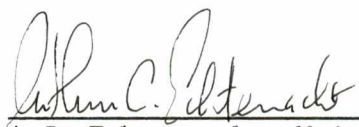
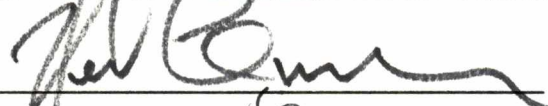


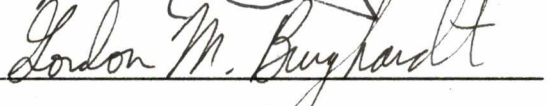
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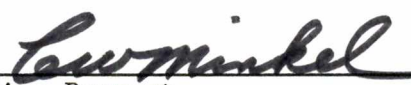

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SELECTED ASPECTS OF THE THERMAL RELATIONS AND ECOPHYSIOLOGY OF THE
LIZARD ANOLIS CAROLINENSIS (SAURIA: IGUANIDAE)

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Mark Angus Wilson

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ABSTRACT

This study was conducted to determine whether certain methods and variables may influence results obtained in tests of the thermal tolerance of reptiles. Knowledge thus gained then allowed an examination of the geographic variation in the thermal tolerance of adult male Anolis carolinensis.

Comparisons of body to ambient temperature were made using animals equipped with thermocouples (a commonly employed method) and animals whose temperature was recorded by insertion of a rapid-reading cloacal thermometer. Individuals with thermocouples averaged 0.5 C below and the individuals without thermocouples 0.4 C above ambient temperature. The cause of lowered temperature in subjects equipped with thermocouples was posited to be due to enhanced evaporative heat loss.

A test was conducted to determine whether acute handling and social stress lead to increased metabolic rate which, in turn, could lead to an unhealthy condition in subordinates of collectively housed lizards, lowering their thermal tolerance. Oxygen consumption and lactate accumulation were measured in individuals subjected to these forms of stress. Both were found to significantly increase rates of aerobic and anaerobic respiration. Collective housing of Anolis, and possibly other reptiles, before thermal testing may increase experimental error.

High temperature tolerance was compared in castrated individuals treated with either testosterone filled or empty silastic tubes, acclimated to 20 ± 2 C for 10 days. While the lizards were heated, the

following responses were noted and body temperatures recorded at the onset of each: Escape movements, panting and onset of muscular spasms. No treatment effect was found for the response temperatures observed. Testosterone does not affect heat tolerance after low temperature acclimation in this species.

Three populations, along a latitudinal gradient, were tested for cold tolerance. After field determinations of the critical thermal minimum (cooling the subject until the loss of the righting response) yielded the appropriate acclimation temperature, the animals were acclimated to 9 C for 48 hours. Laboratory determination of critical thermal minimum revealed that the Tennessee population was significantly more cold tolerant than either Georgia or Florida populations (the latter two were not found to differ): this species consists of physiological races with regard to cold temperature tolerance.

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PART I
INTRODUCTION

Early studies of reptilian ecology in the western Hemisphere dealt with species occupying the desert regions of the southwestern United States. Interest focused on adaptation to the extreme thermal environments inhabited by these species and, as a result, the prevailing attitude concerning reptilian ecology was that physical parameters were of greatest import.

In one such study (Mosauer, 1936), heat tolerance was compared in diurnal lizards and nocturnal snakes in order to test the hypothesis that the lizards, active in the greater heat of daytime, would be more resistant to high temperature. Tests conducted in the laboratory and the field yielded the same result: there was no difference in tolerance to heat between these groups. It was concluded that the difference in time of activity was a result of morphology: a lizard had a distinct advantage over a snake while active because of its ability to keep most of its body off the hot substrate during the day. Since the results of laboratory and field tests were the same and all animals died in a non-lethal state of hydration, it was also concluded that the "lethal" factor in the lives of these reptiles was heat resulting from insolation, not excessive ultraviolet light or loss of body fluid.

In a later study, Cole (1943) compared the lethal temperatures of 10 species of lizards that occupied different habitats in the desert. Species differences in thermal tolerance were found to correlate with habitat. It was also discovered that the color of a lizard influenced its rate of heating; color was previously considered to result

primarily from selection for biotic interaction such as concealment from predation or for mate attraction (Cott, 1939).

Bogert (1949) studied various species of Sceloporus (Sauria, Iguanidae) found in a variety of habitats in Mexico and North America. He concluded that those living in open regions were restricted to terrain where shelter from the sun was available. In contrast, species living in densely forested areas were restricted to areas with ready access to direct solar radiation.

Norris (1967) found that both size and color of a lizard were important in influencing activity. He concluded that upon emergence, dark coloration afforded greater absorption of insolation and hence a rapid increase in body temperature. As body temperature reached a level suitable for activity, a lizard paled, sunlight ceased to affect coloration and background color-matching assumed dominance. It was also concluded that body size, and its influence on the ability to absorb heat through differential surface-to-volume ratio, affected activity: smaller individuals, able to thermoregulate more rapidly, could exhibit continuous activity whereas larger individuals, having more thermal inertia, had a more restricted activity period.

In these studies, and others not mentioned (see Huey, 1982 for a review and list of references), the predominant interest focused on examination of the physical environment as it related to a reptile's ecology. As studies moved away from the desert southwest into neotropical regions and as more detailed laboratory experiments were conducted, attention shifted to the influence of other external and internal factors on the thermal ecology of reptiles.

In comparing the thermoregulation of five species of tropical Cuban anoles, Ruibal (1961) found that it was not only the physical, but the biotic environment that governed a lizard's behavior. Non-heliothermic lizards inhabiting shaded regions were found to be passively dependent upon environmental temperature as opposed to those in intermediate or exposed habitat that actively thermoregulated. Rand and Humphrey (1968) compared lizard species number in open and forested habitat near Belem, Brazil. They found that heliothermic (open habitat) and non-heliothermic (forest) species showed the same degree of homeothermy, and concluded that more species occupied the forest because of its greater area, stability, floristic/faunal diversity and complex structuring rather than because of its thermal environment. Huey (1974) found that Anolis cristatellus (Sauria, Iguanidae) actively regulated body temperature in the open, but passively tolerated body temperature change in adjacent forest. From this he concluded that thermoregulation was adaptive only when costs in time and energy were low.

Not only were these environmental factors found to impact a reptile's ecology, but internal factors were found to do so as well. Regal (1967) showed that lizards in captivity, housed in constantly running thermal gradients, exhibited voluntary hypothermia at night. This not only correlated with the thermal environment a lizard would be exposed to in nature, but also perhaps some physiological need for cooler temperature in a 24 hr cycle. Garrick (1973) demonstrated that testosterone caused a thermophilic response in Dipsosaurus dorsalis

(Sauria, Iguanidae); Lysenko and Gillis (1980) found the same response after feeding in the snake, Thamnophis sirtalis (Colubridae).

Presently, studies in reptilian ecology attempt to incorporate as many of these influencing factors as deemed necessary. "An emerging, synthetic view suggests that reptilian thermal ecology must reflect a complex interaction among biophysical, biotic, economic and phylogenetic concerns" (Huey, 1982).

Cowles and Bogert (1944) provided the framework for most of the investigation into the thermal relations of reptiles that followed their work. The general categories of reaction to temperature included: the lethal minimum and maximum (the low and high temperatures, respectively, that cause death upon short exposure), the critical minimum and maximum (the low and high temperatures, respectively, which prevent locomotion), the voluntary minimum (the low temperature that induces shelter seeking), the basking range (the range in which the animal seeks heat prior to becoming active), the normal activity range (temperature range at which an animal is found active) and the maximum voluntary tolerance (the high temperature at which shelter is sought).

Within the Normal Activity Range, the Mean Selected Temperature (MST) is defined as "the arithmetic average of the body temperature measured from animals in a thermal gradient" (Pough and Gans, 1982). This value is highly correlated to optimal performance in whole-animal physiological systems (e.g. relative velocity, Huey and Webster, 1976; active metabolism, Bennett and Dawson, 1972). It is a measure of

capacity adaptation (Precht et al., 1973), useful in the analysis of the daily activity of a species.

The critical temperatures bound the tolerance range of a species and are measures of resistance adaptation (Precht et al., 1973), useful in analysis, for example, of geographic distribution. Presently termed the Critical Thermal Maximum (CTMax) and Critical Thermal Minimum (CTMin), these are the temperatures that prevent locomotion in a species and thus represent "ecological death" (Pough and Gans, 1982).

Populations of Anolis carolinensis (Sauria: Iguanidae) occupy a vast region in the southeastern United States: the range extending from ca. 24° to 36° N. Latitude and 76° to 100° W. Longitude. Williams (1969) stated that the northern extent of the range of this species was probably limited by cold temperature; this is intuitive given that the genus is of tropical origin. Since members of the genus Anolis have been shown to exhibit thermal differentiation that reflects ecological distribution (Hertz, 1979), A. carolinensis is an ideal subject for an investigation of whether thermal differentiation might reflect geographic distribution.

A frequently employed technique for determination of body temperature is the use of a thermocouple inserted into the cloaca. To date no tests of whether such manipulation, which is necessarily a disturbance to the animal, alters body temperature. The importance of such a test is of obvious significance. Another potential problem inherent in many investigations is that of collectively housing animals during acclimation. Social interaction, under such a circumstance, might affect the physiological performance tested after acclimation.

An additional factor in the thermal relations of reptiles that has not been investigated, is whether testosterone (already implicated as influential in lizard thermoregulation) affects thermal tolerance. If this hormone is shown to have an effect, then the use of both males and females in such experimentation may unnecessarily increase experimental error. Testosterone level in A. carolinensis is high at the time of emergence from winter refugia (Pearson et al., 1976); any influence that it might have on thermal tolerance, at this critical time, would be of import.

The purpose of this work is to first evaluate possible methodological problems inherent in studies of reptilian thermal ecology (Chapters 2 and 3), then to examine the role that testosterone plays in the determination of the CTMax (Chapter 4). The final study of this work will investigate the possibility of the occurrence of physiological races of A. carolinensis with regard to cold temperature tolerance along a latitudinal gradient (Chapter 5). The rationale for each of these studies is considered in the relevant chapter to follow.

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PART II

THERMOCOUPLE EMPLACEMENT LOWERS BODY TEMPERATURE IN

ANOLIS CAROLINENSIS

INTRODUCTION

Recent laboratory studies of reptilian thermoregulation have commonly involved the insertion in the subject's cloaca of a thermocouple connected to a potentiometric device. This method has been used in measurements of critical thermal maximum (e.g., Kosh and Hutchison, 1972; Hertz, 1979), critical thermal minimum (e.g., Kour and Hutchison, 1970; Spellerberg, 1972, 1973), and mean selected temperature (e.g., Licht et al., 1966; Schuett and Gatten, 1980). Little attention has been paid to the possibility that such manipulation might alter an animal's body temperature. In this report, I describe such a phenomenon and suggest a possible mechanism.

MATERIALS AND METHODS

Adult male Anolis carolinensis were obtained from a commercial dealer (Snake Farm, Laplace, LA,) during October, 1985. Prior to testing, animals were housed in aquaria at 25 ± 2 C with a 12 h light/dark cycle and provided with food (crickets) and water ad lib. Four groups of animals were tested: 1) lizards with a thermocouple fastened in the cloaca (tethered); 2) lizards captured with a gloved hand at 72 h intervals for rapid temperature measurement with a free thermocouple (this time interval was deemed necessary to ensure that the lizards were entirely undisturbed: untethered); 3) dead lizards with thermocouple (no physiological control of water loss); 4) lacquer-coated dead lizards with thermocouple (no evaporative water loss). The testing chambers were styrofoam boxes with lids, walls 1 cm thick (28 x 22 x 25 cm ID). Temperature within the boxes resulted from

laboratory air temperature and perhaps conduction from the lab table; there were no radiant heat sources nearby that could create thermal gradients within them. Monitoring of ambient temperature within the chambers was accomplished by fastening a 30-gauge copper-constantan thermocouple to the floor to indicate substrate temperature and suspending another from the lid so that the tip of the probe measured air temperature in the center of the chamber (Table 2.1). Preliminary studies had shown that both substrate and air temperatures in the boxes were uniform or differed no more than 0.1 C. Animals, provided with food, water and wooden perches, were placed in these boxes 5 days prior to experimentation to allow them to habituate to the novel surroundings. On the morning of testing, the wooden perch and water dish were removed to ensure accurate ambient temperature readings. Relative humidity within the chamber was measured one hour prior to testing with a Cole-Parmer Psychrometer. For tethered testing, a 30-gauge copper-constantan thermocouple was placed approximately 1 cm into the previously emptied cloacas of each of 10 lizards and secured in place with surgical tape which also prevented weight loss due to excretion. Each lizard with its thermocouple was then weighed on a Mettler balance to 0.0001 g and replaced in an individual chamber at 12:30 P.M., C.S.T. Ninety minutes later, and at 30 min intervals over 2.0 h, air, substrate and lizard body temperatures were measured with a Micromite thermocouple meter (Thermo Electric Corp.) with resolution to 0.1 C. At the end of this period lizards were reweighed. Ten individuals in the untethered group were tested once in the afternoon on five occasions at 3 day intervals (food and water were provided

TABLE 2.1. Mean ambient (T_a) and body temperatures (T_b), and temperature differences from ambient (mean difference of $T_b - T_a$) in untethered and tethered Anolis carolinensis. Standard deviation in parentheses.

Untethered*				Tethered*			
Mean T_a	Mean T_b	Mean Difference		Mean T_a	Mean T_b	Mean Difference	
25.1 (± 0.5)	25.0 (± 0.6)	-0.1 (± 0.4)		25.2 (± 0.4)	25.1 (± 0.5)	-0.2 (± 0.1)	
24.9 (± 0.3 ; n=3)	25.6 (± 0.6)	0.7 (± 0.3)		25.2 (± 0.4)	24.9 (± 0.2)	-0.3 (± 0.2)	
25.1 (± 0.4 ; n=4)	25.6 (± 0.3)	0.5 (± 0.3)		25.1 (± 0.4)	24.8 (± 0.2)	-0.3 (± 0.3)	
25.1 (± 0.3)	25.7 (± 0.8)	0.6 (± 0.7)		24.5 (± 0.2)	24.0 (± 0.3)	-0.5 (± 0.2)	
25.1 (± 0.3)	25.3 (± 0.5)	0.2 (± 0.3)		24.1 (± 0.1)	23.3 (± 0.3)	-0.9 (± 0.4)	
24.9 (n=1)	25.2	0.3		23.9 (± 0.2)	23.4 (± 0.2)	-0.5 (± 0.2)	
25.0 (± 0.3)	25.5 (± 0.3)	0.5 (± 0.3)		25.0 (± 0.2)	24.3 (± 0.1)	-0.3 (± 0.1)	
24.9 (± 0.3)	24.9 (± 0.4)	0 (± 0.3)		24.7 (± 0.1)	24.3 (± 0.1)	-0.3 (± 0.1)	
24.8 (± 0.2)	25.0 (± 0.3)	0.2 (± 0.3)		24.8 (± 0.1)	23.0 (± 0.8)	-1.8 (± 0.8)	
24.7 (± 0.3 ; n=4)	25.0 (± 0.4)	0.3 (± 0.3)		24.4 (± 0.2)	23.8 (± 0.2)	-0.6 (± 0.2)	
GRAND MEANS	25.3	0.3		24.7	24.1	-0.6	
S.D.	± 0.3	± 0.3		± 0.5	± 0.7	± 0.5	

*Temperature values represent an average of five measurements except where noted in parentheses.

+Values represent averages of five temperature measurements recorded over a 2 hr period at 30 min intervals.

throughout this period). Substrate and air temperatures were recorded just prior to opening the chamber; the lizard was grabbed with a gloved hand, and a thermocouple was inserted into the cloaca. This procedure required an average of 8 s. To determine the degree to which holding the lizard with a gloved hand altered body temperature, 10 lizards were equipped with a thermocouple and placed into test chambers. After 2 h, body temperature was recorded, the chamber opened, and the lizard captured and held for 8 s after which temperature was again recorded. This procedure raised body temperature by 0.1 ± 0.3 C, a handling correction which was subtracted from all subsequent measurements for this test group. Ambient temperature for the dead and dead-sealed groups was measured by placing the substrate probe 1 cm from the lizard and suspending the air probe 2 cm over it. Three animals were killed by intraperitoneal injection of pentobarbital at 11:00 A.M., C.S.T., and subjected to the same temperature monitoring procedure as that of the tethered group. The dead-sealed group consisted of 3 individuals killed as above, coated with lacquer, allowed to dry for 24 h and tested as above.

Mann-Whitney U tests were used to compare the ambient temperatures and percent relative humidities of the tethered and untethered treatments. A Spearman rank correlation test was used to determine whether relative humidity was associated with percentage mass loss in the tethered group; T-tests were used to determine whether mass loss was significantly different from zero in the tethered, dead and dead-sealed treatments.

A Kruskal-Wallis test was used to determine whether heterogeneity occurred within the 4 groups for the difference in body temperature versus ambient temperature (the average of the substrate and air temperatures at the time of measurement) followed by a similar test for heterogeneity among groups. Mann-Whitney U tests were then used to identify differences in body temperature from ambient temperature among adjacent groups (this test was necessary as the repeated measures on the same individuals violated the assumption of independence for many types of parametric statistics).

RESULTS

Ambient temperatures (Table 2.1) and relative humidities (mean = $51 \pm 5\%$) were not found to be statistically different in the tethered and untethered treatments. In addition, relative humidity was not correlated with percentage mass loss in the tethered group ($p < 0.05$).

Eight of the 10 untethered animals had temperatures above ambient and the mean difference for all 10 lizards was $+0.4 (\pm 0.3 \text{ S.D.})^\circ\text{C}$ (Table 2.1). Body temperatures recorded over a 2 h period for tethered animals (thermocouples fastened in place) were always below ambient with a group mean for 10 animals of $-0.5 (\pm 0.5)^\circ\text{C}$. Significant heterogeneity within a treatment was found for only the tethered group; this may have resulted from either difference in hydration state and/or individual differences in susceptibility to stress. Heterogeneity among the treatment groups for difference between body and ambient temperature was highly significant ($p < 0.0001$). The dead vs. dead-sealed groups, (Table 2.2) were not significantly different ($p > 0.05$).

TABLE 2.2. Mean ambient (T_a) and body temperatures (T_b), and temperature differences from ambient (mean difference of $T_b - T_a$) in dead and dead-sealed Anolis carolinensis. Standard deviation in parentheses.

	Dead†			Dead-sealed†		
	Mean T_a	Mean T_b	Mean Difference	Mean T_a	Mean T_b	Mean Difference
	23.9 (± 0.1)	23.8 (± 0.1)	-0.2 (± 0.05)	21.5 (± 0.1)	21.3 (± 0.1)	-0.2 (± 0.1)
	23.8 (± 0.2)	23.7 (± 0.05)	-0.2 (± 0.1)	21.3	21.1 (± 0.1)	-0.2 (± 0.1)
	23.8 (± 0.05)	23.6	-0.2 (± 0.05)	21.3 (± 0.1)	21.2 (± 0.1)	-0.1 (± 0.1)
GRAND MEANS	23.8	23.7	-0.2	21.4	21.2	-0.2
S.D.	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1

†Values represent averages of 5 temperature measurements recorded over a 2 hr period at 30 min intervals.

Living tethered animals lost between 1 and 2% of their total body mass, presumably in evaporative water over a 3.5 h period (Table 2.3). Mass loss in the dead animals was also significant ($p < 0.05$); these animals fitted with thermocouples also had a temperature below ambient (Table 2.2). Dead animals sealed with a coat of clear fingernail lacquer exhibited no loss of mass and produced temperatures only slightly cooler than ambient, (-0.1 C).

DISCUSSION

Monitoring the body temperature of the lizard, Anolis carolinensis, and, perhaps, other reptiles, by means of a cloacal thermocouple fastened in place may produce values for core temperature that average as much as 0.5 C lower than ambient temperature (Table 2.1). Though lower body temperatures in thermocoupled animals have been reported (e.g., Warburg, 1965), the mechanism was not determined. Our results suggest that reduced body temperature is due to evaporative cooling by means of cutaneous water loss. Panting was never previously observed in tethered animals, so the most probable routes of water loss would be evaporation in the vicinity of the cloaca (capillary action under the tape covering the cloaca) and/or evaporation through the skin over the entire body. Many authors (e.g., Minnich, 1970; Heatwole and Vernon, 1977; Mautz, 1980) have found that rates of water loss in reptiles increase with activity. Claussen (1967) found that initial rates of water loss in A. carolinensis and Uta stansburiana after placement into a desiccation apparatus were unusually high compared to those that occurred later. He considered this to be an anomaly that

TABLE 2.3. Mass loss expressed as percent initial mass (grams) in Anolis carolinensis fitted with thermocouples. Only the values from the tethered and dead animals are significantly different from 0.0 ($p < 0.05$). Values in parenthesis are $\pm$ standard deviation.

	Tethered	Dead	Dead-sealed
	0.3	0.3	0.0
	2.0	0.3	0.0
	1.5	0.4	0.0
	0.7		
	0.4		
	0.5		
	1.1		
	1.2		
	5.7		
	2.3		
MEANS	1.6(± 1.6)	0.3(± 0.1)	0.0

might have resulted from increased activity. Activity was not quantified in Claussen's experiment; it is possible that the introduction to a novel environment functioned as a stressor and altered physiological response. I suggest that, in addition to possible elevated evaporative water loss from the cloacal region, there might also be an increased skin permeability related to a stress response. It is possible that the thermocouple functions as a "stressor" similar to the handling "stress" discussed by Greenberg (1983). An increase in cutaneous water loss might result from increase in rate and strength of heart contraction with an attendant increase in blood pressure (Kirby and Burnstock, 1969) or parasympathetic rebound. Either of these could increase blood flow to the periphery, hydrate the resistance barrier of the skin and increase the rate of evaporative water loss (Gans et al., 1968). This, however, was not tested in this study.

Investigators interested in comparing the body temperatures of heterothermic animals to the temperatures of their environment should be aware of the potential error inherent in the thermocouple tethering method. The temperature decrement caused by emplacement of a thermocouple in A. carolinensis, while small, should be of interest to those concerned with the thermal/hydric relations in reptiles, especially when data are compiled from different sources in which different methodologies for the determination of body temperature were employed.

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PART III

AEROBIC AND ANAEROBIC RESPIRATION DURING ACUTE HANDLING AND
SOCIAL STRESS IN ANOLIS CAROLINENSIS

INTRODUCTION

The degree to which intraspecific aggression in reptiles and amphibians influences metabolism has been the subject of recent investigation (e.g. Bennett and Houck, 1983; Pough and Andrews, 1985). Aggression, in these cases, was measured in terms of biting, chasing and display by residents and intruders during territorial encounters. Much of the work has focused on the reliance of anaerobic metabolism during these short-term events (e.g. Bennett et al., 1981), but the importance of aerobic metabolism during such encounters has also been established (Bennett and Houck, 1983). One species of reptile, the lizard Anolis carolinensis (Iguanidae), has been the subject of extensive investigation of aggression, some of it focused on the role of the autonomic nervous system (Greenberg, 1983).

It has been shown that males of this species readily form dominance hierarchies in laboratory settings with dominants remaining green and subordinates becoming more brown. Body color in this species is controlled exclusively by epinephrine, norepinephrine, and melanocyte stimulating hormones (Greenberg and Crews, 1982). With acute stress, the catecholamines, norepinephrine and epinephrine, are released from post-ganglionic nerves and chromaffin tissue of the adrenal gland. Under such circumstances, the levels of these hormones increase 10-30 times (Greenberg, Cox and Howley, unpublished data). Chronic stress results in the secretion of glucocorticoids, predominantly corticosterone in reptiles (Licht and Bradshaw, 1969), by the interrenal tissue of the adrenal gland. The shift from green to brown is effected by the stimulation of beta-adrenergic receptors by

epinephrine. Corticosterone facilitates the methylation of norepinephrine to epinephrine by phenylethanolamine N-methyltransferase (Wurtman and Axelrod, 1965; Wurtman et al., 1967), enhancing this color shift.

Corticosterone was shown to have no effect on whole body oxidative metabolism in the lizard, Calotes versicolor (Agamidae) (Thapliyal and Gupta, 1984), but epinephrine was shown to cause an increase. Coulson and Hernandez (1979) found that epinephrine caused an increase in lactic acid concentration in Alligator mississippiensis (Crocodylia, Alligatoridae). That acute stress causes an increase in both aerobic and anaerobic metabolism appears likely. The purpose of this study was to investigate the roles of acute handling and social stress in the metabolism of the lizard Anolis carolinensis and to attempt to inhibit the influence of norepinephrine and epinephrine using the adrenergic blocking agents, yohimbine and propranolol.

MATERIALS AND METHODS

Animals

The male anoles used in this study ($n = 70$; mean mass 4.761 g, S.D. = 1.127 g, range = 2.450 - 8.143 g) were obtained from commercial dealers in Louisiana and Mississippi, in August 1986 and May 1987, respectively. Prior to experimentation they were housed 3.78-l clear glass jars at 25 ± 2 C, provided with wooden perches, water and food ad libitum, but fasted for two days prior to experimentation (animals in the yohimbine treatment and control were fasted for three days). All measurements were carried out at 25 ± 0.5 C.

Aerobic Metabolism

Ten animals were weighed and placed into individual, clean metabolic chambers made from clear 4.4. cm I.D. plexiglas pipe (volume of 350 ml) in a darkened constant temperature cabinet (B.O.D.) at 25 C and allowed to rest overnight while the chambers were ventilated with humidified room air. In the morning, resting oxygen consumption of each animal was measured in duplicate. Following these measurements, the chambers were removed from the constant temperature cabinet and placed on a laboratory bench behind black screens, and the resting oxygen consumption of animals measured again in duplicate. These measurements were intended to reveal change in resting aerobic metabolism due to room light and noise. Next, the chambers were opened and a second, "intruder" male that had been resting undisturbed in an individual container in the laboratory was introduced into each metabolic chamber occupied by a "resident". The rate of oxygen consumption of the pair was then determined. During this 30 min measurement period, both animals were continually observed through a small window in the black screen and notes taken every 30 sec as to any movements, aggressive displays or encounters, and as to skin color of both animals. These measurements and observations were designed to reveal any increase in oxygen consumption due to aggressive interactions. Finally, to control for possible changes in oxygen consumption brought about simply by handling or by the introduction of an animal into a previously occupied chamber, two additional sets of measurements were made. In the first, five new animals that had been resting in individual containers in the laboratory were introduced into

clean chambers and their resting metabolic rate determined. In the second, five new animals were introduced to chambers previously occupied by other males, and their rates of oxygen consumption determined. In these last two situations, measurements were made with the metabolic chambers resting on a laboratory bench behind the black screen and under room lighting. The animals were visually isolated from other individuals, and their skin color was observed every five minutes. An additional handling control and the yohimbine/propranolol tests were conducted using lizards obtained from Mississippi (a locality ca. 200 miles from the Louisiana source). There was no previous test for possible change in oxygen consumption of the resident due to the opening and closing of the metabolic chamber (for introduction of an intruder), so this was conducted in May after measurements for seasonal difference in oxygen consumption of animals resting in the dark and lighted room. In an attempt to block the potential effects of epinephrine and norepinephrine released during stress, two groups of five animals (with two similar groups of saline controls) were given a .1 cc injection of either propranolol at a dose of 20 mg/kg (Vaughan and Greenberg, 1987) or yohimbine at a dose of 1 mg/kg (Goodman and Gilman, 1970), 30 minutes prior to introduction to metabolic chambers. To ensure that the lizards were stressed, they were vigorously handled for two minutes prior to introduction. Skin color was observed every five minutes. All measurement periods were 30 min, and temperature in each case was 25 C.

Oxygen consumption was determined by a closed system method. Each chamber was sealed and a 10 ml sample of chamber air withdrawn into a

syringe. At the end of the measurement period, a second sample was withdrawn. The oxygen concentration of air samples was determined by pumping the sample through a small tube containing Drierite and Ascarite (to remove water vapor and carbon dioxide, respectively) and into an Applied Electrochemistry S-3A oxygen analyzer. The output of the analyzer was displayed on a strip chart recorder and also passed via an Interactive Structures AI13 analog to digital converter into an Apple IIe computer. This system allowed the oxygen concentration to be read to 0.001%. The rate of oxygen consumption was calculated from the pre- and post-samples of chamber air and the volume of air in each chamber using the equation of Gatten (1985). Values are reported as ml of oxygen consumed (STPD) per gram of body mass per hour.

Anaerobic Metabolism

The reliance of anoles on glycolysis in the same situations described above was determined by measurements of whole body lactate concentration. Ten animals rested overnight in individual containers in the lab at 25 C were quickly frozen in liquid nitrogen. In addition, the pairs of animals used to measure aerobic metabolism were frozen immediately after the second air sample was drawn at the end of the 30 min period of interaction (the resident and intruder could be identified by previously applied markings). Finally, the animals used in measurements of oxygen consumption in clean and previously occupied chambers were also frozen immediately after the second air samples were drawn. In May, additional controls for room light/noise and opening/closing the metabolic chamber were conducted after a

measurement for seasonal difference in whole body lactate concentration in ten animals rested overnight in the laboratory was made. Measurements for the yohimbine and propranolol groups were also conducted at this time. Frozen animals were weighed to 0.001 g and homogenized in cold 1.0 N perchloric acid. The homogenate was centrifuged at 9770 x g for 30 min, then filtered and frozen. The lactate concentration of each animals was determined in duplicate using an enzymatic method (Bergmeyer, 1974) and a spectrophotometer at 340 nm. Extrapolation from absorbance to lactate concentration was achieved by the use of a standard curve, from standards measured along with each set of samples. If samples had an absorbance value above that of the standards, they were diluted and measured again. Lactate concentrations are reported as ug of lactate per gram of body mass.

Statistics

ANOVA was used to first test for heterogeneity, followed by least significant difference tests (LSD) to compare mean VO_2 and whole body lactate accumulation in the handling and social experimental groups; $\pm$ - tests were conducted to determine whether any of the activity levels within a category were significantly different from zero, to test whether introduction to a clean vs. previously occupied chamber, turning brown after being introduced to a chamber, and if propranolol and yohimbine influenced oxygen consumption and lactate accumulation. Spearman rank correlation tests were employed to examine the association of 1) Total percentage of the 30 sec intervals in which movement occurred with oxygen consumption of the pairs, 2) Individual

percentage of the 30 sec intervals in which movement occurred with lactate accumulation, 3) Total percentage of the 30 sec intervals in which either the resident or intruder of the pairs exhibited brown coloration with oxygen consumption, 4) Percentage of intervals in which the intruder exhibited brown coloration with its lactate accumulation, 5) Percentage of intervals in which the resident exhibited brown coloration with its lactate accumulation, 6) Oxygen consumption with the difference in mass (resident - intruder) in the pairs, 7) Total lactate accumulation in both animals in the pairs with difference in mass, 8) Lactate of the resident with difference in mass, 9) Lactate in the intruder with difference in mass. Results were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Activity of Pairs

No movement was observed in pairs of animals in metabolic chambers in $91 \pm 16\%$ of the intervals scored. Movement was observed in a mean of $7\% \pm 10\%$ of the intervals, but this was found to be significantly greater than zero. Aggressive display was observed in only three of the residents and the mean of $2 \pm 7\%$ was not significantly different from zero. Neither oxygen consumption nor total combined lactate accumulation in both animals of a pair was significantly correlated to percentage movement, so activity within the chamber was not considered to have influenced the results obtained in this experimental group. Activity in other treatments, though not quantified, was similarly minimal.

Oxygen Consumption

ANOVA found significant heterogeneity among treatment groups in VO_2 ($p < 0.00001$). Figure 3.1 depicts the results of a Least Significant Difference test of the experimental groups. The month of testing was not found to influence resting oxygen consumption rate, so all experiments were considered comparable. Moving the prospective resident from the B.O.D. to the lighted laboratory significantly increased oxygen consumption, but opening and closing the chamber (a handling factor necessary for the introduction of an intruder) did not significantly increase this over the rate of an animal moved into the laboratory without opening and closing its chamber. The VO_2 of a resident plus intruder was significantly greater than the latter treatment. Placing an animal into a clean or previously occupied metabolic chamber (the handling stress experienced by an intruder) did not significantly increase oxygen consumption (per gram body mass) compared to the rate of paired animals, but the increase was significantly higher than the VO_2 of a lizard following the opening and closing of the chamber. Rate of oxygen consumption of animals introduced to a clean chamber did not differ from animals introduced to a previously occupied one. Six of the ten lizards introduced to an empty chamber turned from green to brown and had a VO_2 significantly greater than the subjects that remained green, so it is possible that the intruder (which turned brown in $85 \pm 30\%$ of the intervals scored) was contributing more to the increase in rate of oxygen consumption than the resident (which remained green in $80 \pm 30\%$ of the intervals) in the pairs, but this could not be determined. The percentage of

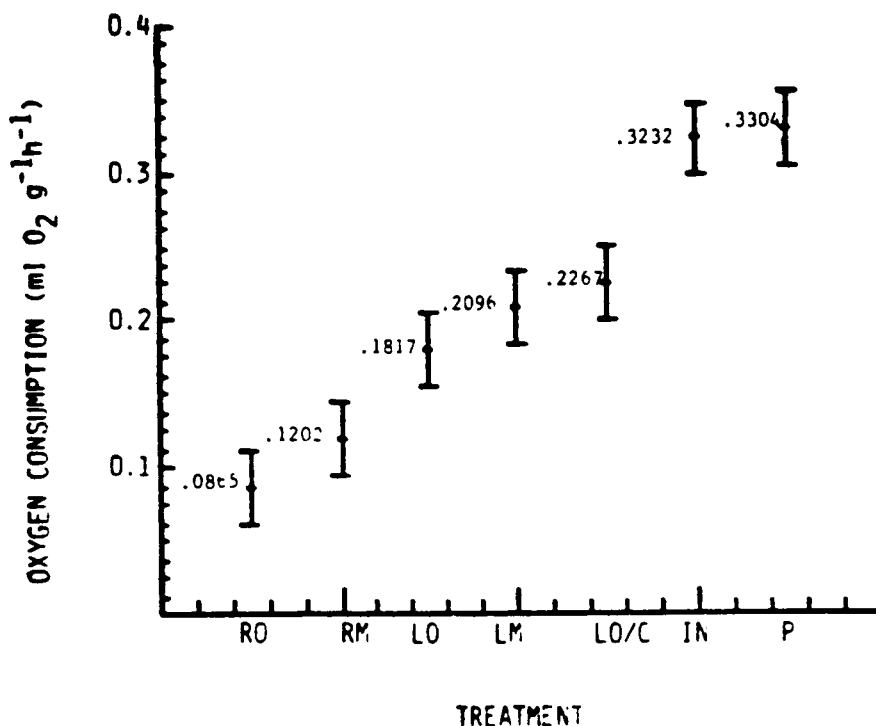


Figure 3.1. 95% LSD intervals (with means) of VO_2 measurements for the residents and intruders during handling and with conspecifics. Resident handling treatments include: Resting in a metabolic chamber in a B.O.D. overnight in October 1986 (RO) and May 1987 (RM); resting in B.O.D. overnight and brought into the laboratory in October 1986 (LO) and May 1987 (LM); the same treatment as in LO and LM, then the metabolic chamber opened and closed (LO/C). The intruder handling treatment (IN) is resting in cups overnight in the laboratory and introduced to a clean or dirty metabolic chamber. (P) is the VO_2 of the animals in pairs ($N=10$ in all but the pairs where $N=20$).

brown coloration scores of both lizards in a pair was not correlated to VO_2 , so whether this bioassay is relevant to oxygen consumption in a social context remains a question. Finally, the correlation between the difference in mass of a resident and intruder with VO_2 was also insignificant.

Lactate Accumulation

ANOVA found significant heterogeneity among treatment means for lactate concentration ($p < 0.00001$). Results of the Least Significant Difference test are shown in Figure 3.2. Month of testing was found not to influence lactate accumulation so, as with the oxygen consumption treatments, all of these tests were considered comparable. Moving the prospective resident into the lighted laboratory from the B.O.D., opening and closing the chamber, and introduction of an intruder all resulted in no significant increase of whole body lactate of the resident. Introduction of an animal to an empty chamber significantly increased lactate, but there was significantly less accumulation in this latter group than when an intruder was introduced to a chamber with a resident. These results indicate that mild handling stress does not significantly affect glycolysis, but that more extreme handling and social stress increases anaerobic metabolism. Lactate levels in animals introduced to a clean or previously occupied chamber were not significantly different. Introduction to an empty chamber and turning brown did not influence lactate level in animals. It seems that there is a greater reliance on aerobic metabolism in individuals experiencing extreme handling stress. The percentage of brown

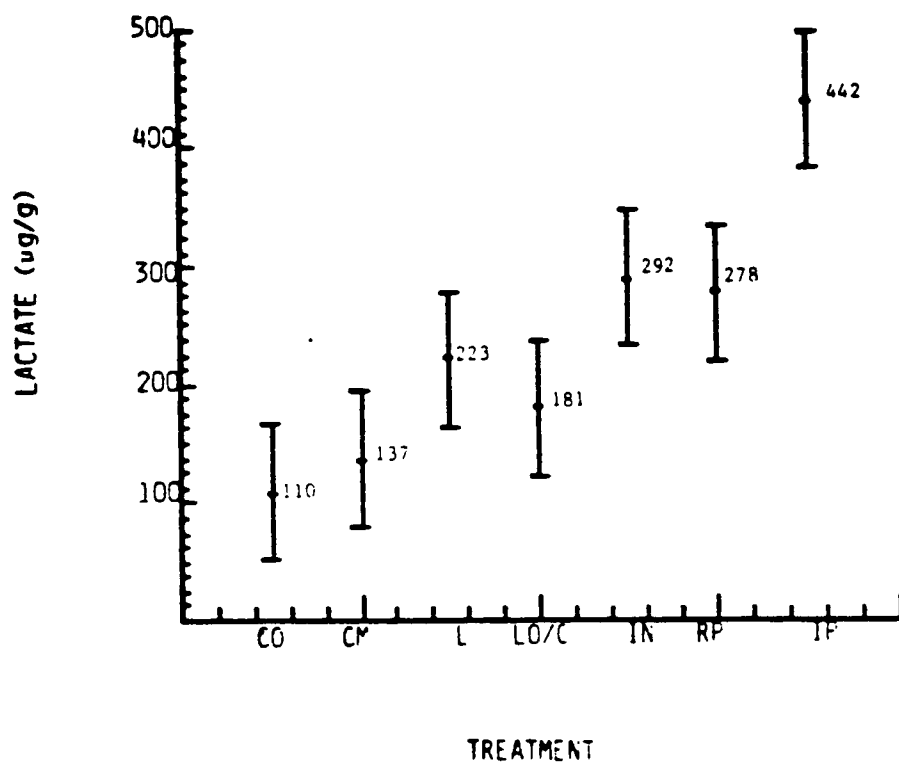


Figure 3.2. 95% LSD intervals (with means) of lactate concentrations (ug/g) for residents and intruders during handling and with conspecifics. Resident treatments include: (L) Resting in the metabolic chamber in a B.O.D. overnight, brought out into the laboratory, air samples taken; (LO/C) the same treatment as in L, then the metabolic chamber opened and closed, air samples taken; (RP) resident of a pair. Intruder treatments include: Resting in cups overnight in the laboratory in October 1986 (CO) and May 1987 (CM), resting in cups overnight in the laboratory and introduced to a clean or dirty metabolic chamber (IN) and intruder of a pair (IP) (N=10 in each treatment).

coloration scores in resident's and intruder's were not correlated to lactate.

Difference in mass of a resident and intruder was significantly negatively correlated to the total lactate of both. The likely cause of this association was the increase in lactate of the intruder because lactate of the resident was not correlated with difference in mass, but lactate of the intruder was (Fig. 3.3). It appears that the larger an intruder is compared to the resident, the greater the rate of glycolysis in the intruder. This may result from a decreased likelihood of the intruder to readily accept subordinate status when it is larger than the resident: i.e., the possibility of an aggressive encounter is greater and this possibly increases the amount of social stress experienced by the intruder. If, however, the intruder is a great deal larger than the resident (the outlier on Fig. 3.3) the degree of reliance upon anaerobic metabolism is much less, possibly because the likelihood of an aggressive encounter is decreased.

Propranolol and Yohimbine Treatments

There was no detectable influence of propranolol or yohimbine on rate of oxygen consumption or lactate accumulation although there were dramatic increases in VO_2 and lactate accumulation (Table 3.1) observed in both experimental groups and controls. It seems likely that these were the result of sympathetic nervous system arousal. The dose of propranolol injected has previously been shown to effectively block the action of epinephrine in this species (Vaughan and Greenberg, 1987), but the dose of yohimbine may not have been sufficient to block alpha-

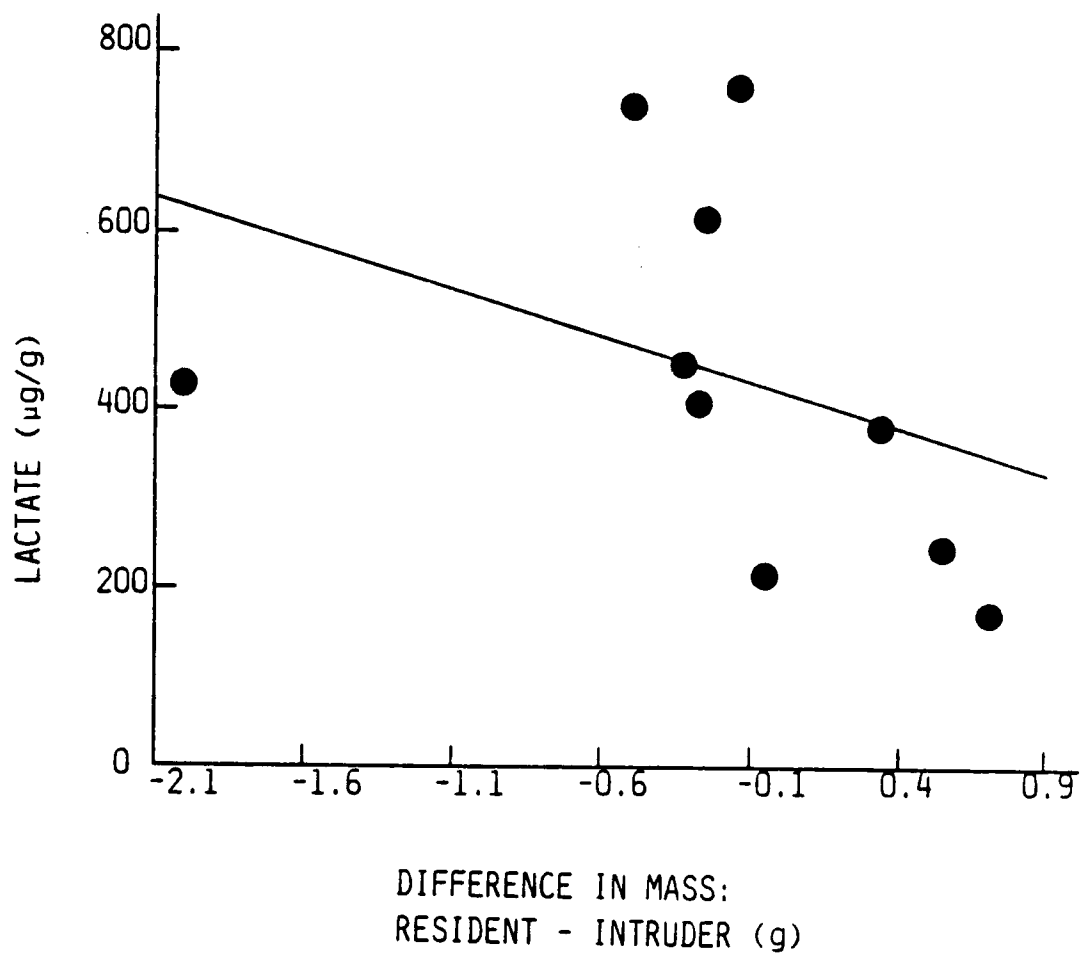


Figure 3.3. The relationship of the difference in mass of a resident-intruder and the lactate concentration of the intruder. The Spearman rank correlation coefficient of -0.66 is statistically significant ($P=0.47$).

TABLE 3.1. Mean oxygen consumption ($\text{ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$) and lactate accumulation (g/g) in stressed Anolis carolinensis after injection of propranolol or yohimbine (with their respective controls).

Treatment	Mean Oxygen Consumption ($\pm$ S.D.)	Mean Lactate Accumulation ($\pm$ S.D.)
Propranolol	.3604 ($\pm$.0558)	602.6 ($\pm$ 196.9)
Saline Control	.3577 ($\pm$.0432)	770.8 ($\pm$ 100.9)
Yohimbine	.3538 ($\pm$.0390)	660.6 ($\pm$ 38.5)
Saline Control	.3716 ($\pm$.0511)	638.6 ($\pm$ 105.8)

receptors (and therefore norepinephrine), though a similar dose of another alpha-blocker, phenotolamine, has been shown effective with other reptiles (e.g. Lillywhite and Seymour, 1978). Such dramatic increases in lactate and VO_2 probably resulted from enhanced muscular contraction (which was undetected) brought about by direct nervous stimulation. Much of the increase in lactate was probably a result of contraction of white skeletal muscle which rapidly undergoes anaerobic respiration (Gleeson et al., 1980).

CONCLUSIONS

Mild disturbances, such as moving a metabolic chamber, necessary for laboratory manipulation of lizards produce significant increases in rate of oxygen consumption (Fig. 3.1), whereas similar disturbance fails to increase reliance on anaerobic metabolism (Fig. 3.2). Stronger disturbance, such as placing an individual into an empty metabolic chamber, results in significant increase in aerobic (compared to animals resting in the laboratory, Fig. 3.1) and anaerobic (compared to animals in cups in the laboratory, Fig. 3.2) metabolism.

Though introducing an animal to a metabolic chamber caused an increase in VO_2 that was not different from the VO_2 of animals in pairs on a per gram basis, an increase in oxygen consumption in a social encounter between two males, in which activity is negligible, was detected (Fig. 3.1: opening and closing a chamber vs. pairs). Intruders experience significantly greater lactate accumulation than residents; this is influenced by their difference in size.

An attempt to block potential metabolic effects of the catecholamines epinephrine and norepinephrine was unsuccessful. Injection of either propranolol or yohimbine had no effect on VO_2 and lactate level. Increases in aerobic and anaerobic metabolism during handling and social stress were likely influenced by nervous stimulation to the musculature.

Acute handling and social stress of lizards have potential influence in laboratory experimentation. If, for example, fighting bouts are staged in a laboratory setting, care should be taken to ensure that handling of both subjects is kept to a minimum because of the possibility of artificially increasing their lactate levels. In addition, an intruder may be predisposed to earlier fatigue than the resident, prior to an aggressive encounter, as a consequence of an increase in anaerobic metabolism experienced when entering a novel environment. Another potential confounding effect of social stress could result from collectively housing animals during acclimation prior to experimentation. If such animals readily form dominance hierarchies in a laboratory setting, such as is known for Anolis carolinensis (Greenberg, 1983), experimental error in tests that follow may be increased due to the enhanced metabolic demand experienced by subordinates during acclimation. For example, if such a demand results in starvation of the animal, this may decrease its thermal tolerance (Larson, 1961). Whether the short term increased metabolic demand due to social stress found in this study extends for a longer time period remains to be tested.

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PART IV

EFFECT OF TESTOSTERONE ON THE CRITICAL THERMAL

MAXIMUM OF ANOLIS CAROLINENSIS

INTRODUCTION

A commonly employed approach in studies of the thermal ecology of ectotherms has been the identification of limits of thermal tolerance, useful in attempts to understand adaptation to extreme conditions. One such measure is the critical thermal maximum (CTMax), "...the arithmetic mean of the collective thermal points at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death" (Lowe and Vance, 1955).

The magnitude of CTMax is affected by thermal acclimation (Ballinger and Schrank, 1970), starvation (Larson, 1961), and there is a circadian rhythm in thermal sensitivity in lizards (Kosh and Hutchison, 1972). Neither sex nor size influences tolerances in lizards (Art and Claussen, 1982), but photoperiod has been shown to affect CTMax in the lizard Anolis carolinensis (Iguanidae) (Licht, 1968; Kosh and Hutchison, 1972). Licht (1968) found that the effect that a long light period had on increasing the CTMax of A. carolinensis occurred only after the acclimation to 20 C and not at higher temperature. Under similar conditions, Pearson et al. (1976) found that androgen production in this species is optimal and that it declines with increasing temperature.

Testosterone has previously been shown to influence thermoregulation in lizards: Garrick (1973) found that it causes a thermophilic response in Dipsosaurus dorsalis (Iguanidae). This study was conducted to determine whether the CTMax of A. carolinensis acclimated to 20 C is affected by testosterone.

MATERIALS AND METHODS

Adult male Anolis carolinensis were obtained from a commercial dealer in Louisiana during October, 1985. Prior to castration, the lizards were held in the laboratory at 25 ± 2 C (12L:12D photoperiod) for ca. 2 months in 3.78 l clear glass jars (visually isolated from one another) supplied with wooden perches; food (crickets) and water were provided ad libitum.

After 10-20 minutes cryoanesthesia, animals were castrated as described by Crews (1974): A 5 mm ventrolateral incision was made, testes palpated through the incision, ligated, removed and the site cauterized. 5-0 or 6-0 silk with a cutting needle was used to close. Subcutaneous 5 mm implants of either empty or testosterone (testosterone propionate) filled silastic tubing (0.025 in. i.d., 0.047 in o.d.) were then made (adapted from Crews et al., 1978). Castrates were acclimated to 20 ± 2 C (12L:12D photoperiod) for 10 days. They were housed, fed and watered as described above, but food was withheld 24 hr prior to testing. There were 20 individuals in each group.

Lizards were heated in a glass bowl with sand substrate under an infrared light (with the intensity of illumination controlled by a rheostat) at a rate of increase in body temperature of ca. 1 C per minute, standard for such tests (e.g., Hertz et al., 1979). All tests were conducted between 12:00 P.M. and 4:30 P.M., E.S.T. to account for any influence of circadian rhythm in heat sensitivity (Kosh and Hutchison, 1972). Body temperature was monitored with a 30-gauge copper-constantan thermocouple inserted ca. 1 cm into the cloaca, taped

at the base of the tail, and connected to a potentiometer (Thermo-Electric micromite).

As the subjects were heated, the following responses were noted and body temperatures recorded: an attempt to escape by jumping or vigorous clawing (experimental voluntary maximum, Ballinger et al., 1970), gape (panting threshold, Heatwole et al., 1973) and onset of muscular spasms (Hutchison et al., 1966). The latter was the indicator of the CTMax. T-tests were used to compare the body temperatures for these responses between the treatment groups. Animals that failed to exhibit all of the responses were excluded from statistical analysis.

RESULTS AND DISCUSSION

Testosterone does not influence the CTMax of A. carolinensis acclimated to 20 C: No significant difference was found for treatment in any of the responses (Table 4.1). This finding is supported by other investigators who observed no sexual differences in CTMax for A. carolinensis and other lizard species (Kour and Hutchison, 1970; Kosh and Hutchison, 1972; Hutchison and Kosh, 1974).

Testosterone production is probably influenced by photoperiod via pineal melatonin secreted during the cool, dark phase (Underwood, 1985a). Melatonin has been shown to have an antigonadal effect in lizards (Thapliyal and Halder, 1979; Underwood, 1985b). This hormone has also been shown to influence thermoregulation: It depresses the mean selected temperature in the turtle, Terrapene carolina triunguis (Emydidae) (Erskine and Hutchison, 1981). Natural conditions of increasing photoperiod and low environmental temperature favor high testosterone and low melatonin levels; it is under those conditions

Table 4.1. Mean temperatures (C, $\pm$ 1 standard deviation) of the experimental voluntary maximum (EVM), panting threshold (PT) and onset of spasms (OS = CTMax) for the treatment groups Testosterone and Blank with associated T-tests. No significant differences in response temperatures between treatments were found ($p > .05$).

	EVM	PT	OS	n
Testosterone	36.6 $\pm$ 1.8	39.0 $\pm$ 1.5	43.5 $\pm$.07	17
Blank	35.7 $\pm$ 2.0	38.8 $\pm$ 1.4	43.2 $\pm$ 1.2	11
P (T-test)	.22	.73	.44	

that A. carolinensis emerges from winter refuge in the early spring. Our study indicates that testosterone does not influence high temperature tolerance at this critical time.

Gonadal androgen is, however, known to influence the aggression involved in the establishment of a dominance hierarchy in A. carolinensis under laboratory conditions (Greenberg et al., 1984). Dominant individuals have been shown to have greater access to thermal resource and food under these circumstances, and both can influence the magnitude of heat resistance. Regal (1968) found that dominant Gerrhonotus multicarinatus (Sauria: Anguidae) selected higher body temperature in a thermal gradient when paired with a subordinate. Bels (1984) showed that dominant A. carolinensis had more access to a thermal resource than subordinates. The availability of high environmental temperature has been shown to enhance heat tolerance (e.g., Art and Claussen, 1982). Bels (1984) also found that socially dominant lizards gained weight while subordinates either gained less or actually lost weight during a seven week period in which they were housed together. Starvation lowers the CTMax of lizards (Larson, 1961).

The known influence of testosterone on aggression may have important effects on the CTMax of lizard species, and therefore may increase the experimental error in thermal tests of individuals that are housed together during acclimation. Although time and effort would be increased, housing animals individually with visual isolation from conspecifics should improve experimental precision.

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PART V

GEOGRAPHIC VARIATION IN THE CRITICAL THERMAL MINIMUM OF
ANOLIS CAROLINENSIS ALONG A LATITUDINAL GRADIENT

INTRODUCTION

Anolis carolinensis Voigt (Sauria: Iguanidae) is the only member of its genus native to the United States where it occupies a vast region in the southeast. The presumed colonizer and progenitor of A. carolinensis is A. porcatius from Cuba (Williams, 1969). Immunological evidence indicates that this colonization took place during the Pliocene (Buth et al., 1980). Genetic analyses suggest the likelihood of at least two colonizations: one in south Florida and one along the central Gulf Coast west of the Florida peninsula. It is hypothesized that dispersal occurred from this latter colonization resulting in the present distribution of most North American populations; the former colonization resulting in those populations presently found in southwestern Florida (Wade et al., 1983).

Figure 5.1 indicates this distribution along with average January temperatures for the southeastern United States. Williams (1969) stated that the distribution of A. carolinensis is probably restricted in the north by temperature. Examination of macroclimatic data for the southeastern U.S. suggests that this may be true and it is intuitive that cold temperature might restrict an ectotherm of tropical origin.

Most studies concerning the thermal relations of this species have been conducted on specimens obtained from a commercial dealer in Louisiana (e.g., Licht, 1968; Kour and Hutchison, 1970; Kosh and Hutchison, 1972; Art and Claussen, 1982) with a few studies of animals obtained elsewhere (e.g., Corn, 1971; Clark and Kroll, 1974). These studies have involved the eccentric, mean selected and critical thermal maximum (CTMax) temperatures and only one was concerned with the

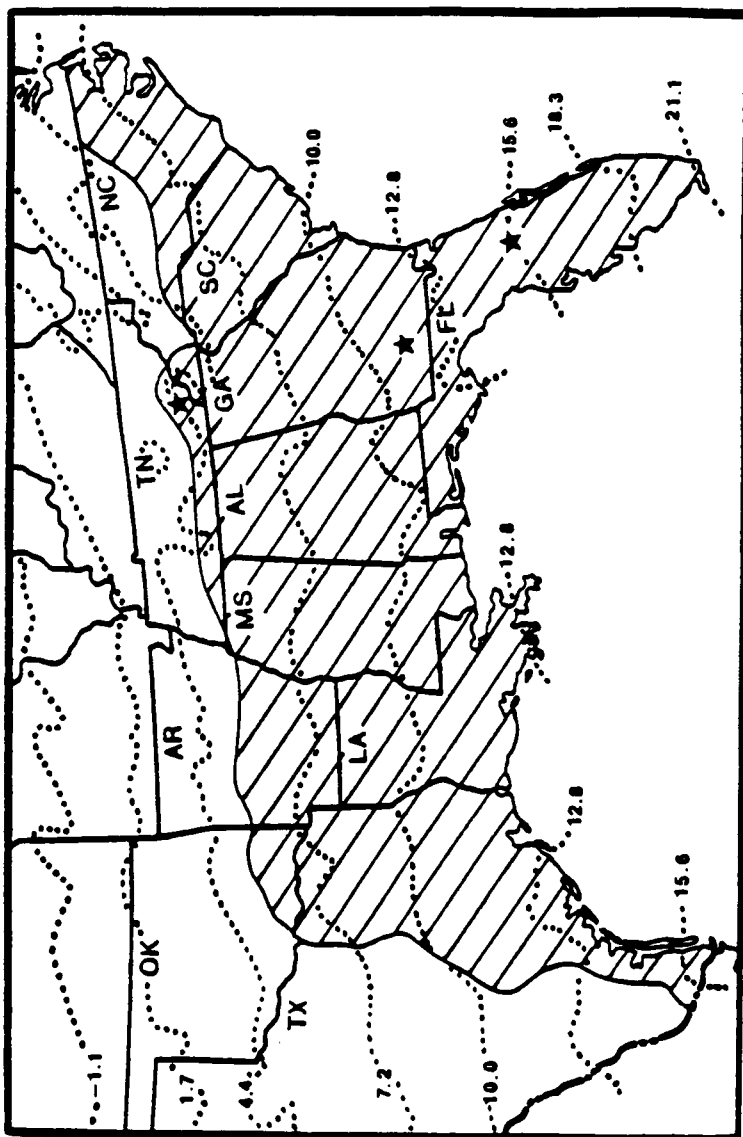


Fig. 5.1. Geographic distribution of *Anolis carolinensis* (cross-hatched) in southeastern United States (Conant, 1975) with average January isotherms (C, dotted lines) (United States Department of Agriculture, 1941). Stars indicate sites from which samples were obtained.

tolerance of A. carolinensis to cold temperature (Kour and Hutchison, 1970). Specimens for this latter study were obtained from Louisiana, as mentioned above, a region well below the northern range extent of this species. To date there exists no information concerning the possibility of geographic variation in the thermal tolerance of A. carolinensis although populations of this species likely experience vastly different winter temperatures. This study was conducted in order to determine whether latitudinally distinct populations of A. carolinensis have differing critical thermal minima (CTMin) when acclimated under the same thermal conditions. For purposes of our study, CTMin is defined as that temperature which caused cold narcosis and prevents locomotion (Cowles and Bogert, 1944).

MATERIALS AND METHODS

At the end of March 1986, adult male A. carolinensis were collected from three populations along a north-south transect: Orlando, Florida ($28^{\circ}26'$ N Lat.), Valdosta, Georgia ($30^{\circ}47'$ N), and near Tallassee along the Little Tennessee River in Tennessee ($35^{\circ}34'$ N). The macrothermal histories of each site are given in Table 5.1. Following the methods of Spellerberg (1973), CTMin tests were conducted on the afternoon of capture in order to obtain a rough estimate of cold tolerance: a temperature range from which a lab acclimation temperature could be extracted.

Lizards were cooled on a glass bowl in an ice-filled styrofoam cooler (walls 4 cm thick, inner dimensions 25 x 30 x 40 cm) until they were unable to right themselves 30 seconds after being placed in a

Table 5.1. Mean daily maximum and minimum temperature (C, range in parentheses) for collection sites of Anolis carolinensis for 8 days prior to and including date of capture, and mean winter (November-February) temperature for the sites. Data obtained from the United States National Oceanographic and Atmospheric Administration, Asheville, North Carolina.

Site	Date of Capture	Mean Daily Maximum	Mean Daily Minimum	Mean Winter
Orlando, Florida	26 March 1986	23.6 $\pm$ 5.78 (15 to 31)	11.8 $\pm$ 5.09 (6 to 20)	16.8
Valdosta, Georgia	28 March 1986	22.50 $\pm$ 6.08 (13 to 28)	5.97 $\pm$ 4.95 (-1 to 12)	14.3
Tallassee, Tennessee	30 March 1986	23.1 $\pm$ 3.00 (19 to 27)	1.0 $\pm$ 4.72 (-7 to 8)	6.5

supine position and stimulated in the pelvic region with a fine brush. Lizard body temperature was monitored with a Thermo Electric Micromite potentiometer with resolution to .1C and a 30 gauge copper-constantan thermocouple inserted ca. 1 cm into the cloaca and taped to the base of the tail.

Spellerberg (1972) found the greatest degree of cold acclimatability in Australian lizards at an acclimation temperature of 1 C below field CTMin values during a 48 hr period. A temperature of 9 C (an intermediate field CTMin value) was chosen for the 48 hr acclimation which was accomplished in an incubator with a 12L:12D light cycle. Lizards were held in 3.78 l clear glass jars provided with wooden perches, food (crickets) and water ad libitum for 14 to 18 days at 25±2 C (12L:12D photoperiod) prior to cold acclimation. The lizards were visually isolated from one another. The CTMin tests that followed were conducted as previously noted. All tests were conducted between 1530 and 1800 hr in order to account for a potential circadian rhythm in cold tolerance (Spellerberg and Hoffman, 1972).

A Kruskal-Wallis test was used to check for heterogeneity and Mann-Whitney U tests were performed to compare interpopulational CTMin values when significant heterogeneity (at the $P < .0001$ level) was evident.

RESULTS

Lizards ranged in mass from 2.23-5.89 g. There was no correlation between CTMin and mass (Spearman rank correlation test, $p > .05$). The CTMin values obtained on the afternoon of capture for the three

population samples are shown in Table 5.2. There is a trend toward increase in cold tolerance with latitude, but previous microthermal histories are unknown, so these results cannot be compared. Table 5.2 also lists the values obtained after cold temperature acclimation. An increase in cold tolerance with latitude is evident although Mann-Whitney U tests found significant differences in CTMin ($P < .05$) only for Tennessee versus Florida and Tennessee versus Georgia; no significant difference was found between Florida and Georgia ($P > .05$).

DISCUSSION

After reviewing the literature on the thermal ecology of 22 species of Anolis, Hertz (1979) concluded that various measures of heat resistance correlated highly with the type of habitat occupied by these species (open, edge or forest), thermal differentiation that reflects ecological distribution. Much of the variability within species was attributed to either difference in acclimatization or to genetic adaptation of different populations. One species, A. roquet showed variation in CTMax that was correlated to subspecific status, so that this variation was thought to have a genetic basis.

Spellerberg (1972) concluded that speciation and dispersion of southeastern Australia reptiles required adaptation to low temperature as the variation in CTMax was small compared to that of the CTMin values. For example, members of the Sphenomorphus species complex were shown to have altitudinal zonation that was correlated with lower temperature tolerances (Spellerberg, 1973). Spellerberg (1975) found that European forms of Lacerta agilis had lower average values of CTMin

Table 5.2. Critical thermal minima (C) of Anolis carolinensis as determined on the afternoon of capture and after 48 h acclimation at 9 C and at a photoperiod of 12L:12D. Mean $\pm$ one standard deviation (range in parentheses). N = 12 for each test.

	Orlando, Florida	Valdosta, Georgia	Tallasse,ee, Tennessee
Afternoon of Capture	11.91 $\pm$ 0.97 (9.8-13.6)	11.39 $\pm$ 1.64 (8.1-14.0)	8.68 $\pm$ 1.21 (7.2-11.8)
Following Acclimation	10.82 $\pm$ 1.21 (8.3-12.3)	9.91 $\pm$ 1.57 (5.9-12.2)	7.41 $\pm$ 1.09 (5.8-9.1)

than English forms and presumed this to have resulted from adaptation to colder winter temperatures on continental Europe.

The variation in average CTMin of Anolis carolinensis following acclimation is likely to be the result of genetic differentiation in low temperature tolerance rather than difference in acclimatization because the previous thermal histories of each were controlled. The lack of a significant difference in CTMin between the Orlando and Valdosta populations may reflect lack of genetic isolation between these two populations as this species is very abundant in this region of the southeast. Another possible explanation for there being no difference in cold tolerance between these populations is that they do not experience significantly different winter temperature (i.e., there has been no differential selection for low temperature adaptation). The latter hypothesis is supported by the relative similarity of long-term macrothermal histories of the two southern sites as compared to the northern locality (Table 5.1). Our CTMin results following acclimation of lizards at 9 C are a much better reflection of long-term mean winter temperatures at the three sites than of macrothermal conditions over the eight days prior to capture. It appears that A. carolinensis, like Lacerta agilis, consists of physiological races with regard to cold tolerance, thermal differentiation that reflects geographic distribution. These data further indicate that members of the genus Anolis are evolutionarily labile with regard to physiological adaptation to thermal environment.

SUMMARY

Critical thermal minima of three populations of Anolis carolinensis along a latitudinal transect were compared. The northernmost population, from eastern Tennessee, experiences colder winter temperatures than populations from southern Georgia and central Florida.

The CTMin of the Tennessee population is significantly lower than the southern populations after all were acclimated to 9 C for 48 hours. The Georgia and Florida populations did not differ in this regard, possibly because of a lack of genetic isolation between them or because the winter temperatures that they experience are not markedly different.

Several investigators have found thermal differentiation in the genus Anolis that is highly correlated to ecological distribution. The physiological difference observed in this study reflects geographic distribution and corroborates the general conclusion that members of this genus exhibit evolutionary lability in physiological adaptation.

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PART VI

SUMMARY

Evidence presented in this work reveals potential sources of experimental error inherent in current methodology employed in studies of the thermal relations of reptiles. The use of a thermocouple inserted into the cloaca of Anolis carolinensis significantly lowers its body temperature from ambient temperature, the likely cause being passive water loss in the vicinity of the cloaca and/or increased cutaneous water loss due to stress. This has been a common method employed in laboratory studies of thermoregulation in reptiles. Care should be taken in cross-species body temperature comparisons when different methodologies were employed, since the results may not be comparable. In addition, it is not known whether this phenomenon extends to larger bodied reptiles with more thermal inertia or to animals that inhabit more xeric habitats that tend to be more water conservative.

Acute handling and social stress were shown to significantly increase both aerobic and anaerobic respiration in A. carolinensis. Whether this enhanced metabolism extends for a longer time period remains to be tested. It is possible that collectively housing the animals during laboratory acclimation, a method frequently utilized in investigations of reptilian thermoregulation, creates unwanted experimental error in the tests that follow: Subordinates may experience starvation due to the social stress. Testosterone, another factor that had been previously shown to influence thermoregulation in reptiles, was not found to effect the thermal tolerance of A. carolinensis acclimated at 20 C. Testosterone may indirectly influence thermal tolerance because of its role in social dominance:

Collectively housing males during acclimation may artificially increase the experimental error in tests that follow because dominants have previously been found to have greater access to heat source which in turn would increase their heat tolerance over subordinates. The results reported herein, however, must be considered tentative because the use of silastic implants, though frequently employed in behavioral experimentation, is a crude method for the application of hormones. This experiment would have been more refined had release rate of the implants been determined.

Results of the experiment concerning the geographic variation in the critical thermal minima of different populations of A. carolinensis reveal that this species consists of physiological races with regard to cold temperature tolerance. The Tennessee population was found to have a significantly lower CTMin than either the Georgia or Florida populations. Members of this species have been shown to be evolutionarily labile with regard to thermal ecology, corroborating the findings of other researchers who studied the thermal relations of other species of Anolis on ecological scales.

VITA

Mark Angus Wilson was born in San Francisco, California on April 15, 1957. Attending elementary and high school in Walnut Creek, California, he entered The University of California at Berkeley in the Fall of 1975. A Bachelor of Arts degree in Biology was awarded him in the Spring of 1980. The following year was spent studying reptile ecology. In the Fall of 1981 he entered The University of Tennessee, Knoxville to pursue a Ph.D. in Zoology. This degree was awarded in the Fall of 1987.