THERMAL PREFERENCE AND THE EFFECTS OF FOOD AVAILABILITY ON COMPONENTS OF FITNESS IN THE BEARDED DRAGON, *POGONA VITTICEPS*.

ALANA C. PLUMMER

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GENERAL INTRODUCTION

Biochemical and physiological processes are temperature dependent (Bartholomew 1982; Huey 1982; Grant 1990), being optimized over a narrow range of body temperatures (T_bs, Table 1-1; Huey 1982; Angilletta *et al.* 2002). Ectotherms that are able to maintain T_b within the optimal range for performance through behavioural thermoregulation benefit by maximizing their net energy gain (Huey and Slatkin 1976; Angilletta *et al.* 2002), and thus, their fitness. Due to the strong link between temperature dependent physiological performance and behavioural thermoregulation, it has been suggested that these traits evolved concurrently in a process called coadaptation (Huey and Bennett 1987; Angilletta *et al.* 2006). The coadaptation hypothesis postulates that a shift in the optimal temperature for a performance (T_o; Table 1-1) away from the preferred body temperature range (T_{set}; Table 1-1) should favour a corresponding shift in T_{set} via coadaptive pressure (Huey and Bennett 1987). The coadaptation hypothesis is the central theme that links the subsequent chapters of this thesis.

Due to this link between physiological performance and behavioural thermoregulation, investigators have in effect equated T_o with T_{set}. Therefore, correctly measuring T_{set} is of central importance for understanding ectotherm thermoregulatory strategies and physiological performance. Nevertheless, to date there have been no clear comparisons between the methods used to determine T_{set}. My first objective of Chapter 1 was to assess whether estimates of T_{set} were altered depending on whether lizards were measured in a thermal gradient or a shuttle box, two commonly used operational measures of T_{set}. I expected the T_{set} generated by the shuttle box to be broader than the T_{set} generated from the thermal gradient. If the estimates of T_{set} were found to differ, my second objective was to determine whether these different T_{set}s changed the interpretation of thermoregulatory

indices. Different estimates of T_{set} may not change the interpretations of d_e - d_b because this metric is relative to the same T_{set}. There may be more implications for interpretations of the mean d_b, mean d_e, and E_x. For E_x, different T_{set}s may change the percentage of time that T_b is within T_{set}.

Correctly measuring T_{set} also has implications for investigations into ectotherm physiology. Locomotion and food assimilation have been the major focus of studies investigating performance optimization by thermoregulatory behaviour because of their close link to fitness (Bennett 1980; Van Damme *et al.* 1991; Irschick 2003). If it is net energy gain that is being maximized within the system and not locomotion, herein lies a problem for interpreting thermoregulatory behaviour. The marked difference in the thermal reaction norms has important implications for how we measure and interpret T_{set}, and thus how we use thermoregulatory indices, as they assume that reptiles thermoregulate using a set-point system. My third objective was to determine which measure of T_{set} best represents optimization of performance. However, regardless of which performance is maximized by thermoregulation, both reaction norms are asymmetric and this asymmetry causes problems when relating deviations or T_b from T_{set} to potential departures in from optimal physiological performance.

Food scarcity, a ubiquitous situation found in nature could results in a shift in T_{set} away from the optimal temperatures for physiological performances. And given the importance of foraging to fitness, as foraging is equated with energy acquisition, the strategies that animals have evolved to deal with low food availability are of interest. Chapter 2 addresses how ectotherms cope in times of food scarcity in terms of changes in thermal preference, metabolic rate, and locomotor performance. Studies have identified

circumstances when voluntary lowering T_b, as generally occurs at dusk or during winter, may be required (Regal 1967; Cogger 1974). However, few studies have investigated the effects of low food availability on thermoregulation and none have documented the effects on T_{set} despite the importance of thermoregulation to ectotherms. In times of decreased food availability, thermoregulating animals may be able to improve the balance between the energy from ingested food and the energy used by the animal by voluntarily reducing T_b (Brett 1971; Lillywhite *et al.* 1973). Indeed, support for this comes from studies that have looked at metabolic rate and documented a decrease in oxygen consumption by fasted or starved animals (Benedict 1932; Roberts 1968; Gatten 1980). Locomotor performance can be an important component of fitness (Irschick and Garland 2001; Lailvaux and Irschick 2006; Husak *et al.* 2008), but to date, there has been no study documenting decreased sprint speed in lizards as a result of low food availability. Overall, I expected: (1) lizards to select lower T_bs and, therefore, to have lower standard metabolic rates when fasted; and (2) lizards to exhibit decreased locomotor performance when experiencing low food availability.

As a final note, this thesis is written in manuscript format with each chapter meant to stand alone as an independent article. As such, there is some duplication of methodology, results, and references because they pertain to both chapters. In particular, the thermal preference data determined from the measurements of T_{set} in a thermal gradient and shuttle box that are presented in Chapter 1 correspond to the thermal preference data under fed conditions presented in Chapter 2. References for the General Introduction can be found in the Literature Cited sections of Chapters 1 and 2.

CHAPTER 1:

THERMAL PREFERENCE IN BEARDED DRAGONS

ABSTRACT

Thermoregulatory indices are based on an estimation of the preferred body temperature range (T_{set}), but few studies have compared the different methods of measuring T_{set} or how these methods influence our understanding of the relationship between thermoregulation and physiological performance. For the bearded dragon, *Pogona vitticeps*, T_{set}s were measured within a thermal gradient and shuttle box. Additionally, performance curves were determined for relative sprint speed and gut passage time. The shuttle box T_{set} was broader than the T_{set}s from the thermal gradient. Of the indices examines, only the effectiveness of thermoregulation may remain unaffected by differing methods of measuring T_{set}. The optimal T_bs for both performances were best described by the 50% T_b distribution. The T_b distributions were negatively skewed, suggesting support for net energy gain being maximized by thermoregulation. This study highlighted the need for more meaningful measures of thermoregulation than those provided only by temperature-dependent performances.

RÉSUMÉ

Les indices de thermorégulation sont basés sur une évaluation de l'étendue des températures corporelles préférées (T_{set}), mais peu d'études ont comparé les différentes méthodes de mesurer T_{set} ou comment ces méthodes peuvent influencer notre compréhension de la relation entre la thermorégulation et le performance physiologique. Chez le dragon barbu, *Pogona vitticeps*, les valeurs de T_{set} ont été mesurées dans un gradient thermique et une boîte de navette. En plus, les courbes de fonctionnement ont été déterminées pour la vitesse de pointe relative et le temps de passage d'intestin. Le T_{set} établi dans la boîte de

navette était plus étendu que les T_{set}s dans le gradient thermique. Des indices examines, seulement l'efficacité de la thermorégulation peut demeurer inchangée par des méthodes différentes de mesurer T_{set}. Les valeurs de T_o pour les deux fonctionnements ont été mieux décrites par la distribution de T_b de 50%. Les distributions de T_b ont été négativement faussées, suggérant qu'il y a un certain soutien pour l'acquisition d'énergie maximisée par la thermorégulation. Cette étude a accentué le besoin de mesures de thermorégulation plus significatives que celles fournies seulement par des exécutions dépendantes de la température.

INTRODUCTION

Biochemical and physiological processes are temperature dependent (Bartholomew 1982; Huey 1982; Grant 1990), being optimized over a narrow range of body temperatures (T_bs, Table 1-1; Huey 1982; Angilletta *et al.* 2002). Ectotherms that are able to maintain T_b within the optimal range for performance through behavioural thermoregulation benefit by maximizing their net energy gain (Huey and Slatkin 1976; Angilletta *et al.* 2002), and thus, their fitness. Locomotion and, to a lesser extent, food assimilation have been the major focus of studies investigating performance in ectotherms because of the supposed close link between these physiological processes and fitness (Bennett 1980; Van Damme *et al.* 1991; Irschick 2003). Given the ecological importance of locomotion through its roles in the escape from predation and the enhancement of foraging success (Christian and Tracy 1981; Pough 1989; Irschick and Garland 2001), there has been a tight link suggested between locomotor performance and fitness (Arnold 1983). Recent work, however, has suggested that animals do not often run at the highest levels of their performance capacity in nature (Irschick and

Losos 1998) suggesting that the link between locomotion and fitness may not be as strong as previously believed.

Net energy gain may be more tightly linked to fitness than is locomotion. Natural selection is a process that favours genotypes that are capable of optimizing net energy gain and growth (Stearns 1992). For example, an increase in size results in increased rates of survival and reproductive output, and thus, individuals that achieve larger size earlier in life glean higher survival and fitness (Sauer and Slade 1987). For animals with indeterminate growth, such as ectothermic vertebrates, the effect of size may be more important than in animals with determinate growth because of the wider range of sizes between mature individuals (Andrews 1982). For instance, amphibians that attain full body size rapidly do so by allocating a large proportion of ingested energy to growth, and not only reach sexual maturity sooner, but also avoid gape-limited predators (Pough 1980; Lillywhite *et al.* 1973). Additionally, female marbled salamanders (*Ambystoma opacum*) that are fed higher energy diets achieve larger body sizes, and consequently achieve greater reproductive success (Scott and Fore 1995). Finally, as female size increases so too does egg and clutch size in many lizards (Pianka 1986; Cox *et al.* 2003).

If it is net energy gain that is being maximized within the system and not locomotion, herein lies a problem for interpreting thermoregulatory behaviour. The seminal cost-benefit model of thermoregulation by Huey and Slatkin (1976) is based on energetics, but the thermal reaction norms used in their model may be more typical of locomotion than of net energy gain (Dubois *et al.* 2008). This is a potential problem, because how we interpret the effect of thermoregulatory behaviour on performance depends on the shape of the thermal reaction norm. In this chapter, I examine how meaningful our measurements of

thermoregulation are in light of different measuring techniques and which performance

(locomotion or net energy gain) is maximized by thermoregulatory behaviour.

Preferred Body Temperature and Interpretation of Thermoregulatory Indices

To describe the thermoregulatory strategy of reptiles and to quantify variation in

geographical, reproductive and seasonal thermoregulation, researchers use indices of

thermoregulation (Hertz *et al.* 1993; Christian and Weaver 1996; Díaz 1997; Rock *et al.*

2002; Díaz and Cabezas-Díaz 2004). These indices require three types of data. First, the T_b

of active individuals must be measured, which is usually accomplished by inserting a

thermocouple into the cloaca or by using temperature-sensitive radio-transmitters. Second, T_e

(Table 1-1) must be quantified, which is measured either by heat-exchange mathematical

models or by using hollow copper representations of the study animal placed randomly in the

available habitat. T_e represents the T_bs available within the environment of a randomly

moving, thermoconforming animal. Finally, the preferred T_b range (T_{set}, Table 1-1), must be

identified. It is assumed that T_{set} represents the optimal temperature for performance (T_o,

Table 1-1). T_{set} is usually determined as the central 50% of the T_b distribution in a laboratory

thermal gradient, a value that is completely arbitrary.

The most commonly used indices are based on the extent to which reptiles maintain

T_b close to T_{set} (accuracy of T_b or mean d_b, Table 1-1) and the quality of the thermal habitat

(mean d_e, Table 1-1; Hertz *et al.* 1993). Mean d_b is the absolute value of the mean deviations

of T_b from T_{set}. If T_b is above T_{set}, mean d_b will be calculated as the difference between the

upper set-point of T_{set} and T_b. If T_b is below T_{set}, mean d_b will be calculated as the difference

between the lower set-point of T_{set} and T_b. If T_b is within T_{set}, d_b=0. Mean d_e is the absolute

value of the mean deviation of T_e from T_{set}. Hence, a high mean d_e represents a low thermal

quality habitat and a mean $d_e = 0$ represents a thermally ideal habitat. The effectiveness of thermoregulation (d_e-d_b, Table 1-1; Blouin-Demers and Weatherhead 2001) measures the deviation from thermoconformity. A positive value indicates an individual that thermoregulates, a value of 0 represents a thermoconformer, and a negative value indicates an individual that actively avoids thermally favourable habitats (Blouin-Demers and Weatherhead 2001). Finally, thermal exploitation (E_x, Table 1-1; Christian and Weavers 1996) indicates the amount of time an individual maintains T_b within T_{set} as a percentage of the time available to do so, as indicated by T_e.

The indices of thermoregulation mentioned above are commonly used and take into consideration T_{set}. Thus, measuring T_{set} correctly is of central importance to the study of thermoregulation. It is possible that a change in T_{set}, depending on how it is measured, could alter our interpretations of these indices. Ideally, T_{set} is determined in an environment with limited ecological constraints, such as the laboratory. The majority of studies investigating thermoregulation have used thermal gradients in which an animal may select any T_b from a range of environmental temperatures, and T_{set} is determined from a percentage of the T_b distribution (e.g., the central 50% or 80%; Huey 1982; Hertz *et al.* 1993; Blouin-Demers and Nadeau 2005). The percentage of T_bs used to determine T_{set} is arbitrary (Wills and Beaupre 2000) and it may not be as easily linked to the biology of an organism as other methods for determining T_{set}.

While thermal gradients can give researchers useful information on the preferential T_b of an animal, it is an incomplete picture of thermoregulation because most vertebrates, including lizards, use a set-point system (Berk and Heath 1975; Crawshaw 1980). Within this system, animals avoid T_bs above and below upper and lower set-points (USP and LSP, Table 1-1). Maintenance of T_b within the range of USP and LSP allows animals to engage in other

activities without devoting continuous time and energy, because animals are indifferent to T_bs within T_{set}. The set-point system is most readily tested using the shuttle box method. In a shuttle box, animals make a choice about when to begin cooling (reaching USP) or heating (reaching LSP), suggesting that this method more closely mirrors natural thermoregulatory behaviour. To date, no clear comparison has been made between the T_{set}s produced be either the thermal gradient or shuttle box methods, despite the importance of T_{set} in calculating the indices of thermoregulation.

The first objective of my research was to assess whether estimates of T_{set} are different depending on the method of measurement. To do this, I measured T_{set} in both a thermal gradient and a shuttle box under controlled conditions. As the shuttle box directly measures T_bs when an individual switches its behaviour to begin heating or cooling rather than by using an arbitrary percentage of the T_b selected in the thermal gradient, I expected the shuttle box to yield a broader T_{set} than the thermal gradient. If the estimates of T_{set} were found to differ, my second objective was to determine whether these different T_{set}s changed the interpretation of thermoregulatory indices. Different estimates of T_{set} may not change the interpretations of d_e - d_b because this metric is relative to the same T_{set}. There may be more implications for interpretations of the mean d_b, mean d_e, and E_x. For E_x, different T_{set}s may change the percentage of time that T_b is within T_{set}.

Thermal Sensitivity of Physiological Performance

Due to the strong link between temperature dependent physiological performance and behavioural thermoregulation, it has been suggested that these traits evolved concurrently in a process called coadaptation (Huey and Bennett 1987; Angilletta *et al.* 2006). Here, a shift in the T_o for a performance away from T_{set}, should favour a corresponding shift in T_{set} via coadaptive pressure (Huey and Bennett 1987). Support for the thermal coadaptation

hypothesis has been found in ectotherms, including lizards (Angilletta *et al.* 2002) and

snakes (Blouin-Demers *et al.* 2003). Thus, because of the link between physiological

performance and behavioural thermoregulation, investigators have in effect equated T_o with

T_{set}.

Commonly used thermoregulatory indices assume that deviations above and below

T_{set} are equivalent in terms of their effects on an organism. Locomotor performance, a

physiological characteristic that exhibits thermal dependence (Huey 1982), has been

commonly used to quantify the optimal range of performance. However, locomotor

performance curves are generally asymmetric with an increase in performance as a function

of temperature until an optimum performance temperature (T_o) or range of temperatures is

reached, after which there is a sharp decline in performance (Huey 1982; Huey and Bennett

1987). Thus, a deviation of T_b above T_{set} suggests a greater reduction in performance than a

deviation of T_b below T_{set}.

Several characteristics of digestive physiology also exhibit thermal dependence

(Skoczylas 1978). One such characteristic is gut passage time and three trends have been

found in lizards to date: (1) a decrease in passage time with an increase in T_b (Waldschmidt

et al. 1986); (2) a decrease in passage time with an increase in T_b at lower T_bs and a plateau

in gut passage time at higher T_bs (Ji *et al.* 1995); and (3) a decrease in passage time with an

increase in T_b at lower temperatures and an increase in passage time again at higher T_bs (Van

Damme *et al.* 1991; Beaupre *et al.* 1993). Thus, in general, increases in T_b result in decreases

in gut passage time. This increase in T_b may also increase digestive enzyme activity, but

because of the decrease in gut passage time, the exposure of these enzymes to food may be

reduced and result in decreased enzymatic performance (Harwood 1979; Waldschmidt *et al.*

1986; Zhang and Ji 2004). Thus, digestive efficiency is relatively temperature independent.

Another temperature dependent process, food consumption, increases approximately linearly until the critical thermal maximum (CT_{max}, Table 1-1; Harwood 1979; Waldschmidt *et al.* 1986). Metabolic rate also increases steadily until the upper lethal temperature (Thompson and Withers 1992), but because the Q_{10} (Table 1-1) for food consumption is higher (has a steeper slope) than the Q_{10} for standard metabolic rate (SMR), the net energy function (food consumed - SMR) should also increase as a function of T_b (Dubois *et al.* 2008). Thus, the reaction norm for net energy gain may have a very different shape than the reaction norm for locomotion.

If it is net energy gain and not locomotion that is being maximized in ectotherms, as suggested by Huey and Slatkin (1976), then the marked difference in the thermal reaction norms has important implications for how we measure and interpret T_{set}, and thus how we use thermoregulatory indices, as they assume that reptiles thermoregulate using a set-point system. My third objective was to determine which measure of T_{set} best represents optimization of performance. Thus, I investigated whether the T_{set} established in a thermal gradient (either the 50% or 80% T_b distribution) or a shuttle box better reflected the optima for sprint speed and gut passage time. In a system where net energy gain is maximized, we may expect ectotherms to maintain T_b close to CT_{max} in a thermal gradient, as measured by the 95% quartile (Dubois *et al.* 2008), rather than to let T_b fluctuations within a thermally neutral zone. This would lead to T_b distributions skewed to the left rather than normally distributed.

MATERIALS AND METHODS

Study Species and Maintenance

The central bearded dragon, *Pogona vitticeps* (Ahl 1926), occupies an expansive range of environments encompassing the eastern half of Southern Australia, the south-eastern Northern Territory and the interior of all the eastern states (Cogger 2000). A semi-arboreal species, *P.vitticeps* can be found in open habitats with heterogeneous vegetation (Melville and Schulte 2001). Because of the variety of habitats, individuals of this species experience a wide range of temperatures, making it an ideal species for the study of thermoregulation. *P.vitticeps*, like the majority of agamids, is a diurnal, insectivorous, sit-and-wait predator (Huey and Pianka 1981).

Male and female *P. vitticeps* were obtained from breeders from two clutches. Lizards were measured for snout-vent length (SVL) using electronic digital calipers and weighed in a cloth bag on a precision scale. Twelve individuals were used for each experiment. Lizards were maintained in opaque plastic terraria filled with 5 cm of sand, a basking rock, a refuge, and a bowl of water. Terraria were housed in environmental chambers (Constant Temperature Control Ltd. Model ER 600; 12L:12D). The temperature was maintained at 30˚C during the photophase and decreased to 20˚C during the scotophase. Lizards were provided with ad lib water and fed 5% of their body mass in crickets dusted with vitamin-mineral powder every 2 days. The University of Ottawa Animal Care and Veterinary Service approved all manipulations, under protocol BL-204.

Preferred Body Temperature and Interpretations of Thermoregulatory Indices

Measuring Internal T_b

I measured the internal T_b of lizards using T-type thermocouple 36 ga wire (Omega Engineering Inc.) inserted 2 cm in the cloaca and secured externally with tape. The trailing

thermocouple wire was connected to an Onset HOBO data logger (U12-014). T_b

measurements were taken every 3 seconds.

Thermal Preference

I measured T_{set} (n = 12: 7 females, 5 males) the day following feeding. I used two

techniques to determine T_{set}: a shuttle box and a thermal gradient. Both the shuttle box and

thermal gradient were constructed of stainless steel (each measuring 1.0 x 0.5 x 0.5 m) with

the base covered with 2-3 cm of sand to provide traction. Both were housed in an

environmental chamber with an ambient temperature (T_a, Table 1-1) of 20°C (12L:12D).

This temperature was selected because it is ecologically relevant for this species and is low

enough to be uncomfortable, but not lethal, thus encouraging lizards to use the heating pads

provided. Lizards were placed in the shuttle box and thermal gradient 24-48hr prior to testing

to adjust to testing conditions. I used 12 hours of T_b recording for each lizard and each

technique to estimate T_{set}.

In the shuttle box, a heating pad (45°C) could be activated and deactivated by the

lizard via infrared (IR) photosensors and emitters (Fairchild Semiconductor QED). The

temperature of 45°C was selected because it is outside the tolerable range for *P.vitticeps*

(Warburg 1965), which limited the time that an individual could spend on the heating pad.

This ensured that an individual had to shuttle (it was either heating or cooling) to maintain its

preferred body temperature range.

The shuttle box measurements were video recorded for 4 lizards. While reviewing the

recordings, all changes in behaviour were documented and compared to the T_b profile. For

example, postural changes (when a lizard lifted its head off the pad or moved its body

slightly), when a lizard passed over the pad but did not remain on it, and 'cheating'

behaviour (when a lizard was laying only half on the pad) were recorded. Shuttling events

were then defined, which included a peak (when the lizard left the pad) and trough (when the lizard moved onto the pad), which excluded any cheating behaviour. Only cheating behaviour allowed lizards to remain for prolonged periods of time on the heating pad without shuttling. Peaks and troughs each had a corresponding T_b and time. Shuttling behaviour was defined as an event on the T_b profiles that resulted in a minimum of a 10°C change in temperature over at least 7 minutes (Fig. 1-1). With this information, the upper (or lower) temperature set-point was determined by averaging the peaks (or troughs) for each individual. The mean of the individual averages (n = 12) was then determined to give the USP and LSP of T_{set} for *P. vitticeps*.

The thermal gradient was established using a heating pad (10.16 cm; set at 45°C) and the ambient temperature to produce a smooth thermal gradient. The central 50% and 80% of T_b distributions, designated by the 25% and 75% and the 10% and 90% quartiles respectively, were determined for each individual (n = 12) and then averaged to give T_{set} for *P. vitticeps*.

Thermal Sensitivity of Physiological Performance

Defining Critical Thermal Minimum (CT$_{min}$)

Eleven lizards were used to determine the T_b at which locomotor activity ceased (or CT_{min}, Table 1-1). Lizards were placed in an environmental chamber and given sufficient time for their T_b to reach the T_a of 15°C. A program was then initiated to decrease the T_a of the chamber at a rate of 1°C every 30 min from 15°C to 4°C (the lowest temperature reached by these chambers). Lizards were tested every 15 minutes for a righting response. The T_b at which the righting response ceased twice in a row was recorded as the CT_{min}. The mean T_b at

which the righting response ceased was $7.45 \pm 0.34°C$. The CT_{min} was used as the bound to the lower end of the performance curve for sprint speed.

The CT_{max} was not measured for *P. vitticeps* in this study because of concerns for the well being of the animals and the need for them in further experiments. Instead, I used the value of 43.5°C established by Warburg (1965) for the sister species, *Pogona barbata*. I chose to approximate the upper limit of performance using *P. barbata* because *P. vitticeps* and *P. barbata* exhibit similar central 50% distributions of selected body temperatures in a thermal gradient (Schäuble and Grigg 1998; current study). Additionally, species of the same genus show limited variation in CT_{max} (Spellerberg 1972a, b).

Sprint Speed

The thermal sensitivity of sprint speed was examined at six temperatures (15, 20, 25, 30, 35 and 38°C), ordered randomly. Lizards were held for 2 hours prior to testing within the temperature controlled environmental chambers to allow for sufficient time for T_b to reach T_a. Sprint speed was measured by chasing lizards at a consistent pace down a plastic race track (20.3 cm wide) for 1 m. The track was filled with 3 cm of sand and soil for traction. Lizards were measured the day following feeding (n = 12: 7 females, 5 males). Individuals were measured three times between 1000h and 1400h and remained at the test temperature for at least 20 min between trials to recuperate. Lizards were tested in random order and the T_b of lizards was measured prior to each trial ($\pm1°C$; Control Company Traceable thermometer). These procedures were repeated at each temperature. Trials were videotaped for subsequent observation and analysis.

Gut Passage Time

Lizards were fasted for 3 days at 30ºC prior to measurements of gut passage time (n = 12: 7 females, 5 males). Coloured beads were inserted into the abdomens of the crickets to serve as fecal markers: a green bead denoted the first cricket while a red bead indicated the last cricket that was fed to the lizard. I recorded when the green and red markers were swallowed (t_0) and passed (t_1). Lizards were reluctant or refused to eat the marked crickets at lower T_as. Thus, lizards were fed the marked crickets at 30ºC regardless of the testing temperature and immediately placed in the environmental chamber at the test temperature. All lizards were fed between 1000h and 1400h. Lizard terraria were checked for fecal samples every 60 min during the photophase. Pilot studies at different T_as indicated that lizards were inactive during the scotophase and did not defecate. During experimentation, lizards were maintained on paper towels rather than the soil and sand substrate used for maintenance protocols because pilot studies indicated that lizards ingested some of the latter and this could have affected results for gut passage time. Lizards were measured once at each temperature.

Curve Fitting

For each individual, the maximum sprint speed over the three trials for each test temperature was divided by the maximum sprint speed over all test temperatures to give the relative sprint speed for each test temperature. These values were then expressed as a percentage. There was little variation in T_b over the three trials for a given test temperature (mean difference between maximum and minimum T_b s was $0.60 \pm 0.07°C$) and therefore, mean T_b was used during analysis.

For each individual, the minimum (or fastest) gut passage time (either from the green or red beads) for each test temperature was divided by the minimum gut passage time over

all test temperatures to give the relative gut passage time for each test temperature. These values were then expressed as a percentage. It took between 1 and 2 hours for lizards to reach the T_a within the experimental chamber, but because the duration of passage time was on a scale of days, the T_b of the lizard was equated to the T_a. Relative performances (sprint speed and gut passage time) were used rather than absolute performances to control for individual differences and to facilitate comparisons with previous studies.

Based on previous research, performance as a function of T_b was fit to the following nonlinear regression models: logistic-exponential (Eq.1; Stevenson *et al.* 1985), exponential-exponential (Eq. 2; Stevenson *et al.* 1985); Logan 10 (Eq. 3; Logan *et al.* 1976); quartic (Eq. 4); and sextic (Eq.5). In the equations below, S is a scaling factor, k_1, k_2, k_3, δ, γ, φ and ρ are fitted parameters, CT_{min} and CT_{max} are the critical thermal minimum and maximum, T_b is the mean body temperature, and A-F are coefficients. The CT_{min} and CT_{max} were set at 7.45°C and 43.5°C, respectively. Experimental data points were given a weight of 1 and the CT_{min} and CT_{max} were given a weight of 5. The error sum of squares was assessed for goodness of fit, as non-linear curve fitting can be sensitive to starting parameters (van Berkum 1986; Motulsky and Ransnas 1987). The corrected Akaike's information criterion (AIC_c), $\Delta\, AIC_c$ and Akaike weight (w_i) were compared between the nonlinear regression models to determine which provided the best fit (Burnham and Anderson 2004; Angilletta 2006).

$$\text{Performance} = S\left(\frac{1}{1 + k_1 e^{-k_2(T_b - CT_{min})}}\right)\left(1 - e^{k_3(T_b - CT_{max})}\right) \tag{1}$$

$$\text{Performance} = S\left(1 - e^{-k_1(T_b - CT_{min})}\right)\left(1 - e^{-k_2(T_b - CT_{max})}\right) \tag{2}$$

$$\text{Performance} = \frac{\rho}{\gamma}\left(\frac{1}{1+\dfrac{\left(\dfrac{\rho}{\gamma}-\varphi\right)}{\varphi}e^{-\rho T_b}}\right)-\left(e^{-\left(\frac{CT_{max}-T_b}{\delta}\right)}\right) \tag{3}$$

$$\text{Performance} = Ax + Bx^2 + Cx^3 + Dx^4 \tag{4}$$

$$\text{Performance} = Ax + Bx^2 + Cx^3 + Dx^4 + Ex^5 + Fx^6 \tag{5}$$

Data for each individual for both sprint speed and gut passage time performances were then fitted to the selected model to determine (1) the T_b for maximal performance (T_o) and (2) the range of T_bs for which performance was 80% of the maximum (the lower and upper bounds of the 80% performance breadth, B_{80} (Table 1-1); Stevenson *et al.* 1985). The average over all individuals gave the mean T_o and mean B_{80} for *P. vitticeps* for relative sprint speed and relative gut passage time.

Previous Research Documenting Gut Passage Time

Gut passage time data from five previous studies (4 lizards and 1 snake study) were reanalyzed using the curve fitting methods described above. CT_{min} and CT_{max} were known for these species. For *Eremias brenchleyi* (Xu and Ji 2006), *Eumeces elegans* (Du *et al.* 2000), *Lacerta vivipara* (Van Damme *et al.* 1991; Gvoždík and Castilla 2001), and *Takydromus sexlineatus* (Zhang and Ji 2004) the documented means were used, while individual values were used for *Thamnophis elegans vagrans* (Stevenson *et al.* 1985; Huey *et al*. 1989). For each species, each measurement of gut passage time was calculated as a function of the minimum (or fastest) gut passage time and then expressed as a percentage.

Usually, the model with the lowest SSE, AIC_c value and/or highest w_i values is taken

to be the model most likely to fit the data. However, these potential models were discounted

if the resulting curve was multimodal or if the curve crossed the x-axis prior to CT_{min}. In the

event that this occurred, the curve with the next best fit was chosen.

Statistical Analyses

To test for significant differences between means for males and females for the

thermoregulatory parameters measures *t*-tests were used. Paired *t*-tests were used to test for

statistical differences between means of T_os for sprint speed and gut passage time and B_{80} for

sprint speed and gut passage time. The analyses were conducted with JMP Version 5.0.1a

(SAS Institute Inc. 1989-2002). Assumption of normality and homogeneity of variance were

checked and data were logarithmically transformed when necessary. Statistical tests were

considered significant at $\alpha = 0.05$. Means are reported as ± 1 SE.

RESULTS

Preferred Body Temperature and Interpretations of Thermoregulatory Indices

Thermal Preference

The T_b distributions in the thermal gradient were not normally distributed (Fig.1-2;

Kolgomorov-Smirnov tests: all D's > 0.08, all p < 0.01). The mean skewness across all

individuals was -1.33 ± 0.27. Males and females did not differ for any of the parameters

measured, therefore the data were pooled (thermal gradient: mean T_b: t (10) = 0.23, $p = 0.82$;

10% quartile, t (10) = 0.88, $p = 0.40$; 25% quartile, t (10) = 0.50, $p = 0.63$; 75% quartile, t

(10) = 1.38, $p = 0.20$; 90% quartile, t (10) = 1.36, $p = 0.20$; 95% quartile: t (10) = 1.17, $p =$

0.27; shuttle box: mean T_b, t (10) = 1.33, $p = 0.21$; USP, t (10) = 2.02, $p = 0.07$; LSP, t (10) =

0.10, $p = 0.92$). Averaged across all individuals, the mean 10%, 25%, 75%, 90% and 95% quartiles in the thermal gradient were 29.9 ± 1.2°C, 32.3 ± 1.3°C, 38.4 ± 0.3°C, 39.2 ± 0.3°C, and 39.7 ± 0.3°C respectively (Figs. 1-2, 1-3). Averaged across all individuals, the mean LSP and USP in the shuttle box were 25.6 ± 1.6°C and 40.4 ± 0.5°C, respectively (Fig. 1-3). The mean T_b in the thermal gradient and shuttle box were 35.2 ± 0.5°C and 33.2 ± 2.4°C, respectively. The shuttle box yielded a broader T_{set} that approximated the 96% central distribution (25.9 ± 1.2°C - 40.2 ± 0.3°C) of the T_b in the thermal gradient.

Thermal Sensitivity of Physiological Performance

Sprint Speed and Gut Passage Time

The curves that best described sprint speed and gut passage time were quartic and exponential-exponential, respectively (Table 1-2). Males and females did not differ in T_o for either relative sprint speed ($t (10) = 0.66$, $p = 0.52$) or relative gut passage time ($t (10) = 0.31$, $p = 0.77$). The optimal performance temperature was significantly higher for relative gut passage time (mean $T_o = 35.4 ± 0.5°C$) than for relative sprint speed (mean $T_o = 33.7 ± 0.4°C$; paired $t (11) = 3.39$, $p = 0.0060$; Fig. 1-4).

Males and females did not differ in the lower bound of B_{80} for either relative sprint speed ($t (10) = 0.83$, $p = 0.43$) or relative gut passage time ($t (10) = 0.63$, $p = 0.55$). The lower bound of B_{80} was significantly lower for relative gut passage time (mean lower $B_{80} = 24.5 ± 0.5°C$) than for relative sprint speed (mean lower $B_{80} = 27.4 ± 0.5°C$; paired $t (11) = 4.29$, $p = 0.0013$). Males and females did not differ in the upper bound of B_{80} for either relative sprint speed ($t (10) = 0.26$, $p = 0.80$) or relative gut passage time ($t (10) = 0.49$, $p = 0.64$). The upper bound of B_{80} was significantly higher for relative gut passage time (mean

upper $B_{80} = 40.3 \pm 0.3°C$) than for relative sprint speed (mean upper $B_{80} = 38.4 \pm 0.2°C$; paired t (11) = 6.24, $p < 0.001$).

Previous Research Documenting Gut Passage Time

The results from the nonlinear regression modeling are given in Table 1-3. For *E. brenchleyi* and *E. elegans* the most likely model was multimodal, so the next best model, logistic-exponential and sextic, respectively, were used. The sextic model for *L. vivipara* was multimodal and the quartic model crossed the x-axis prior to reaching the CT_{min}, and therefore the logistic-exponential model was chosen. The quartic and Logan 10 models for *T. sexlineatus* crossed the x-axis prior to CT_{min}, and thus the logistic-exponential model was used. Finally, for *T. elegans vagrans* the quartic and Logan-10 models crossed the x-axis prior to CT_{min}, so the exponential-exponential model was used. In all cases, there was a decrease in the mean or individual values for relative gut passage time prior to reaching the CT_{max} (Fig.1-5).

DISCUSSION

Preferred Body Temperature and Interpretations of Thermoregulatory Indices

T_{set} for *P. vitticeps* using the thermal gradient had a range comparable to those established for other lizards. The 50% distributions for *P. barbata* in summer and autumn were 29.2 ± 0.6 - $33.1 \pm 1.1°C$ and 32.0 ± 0.7 - $34.8 \pm 0.8°C$, respectively (Schäuble and Grigg 1998), and 37.0 - 39.5°C for *Dipsosaurus dorsalis* (DeWitt 1967). Additionally, T_{set} established for *D. dorsalis* using the 68% (36.1 - 40.1°C; DeWitt 1967) and 95% (33.2 - 41.8°C; DeWitt 1967) distributions are comparable to the T_{set}s established for *P. vitticeps* in

this study (50% T_b distribution: 32.3 ± 1.3 - 38.4 ± 0.3°C; 80% T_b distribution: 29.9 ± 1.2 - 39.2 ± 0.3°C).

Few studies have used the shuttle box method to establish T_{set}, but shuttling data collected for *D. dorsalis* show a similar trend to the thermal gradient T_{set}s established in this study, rather than the T_{set} from the shuttle box (shuttle box T_{set}: 25.6 ± 1.6 - 40.4 ± 0.5°C (current study); 36.1 ± 0.84 - 40.6 ± 0.35°C (Kingsbury 1999); 37.3 ± 0.49 - 41.2 ± 0.39°C (Kluger *et al.* 1973); 36.4 ± 0.19 - 41.7 ± 0.28°C (Berk and Heath 1975); 34.5 ± 0.24 - 43.6 ± 0.49°C (Barber and Crawford 1979)).

While the USP appears consistent across the aforementioned species, the LSP is more variable when comparing the results of this study to those previously mentioned. A typical locomotor performance curve suggests that a T_b above T_{set} results in a greater reduction in performance than a T_b below T_{set}. Thus, it is possible that animals may be more passive to changes in T_b at the lower bound of T_{set}, as a deviation of T_b below T_{set} incurs less physiological cost in terms of decreased performance and fitness. This could explain the more variable LSP noted in my results. Overall, the T_{set} determined using the shuttle box was broader than the T_{set} determined using the thermal gradient. This is not altogether surprising given that the shuttle box directly measures the set-points, rather than a researcher selecting an arbitrary percentage of T_bs from a distribution as estimations of the set-points.

Disparities between T_{set} measured using the thermal gradient and shuttle box methods would likely not affect interpretations of the effectiveness of thermoregulation (d_e-d_b) but could have an effect on the accuracy of thermoregulation, habitat thermal quality, and thermal exploitation. The accuracy of T_bs (mean d_b) and thermal quality of a habitat (mean d_e) both use departures from T_{set} by field T_bs and T_e, respectively, to describe

thermoregulatory behaviour. Shifts in T_{set} could allow more T_bs to fall closer to or within the preferred thermal range, suggesting higher accuracy of T_bs and higher thermal quality habitats and vice versa. However, because the effectiveness of thermoregulation (d_e-d_b; Blouin-Demers and Weatherhead 2001) evaluates the overall difference between values of d_b and d_e, even if both values are over/underestimated, the interpretation would remain the same. This would not be the case, however, for E_x where shifts in T_{set} could increase or decrease the percentage of time a lizard was able to maintain T_b within T_{set} when T_e was within T_{set}.

By using both a thermal gradient and shuttle box, I showed that the T_{set}s determined using these methods differed and to an extent where interpretations of some thermoregulatory indices could vary depending on the T_{set} used to calculate them. This study stressed the potential shortcomings of thermoregulatory indices and suggests the use of more meaningful measures of thermoregulation. One possibility is the use of reaction norms to measure the improvement in performance gained by thermoregulation (e.g., Hertz *et al.* 1993, Blouin-Demers and Weatherhead 2008).

Thermal Sensitivity of Physiological Performance

The thermal reaction norm for relative sprint speed accords well with previously published works, showing the stereotypical asymmetric curve (Fig.1-3; Huey 1982; Huey and Bennett 1987; Angilletta *et al.* 2002). Additionally, the mean T_o (33.7 ± 0.4°C) and mean B_{80} (27.4 ± 0.5 - 38.4 ± 0.2°C) agree well with other estimates from various inland skink species in Australia (Huey and Bennett 1987) and *Sceloporus undulatus* (Angilletta *et al.* 2002).

In this study, relative gut passage increased with an increase in T_b at lower temperatures and decreased at higher temperatures. This trend was also seen in Van Damme

et al. (1991) and Beaupre *et al.* (1993). The T_o for gut passage time in *P. vitticeps* (35.36 ± 0.48°C) was higher than those for *L. vivipara* (30.2 ± 1.0°C; Van Damme *et al.* 1991) and *T. e. vagrans* (30.0°C; Stevenson *et al.* 1985). The B_{80} for *P. vitticeps* was also broader (24.5 ± 0.5 - 40.3 ± 0.3°C) than those described for *L. vivipara* (24.9 - 35.6°C; Van Damme *et al.* 1991) and *T. e. vagrans* (24.0 - 35.5°C; Stevenson *et al.* 1985). Variation in the natural thermal environment may, in part, explain the variation in the optimal temperatures and performance breadths that we see between these species.

Neither the thermal gradient nor the shuttle box T_{set} appear to be good approximations for the T_os for either sprint speed or gut passage time. However, out of the 3 T_{set}s determined in this study, the central 50% of the T_b distribution seems to be the best approximation for the performances measured. While the T_o for gut passage time was closer to the 95% quartile than the T_o for sprint speed, the T_os for both physiological performances seemed to be most closely approximated the mean T_b within central 50% T_b distribution. I had expected that if net energy gain were maximized through thermoregulation, animals would maintain their T_b close to CT_{max} rather than fluctuate within T_{set}. However, even though the mean T_b from the 50% T_b distribution best described the T_o for gut passage time, this distribution was negatively skewed, suggesting that there is some support for net energy gain being maximized by thermoregulation in *P. vitticeps*.

For two of the studies that were reanalyzed, the T_o for gut passage time and mean T_b have been previously documented. As mentioned previously, the T_o gut passage time for *L. vivipara* was given as 30.2 ± 1.0°C (Van Damme *et al.* 1991). *L. vivipara* selects a range of temperatures between 29.9°C and 34°C (Van Damme *et al.* 1986) within a thermal gradient and exhibits a mean field T_b of 29.9°C (Van Damme *et al.* 1987). T_o for *T. e. vagrans* was given as 30.0°C. *T. e. vagrans* selects a mean T_b of 29.6°C in the lab thermal gradient and

exhibits an active mean field T_b of 30.1°C in autumn (Peterson 1987). The close link between the mean T_b and T_o for gut passage time in *T. e. vagrans* shows a similar trend to *P. vitticeps*.

Dubois *et al.* (2008) did document that the upper 95% of the thermal gradient approximated the T_o for net energy gain in absorptive *Glyptemys inscultpa*. The authors used metabolic rate as a proxy for net energy gain rather than gut passage time and argued that the increase in metabolic rate with increasing T_b can be used as an estimate for increases in net energy gain. The relationship between T_b and metabolic rate indicates a steady increase in metabolic rate until the upper lethal temperature (Thompson and Withers 1992). From the present study, the thermal reaction norm indicated an increase in relative passage time with an increase in T_b at lower temperatures and a decrease in relative passage time again at higher temperatures rather than a vertical drop. Reanalysis of previous studies documenting gut passage time showed similar trends to my own research in that there was evidence of a decrease in relative gut passage time at higher T_bs but prior to CT_{max}. Thus, the differing conclusions from Dubois *et al.* (2008) and the present study may result from the different physiological performances used to estimate net energy gain.

Another possible reason for the variation in results found between Dubois *et al.* (2008) and this study is that the role of thermoregulation in optimizing physiological performance may vary between the model systems. As mentioned by Dubois *et al.* (2008), speed is not used to capture prey or elude predators in *G. inscultpa*. However, this turtle's ability to process food may be constrained by the thermal environment as it inhabits cooler northern climates. For this species, the role of thermoregulation may be more important for optimizing net energy gain than for locomotion. *P. vitticeps*, on the other hand, may rely more heavily on burst movements for both prey capture and predator avoidance, making

locomotor performance more important for this species than for *G. insculpta*. As *P. vitticeps* is a desert species, reaching optimal temperatures may not be a limiting factor. However, as no field estimates of thermoregulation for this species currently exist, the thermal restrictiveness of its habitat relative to that of a more northern species can only be presumed.

It is possible that for *P. vitticeps*, behavioural thermoregulation attempts to maximize multiple performances, rather than either net energy gain or locomotion. This would seem to be supported by both the relative sprint speed and gut passage time T_os being found within the measured T_{set}s. Indeed, thermoregulation has been shown to maximize multiple performances (sprint speed, endurance and the rate of metabolizable energy intake) in *Sceloporus undulatus* (Angilletta *et al.* 2002).

I was not able to detect sex-specific differences for measures of thermal preference or physiological performance due to low sample sizes. Other studies have noted sex-specific differences for measures of thermoregulation (Pentecost 1974; Sievert and Hutchison 1989), however, overall these gender differences appear to relatively uncommon in desert lizards (Huey and Pianka 2007). How widespread gender differences are for optimal temperatures for performance and the 80% performance breadth is unknown because to date many studies to date have not specifically addressed this question. Regardless of low power to detect sex-specific differences, I was still able to address the main objectives of this study.

Overall, I found that of the 3 measurements of T_{set}, the 50% T_b distribution best approximated the T_os for sprint speed and gut passage time. I also found some support for net energy gain being maximized by thermoregulation in that while the T_o for gut passage time fell within T_{set}, the distribution of T_{set} was negatively skewed as would be expected in a system where net energy gain is maximized. Regardless of whether it is locomotor performance or net energy gain being maximized, there is still the issue of the asymmetry of

the thermal reaction norms that describe relative sprint speed and relative gut passage time.
A deviation in T_b above T_{set} results in a greater reduction in performance than a deviation in
T_b below T_{set}, yet the thermoregulatory indices assume that deviations in T_b above and below
T_{set} are equivalent. Again this study highlights the need for more meaningful measures of
thermoregulation than those provided only by temperature-dependent performances. For
example, comparisons of the thermal reaction norms for a given performance between
thermoregulating animals and non-thermoregulating animals (determined using T_e) would
quantify the improvement in performance of a temperature-dependent process due to
thermoregulation (Hertz et al. 1993; Blouin-Demers and Weatherhead 2008).

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TABLES

Table 1-1: Definitions of terms to describe thermoregulation.

Term	Definition
B_{80}	Range of T_bs for which performance was 80% of the maximum.
CT_{max}	The high temperature when locomotor activity becomes disorganized and animals lose their ability to escape from conditions that will become lethal (corresponds to ''critical maximum'' of Cowles and Bogert 1944).
CT_{min}	The low temperature that produces cold narcosis and prevents locomotion (corresponds to ''critical minimum'' of Cowles and Bogert 1944).
d_e - d_b	Effectiveness of thermoregulation (Blouin-Demers and Weatherhead 2001).
E_x	Thermal exploitation measured as the percentage of time from which T_b is within T_{set} when d_e=0 (Christian and Weavers 1996).
LSP	Lower set-point: Lower bound of T_{set} at which thermoregulatory responses occur. Originally postulated by Cowles and Bogert 1994 as the minimum voluntary temperature.
mean d_b	The accuracy of body temperature measured as the mean deviation of T_b from T_{set} (Hertz *et al.* 1993).
mean d_e	The thermal quality of a habitat measured as the mean deviation of T_e from T_{set} (Hertz *et al.* 1993).
Q_{10}	A temperature coefficient that quantified the rate of change of a biology process as a result of an increase in temperature of 10ºC.
T_a	The ambient temperature of an environment.
T_b	The body temperature of a representative sample of study animals.
T_e	Operative environmental temperature: distribution T_bs available to a nonthermoregulating animal (Hertz *et al.* 1993).
T_o	Optimal T_b: T_b for maximal performance.
T_{set}	The set-point or preferred temperature range of an active individual (Hertz *et al.* 1993).
USP	Upper set-point: Upper bound of T_{set} at which thermoregulatory responses occur. Originally postulated by Cowles and Bogert 1994 as the maximum voluntary temperature.

Table 1-2: Non-linear models describing the thermal performance of relative sprint speed and relative gut passage time. Using the corrected Akaike's information criterion (AIC_c), the model with the lowest AIC_c is the model most likely to describe the data. K is the number of parameters in the model, SSE is the residual sum of squares and w_i the Akaike weight. Models in bold were used to calculate T_o and B_{80} for *P. vitticeps*. Stevenson *et al.* 1985[1]. Logan *et al.* 1976[2].

Performance (N)	Model	K	SSE	AIC_c	w_i
Relative Sprint	Logistic-exponential[1]	7	10545.73	77.33	<0.001
Speed (12)	Exponential-exponential[1]	6	13811.73	65.53	0.20
	Logan 10[2]	6	10603.03	64.15	0.39
	Quartic	6	10423.05	64.07	0.41
	Sextic	8	10387.33	99.25	<0.001
Relative Gut	Logistic-exponential[1]	7	6710.90	74.97	<0.001
Passage Time	**Exponential-exponential[1]**	6	5438.20	60.68	0.39
(12)	Logan 10[2]	6	6714.29	61.77	0.22
	Quartic	6	5459.29	60.70	0.39
	Sextic	8	4726.72	95.14	<0.001

Table 1-3: Non-linear models describing the thermal performance of relative gut passage time from previous research. AIC_c is the corrected Akaike's information criterion, K is the number of parameters in the model, SSE is the residual sum of squares and w_i the Akaike weight. Models in bold were used in figures. Stevenson *et al.* 1985[1]. Logan *et al.* 1976[2].

Species (N)	Model	K	SSE	AIC_c	w_i
Eremias	**Logistic-exponential**[1]	7	149.60	38.89	0.023
brenchleyi	Exponential-exponential[1]	6	203.51	45.78	<0.001
(70)	Logan 10[2]	6	192.23	44.04	0.0017
	Quartic	6	172.91	40.82	0.0086
	Sextic	8	107.42	31.38	0.96
Eumeces	Logistic-exponential[1]	7	549.52	87.70	<0.001
elegans	Exponential-exponential[1]	6	432.27	75.47	0.079
(95)	Logan 10[2]	6	755.41	98.50	<0.001
	Quartic	6	386.02	70.80	0.82
	Sextic	8	380.64	74.94	0.10
Lacerta	**Logistic-exponential**[1]	7	50.29	-49.17	<0.001
vivipara	Exponential-exponential[1]	6	228.52	41.96	<0.001
(142)	Logan 10[2]	6	336.45	65.82	<0.001
	Quartic	6	32.05	-79.17	<0.001
	Sextic	8	0.40	-344.59	1
Takydromus	**Logistic-exponential**[1]	7	590.62	89.63	0.17
sexlineatus	Exponential-exponential[1]	6	1214.96	116.10	<0.001
(92)	Logan 10[2]	6	610.03	88.57	0.29

	Quartic	6	595.38	87.60	0.47
	Sextic	8	584.30	91.60	0.064
Thamnophis	Logistic-exponential[1]	7	992.75	60.88	0.0039
elegans	**Exponential-exponential**[1]	6	4011.79	58.36	0.014
vagrans	Logan 10[2]	6	1145.30	51.28	0.47
(13)	Quartic	6	1108.49	51.10	0.51
	Sextic	8	1063.41	76.87	<0.001

FIGURES
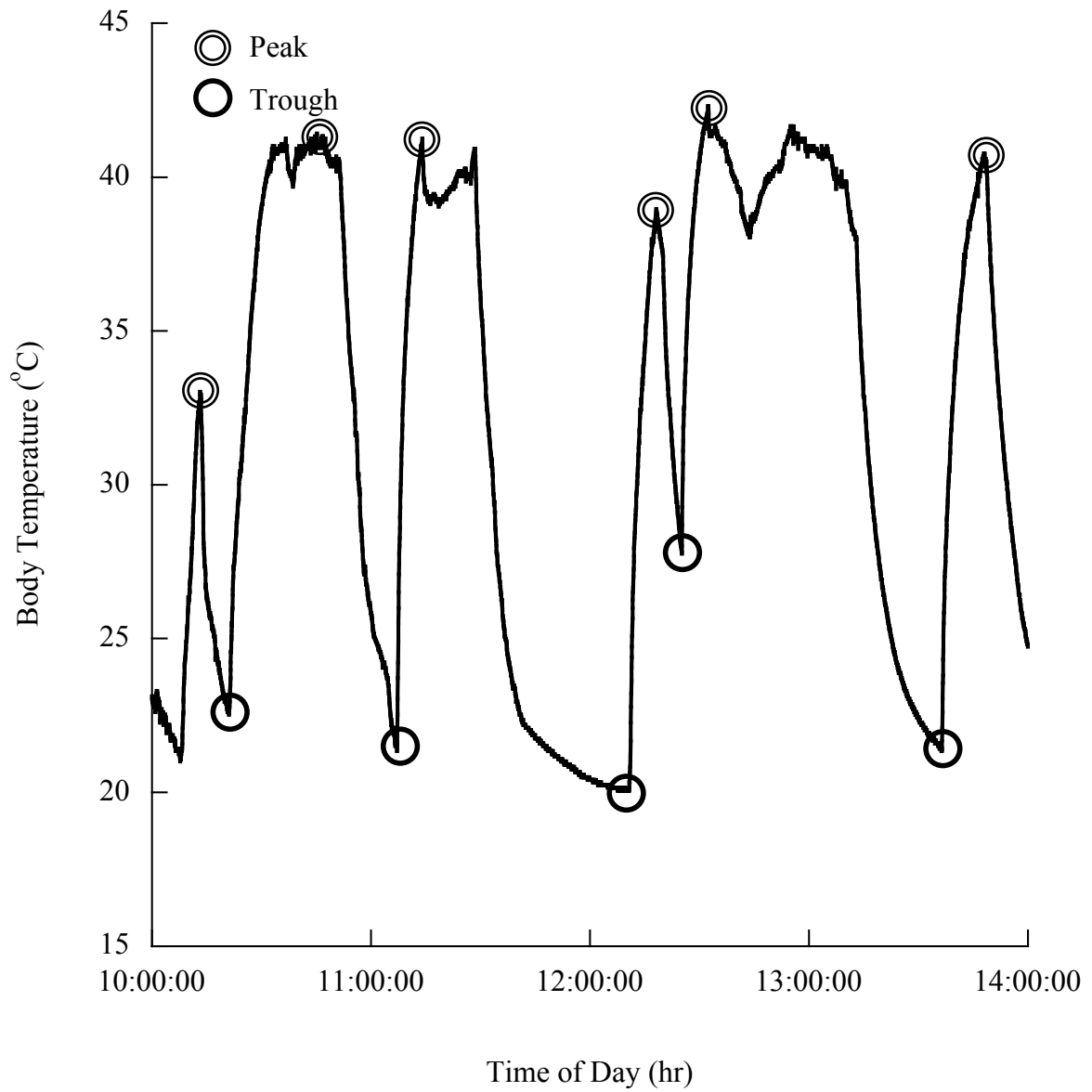
Figure1-1: Representative body temperature profile from individual male *P. vitticeps* in a shuttle box.
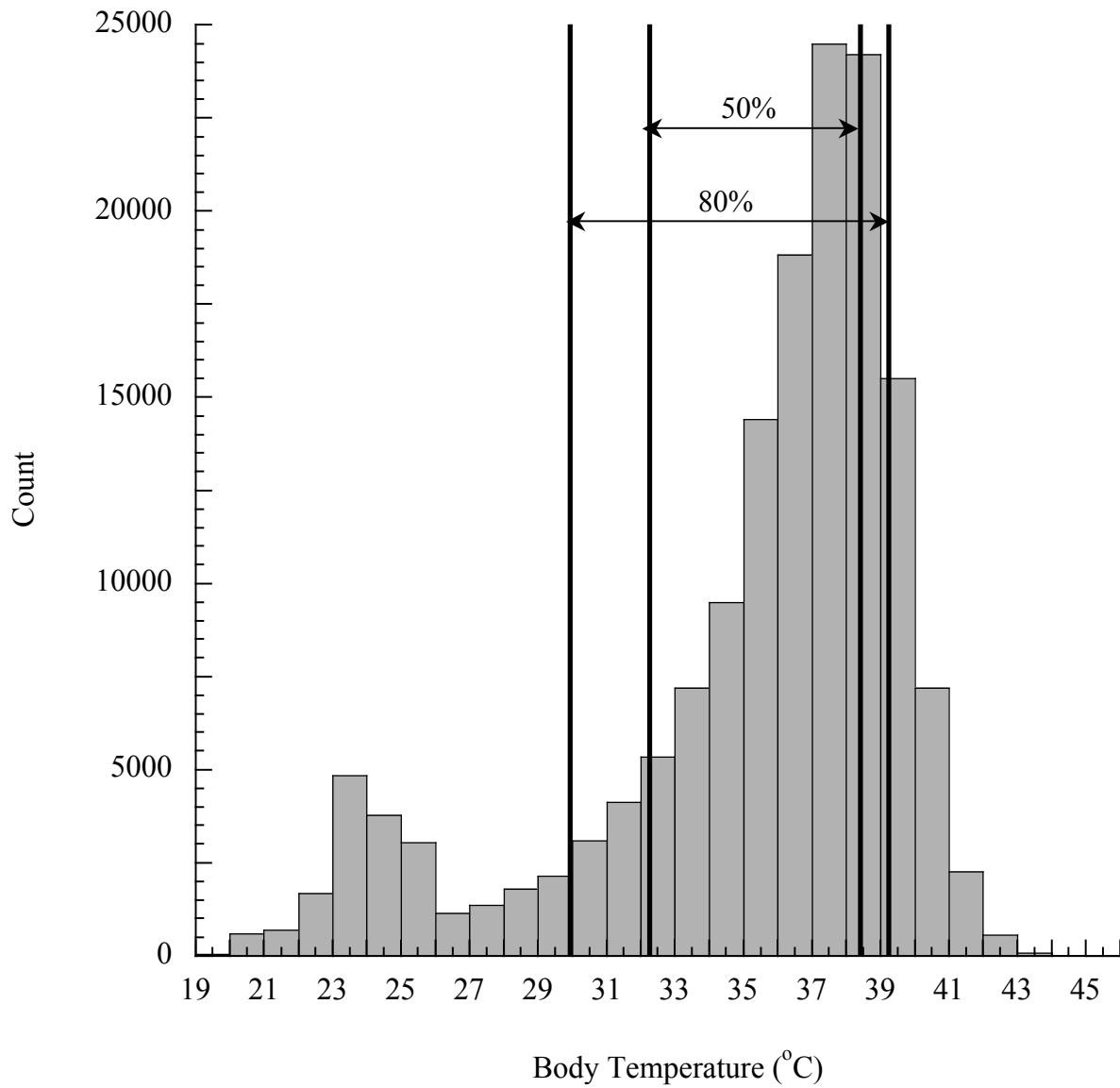
Figure 1-2: Distribution of body temperatures as measured in a thermal gradient for *P. vitticeps* (n=12). Vertical lines delineate the T_{set}s given by the central 50% and 80% T_b distributions.
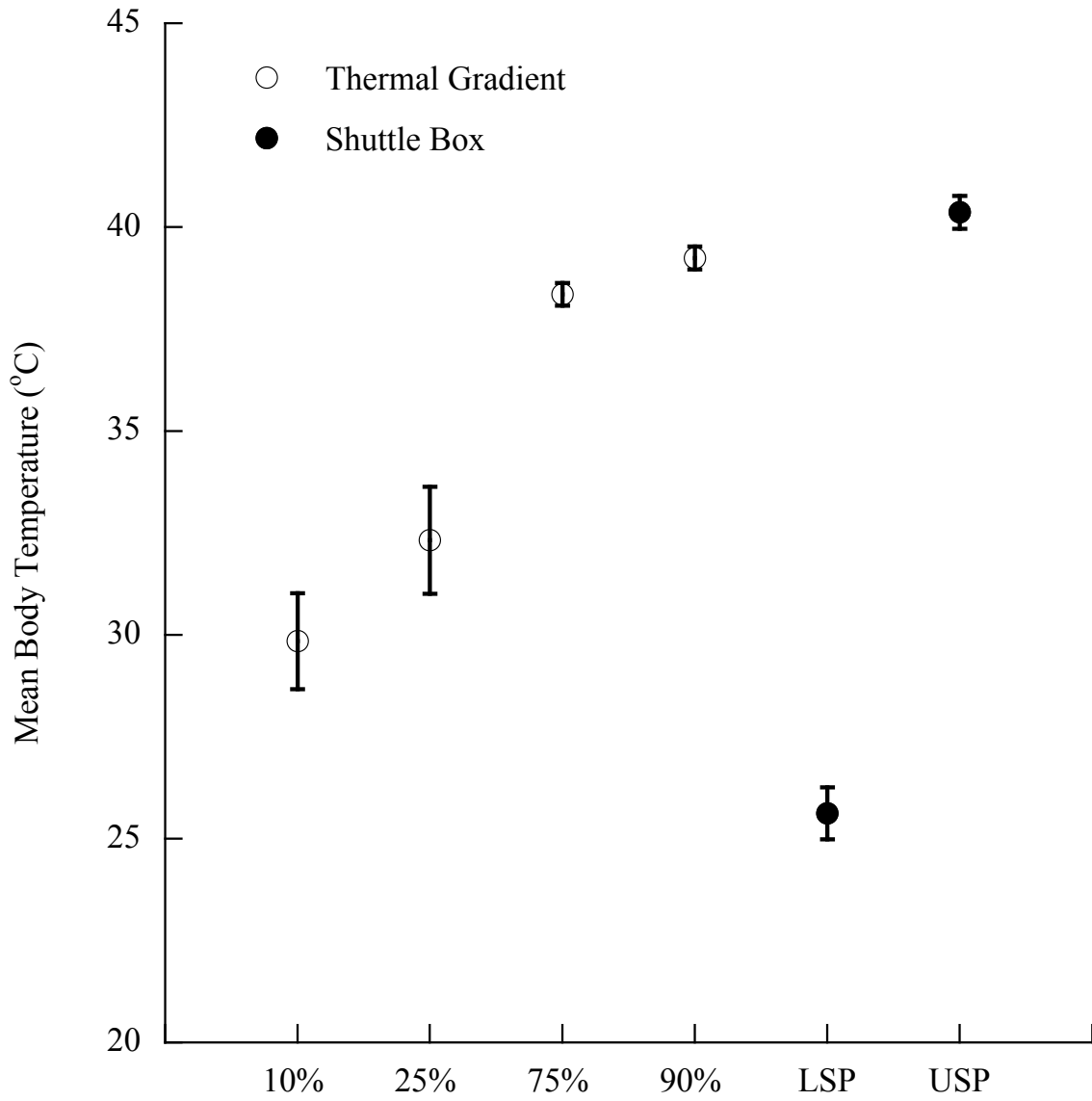
Figure 1-3: Parameters in thermal biology measured in a thermal gradient (% quartiles) and the shuttle box (set-points) for *P. vitticeps*. Means±SE (n=12).
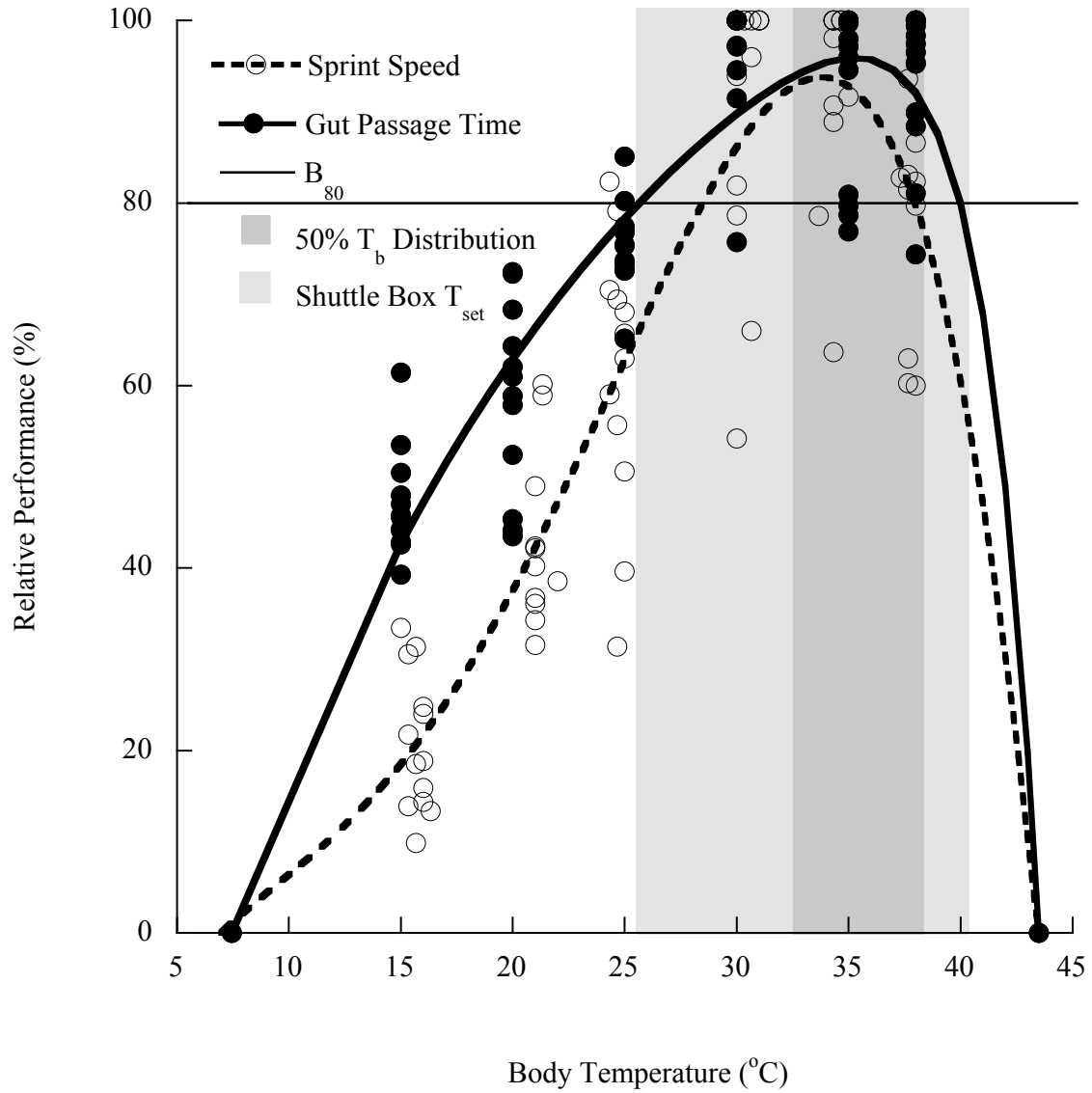
Figure 1-4: Thermal performance curves for relative sprint speed and relative gut passage time as a function of body temperature (°C) in *P. vitticeps*. The uppermost point of each curve represents T_o (n = 12).
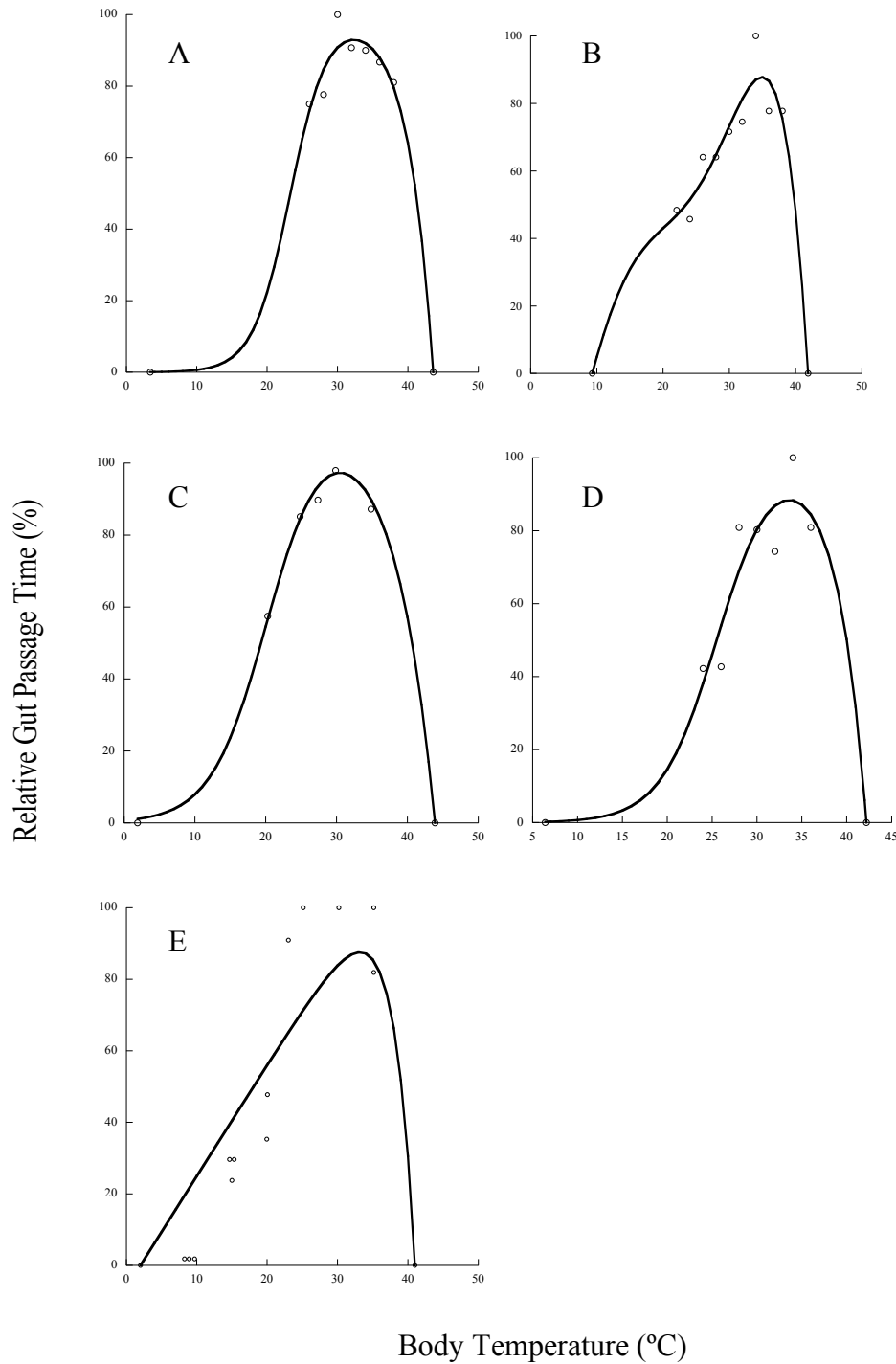
Figure 1-5: Thermal performance curves for relative gut passage time as a function of body temperature (°C) from previous research. A) *Eremias brenchleyi*; B) *Eumeces elegans*; C) *Lacerta vivipara*; D) *Takydromus sexlineatus*; and E) *Thamnophis elegans vagrans.*

CHAPTER 2:

EFFECTS OF FOOD AVAILABILITY ON THERMMOREGULATION, METABOLISM AND LOCOMOTOR PERFORMANCE IN THE BEARDED DRAGON, *PROGONA VITTICEPS*

ABSTRACT

There is a close link between energy acquisition and fitness. Because variation in food availability is ubiquitous in nature, the mechanisms that animals have evolved to cope with food scarcity are of interest. For *Pogona vitticeps*, I examined the effects of feeding treatment (fed vs. fasted) on: behavioural thermoregulation, as measured by the preferred body temperature range (T_{set}) from a thermal gradient and shuttle box; metabolic rate at 20, 30 and 38ºC; and sprint speed from 15-38ºC. Fasted lizards exhibited significantly lower body temperatures (T_bs) and VO_2, and had lower Q_{10} values. These results suggest that voluntarily lowering T_b may improve the energy balance of thermoregulating animals in times of food scarcity. Sprint speed did not vary between fed and fasted treatments. Animals may sprint as fast as possible regardless of energy reserves. Other locomotor performances, such as endurance, may be more affected by food availability in terrestrial ectotherms.

RÉSUMÉ

Il y a un lien étroit entre l'acquisition d'énergie et l'aptitude. Puisque la variation de la disponibilité de nourriture est omniprésente en nature, les mécanismes développés par les animaux pour faire face à la pénurie de nourriture sont d'intérêt. Les objectifs pour cette étude étaient d'examiner les effets du rationnement de la nourriture sur la thermorégulation, le métabolisme et la locomotion chez le dragon barbu, *Pogona vitticeps*. Pour *Pogona vitticeps* j'ai examiné les effets d'alimenter le traitement (alimenté contre à jeûn) dessus: thermorégulation comportementale, comme mesurée par les températures corporelles préférées (T_{set}) dans un gradient thermique et une boîte de navette; le taux métabolique à 20, 30 et 38ºC; et la vitesse de pointe de 15-38ºC. Les lézards à jeûn ont eu les températures corporelles (T_bs) et VO_2 significativement inférieur, et ont eu les valeurs des Q_{10} inférieur.

Ces résultats, suggèrent que la capacité à abaisser volontairement la T_b puisse améliorer l'équilibre énergétique des animaux thermorégulant en période de disponibilité réduite de nourriture. La vitesse de pointe n'a pas varié entre les traitements alimentés et à jeûn. Les animaux peuvent sprinter aussi rapidement que possible indépendamment de leurs réserves d'énergie. D'autres fonctions locomotrices, telles que l'endurance, peuvent davantage être affectées par la disponibilité de la nourriture chez les ectothermes terrestres.

INTRODUCTION

Energy can be partitioned for use in maintenance, growth or reproduction and the increased energy required for reproduction suggests a link between the rate of energy acquisition and fitness (Schoener 1971; Scott and Fore 1995). For many animals, energy acquisition is equated with foraging and given the importance of foraging to fitness, we expect animals to have evolved strategies to deal with low food availability. Food availability can have important implications for many aspects of ectotherm behaviour and physiology, including thermoregulation (Nagy 1972; Sievert 1989), metabolism (Benedict 1932; Roberts 1968; Forsberg 1997), locomotion (Kenward 1978; Guderley 2004), and growth (Angilletta 2001; Taylor *et al.* 2005).

To date, research investigating food availability and thermoregulation has focused on the benefits of maintaining high body temperatures (T_bs; Table 2-1) following feeding (postprandial thermophily; Regal 1966; Gatten 1974; Blouin-Demers and Weatherhead 2001). Postprandial thermophily has been observed in members of the major reptilian taxa (Regal 1966, Gatten 1974, Lang 1979, Lysenko and Gillis 1980). Benefits to maintaining these high T_bs include an increase in energy available for growth, reproduction, and maintenance (Angilletta 2001) and a reduction in the time required for digestion (Cowles and

Bogert 1944; Wang *et al.* 2003). The decreased duration of digestion may be advantageous because it increases the time available for other activities (Pianka and Pianka 1970) and decreases the exposure time required to increase T_b, a period which could increase the risk of predation (Wang *et al.* 2003).

Because post-absorptive individuals have been shown to maintain higher T_bs, it follows that fasting individuals should maintain lower T_bs. Studies have identified circumstances when voluntary lowering T_b, as generally occurs at dusk or during winter months, may be required (Regal 1967; Cogger 1974). Few studies, however, have investigated the effects of low food availability on thermoregulation and none have documented the effects on the preferred T_b range (T_{set}; Table 2-1) despite the importance of thermoregulation to ectotherms. For those studies that have looked at food availability, it was found that food-rationed lizards in a thermal gradient selected lower T_bs than satiated individuals (Bradshaw *et al.* 1980; Brown and Griffin 2005). Moreover, there was a non-significant decrease in thermoregulatory precision, measured by the variance around the mean T_b, in fasted versus fed collared lizards (*Crotaphytus collaris*; Sievert 1989). Chuckwallas (*Sauromalus obesus*) were found to abandon thermoregulatory activity during periods of starvation in the field (Nagy 1972). In times of decreased food availability, thermoregulating animals may be able to improve the balance between the energy from ingested food and the energy used by the animal by voluntarily reducing T_b (Brett 1971; Lillywhite *et al.* 1973).

As is the case with thermoregulation studies, to date many metabolic studies have focused on post-absorptive animals and more specifically on the increase in oxygen consumption accompanying digestion and nutrient assimilation, a concept referred to as specific dynamic action (Kleiber 1961). Comparatively few studies have dealt with the

effects of low food availability on standard metabolic rates (SMR; Table 2-1), despite the fact that such research could aid our understanding of how animals respond to periods of low food availability. Those studies that have examined the effects of fasting used measurements of oxygen consumption to quantify the energy requirements of physiological processes in aerobic animals. Studies documenting a resulting decrease in oxygen consumption by fasted or starved animals compared to fed ones have come from fish (Beamish 1964; Yang and Somero 1993; Forsberg 1997), caimans (Gatten 1980), lizards (Roberts 1968), snakes (Benedict 1932), and turtles (Rapatz and Musacchia 1957; Belkin 1965; Sievert *et al.* 1988).

Locomotor performance can be an important component of fitness as an animal's ability to escape predators, capture prey and interact with conspecifics are key to fitness (Irschick and Garland 2001; Lailvaux and Irschick 2006; Husak *et al.* 2008). Support for an effect of low food availability on locomotor performance is found in endotherms. Fasting results in flight muscle catabolism in birds, which could affect their ability to flee from predators (Grammeltvedt 1978; Swain 1992). Indeed, Kenward (1978) observed that woodpigeons with less supracoracoideus muscle dry mass were caught more often by goshawks.

Locomotor performance, in the form of sprinting is largely an anaerobic process in reptiles and requires the nearly exhaustive catabolism of muscle glycogen catabolism to fuel bursts activity (Gleeson 1991). The result is the accumulation of lactic acid in the blood (Black 1961) and is linked to exhaustion in reptiles. Reptiles are capable of replenishing, almost completely, muscle glycogen stores during recovery from burst activity (Gleeson and Dalessio 1989), but how might an already low supply of muscle glycogen, as a result of low food availability, be reflected in overall sprint performance?

Studies from ectotherms, however, give mixed support for the effects of low food availability on locomotor performance. For example, cod show an increase in sprint swimming speed following feeding but no change following a starvation period (Guderley 2004). In studies done on reptiles, the burden of carrying a clutch has been shown to impair locomotor performance in gravid lizards (Bauwens and Thoen 1981; Cooper *et al.* 1990; Miles *et al.* 2000), but the relationship between locomotor performance and decreased body condition, which could result from low food availability, is unclear. While it has been shown that food-deprived lizards are less active and lose more weight compared to fed lizards, as indicated by their tendency to spend more time in refuge (Dunlap 1995), I am unaware of any study documenting decreased sprint speed in lizards as a result of low food availability.

The objectives for this study were to quantify the effects of food availability on several aspects of ectotherm physiology. I expected: (1) lizards to select lower T_bs and, therefore, to have lower standard metabolic rates when fasted; and (2) lizards to exhibit decreased locomotor performance when experiencing low food availability.

MATERIALS AND METHODS

Study Species and Maintenance

The central bearded dragon, *Pogona vitticeps* (Ahl 1926), occupies an expansive range of environments encompassing the eastern half of Southern Australia, the south-eastern Northern Territory and the interior of all the eastern states (Cogger 2000). A semi-arboreal species, *P. vitticeps* can be found in open habitats with heterogeneous vegetation (Melville and Schulte 2001). Because of the variety of habitats, individuals of this species experience a wide range of temperatures, making it an ideal species for the study of

thermoregulation. *P. vitticeps*, like the majority of agamids, is a diurnal, insectivorous, sit-and-wait predator (Huey and Pianka 1981).

Male and female *P. vitticeps* were obtained from breeders from two clutches. Lizards were measured for snout-vent length (SVL) using electronic digital calipers, and weighed in a cloth bag on a precision scale. Lizards were maintained in opaque plastic terraria filled with 5 cm of sand, a basking rock, a refuge, and a bowl of water. Terraria were housed in environmental chambers (Constant Temperature Control Ltd. Model ER 600; 12L:12D). The temperature was maintained at 30°C during the photophase and decreased to 20°C during the scotophase. Lizards were provided with ad lib water and fed 5% of their body mass in crickets dusted with vitamin-mineral powder every 2 days under the fed treatment and every 4 days under the fasted treatment. Under the fed treatment (n = 12: 7 females, 5 males) lizards were tested the day following feeding. Under the fasted treatment (n = 10: 6 females, 4 males) lizards were tested 3 days following feeding. The University of Ottawa Animal Care and Veterinary Service approved all manipulations, under protocol BL-204.

Thermal Preference

Internal T_bs of lizards were measured using T-type thermocouple 36 ga wire (Omega Engineering Inc.) inserted 2 cm in the cloaca and secured externally with tape. The trailing thermocouple wire was connected to an Onset HOBO data logger (U12-014). T_b measurements were taken every 3 seconds.

I measured T_{set} under both fed (n = 12) and fasted treatments. Lizards were kept on the respective diet for 3 weeks prior to testing. I used two techniques to determine T_{set}: shuttle box (fasted n = 9) and thermal gradient (fasted n = 10). Both the shuttle box and thermal gradient were constructed of stainless steel (each measuring 1.0 x 0.5 x 0.5 m) with the base covered with 2-3 cm of sand to provide traction. Both were housed in an

environmental chamber with an ambient temperature (T_a, Table 2-1) of 20°C (12L:12D). This temperature was selected because it is ecologically relevant for this species and is low enough to be uncomfortable, but not lethal, thus encouraging lizards to shuttle. Lizards were placed in the shuttle box and thermal gradient 24-48hr prior to testing to acclimate to testing conditions. I used 12 hours of T_b recording for each lizard and each technique to estimate T_{set}.

In the shuttle box, a heating pad (45°C) could be activated and deactivated by the lizard via infrared (IR) photosensors and emitters (Fairchild Semiconductor QED). The temperature of 45°C was selected because it is outside the tolerable range for *P. vitticeps* (Warburg 1965), which limited the time that an individual could spend on the heating pad. This ensured that an individual had to shuttle (it was either heating or cooling) to maintain its preferred body temperature range.

The shuttle box measurements were video recorded for 4 lizards. While reviewing the recordings, all changes in behaviour were documented and compared to the T_b profile. For example, postural changes (when a lizard lifted its head off the pad or moved its body slightly), when a lizard passed over the pad but did not remain on it, and 'cheating' behaviour (when a lizard was laying only half on the pad) were recorded. Shuttling events, which included a peak (when the lizard left the pad) and trough (when the lizard moved onto the pad), and excluded any cheating behaviour, were then defined. Only cheating behaviour allowed lizards to remain for prolonged periods of time on the heating pad without shuttling. Peaks and troughs each had a corresponding T_b and time. Shuttling behaviour was defined as an event on the T_b profiles that resulted in a minimum of a 10°C change in temperature over at least 7 minutes (Fig. 2-1). With this information, the upper (or lower) temperature set-point was determined by averaging the peaks (or troughs) for each individual. The mean of

the individual averages was then determined to give the USP and LSP (Table 2-1) of T_{set} for *P. vitticeps*.

The thermal gradient was established using a heating pad (10.16 cm; set at 45°C) and the ambient temperature to produce a smooth thermal gradient. The central 50% and 80% of T_b distributions, designated by the 25% and 75% and the 10% and 90% quartiles respectively, were determined for each individual and then averaged to give T_{set} for *P. vitticeps*.

Metabolic Rate

Lizard oxygen consumption was measured using an open flow-through respirometry system. Each lizard (n = 8; 4 males and 4 females) was placed in a separate airtight 2 L container, which was then housed in an environmental chamber during experimentation. The respirometry system was held outside the chamber to draw in outside air. Incurrent air was scrubbed of CO_2 and H_2O using sequential columns of Drierite, Ascarite and Drierite (DAD) and the dry, CO_2-free air was pushed into a multiplexer (ROXY 8, Sable Systems International) at 300 mL/ min using polyethylene tubing. From the multiplexer, the dry, CO_2-free air then moved to a flow meter (Flow-Bar 8, Sable Systems International) and then, using 3 m long tubing, into 5 airtight containers. The excurrent air from the containers was fed back into the multiplexer and subsampled at 100 mL/ min before being sent into the CO_2 analyzer (LI-7000, LI-COR[®] Biosciences). The excurrent air was then scrubbed of CO_2 and H_2O using DAD columns before being sent to the O_2 analyzer (OXZILLA II, Sable Systems International). The O_2 and CO_2 analyzers were zeroed, and baseline measurements of O_2 were established before each lizard was measured.

Lizards were divided into two groups of 4, with each group containing fed and fasted individuals. Both groups experienced 6 weeks under either the fed (n=3) or fasted (n=5)

treatment and the rate of oxygen consumption (VO_2; Table 2-1) for the 2 groups were measured one day apart. Fed and fasted treatments followed the procedure described under *Study Species and Maintenance* with the exception that during the week of measurements, the lizards under the fed treatment were not fed the day before measuring (as had been done in previous thermal preference and sprint speed experiments). This was done to reduce the chances that lizards from different feeding treatments would be at different stages of digestion.

Each lizard was measured at three T_as, and all T_as were recorded on the same evening. The order of T_as was 20, 30, and 38°C for the first group; and 38, 30, and 20°C for the second group to control for order effects. Onset HOBO data loggers (U12-014) and iButtons (Maxim Integrated Products © 2009) were placed in each airtight container to monitor the changes in temperature every minute. VO_2 were measured from 2100h to 0600h with each T_a measured for 3 hrs. From each 3 hr block, two measurements per lizard of VO_2 that had closely matching test T_as were used during analysis. Thus, a total of 12 min per lizard per test T_a was used. Body mass for each lizard was determined as described above.

Sprint Speed, Body Condition and Growth Rate

The thermal sensitivity of sprint speed was examined under fed (n = 12: 7 females, 5 males) and fasted (n = 10: 6 females, 4 males) treatments at six temperatures (15, 20, 25, 30, 35 and 38°C) ordered randomly. Lizards were maintained under the respective diets for 6 weeks prior to testing. Immediately prior to testing, lizards were held for 2 hours within the temperature controlled environmental chambers to allow for sufficient time for T_b to reach T_a. Sprint speed was measured by chasing lizards down an ABS track (20.32 cm diameter) for 1 m. The track was filled with 3 cm of sand and soil for traction. Chasing was done by

the same experimenter and at a consistent pace. Individuals were measured three times in one day, allowing at least 20 min between trials to recuperate. Lizards were tested in random order and the T_b of lizards was measured prior to each trial (± 1°C; Control Company Traceable thermometer). These procedures were repeated at each temperature. All trials took place between 1000h and 1400h. Trials were videotaped for subsequent observation and analysis.

At each temperature, three measurements of SVL and body weight were taken for each individual. An average of the three measurements was taken to give the mean SVL and mean body weight for each individual. To obtain body condition, I divided the residuals from the regression of $\log_{10}$(body mass) on $\log_{10}$(SVL) by the predicted mass and then expressed this value as a percentage. Growth rate was calculated over a 20-day period during the sprint speed trials for both the fed and fasted treatments. Sprint speed was measured in SVL (mm) per second.

Statistical Analyses

I used t-tests to test for differences in the means between males and females for the measured thermoregulatory parameters. Paired t-tests were used to test the differences between fed and fasted treatments for the thermoregulatory parameters measured, body condition and growth rate. Unless stated otherwise, t-tests were two-tailed.

Only main effects and interactions of potential interest were included in the repeated measures ANCOVA model as there were insufficient degrees of freedom to run a full model. To control for individual effects in the repeated measures design, individual was included as a random effect. The slope of the regression of $\log_{10}$(VO$_2$) on $\log_{10}$(body mass) was not significantly different from 1 and therefore, mass-specific metabolic rates were not used in the analysis. The model included feeding treatment, individual, sex, day and T_a as main

effects and $\log_{10}$(body mass) as a covariate, as well as the following interactions: feeding treatment × $\log_{10}$(body mass); feeding treatment × T_a; feeding treatment × day; and sex × $\log_{10}$(body mass). The model was fit using the restricted maximum log-likelihood (REML) procedure and the type III sums of squares were used to test the significance of the independent variables.

Repeated measures MANOVA was used to determine the effect of sex and feeding treatment on the absolute sprint speed at the 6 measured temperatures. Due to the small sample size, missing values for absolute sprint speed were substituted with the mean absolute sprint speed at each temperature, giving a balanced design.

Analyses were conducted with JMP Version 5.0.1a (SAS Institute Inc. 1989-2002) and ExpeData (Sable Systems International 2002-2005 Version 1.1.25). The assumptions of normality and homogeneity of variance were checked and data were transformed when necessary. Statistical tests were considered significant at $\alpha = 0.05$ unless otherwise stated. Means are reported as ± 1 SE.

RESULTS

Thermal Preference

Under the fed treatment, males and females did not differ for any of the parameters measured, therefore, the data were pooled (thermal gradient: mean T_b, t (10) = 0.23, $p = 0.82$; 10% quartile, t (10) = 0.88, $p = 0.40$; 25% quartile, t (10) = 0.50, $p = 0.63$; 75% quartile, t (10) = 1.38, $p = 0.20$; 90% quartile, t (10) = 1.36, $p = 0.20$; shuttle box: mean T_b, t (10) = 1.33, $p = 0.21$; USP, t (10) = 2.02, $p = 0.07$; LSP, t (10) = 0.10, $p = 0.92$). Averaged across all individuals, the mean T_b in the thermal gradient was 35.2 ± 0.5°C and the mean 10%,

25%, 75% and 90% quartiles were 29.9 ± 1.2°C, 32.3 ± 1.3°C, 38.4 ± 0.3°C and 39.2 ±

0.3°C, respectively (Figs. 2-2, 2-3). Averaged across all individuals, the mean T_b in the

shuttle box was 33.2 ± 2.4°C and the mean LSP and USP were 25.6 ± 1.6°C and 40.4 ±

0.5°C, respectively (Fig. 2-3).

Under the fasted treatment, males and females did not differ for any of the parameters

measured, and therefore the data were pooled (thermal gradient: mean T_b, t (8) = 1.22, p =

0.26; 10% quartile, t (8) = 1.51, p = 0.17; 25% quartile, t (8) = 0.99, p = 0.35; 75% quartile, t

(8) = 1.06, p = 0.32; 90% quartile, t (8) = 1.34, p = 0.22; shuttle box: mean T_b, t (7) = 0.16, p

= 0.88; USP, t (7)= 0.33, p = 0.75; LSP, t (7) = 0.32, p = 0.76). Averaged across all

individuals, the mean T_b in the thermal gradient was 31.7 ± 0.8°C and the mean 10%, 25%,

75% and 90% quartiles were 26.8 ± 1.3°C, 28.8 ± 1.4°C, 34.7 ± 0.5°C and 35.7 ± 0.6°C,

respectively (Fig.2-2, 2-3). Averaged across all individuals, the mean T_b in the shuttle box

was 27.8 ± 0.6°C and the mean LSP and USP were 21.9 ± 0.6°C and 33.9 ± 0.7°C,

respectively (Fig. 2-3).

Fed individuals had a higher mean T_b than fasted individuals in the thermal gradient

(one-tailed paired t (9) = 2.69, p = 0.012). The mean difference between the fed and fasted

treatments was 3.2 ± 1.2°C. As in the thermal gradient, fed individuals exhibited a higher

mean T_b compared to fasted individuals in the shuttle box (one-tailed paired t (8) = 6.41, p =

0.0001).

There was no significant difference in 10% or 25% quartiles for the fed or fasted

treatments (10%: paired t (9) = 1.09, p = 0.31; 25%: paired t (9) = 1.09, p = 0.30). However,

the 75% and 90% quartiles were both significantly lower under the fasted treatment (75%

quartile: paired t (9) = 4.58, p = 0.0013; 90% quartile: paired t (9) = 4.03, p = 0.003). Thus,

both the 50% and 80% T_b distributions were narrower under the fasted treatment with lizards selecting lower T_bs on the upper bounds of these T_b distributions.

Both the LSP and USP were significantly lower under the fasted treatment (LSP paired t (8) = 3.94, p = 0.0066; USP paired t (8) = 8.49, p < 0.0001). Thus, the T_{set} determined by the shuttle box was narrower under the fasted treatment and shifted toward lower selected T_bs.

Metabolic Rate

In the ANCOVA model, $\log_{10}(VO_2)$ was measured as the dependent variable with feeding treatment, individual, sex, day and T_a as main effects and $\log_{10}$(body mass) as the covariate and the following interactions: sex × $\log_{10}$(body mass); feeding treatment × $\log_{10}$(body mass); feeding treatment × T_a; feeding treatment × day. The 2 latter interactions were not statistically significant and thus were removed from the model with no significant effect on model fit.

There was a significant interaction between feeding treatment and $\log_{10}$(body mass) ($F_{1,33}$ = 16.87, p = 0.0002). The separate regression equations for the fed and fasted treatments describing this relationship indicated that $\log_{10}(VO_2)$ increases with $\log_{10}$(body mass) but at a slower rate for lizards under the fasted treatment. The equations are given below:

Fed: $\mathrm{Log}_{10}(VO_2)$ (mL h^{-1}) = -0.34 + 0.56($\log_{10}$(body mass))and

Fasted: $\mathrm{Log}_{10}(VO_2)$ (mL h^{-1}) = -0.37 + 0.53($\log_{10}$(body mass)).

As expected, there was a significant effect of T_a on $\log_{10}(VO_2)$ ($F_{2,33}$ = 129.39, p < 0.0001). Post-hoc analysis using Tukey-Kramer HSD indicated that $\log_{10}(VO_2)$ was significantly different between all levels of T_a (q = 2.42, p = 0.05). The mean oxygen

consumption at 20, 30 and 38°C was 0.37 ± 0.04 mL h^{-1}, 0.77 ± 0.03 mL h^{-1}, and 1.09 ± 0.06 mL h^{-1}, respectively (Fig. 2-4). The Q_{10}s (Table 2-1) for the fed and fasted treatments were 2.38 and 1.89, respectively, from 20-30°C, and 1.56 and 1.48, respectively, from 30-38°C.

There was a significant interaction between sex and $\log_{10}$(body mass) ($F_{1,33} = 5.15$, $p = 0.03$). The separate regression equations for the males and females describing this relationship indicated that $\log_{10}$(VO$_2$) increases with $\log_{10}$(body mass) but at a slower rate for males. The equations are given below:

Females: $\log_{10}$(VO$_2$) (mL h^{-1}) $= -0.38 + 0.55(\log_{10}$(body mass))and

Males: $\log_{10}$(VO$_2$) (mL h^{-1}) $= 0.21 + 0.26(\log_{10}$(body mass)).

Sprint Speed, Body Condition and Growth Rate

Absolute sprint speed at the six measured temperatures varied with respect to sex (MANOVA: $F_{1,20} = 6.32$, $p = 0.021$) but not treatment (MANOVA: $F_{1,20} = 0.93$, $p = 0.35$). Post-hoc tests using the Bonferroni correction ($\alpha = 0.025$) indicated that males were significantly faster than females (MANOVA: $F_{1,22} = 6.63$, $p = 0.017$).

There was no effect of sex on body condition for either the fed or fasted treatments (fed: t (70) $= 0.015$, $p = 0.99$; fasted: t (58) $= 1.13$, $p = 0.26$). Body condition did not vary between the treatments (paired t (59) $= 0.89$, $p = 0.38$). Within the range of body size tested, there was no effect of initial SVL on growth rate ($F_{1,20} = 2.96$, $p = 0.10$). Lizards under the fed treatment grew at a faster rate (0.2 ± 0.02 mm/day) than those under the fasted treatment (0.09 ± 0.02 mm/day; paired t (9) $= 3.72$, $p = 0.0024$).

DISCUSSION

A comparison of the mean T_b using both the thermal gradient and the shuttle box showed a decrease in selected T_bs under fasted conditions. A decrease in selected T_b under a fasting treatment (using a thermal gradient) was also found in *Anolis carolinensis* (Brown and Griffin 2005). The decrease in selected T_bs for *A. carolinensis* was smaller compared to this study ($3.2 \pm 1.2°C$), with a mean decrease of $0.4 - 0.7°C$ in females and $0.6 - 2.1°C$ in males. The selected T_bs of *A. carolinensis* were measured only at 1400h, 1600h and 1800h, while this study measured selected T_b from 0700h – 1900h, which could account for the larger range in the temperature decrease. The decrease in T_b selected in a thermal gradient in my study was more similar to the results of Bradshaw *et al*. (1980), who found that the Australian lizard, *Lialis burtonis*, decreased its T_b from the mean preferred T_b of $35.1 \pm 0.11°C$ to $32.2 \pm 0.25°C$ when starved.

To my knowledge, no other study has examined how fasting in reptiles affects T_{set}. Both the 50% and 80% T_b distributions were narrower under the fasted treatment, but with lizards selecting lower T_bs only on the 75% and 90% quartiles of these T_b distributions in the thermal gradient and not the 10% and 25% quartiles. However, in the shuttle box both the USP and LSP were significantly lower under the fasted treatment. Why the difference in the two measurements of T_{set} occurs is unknown, but the overall trend of a decrease in selected T_bs under fasted conditions supports the predictions of this study.

For an increase in 10°C, lizards with low food availability consumed less oxygen. These results, combined with the results from the thermal preference experiments, suggest that the decrease in the mean T_b and the narrowing of T_{set} under fasting treatment could be a means to conserve energy, especially for ectotherms with high preferred T_bs. With metabolic

rate decreasing with decreasing temperature, the ability to voluntarily reduce T_b may improve the energy balance of thermoregulating animals in times of decreased food availability (Brett 1971; Lillywhite *et al.* 1973).

Interestingly, there was no significant difference between sprint speeds in lizards under the fed or fasted treatment. It is possible that there is no effect of decreased food availability on sprint speed. By definition, sprinting is a short-term behaviour. It may be that animals will sprint as fast as possible because the benefit to escape a predator, for example, is immediate and relatively low-cost compared to the cost of not escaping the predator. However, it is also possible that there is an effect of food availability but that the fasted treatment was not powerful enough to elicit a significant decrease in sprint speed. While there was no significant difference in body condition, lizards grew at a slower rate under the fasted treatment suggesting that the lizards were putting their acquired energy towards maintenance. Thus, there was a physiological effect of the treatment on the animals, but it may not have been powerful enough to elicit a response in sprint speed.

Other components of locomotor performance, such as endurance, may be more affected by decreased food availability than sprint speed. Endurance mobilizes lipids, a main source of metabolic fuel, but when lipid reserves are low the glycolysis of muscle proteins may be used as an energy source (Munro 1964; Cherel *et al.* 1987). A situation could arise where in the absence of sufficient lipid stores muscle proteins would be mobilized. One such situation could involve low food availability. For example, endurance, but not sprint swimming, has been shown to decrease in starved cod (Guderley 2004). Further work into the possible effect of low food availability on endurance in reptiles is needed to confirm this.

While I could not detect an effect of food availability on sprint speed in *P. vitticeps*, I did find that males sprinted faster than females at all test temperatures and under both

feeding treatments. The idea that improved locomotor performance can lead to a defense advantage in territorial species in not uncommon. Here, faster males may have an additional fitness benefit, as they may be able to defend a larger homerange and/or more mates when compared to their slower competitors (Husak *et al.* 2006; Peterson and Husak 2006; Husak *et al.* 2008). Male *P. vitticeps* do exhibit territorial behaviour, but whether or not improved locomotor performance results in a territorial and/or mating advantage in this species is currently unknown.

In addition, I was also not able to detect sex-specific differences for measures of thermal preference or body condition due to low sample sizes. One study noted sex-specific differences for the effect of fasting on measures of thermoregulation (Brown and Griffiths 2005), however these differences were small. Regardless of the low power to detect sex-specific differences, I was still able to address the main objectives of this study.

Overall, the decrease in selected T_bs and VO_2 under the fasted treatment provided evidence that behavioural thermoregulation is a means to regulate energy consumption in times of limited energy resources. However, more work is needed to elucidate potential effects of food availability on locomotor performance in terrestrial ectotherms. In lizards, looking at more active predators rather than sit-and-wait predators may provide more insight into possible effects on endurance in these animals.

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Table 2-1: Definitions of terms to describe thermoregulation and metabolism.

| Term	Definition
LSP	Lower set-point: Lower bound of T_{set} at which thermoregulatory responses occur. Originally postulated by Cowles and Bogert 1994 as the minimum voluntary temperature.
Q_{10}	A temperature coefficient that quantified the rate of change of a biology process as a result of an increase in temperature of 10ºC.
SMR	Standard metabolic rate: Metabolic rate of a resting and fasted ectotherm for a given body temperature.
T_a	The ambient temperature of an environment.
T_b	The body temperature of a representative sample of study animals.
T_{set}	The set-point or preferred temperature range of an active individual (Hertz *et al.* 1993).
USP	Upper set-point: Upper bound of T_{set} at which thermoregulatory responses occur. Originally postulated by Cowles and Bogert 1994 as the maximum voluntary temperature.
VO_2	Rate of O_2 consumption.
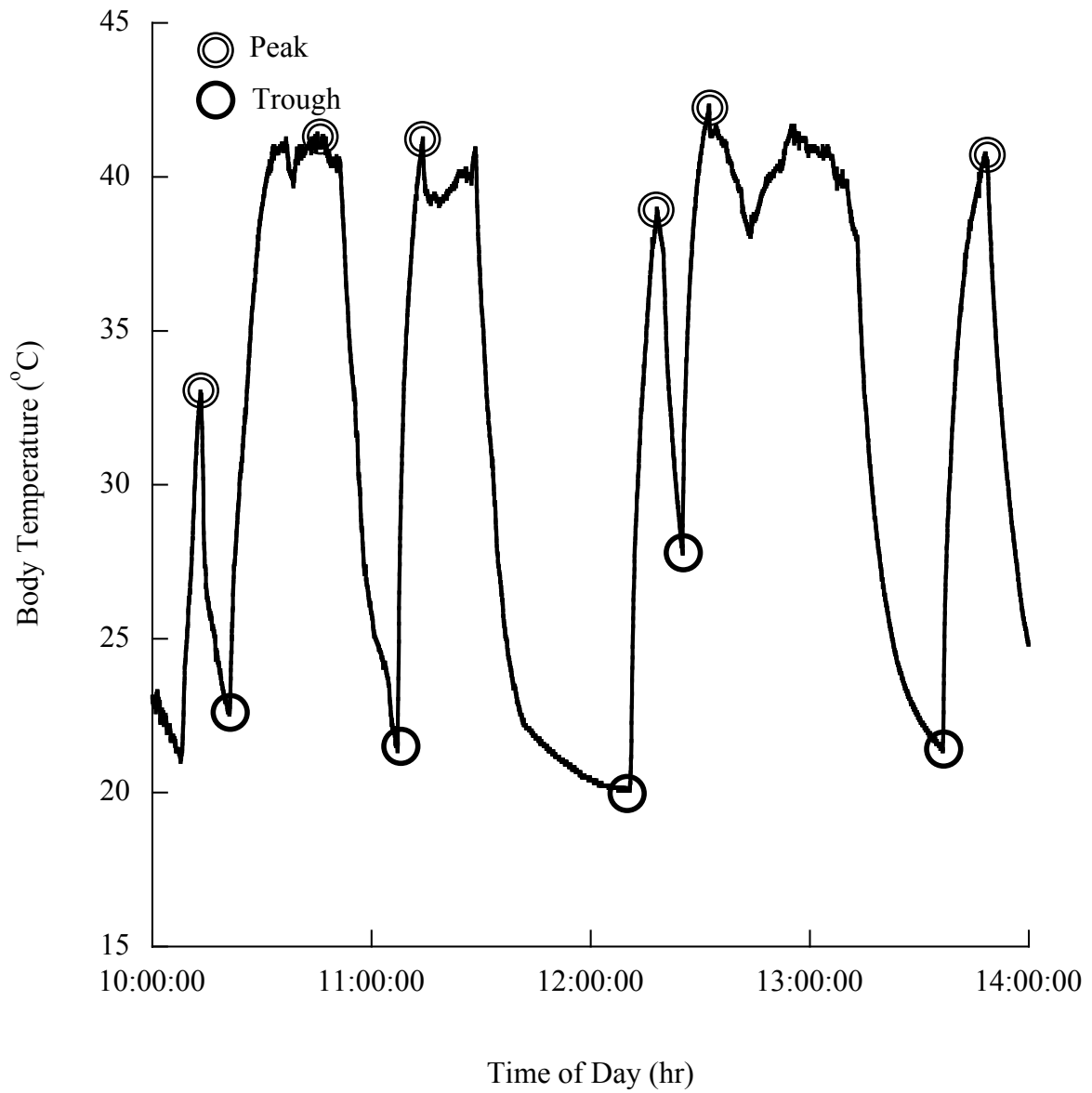
Figure 2-1: Representative body temperature profile from individual male *P. vitticeps* in a shuttle box.
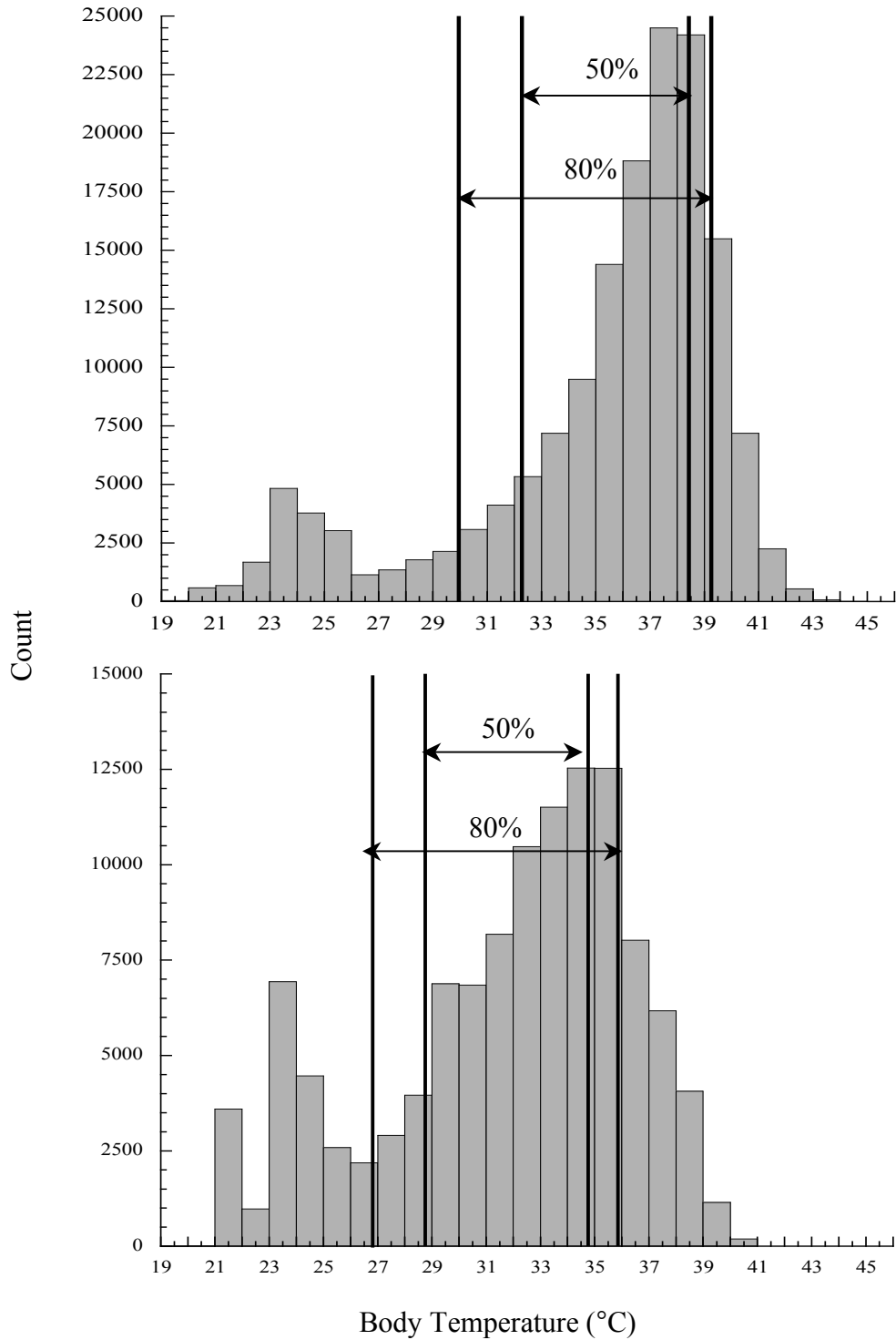
Figure 2-2: Body temperature distributions from thermal gradient for (A) fed (n = 12) and (B) fasted (n = 10) *P. vitticeps*. Vertical lines delineate the T_{set}s given by the central 50% and 80% T_b distributions.
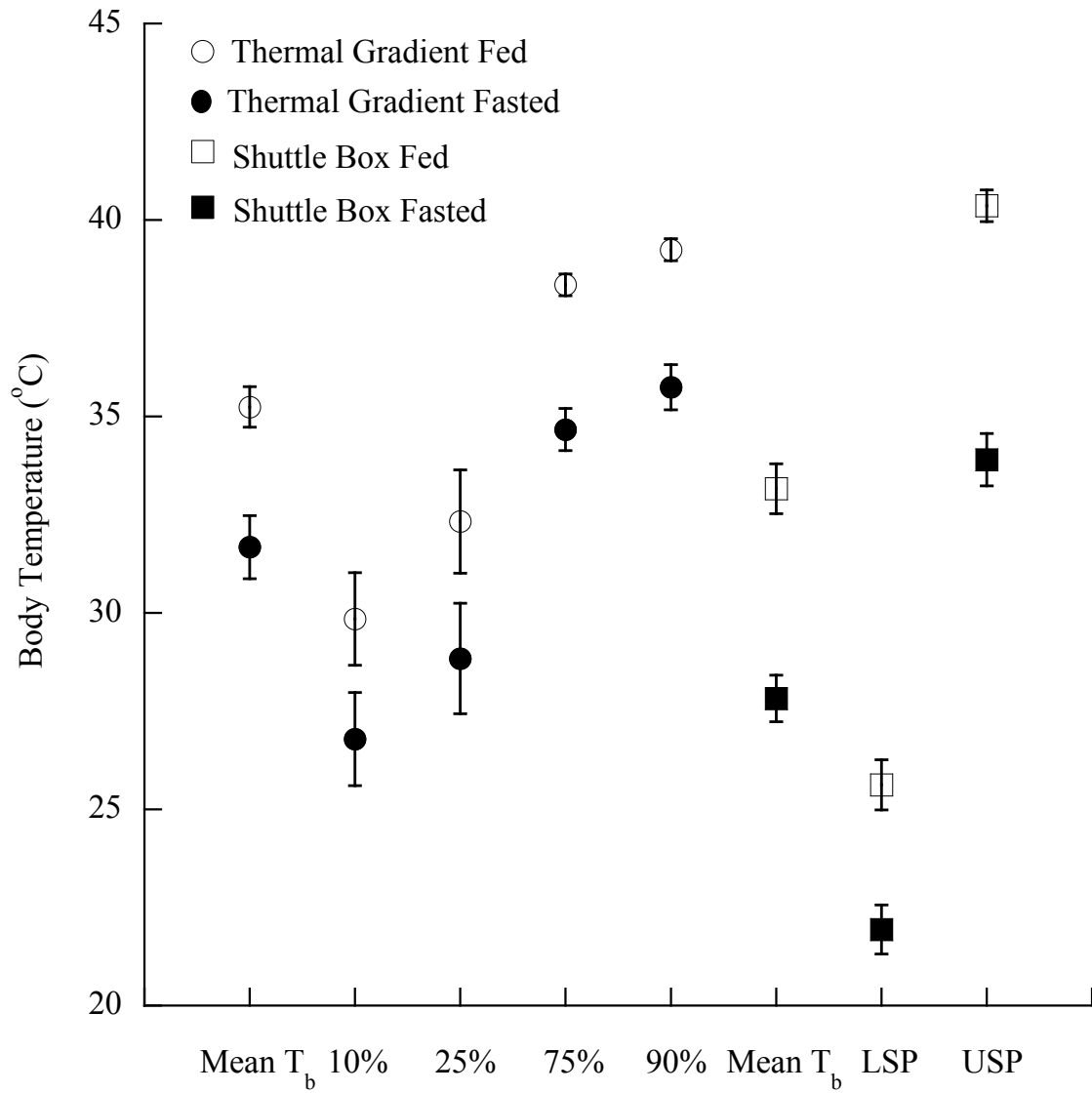
Figure 2-3: Parameters in thermal biology measured by a thermal gradient (% quartiles) and shuttle box (set-points) methods under fed (n = 12) and fasted treatments (n = 10) for *P. vitticeps*. Means±SE.
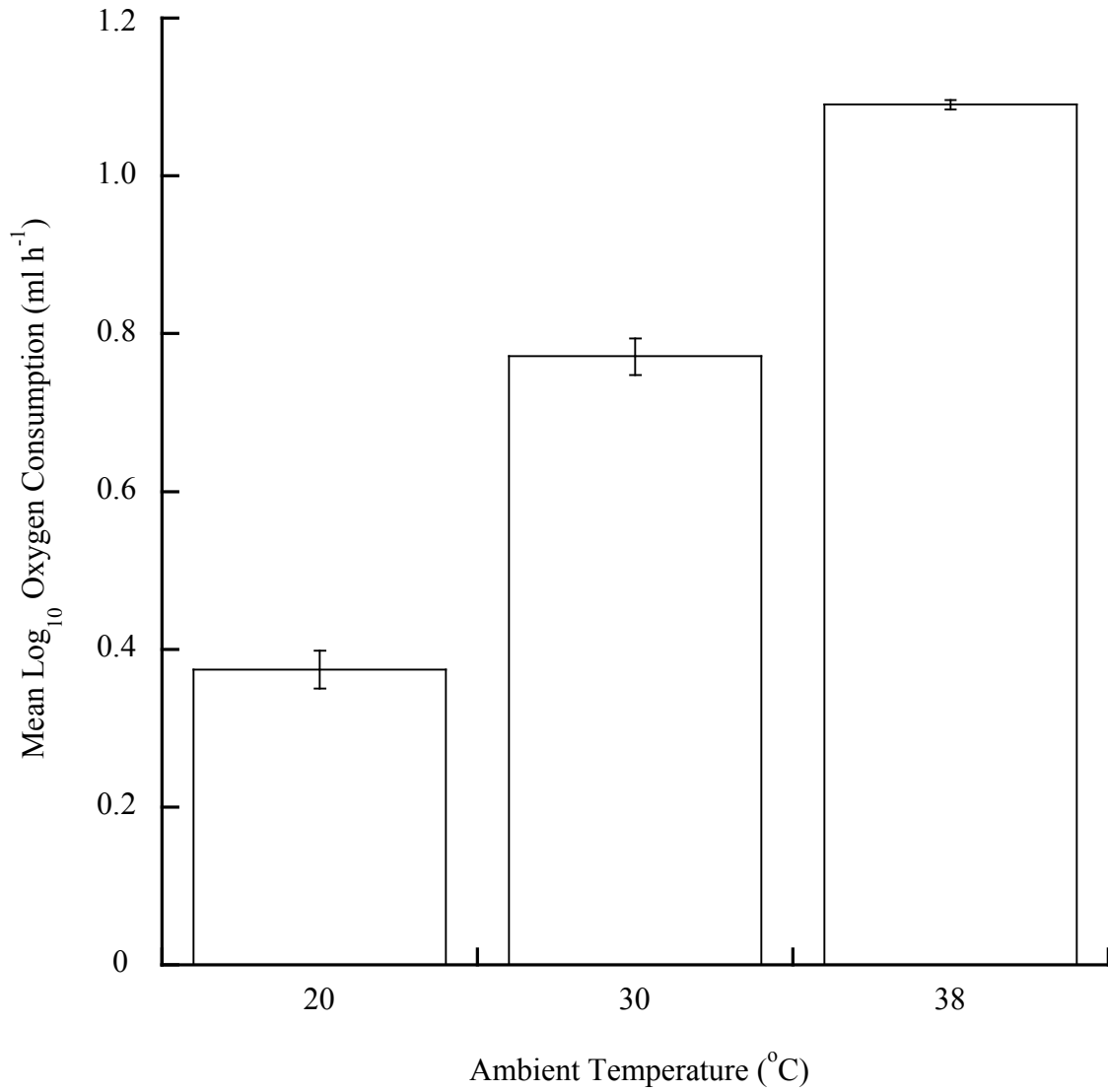
Figure 2-4: Mean oxygen consumption (ml h^{-1}) as a function of body temperature for *P. vitticeps* (n=8). Significant difference in oxygen consumption between males and females is not shown. Means±SE.

APPENDIX A: Synopsis for CTCL Model ER 600 Environmental

Chamber Operation and Maintenance Manual

Modes of Operation

There are four modes of operation for the Set-point Programmer. p.1-2 [1]

Base Mode runs from day-to-day when no program is running. You can select a program to run while in this mode.
Program Run Mode can view the status or information of a program is this mode. In this mode, a program can either be running, held or waiting on a delay to start.
Program Define Mode allows you to enter this mode through Base Mode or Program Run Mode (while a program is running) to edit a program.
Controller Define Mode allows you to define the controller characteristics.

Both the *Program Define Mode* and *Controller Define Mode* need to be entered through the Lock Code. To enter either of these modes press and hold the RUN/HOLD button until the display screen reads ABORTED. Next press and release both the SCROLL and UP keys. The screen should read UNLOCKED. Use the UP key to reach 10 and then press the SCROLL key to enter the code.

Parameters

Temperature
A set-point value or temperature is set whenever an incubator or environmental chamber is active. It can be set without designing or running a program. However, if a program is running, you can only adjust a set-point value in *Base Mode*. See *Aborting a program* under **Quick Programming References**.

Lights
To adjust the lighting, the Set-Point Controller must be in *Program Run Mode* or *Program Define Mode*. Lighting is referred to as events. The events are displayed in binary on the Set-Point Controller. There are four lights that can be adjusted to be either on or off using the UP and DOWN keys, respectively. When all lights are turned on, the screen will read 1111. When all lights are turned off, the screen will read 0000.

Quick Programming References

Designing a program is done in *Program Define Mode* which can be accessed from *Base Mode* or while a program is currently running. p.8-1 To enter *Program Define Mode*, you must enter via Lock Code (see above). To display and define different parameters, use the

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[1] Pages refer to page numbers in Operation and Maintenance Manual

SCROLL key to navigate through *Program Define Mode*. To exit *Program Define Mode*, press the MODE key until EXIT appears on the display. Then press the SCROLL key. To select a different program to design or run, use the PROF key while in *Program Define Mode*. To change a segment within a program, use the RUN/HOLD key.

Once you are in *Program Define Mode* different parameters can be set. The name of the parameter being set is shown in the display panel. There are three types of parameters that can be set:

1. Parameters common to all programs (Global) p. 8-2
When setting these parameters, the letter A appears in the program number display box. The segment number remains blank as the parameters are global. The list of global parameters is shown on p. 8-2.

2. Parameters common to a program as a whole p.8-3
There are 8 available programs that can be designed. When defining the parameters shown on p. 8-3, the number of the program is displayed in the program number box. The segment number box remains blank.

3. Parameters specific to one segment within a program p.8-5
There are 16 segments that can be designed within a program. This means that any program you design can have a maximum of 16 segments only. When defining the parameters shown on p.8-5, the number of the program (1-8) is displayed in the program number box. The number of the segment being defined (1-16) is displayed in the segment number box. Parameters specific to one segment within a program are the most commonly used parameters. Once defined, they can be adjusted as needed.

Designing a program with parameters specific to one segment within a program:
1. You must be in *Program Define Mode*. Abort any program that might be running (see *Aborting a program*).
2. Press the PROF key to select a program number.
3. Press the PROF key until the desired number of segments is reached.
4. Press the RUN/HOLD key.
5. Use the SCROLL key to navigate through the parameters to be set.
    Final Set-Point (Final SP) is the temperature at the end of the segment. See *Temperature*.
    Segment Time or Ramp Rate is the duration of the segment. The unit of time depends on the setting of the Pre-x60 parameter. To express the segment in hours: minutes, the Pre-x60 parameter is set to OFF.
    Event is the lighting during the segment. See *Lighting*.
6. Press the MODE key until it reads EXIT?
7. Press the SCROLL key.
8. Press but do not hold down the RUN/HOLD key.

Aborting a program: p.3-3
1. Hold down RUN/HOLD to abort the program. The display will read ABORTED.

Restarting a program that is already running:
1. Hold down RUN/HOLD to abort the program. The display will read ABORTED.
2. Press the SCROLL and UP keys simultaneously to unlock the controls. The display will read UNLOCKED.
3. Use the UP key to enter 10.
4. Press the SCROLL key.
5. Press the MODE key until it reads EXIT?
6. Press the SCROLL key.
7. Press but do not hold down the RUN/HOLD key.
The program is reset.

Edit a running program:
1. Hold down RUN/HOLD to abort the program. The display will read ABORTED.
2. Press the SCROLL and UP keys simultaneously to unlock the controls. The display will read UNLOCKED.
3. Use the UP key to enter 10.
4. Press the SCROLL key.
5. Press the SCROLL key again to cycle through the parameters and alter those that you wish using the UP and DOWN keys.
6. Press the MODE key until it reads EXIT?
7. Press the SCROLL key.
8. Press but do not hold down the RUN/HOLD key.

Adjusting a set-point value without designing a program: p.4-1
1. In *Base Mode,* press the SCROLL key until SETPOINT appears on the display.
2. Use the UP and DOWN keys to adjust the set-point value to the desired temperature.
3. Press the SCROLL key again to activate this temperature.