of these variables of lesser, but frequently significant, importance. The equations for OC are also those in which body weight or physiological body weight appear most frequently as significant independent variables.

For conforming bats, DIF was not considered for an independent variable but was replaced by T_i alone. In 5 of the 6 multiple regression models for OC in conforming bats, the most important independent variable was T_i (linear or curvilinear) with T_a (curvilinear) as the most important independent variable

in the remaining equation. It is of particular interest that although T and T are very strongly interrelated in conforming individuals, both of these variables appear in many of the equations for conforming bats. T_i and T_a, thus, exhibit both significant cooperative and separate relations to OC. Weight appears as an independent variable in only two of the OC equations for conformers and is a minor variable when it appears.

Although we had expected OC to be the best predictive independent variable for HR in regulating bats, this was not the case with several other variables serving as the best parameter for explaining data variability. Among conforming bats, HR is best predicted from T_b in most cases with T_b being the only significant independent variable in pregnant conforming *M. thysanodes*.

In several cases, too few data were available for RR to perform multiple regression analysis. Although many independent variables contribute significantly to the multiple regression models, DIF is the best variable for prediction in pregnant regulators while OC is the best parameter for prediction in post-lactating regulators. Among conforming pregnant individuals, the most important independent variable is the square root of T with the square root of T exerting a separate secondary influence.

In modeling EWL, we had assumed RR would be a significant independent variable. It is included, however, only as a variable of minor importance in two of the twelve equations. Variation in EWL then presumably results from variation in depth of breathing rather than breathing rate. Secondly, we predicted that EWL would be a strong function of T^2 and, in fact, this parameter is a significant variable in most models.

After examining data for the entire range of exposure temperatures (T's from 16 to 40°C), secondary multiple regression analysis of data in the T_a range from 16 to 36 C yielded models with much higher r^2 values indicating that a large portion of inexplicable data variability occurs during exposure to the abnormally high T_a of 40°C.

Relationships to ambient temperature

The relationships of most of the measured physiological parameters to T_a are cuvilinear. By setting the first derivative of the second order equation equal to zero and solving that equation, one finds the T_a

Table 3. Ambient temperatures at which physiological responses reach minimum levels in regulating *M. thysanodes* and *M. lucifugus* in varying reproductive conditions. Regulated T_i is also given

RC	VO	OC	HR	RR	EWL	OP	Tb*
		Myotis thysanodes					
PR	37.5	37.1	31.7	36.8	24.7	--	31.9
LA	33.8	34.2	--	--	24.8	32.8	29.8
PL	37.4	37.3	32.1	33.8	--	44.8	31.9
		Myotis lucifugus					
PR	34.3	34.3	31.8	33.0	24.6	36.8	29.5
LA	35.1	35.1	--	37.5	22.5	33.2	27.0
PL	32.8	33.0	33.5	--	23.2	31.4	29.7

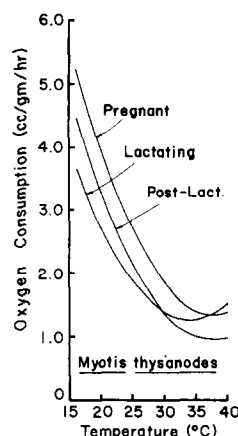* from Studier & O'Farrell (1972)

Fig. 3. Weight specific oxygen consumption of adult female *Myotis thysanodes* of varying reproductive conditions as a function of ambient temperature.

at which the physiological responses are minimal (Studier *et al.*, 1975). This has been completed for the second order equations found in Tables 2 and 4 with values shown in Table 3. The temperatures shown in Table 3, then, represent the thermal neutral point for each of the physiological parameters measured.

The observation that minimal VO and OC are reached at T_i's in excess of controlled T_i in regulating *M. thysanodes* and *M. lucifugus* is unusual in endothermic bats (Leitner, 1966; Leitner & Nelson, 1967) and, it is reflective of our previous observation that regulating individuals enter partial hyperthermia at T_i's in excess of 20°C for *M. lucifugus* and 24°C for *M. thysanodes* (Studier & O'Farrell, 1972). Additionally, these high T_i's at which minimal maintenance energy expenditure occur are behaviorally selected by bats of both species in their natural roosts (Studier & O'Farrell, 1972). The diurnal verticle movements by bats in this case and other studies at roost sites (Licht & Leitner, 1967a; Wilson, 1971), therefore, appear to be behavioral microhabitat selection resulting in minimal metabolic stress during the bulk of the roosting period.

The relationship of VO to T_i in regulating *M. thysanodes* is shown in Fig. 3. These curvilinear relationships are characteristic for regulating bats and other mammals (Leitner, 1966; Leitner & Nelson, 1967; Carpenter & Graham, 1967; McNab, 1969). Since in *M. thysanodes* the level of controlled T_i in lactating regulators is about 2°C lower than the controlled T_i in pregnant regulators (Table 3), if thermal conductance doesn't change, the relationship for lactating regulators should simply be shifted to the left about 2°C from the relationship for pregnant regulators. This appears to be the case. Such a leftward shift due to decreased controlled T provides a considerable energetic savings among lactating individuals. Similar reasoning would indicate that the VO to T_i relation during the post-lactation period should return to be identical to the VO to T relation in pregnant regulators since T returns to the same level. Although minimum T_i for VO occurs at the same T_i in post-lactating and pregnant bats, the relationship has shifted downward indicating a change in thermal conductance has occurred. The decreased rate of heat loss allows a higher T at reduced metabolic cost. The change in thermal conductance may

Table 4. Best regression equations for the measured parameters as functions of T_i. Values are given as in Table 2. Abbreviations and symbols are defined in Table 1 except for OP = oxygen pulse (cc/beat) and EHL = evaporative heat loss (= metabolic heat production/ evaporative heat loss). Coefficients for OP are times 10^3

		Myotis thysanodes						*Myotis lucifugus*					
DV	RC	a	b	c	r^2	F	df	a	b	c	r^2	F	df
OP	PR		-0.0331 (0.0055)	1.79 (0.16)	0.337	36.1	1,71	0.00151 (0.00054)	-0.111 (0.030)	2.51 (0.38)	0.338	33.1	2,130
OP	LA	0.00244 (0.00085)	-0.160 (0.048)	3.08 (0.65)	0.430	11.3	2,30	0.00256 (0.00060)	-0.170 (0.033)	3.22 (0.43)	0.632	34.3	2,40
OP	PL	0.000960 (0.000701)	-0.0861 (0.0390)	2.16 (0.51)	0.556	23.2	2,37	0.00212 (0.00055)	-0.133 (0.031)	2.41 (0.40)	0.310	16.4	2,73
EHL	PR		0.0413 (0.0033)	-0.593 (0.104)	0.427	154.0	1,207	0.00162 (0.00050)	-0.0465 (0.0282)	0.502 (0.368)	0.427	88.2	2,237
EHL	LA		0.0383 (0.0077)	-0.317 (0.245)	0.381	24.6	1,40		0.0459 (0.0052)	-0.688 (0.163)	0.550	78.1	1,64
EHL	PL		0.0895 (0.0236)	-1.426 (0.695)	0.165	14.4*	1,73		0.0634 (0.0083)	-0.888 (0.247)	0.256	57.9	1,168

result from increased insulation due to molt or fat deposition or may reflect decreased peripheral blood flow. Since body fat levels. however, bear no relationship to T or VO for the whole sample or within any reproductive category, it is unlikely that integumentary fat deposits contribute significantly to increased thermal insulation. Relative roosting energy budgets throughout the summer period for both species is discussed later in this paper.

While the relation of T to VO or OC results in equations with rather good predictive value. the relation of HR to T, although significant, has little predictive value. Even with this warning in mind, however, it appears that minimum HR in regulators generally occurs at a T considerably lower than the T at which minimum VO or OC occurs. Behavioral selection of roosting T for minimal VO or OC, therefore, is associated with an above-minimal HR.

The predictive value of the relation of breathing rate (RR) to T is also poor. It seems that RR. OC and VO may be minimal at the same T. The relations of HR and RR do agree with previous studies of these relations in other bats (Leitner, 1966; Leitner & Nelson, 1967; Carpenter & Graham, 1967).

Evaporative water loss (EWL) reaches minimal levels at T's in the lower to middle 20s in both species (Fig. 4). These T's are well below the controlled T of regulating individuals but fall in the T range in which the bats begin partial hyperthermia (Studier & O'Farrell, 1972). It also is apparent that EWL is rising while RR is falling between T's in the low 20s to mid-30s indicating further that the increased EWL results from increased depth of breathing rather than increased breathing rate. The strong observed relation of EWL to T agrees with other studies of EWL in bats (see Studier, 1970).

In nearly all cases, oxygen pulse decreases in curvilinear fashion reaching minima at T's which roughly approximate the T's where VO and OC are minimal (Table 3). Since OP = OC/HR = (stroke volume) (arterio-venous difference in oxygen content). as T deviates from the T where OP is minimal, stroke volume of A-V difference or both must increase. With knowledge of OP and HR, the contribution of HR to oxygen transport can be calculated by the method of Bartholomew & Tucker (1963). Results of this
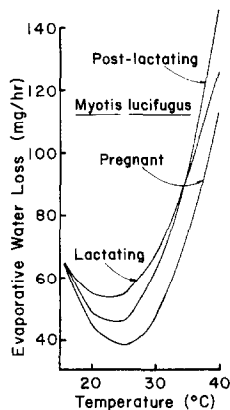
Table 5. Percentage of heart rate contribution to oxygen transport in *Myotis thysanodes* and *M. lucifugus* of differing reproductive status

RC	15–20°C	20–25°C	25–30°C
		Myotis thysanodes	
PR	63.1	55.4	37.1
PL	41.9	34.8	22.3
		Myotis lucifugus	
PR	41.8	36.5	27.3
LA	17.6	18.9	26.8
PL	29.7	26.3	24.3

analysis are depicted in Table 5. The contribution of heart rate to oxygen transport in these bats is markedly lower than values previously reported for bats (Leitner, 1966; Leitner & Nelson, 1967). The *Myotis* presently under consideration, however, are much smaller than the bats previously studied. It appears that the contribution of HR to oxygen transport in *M. lucifugus* is less than that in *M. thysanodes*, particularly at lower T ranges. HR contributes a greater and greater percentage to oxygen transport as T lowers except in lactating *M. lucifugus* where the HR contribution increases with increasing T range.

The fraction of evaporative heat loss (EHL) to metabolic heat production (MHP) as a function of T has been given in Table 3. EWL becomes increasingly more important in linear fashion as a mechanism of metabolic heat dissipation as T increases in this study as well as others (Licht & Leitner, 1967b; Procter & Studier, 1970). Although the correlation coefficients are quite low. the T's at which EHL equals MHP has been calculated (Table 6). Since when T equals or exceeds T, EWL is the only significant mechanism of heat loss, one expects EHL to equal MHP at T's equal to controlled T in regulators. With the exception of the dubious values for post-lactating bats (r^2 equals 0.165 and 0.256), the T's where EHL equals MHP are considerably above controlled T in regulators. These high T's are again reflective of hyperthermia in both species and tend to reflect the highest tolerable hyperthermic T_b for these species. The high T_a's at which EHL equals MHP help conserve water by allowing radiation and conductance to aid heat dissipation at

Fig. 4. Evaporative water loss in adult female *Myotis lucifugus* in various reproductive states as a function of ambient temperature.

Table 6. Ambient temperatures at which evaporative heat loss equals metabolic heat production in *Myotis thysanodes* and *M. lucifugus*. Abbreviations as previously used

Rep. Cond.	M. thysanodes	M. lucifugus
Pregnant	38.6	37.0
Lactating	34.4	36.8
Post-lactating	27.1	29.8

high T_a's. It would also seem apparent that, like other bat species (Licht & Leitner, 1967b), these *Myotis* can dissipate well over 100% of MHP by EWL.

Roosting energy budgets

Since we have recorded daily fluctuations in roost site temperatures (Studier & O'Farrell, 1972), as well as diurnal activity patterns (O'Farrell & Studier, 1973, 1975), it is possible to construct a reasonably accurate energy budget for roosting bats of these two species. For ease of calculations, T_a has been simplified as follows. Bats return to the maternity roost by 0400 hr when the T_a averages about 16°C during the spring (pregnant) and fall (post-lactation) and 20°C during the summer (lactation). T_a begins to rise at a rate of 8°C/hr commencing at 0730 hr in the spring and fall and 0800 hr in the summer. This T_a rise continues until optimal T_a for minimal OC is exceeded, after which time bats initiate behavioral thermoregulation by selecting the thermal microclimate corresponding to the optimal T_a for minimal OC. They remain at this optimal T_a for the remainder of the roosting period. For example, a pregnant *M. thysanodes* is considered to spend 3.5 hr at a T_a of 16°C (0400–0730 hr), 0.5 hr at 20, 24, 28, 32 and 36°C and the remaining 10.0 hr at its optimal T_a of 37.1°C (1000–2000 hr) at which time the bat exits the maternity roost to begin its nightly activity. The relationships of OC to T_a given in Table 2 were used to calculate total roost time oxygen consumption which was converted to Kcal using 4.825 Kcal/l. of oxygen consumed as an energy equivalent (Ganong, 1973). Results of this analysis are given in Table 7. For roost maintenance energy, it is apparent that pregnant regulators require the largest amount of energy. The large drop in roost maintenance energy requirement during lactation in *M. thysanodes* appears to be the result of the previously discussed drop in T in this species during lactation. Post-lactation energy requirements are lower than pregnancy requirements due apparently to marked changes in thermal conductance in both species allowing considerably increased insulative properties. Daily energy demand in conformers is much less than that of regulators. In our previous study (Studier & O'Farrell, 1972), we found that many bats, particularly during pregnancy, exhibited an unusual thermoregulatory pattern we described as

"shifting". Shifters were neither strict conformers nor strict regulators but regulated at some T_a's and conformed at others. The ability to conform at the low T_a during the beginning of the daily roosting period is an extreme energy conserving mechanism. A pregnant *M. thysanodes* which conformed at 16°C and regulated at all higher T_a's would have a roosting energy requirement of 0.76 Kcal as opposed to an energy demand of 1.41 Kcal in an individual which regulated throughout the sub-optimal T_a range.

SUMMARY

Physiological responses differ between *Myotis lucifugus* and *M. thysanodes* when measured throughout the summer roosting period.

1. Whole bat oxygen consumption (OC), weight specific oxygen consumption (VO), body temperature (T_t), heart rate (HR), breathing rate (RR), and evaporative water loss (EWL) are significantly different in regulating and conforming individuals and are significantly related to reproductive condition (pregnant, lactating, post-lactating).

2. OC, VO, T, HR, RR and EWL are not significantly related to body composition (fat or water index, percentage fat, water, non-fat organic, or mineral), spleen or adrenal weights, or adult age class.

3. Multiple regression equation models of the physiological parameters show general lack of uniformity and a large number of interrelationships.

4. The relationships of OC, VO, T_t, HR, RR and EWL to ambient temperature (T_a) are nearly curvilinear yielding optimal T's where each parameter is minimal. OC, VO and RR reach minimal values at similar T's which are greater than controlled T. HR reaches minimal values at T's which approximate controlled T. EWL reaches minimal values in T_t's in the low 20s°C which are the T's at which partial hyperthermia is initiated.

5. Oxygen pulse (cm^3 of oxygen consumed/heart beat) shows a curvilinear relation to T_a reaching minimal values at T's approximately equal to those of OC, VO and RR. The contribution of HR to oxygen transport is generally less than 50% and increases as T_a range lowers except during lactation.

6. The fraction of evaporative heat loss to metabolic heat production is directly and linearly related to T_a reaching 100% at T's considerably higher than normally controlled T_t due to hyperthemic T at high T's.

7. Roosting metabolic energy demand is considerably reduced in conformers in comparison to regulators and is also a function of reproductive condition. The ability of individuals to conform at low T's and then shift to regulation at other T_a's results in a large energy savings in roosting energy budget.

Table 7. Estimated kilocalories required for maintenance metabolism during the roosting period in *Myotis thysanodes* and *M. lucifugus*

Rep. Cond.	M. thysanodes	M. lucifugus
Regulators		
Pregnant	1.41	1.34
Lactation	1.09	1.24
Post-lactation	1.22	1.06
Conformers		
Pregnant	0.69	0.61
Lactation	0.62	0.72
Post-lactation	0.57	0.48

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