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ASPECTS OF THE PHYSIOLOGY OF THE ANGULATE TORTOISE,
CHERSINA ANGULATA, WITH SPECIAL EMPHASIS ON THE
INFLUENCE OF BODY SIZE

by

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To my parents

DEO SOLI GLORIA

ERRATA

- p. 29 After the last paragraph insert: The tortoises were placed in the chamber so that they rested with their heads next to the airstream exit.
- p. 63 line 14 Add: The energy contents of the carrot tops and faeces, which had been dried to constant mass at 49-62°C and ground finely, were obtained by bomb calorimetry (CP500 Calorimeter, Digital Data Systems).
- p. 83 line 9 Add after "midline.": The tortoises were usually cooled down at ambient temperatures of about 10-15°C to anaesthetise them before surgery. The use of a chemical anaesthetic was not considered safe.

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CHAPTER 1

GENERAL INTRODUCTION

Twelve species of tortoise occur in southern Africa, approximately one quarter of the total number of species extant. The Cape Province of South Africa is richly endowed with tortoise species (at least 11 species) (Grieg & Burdett 1976). Given this unusual diversity, they deserve intensive study to determine what factors have allowed the diversification to occur. The angulate tortoise (*Chersina angulata* Schweigger) is distributed widely in the Cape Province of South Africa at low altitudes. It is found in a variety of habitat types with rainfall figures ranging from 100mm per annum to 700mm per annum (Grieg & Burdett 1976). It is a small to medium-sized tortoise (up to 300mm in total length) and is one of the common species in the southwestern Cape (Grieg & Burdett 1976; Branch 1984).

The genus *Chersina* is found in mid- to late-miocene deposits at Langebaanweg in the Cape Province, and the apparently single fossil species appears to be very similar to the angulate tortoise (Hendey 1981). Tortoises have remained almost unchanged since the Tertiary period, according to the fossil record (Romer 1966). This suggests that the biochemical and physiological make-up of tortoises must have enabled them to survive large environmental changes. Indeed their resilience under extreme environmental conditions is remarkable (eg. Nagy & Medica 1986), and their survival must in part be due to their specific physiology.

Physiological studies on tortoises

Early physiological work on tortoises concentrated on their metabolism (Hughes et al. 1971; review by Bennett & Dawson 1976) and their water relations (Bentley & Schmidt-Nielsen 1966; Schmidt-Nielsen & Bentley 1966; Cloudsley-Thompson 1970; review by Minnich 1982). This work stimulated interest in comparative reptilian physiology, relating similarities and differences between species in their physiology to their form, habits and habitat type. In the 1970s much work was done on the rates of heating and cooling in tortoises (Bethea 1972; Craig 1973; Voigt 1975; Voigt & Johnson 1977). Unfortunately, because of the way in which the results were calculated and reported, they could not be easily compared with other studies involving tortoises or other reptiles (Smith 1976). Attention in this field has now been drawn to physiological control of heat exchange (Bartholomew 1982). The initial work confirming the fact that in turtles physiological control of heat exchange does occur was done by Weathers & White (1971), but only a study by Voigt & Johnson (1977) has looked at this in some detail in tortoises. The effect of body size on the efficiency of physiological control mechanisms is a new area of research in this field (Turner 1987).

Apart from the work mentioned above, the Testudinidae as a group were neglected in many areas of physiology until the late 1970s and early 1980s. This is surprising, considering

the uniqueness of this group and their unusual anatomy. Recently a few detailed studies have been done on tortoises regarding their energy budgets and energy utilisation (Hamilton & Coe 1982; Nagy & Medica 1986), circadian rhythms in energy use (Kirsch & Vivien-Roels 1984), and respiration (Glass, Burggren & Johansen 1978; Benchetrit & Dejours 1980). However, only one or two species of tortoise have been investigated in these areas of physiology.

Recently, much interest has centred on the effect body size has on physiological rates in animals, and how this affects the ecology of these animals (Peters 1983). This has led to the calculation of general relationships among animals of different sizes within a taxon and specific physiological rates. Mathematically, the relationships generally take the form of power equations (Peters 1983). Often broad taxa have been used in formulating such relationships (eg. ectothermic vertebrates, or reptiles as a whole; Peters 1983). Theories have been put forward as to why such relationships hold, as well as others arguing against the validity of these relationships (Heusner 1982a,b; review by Peters 1983). Surprisingly, work investigating allometry in tortoises has been neglected, in spite of being such unusual animals.

In many respects tortoises are good subjects for physiological studies because they are reasonably hardy, fairly easy to maintain, and more especially, because they are the

only terrestrial vertebrates in which most of the body is confined to a rigid external shell. This prevents certain postural body changes, unlike other vertebrates with hard dermally-derived external layers, and therefore most of the tortoise's body surface area exposed to environmental conditions is fixed. This can, to a certain extent, facilitate investigation of the effects body size has on the physiology of tortoises. The hemispherical shape of tortoises gives these animals small surface area to volume ratios (Bartholomew 1982). This has implications for rates of heat exchange, since larger surface area to volume ratios enables greater mass-specific rates of heat exchange.

Mean body mass is difficult to obtain for a single tortoise, and this is a drawback in using these animals in physiological studies involving allometry. The reason for this difficulty is that the precise mass of a tortoise at any time depends on the amount of urine stored in the urinary bladder. The amount of urine stored in the bladder may account for as much as ten per cent of the mass of the tortoise (pers. obs). This is not unusual (see Nagy & Medica 1986). Tortoises store water in the urinary bladder for use when necessary and as a sink for excess dietary ions (Mahmoud & Klicka 1979; Minnich 1982; Nagy & Medica 1986). The tortoise may void this urine when frightened or threatened as a defence mechanism. However, the amount of dilute urine stored in the bladder depends on the state of hydration of the tortoise. This depends on factors such as

whether it has eaten or drunk recently (review by Minnich 1982), whether it has voided urine through fright, and the amount of urine that has been voided, because not all may be voided at the same time (pers. obs).

The changes in mass of several of the study animals over two years can be seen in Fig. 1. It is likely that most of these fluctuations in mass are to a large degree due to the state of hydration of the tortoise. The urine stored in the urinary bladder is metabolically inactive, and this, for example, will affect the results to some degree when body mass is an experimental variable. Therefore there is a compromise between relatively fixed body shape and labile body mass when using tortoises for physiological studies.

Aims of this study

Until recently not much of the physiology of the angulate tortoise had been investigated, and the work done focused on its thermoregulation (Craig 1973; Perrin & Campbell 1981). However, during the last few years research dealing with more of its thermoregulation and water relations was begun (S. Els pers. comm.).

This study aims to examine the relationships between body size and the rates of resting metabolism, evaporative water loss and heat transfer. The relationship of the changes in certain physical properties to the corresponding changes in physiological properties, due to increases in body size,

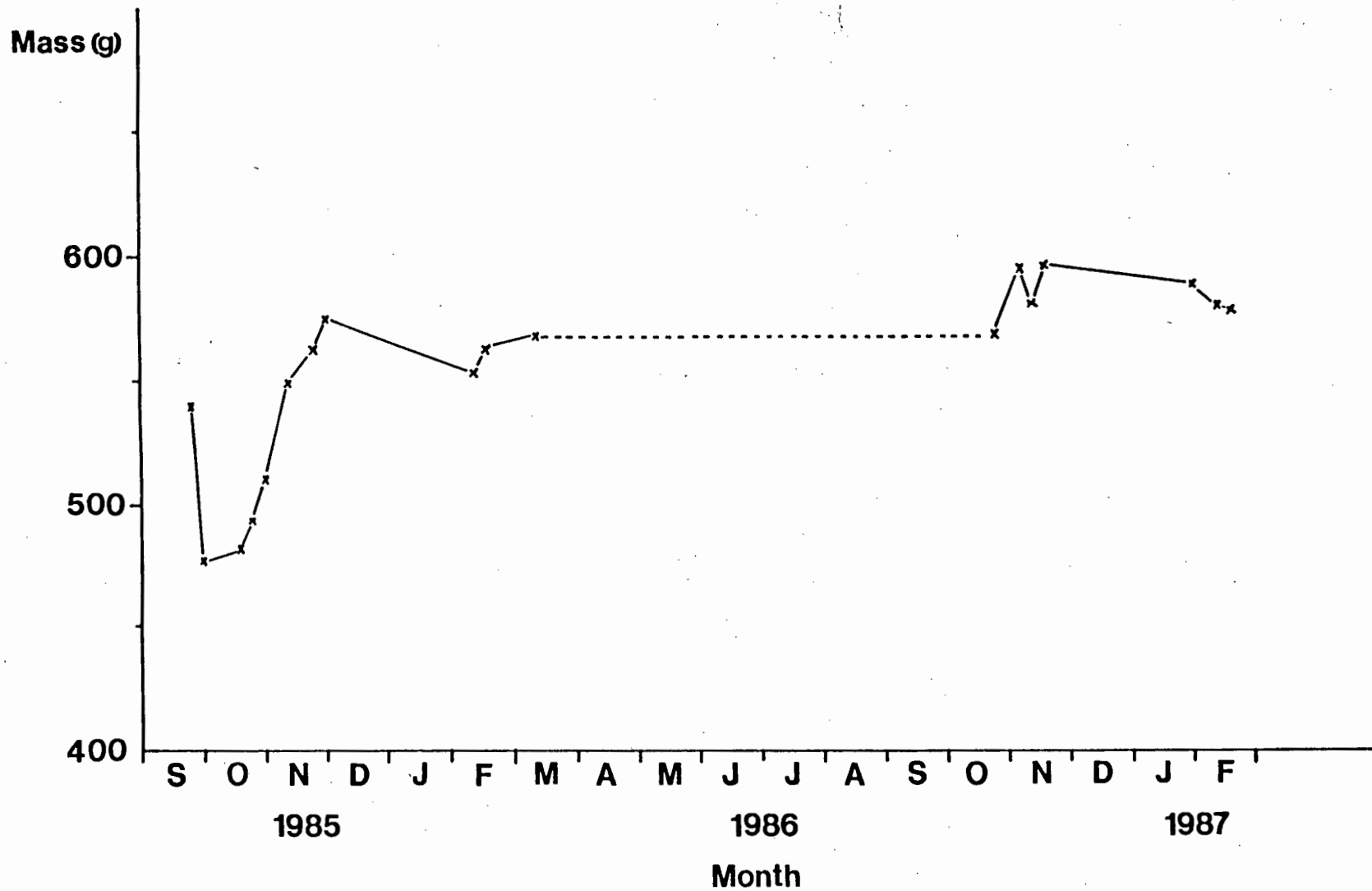


Figure 1 Change in mass over two summer seasons of a male tortoise used in experiments investigating resting metabolic rates, rates of heat transfer, and rates of evaporative water loss. The scale of the x-axis is not precise. Months are initialled and start at September 1985, ending at February 1987.

will be investigated. Aspects of a theory of biological similarity will be discussed in the light of the results.

Other physiological processes, such as respiration and assimilation efficiency, will be looked at without reference to body size.

Environmental and experimental conditions

The experimental conditions with respect to temperature, relative humidity and photoperiod were kept close to those found in the natural environment of the tortoises. Appendix 1 contains details of these climatic variables. Details of the tortoise husbandry practised during the duration of these experiments are given in Appendix 2.

Statistical Tests

Data were tested for departure from normality usually by a subjective graphical method (Zar 1984). When the data did not appear to come from a normal distribution, non-parametric statistics were used. Regression equations were obtained for untransformed data, as stipulated by Zar (1968). All statistical tests were done on untransformed data. The significance level used was a probability of 0.05. Statistics have not been given usually where the sample sizes were too small for a rigorous statistical analysis.

Statistical tests followed Zar (1984). Most regressions and statistics were calculated using Statpro programs (Wadsworth Professional Software).

Many allometric relationships have been described by the power equation of the form $Y = aX^b$ (Peters 1983). When logarithms are taken of both sides of the equation a linear relationship results, with a slope of b . Where power equations were used to express relationships between two properties of the angulate tortoises under investigation, they were of the form $Y = aX^b + c$, except where it was biologically meaningful for the equation to pass through nought. In the latter cases the form $Y = aX^b$ was used.

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CHAPTER 2

SCALING OF PHYSICAL DIMENSIONS

There has been much controversy in the literature over the allometric scaling of heat exchange and metabolic rates of animals (Hemmingsen 1960; Heusner 1982a; Feldman & MacMahon 1983; Peters 1983; Prothero 1984, 1986; Turner 1985, 1987). Do these rates scale to a specific function of body size, and can animals be considered isomorphic over wide-ranging taxa? It has often been argued that heat exchange and metabolism scale to body mass as a power function with a constant exponent (slope). The mass coefficient is stated to be constant for a specific taxon. However, aspects of this hypothesis have recently been disputed on strong theoretical and empirical grounds by Heusner (1982a,b) for metabolic rates, and Turner (1985, 1987) for rates of heat exchange.

Heusner (1982a,b) used dimensional analysis to show that only isomorphic animals would have a constant mass coefficient. Dimensional analysis expresses secondary quantities such as energy metabolism and heat exchange in terms of primary quantities such as mass and length. Animals show similarity when the ratios of the primary quantities are the same in all individuals. The properties of the animals that are size-independent must also be constant in all individuals, eg. density and temperature. All animals have both size-dependent (extensive) properties and size-independent (intensive) properties, and secondary quantities are products of both extensive and intensive properties (Heusner 1982b). Heusner suggested that energy metabolism is a product of an extensive property (M^b) and a conjugated

intensive property, which he assumed to be the mass coefficient since energy metabolism is expressed in the form aM^b .

Heusner showed from dimensional analysis that animals are biologically similar, ie. have the same form in space and time, when the power exponent (b) of the allometric function is 0.67. The mass coefficient (a) must be independent of mass. When the exponent does not equal 0.67 then the coefficient will not be mass-independent. Therefore, Heusner suggested that the equation usually used to relate standard energy metabolism to body mass for a wide range of animal taxa is incorrect. This has been shown empirically for many species as diverse as mammals and protozoa, covering a range of many magnitudes of body size (Haysson & Lacy 1985; Prothero 1986).

Heusner suggested that within a species, mature mammals are biologically similar with respect to energy metabolism. Energy metabolism in mature individuals of a species scales with mass to a power of 0.67 (Brody 1974; Heusner 1982a). Heusner also suggested that in young, growing individuals the exponent is greater than 0.67, since growing animals change form. The opposite is true for senescing individuals, due to the deterioration of body structures. The mass coefficient differs according to structural and functional differences between species, and between different age groupings within a species.

More recently, Turner (1985) has suggested that the exponent relating organismic properties to body size need not necessarily be 0.67 in isomorphic animals. He shows that the magnitude of the exponent may also depend on other factors, such as environmental conditions, and major modes of energy transfer and their interaction with the properties of the animals, not just upon the properties of the animals themselves as Heusner assumed. The interaction between the properties of the animals and the properties of the environment may change with a change in any of the properties of the animals, such as body mass. This will result in more complex scalings than just a simple power function with an exponent of 0.67. For example, the rate of heat transfer from eggs scales to mass with an exponent of 0.60 with only two modes of heat transfer. This exponent is a composite number resulting from small eggs losing heat mainly by radiation and large eggs losing heat mainly by convection, since heat loss via radiation scales with a different exponent to mass than heat loss via convection (Turner 1985).

Methods

The angulate tortoises used in this study were the same individuals used to determine rates of heat transfer (Section 5). The masses reported for these individuals are the same as those reported in Section 5.

The functional surface areas of the tortoises were assumed to be equivalent to the surface areas of the "shells"; the openings through which the appendages protrude were assumed to be covered by smooth, flat shell. These assumptions were thought to enable a reasonable estimate of the functional surface area, since during the experiments determining rates of cooling, the appendages were withdrawn (Section 5). This was often the case during metabolic rate determinations at 20°C as well (Section 3). Surface area was calculated by triangulation. The surface of the shell was divided into triangular areas. Precision calipers were used to measure the sides of the triangles to the nearest 0.5mm. The area of each triangle was calculated as $(s(s-a)(s-b)(s-c))^{1/2}$, where $s=1/2(a+b+c)$ and a, b, c are the lengths of the sides in mm. Total surface area of the "shell" was then calculated by summing the areas of all the triangles (calculation was done using the software package Lotus 1-2-3, Lotus Development Corporation).

The volumes of tortoises were measured by displacement of water (Archimedes principle), according to the method of Hoyt (1976). Lead weights were taped to the tortoises, since angulate tortoises are less dense than water. The tortoises were placed in large beakers which had been filled to the level of an outlet tube with deionised water. Displaced water flowed through the outlet tube and was collected in a preweighed container. The volume of the displaced water was calculated by weighing, and by assuming that 1cm^3 of

deionised water has a mass of 1g. The volumes of the weights and holding materials were obtained by displacement of water, and subtracted from the total volume displaced, the remainder being the volume of the tortoise. Volumes were calculated as the mean of the two lowest, most consistent replicates, subjectively chosen from the four or five replicates done for each individual.

Shell masses were obtained by weighing shells with Pesola spring balances. The shell mass, as a percentage of the total body mass, was calculated from the total shell length and data for mass given by Branch (1984).

Results

The masses, surface areas, volumes and densities of the four sizes of tortoises are given in Table 1.

The relationships between the various extensive properties of angulate tortoises are set out below.

Surface area (SA; cm³) scales with mass (M; g) as:

$$SA = 4.865M^{0.693} \quad (1)$$

The regression is highly significant ($F_{1,2} = 3554.64$, $P < 0.005$, $R^2 = 1.000$). The exponent is significantly different from 0.670 (Student's t-test, $t_3 = 7.60$, $P < 0.0025$).

Table 1 Masses (g), surface areas (SA; cm²), volumes (V; cm³), surface area to volume ratios (SA:V) and densities (D; gcm⁻³) of tortoises used for allometric scaling of physical properties

Mass	SA	V	SA:V	D
100	112.05	105	1.07	0.95
305	260.31	329	0.79	0.93
590	408.53	594	0.69	0.99
1010	586.40	1076	0.54	0.94

The relationship between volume (V; cm³) and mass (M; g) is:

$$V = 1.059M - 4.985 \quad (2)$$

The regression is very significant ($F_{1,2} = 1118.34$, $P < 0.01$, $R^2 = 0.994$). The slope cannot be distinguished statistically from 1.000 (Student's t-test, $t_3 = 1.874$, N/S).

Increase in surface area (SA; cm²) with volume (V; cm³) is stated by the equation:

$$SA = 4.973M^{0.685} \quad (3)$$

The regression is highly significant ($F_{1,2} = 771.53$, $P < 0.005$, $R^2 = 1.000$). The exponent is not significantly different to 0.670 (Student's t-test, $t_3 = 0.867$, N/S).

The decrease in the surface area to volume ratio (SA:V) with increasing mass (M; g) is stated by the equation:

$$SA:V = 3.825M^{-0.276} \quad (4)$$

The regression is very significant ($F_{1,2} = 162.61$, $P < 0.01$, $R^2 = 0.994$). The slope is significantly different to -0.330 (Student's t-test, $t_3 = 5288.96$, $P < 0.0005$).

Shell masses were between 34 and 39% of the total body mass (143-230mm long). No consistent pattern of mass change was found with increasing body mass.

Discussion

Angulate tortoises of different sizes cannot be strictly regarded as isomorphic. For isomorphy, density must be constant and surface area must scale with volume to the power of 0.67 (Heusner 1982b; Turner 1985). Volume must scale directly with mass for constant density. The criteria for

isomorphy are, however, fulfilled in terms of surface area and volume in the angulate tortoises considered.

Nevertheless, density is not constant, and the exponent of the equation relating surface area to mass is close to, but significantly greater than, 0.67. For isomorphy, the surface area to volume ratio should be inversely related to increasing mass, with $b=-0.33$. This is not true for the study species, because density varies throughout the size range. Therefore the exponents of the equations derived from the results of this study should differ slightly from those for isomorphic species. The deviations from isomorphy could be primarily due to fluctuations in mass and/or gut content, and the fact that weighing was not done regularly, causing bias in calculating mean body mass. The small sample size contributed to the uncertainty in determining whether angulate tortoises can be considered isomorphic over a large size range.

No apparent trend in relative shell mass was found with increasing size. However, a wider range of sizes and a greater sample size are needed. Also, dry shell masses were recorded, whereas the reference total body masses were wet masses. In other tortoise species, younger tortoises have relatively lighter shells than older ones (Hughes, Gaymer, Moore & Woakes 1971; Bennett & Dawson 1976). Rates of heat transfer may be slightly affected by relative shell mass.

All the results are only approximate because of the variability in measurement between different replicates for the same individual. The body mass of each tortoise varied even over relatively short periods of time. Inflation of the tortoises' lungs may have varied between replicates, which in turn probably contributed to differences between replicate volume, and therefore density, measurements. The true surface areas of the tortoises are undoubtedly larger, owing to the presence of many small ridges (growth rings) and some small projections on the surface of the shells. However, it is doubtful if these small ridges significantly affect the functional surface area of this species in terms of its physiology. Nevertheless, since measurements on the different individuals were done under similar conditions, the results should be qualitatively comparable. Therefore, it seems reasonable to assume that the predictions from the theory for isomorphic animals can be cautiously tested using the angulate tortoise as a study animal. Rates of heat transfer, evaporative water loss and resting metabolic rate should scale as a power function of mass if they are directly proportional to surface area (equation 4). Mass-specific rates should decrease with increasing body mass.

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CHAPTER 3

RESPIRATION AND SCALING OF RESTING METABOLIC RATE

The energy flow through an organism is described as the metabolism of the organism. In air-breathing animals it is approximated by the rate of oxygen consumption, and is recorded usually as such, because in general in these animals energy is released directly or indirectly through oxidative processes (Schmidt-Nielsen 1979). The range in activity found in an animal depends on its metabolism in a broad sense, as do the types of activity an animal can undertake (eg. can it swim or fly). The minimum amount of energy needed by an animal to survive at any point in time is approximated by the energy used by that animal at rest. Any further activity would require additional energy release. However, to survive over a long time period an animal would have to perform other activities such as procuring food, and so the amount of energy needed to exist can be obtained by determining the energetic cost of free existence for the animal.

The resting metabolic rate (RMR) of an ectothermic reptile (Schmidt-Nielsen 1979) is defined as the metabolic rate of a resting, fasting reptile at a specified temperature and at a given period of the circadian rhythm exhibited by its metabolism (Hemmingsen 1960; Bennett & Dawson 1976; Aschoff 1981; Peters 1983; Prothero 1984). RMR increases with size (Peters 1983). Mass-specific RMR, i.e. RMR per gram body tissue, decreases with body size (Schmidt-Nielsen 1979). Although overall metabolic rate is more important ecologically, as only whole organisms are

ecological "units", the value of mass-specific metabolic rate is that it allows interspecific comparisons to be made. Therefore specific metabolic rate is used in this paper. The RMR of an individual ectotherm varies according to the age, sex, condition with respect to nutrition, state of thermal acclimation, photoperiod history, and period within the circannual and circadian rhythm of the metabolism, of the individual (Bennett & Dawson 1976; Kuchling 1981; Bartholomew 1982; Peters 1983; Prothero 1984). These conditions have to be taken into account before RMR can be measured with some degree of accuracy.

The RMRs of the angulate tortoise *Chersina angulata*, over the range of temperatures normally encountered by this species in its habitat in the south-western Cape Province, has been investigated in this section. Whether there is any consistent change in RMR as body size increases has also been investigated at a specified ambient temperature (20°C). Body mass was the index of body size used here. Patterns of breathing, as obtained from continuous chart recordings of oxygen consumption, have been discussed.

Methods

Ten *C. angulata* were obtained during the summer of 1985/1986, eight of which were caught in the Cape of Good Hope Nature Reserve. The latter were housed in cages made from wood and chicken mesh, measuring approximately 300 mm x 395 mm, in a heated room (18-24°C). Photoperiod was kept

constant at twelve hours light and twelve hours dark (12L:12D), "sunrise" being at 06h00 local time. Light (and some heat) was provided by a 300W halogen lamp placed above the cages, which simulated natural sunlight within the visible wavelengths.

The tortoises were weighed before each experiment. All tortoises were adult individuals, as indicated from growth curves and other data (Branch 1984), and all were in a length range exceeding 150 mm. Mean masses are indicated in Fig. 5. Food was withheld from individual tortoises at least forty-eight hours before the experiments. This is probably the minimum time period required for the metabolic rate of these tortoises to return to resting levels after the increase caused by the digestive processes (specific dynamic action) (Benedict 1932, quoted in Bennett & Dawson 1976; Bartholomew 1982). Glucose levels in the blood are also reduced significantly after forty-eight hours of fasting in *Testudo graeca*, reaching a minimum plateau after four to five days (Vladescu 1965). Stable levels in the metabolic rate and blood glucose appear to be maintained in tortoises for at least eight and ten days, respectively, after fasting is started (Benedict 1932, quoted by Bennett & Dawson 1976; Vladescu 1965). Results obtained during this time period should therefore be valid. The first two sets of experiments were designed to determine the effect of ambient temperature (T_a) on metabolic rate. The effect that acclimation to the experimental apparatus over 24 hours had on metabolic rate

was also studied in the second set. The main differences between the first two sets of experiments were the duration of the experiments ("non-acclimated" phase and "acclimated" phase, respectively), the time of year during which the experiments were undertaken (summer versus winter), and the fact that the tortoises were kept indoors under a "summer" photoperiod during the winter in which the "twenty-four" hour experiments were done.

Other tortoises used in experiments relating RMR to body size were obtained from the Cape of Good Hope Nature Reserve during the summer of 1986/1987 and the beginning of winter 1987. Their masses ranged from 41-798g. These tortoises were housed in the outside pen, exposed to the external environmental conditions. About four days before oxygen consumption determination, each tortoise was moved indoors, starved, and exposed to similar light conditions as described above. Ambient temperature varied between 17°C and 23°C. The 89g and 314g tortoises had implanted thermocouples (see Fig. 6 for an indication of mean masses).

Apparatus

The apparatus used to determine oxygen consumption at rest is illustrated schematically in Fig. 1. The cylindrical plexiglass chamber was designed to minimise the volume of dead space and to restrain the tortoises in a horizontal plane without unduly stressing them (as would happen with a mask). Limited movement in the vertical plane could occur.

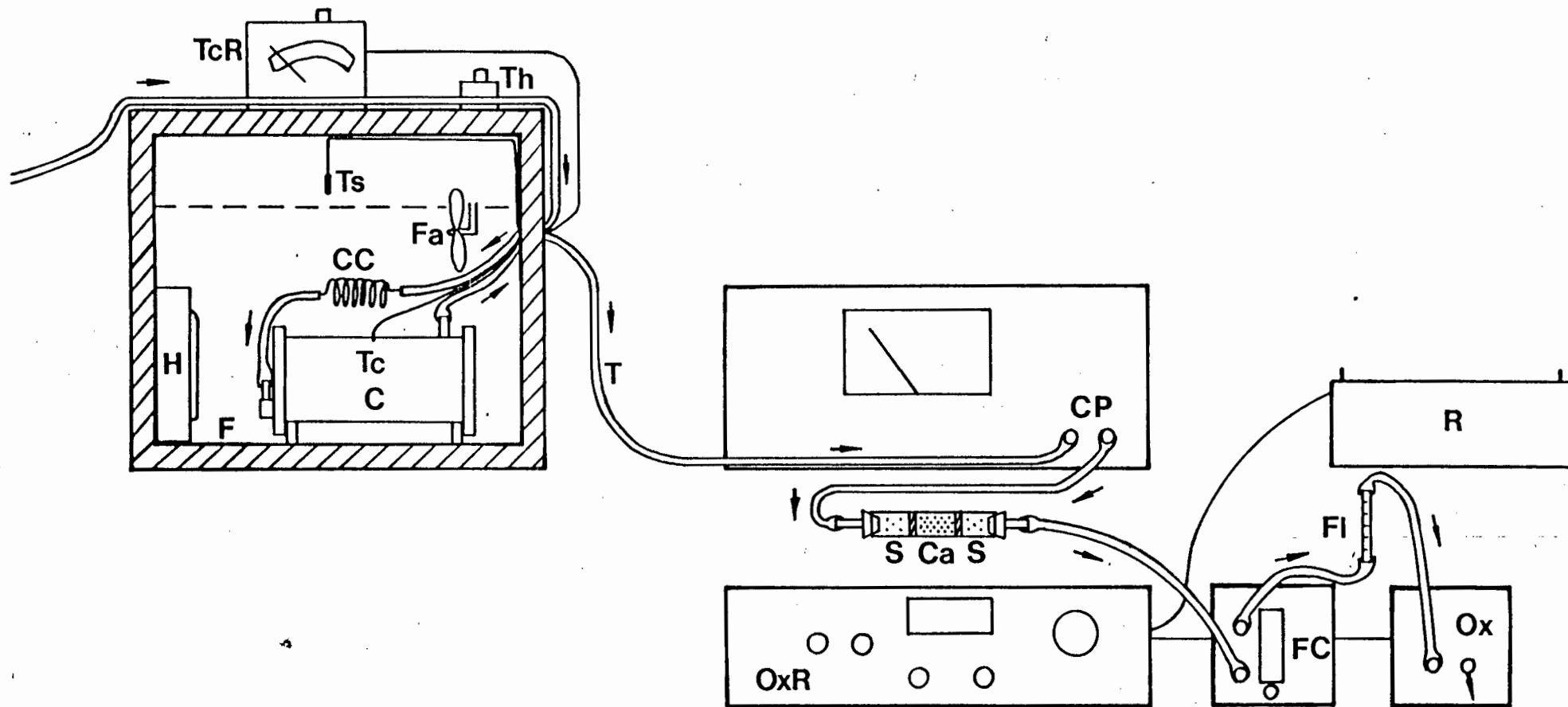


Figure 1 Schematic representation of the apparatus set-up for the resting metabolic rate determinations. C = metabolic chamber; Ca = soda lime; CC = cooling coil; CP = pump; F = refrigerator; Fa = fan; FC = flow control; Fl = flowmeter; H = heater; Ox = oxygen sensor; OxR = oxygen analyser; R = chart recorder; S = silica gel; T = tubing; Tc = thermocouple; TcR = thermocouple reader; Th = thermostat; Ts = thermistor probe. Arrows indicate direction of airflow.

The volume of the chamber was approximately 2.5l. The chamber was tested for leaks. The removable end of the chamber was sealed by a greased rubber ring and four rods and bolts. The chamber legs had sponge bottoms to help dampen vibrations from the refrigerator. The refrigerator, in conjunction with a thermostatically controlled antagonistic heater, provided a constant temperature ($\pm 0.2^{\circ}\text{C}$) inside the metabolic chamber. Ambient temperature was measured by a thermocouple inserted through the top of the metabolic chamber. The hole was sealed with clear adhesive glue. The T_a was displayed by a digital thermocouple reader (BAT-12, Bailey instruments). Air was circulated by a fan to distribute the heated air evenly. The copper cooling coil allowed the airstream through the chamber to equilibrate with the refrigerator temperature. The refrigerator had a plexiglass window which was blacked out during the experiments to avoid disturbing the tortoise. There was no lighting in the chamber. In the experiments investigating the effects of body size on resting metabolism, the refrigerator was placed in a controlled environment room where the temperature was controlled to about $\pm 1^{\circ}\text{C}$ and the relative humidity ranged between 35-56%. The 12L:12D photoperiod was kept in the controlled environment room, and the refrigerator was not blacked out.

Air from outside the building was drawn through the chamber by a pump (ADC-225-MK3, Analytical Development Company) and then pushed by the pump through a flow control (R-

1, Applied Electrochemistry) to an oxygen sensor (N-22, Applied Electrochemistry). Water vapour and carbon dioxide were removed from the air by silica gel and soda lime, respectively. The output of the oxygen analyser (S-3A/1, Applied Electrochemistry) was amplified and graphically recorded by a two-channel chart recorder (Pederson MR). Minor changes in the configuration of the apparatus were made for the experiments testing the effects of body size on RMR. Air was drawn through the whole apparatus, including the oxygen sensor. Most of the silicone tubing used in the first set of experiments was replaced by latex tubing, and the length of tubing was shortened. Connections were also glued together. The chart recorder (Kipp & Zonen BD 41) had an attached amplifier.

Flow-rate of air was between 148 and 159ml min⁻¹. The flowmeter (2-A-150, Fisher Controls Limited) was calibrated from a chart for air at 15°C and 760mmHg (Fisher Controls Limited) for the first set of experiments, and by a calibrated mass flowmeter (Teledyne Hastings-Raydist ECPR-1A) for the experiments investigating allometry. Barometric pressure was usually recorded at the beginning and the end of the experiment, and the mean used. Sometimes the barometric pressure was only recorded at the beginning or within 1½ hours of the end of the experiment. Pressure changes during the experiments did not appear to have an appreciable effect on the volumes of oxygen used.

Data collection

Data were collected after at least a two hour settling period in the apparatus (unless a consistent breathing pattern was obtained earlier) in the experiments investigating the effect of T_a on metabolic rate, and data were discarded if the animal was active for more than four hours in the initial period (four points). In the experiments investigating allometry, data were collected regardless of the settling period. However, the data used in the results section were only collected a relatively long period after resting levels were reached.

Results were obtained from the period during which oxygen consumption was at a minimum. The mean time taken for each trial in the first set of experiments to determine the effect of T_a on metabolic rate was about five hours. The data collection period varied between 19-149 minutes. The experiments done to determine oxygen consumption in the acclimated phase were more than 24 hours long, except for one which took $17\frac{1}{2}$ hours. The duration of the data collection period varied from 24 minutes to 264 minutes. The regressions obtained for the above experiments were calculated using a BMDP non-linear regression computer package (BMDP Statistical Software).

The mean time taken for each experimental run during the allometry study was about seven hours, and the duration of the data collection period varied from 31 to 149 minutes. During this study, in all but one case, three replicate

experiments were done per tortoise. Two replicates were carried out for the one exception. It was found that the oxygen consumption during the first set of replicates was significantly higher than in either of the other two sets (Wilcoxon paired-sample test, $T_8^{1,2} = 0.0$, $T_8^{1,3} = 1.0$, $P < 0.05$ in both cases). There was no significant difference in oxygen consumption between the second and the third sets of replicates (Wilcoxon paired-sample test, $T_8^{2,3} = 12$, N/S). This seemed to indicate that the tortoises only became acclimated to the experimental apparatus to some degree after the first set of replicates. Therefore, only the lowest oxygen consumption result from the second and third replicate sets was used for each individual in calculating the allometric relationships.

Any "drift" in the baseline oxygen percentage and chart recording during the experiment was assumed to be linear. The recording baseline was corrected for drift. In the allometry experiments, visual readings were taken from the oxygen analyser and recorded. This was used to correct more accurately for baseline drift during the experimental runs. Integration of the area under the recorder trace to obtain oxygen consumption was done gravimetrically. The area under the trace was cut out and weighed. This was compared to a mean mass of an area of the same type of chart paper, representing a known volume of oxygen under the chart recorder settings. The volume of oxygen represented by the area under the experimental trace was then calculated by ratio. All

volumes were corrected to STPD (0°C ; 1013mBars; i.e. 0°C ; 1 atmosphere).

Results

Circadian rhythm

A circadian rhythm in RMR appeared evident from the chart traces of oxygen consumption obtained from the twenty-four hour runs. Where RMRs were determined during both the diurnal and nocturnal periods ($n=9$), mass-specific oxygen consumption was greater generally during the day than during the night, diurnal rates being up to fifteen per cent greater. The three exceptions did not have exceptionally raised RMRs at night; in two of the cases the nocturnal mass-specific RMRs differed from the diurnal ones by up to ten percent. The third individual possibly had a raised mass-specific RMR at night because it had not settled in the chamber. The high metabolic rates of the other two exceptions were probably due to the same cause, since these runs were started late in the afternoon.

Table 1 shows the period of day during which the greatest mass-specific RMRs were recorded. The rates were determined from runs in which RMRs were recorded from the morning, afternoon and evening periods during each run. Mass-specific RMRs are highest during the morning. The periods of the day when high oxygen consumption values were recorded for several tortoises are shown in Fig. 2. The periods of high

oxygen consumption started in the early hours of the morning and ended usually during the mid-afternoon or earlier. However, the difference in RMRs obtained between 0h00-12h00 and between 12h00-24h00 were not significant (Wilcoxon paired-sample test, $T_7 = 3.5$, N/S).

Table 1 Minimal values of mass-specific oxygen consumption ($\text{mlO}_2\text{g}^{-1}\text{body mass h}^{-1}$) of angulate tortoises during different time periods over twenty-four hours (T_a is the ambient temperature in degrees centigrade to which the tortoise was exposed; * is the period of peak specific RMR; individual tortoises are labelled under Tor No).

$T_a(^{\circ}\text{C})$	Tor No	Time Period			
		0h01-6h00	6h01-12h00	12h01-18h00	18h01-24h00
15	10	0.007	*0.008	0.006	0.007
20	8	-	*0.013	0.007	0.012
30	6	0.020	*0.022	0.021	-
35	9	0.020	0.023	0.026	*0.029*

* Possibly high (tortoise not settled?)

When tortoises were active during runs relatively high values of oxygen consumption were recorded. Therefore periods of high oxygen consumption in the twenty-four hour

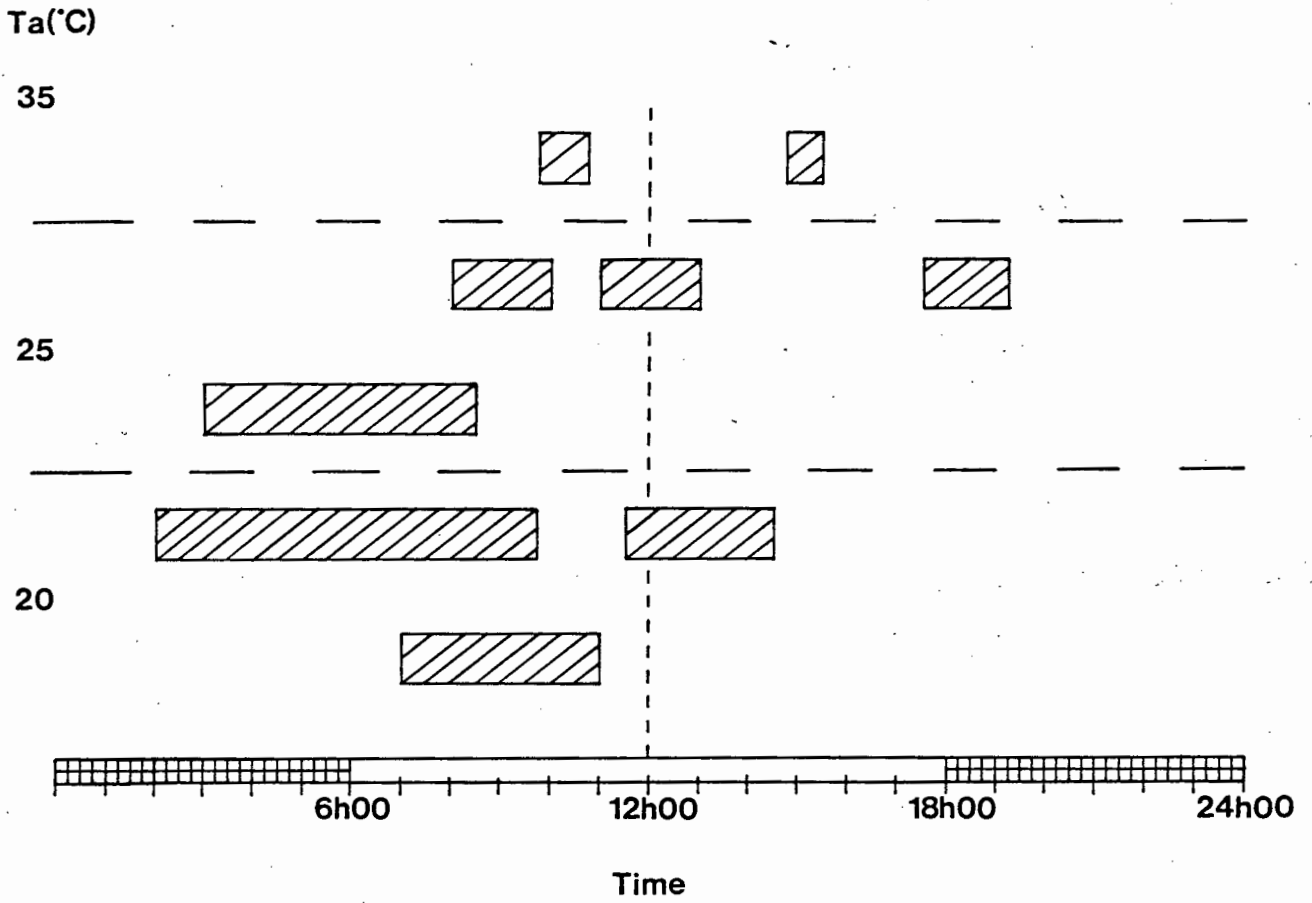


Figure 2 Periods of the day during which high total metabolic rates were recorded for angulate tortoises at different ambient temperatures (N=5).

runs are likely to reflect activity. Activity occurred primarily during daylight hours, and the circadian rhythm in oxygen consumption seemed to persist over a twenty-four hour period without the entraining influence of photoperiod. It was found that mass specific oxygen consumption values during the afternoon and evening were relatively low and similar. The differences in mass-specific resting oxygen consumption between these two periods were not significant (Wilcoxon paired-sample test, $T_s = 1.5$, N/S). Therefore values obtained during the afternoon or evening were used in calculating RMRs.

Patterns of respiration

Figure 3 gives representative traces of oxygen usage at different T_a s. Figure 4 shows different patterns of oxygen usage at 20°C, by various sizes of tortoises. The interpretation of the trace patterns is difficult. At the higher T_a s oxygen consumption increased (height of trace) and the frequency of breaths increased, as indicated by the more irregular trace. Definite increases in oxygen concentration and a time lag in rate of increase due to the slow flow-rate can be seen. It is possible that these increases represent apnoeic periods. If this is so, apnoeic periods of up to 30 minutes were common under these conditions. However, the peaks possibly represent periods of frequent breathing, and the increases in the percentage of oxygen periods of less

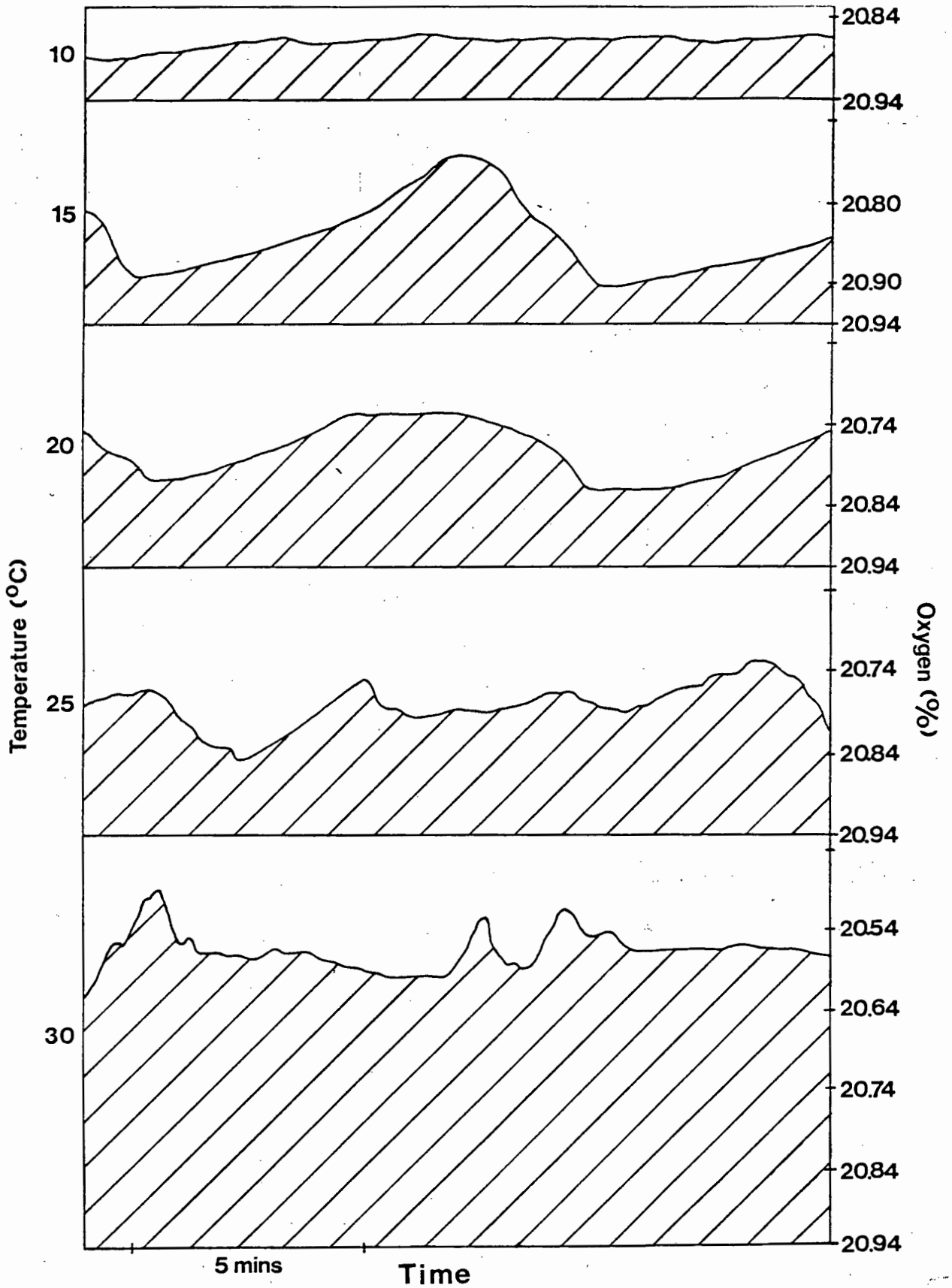


Figure 3 Respiratory patterns of angulate tortoises at different ambient temperatures, based on changes in per cent oxygen in the expired air. Percentage of oxygen in fresh air was assumed to be 20.94%

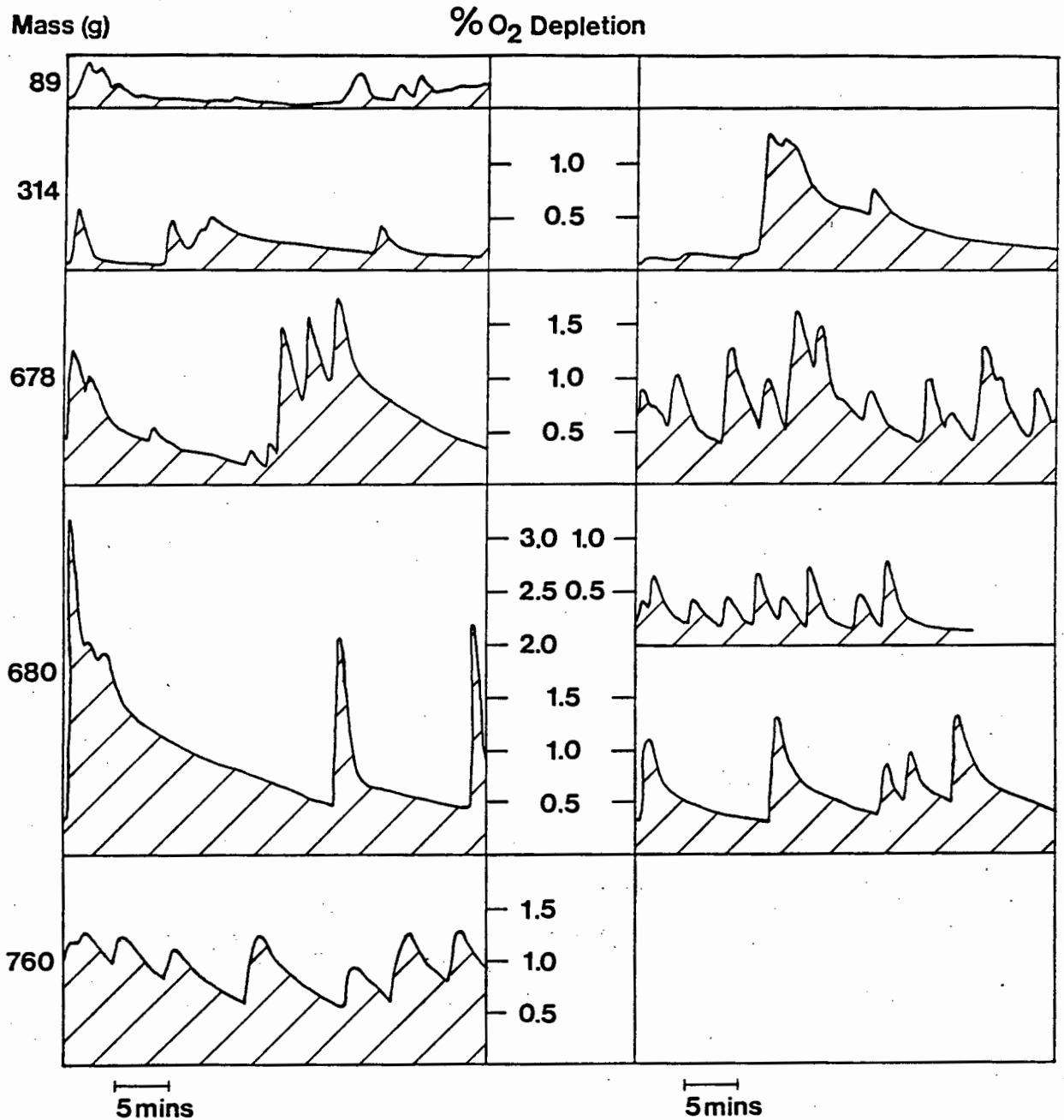


Figure 4 Respiratory patterns shown by angulate tortoises. Variation between tortoises of different mass are shown vertically. Individual differences are shown horizontally, each block representing a period of a single experiment. The height of each trace indicates the percentage drop in the per cent of oxygen in the airstream when the tortoise exhales.

frequent or shallow breathing. Variability in respiratory patterns within individuals can be seen in Fig. 4. Sometimes long periods of "apnoea" are present, while at other times breathing appears to be regular.

The lengths of the "apnoeic" plateaux at different T_a s are reported in Table 2. Since these results are for the twenty-four hour experiments, body temperature should have been in equilibrium with T_a .

Table 2 Length of "non-ventilatory" periods during breathing of angulate tortoises at three ambient temperatures (T_a ; °C). N is number of tortoises, \bar{X} is mean length of "non-ventilatory" period in minutes; s is S.D. in minutes; n is number of "non-ventilatory" periods. All times are to the nearest minute.

N	T_a (°C)	\bar{X}	s	n
1	25	31	4	7
3*	30	32	7	35
1	35	20	5	9

* \bar{X} is mean of means, s is S.D. of means. One highly variable run omitted (\bar{X} =66 minutes, s=20 minutes); settling time for this tortoise was about double the settling times recorded for other tortoises.

An "apnoeic" period of at least 46 minutes at 10°C and one of at least 44 minutes at 20°C were recorded during the first set of experiments studying the effect of T_a on resting oxygen consumption.

Resting metabolic rates

Mass-specific oxygen consumption at rest during summer ($\dot{V}O_2$; $\text{mlO}_2\text{g}^{-1}\text{h}^{-1}$) increased in a power relationship with increasing ambient temperature (T ; °C; Fig. 5). The relationship was stated by the equation:

$$\dot{V}O_2 = 0.0008T^{1.279} - 0.005 \quad (1)$$

The regression was highly significant ($F_{1,30} = 47.02$, $P < 0.005$, $R^2 = 0.619$). The data were heteroscedastic; the variability in mass-specific oxygen consumption increased at higher temperatures. Most of the variability was almost certainly the result of the tortoises displaying different levels of activity.

The Q_{10} between 10 and 20°C was 3.2, and between 20 and 30°C it was 1.8 (calculated as in Schmidt-Nielsen 1979).

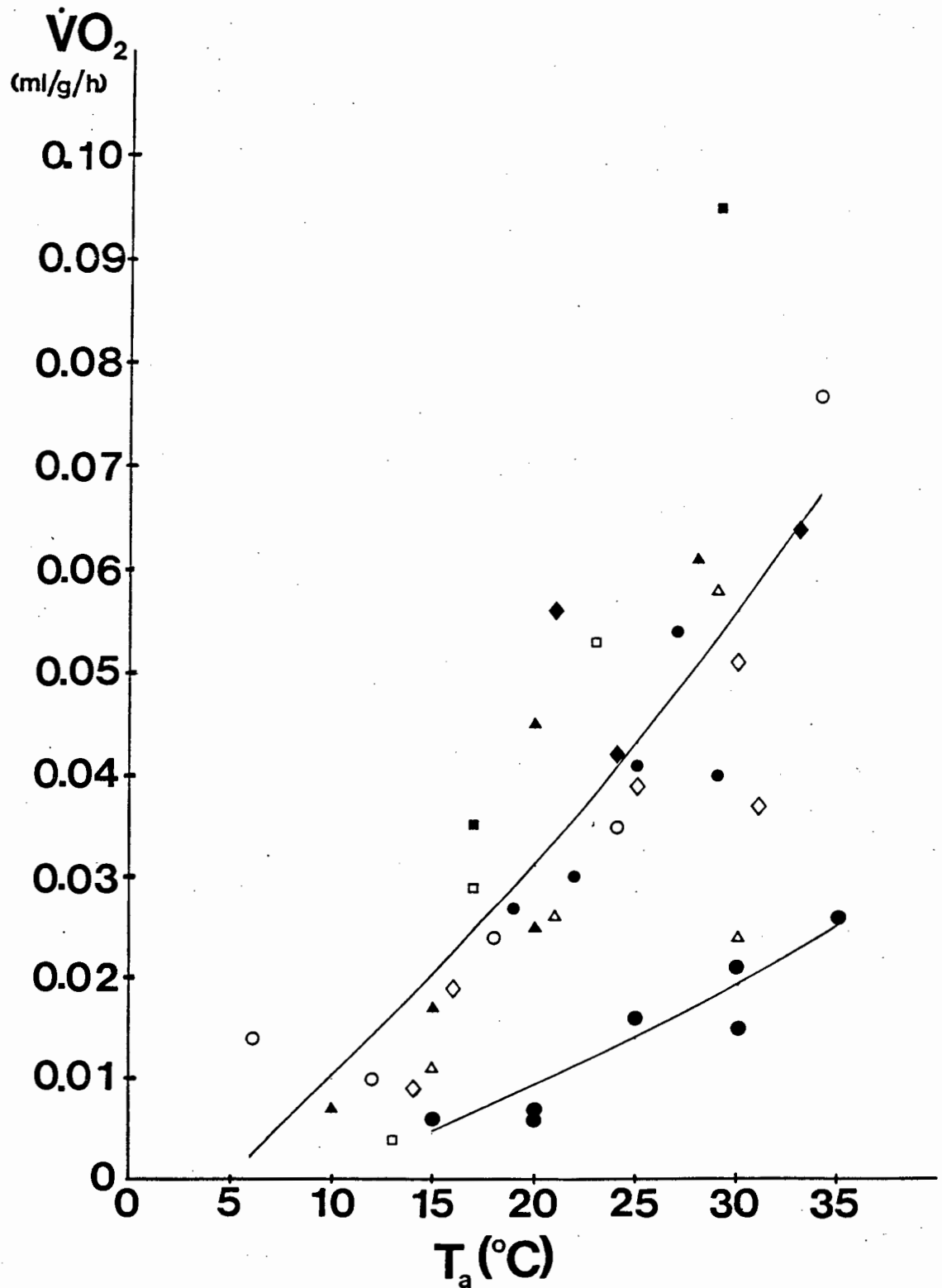


Figure 5 Mass-specific oxygen consumption ($\dot{V}O_2$; $\text{mlO}_2 \text{ g}^{-1} \text{ h}^{-1}$) of angulate tortoises at rest at various ambient temperatures (T_a ; degrees centigrade). Individual tortoises are coded by symbols (\blacktriangle 499g; \bullet 534g; \square 550g; \blacksquare 579g; \blacktriangle 645g; \circ 679g; \blacklozenge 749g; \diamond 1016g). The large dots and separate regression curve are for the 24-h experiments.

The relationship between mass-specific resting oxygen consumption ($\dot{V}O_2$; $\text{mlO}_2\text{g}^{-1}\text{h}^{-1}$) and ambient temperature (T ; $^{\circ}\text{C}$) in the acclimated phase during winter was best approximated by a power function between 15 and 35°C (Fig. 5), stated by:

$$\dot{V}O_2 = 0.00005T^{1.758} - 0.001 \quad (2)$$

One datum was omitted (30°C ; oxygen consumption was approximately twice that of the next highest). The regression was highly significant ($F_{1,6} = 37.056$, $P < 0.005$, $R^2 = 0.974$). The exponent of equation (1) was significantly less than that of equation (2) (Student's t -test, $t_{31} = -1391.176$, $P < 0.0005$). The mass coefficient of equation (1) was significantly greater than that of equation (2) (Student's t -test, $t_{31} = 2.178$, $P < 0.025$). The RMR in summer during the "non-acclimated" phase between 15 and 30°C was 3 to 4.2 times greater than that in winter during the acclimated phase, i.e. an average of about 3.5 times greater.

Allometry

At 20°C, resting oxygen consumption increased exponentially with body mass. The relationship between resting oxygen consumption ($\dot{V}O_2$; mlO₂h⁻¹) and body mass (M; g) was:

$$\dot{V}O_2 = 0.948e^{0.0033M} \quad (3)$$

The regression was highly significant ($F_{1,7} = 30.54$, $P < 0.005$, $R^2 = 0.814$). The oxygen consumption values, when converted to mass-specific values, for tortoises of masses between 698g and 798g from this equation were between 56% and 90% higher than the equivalent value from equation (2) at 20°C.

The relationship between resting oxygen consumption ($\dot{V}O_2$; mlO₂h⁻¹) and body mass (M; g) for tortoises under 450g (165mm total length) was best represented as an exponential function:

$$\dot{V}O_2 = 0.559e^{0.0045M} \quad (4)$$

The regression was highly significant ($F_{1,2} = 364.83$, $P < 0.005$, $R^2 = 0.997$). The slope was significantly greater than that of equation (3) (Student's t-test, $t_3 = 2.756$, $P < 0.05$). The relationship between resting oxygen consumption ($\dot{V}O_2$; mlO₂h⁻¹) and body mass (M; g) for tortoises between 678g and 798g was:

$$\dot{V}O_2 = 0.00006M^{1.841} \quad (5)$$

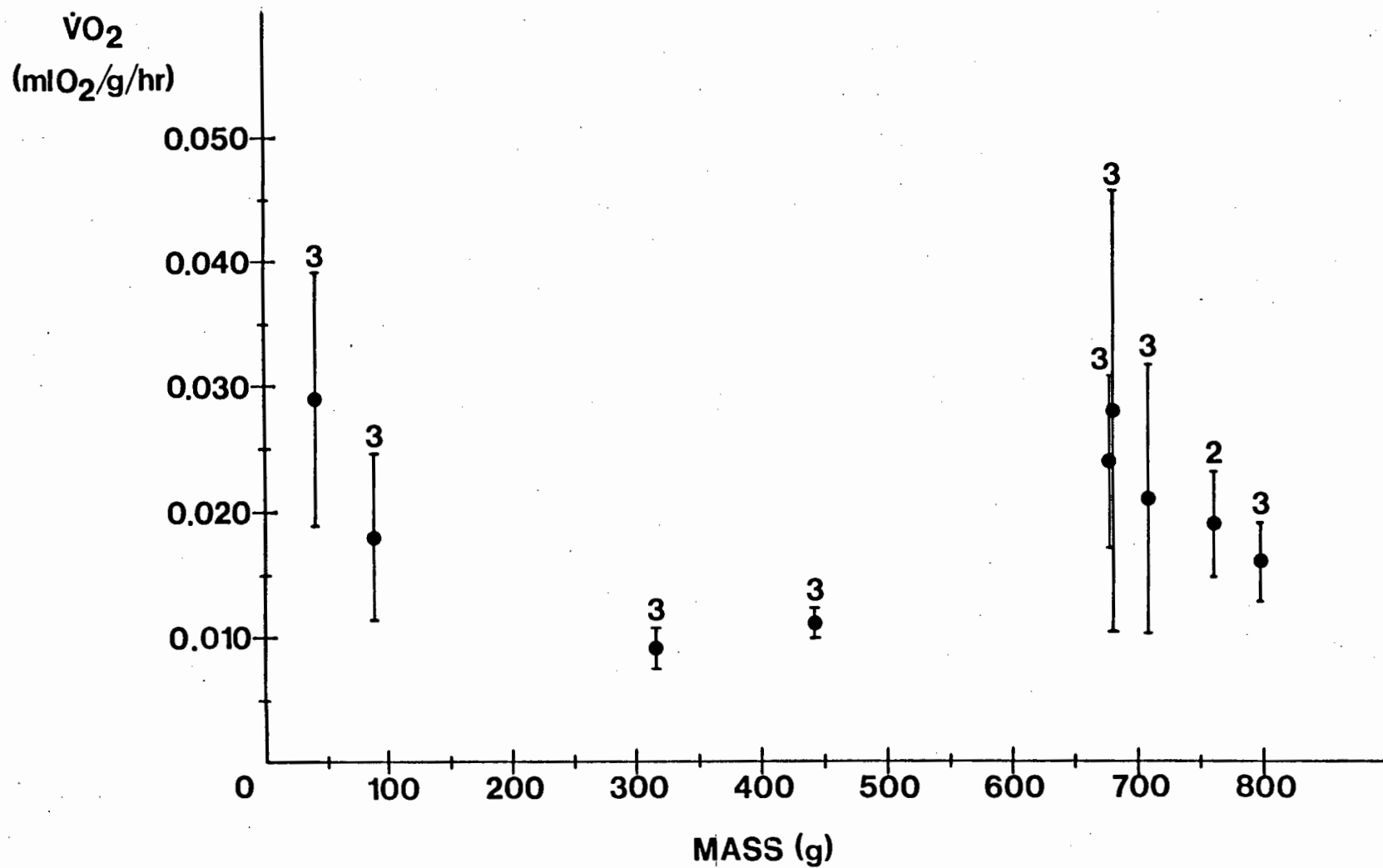


Figure 6 Mass-specific oxygen consumption of different sizes of angulate tortoises. The mean value for each individual is indicated by a dot; the vertical lines indicate \pm one standard deviation. The number above each line is the number of replicates done for that individual.

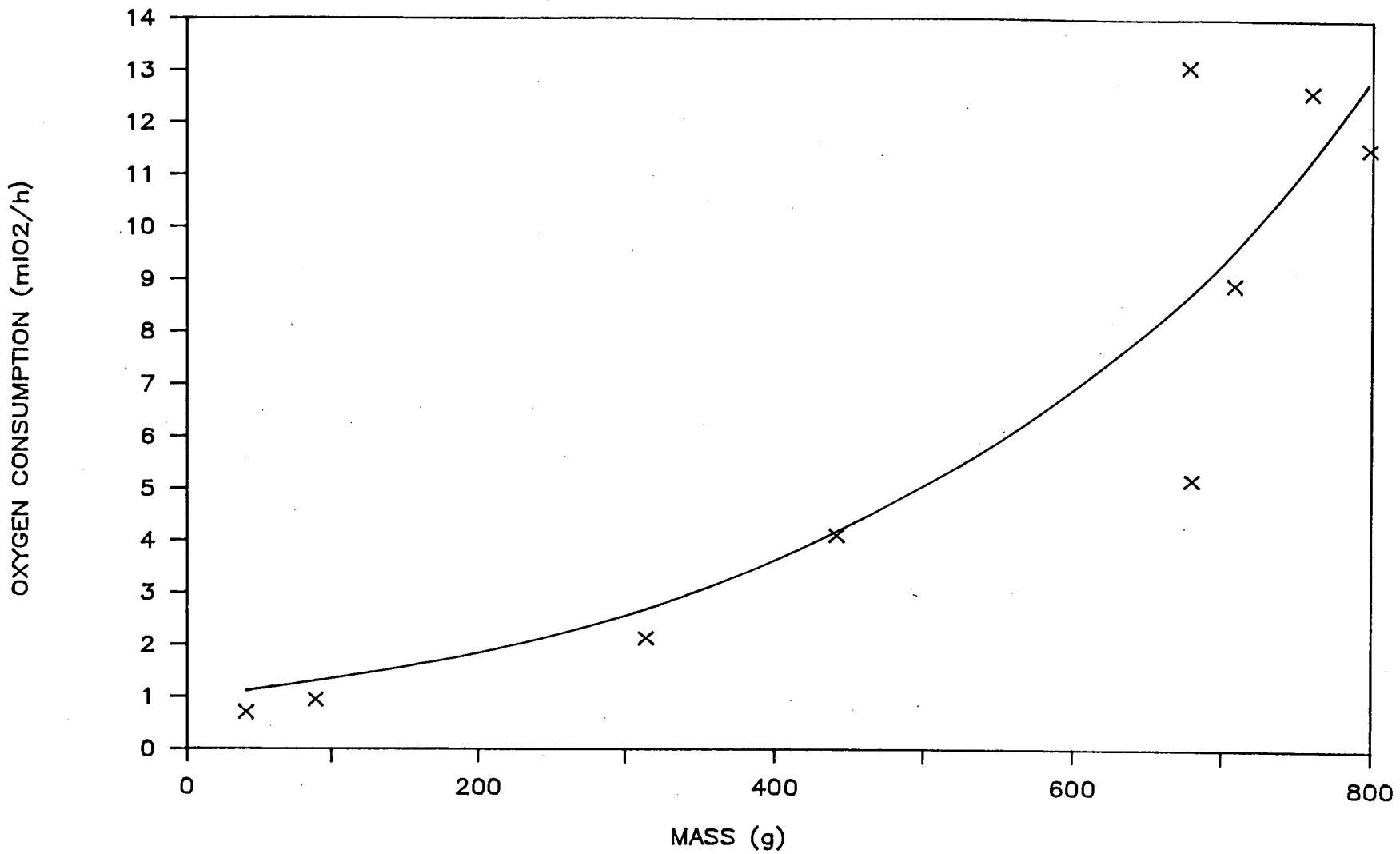


Figure 7 Oxygen consumption at rest ($\text{ml O}_2 \text{ h}^{-1}$) of angulate tortoises of different mass (grams). The curve represents equation (3).

The regression fit was not significant ($F_{1,3} = 0.66$, N/S). The range in mass for this equation is very small, and so the reliability of the equation for mature tortoises is uncertain. Figure 6 is the graph of mass-specific RMR on mass, and Fig. 7 is the plot of total resting metabolic rate against mass.

Discussion

High oxygen consumption in the angulate tortoise occurred generally during daylight hours, often beginning in the early hours of the morning and ending during early- to mid-afternoon, and occasionally for a short period in the early evening. The results did not indicate any precise pattern of movement during the twenty-four cycle, as has been recorded for another species (Cloudsley-Thompson 1970), except that activity mainly occurred during the morning and early afternoon. However, it cannot be certain whether the general pattern of oxygen consumption found was biologically meaningful, because the tortoises were in an unnatural environment, where stress must have played some role in determining patterns of oxygen use. The differences in oxygen consumption between the morning and the afternoon/evening hours were not statistically significant, but it was felt that the small sample size precluded rigorous statistical analysis.

Similar patterns of oxygen consumption to those found for *Chersina angulata* have been recorded for the tortoise *Testudo hermanni* at three T_a s by Kirsch & Vivien-Roels (1984). They found that *T. hermanni* shows a daily rhythm in oxygen consumption, with peak values during the day. The peaks start in the pre-dawn hours and attain a maximum during the morning. At higher temperatures the peaks attain greater amplitude. At low temperature (8°C) no rhythm exists. Cloudsley-Thompson (1970) found that activity in the desert tortoise *T. sulcata* shows a daily rhythm, peaking during daylight hours (the peak in activity is bimodal). Kirsch & Vivien-Roels (1984) observed that *T. hermanni* is active during high oxygen consumption periods, and so the daily rhythm in oxygen consumption appears to be related to their activity period. The length of time the rhythm in metabolic rate persists in the angulate tortoise under constant photoperiod conditions was not determined. With *T. hermanni* the peaks decline after a couple of days at 18°C (Kirsch & Vivien-Roels 1984). The amount and duration of activity in the desert tortoise declines in constant dark conditions after a few days (Cloudsley-Thompson 1970).

Brumation in *T. hermanni* appears to be related to an endogenous circannual rhythm in its metabolic rate (Kuchling 1981). There are also significant variations in oxygen consumption during the brumation period. However, the maximum difference between the mean values recorded during brumation were in the order of 67%. There appeared to be a

circannual rhythm in the RMRs of angulate tortoises. Mass-specific metabolic rates in summer were three to four times greater than winter (from equations (1) and (2); Fig. 5). This occurred even though the tortoises were kept indoors during the experiments, where T_a changes would not be as severe as outside, and under a "summer" photoperiod. The tortoises had, however, been exposed for about four weeks to the natural autumn/ winter environmental conditions until a couple of weeks before the experiments started. This is necessary to initiate the endogenous changes in the metabolic rate which accompany brumation in *T. hermanni* (Kuchling 1981). Kuchling showed that in *T. hermanni* the endogenous circannual rhythm overrode forced changes in the state of activity of the tortoise. Mass-specific metabolic rates were initially significantly higher in tortoises forced into hibernation during the wrong time of the year, and it possibly took a month for normal hibernating metabolic rates to be reached (Kuchling 1981). The complicating factors in determining whether a circannual rhythm exists in the angulate tortoise, however, were the extents to which (1) acclimation to captivity and the experimental conditions, and, (2) disturbance, affect RMRs in these tortoises. During the summer experiments, oxygen consumption was measured relatively soon after the tortoises had settled. With respect to the duration of the experimental runs, Kirsch & Vivien-Roels (1984) suggested that even after 24 hours, acclimation levels were not reached in *T.*

hermanni. During my "twenty-four" hour experiments, the decrease in RMR due to acclimation could be calculated. The experiment showing the greatest decrease had an initial RMR that was 2.3 times greater than the final recorded RMR; generally the difference was much less (the mean figure calculated for the experiments being 1.4 times). Therefore acclimation could not account for all the difference in metabolic rates; it probably accounted for about half the difference. Disturbance during transport does significantly raise blood glucose concentrations (Kuchling 1981), and therefore metabolic rates are increased. Disturbance did occur during the transport of tortoises before experiments, but it was similar for both experimental periods.

Supporting evidence for a circannual rhythm in the metabolic rates of angulate tortoises was that the mass-specific resting oxygen consumption for tortoises between 698g and 798g mass during late winter/early spring (from equation (3)) were only between 56%-90% higher than the value for tortoises during winter at 20°C obtained from equation (2). The experimental lengths during late winter, however, were more similar to those of the summer experiments. The effect of acclimation to captivity could not be assessed. It is possible that the tortoises merely adapted to sitting in relatively small cages, and their metabolic rates decreased as a result of this adaptation to the cages. If this was the case, then it takes these tortoises many months to show a decrease in metabolic rate due to acclima-

tion to their living conditions. The tortoise pen and room were generally visited only once or twice a day, for cleaning and feeding purposes, or to return tortoises after experiments. This ensured as little habituation of tortoises to humans as possible.

It is realised that RMRs (and therefore equations (1) and (2)) are strongly influenced by differences in mass (Robinson, Peters & Zimmermann 1983; Andrews & Pough 1985; Wright 1986). However, at least 62% of the variation in equations (1) and (2) was explained by temperature effects alone, which suggests that equations (1) and (2) are valid. In summary, there is a strong possibility, from the evidence, for an endogenous circannual rhythm in angulate tortoises, with a reduced RMR during winter.

It has been shown that turtles and tortoises breathe in phases separated by an apnoeic period, i.e. the respiratory period consists of a ventilatory period and a period of breath-holding (Cloudsley-Thompson 1974; Wood & Lenfant 1976; Glass, Burggren & Johansen 1978; Benchetrit & Dejours 1980; Milsom & Jones 1980). The ventilatory period begins with an active expiratory phase which precedes an active inspiratory phase. There may be one to several breaths during the ventilatory period. The final inspiration is followed by the period of breath-holding. The breath-hold length is regulated by the chelonian to control its respiration (Benchetrit & Dejours 1980; Milsom & Jones 1980).

The results for breath-hold length in this study were surprising. Benchetrit & Dejours (1980) recorded apnoeic periods of three seconds to eight minutes (mean 43 seconds) for *Testudo horsfieldi* between 23 and 25°C. Glass et al. (1978) recorded a mean non-ventilatory period of 42 seconds at 25°C for *T. pardalis*. These are much lower than those recorded for *C. angulata* (eg. 31 minutes at 25°C) from oxygen depletion traces. However, Cloudsley-Thompson (1974) found that individuals of *T. graeca* hold their breaths for 15-20 minutes at T_a s of 26 and 27°C.

This large discrepancy in breathing patterns could be explained in three ways. Firstly, tortoise species in temperate and outer-subtropical areas tend to undergo brumation during winter (Auffenberg & Iverson 1979; Kuchling 1981). Variations in tortoise (and other reptilian) metabolic rates are temperature-specific and season-specific (Bennett & Dawson 1976; Kuchling 1981; Kirsch & Vivien-Roels 1984). During the time of year that my experiments took place (May/June), angulate tortoises are normally inactive (Branch 1984; pers. obs). If the natural inactivity of angulate tortoises is endogenously controlled, the slow respiration due to the low metabolic rate would then account for the long apnoeic periods. The long "apnoeic" periods recorded at low ambient temperatures may have been due to the effect low temperatures have on the metabolism of ectotherms. The other possible reasons are that either the methods used in this study, and by Cloudsley-Thompson (1974), were not sensitive

enough to record all breaths, or else the methods used by Benchetrit & Dejours (1980) and Glass et al. (1978) (eg. masks) were stressful to the tortoises studied, which would increase their ventilation rates. However, not enough details of method and equipment were given by the authors to determine how stressful the conditions were. More sensitive recordings of pressure changes or gas exchange under non-stressful conditions are required before any firm conclusions can be reached regarding differences in ventilatory rates between tortoise species.

The mass-specific RMR of angulate tortoises were within the same order of magnitude as those recorded for other tortoise species (Table 3). A relatively constant Q_{10} over a wide range in temperatures has been found in *T. hermanni* and in *G. carbonaria* (approximately 3.5 and 2.0 respectively) (Kirsch & Vivien-Roels 1984; calculated from data in Santos-Pinto et al. 1985). In *C. angulata* the Q_{10} changed continuously, as is probable for all tortoise species, but was less at higher temperatures (10-20°C: 3.2; 20-30°C: 1.8; equation (1)).

Heusner (1982a, b) suggested that the RMRs of different age groupings within a species would have allometric scalings with different mass exponents, but that it would be 0.67 for mature individuals of any isomorphic species. The exponent for growing individuals would be greater than 0.67. The mass coefficient would also change with structural or

Table 3 Mass-specific resting metabolic rates ($\dot{V}O_2$; $\text{mlO}_2\text{g}^{-1}\text{h}^{-1}$) of different tortoise species at various ambient temperatures (T_a) or body temperatures (T_b). Mass is in grams, temperatures are in degrees centigrade, N is the number of individuals tested

Species	Mass(g)	N	T_a ($^{\circ}\text{C}$)	T_b ($^{\circ}\text{C}$)	$\dot{V}O_2^a$	Reference ^b
<i>Chersina angulata</i> ^c	50	-	20	-	0.027	1
	100	-	20	-	0.016	1
	300	-	20	-	0.010	1
	400	-	20	-	0.010	1
	700	-	20	-	0.014	1
<i>Geochelone elephantopus</i>	69000,	2	20	-	0.014	2
	132000					
<i>G. gigantea</i>	534	1	-	22.9	0.027	4 ^d
	772	1	-	28.2	0.047	4 ^d
	1378	1	-	26.2	0.049	4 ^d
	1806	1	-	27.5	0.046	4 ^d
	19550	1	-	24.0	0.017	4 ^d
	80000	1	20	-	0.015	4 ^d
<i>G. denticulata</i>	4200	1	20	-	0.014	2
	4200	1	30	-	0.035	2
<i>G. polyphemus</i>	5450	1	20	-	0.016	2
	5430	1	30	-	0.030	2
<i>G. carbonaria</i> ^e	1600	-	15	-	0.024	6
	10300	-	15	-	0.007	6
	1600	-	20	-	0.032	6
	10300	-	20	-	0.016	6
	1600	-	30	-	0.063	6
	10300	-	30	-	0.036	6
<i>Testudo graeca</i>	225	1	18	-	0.027	3
<i>T. hermanni</i>	265-474	4	8	-	0.003	5 ^f
	265-474	4	18	-	0.011	5 ^f
	265-474	4	28	-	0.033	5 ^f
	672	1	-	24.6	0.030	4 ^d

- ^a Oxygen consumption at rest in $\text{mlO}_2\text{g}^{-1}\text{h}^{-1}$
- ^b
- 1 This study
 - 2 Benedict (1932), quoted by Bennett & Dawson (1976)
 - 3 Dontcheff & Kayser (1937), quoted by Bennett & Dawson (1976)
 - 4 Hughes *et al.* (1971). Data selected from this paper on basis of low values for inactive animals over a large mass range
 - 5 Kirsch & Vivien-Roels (1984)
 - 6 Santos-Pinto *et al.* (1985)
- ^c Theoretical values from equation (3)
- ^d Oxygen consumption at normal temperature and pressure; T^a ranged from 21.3°C to 29.2°C
- ^e Theoretical values from allometric equation; smallest tortoise used in experiments was 1600g. Body temperatures in equilibrium with T
- ^f Mean of recorded values for four (possibly five) tortoises. Body temperatures in equilibrium with ambient temperatures

functional differences. Therefore the allometric equations for heteromorphic species, or for different age groupings within a species, would have different mass coefficients. How do the results of this study compare with those expected from theory dealing with metabolic rates in isomorphic animals? The RMR of angulate tortoises increased exponentially with body size, not as a power function which is typical of most animals. The best fit curve for relatively fast-growing tortoises was also an exponential function. This suggests that a power function is not necessarily the only form of equation that describes the allometry of RMR. Juvenile reptiles and mammals have different allometric scalings to adults (Brody 1974; Andrews & Pough 1985). This can be seen from differences between intraspecific and interspecific scalings (Feldman & MacMahon 1983; Andrews &

Pough 1985), and has been shown empirically for mammals (Brody 1974). Empirical evidence for reptiles is lacking (Andrews & Pough 1985). The juvenile angulate tortoises showed a different metabolic scaling at rest to the adults (Fig. 6). However, juvenile mammals have relatively higher RMRs than adults (Brody 1974), and this is implied in reptiles from the intraspecific slopes. The discrepancy shown by the results for the study animal, in which the juveniles had relatively lower RMRs (Fig. 6; Table 3), was in part due to the adults showing more stress than the juveniles. This is noticeable in the respiratory traces (Fig. 4). The younger animals were more relaxed, judging by their longer apnoeic periods. Tortoises that are stressed can be heard to breathe, and appear to breathe more rapidly (pers. obs). However, the traces for some of the larger tortoises show the characteristic long periods of apnoea. Therefore some of the discrepancy may have been real. The shape of the graph of mass-specific RMR against mass is hyperbolic. The form of the relationship is influenced strongly by the covariance between body mass and mass-specific RMR, since the mass term is found in both variables (Heusner 1982a). Differences in relative shell masses and urine volumes between different-sized individuals would affect the shape of such a curve. Angulate tortoises show a distinct slowing in growth rate between a total length of 130mm and 160mm (Fig. 5B in Branch 1984). This corresponds to a mass of about 350g to 450g. If younger tortoises do have comparatively larger shells than

adults (Section 2), the relative effects of a large shell mass and a slowing metabolism may have contributed to the minimum occurring at this size. Another possibility is that the small individuals used in this study had unusually low metabolic rates. Owing to the small sample size this is a possibility. Differences between the juvenile and the adult tortoises in acclimation or nutritional state could have affected the results.

Mature tortoises grow throughout their lives. The theory put forward by Heusner for mature isomorphic animals does not hold for them, because the assumption of no further structural development is not fulfilled. However, the exponent for mature tortoises was much greater than the proposed 0.67. The much steeper slope was probably the result of certain individuals showing consistently high oxygen consumptions, due to the restrictive environment of the metabolic chamber or to individual variability in activity (Fig. 6). The tortoises seemed to rest with their appendages withdrawn into their shells. From observation, it appeared that exhalation and inhalation occurred when the tortoise extended its neck and limbs. The restrictive environment probably affected breathing movements, ie. extension of limbs and neck during inhalation. Andrews & Pough (1985) have shown that the slope for mature squamate reptiles is 0.80, which is greater than that suggested by theory. Whether these reptiles are isomorphic is not known. Heusner (1982a, b) backed up his theory empirically with results

obtained for mammals. Poikilotherms may show physiological system scalings that are different to mammals. For example, there may be differences in the use of anaerobic metabolism between mammals and reptiles (Bartholomew 1982). As has been pointed out (eg. Schmidt-Nielsen 1979), factors such as the allometric scaling of the circulatory system, and differing rates of the intermediate metabolic reactions, may play a major role in determining the scaling of RMR. These may vary between species.

The slope of the equation for the smaller tortoises would have been most affected by fluctuations in flow-rate and drift in the oxygen analyser and chart recorder.

It is realised that the confidence in the interpretation of the results in this paper is somewhat restricted by the small sample sizes and small number of replicates. Future studies should incorporate a larger number of tortoises, and more replicates per individual.

The results also indicate the importance of using absolute RMR, as opposed to mass specific RMR, when comparing differences between species in metabolic rates. If mass-specific metabolic rates are used, then a specific body mass must be used. Extrapolations to a body mass greater than that found in a species, or even outside the range of that used to establish the metabolic relationship, must be done with extreme caution.

The results obtained bring out a few interesting points about experimental procedure with tortoises. RMR decreases

with time as the tortoises become acclimated to the experimental conditions (Methods; Fig. 3). This has been pointed out previously (Kirsch & Vivien-Roels 1984). These authors suggested that the standard metabolic rate should be calculated from data obtained during the period between forty-eight and seventy-two hours after the start of the metabolic determinations. However, this leads to excessive experimental time being used, and results obtained after a few hours should suffice if they are divided by an acclimation factor. In angulate tortoises, this factor is 1.4.

There is a danger, especially with flow-through systems, of taking readings too soon, before the animal has settled. If the animal struggles for a long time an oxygen debt may develop, which possibly takes hours to repay in reptiles (Bartholomew 1982), and therefore oxygen consumption will be raised for a long period afterwards. Reptiles seem to rely on anaerobic respiration only during "burst" and forced activity (Bennett & Dawson 1976; Bennett 1978; Bartholomew 1982), but it may occur during struggling. Nevertheless, there does not seem to be many precise data on the relative importance of anaerobic respiration and the magnitude of the oxygen debt incurred during activity in tortoises. The recovery from oxygen debt may partly explain the lower oxygen consumptions found towards the ends of the twenty-four runs, together with the lowering of stress as the tortoises became used to the experimental environment.

As can be seen from the respiratory patterns, it is necessary to take samples over many minutes, and preferably hours, using flow-through systems, so that both ventilatory and non-ventilatory periods are adequately represented to avoid over-estimating or under-estimating RMR. Another source of error lies in the fact that the body temperatures of the tortoises may not be in equilibrium with the T_a , thereby increasing variability within the readings. This is overcome by allowing longer experiments.

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CHAPTER 4

ASSIMILATION EFFICIENCY

The amount of energy required by an unrestrained animal to exist over a specified period of time without impairing its activity or depleting its mass can be calculated from the amount of energy it consumes and the amount it egests. The amount of food an animal absorbs through its digestive tract as a percentage of the food it consumes is known as the assimilation efficiency of the animal. When the amount absorbed is expressed in energy terms (units are kilojoules, or kJ), the assimilated energy is obtained, and this is a measure of the energetic cost of free existence provided the animals do not lose mass or gain it in appreciable amounts. Of course some energy is lost in the urine, but usually in reptiles this is not so great a quantity as to make the assimilated energy an unsuitable index of daily energy expenditure (see Nagy & Medica 1986). This is especially true where urine collection may be unreliable, as in tortoises. The term "assimilation efficiency" (= digestive efficiency) is used loosely for convenience, since most authors use it (see Harlow, Hillman & Hoffman (1976) for a discussion about usage).

In herbivorous reptiles the use of solid markers in digestion trials have been used (Hamilton & Coe 1982). However, in the angulate tortoise, being a small tortoise, this method is not satisfactory, and so a continuous feeding method was utilised (Maynard 1951). The latter is the most appropriate method when the animals have complex digestive systems, such as those of many herbivores (Maynard 1951). It

is necessary that the animals can maintain energy balance on the food provided, and that the experimental period is long enough to avoid periodic fluctuations in feeding or defecation. Several animals are required for a digestion trial to minimise individual variability (Maynard 1951).

The assimilation efficiency of the angulate tortoise on a single type of food was investigated, and this is related to the total energy requirements for this species to maintain an existence metabolism.

Methods

For the assimilation efficiency experiments four tortoises of similar mass (range 542-765g) were placed singly in roughly rectangular enclosures in a pen open to the elements. The pen had a concrete floor. Each tortoise was weighed before and after the feeding trials. All tortoises were adult individuals, as indicated from growth curves and other data (Branch 1984), and all were in the total length range of over 150mm. Each tortoise was fed carrot tops *ad lib.* for a period of about three weeks before the actual trials started. New carrot tops were fed every day and no water was provided; however tortoises could drink when it rained (although they were not seen to do so). The fresh food fed was weighed to obtain the wet mass. A representative sample of the food fed to each tortoise each day was weighed, dried at 49-62°C to constant mass, and weighed again to get the wet:dry mass. The proportion of leaves to

stem roughly similar to that actually eaten by the tortoise was sampled. The tortoises were only occasionally seen to chew at the stems. The dry mass eaten by each tortoise was calculated as:

$$\{[\text{Wet mass fed (g)}] \times [\text{dry:wet mass ratio}]\} - \text{dry mass collected (g)} \quad (1)$$

Dry mass assimilation efficiency (DMAE; %) was calculated as reported by Hamilton & Coe (1982):

$$\text{DMAE} = \frac{\text{consumption} - \text{defaecation}}{\text{consumption}} \times 100\% \quad (2)$$

All masses were in grams.

Energy assimilation efficiency (EAE; %) was calculated by replacing the masses of the components in equation (2) with their respective energy values (kJ).

The trials were carried out in February/March 1986 during the latter part of the Cape summer, when it could be expected that the tortoises were feeding well before the cold, wet winter months. During the latter period the tortoises are largely inactive (Branch 1984; pers. obs).

Results

The mean dry mass of carrot tops eaten by each tortoise per day, the mean dry mass of faeces produced per day, and their respective energy contents in kilojoules are given in Table 1. Faeces were not produced every day, so a theoretic-

cal daily mean was obtained from the data. The overall mean dry mass of carrot tops eaten and faeces produced per day, and their respective energy values are also given. The mean dry mass as a percentage of wet mass for the carrot tops used was 17.95%.

Table 1 Mean dry masses and energy content of carrot tops eaten and faeces produced per day by tortoises (Individual tortoises are labelled under No.; \bar{X} = mean dry mass in grams per day; s = standard deviation; kJ = energy content in kilojoules per gram dry mass; n = number of replicates per tortoise)

No.	Food (Carrot Tops)						Faeces				
	\bar{X}	s	n	kJ	s	n	\bar{X}	s	kJ	s	n
3	1.15	0.47	14	16.20	0.06	6	0.10	0.07	17.03	0.52	6
6	1.04	0.40	21	15.95	0.14	6	0.16	0.40	16.31	0.67	7
10	0.89	0.43	14	15.88	0.12	6	0.11	0.21	16.26	0.92	7
11	0.96	0.41	21	16.01	0.15	6	0.06	0.11	14.26	0.66	6
Mean.	1.01	0.11		16.01	0.14		0.11	0.04	15.97	1.19	

The dry mass and energy assimilation efficiencies of *C. angulata* on carrot tops are provided in Table 2. Also shown

Table 2 Dry mass assimilation efficiency (DMAE; %), energy assimilation efficiency (EAE; %), mean energy absorbed per day (E absorbed; kJday⁻¹), and mean energy absorbed per kilogram per day (E absorbed kg⁻¹; kJkg⁻¹body mass day⁻¹) for angulate tortoises feeding on carrot tops.

Mass	DMAE	EAE	E absorbed	E absorbed kg ⁻¹
0.564	91.3	90.9	16.927	30.012
0.660	84.6	84.3	13.978	21.179
0.765	87.6	87.3	12.344	16.136
(0.542	93.8	94.4	14.514	26.779)*
Mean	0.663	87.8	14.416	22.442
+ SD		3.35	2.3227	7.0237
(First 3)				

* Died later the same year

are the energy values of the food absorbed by each individual, and energy absorbed per kilogram body mass for inter-specific comparison.

Dry mass assimilation efficiency and energy assimilation efficiency were both 88%. Mean energy absorbed was 14.4kJ, and per kilogram it was 22.4kJ.

Discussion

The assimilation efficiency of angulate tortoises on carrot tops is very high, in terms of both dry mass and energy

(Table 2). Other herbivorous reptiles have dry mass assimilation efficiencies ranging between 30 and 70% (Nagy & Shoemaker 1975; Hamilton & Coe 1982; Nagy & Medica 1986). Energy assimilation efficiencies vary between 34.5 and 86.3% (Throckmorton 1973; Ruppert 1980; Hamilton & Coe 1982; Nagy & Medica 1986). It seems likely that the high assimilation efficiency found in this study is attributable to a low cellulose and fibre content of carrot tops, since an inability to digest cellulose has been suggested as a reason for the low assimilation efficiency of the Aldabra giant tortoise (Hamilton & Coe 1982). Only between 24 and 25% of the nutrient holocellulose is broken down in the diet of this giant tortoise (Hamilton & Coe 1982). A relatively high fibre content in the diet may decrease assimilation efficiency markedly (Harlow *et al.* 1976). It is assumed that carrot tops are low in fibre. Of course, carrot tops are not the natural diet of angulate tortoises, and many fynbos plants have high fibre and cellulose contents (eg. Specht & Moll 1983), so the assimilation efficiency of this species on its natural diet should be considerably lower.

When compared with other tortoise species (Table 3), the angulate tortoise has a lower requirement of assimilated energy (= existence metabolism, or daily energy expenditure) than the other tortoises. In terms of size, larger species have lower mass-specific energy requirements (Peters 1983). Based on the expected mass-specific energy requirements, the values for angulate tortoises would have been between the

values recorded for the other two species (Table 3; mean mass of *Gopherus agassizii* was 521g, that of *Geochelone gigantea* was ≈ 17 kg).

Table 3 Dry mass assimilation efficiency (DMAE; %), energy assimilation efficiency (EAE; %) and mean existence metabolism (EM; $\text{kJkg}^{-1}\text{day}^{-1}$) for different species of tortoise

Species	DMAE	EAE	EM ^a	Reference
<i>Chersina angulata</i>	87.8	87.6	21.8 ^b	This study
<i>Geochelone gigantea</i>	30.4	34.5	26.3 ^c	Hamilton & Coe (1982)
<i>Gopherus agassizii</i>	61.6	54.3	39.7 ^d	Nagy & Medica (1986)

^a During dry season

^b From mean values for 660g tortoise (Table 2)

^c Calculated from ash-free dry masses

^d Calculated from data for force-fed individuals (similar figure to annual, weighted, mean metabolisable energy for free-ranging tortoises in the same study)

Within the size range of angulate tortoises, the largest individual had the lowest mass-specific energy requirements for free existence, and vice versa for the smallest individ-

ual. This is the typical pattern, because smaller individuals generally grow faster relative to larger ones (Peters 1983; Branch 1984).

The median of recorded maximum and minimum temperatures during the digestion trials was 22°C. The resting metabolic rate of a tortoise of 660g mass during summer is approximately $580\text{mlO}_2\text{day}^{-1}$ (equation 1, Chapter 3). In energy terms this is 11.668kJday^{-1} , or $17.679\text{kJkg}^{-1}\text{day}$, taking the energy value of one litre of oxygen to be 20.083kJ (Schmidt-Nielsen 1979). The daily energy expenditure of reptiles is usually between 1.7 and 3 times the resting metabolic rate (Nagy & Shoemaker 1984; Peters 1983). For tortoises which have a heavy shell but are generally slow-moving, a practical estimate would be twice the resting metabolic rate. Therefore in daily energy terms, as obtained from oxygen consumption figures, a 1kg angulate tortoise should require 35.358kJday^{-1} during summer. This figure is greater than that obtained for angulate tortoises from calorific figures for daily food absorption ($21.8\text{kJkg}^{-1}\text{day}^{-1}$; Table 3). The energetic cost of free existence is 1.2 times the resting metabolic rate during summer, or 4 times the resting metabolic rate during winter. These figures are similar for a 660g and a 1kg tortoise, owing to the way the results were calculated (Table 2; see equations (1) and (2), Chapter 3). The angulate tortoises under study were confined in a small pen and fed by hand, and therefore were not in their natural habitat. This would lower the locomotion costs. It is

possible that the daily energy expenditure is lower than that for many other reptiles because of the insulating and protective effects of the shell mentioned above, and because tortoises move slowly. Nevertheless under natural free-ranging conditions, transport costs for tortoises are probably relatively high compared to other reptiles. The metabolisable energy efficiency of an animal takes into account energy lost through the urine, and it is a better estimate of daily energy requirements (Nagy & Medica 1986). Energy loss in the urine is low. In *G. agassizii* it is 3% of the energy consumed (Nagy & Medica 1986). Assuming the same proportion lost in *C. angulata* as is lost in *G. agassizii*, the daily energy expenditure in *C. angulata* would be 20.766kJ.

Most of the tortoises (three of the four) gained mass during the experiment. However, as Nagy & Medica (1986) have pointed out, under the experimental conditions it is not possible to say how much of the mass gain was due to water intake and storage, and therefore it is not known if these tortoises were in positive energy balance or not. A small loss in mass does not significantly affect energy or dry mass assimilation efficiency (Nagy & Medica 1986). Carrot tops have a high water content ($\approx 82\%$ by mass), and the relatively low consumption of food in dry mass terms compared to the other species may be partly due to this fact; Nagy & Medica (1986) have shown that dry matter intake is low when desert tortoises (*G. agassizii*) eat succulent green plants.

The energy content of carrot tops is similar to that found by these authors for the natural summer food of *G. agassizii* (16.0 and 16.8kJg⁻¹ respectively).

Gut passage time in angulate tortoises is not known, but it is presumed that the collection periods (14 and 21 days) were long enough to enable representative samples of faeces to be collected. Other tortoise species have gut passage times ranging from approximately 8 to 20 days (Hamilton & Coe 1982; Nagy & Medica 1986). A carmine dye added to the food of the angulate tortoises was not visible in the faeces. Hamilton & Coe (1982) and Nagy & Medica (1986) have shown that food consumption may vary greatly according to the environmental conditions, time of year and availability of different food types. It is therefore necessary to monitor food consumption by the angulate tortoise throughout the year before reliable qualitative comparisons can be made with other species. In fact the tortoise *G. agassizii* may be under negative energy balance during certain times of the year, which is corrected during other parts of the year (Nagy & Medica 1986). This may also occur in *C. angulata*.

Eggs were laid by female tortoises studied for this thesis between October and March, so it appears that these trials took place towards the end of the breeding season, and there would be less demand on the food reserves then. However, the tortoises probably would be feeding well before the winter brumation, and possibly to replace food stores used during the reproductive period. Lack of specific

nutrients in the diet can affect assimilation efficiency (Maynard 1951). It is not known whether the tortoises were affected by a lack of specific nutrients in carrot tops (or in the general food fed to them). The experimental period was, however, well within the limits in which this species survives naturally, with little or no access to such nutrients, i.e. in winter (Branch 1984). Hamilton & Coe (1982) found consumption did not differ between sexes in *G. gigantea*. Although both sexes were used in the experiments reported here, the sample size and mass range, *inter alia*, were not suitable to test for differences between sexes.

It has been shown in a herbivorous lizard that an increase in body temperature above a critical minimum concomitantly causes an increase in assimilation efficiency (Harlow *et al.* 1976). For reptiles direct solar radiation can be a more important avenue of heat gain as far as raising body temperature is concerned than conductance during the earlier hours of the day, and enables the reptiles to enjoy a longer period of time at optimal body temperatures for digestion (Stevenson 1985; McFarland, Pough, Cade & Heiser 1985). Much of the pen in which the tortoises were kept was fairly well shaded, and although the measured ambient temperature range (15+34°C) encompassed the probable preferred body temperature range of the angulate tortoise (Perrin & Campbell 1981; see Branch 1984), it is possible that the solar radiation was not intense enough in the enclosures to enable the most efficient body

temperatures for digestion to be kept for prolonged periods. This might have reduced the consumption rates of the tortoises and the food passage time, and this may have been a further factor in the low total energy intake found during these assimilation trials.

My experiment has shown that it is difficult to estimate realistically the energy balance of free-ranging tortoises in their natural environment from values obtained for tortoises in captivity. Future work should concentrate on providing a more natural environment for the tortoises, allowing free-ranging activity, utilising natural food and a larger sample size, determining passage times of food in the gut, and paying attention to the body composition of the tortoises.

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CHAPTER 5

SCALING OF RATES OF HEAT TRANSFER

Tortoises are ectothermic reptiles, and therefore their deep body temperatures are determined to a large extent by ambient conditions. The deep body temperature of a tortoise at any time is determined by the various heat flows to and from the body of the tortoise, and the ability of the tortoise body to store heat. The heat flow can be described by the equation (modified from Schmidt-Nielsen 1979):

$$H_{\text{tot}} = H_m + H_s - H_e + H_c + H_r \quad (1)$$

where H_{tot} is total heat contained in the body at any one instant; H_m is heat produced by metabolism - heat lost due to work; H_s is heat storage in the body; H_e is heat lost through evaporation; H_c is lumped convective and conductive heat exchange; H_r is radiative heat exchange (this assumes that no other factors occur which could affect heat exchange, such as water vapour condensation on the body surface).

Deep body temperature is an index of the heat contained within the body core at any time. Body temperature varies throughout the body (Schmidt-Nielsen 1979), but the core of the body produces the most endogenous heat. Because deep body temperature does not fluctuate as much as temperature elsewhere in the body, it is used as representative of body temperature (Schmidt-Nielsen 1979). In chelonians, deep body temperature is the most practical and reliable temperature measurement (Webb & Johnson 1972; Webb & Witten 1973; Voigt & Johnson 1977; Hutchison 1979). The relationship between

deep body temperature and overall heat transfer between an ectotherm and its environment can be represented in linearised form by (Bakken 1976b):

$$T_b = T_e + M^*/K_o - \tau dT_b/dt \quad (2)$$

where T_b is deep body temperature ($^{\circ}\text{K}$); T_e is the operative environmental temperature ($^{\circ}\text{K}$); M^* is the effective net metabolic heat production (W animal^{-1}) (ie. metabolic heat production minus heat lost through evaporation and work); K_o is the overall thermal conductance of the ectotherm ($\text{W }^{\circ}\text{K}^{-1} \text{ animal}^{-1}$) (takes into account conductance of the skin of the animal, the heat transfer coefficient between the animal surface and the ground, the convective and thermal radiation conductances between the animal surface and the external medium, the conductance of heat through the animal tissues); τ is the time constant (equal to $1-1/e \approx 63\%$ of the T_b response to a sudden change in T_e or in a physiological parameter to occur). The operative environmental temperature is equal to the temperature an animal would have with no physiological thermoregulation when in thermodynamic equilibrium with its environment (Bakken 1976b), and by definition with no behavioural thermoregulation (see Bakken 1976b; also for the theoretical argument for the linearisation of heat transfer processes and for details of the linearisation procedure).

Equation (2) describes the heat flow equation (1) at any point in time. T_b is an index of H_{tot} , $(T_e - \tau dT_b/dt)$ is an index of H_c and H_r , and M^*/K_o is an index of H_m , H_e and H_s .

The time constant may be calculated from the slope of a plot of $\log(T_b - T_b^{eq})$ vs time (Bakken 1976a). It is equal to the negative reciprocal of the slope. T_b^{eq} is the temperature of the animal when at equilibrium with T_e in a heating or cooling experiment. The time constant is important in the thermal physiology of ectotherms, because the thermal conductance of the animal at any body temperature can be calculated from it if M^* is known. It also facilitates interspecific comparison of rates of heat transfer. A change in t during heating and cooling is indicative of physiological thermoregulation (Smith 1976), or behavioural thermoregulation (see Voigt & Johnson 1977). A useful feature of t is that it is independent of the magnitude of the temperature difference between the cold and hot environments used during transient temperature determinations (see below). This is because τ is a ratio of the form (Bakken 1976a; Smith 1976):

$$\tau \propto \frac{T_2 - T_1}{\frac{t_2}{t_1}} \quad (3)$$

where t_1 , t_2 are two arbitrary times during a temperature transient;

T_1 and T_2 are the temperature values for $(T_b - T_b^{eq})$ at t_1 and t_2 respectively

It may not be possible to calculate a generalised K_o for an ectotherm from heating or cooling curve experiments in order to compare the thermal conductances of different ectotherm species during heating or cooling because of the reasons stated below (Bakken 1976a, 1976b). This is especially true if physiological thermoregulation occurs, unless this is constant throughout the experiment. Comparison of differences in resistance to heat flow (the reciprocal of thermal conductance) between various ectotherms is therefore best done by comparing thermal time constants. The major difficulty in using τ lies in the fact that metabolic rate and rate of evaporative water loss does not remain linear with an increase or decrease in body temperature. This can be minimised by taking an average value for these rates over a section of the experimental period, or by using a computer program to calculate T_b^{eq} (Bakken 1976a). However T_b^{eq} can be measured directly. There are several problems with measuring T_b^{eq} directly, most of which are minimised using the iterative computer program method (Bakken 1976a). These problems all affect T_b^{eq} , and they can have serious effects on the determination of τ . They are listed below:

- 1) The most important factor is the constancy of the general thermal environment. Unfortunately this depends on the ability of the equipment to maintain the thermal environment accurately.

- 2) The animal may: 1. change posture, changing the value of K_o , or;
2. start a thermoregulatory response.
- 3) The metabolic rate and rate of evaporative water loss of the animal may vary with T_b in a non-linear way (eg. both rates may increase at higher body temperatures).
- 4) Activity causes an increase in metabolic rate (and evaporative water loss), but not all the heat produced is dissipated as work, because it takes time for the metabolic rate to return to resting levels (Bartholomew 1982a). Some heat will be removed by evaporation of water. This will affect τ , and K_o if the animal is active near the time T_b^{eq} is determined (Bakken 1976a).

Reptiles use both behavioural and physiological mechanisms to control their body temperatures. The behavioural means, in possible decreasing order of importance, include: the choice of habitat; utilisation of different microhabitats, eg. by seeking shade when the radiative heat load is very high, by burrowing, or by climbing; timing activity to the most favourable times of the day in terms of heat load; postural adjustments, eg. body shape changes, orientation to the sun and wind, and elevation off the substrate, and conduction to the substrate (Bartholomew 1982b, Stevenson 1985b).

Physiological mechanisms used by reptiles to control heat flow include controlling blood flow through the cardiovascular system to the appendages and the skin surface

by vasomotor control, shunts, and independent control of cardiac output (i.e. independent of oxygen requirements); counter-current systems; evaporative cooling; increasing the metabolic rate through hormones, i.e. thyroxine; changing skin colour and therefore absorption of heat from specific wavelengths of light in the visible range; and hypothermic or hyperthermic tolerance (Bartholomew 1982b, Stevenson 1985b). Increased body heat production caused by a raised metabolic rate results from activity, and the metabolic rate of a reptile increases with increasing T_e because the Q_{10} of chemical reactions is often greater than one. It is not known, however, to what extent reptiles can control their body temperature physiologically (Bartholomew 1982b, Stevenson 1985b).

Behavioural thermoregulation is the most important means by which reptiles control their body temperatures, in the sense that the range of body temperatures obtained by behavioural mechanisms alone is far greater than those allowed purely by physiological control of body temperatures (Bartholomew 1982b, Stevenson 1985a). However both behavioural mechanisms and physiological mechanisms are closely interlinked (McFarland, Pough, Cade & Heiser 1985), and reptiles can control physiologically the rate of heating and cooling to some degree.

Different sizes of the angulate tortoise (*Chersina angulata*) were used in this study. The aims of these experiments were to determine how rates of heating and cooling are

affected by body size and ambient conditions, and to compare these rates with other reptiles. The results are discussed in relation to recent theory on the causes of differences in rates of heat transfer between animals of different sizes. It was decided to both calculate and directly measure T_b^{eq} . If the factors in 2) 1. and 3) above make a minor contribution to the overall heat transfer between the angulate tortoises and the environment, then the accuracy to which the T_b^{eq} is determined is increased. This appears to be the case. Then by keeping the environment as constant as possible we can determine whether the properties of the tortoises change with increasing size, such as when the tortoises grow. The angulate tortoise can thermoregulate behaviourally by withdrawing its head and legs into its shell.

Methods

Experiments took place between the months of April and September during 1987. Tortoises of four different sizes were used in the heating and cooling experiments. The masses of the tortoises ranged from about 100g to 1010g. The masses were approximate only (to the nearest five grams), because mass changes did occur during the experimental period. The tortoises were kept under semi-natural environmental conditions in an outside pen until a few days (not less than forty-eight hours) before a specific experimental run, during which time they were kept indoors under a 12L:12D photoperiod regime (light being provided by a 60W light bulb

and some natural sunlight). They were starved during this period to prevent urination and defecation during the experiments. Each tortoise was not kept under a specific temperature regime. After an experiment the tortoise was released in the outside pen. A copper-constantan thermocouple, with the tip coated with dental cement, was surgically implanted under aseptic conditions into the abdominal cavity of each tortoise from the plastron surface midline. The thermocouple was cemented in place with dental cement, which was then coated with clear adhesive glue. Masking tape was sometimes placed over the glue for further protection. The tips of the thermocouples had diameters of between 0.4 and 0.9 mm. Thermocouples were calibrated either before or after the experiments (Appendix 3 (1) and (2)).

Body temperature transients of ectotherms are measured by taking the animals out of an environment with a specific temperature and placing them in another environment with a different temperature, and continuously recording the change in body temperature. The experimental procedure used here involved transferring a tortoise from a relatively stable high temperature ($34.1 \pm 0.1^{\circ}\text{C}$) to a relatively stable low temperature ($11.7 \pm 0.1^{\circ}\text{C}$) for the cooling experiment, and *vice versa* for the heating experiment, and monitoring at intervals the change in deep body temperature of the tortoise, as well as generally the change in ambient temperature. The cold environment was provided by a temperature controlled cabinet placed in a controlled environment room.

Ambient temperature was controlled to $\pm 0.1^{\circ}\text{C}$ of the set temperature during the runs done under near-free convection conditions. The relative humidity in the cabinet was below 15%. The hot environment was provided by a controlled environment room which controlled temperature to about $\pm 0.2^{\circ}\text{C}$. Relative humidity in this room ranged between approximately 21-27%. Weathers (1972) has shown that high relative humidity may affect the rate of heating during warming experiments on reptiles, but that relative humidity seems to have negligible effect on cooling rates. The relative humidity during the heating experiments was too low to have an effect on the heating rates of the tortoises (see Weathers 1972).

The tortoise was placed in an all-glass terrarium with an insulative polystyrene layer on the outside bottom of the terrarium for both the heating and the cooling experiments. The terrarium used in the cooling experiments was also insulated inside along the bottom and part of the sides by felted fibre, which has a similar conductance to air. This reduced the effect of conduction from the tortoise to the terrarium. To ensure conditions as close to free convection as possible the terrarium in the cooling runs had a glass lid with a one centimetre gap left to allow exchange of air in the terrarium with that of the cabinet. A lid was required as a windshield because the cabinet had a fan to distribute the heated air.

The two terraria were kept under the constant conditions during the experimental period, and therefore were equilibrated to the environmental conditions. Glass terraria were used so that the tortoise would be visible at all times. Another factor was that glass has a higher thermal conductance than other substances such as plastic and so would equilibrate faster to the environmental conditions during the experiment. Later, in other experiments, the glass lid of the terrarium was removed during cooling experiments and the fan allowed to blow air over the tortoises. Airflow was not laminar. The ambient temperature was set to $11.6 \pm 0.15^\circ\text{C}$, and controlled to $\pm 0.35^\circ\text{C}$.

Windspeed was $\approx 20\text{cms}^{-1}$ during the cooling experiments under near-free convection conditions, and ranged between 21 and 40cms^{-1} during the heating experiments. Direct windspeed from the fan was about 3ms^{-1} , but during the forced convection cooling experiments, measured windspeeds were between 0.4 and 1.9ms^{-1} , depending on the position of the unidirectional hotwire anemometer (Wallac OY thermo-anemometer) relative to the fan. Much of the variation was due to turbulence. Measured windspeeds and relative degree of turbulence under the three experimental conditions over a one minute period are plotted in Fig. 1. Environmental measurements were taken some time after the last experiment. Each tortoise was generally weighed after the experiment. It should be noted that measured ambient temperatures in the terraria were elevated (during cooling) or lowered (during

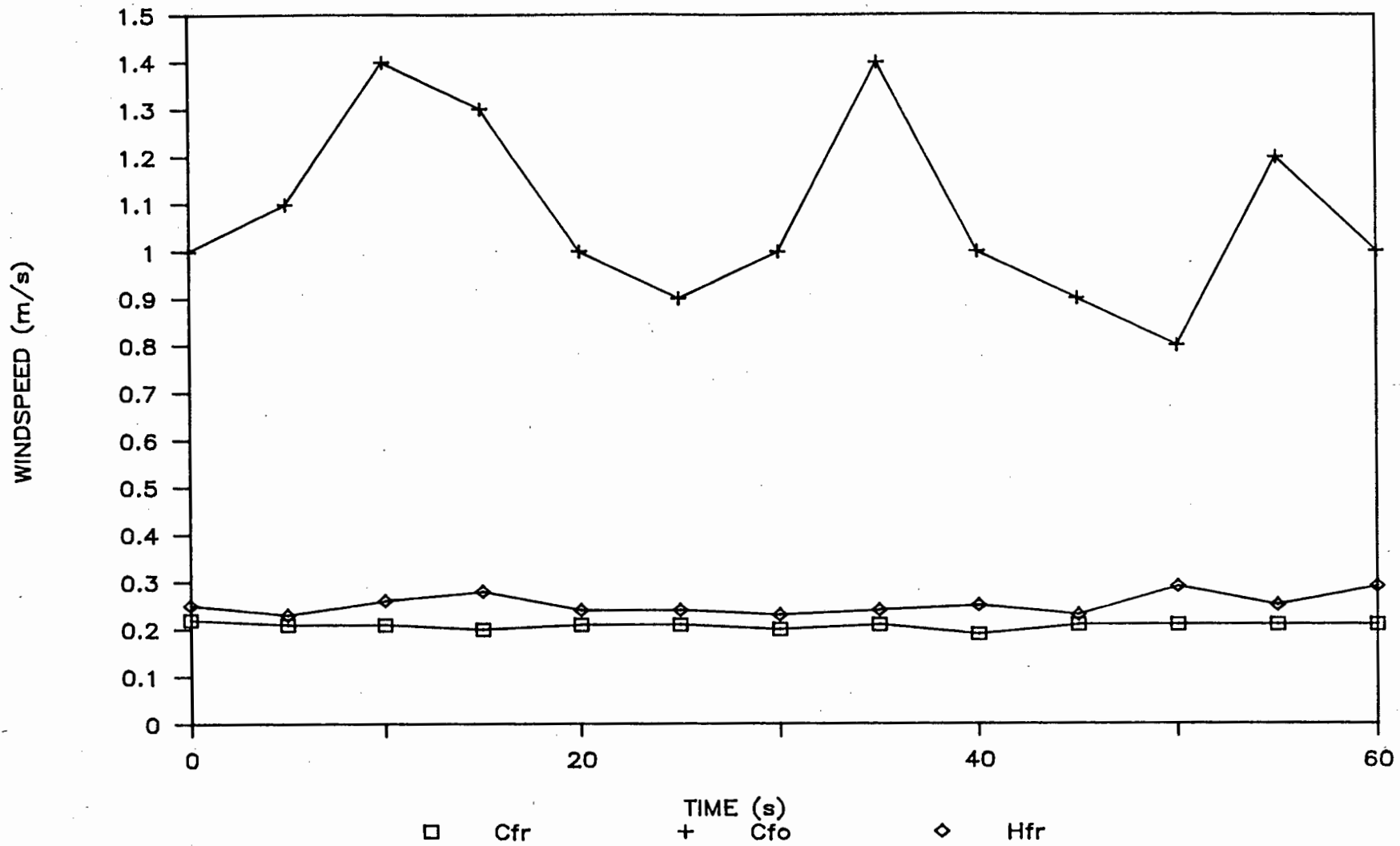


Figure 1 Indication of turbulence during heating and cooling experiments. Cfr: cooling, near-free convection; Cfo: cooling, forced convection; Hfr: heating, near-free convection.

heating) in relation to the set-point for up to several hours during the experiment. The maximum measured deviation from the set ambient temperature after three hours was $\approx 1^{\circ}\text{C}$. The magnitude of the measured deviation from set ambient temperature varied according to how close the tortoise was to the thermocouple. However, even when the tortoise was next to the thermocouple tip, the maximum deviation measured by the ambient thermocouple was only a few degrees centigrade, and it became less with time. It was felt that these deviations were not serious (see Discussion), especially since all the tortoises were subjected to the same conditions.

The ambient temperatures in the terraria were recorded by thermocouples with white enamel paint on their tips at the same time as deep body temperatures. Temperature readings were taken visually from two Bailey digital recorders (BAT-12, Bailey instruments). The transfer time between the two environments took about one to two minutes. In the heating experiments the low environmental temperature was adjusted to obtain similar initial body temperatures for all tortoises.

In the cooling experiments, but not the warming ones, the tortoises were equilibrated to the initial temperature for at least twelve hours before experiments began. To standardise, in case of any circadian rhythms in heat flow, T_b^{eq} was determined the next day at approximately the same time as the experiment was begun the day before. During each equilibration period and experimental run the tortoise was kept

under constant light conditions. The circadian rhythm in the metabolism of the tortoise was probably kept in phase with the diurnal light-dark phase during this time because it appears that the activity patterns of tortoises and metabolic rates of many animals only drift out of phase of the diurnal light cycle after several days in constant photoperiod conditions (eg. Heusner & Jameson 1981; Cloudsley-Thompson 1970). Reasonable care was taken to keep possible disturbance low.

Time constants were calculated by an iterative computer method based on the method of Bakken (1976a). The method plots a graph of T_b versus time, but the region of the curve used to calculate τ has to be chosen visually. Care was taken to choose the region of the curve mimicking a first order (exponential) curve. However, within this region the largest possible range in T_b was taken because it was found that the calculation of T_b^{eq} is more accurate the greater the number of data points used (see also Bakken 1976a). Therefore any effect caused by the animal being disturbed on handling, and being active at the beginning of the run were minimised (eg. see Fig. 2). The calculated T_b^{eq} was checked with the measured T_b^{eq} in cases where the latter was determined. Values of τ were reported to the nearest 60 seconds, the shortest time scale actually used between T_b readings. Where replicates were done they usually did not differ by more than 8% in τ (N=7 out of 12 possible tortoise/condition combinations; up to 3 replicates per com-

bination). The reported value of τ was obtained from the experimental replicate that best met specific criteria, such as the calculated T_b^{eq} being close to the measured T_b^{eq} , and least observable change in ambient temperature during the experiment.

Results

Behaviour

During the cooling runs the tortoise generally withdrew its head, limbs and tail into its shell within several minutes of being transferred to the cold environment, with only its claws and tips of its limbs visible. It usually remained in this position for the duration of the experiment. Therefore its surface area could be approximated by that of the shell.

During the warming experiments a different behaviour was observed. Initially the tortoise would sit with parts of its head and limbs protruding from the shell. Later it would become active, moving around the terrarium, and would show behavioural thermoregulation; the greatest extent to which the latter was taken was the tortoise resting with both pairs of ~~back and front~~ limbs splayed, the tail drooping, and the head and neck extended with the chin resting on the terrarium bottom. Degrees of behavioural thermoregulation were encountered: different combinations of appendages would be extended at certain times, such as the hind pair of legs only, or one leg only, or all legs extended with the head

withdrawn. As body temperature increased various appendages were withdrawn into the shell. Only one or two of the appendages may be kept extended as body temperature approached ambient temperature. Finally, as body temperature reached equilibrium with ambient temperature, often all of the appendages would be extended.

Rates of heat exchange

Figures 2 to 4 are body temperature/time response curves for the tortoises studied under the different experimental conditions. ^{See Table 1 for the start and end ambient temperatures.} In general the graphs take the form of exponential curves; however all show second order responses, especially at the beginning of the body temperature transients, when the tortoises were disturbed and transferred from one environment to the other, and during the settling period in the new environment. The effect activity and behavioural thermoregulation had on the rates of heating can be seen in Fig. 5 (a). The effect activity had on the slope of the curve was more pronounced in the small tortoise than the others. There was very little noticeable effect of either activity or basking in the two largest tortoises. Activity initially tended to cause an increase in the slope of the curve, but later there was a decrease in slope, while "basking" caused no major change in the slope, only slightly increasing it in Fig. 2 (c) for example. Figure 5 (b) and (c) are semi-logarithmic plots of absolute differences between body temperature and equilibrium body temperature.

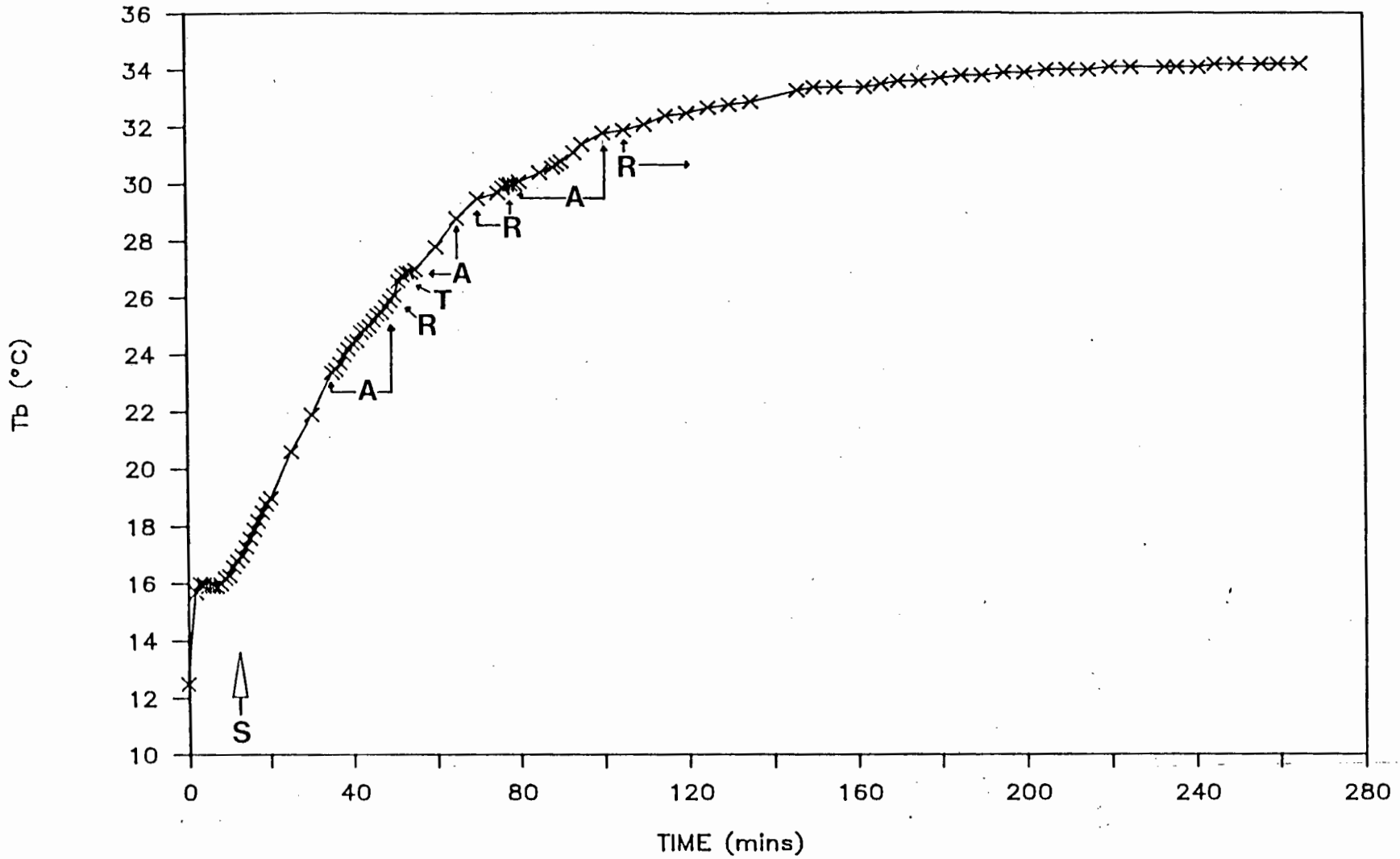


Figure 2 (a) Heating curves for tortoises under conditions of near-free convection. Body temperature (T_b) is in degrees centigrade, time in minutes (100g tortoise). 'A' indicates period of activity; 'R' shows period when tortoise is resting; 'S' is a region of the curve not mimicking a first order system; 'T' indicates period of pronounced behavioural thermoregulation.

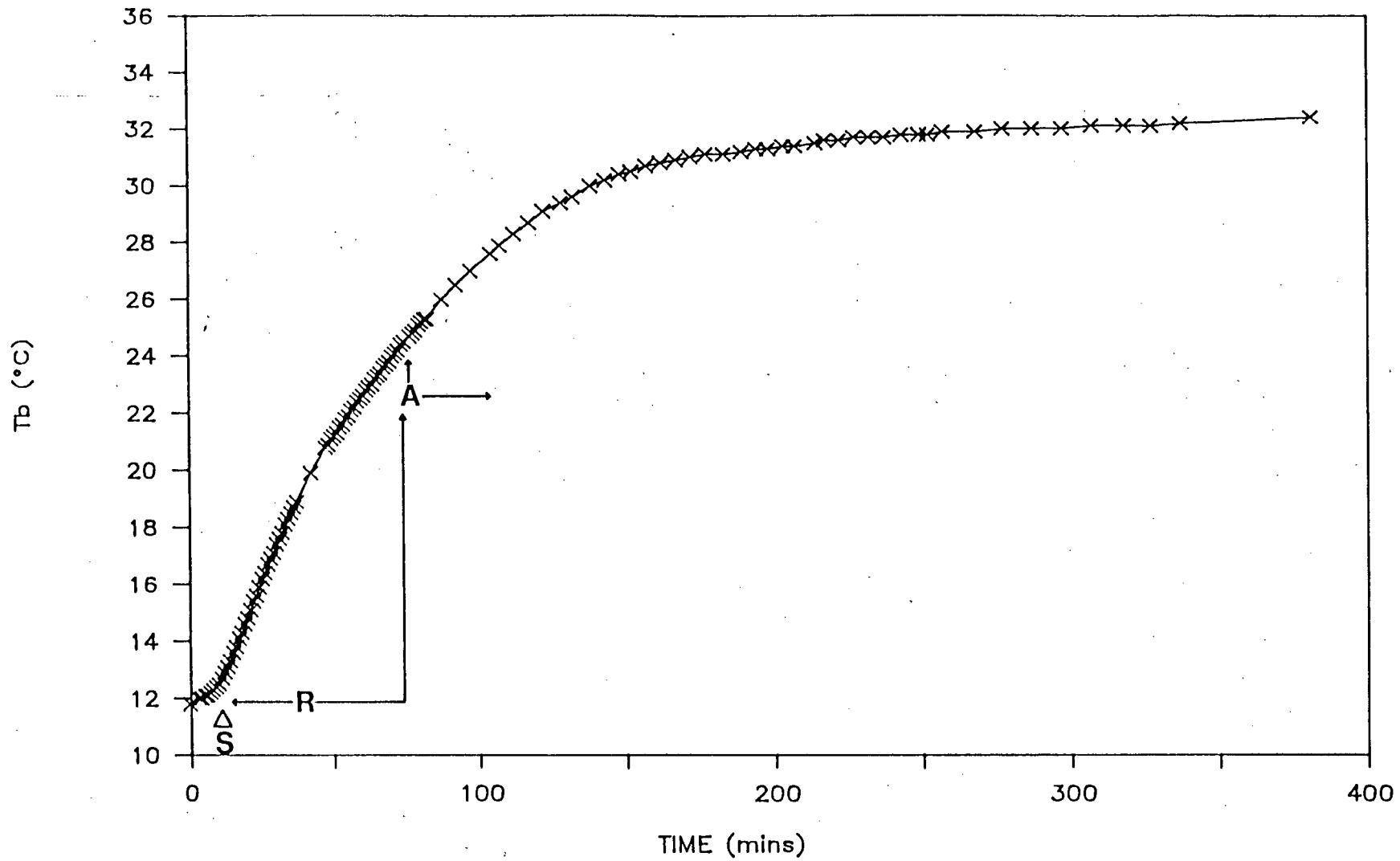


Figure 2(b) Heating curves for tortoises under conditions of near-free convection. Body temperature (T_b) is in degrees centigrade, time in minutes (305g tortoise). Symbols are the same as in Fig. 2 (a).

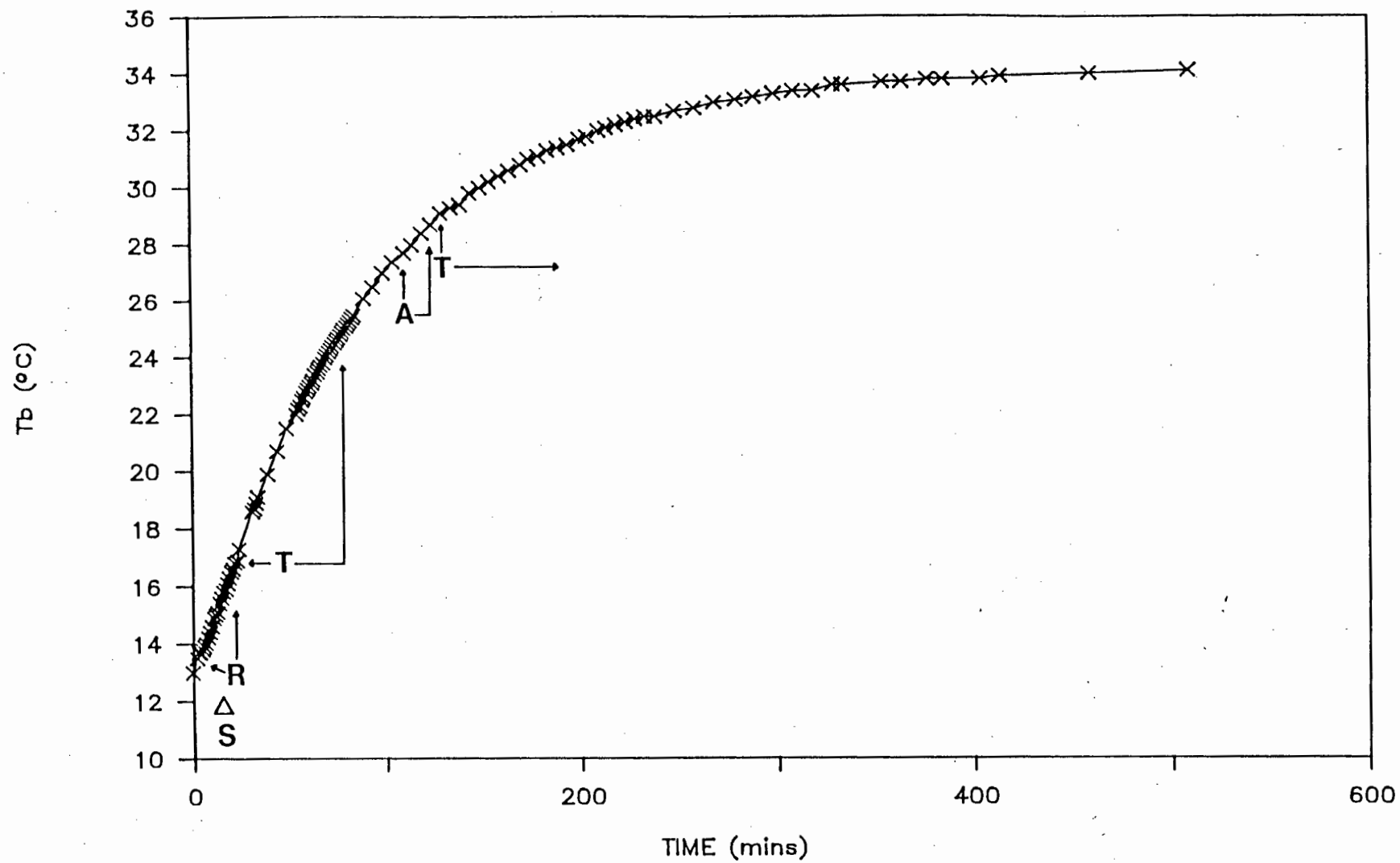


Figure 2 (c) Heating curves for tortoises under conditions of near-free convection. Body temperature (T_b) is in degrees centigrade, time in minutes (590g tortoise). Symbols are the same as in Fig. 2 (a).

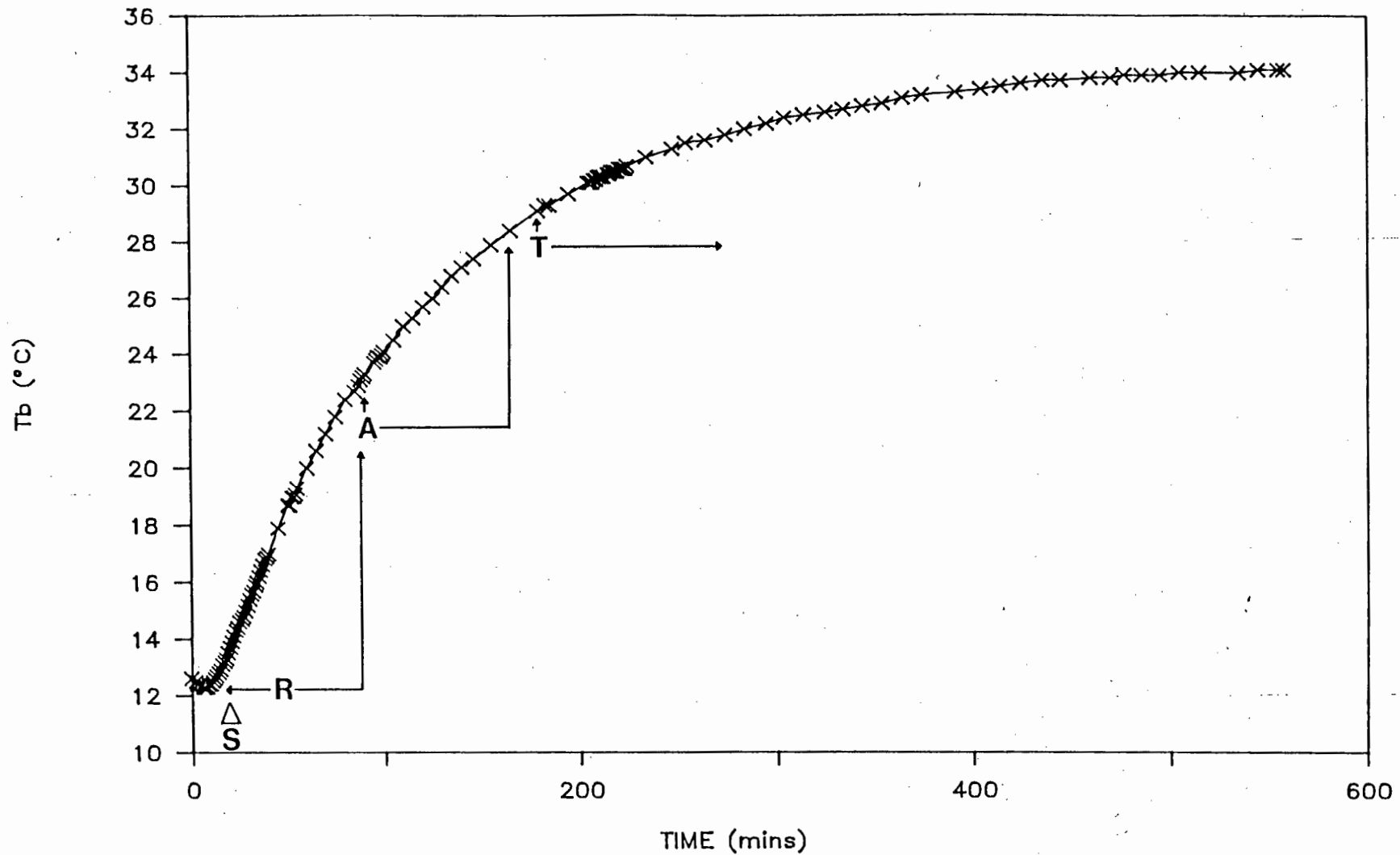


Figure 2 (d) Heating curves for tortoises under conditions of near-free convection. Body temperature (T_b) is in degrees centigrade, time in minutes (1010g tortoise). Symbols are the same as in Fig. 2 (a).

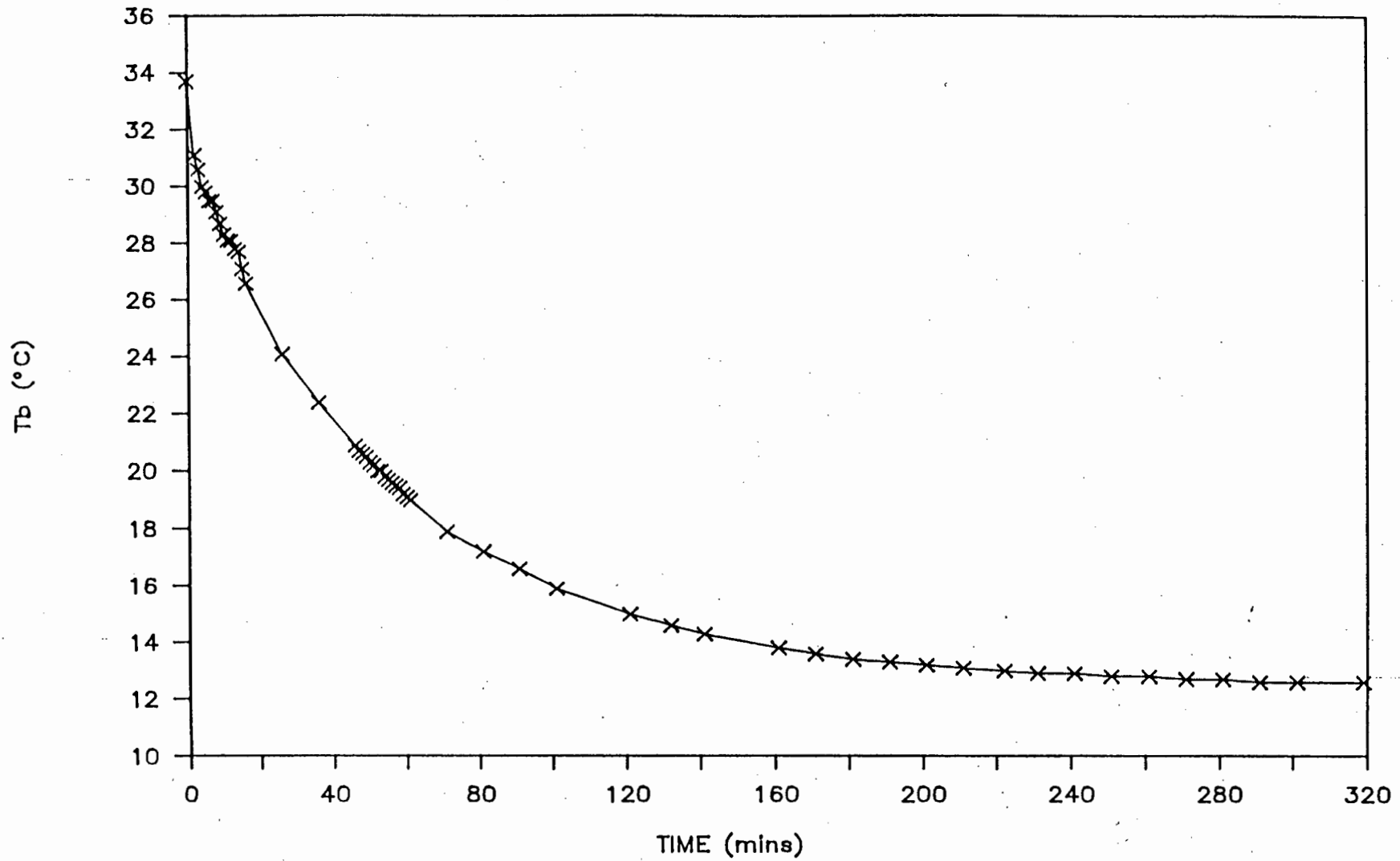


Figure 3 (a) Cooling curves for tortoises under condition of near-free convection (100g tortoise). Labels and symbols are the same as in Fig. 2 (a).

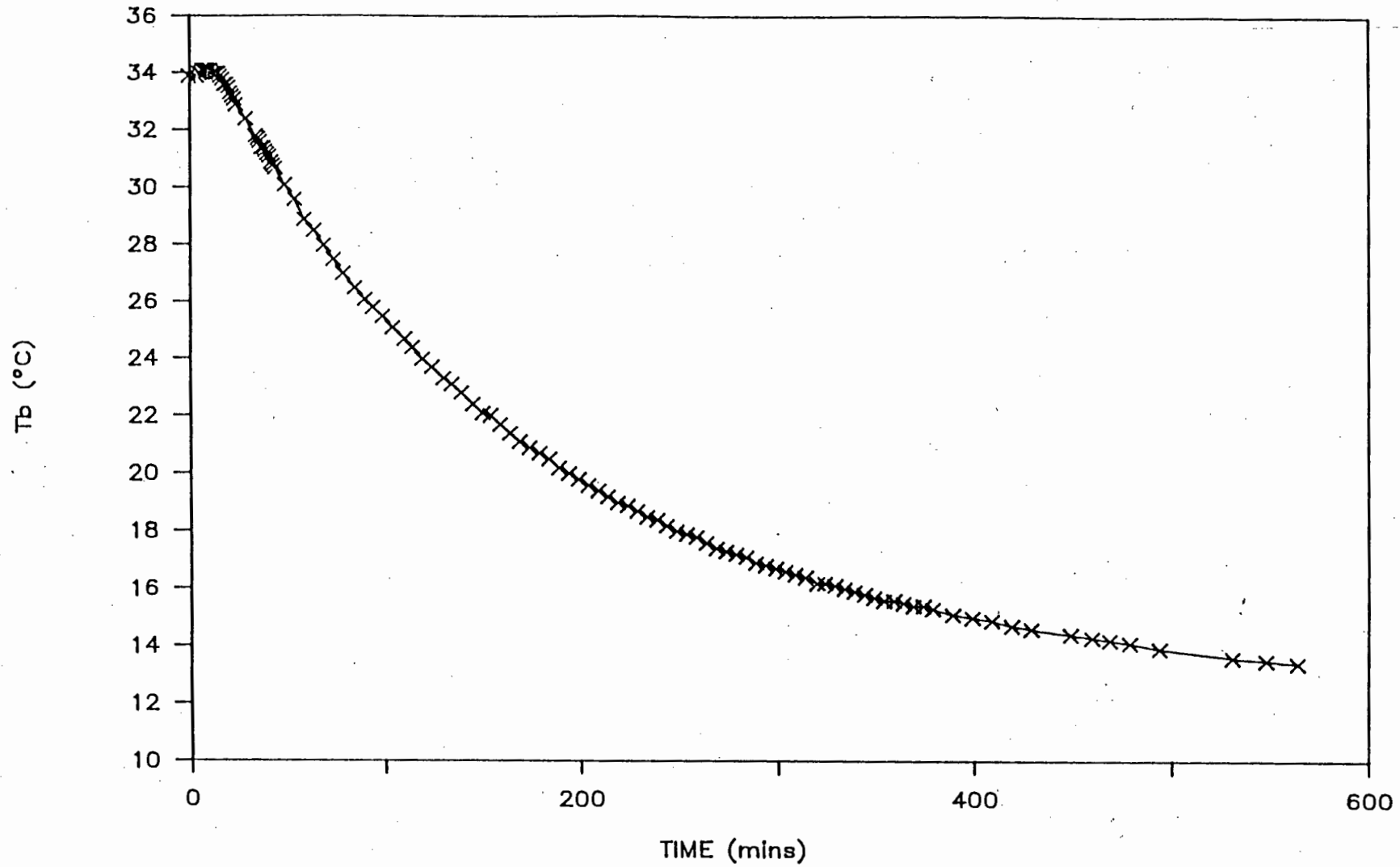


Figure 3 (b) Cooling curves for tortoises under condition of near-free convection (305g tortoise). Labels and symbols are the same as in Fig. 2 (a).

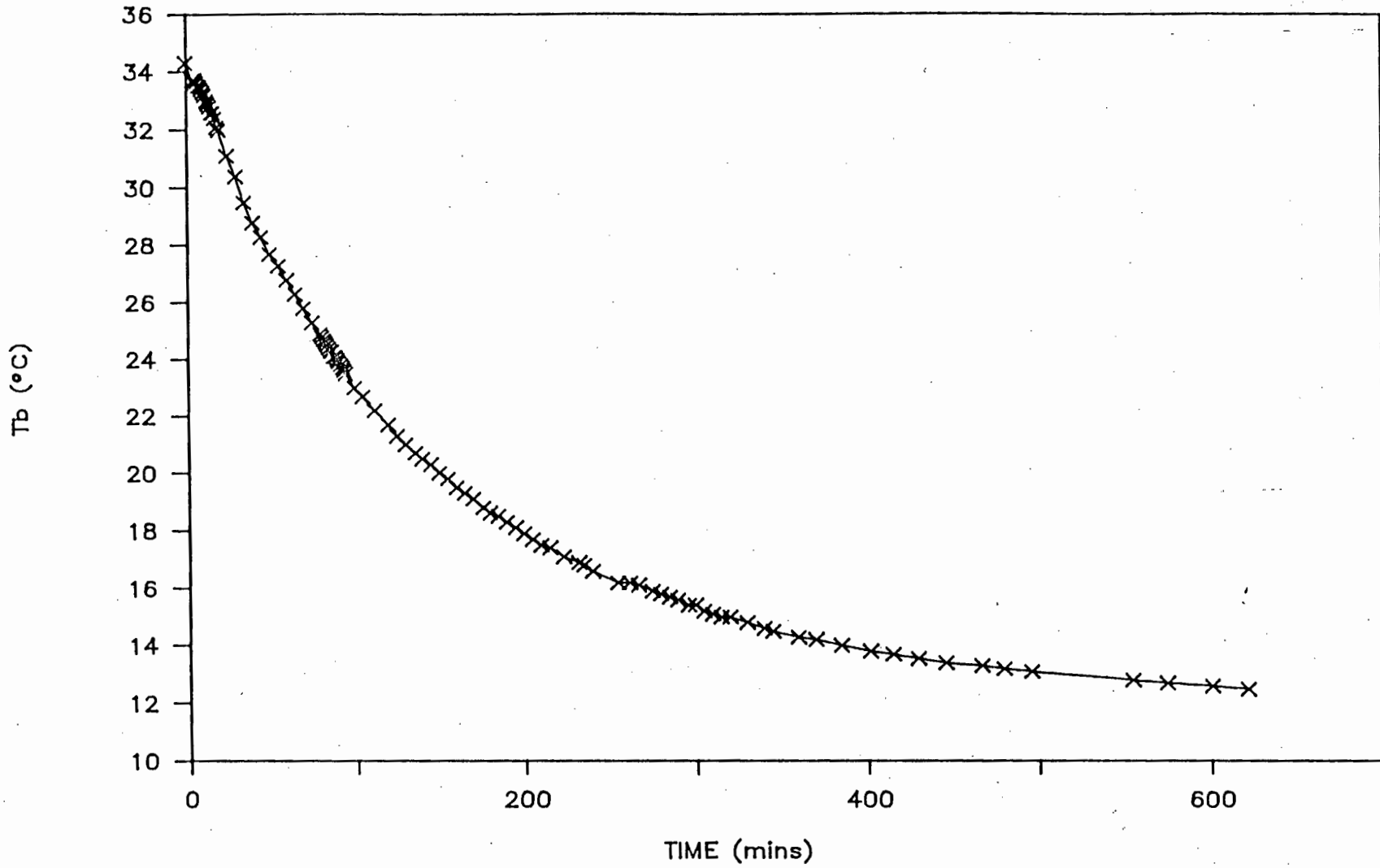


Figure 3 (c) Cooling curves for tortoises under condition of near-free convection (590g tortoise). Labels and symbols are the same as in Fig. 2 (a).

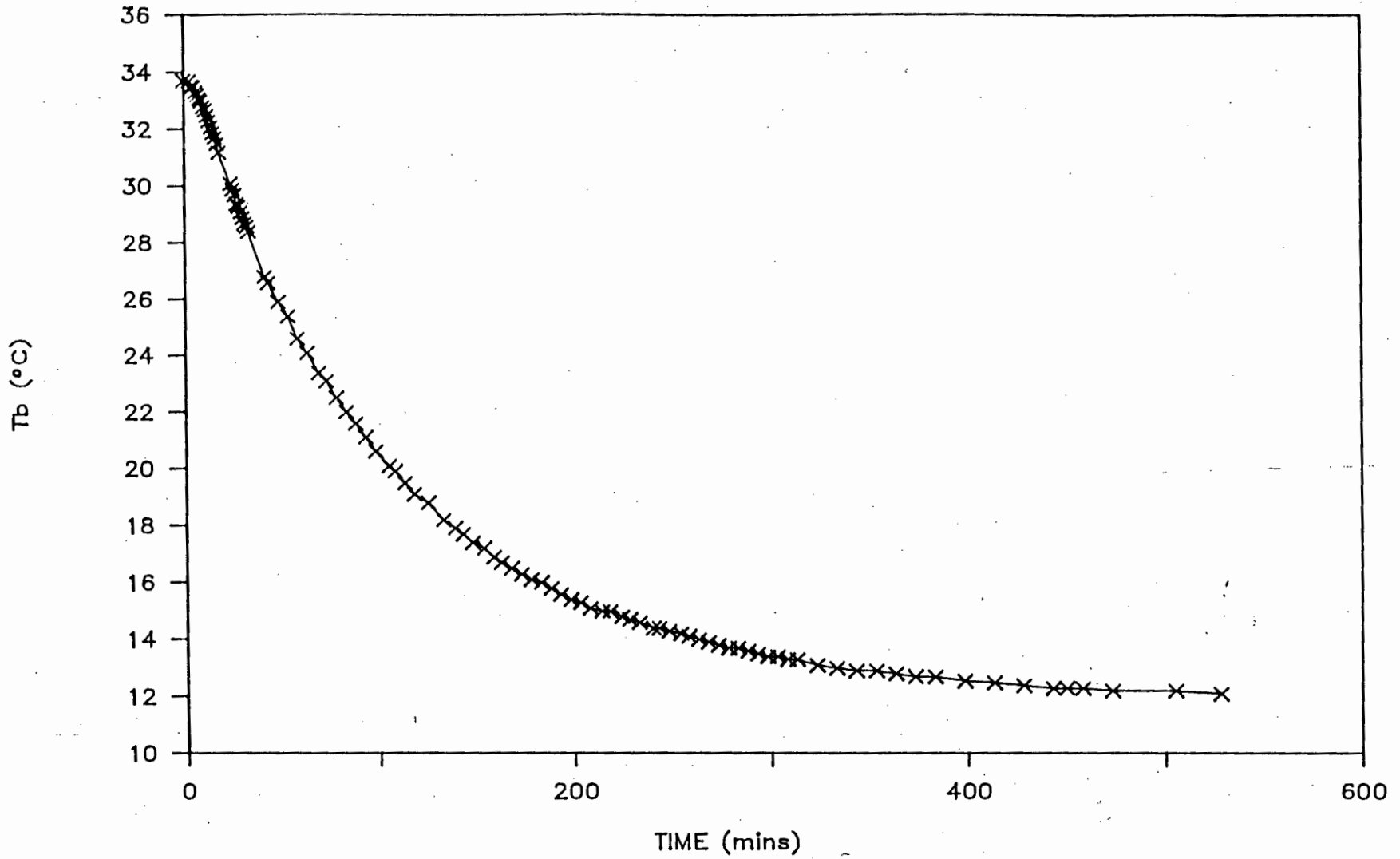


Figure 3 (d) Cooling curves for tortoises under condition of near-free convection (1010g tortoise). Labels and symbols are the same as in Fig. 2 (a).

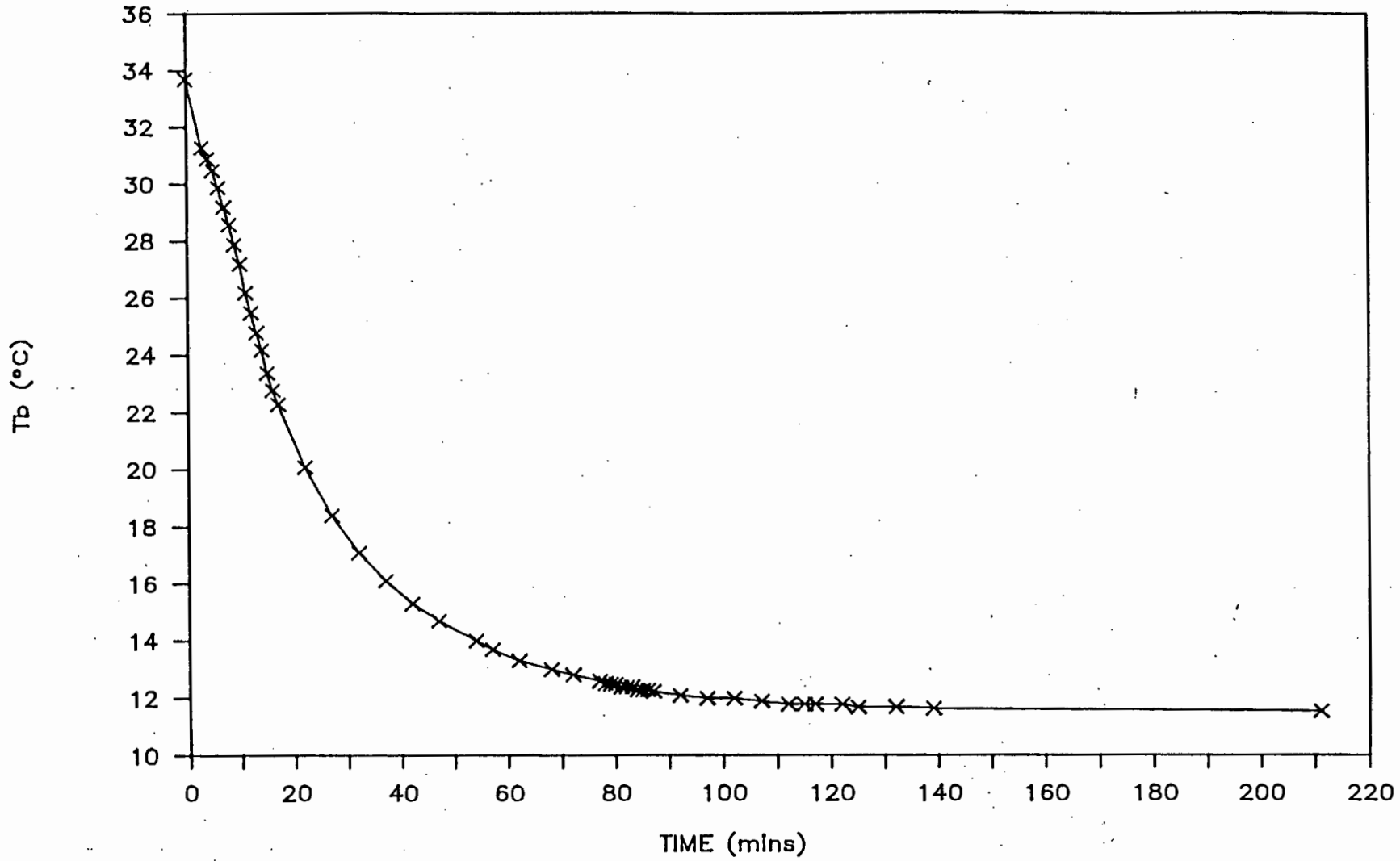


Figure 4 (a) Cooling curves for tortoises under condition of forced convection (100g tortoise). Labels and symbols are the same as in Fig. 2 (a).

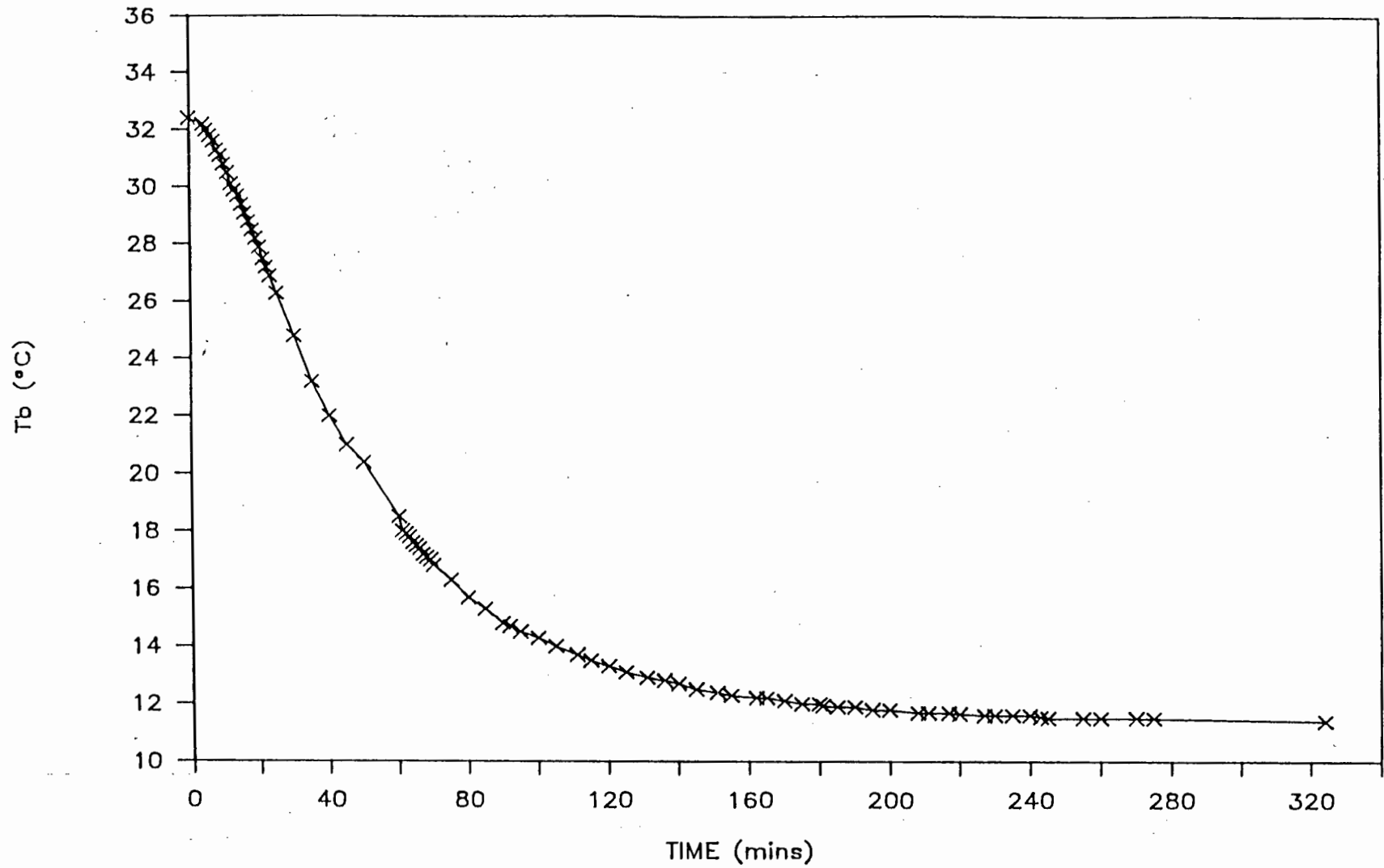


Figure 4 (b) Cooling curves for tortoises under condition of forced convection (305 tortoise). Labels and symbols are the same as in Fig. 2 (a).

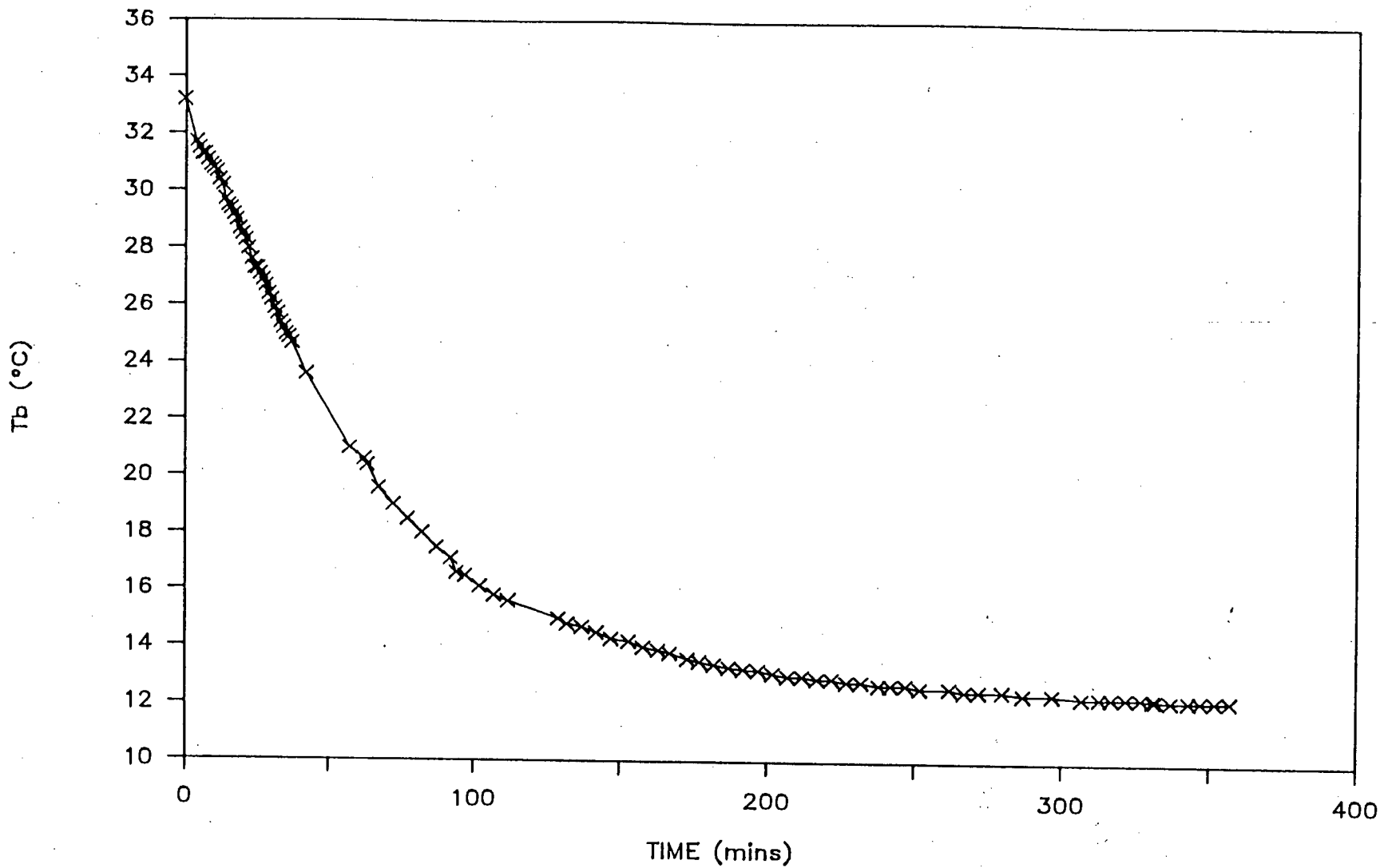


Figure 4 (c) Cooling curves for tortoises under condition of forced convection (590g tortoise). Labels and symbols are the same as in Fig. 2 (a).

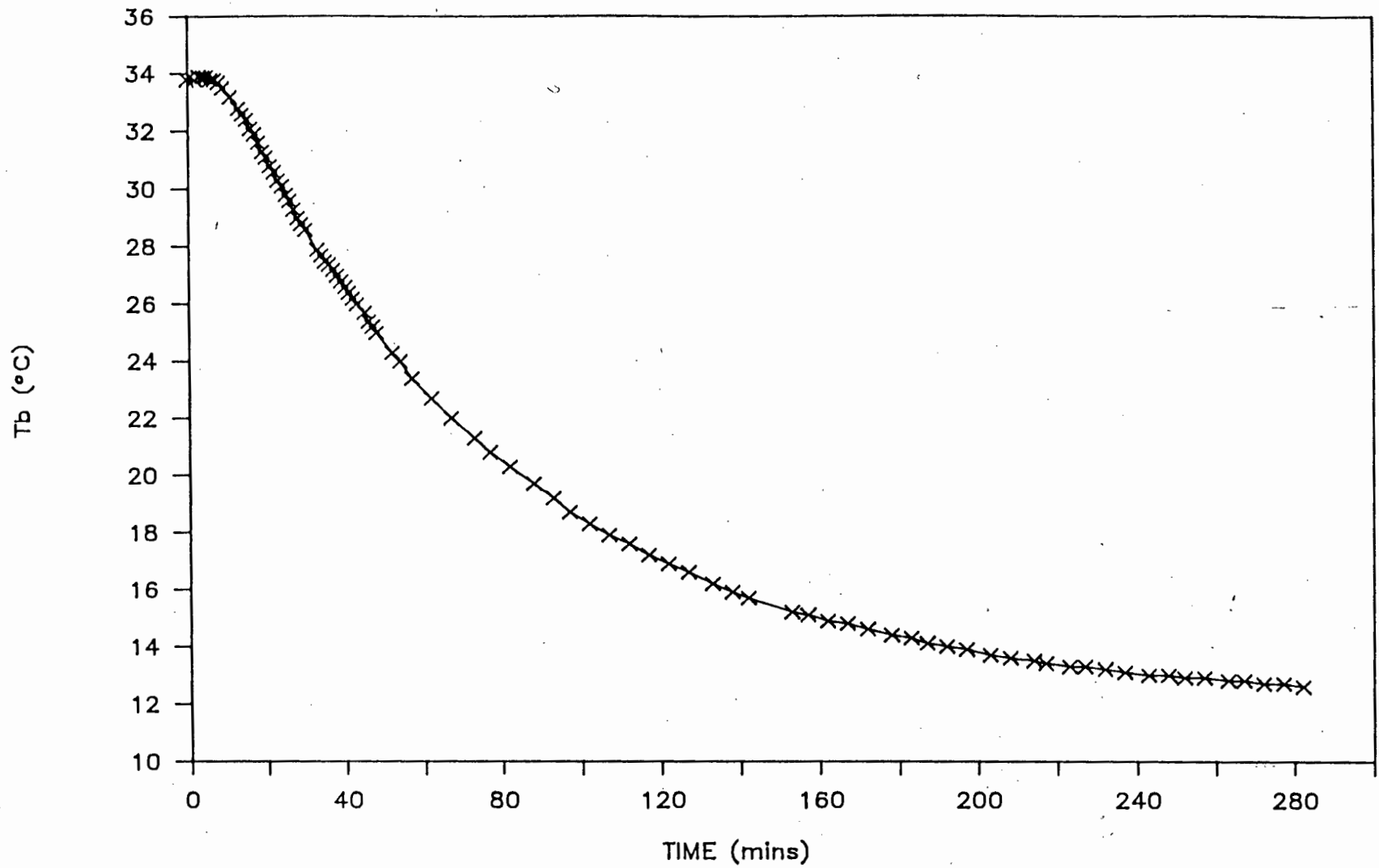


Figure 4 (d) Cooling curves for tortoises under condition of forced convection (1010g tortoise). Labels and symbols are the same as in Fig. 2 (a).

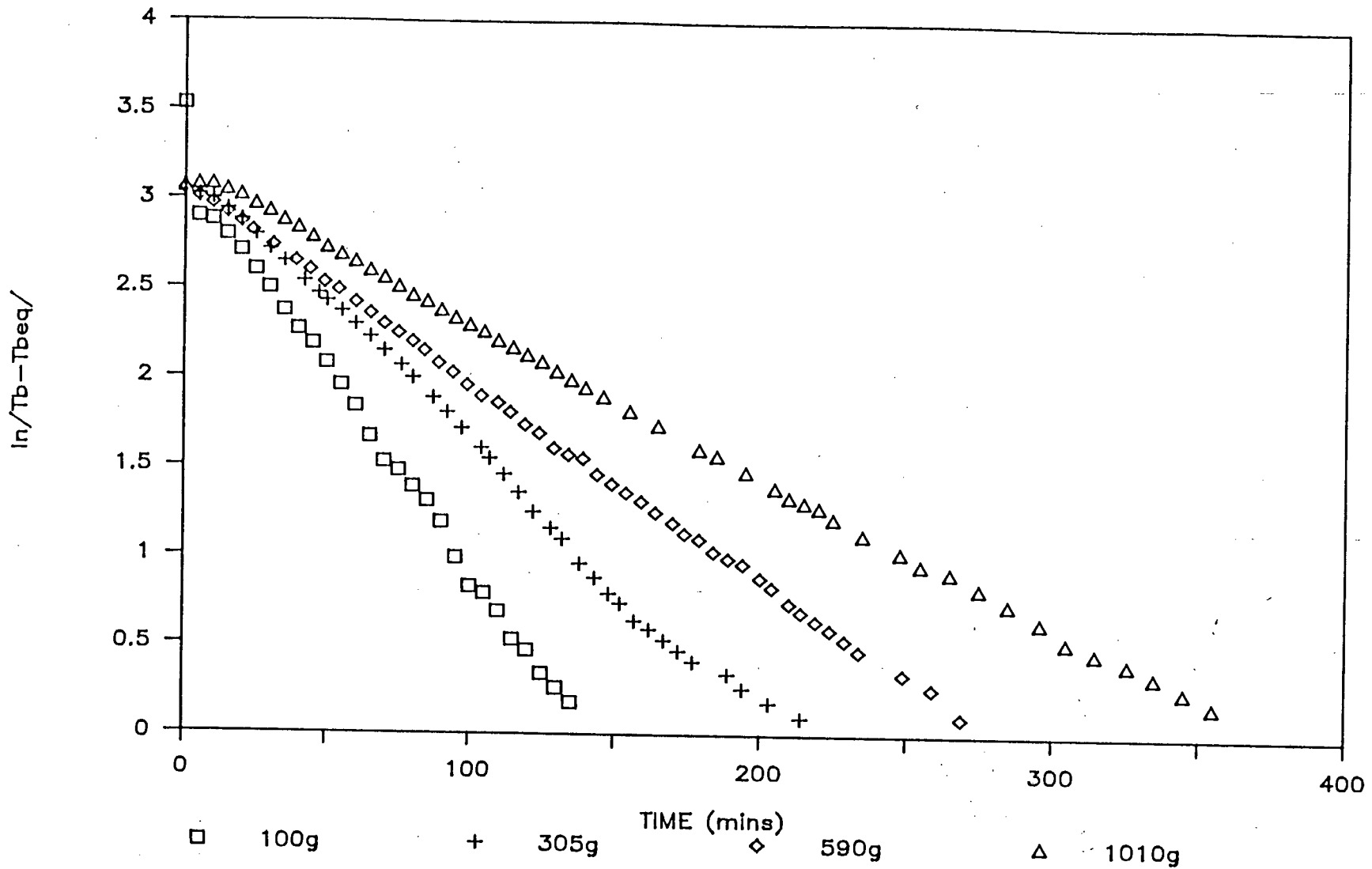


Figure 5 (a) Semi-logarithmic plot of absolute difference between body temperature and equilibrium body temperature of tortoises against time. Heating, condition of near-free convection. Major downward fluctuation in curves due to activity. Temperatures are in degrees centigrade.

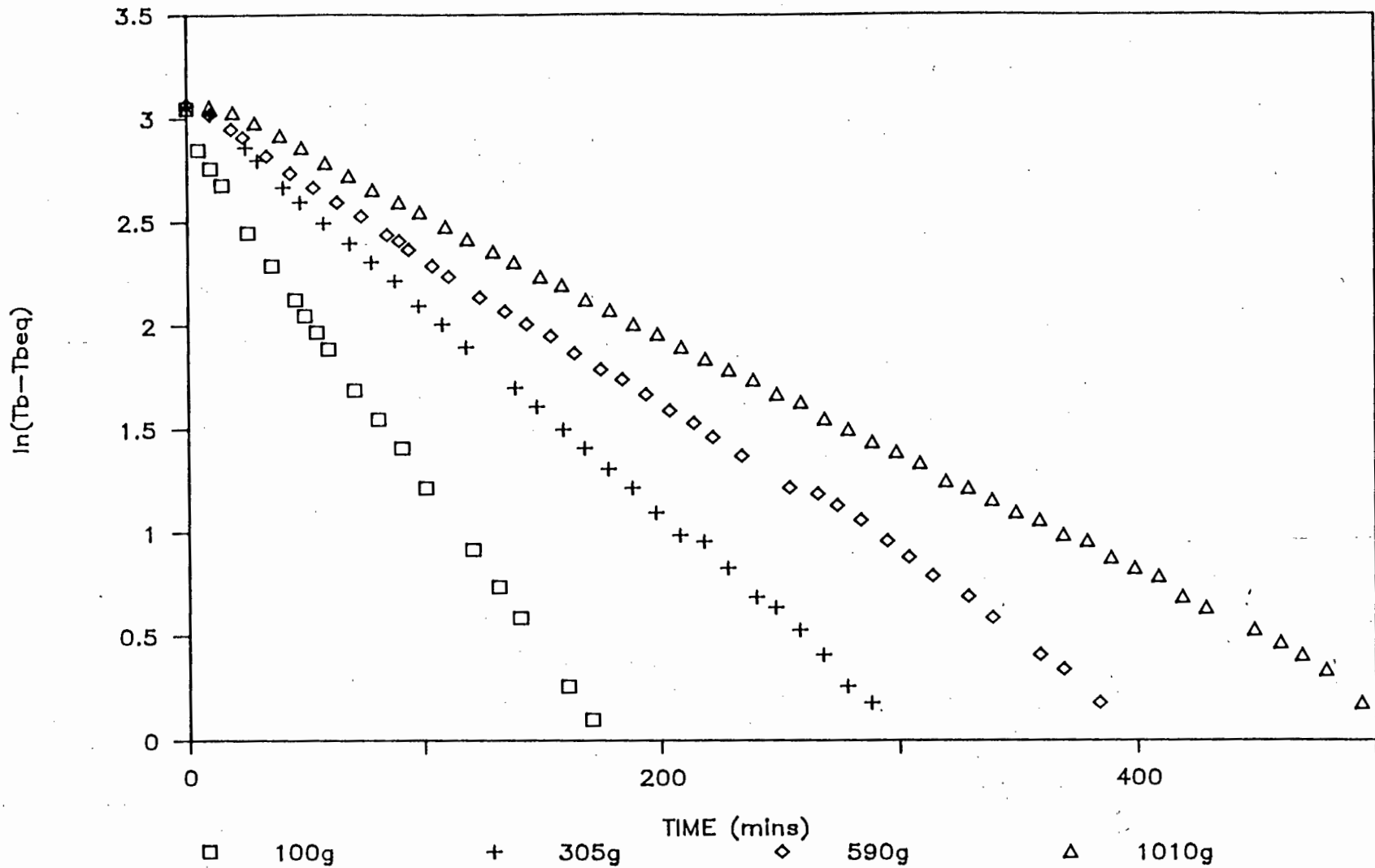


Figure 5 (b) Semi-logarithmic plot of absolute difference between body temperature and equilibrium body temperature of tortoises against time. Cooling, condition of near-free convection. Temperatures are in degrees centigrade.

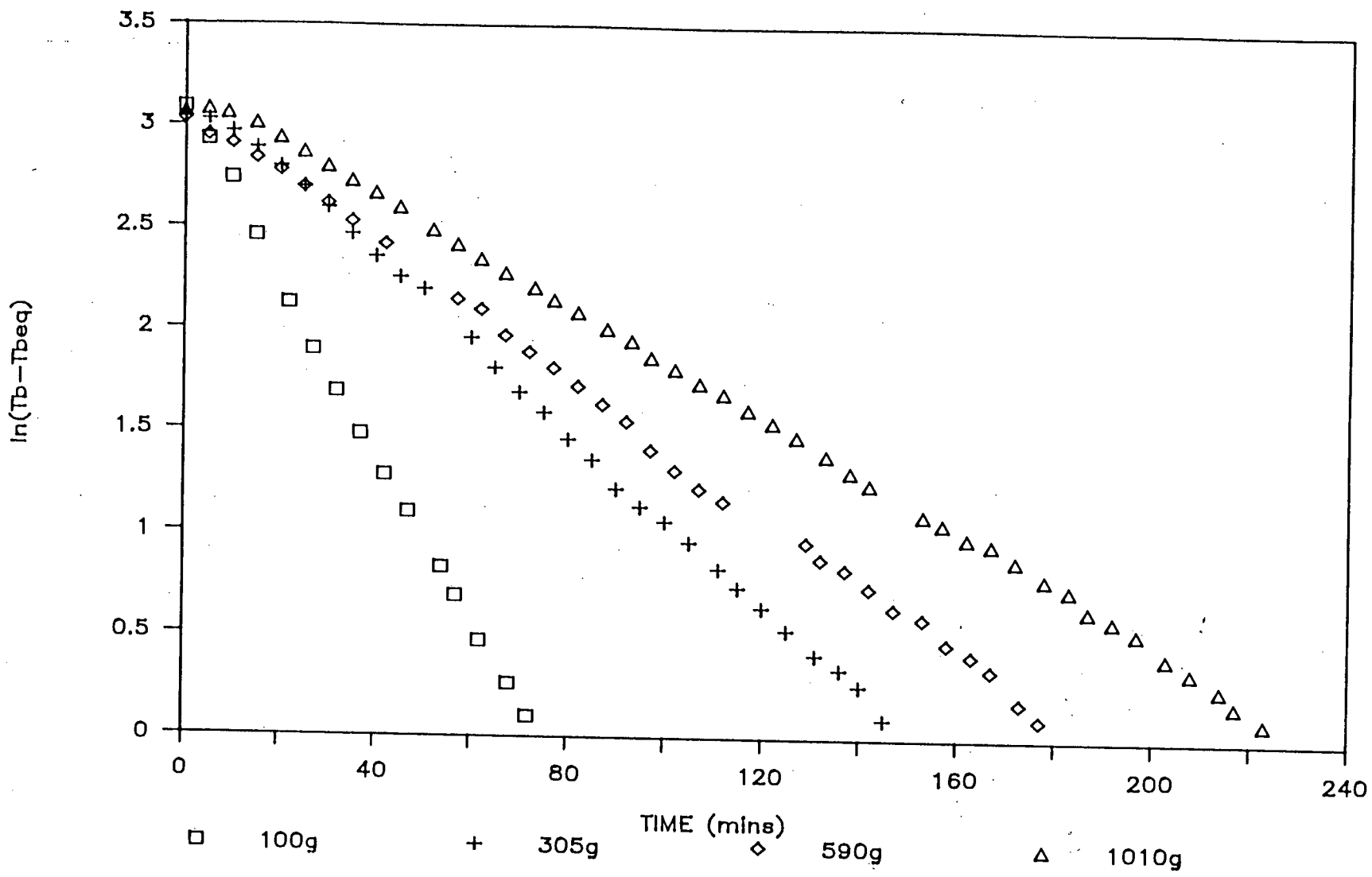


Figure 5 (c) Semi-logarithmic plot of absolute difference between body temperature and equilibrium body temperature of tortoises against time. Cooling, condition of forced convection. Temperatures are in degrees centigrade.

If the tortoises acted as ideal inanimate objects the graphs would be straight lines, provided the environmental conditions were constant.

Table 1 reports the results of the experiments investigating rates of heat transfer under three experimental conditions and with four sizes of tortoise.

Table 1 Time constants (τ) calculated from rates of heat transfer for tortoises of various body masses exposed to a step change in ambient temperature. Temperatures are in degrees centigrade. (Mass is tortoise mass in grams; T_b^0 is initial body temperature of tortoise; τ is the time constant in seconds; T_a is ambient temperature; T_b^{eq} is the calculated equilibrium body temperature; ceq-meq is the maximum difference between calculated and measured body temperatures at equilibrium; range is the total change in body temperature).

Condition	Mass(g)	T_b^0	τ (s)	T_a	T_b^{eq}	ceq-meq	Range
Cooling: minimal turbulence	100	33.7	3600	11.7	12.5	-	21.2
	305	33.7	5940	11.7	12.4	0.5	21.3
	590	34.3	8340	11.7	12.8	1.1	21.5
	1010	33.9	10200	11.7	12.7	0.3	21.2
Cooling: very turbulent	100	33.6	1380	11.7	11.7	0.1	21.9
	305	32.4	2760	11.7	11.4	0.0	21.0
	590	33.2	3540	11.7	12.4	-	20.8
	1010	33.8	4380	11.7	12.2	-	21.6
Heating: low turbulence	100	12.5	2580	34.1	34.1	-	21.6
	305	11.8	3840	34.1	32.6	0.0	20.8
	590	13.0	5340	34.1	34.1	0.1	21.1
	1010	12.6	6960	34.1	34.1	-	21.5

The tortoises warmed faster than they cooled during temperature transients between ambient temperatures of approximately 34.2°C and 11.7°C (Fig. 6). The effect of windspeed on rates of heat transfer can be seen in Fig. 6: time constants in turbulent conditions with a windspeed of about 1.2ms⁻¹ were less than half those in conditions of near free convection.

The allometric equations for the time constants during cooling and heating are (where τ is in seconds; M is mass in grams):

- 1) Cooling, near free convection

$$\tau = 475M^{0.445} \quad (1)$$

The regression is highly significant ($F_{1,2} = 641.62$, $P < 0.005$, $R^2 = 0.993$).

- 2) Cooling, forced convection

$$\tau = 191M^{0.455} \quad (2)$$

The regression is very significant ($F_{1,2} = 131.75$, $P < 0.01$, $R^2 = 0.985$).

- 3) Heating, near free convection

$$\tau = 300M^{0.453} \quad (3)$$

The regression is highly significant ($F_{1,2} = 351.04$, $P < 0.005$, $R^2 = 0.997$)

The slope of equation (1) is not significantly less than that of equation (2) (Student's t-test, $t_3 = -0.035$, N/S),

nor is the slope of equation (1) significantly less than that of equation (3) (Student's t-test, $t_3 = -0.028$, N/S). The coefficient of equation (1) is significantly greater than that of equation (2) (Student's t-test, $t_3 = 1001.199$, $P < 0.0005$), and is significantly greater than that of equation (3) (Student's t-test, $t_3 = 616.936$, $P < 0.0005$). The slope of equation (1) is not significantly greater than that relating the time constants for the cooling of birds' eggs with mass under conditions of free convection (eggs: $b = 0.402$; Turner 1985; Student's t-test, $t_3 = 0.152$, N/S). The slope of equation (2) is not significantly greater than 0.43 (Student's t-test, $t_3 = 0.106$, N/S; Table 3). Figure 6 is the plot of body mass vs τ . When both variables are transformed to base 10 logarithms and plotted against each other a linear relationship is obtained, with the slope approximately equal to the exponent of the untransformed data plot (Fig. 7).

The relative effect of high windspeed on cooling rates is similar for all body sizes, except for the smallest size studied where it had a greater effect (Table 2: τ_{cfr}/τ_{cfo}).

Table 2. Ratios of time constants for tortoises of various body sizes under the different experimental conditions. (Mass is mass of tortoise in grams; τ_{hfr} is the time constant (seconds) determined for warming experiments; others are time constants (seconds) determined for cooling experiments: τ_{cfr} for conditions close to free convection, τ_{cfo} for turbulent conditions).

Mass (g)	$\tau_{\text{cfr}}/\tau_{\text{hfr}} (\phi)$	$\tau_{\text{cfr}}/\tau_{\text{cfo}}$
100	1.40	2.61
305	1.55	2.15
590	1.56	2.36
1010	1.47	2.33

Figure 7 also indicates this effect.

The ratio $\tau_{\text{cooling}}:\tau_{\text{warming}}$ (i.e. $\tau_{\text{cfr}}/\tau_{\text{hfr}}; = \phi$) under near free convective conditions is similar for all body sizes (Table 2). The relationship between ϕ and body mass is slightly hyperbolic (Fig. 8).

Table 3 reports the exponents relating time constants to body mass and the time constants during cooling under conditions of forced convection ($\geq 1\text{ms}^{-1}$) for three species of reptile.

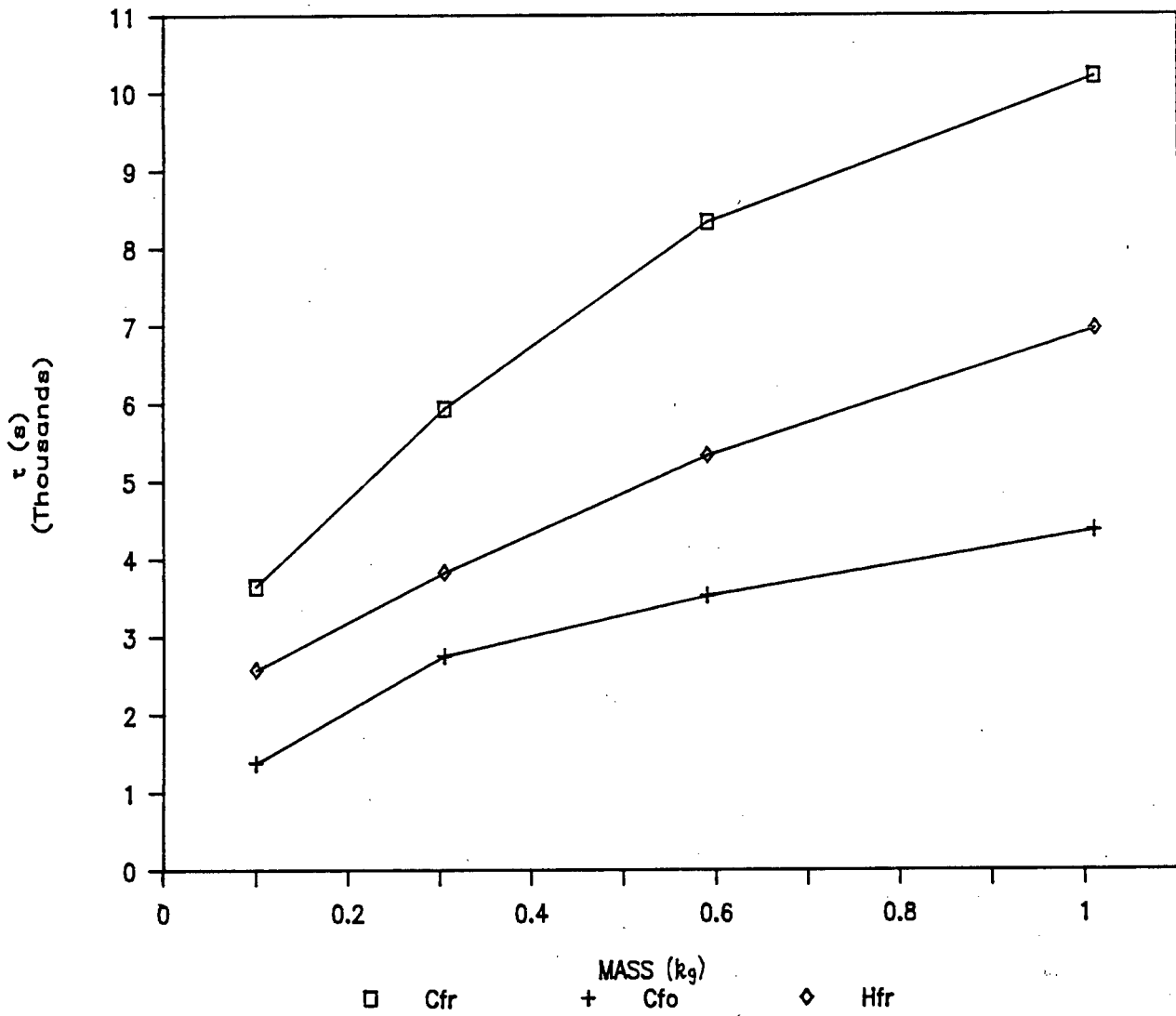


Figure 6 Plot of τ against tortoise mass. τ is in seconds, mass in kilograms. Cfr: cooling, near-free convection; Cfo: cooling, forced convection; Hfr: heating, near-free convection

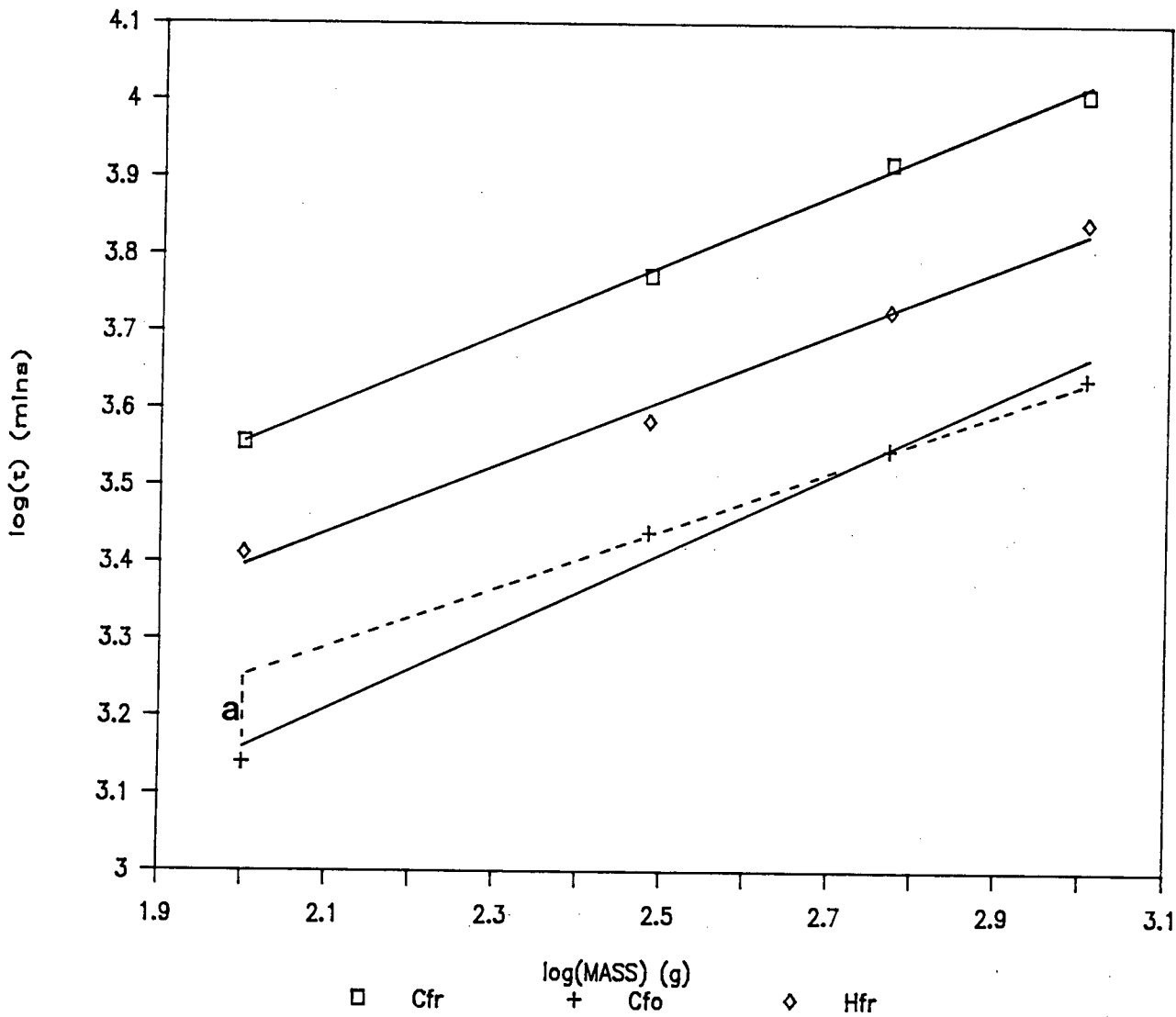


Figure 7 Log-log plot of τ against tortoise mass. Legend and symbols as in Figure 6. The best fit lines were obtained by least squares. a is the possible deviation of τ under the condition of forced convection for the smallest tortoise, due to convection having the greatest effect on heat transfer in small animals.

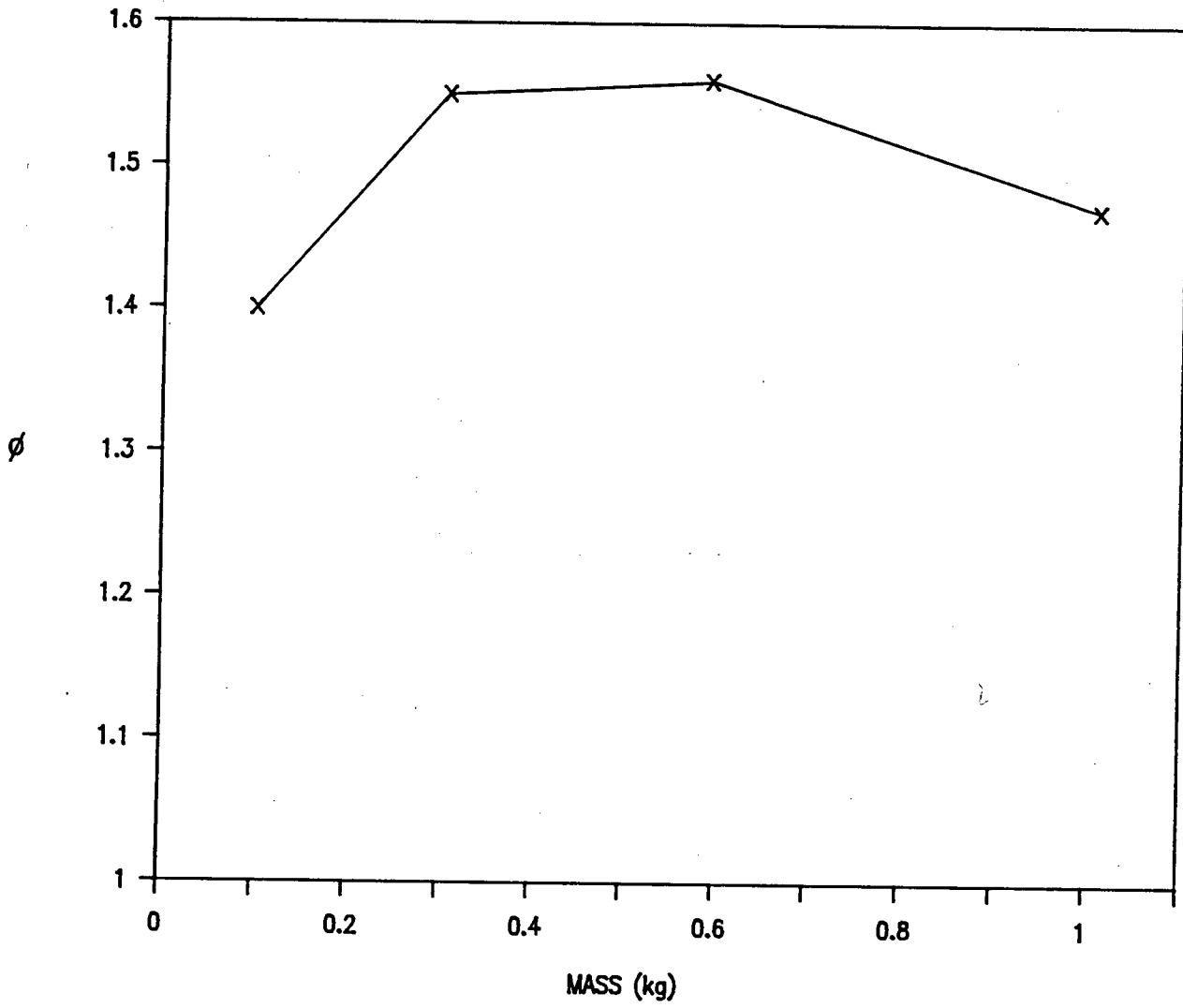


Figure 8 Plot of ϕ against tortoise mass. Mass in kilograms.
 $\phi = \tau_{cfr} / \tau_{hfr}$

Table 3 Exponents (b) of relationships relating time constants to body mass, and time constants for 1kg individuals (τ ; seconds), for different reptiles cooling under conditions of forced convection. Time constants were calculated from given allometric equations.

Species	b	τ	Reference
Testudinidae <i>Chersina angulata</i>	0.46	4582	This study
Alligatoridae <i>Alligator mississippiensis</i>	0.43	2061	Smith (1976)
Crocodylidae <i>Crocodylus niloticus</i>	0.43	3664	Loveridge (1984)

The exponents are similar, and that for the angulate tortoise cannot be distinguished statistically from the others (see above).

Discussion

Variable results have been found for the comparison of rates of heating with rates of cooling in different tortoise species. Some seem to cool faster than they warm, some cool and heat at similar rates, and others heat faster than they

cool (Table 4). However it now appears that most reptiles above about 20g mass, including tortoises, heat faster than they cool (see Turner 1987a), and circulation changes have been shown in several reptile species when placed in a step change in temperature, which implies that the rates of heat transfer are regulated physiologically. These changes include vasoconstriction and vasodilation, and greater heart rates during heating compared with cooling (Weathers & White 1971; Voigt 1975; Smith 1976; Bartholomew 1982b; Smith, Standora & Robertson 1984). The first confirmation that turtles employ physiological control of body temperature was the study by Weathers & White (1971). There is an increase in both carapace and cutaneous blood flow during heating in terrapins, and a decrease during cooling.

Subsequently, Voigt (1975) demonstrated physiological control of heat exchange in the tortoise *Gopherus agassizii*. It appears that where physiological thermoregulation occurs during transient temperatures under a step change in temperature, the type of thermoregulatory response remains constant throughout the duration of the experiment (eg. Voigt 1975; Smith *et al.* 1984), which facilitates comparison of τ between species.

Unfortunately, not all results are comparable, since different conditions and methods of monitoring body temperature changes were used; more especially not all are comparable because of different methods of reporting the heat transfer rates (Bakken 1976a; Smith 1976).

Table 4 Comparison of rates of cooling (T_c) and rates of heating (T_h) in various tortoise species

Species	T_c/T_h	Condition	Reference
<i>Chersina</i>	>1	Laboratory	Craig (1973)
<i>angulata</i>	<1	Laboratory	Perrin & Campbell (1981)
<i>Gopherus</i>	<1	Field	Voigt (1975)
<i>agassizii</i>	=1	Laboratory	Voigt (1975)
<i>G.</i> <i>berlandieri</i>	<1	Laboratory	Voigt & Johnson (1977)
<i>G.</i> <i>carbonaria</i>	<1	Laboratory	Weathers & White (1971)
<i>G.</i> <i>polyphemus</i>	>1	Laboratory	Spray & May (1972)
<i>Terrapene</i> <i>carolina</i>	>1	Laboratory	Spray & May (1972)
<i>T.</i> <i>ornata</i>	>1	Laboratory	Bethea (1972)

The only similar study to this one for tortoises was that of Voigt & Johnson (1977). They found that *Gopherus berlandieri* warmed faster than it cooled. Unfortunately, they do not state whether conditions of free or forced convection prevailed.

Free convection only applies under windspeed conditions of 10cms^{-1} or less (Foley & Spotila 1976). At windspeeds of 50cms^{-1} much of the boundary layer of air will have been removed from around the tortoise (Foley & Spotila 1976), which increases the heat gradient between the surface of the

tortoise and the immediate surrounding air, and causes an increase in the rate of heat loss. Windspeeds between 1 and 4ms^{-1} do not change the results significantly (Loveridge 1984; Turner 1987a).

The rates of warming recorded for the angulate tortoise in this study are probably underestimates. Cloudsley-Thompson (1974) found that in *Testudo graeca* that heat loss through evaporative water loss exceeded the heat produced by metabolism (ie. M^* is negative; equation (2)) until a cloacal temperature of 33°C . The angulate tortoise is probably similar in this respect. However no measurements of evaporative water loss are available over the range of temperatures encountered during the heating and cooling experiments, so M^* cannot be calculated. No inflexion was found in the cooling or warming curves as reported by Craig (1973) and Perrin & Campbell (1981).

Time constants for angulate tortoises cooling in air under conditions of forced convection are about double that of other reptiles (calculated from Bell 1980; Table 3). The small surface area to volume ratio of tortoises (Bartholomew 1982b) would contribute to the greater time constants.

The time constants for cooling rates of eggs (as isomorphic bodies) scale with mass to the power of 0.402, instead of the expected 0.33 (Turner 1985). This results from the fact that external resistance to heat flow varies according to whether radiation or convection is the main mode of heat transfer, which itself depends upon the size of the egg (Turner 1985). The exponent relating the time constants for angulate tortoises cooling under conditions of near-free convection ($b = 0.445$) cannot be distinguished statistically from that for birds' eggs cooling under free convection conditions. This implies that heat is lost from tortoises during cooling in a manner analogous to that from bird's eggs. Time constants for reptiles cooling under conditions of forced convection scale with mass in a similar way to eggs under conditions of free convection (Table 3). This does not necessarily imply that these animals are isomorphic, but is probably a composite result of different resistances to heat transfer, as discussed above. Interestingly, the slope of the graph relating τ to mass does not change even when windspeed is increased dramatically (Loveridge 1984; this study). This suggests that if any physiological control is taking place the type of control is constant throughout the experiment for all sizes of animal tested within a species. In air, the external resistance to heat flow from small reptiles should depend largely upon the convective conditions at the time, whereas radiation should be the main avenue of heat loss in larger reptiles, assuming

negligible heat loss through conduction to the substrate (Turner 1987a). This is noticeable in Table 2 (also Fig. 7), where the ratio $\tau_{cfr} : \tau_{cfo}$ is least for the smallest tortoise.

Internal resistance to heat flow may vary with the effectiveness of blood flow in dissipating heat (or vasoconstriction to prevent heat loss), which in itself depends on body size (Turner 1987a). The data obtained cannot tell us what role blood flow played in controlling rates of heat flow during the experiments.

It has been shown experimentally and in theory that blood flow to the body surface and appendages of reptiles is important in thermoregulation of reptiles (Bartholomew 1982b; Turner 1987a). In fact many animals regulate their body temperatures to a large degree through their limbs (Schmidt-Nielsen 1979). Small reptiles with appendages should rely heavily on adjustments of blood flow to their appendages to control heat exchange, whereas large reptiles could possibly control rates of heat transfer by controlling the flow of blood to the surfaces of their torsos (Turner 1987a). The importance of blood flow in controlling rates of heat transfer in terrestrial reptiles will depend upon the relationship between blood flow resistance and conduction resistance to heat flow within the body, and the magnitude of the external resistance to heat flow between the reptile and its environment. When internal resistance to conduction is high blood flow will become important in controlling heat exchange between a reptile and its environment (Turner

1987a). Therefore it is possible that blood flow to the appendages, and possibly to the shell, will be important in angulate tortoises in increasing their body temperatures during warming. As Turner & Tracy (1983) point out, there is no reason why tortoises should not use their limbs for physiological and behavioural thermoregulation when they are splayed during basking. Increasing blood flow to the limbs should increase the capacity of the tortoise to gain thermal energy and to lose body heat. However it is not known for all body sizes whether tortoises rely on changing blood flow to the appendages to control their body temperatures.

Two interesting lines of observation suggest the importance of appendages in the thermoregulation of tortoises. Assuming that the shell is not as well vascularised as the appendages, the effect of changing blood flow to the appendages on the transfer of heat would be greater than changing blood flow to the shell. This also assumes that the resistance to heat exchange of conduction through the shell and body tissues is greater than that of blood flow. If this is true, the shell could be an "insulator" relative to the appendages. Within minutes of being placed in the cool environment, in the present investigation, the angulate tortoises withdraw their appendages into their shells, seemingly to conserve heat. During heating they extended their appendages, including exposing the relatively thin skin of their necks, to gain heat and increase their body temperature. This behaviour has been shown to occur in the

laboratory in *Gopherus berlandieri* and *G. polyphemus* (Spray & May 1972; Voigt & Johnson 1977).

In theory at least, there should be a certain size in which the role of blood flow in the control of heat exchange is maximised (Turner 1987a). This has been shown to be true for the alligator (*Alligator mississippiensis*; Turner & Tracy 1985) but the size at which \emptyset is a maximum will vary depending upon the species, since internal resistance to heat flow will vary between species, and with the external conditions. Whether \emptyset is maximised at a body size of between 600-700g in angulate tortoises, as indicated in Fig. 8, is equivocal, due to the small sample size. However, support for this hypothesis comes from data for the tortoise *Gopherus berlandieri* (Fig. 9). Between 740 and 1009g \emptyset falls with increasing body mass, indicating that if a maximum in \emptyset is reached it occurs before a size of 1kg. Whether this maximum is caused by relative differences in the effect of blood flow or conduction in the transfer of heat during heating and cooling, and whether it stems from differences in body size, will have to await further quantification. This is another reason why overall thermal conductance may not be valid, because the thermal conductance is probably size-dependent. It seems likely that when the angulate tortoises withdrew their heads into their shells at relatively high body temperatures (between 27 and 32°C) they are using the shell as ^{"relative"} insulation to retard further rise in head temperature. This has been noticed in other chelonians

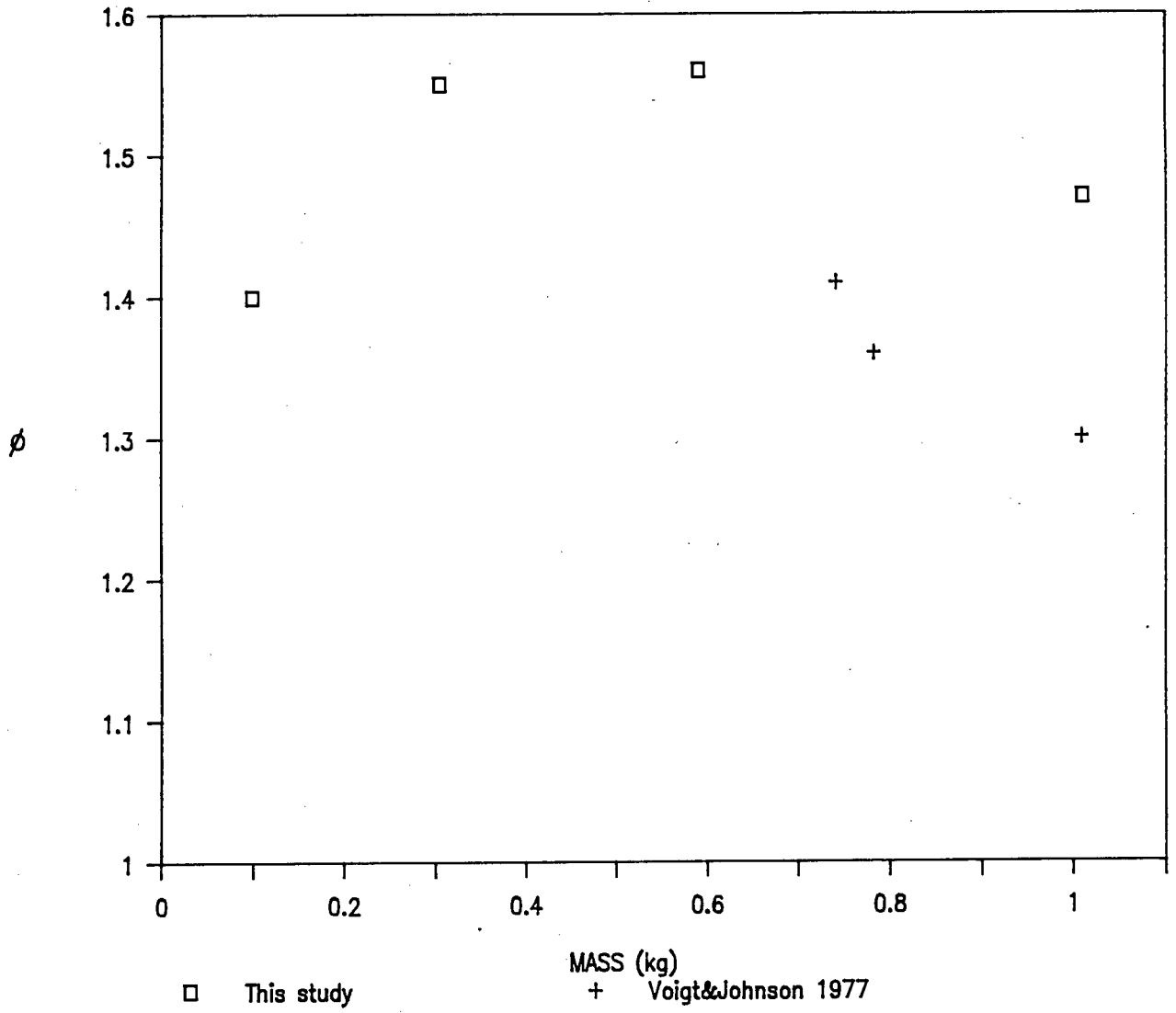


Figure 9 Plot of ϕ against tortoise mass. Mass in kilograms.

(Webb & Johnson 1972; Voigt & Johnson 1977), and control of head temperature seems more critical than body temperature in turtles (Webb & Johnson 1972).

To enable comparisons to be made between the different body sizes it has to be assumed that all the animals behaved in the same way during the experiments. However, there was some individual variation in activity during the heating experiments, and so confidence in the accuracy of θ and in the comparisons between the time constants for heating is somewhat lessened. Of course, individual differences are unavoidable.

Because conduction to the substratum would have played a role in heat transfer in these experiments (eg. heat gained in the warming experiments via conduction to the limbs in all the tortoises) and because of differences in conditions between the heating and the cooling experiments, the absolute value of θ would not be accurate, and θ cannot be directly compared with that obtained for other reptiles. The effect of conduction to the substratum can be great (Stevenson 1985b). However the relative rates of cooling and heating should be relatively comparable with experimental data from other reptiles, and qualitatively compatible with theories on heat transfer rates.

Thermal conductance depends in part upon the properties of the external layers of the animal. If the external surface varies in insulating properties in different regions of the body then a single K_0 at a specific body temperature

cannot be assumed. In reptiles this problem is usually overcome by assuming the skin has negligible resistance to heat flow when compared to other factors (Bakken 1976b). The conductivity of the shell of the tortoise will differ from that of the skin, and therefore K_0 will vary over the tortoise body. However when cooling the tortoise tends to pull its head and its legs into its shell, and most of the visible parts are heavily scaled. Therefore it may be possible to use a single K_0 for the tortoise during cooling curve determinations if any physiological thermoregulation is constant. Differences between species of tortoise in τ should be determined from cooling experiments, because during these the tortoises are not active, therefore reducing error through activity, behaviour and evaporative water loss. The validity of using the time constant in studying the physiology of ectotherms, especially in predicting thermal responses in the field, has been questioned (Turner 1987b). This is because thermal responses in these animals may be determined more accurately when they are considered mathematically as second order systems. It appears that transient body temperatures of large angulate tortoises appear to be better represented as a second order system (Fig. 10; cf. Fig. 1 in Turner 1987b). A first order system would be represented by a straight line, τ being equal to the negative reciprocal of the slope. Therefore τ would not be an accurate representation of the slope of the graph for the large tortoise. Transient body temperatures of small

ectotherms behave more like first order systems, and τ may be used adequately (Turner 1987b). This can be seen in Fig. 10, where the graph for the small tortoise approximates a straight line better than the graph for the large tortoise. In calculating τ in this study care was taken to avoid portions of the curves definitely not mimicking a first order function.

Dead angulate tortoises were not used as controls because previous studies have shown that they heat and cool at similar rates (Craig 1973; Perrin & Campbell 1981). The fact that the graph lines in Fig. 5 are reasonably straight seems to indicate no major problem in the thermal characteristics of the equipment used.

The work reported in this chapter would be improved by: using a wind tunnel to ensure airflow over the tortoises was laminar during both the heating and the cooling experiments; ensuring that the environment temperatures were precisely the same during the heating and cooling experiments; ensuring that the body temperatures of the tortoises were in equilibrium with the ambient temperatures at the beginning of both the heating and the cooling experiments, and; using equipment that controls ambient temperature more precisely. Future work on rates of heat transfer should utilise a method by which changes in blood flow to the limbs and shell can be tracked during the experiments.

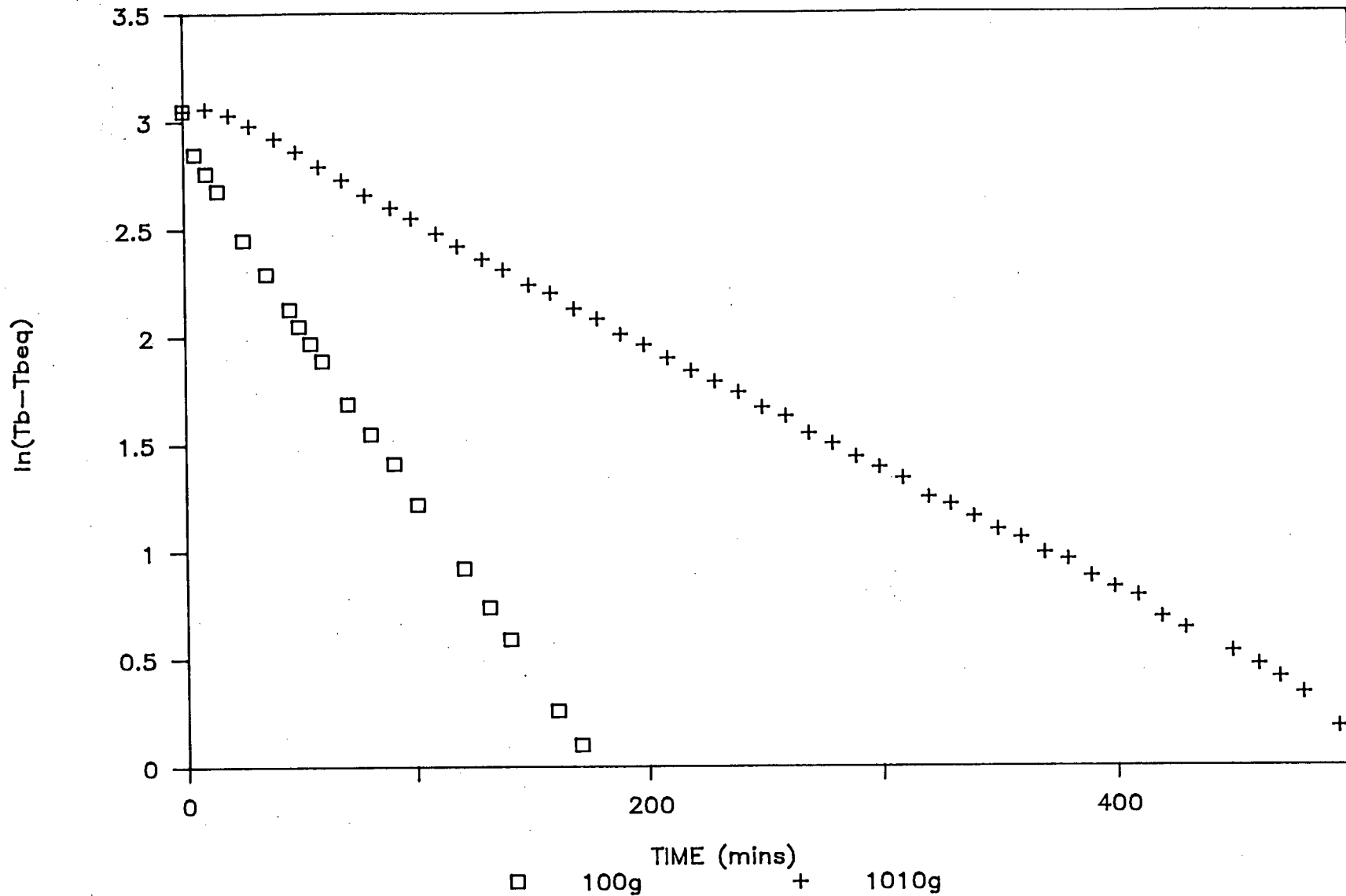


Figure 10 Semi-logarithmic plot of absolute differences between body temperature and equilibrium body temperature of tortoises against time. Temperatures are in degrees centigrade. See text for further explanation.

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CHAPTER 6

EVAPORATIVE WATER LOSS

Reptiles have skins that are relatively impermeable to water (Schmidt-Nielsen 1979). It has often been claimed that the permeability of the skin of a specific reptile, and therefore the rate of evaporative water loss (EWL), could be correlated with the availability of moisture in its habitat. Reptiles with lower rates of evaporative water loss should be found in more xeric habitats. More recent evidence points to the microhabitat in which the reptile lives, rather than broad habitat type, as being the primary factor determining whether a reptile with a certain skin resistance is able to remain in water balance and therefore exist within that microhabitat (Gans, Krakauer & Paganelli 1968; Davis, Spotila & Scheffler 1980; Mautz 1982).

Tortoises obtain much of their water from their food, and can withstand long periods of dehydration (Minnich 1982). The angulate tortoise appears to be able to withstand long periods without drinking if the water content of the food is high (see Section 1). This tortoise is found in a wide range of habitats, ranging from xeric (under 100mm rainfall annually) to mesic (700mm rainfall annually) in the Cape province of South Africa (Greig & Burdett 1976). Since it inhabits both xeric and mesic habitats, and since in the western Cape Province of South Africa it has to tolerate long, hot summers with little rainfall, it could be worthwhile to determine its EWL. This could then be compared to the EWLs of other species of tortoise which live in comparable or different habitat types.

Body water in tortoises is lost through the urine, faeces, skin, respiratory tract, cloaca and moist corneal surfaces (Mautz 1980, 1982). Water lost through the urine and faeces was not obtained in the experiments reported below because it does not contribute to EWL. Also, it is difficult to accurately measure such loss per unit time, since tortoises store water in their urinary bladder for long periods, and do not necessarily release all their urine at the same time (Mahmoud & Glicka 1979; pers. obs). Evaporative water loss in reptiles is affected by temperature, humidity, windspeed, metabolic rate, activity, time of diel cycle in which the measurements are taken, state of acclimation of the reptiles, body shape and body size (Foley & Spotila 1978; Mautz 1980, 1982). These factors should be controlled as far as possible in water loss experiments.

This study aims to determine the total EWL of angulate tortoises. It also aims to determine the scaling of EWL to body mass.

Methods

Six angulate tortoises were used in the first set of EWL experiments. The range in mean mass was 591-737g. Unfortunately, due in part to the design of the experimental chambers, the tortoises were subjected to a range in ambient temperature and water vapour pressure within the ranges specified in Table 1. Also, it was only realised some time after the experiments had ended, that the readouts of the environment rooms were not calibrated accurately, and so calibration of the environmental conditions were done months after the experiments. However, the latter measurements are specified in Table 1.

The tortoises were kept in a controlled temperature room during the experiments at the experimental temperature. They were fed their normal diet. The tortoises generally gained mass at some stage during the experiments, and then lost it again. Each tortoise was starved for at least forty-eight hours before the experimental run. Total EWL was calculated by two methods, both of them gravimetric. (a) The tortoise was weighed before and after each run. (b) Water vapour was collected by drawing air with a pump (ADC 124, Analytical Development Company) through two metabolic chambers and two series of preweighed drying tubes and silicone tubing of equal corresponding size (Figure 1). The drying tubes contained silica gel. The one series was used as the control, the other series being the experimental apparatus. The

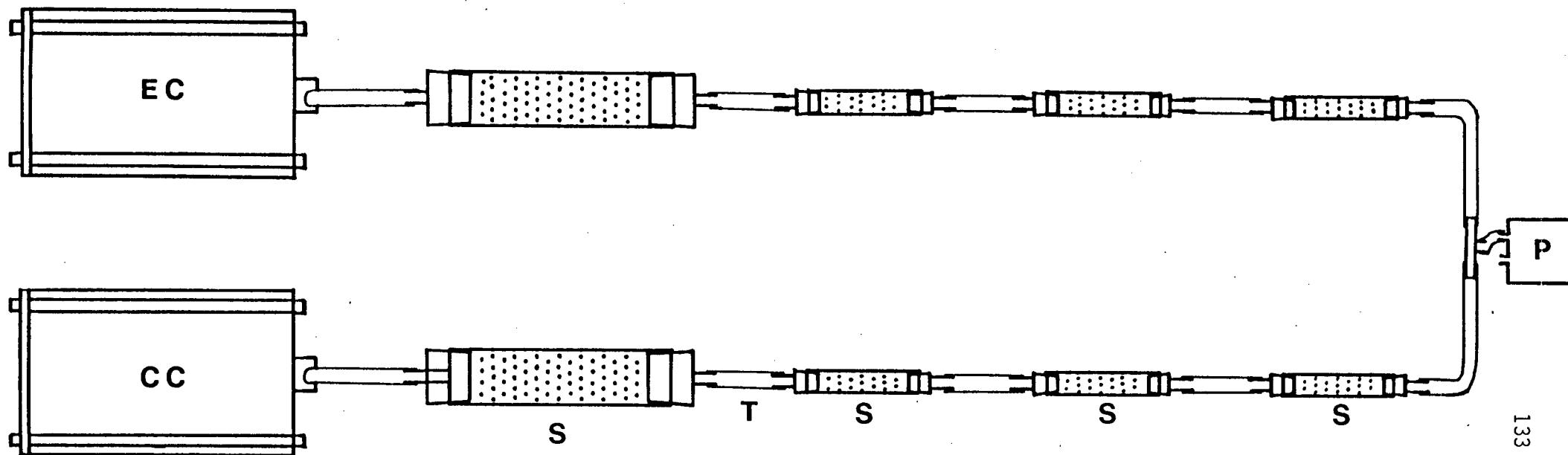


Figure 1 Apparatus used to determine evaporative water loss rates for the study animal under two temperature and two relative humidity ranges. EC = experimental chamber; CC = control chamber; P = pump; S = drying tube containing silica gel; T = silicone tubing.

drying tubes were reweighed at the end of the experimental run, and the difference in mass in each set before and after the run was the amount of water collected. The difference in mass (in grams) of water collected between the two series of drying tubes was the amount of water lost by the tortoise during that period. The time period of each run was approximately four hours. Water loss was measured in $\mu\text{gH}_2\text{Og}^{-1}\text{body mass h}^{-1}$ (see Mautz 1982). The loss in mass of the tortoise was considered to be due entirely to the loss of water (loss in mass due to carbon dioxide release was assumed to be negligible). In fasting reptiles release of carbon dioxide does not contribute to mass loss (Mautz 1982). The mass of water lost or collected was divided by the initial mass of the tortoise to calculate the mass-specific rate of water loss. Between two and nine replicates were done for each tortoise under each experimental condition.

Two sets of experiments were carried out to determine the allometric scaling of total EWL. The first set was done at the same time as the experiments investigating the allometry of resting metabolic rate (Section 3). T_a was $20.0 \pm 0.7^\circ\text{C}$, conditions of free convection prevailed, and the relative humidity of the air in the controlled environment room ranged continuously between 35% and 56%.

The second set of experiments was carried out in the same controlled environment room as the first set, at the same control room settings. T_a in the room was $20.6 \pm 1.5^\circ\text{C}$, and relative humidity (RH) ranged continuously between 42% and

72%. The nine tortoises were placed separately in open plastic containers, measuring approximately 275x345mm, on the floor of the room. The tortoises were left in the containers for about 25 hours (see Appendix 3 (3)).

Each tortoise was weighed at the beginning of the experiment, and then weighed again at the end, to determine the rate of total EWL. The time was recorded at each weighing. The tortoises were not equilibrated to the experimental conditions beforehand.

Data for trials in which the tortoise defecated or urinated were not included in the analysis for any of the sets of EWL experiments. Data for a tortoise with major, visible scale lesions were excluded from the analysis. Its rate of EWL was very high.

Results

The results for mass-specific water loss are set out in Table 1. There was no significant difference between the two methods of measuring EWL (Paired sample t-test, $t_{16} = 0.191$, N/S). Since the standard deviations of the results for EWL obtained by measuring the loss in tortoise mass were generally less than those of the other method, further data analysis was confined to results obtained by the mass loss method.

Table 1 Evaporative water loss of angulate tortoises. The mean mass of the tortoises was 664g (range 591-737g). All values are means of means (+/- one standard deviation) to the nearest 10ug. X_1 is mean water loss ($\text{ugg}^{-1}\text{h}^{-1}$) calculated from loss in mass of the tortoises during the experimental periods; X_2 is mean mass of water ($\text{ugg}^{-1}\text{h}^{-1}$) collected in the drying tubes; T_a and VPD are ambient temperature ($^{\circ}\text{C}$) and vapour pressure deficit (mmHg) ranges, respectively, of the controlled temperature room; N is number of tortoises.

N	T_a	VPD	X_1	X_2
6	13.5-19.9	2.598-10.738	80 (<u>+40</u>)	70 (<u>+60</u>)

Trials were undertaken at a T_a of approximately 14.5°C and at two ranges in RH of about 36-39% and 48-66%, in which a petridish with a weighed amount of water and a filter paper evaporating wick was placed in the experimental apparatus. The amount of water vapour collected by the silica gel was within 13% of the measured water loss, although the error was generally about 10%. It appears that the silica gel does not trap all the water vapour moving through it. Water loss rates of the angulate tortoises, as measured by this method, were sometimes greater, and sometimes less, than that calculated gravimetrically from the loss in mass of the tortoises.

Total EWL increases with body size. The scaling of total EWL (mgh^{-1}) to body mass (M ; g) is (Fig. 2):

$$\text{EWL} = 0.2558M^{0.797} \quad (1)$$

The regression fit is significant ($F_{1,7} = 9.221$, $P < 0.025$, $R^2 = 0.902$). The difference between the mass exponent and the theoretical exponent of 0.67 is very highly significant (Student's t -test, $t_8 = 6.195$, $P < 0.0005$).

The allometric scaling of total EWL (mgh^{-1}) obtained during the 25 hour experiments is (Fig. 3):

$$\text{EWL} = 12.1392e^{0.0018M} \quad (2)$$

Mass (M) is in grams. The regression is highly significant ($F_{1,7} = 47.64$, $P < 0.005$, $R^2 = 0.899$).

The best fit curve for the tortoises under 450g is:

$$\text{EWL} = 1.406M^{0.517} \quad (3)$$

where EWL is in mgh^{-1} and mass (M) is in grams. The regression is not significant ($F_{1,2} = 11.08$, N/S).

The relationship between EWL (mgh^{-1}) and mass (M ; g) for tortoises larger than 450g is:

$$\text{EWL} = 0.0005M^{1.731} \quad (4)$$

EWL
(mg/h)

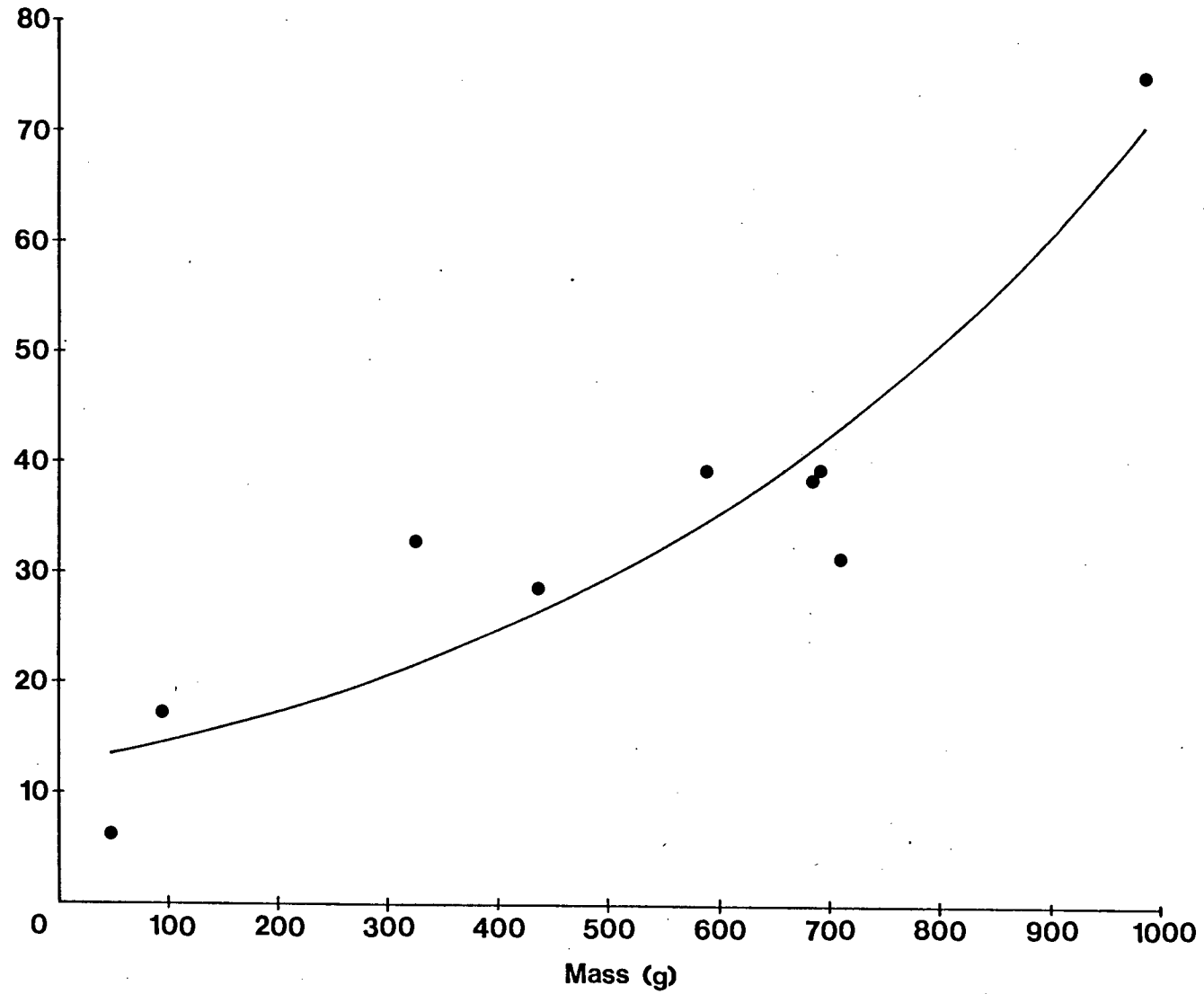


Figure 3 Graph of total evaporative water loss (EWL; mgh⁻¹) against tortoise mass (grams). Data are from the twenty-five hour experiments. The curve represents equation (2).

The regression is not significant ($F_{1,2} = 11.35$, N/S).

The results used to determine equations (1) and (2) are shown graphically in Figures 2 and 3. The rates of total EWL for a 664g tortoise obtained from equations (1) and (2) (70 and $60 \text{ ug g}^{-1} \text{ h}^{-1}$, respectively) are close to that reported in Table 1, under similar conditions.

Discussion

The mean evaporative water loss values for angulate tortoises ($\bar{X}=80 \text{ ug g}^{-1} \text{ h}^{-1}$) are within the same order of magnitude of those of other tortoise species, such as *Gopherus agassizii* and *Geochelone sulcata* (Schmidt-Nielsen & Bentley 1966; Cloudsley-Thompson 1970; Table 2). Although it has been stated that evaporative water loss is greater in reptiles from wetter areas than drier areas (eg. Schmidt-Nielsen & Bentley 1966), this is not necessarily the case (Mautz 1982). The microhabitat in which tortoises are found depends largely upon the vegetation types in their habitat, since tortoises are terrestrial and confined to the surface of the ground or burrows. Angulate tortoises are found in xeric to mesic "heathland" areas, with thick shrub, but very few trees. Angulate tortoises have total EWLs intermediate between a desert species, *Gopherus agassizii*, living in a xeric habitat where vegetation is sparse, and a species, *Terrapene carolina*, living in a mesic habitat with trees and

Table 2 Evaporative water loss rates for different species of tortoise. Mass is in grams, T_a is ambient temperature ($^{\circ}\text{C}$), R.H. is relative humidity (%), TWL is total evaporative water loss, RWL is respiratory water loss. All water loss values are in $\mu\text{g g}^{-1}\text{h}^{-1}$. R is reference (see below)

Species	Mass	T_a	RH	TWL	RWL/TWL(%)	R
<i>Chersina angulata</i>	650	14-20	36-85	80	-	5
<i>Geochelone sulcata</i>	305	25	-	260	44-48	1
<i>Gopherus agassizii</i> *	1770	23	-	71	24	4
	1770	35	-	140	48	4
<i>G. polyphemus</i> *	1328	32	30	441	-	2
<i>Terrapene carolina</i>	452	21	45	512	24	3

1 Cloudsley-Thompson (1970)

2 Ross (1977)

3 Spotila & Berman (1976)

4 Schmidt-Nielsen & Bentley (1966)

5 This study

* Mean mass used; however difference in mass between largest and smallest tortoise was 1875 grams

* Mean mass used; range in mass was 305-2961g

grassland (eg. Nagy & Medica 1986; Schmidt-Nielsen & Bentley 1966).

A rise in body temperature has been shown to cause an increase in total EWL in tortoise species and in other reptiles (Table 2; Davis et al. 1980). High relative humid-

ity reduces the saturation deficit, causing a decrease in the rate of EWL (Gans et al. 1968). In terrapins, the water vapour saturation deficit is more important than T_a in influencing rates of EWL (Foley & Spotila 1978). The relatively large standard deviations found in the total EWL found in my study ~~is~~^{are} presumably partly due to the wide range in body temperatures and water vapour saturation deficits.

In snakes, EWL is insensitive to RH of below 73% (Gans et al. 1968). EWL in lizards shows a similar insensitivity to RH (Mautz 1980). The increased rates of EWL of snakes at high temperatures is probably due to an increase in the blood flow to the skin. This shortens the distance for the diffusion of water in the skin barrier (Gans et al. 1968). However, much of the variation in EWL found in my study is probably caused by greater activity at the higher temperatures. This will be discussed more fully later.

It is interesting that an exponential relationship was found between EWL and body mass during the 25 hour experiments, and that the mass exponent in equation (1) was significantly greater than 0.67. There are two possible explanations for this phenomenon. Small tortoises have relatively large surface areas (Section 2), so their absolute EWL will be ^{higher than expected} ~~relatively high~~ (Bentley & Schmidt-Nielsen 1966; cf. Dunson & Bramham 1981; Table 2). This leads to a higher elevation of the left hand tail of the graph (Figure 2). Also, the results suggest that respiratory water loss was a major component of total EWL during these experiments.

Activity has the greatest effect on total EWL rates (Mautz 1982). This is clear in the experiments done concomitantly with resting oxygen consumption measurements, where tortoises with relatively high average oxygen consumptions have relatively high EWL rates (eg. 680g, Figure 2; cf Figure 6, Section 3). It is likely that the increase in EWL during activity is due to an increase in respiratory water loss (see results in Mautz 1982, although skin vasodilation may play a role). The increase in respiratory water loss would result from greater respiratory rates. If one or two of the larger animals in each group were more active than the others, as appears to be the case from the results (Figure 2), the equation will be exponential in form, or the power exponent will increase. There is still some doubt as to when cutaneous or respiratory water loss is a more important avenue of water loss in reptiles (Davis et al. 1980).

Lower total mass-specific EWL rates occurred during the longer experiments, as shown earlier (cf. results for a 664g tortoise from Table 1, equation (1) and equation (2): 80, 70 and 60 $\text{ugg}^{-1}\text{h}^{-1}$ respectively). These results suggest that activity was the primary factor influencing EWL rates in the angulate tortoises, lower rates resulting through the lowering of stress as the tortoises became acclimated to the experimental conditions.

Equation (2) is considered to be the more appropriate of the two allometric equations because the masses are more evenly spaced, the mass range is greater, and the experi-

ments were of longer duration, which would reduce the effects of activity. The best fit equation (significant at the 5% level) from the first set of results for the tortoises under 450g is also an exponential function.

With respect to avenues of evaporative water loss other than cutaneous and respiratory water loss, only ocular water loss is important (Mautz 1982). Ocular water loss in the experimental angulate tortoises would probably be minimal because the tortoises spent much of the time with their eyes closed. A more significant error may be introduced when the rate of flow of air through the chamber is slow, causing a build up of water vapour in the chamber and therefore causing a decrease in the saturation deficit within the chamber (Gans *et al.* 1968). This may have occurred during these experiments. If the relative humidity is high, it appears water vapour input can be relatively large in tortoises (Nagy & Medica 1986). It is important that the body temperature of the tortoises are in equilibrium with the ambient temperature, or else the amount of respiratory water lost may be different to the actual value at equilibrium body temperature (Mautz 1982). However the error is probably small. The body temperatures of the study animals were not at equilibrium with T_a initially during the allometry experiments, but at the end they would be close to, or at, equilibrium. Since all the tortoises were exposed to the same conditions, it is assumed that the comparative effect

of differences in EWL rates due to the non-equilibria of body temperatures is small.

In conclusion, it appears that the longer the duration of the experiment, the lower the mass-specific total EWL. Activity increases rates of EWL markedly. The allometric scaling of the rate of EWL for this species is influenced to a large degree by activity. Future work should concentrate on utilising accurately controlled experimental conditions in determining EWL rates of angulate tortoises. It will, however, be difficult to remove the variable of activity in the experimental design. It would be interesting to do experiments at widely-differing ambient temperatures and water vapour saturation deficits, to determine how these variables affect the total EWL of angulate tortoises, and to investigate the relative importance of respiratory and cutaneous EWL under these conditions.

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CHAPTER 7

CONCLUSION

cantly different from the expected 0.67. The slopes of the equations relating physiological rates to body mass in juveniles are generally greater than those for all individuals. These results indicate that there are significant physiological differences between different ages of tortoise, although certain of their important physiological properties, eg. surface area, volume and mass, scale superficially in a similar way. Some of these differences result from external conditions acting upon these properties in different ways with a change in size. For example, physiological rates dependent upon surface area are relatively greater in smaller tortoises, where the magnitude and the type of the environmental conditions are the greatest determinants of these rates. Some differences, eg. in resting metabolic rate, probably result from differences in the internal properties of the tortoises, eg. in the oxygen transport system.

The angulate tortoise shows a circadian rhythm, and probably a circannual rhythm, in resting metabolic rate, with the peak resting oxygen consumption being found during their active periods, ie. during the morning/early afternoon and summer, respectively.

This species' breathing cycle appears to have long apnoeic periods of about 30 minutes at 25°C when totally at rest. The duration of the non-ventilatory period decreases with increasing ambient temperature. The resting metabolic rate of a 650g tortoise during summer is about $0.037 \text{ ml O}_2 \text{ g}^{-1}$

l^{-1} (about $0.026 \text{ mlO}_2\text{g}^{-1}\text{h}^{-1}$ in the acclimated phase), and during winter approximately $0.011 \text{ mlO}_2\text{g}^{-1}\text{h}^{-1}$. This is equivalent to $580 \text{ mlO}_2\text{day}^{-1}$ ($405 \text{ mlO}_2\text{day}^{-1}$) and $175 \text{ mlO}_2\text{day}^{-1}$ respectively. The energetic cost of free existence during late summer is 1.2 times (1.7 times) the summer RMR and 4 times the winter RMR, or 14 kJday^{-1} .

Rates of heat transfer scale as a power function of body mass. The value of the exponent is not significantly different to the theoretical and empirical value for birds' eggs, which are isomorphic over their size range. The types of external conditions determine the magnitude of the mass exponent and the mass coefficient. The physical properties of the tortoise and its physiological responses under the specified environmental conditions partly determine the value of the mass coefficient. This tortoise species has much slower mass-specific rates of heat exchange than other reptiles, in the order of 100%, because it has a relatively small surface area to volume ratio. The angulate tortoise behaviourally exploits its shell as an insulator for its head and limbs under low (12°C) and high (34°C) ambient temperatures. Rates of heat exchange were affected by metabolic heat production and evaporative water loss during activity in small tortoises, but not in large individuals. Tortoises of about 600g mass appear to utilise physiological thermoregulatory mechanisms most efficiently.

Total evaporative water loss rates of this species of tortoise are primarily affected by activity, as has been

found for other species of reptiles. At 20°C, a 650g tortoise loses about 60 $\mu\text{gH}_2\text{Og}^{-1}\text{h}^{-1}$ when relatively inactive.

Most of the above conclusions should, however, be treated with some caution because of the small sample sizes used in obtaining the results and the various shortcomings in the methodology.

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APPENDICES

Appendix 1 Environmental and experimental conditions

The environmental conditions used in the experiments are close to those recorded at the Cape of Good Hope Nature Reserve, from where most of the tortoises were obtained; the mean ambient temperatures and relative humidities recorded there over many years are as follows (Weather Bureau 1986):

Table 1 Temperatures and relative humidities recorded at the Cape of Good Hope Nature Reserve

<u>Time</u>	<u>Temperature(°C)</u>			<u>Relative Humidity(%)</u>
	<u>Minimum</u>	<u>Mean</u>	<u>Maximum</u>	<u>Mean</u>
08h00		14.5		83
14h00	12.7	17.2	18.5	74

Maximum range in ambient temperature recorded is 0°C-36.1°C (Weather Bureau 1986).

Photoperiod conditions, hours light:hours dark (L:D), in the Cape Peninsula during the months in which experiments were done are as follows (Weather Bureau, pers. comm.):

Table 2 Approximate photoperiods for the Cape Peninsula region

<u>Day and Month</u>	<u>Photoperiod</u>
1 May	11L:13D
25 June	10L:14D
10 August	11L:13D
10 September	12L:12D
3 October	13L:11D
2 December	14L:10D

A photoperiod similar to the natural daylength at the beginning of each experimental period was used during the pre-experimental phase.

Reference

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Appendix 2 Husbandry

When the tortoises were not being kept under standardised environmental conditions during experiments they were kept together in a wire-mesh pen with a concrete floor which was exposed to the ambient weather conditions. The concrete floor facilitated cleaning and the collection of faeces for the assimilation efficiency experiments. A quarter of the pen was covered by a roof. During winter the tortoises were either kept in a heated room adjacent to the pen or else left outside (later cardboard boxes were added to the outside pen as shelters). It was preferable for the tortoises to be left outside, because they were then exposed to the natural weather conditions.

The tortoises were fed leafy green vegetables and fruit generally six days a week, and later the vegetable leaves were coated with supplementary vitamin syrup once or twice a week. During winter, feeding was restricted to about five days of the week because angulate tortoises rarely feed during this season, due to the fact that the winters are cold and wet, tortoises do not seem to feed while it is raining, and activity in these tortoises is correlated to ambient temperature (Branch 1984; S. Els pers. comm.; this study). The tortoises used in the present study did not appear to be active on days during which the maximum daily air temperature did not exceed 15°C. This was ascertained from measurements of air temperature in the pen and observa-

tions at feeding time. Tortoises obtain much of their water from their food (Mahmoud & Glicka 1979; review by Minnich 1982); the specimens used in this study were seldom observed drinking although they had access to water when it rained.

When kept indoors tortoises were usually housed singly in cages made from wood and chicken mesh, measuring approximately 300mm x 395mm, or in plastic containers measuring about 276mm x 345mm. Bedding of kikuyu grass was provided in the cages; the tortoises were not seen eating this grass and so it was considered suitable bedding. The plastic containers were lined with absorbent paper. Bedding was changed and the containers cleaned regularly.

Appendix 3 Photographs

(a)



(b)



(1) Angulate tortoise with implanted thermocouple.

(a) Dorsal view. (b) Ventral view



(2) The angulate tortoises used in the experiments investigating rates of heat transfer



(3) Size range of angulate tortoises used in the evaporative water loss experiments