The paradox of nocturnality in lizards

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Harvard Law of Animal Behaviour:

"Under the most closely defined experimental conditions,
the animal does what it damned well pleases"

Anon.

Hoplodactylus maculatus foraging on flowering *Phormium cookianum*

VICTORIA UNIVERSITY OF WELLINGTON

Te Whare Wānanga o te Ūpoko o te Ika a Māui

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Except where specific reference is made in the main text of the thesis, this thesis contains no material extracted in whole or in part from a thesis, dissertation, or research paper presented by me for another degree or diploma. No other person's work (published or unpublished) has been used without due acknowledgment in the main text of the thesis. This thesis has not been submitted for the award of any other degree or diploma in any other tertiary institution. All chapters are the product of investigations in collaboration with other researchers, and co-authors are listed at the start of each chapter. In all cases I am the principal author and contributor of the research. I certify that this thesis is 100,000 words or less, including all scholarly apparatus.

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Abstract

Paradoxically, nocturnal lizards prefer substantially higher body temperatures than are achievable at night and are therefore active at thermally suboptimal temperatures. In this study, potential physiological mechanisms were examined that may enable nocturnal lizards to counteract the thermal handicap of activity at low temperatures: 1) daily rhythms of metabolic rate, 2) metabolic rate at low and high temperatures, 3) locomotor energetics, and 4) biochemical adaptation. A multi-species approach was used to separate evolutionary history of the species from any potential links between physiology and activity period. Four to eight species of lizards, encompassing nocturnal and diurnal lizards from the families Diplodactylidae and Scincidae, were used for all physiological measurements.

Three daily patterns of metabolic rate ($\dot{V}O_2$) were apparent depending on the species: 24 h cycles, 12 h cycles, and no daily cycle. The daily patterns of $\dot{V}O_2$ and peak $\dot{V}O_2$ did not always coincide with the activity period of the species. All nocturnal lizards tested had a lower energetic cost of locomotion (C_{min}) than diurnal lizards. Diurnal lizards from New Zealand also had low C_{min} values when compared with nocturnal geckos and diurnal lizards from lower latitudes. Thus, a low C_{min} appears to be related to activity at low temperatures rather than specifically to nocturnality. However, more data are required on lizards from high latitudes, including more New Zealand lizards, before the generality of this pattern can be confirmed. Also, based on correlations with lizards active at warmer temperatures, a low C_{min} only partially offsets the thermal handicap imposed on lizards that are active at low temperatures. Nocturnal lizards were found to have lower thermal sensitivities of metabolism (lower Q_{10} values) than diurnal lizards, indicating that their energy-dependent activity was not as sensitive to changes in environmental temperature.

The similarity of other metabolic processes among species with differing activity periods may be partly explained by the ability of nocturnal species to thermoregulate to

achieve higher temperatures during the day. The amplitudes of daily $\dot{V}O_2$ cycles and mass-specific $\dot{V}O_2$ did not differ among nocturnal and diurnal New Zealand lizards at low temperatures. The specific activity of the glycolytic enzyme lactate dehydrogenase (LDH) isolated from the tail muscle of lizards was also comparable among nocturnal and diurnal lizards over a range of biologically relevant temperatures. Thus, activity of lizards at low temperatures is not enabled by lower energy requirements over a 24 h period, elevation of metabolic rates, or biochemical adaptation of LDH to specific temperatures.

These results confirm that locomotion is more efficient in nocturnal lizards and diurnal lizards from New Zealand than lizards from elsewhere, but that other metabolic processes do not appear to differ among species. Additional physiological and behavioural adaptations may exist that complement the increased efficiency of locomotion, thus enabling nocturnal lizards to be active at low temperatures.

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CHAPTER 1
The paradox of nocturnal lizards: introduction & overview

1.1 The paradox

All biological processes are influenced by body temperature. In general, endotherms regulate their body temperatures through metabolic processes independently of ambient temperature, maintaining stable body temperatures required for physiological processes. Ectotherms gain their heat from external sources and many behaviourally regulate their body temperatures (Hochachka and Somero, 2002). Thus, ectotherms have the advantage of lower energy requirements than endotherms (Pough, 1980), but this comes at a cost as they are often unable to achieve optimal temperatures for biological processes (Hochachka and Somero, 2002). Nocturnality often involves activity at low temperatures, especially in temperate regions where ambient night temperatures can be extremely low. Nevertheless, many temperate reptiles have evolved nocturnality, including around half of the 80+ currently proposed lizard species in New Zealand (Hitchmough et al., (in press)).

In lizards, nocturnality presents a paradox. Nocturnal lizards prefer substantially higher body temperatures than are achievable during their active phase at night, and are active at thermally suboptimal temperatures. For example, many nocturnal lizards actively thermoregulate to reach high temperatures (25 °C+) during the day (e.g., Werner and Whitaker, 1978; Huey and Bennett, 1987; Tocher, 1992; Kearney and Predavec, 2000), but some nocturnal lizards can remain active at body temperatures as low as 10 °C (e.g., Werner and Whitaker). Both nocturnal and diurnal lizards have similar optimal temperatures for sprinting, implying that locomotory performance in nocturnal lizards is thermally suboptimal at night (Huey et al., 1989). Therefore, it is reasonable to infer that nocturnal lizards must gain an advantage from being nocturnal and must have some

physiological mechanism(s) to overcome the thermal handicap of activity at low temperatures.

Nocturnality presumably evolved in gekkotan lizards as an adaptation to their diet and inferior competitive ability to other lizards, such as iguanids (Vitt et al., 2003). However, the reasons for why they became nocturnal do not explain how they manage to remain active at suboptimal, low night temperatures. Few studies have delved into this interesting question, and those that do are primarily focused on the locomotor energetics of nocturnal lizards (e.g., Autumn et al., 1994; Farley and Emshwiller, 1996; Autumn et al., 1997; Autumn, 1999; Autumn et al., 1999). Research on locomotor energetics shows that nocturnal geckos have substantially lower energetic costs of locomotion than diurnal lizards, which partially, though not completely, offset reduced aerobic capacity at low temperatures (e.g., Autumn et al., 1994; Farley and Emshwiller, 1996; Autumn et al., 1997; Autumn et al., 1999).

The lizard families in New Zealand (Scincidae and Diplodactylidae[1]) (Gill and Whitaker, 2001; Han et al., 2004) provide an ideal model system in which to study nocturnality. Each family consists of two genera, one predominantly nocturnal (*Cyclodina* skinks and *Hoplodactylus* geckos) and the other predominantly diurnal (*Naultinus* geckos and *Oligosoma* skinks). Each family has a different evolutionary history with regard to nocturnality. Geckos are ancestrally nocturnal (Vitt et al., 2003), which means the diurnal geckos in New Zealand are secondarily diurnal. Conversely, skinks are ancestrally diurnal (Vitt et al., 2003), but some species have evolved nocturnality or are crepuscular (active in the twilight). Although the phylogenetic relationships of New Zealand lizards are not completely resolved, it is apparent that rapid speciation has occurred in both the skinks and geckos (Hitchmough, 1997; Hickson et al., 2000). The advantage of analysing closely related species is that

[1] The New Zealand geckos have recently had a family name change from Gekkonidae to Diplodactylidae (Gill and Whitaker, 2001; Han et al., 2004). Diplodactylidae is used throughout this thesis where data are not already published as Gekkonidae.

differences related to evolutionary history are minimised and can be separated from differences due to activity period.

Before discussing the reasoning and methodology behind addressing the nocturnality paradox, it is important to define some key terms. Throughout this thesis nocturnal species are defined as those that emerge and are active during the scotophase (dark), diurnal species as those that emerge and are active during the photophase (light), and crepuscular species as those that emerge and are active during the twilight (dawn and dusk). However, many nocturnal lizards also emerge during the photophase (e.g., *H. maculatus*; Werner and Whitaker, 1978), and some diurnal lizards take advantage of beneficial environmental conditions, emerging during the scotophase (e.g., *Oligosoma striatum* and *O. zelandicum*; Neilson et al., 2004). Thus, the definition of a species as nocturnal or diurnal, made in relation to activity phase, is not absolute. Despite this ambiguity, 'nocturnal' species forage widely at low body temperatures at night and rarely move large distances during the day. Conversely, 'diurnal' species may emerge at low ambient and body temperatures (e.g., at dawn) but never roam widely until higher body temperatures are attained. This thesis explores possible physiological adaptations of nocturnal lizards that may enable them to be active at low temperatures.

1.2 Thesis structure

The ability of nocturnal lizards to remain active at low night temperatures is investigated by comparing four aspects of reptilian physiology among nocturnal and diurnal lizards. A multi-species approach is used to separate evolutionary history from potential links between physiology and activity period. The physiological measures investigated are daily rhythms of metabolic rate (Chapter 3; accepted with revisions), metabolic rate at low and high temperatures (Chapter 4), locomotor energetics (Chapter 5), and biochemical adaptation (Chapter 6; in press).

Before beginning research on the nocturnality paradox the validity of the assumption that the rate of oxygen consumption is not higher during an animal's first exposure to

experimental procedures was tested. This research has been published and the manuscript is reproduced in Chapter 2. A further test of this assumption is outlined briefly in Chapter 5. Since this thesis is organised as a series of independent manuscripts for publication, there is some repetition of general information in the individual chapters. Chapter 7 includes a synthesis of all chapters, providing a synopsis of what is currently known about the nocturnality paradox and suggesting future directions for research. Due to a lack of published studies on basic biology of many species, a published field guide (Gill and Whitaker, 2001) is used throughout this thesis as a basis species activity periods. In Chapter 7 the dichotomy between published accounts of species activity periods and their physiology is evaluated. The appendices include statistical and methodological information and publications arising from this research.

1.3 What physiological mechanisms could explain the nocturnality paradox?

1.3.1 Daily rhythms of metabolic rate

Many biochemical, physiological and behavioural parameters exhibited by animals have daily fluctuations (Sheeba et al., 1999; Wagner-Smith and Kay, 2000). Daily fluctuations in the rate of oxygen consumption ($\dot{V}O_2$) may, among other functions, serve as an energy conserving mechanism during the inactive part of the day (reviewed by Bennett and Dawson (1976)). Since some nocturnal species thermoregulate to high temperatures throughout the day and are active at low temperatures at night (e.g., Werner and Whitaker, 1978; Tocher, 1992; Rock et al., 2000, 2002), they may have less pronounced patterns of phase and amplitude of $\dot{V}O_2$ at a given temperature than diurnal species. Less pronounced cyclical patterns in $\dot{V}O_2$ may in turn be part of an energy conserving mechanism employed by nocturnal lizards.

Question: *Do nocturnal lizards have less pronounced daily rhythms of $\dot{V}O_2$ than diurnal lizards?*

1.3.2 Absolute levels of metabolic rate

Measures of metabolism in physiological ecology can identify potential energetic constraints that operate on individual organisms, as well as provide mechanistic explanations for large scale ecological and evolutionary patterns (Zaiden, 2003). Physiological processes, such as rate of oxygen consumption ($\dot{V}O_2$), generally increase with temperature (e.g., Bennett and Dawson, 1976; Withers, 1992), but some reptiles show different patterns, in that their $\dot{V}O_2$ has a temperature independent plateau (e.g., garter snakes *Thamnophis sirtalis parietalis*; Aleksiuk, 1971). Nocturnal lizards may have a greater temperature range over which metabolic activity can take place, and may also have a lower thermal sensitivity of $\dot{V}O_2$ than diurnal lizards, resulting in overall greater metabolic stability and less dependence of $\dot{V}O_2$ on body temperature.

Question: *Do nocturnal lizards have higher $\dot{V}O_2$ at low temperatures compared with diurnal lizards?*

1.3.3 Locomotor energetics

Nocturnal geckos have evolved a low energetic cost of locomotion (C_{min}; energy required to move a gram of body mass over one kilometre) which increases maximum aerobic speed and partially offsets the decrease in maximum $\dot{V}O_2$ caused by activity at low night temperatures (Autumn et al., 1994; Farley and Emshwiller, 1996; Autumn et al., 1997; Autumn et al., 1999). All lizards are ancestrally diurnal, and nocturnality has arisen independently in geckos, snakes, and skinks (Pianka and Vitt, 2003; Vitt et al., 2003). If a low C_{min} is present in all nocturnal squamates, then the evolution of a low C_{min} and nocturnality may be connected. However, if a low C_{min} is not present in other nocturnal squamates, C_{min} may have evolved independently of nocturnality, or may even appear in lizards that are active at low temperatures (i.e., nocturnal lizards, as well as diurnal lizards from temperate regions).

Question: *Do nocturnal lizards have a lower energetic cost of locomotion than diurnal lizards?*

1.3.4 Biochemical adaptation

Lactate dehydrogenase (LDH) is a key metabolic enzyme involved in the glycolytic pathway and is correlated with endurance (Guderley, 2004). There are multiple LDH isozymes, each with different temperature profiles (Conn et al., 1987). Thus, if different isozymes are expressed as the temperature changes, LDH can function over a wide range of temperatures. Many ectothermic species show temperature adaptation of enzymes. For example, fish adapted to polar conditions have higher total metabolic enzyme activity of LDH than those adapted to tropical conditions (Hochachka and Somero, 2002; Kawall et al., 2002). The locomotor performance of reptiles may also be less dependent on attaining a 'preferred' or 'optimal' body temperature range than previously thought (Seebacher et al., 2003). Nocturnal lizards may respond to low temperatures during their activity period by changing their biochemical characteristics rather than attempting to maintain stable body temperatures. It is also possible that the metabolic enzymes have temperature optima that coincide with lower temperatures.

Question: *Do nocturnal lizards have higher LDH activity at all temperatures compared to diurnal lizards?*

1.4 Summary of approach and some experimental limitations

The paradox of nocturnal lizards is explored here by using an integrated physiological and phylogenetic approach. The physiological measures that are investigated include daily rhythms of $\dot{V}O_2$, absolute values of metabolic rate, locomotor energetics and biochemical adaptation among nocturnal and diurnal lizards. The New Zealand lizards are an ideal group in which to study the nocturnality paradox as both lizard families have a different evolutionary history in relation to nocturnality, enabling differences in activity period to be separated from differences in phylogeny.

Around 41% of the extant herpetofauna in New Zealand is restricted to offshore islands, which is especially true of endangered and rare species (Towns and Daugherty, 1994).

Thus, as the research presented here includes physiological measures of endangered and rare lizard species, much of the research was restricted to offshore islands. Undertaking physiological measurements on islands was in some cases restrictive and imposed limitations on both experimental design and timing of experiments. For example, the metabolic experiments were not able to be undertaken at the same time of year (to limit seasonal variation) on islands and the mainland. To help counter this problem mainland lizards were acclimated to spring conditions in the laboratory and the common gecko *H. maculatus* was measured at all sites as a control. Also, due to unforeseen circumstances, such as a gecko shorting the solar panel battery storage, electricity was limited to day hours on the islands, with any night measures of metabolic rate requiring the use of a portable generator and limited gasoline supplies. Therefore, metabolic experiments were restricted to a few overnight experiments, and, to enable a statistically robust sample size to be obtained, were only undertaken at one temperature. Subsequently metabolic rates were not all standard or resting, and comparisons had to be made of these different measures.

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CHAPTER 2

Conditioning reduces metabolic rate and time to steady-state in the lizard *Naultinus manukanus* (Reptilia: Gekkonidae)[1]

2.1 Abstract

The rate of oxygen consumption ($\dot{V}O_2$) is commonly used as a measure of whole organism metabolic rate, but requires the animal to be motionless and at rest. Few studies have measured whether animals that appear motionless are truly at rest, or whether being in a novel environment elevates metabolic rate. I investigated whether conditioning of the gecko *Naultinus manukanus* to experimental procedures influenced the $\dot{V}O_2$ and probability of achieving a constant rate of oxygen consumption. Metabolic rate was measured at 24 °C in 22 individuals until a steady-state was achieved, or for 80 minutes if no steady-state was reached, once a day on five consecutive days (five trials). Geckos in the first trial, when compared with subsequent trials, had a significantly higher mass-adjusted $\dot{V}O_2$ (0.89 ± 0.06 vs. 0.67 ± 0.05 ml O_2 h^{-1} respectively), and time to reach a steady-state $\dot{V}O_2$ (66 ± 8 vs. 47 ± 3 min respectively), as well as a significantly lower probability of reaching a steady-state $\dot{V}O_2$ (24% vs. 74% respectively). In conclusion, there may be hidden inaccuracies in studies that do not condition animals and that at least one conditioning trial should be used to obtain a metabolic rate at rest for small lizards.

[1] **Based on:** Hare, K. M., Pledger, S., Thompson, M. B., Miller, J. H., and Daugherty, C. H. 2004. Conditioning reduces metabolic rate and time to steady-state in the lizard *Naultinus manukanus* (Reptilia: Gekkonidae). *Comparative Biochemistry and Physiology, Part A. 139*: 245-250.

2.2 Introduction

Rates of oxygen consumption ($\dot{V}O_2$) are commonly used as an index of metabolic rate in physiological studies that measure, for example, specific dynamic action (e.g., Overgaard et al., 1999; Wang et al., 2001), reproductive energetics (e.g., Thompson and Russell, 1998; Robert and Thompson, 2000a), and the effects of environmental parameters on whole organism energetics (e.g., aestivation (Kennett and Christian, 1994), season (Heusner and Jameson, 1981), sloughing (Taylor and Davies, 1981), thermal acclimation (Tocher and Davison, 1996), and time of day (Currens et al., 2002)). Measures of $\dot{V}O_2$ are generally made on animals that are assumed to be at rest because they are sitting quietly within the experimental apparatus during measurements (Andrews and Pough, 1985). Consideration, however, is rarely given to the possibility that the animal has an elevated metabolic rate from being in a novel environment. Although some researchers have made reference to, or have tried to account for, possible effects of novel environments (e.g., Snyder, 1975; Snyder and Weathers, 1976; Tsuji, 1988; Tocher and Davison, 1996; Hopkins et al., 2004), no one has systematically quantified the effects of novel environments on metabolic rates. Reptiles may remain motionless and apparently at rest, but still have elevated $\dot{V}O_2$. For example, the use of a mask to measure $\dot{V}O_2$ of a monitor lizard (*Varanus gilleni*) approximately triples its metabolic rate, compared to the rate measured in an unrestrained animal in a chamber, even though the lizard appears to be at rest under both conditions (Bickler and Anderson, 1986).

The ability to measure an accurate metabolic rate is essential to inter-specific comparisons and the subsequent analyses of, for example, scaling relationships (Andrews and Pough, 1985). Hence, factors that may elevate metabolic rate need to be identified. As part of my metabolic study with lizards, I wanted to ensure that the measurements of metabolism were made under resting conditions. Therefore, five trials were conducted using flow-through respirometry to measure $\dot{V}O_2$ in unrestrained *Naultinus manukanus* to ask the following questions: 1) Is steady-state $\dot{V}O_2$ (as indicated by a horizontal steady value vs. time on the recorded trace) measured during

earlier trials significantly higher than steady-state $\dot{V}O_2$ measured at later trials? 2) If so, what is the minimum number of trials needed before steady-state $\dot{V}O_2$ values do not differ significantly among trials? 3) Are the $\dot{V}O_2$ measurements more likely to reach a steady-state within a given time frame if the number of conditioning trials is increased? 4) Do animals show individual variation in relation to the metabolic rate obtained and the probability of reaching a steady-state?

2.3 Materials and methods

2.3.1 Animal collection and husbandry

Naultinus manukanus is an arboreal, diurnal, viviparous gecko from the Marlborough region of New Zealand (Gill and Whitaker, 2001). Twenty-two adult *N. manukanus* (10 males and 12 non-pregnant females) were collected from Stephens Island (Takapourewa), New Zealand (40°35'S 173°55'E) from 9 to 14 March 2003. Adult males were distinguished from females by inspection of the ventral tail base for protruding hemipenal sacs (Gill and Whitaker, 2001). Reproductive status of females was determined by abdominal palpation (see Cree and Guillette (1995) and Wilson and Cree (2003) for information on accuracy of this procedure in other New Zealand geckos).

Metabolic rates of all geckos were measured between 14 April and 2 May 2003. Geckos were kept at Victoria University of Wellington (VUW) in groups of three in metal enclosures (700 x 580 x 350 mm) with lids covered with 1 mm square mesh (eight pregnant geckos were not included in the study due to the possibility of elevated metabolic rate during pregnancy (DeMarco, 1993; Robert and Thompson, 2000a)). Each enclosure had grass, periodically changed leaf litter and fresh tree foliage (*Coprosma repens*) for cover. Water was provided *ad libitum* in shallow dishes. The temperature of the room ranged from 18 to 23 °C, and photoperiod was on a 12:12 light:dark cycle (on at 0600 h). Lizards were fed *ad libitum* with mealworm larvae (*Tenebrio molitor*), every

9 to 12 days with blowflies (*Lucilia sericata*), and intermittently with moths (Lepidoptera).

Geckos were fasted for at least 72 h prior to the first measurement of metabolism. This is more than enough time to ensure a post-absorptive state in small lizards (Coulson and Hernandez, 1980; Robert and Thompson, 2000b). All lizards defecated within this time period and did not defecate during or after the respirometry trials. During the fasting period, the lizards were housed individually in 2 L plastic containers with a square of wire mesh (50 x 50 mm) in the lids for ventilation. Water was provided *ad libitum* on saturated paper towels.

2.3.2 Metabolic experiments

All $\dot{V}O_2$ were measured between 0700 h and 1730 h. Because the lizards are diurnal, this represents their active phase. The $\dot{V}O_2$ was measured for each lizard once a day for five consecutive days (five trials), and food was withheld during the five day period. Seven or eight lizards were placed within the experimental apparatus (Appendix 1A) on the morning of the day of $\dot{V}O_2$ measurement, and none were removed until the last measurement was taken at the end of the day. Therefore, each lizard spent 9-11 h in a chamber within the incubator each day. The time of day that each lizard was measured was randomised. The lizards were housed in the 2 L plastic containers for the rest of the time during the five-day experiments.

All lizards were thermally equilibrated to the experimental temperature (24 ± 0.5 °C) within the experimental apparatus (chamber and water bath) for at least 1 h prior to measurements. The temperature chosen for experiments is close to the mean temperature of basking *N. manukanus* (23.8 °C, range = 16.5 to 31.1 °C, mode = 25 to 26 °C; Werner and Whitaker, 1978). Temperature within the incubators was measured at 15 min intervals using data loggers accurate to 0.3 °C (StowAway® TidbiT®, Onset™ Computer Corporation, Massachusetts, USA).

Geckos were kept in individual clear Perspex™ respirometry chambers (0.084 L) within a water bath incubator during measurements of $\dot{V}O_2$. The incubator was completely enclosed with opaque sides, and a quiet room was used so that geckos were not disturbed by the presence of the researcher or by background noise. A reference chamber (no lizard present) was also included within the incubator to obtain the baseline oxygen concentrations. Measurement of oxygen concentration in the reference chamber was taken at the beginning and end of each $\dot{V}O_2$ measurement. Metabolic measurements were recorded until a steady-state was obtained (as indicated by the lowest horizontal steady value on the recorded trace) for at least 5 min, or up to a total of 80 min if no steady-state occurred.

Air was drawn through the respiratory and reference chambers at 12 ml min^{-1} from outside the building using a flow controller and pump (Sable Systems International Inc., Las Vegas, Gas Analyzer Sub-sampler). A soap bubble flow-through system was used to calibrate the flow controller (Long and Ireland, 1985). Air was not dried prior to being passed over the geckos since some New Zealand lizards have relatively high rates of water loss for their size (Neilson, 2002). However, the excurrent air from the chamber passed through a column of self-indication Drierite®, soda lime and then Drierite® again before entering the oxygen analyser (a two-channel Sable Systems FC-2). The scrubbed chamber air was continually compared with the scrubbed air from outside the building to ensure that atmospheric oxygen (20.94%) was used for the experiments.

Output from the oxygen analyser was recorded using Sable Systems (UI2) and MS Windows software. The animals were weighed immediately after removal from the chamber on a Sartørius™ top-loading balance. Barometric pressure was recorded at the beginning and end of each measurement series and the average pressure used in $\dot{V}O_2$ calculations. The steady-state $\dot{V}O_2$ was calculated with DATACAN (Sable Systems Inc. USA) using equations of Withers (1977).

2.3.3 Statistical analysis

Data were analysed using the statistics programme *R* (Ihaka and Gentlemen, 1996; Version 1.5.1). Statistical significance was assumed at $P < 0.05$. Data are expressed as mean ± 1 SE unless otherwise stated.

Only data from those individuals that reached a steady-state of oxygen consumption were used for $\dot{V}O_2$ analyses. It is impossible to get a true measure of oxygen consumption from individuals that do not reach a steady-state. Analyses of covariance (ANCOVA; adjusted for individual effects by subtracting the estimated individual coefficients) were used to investigate the categorical variables of trial (at five levels), time of day (at four levels) and sex on the dependent variable $\dot{V}O_2$. The levels used for time of day are 0700-0900 h, 0900-1100 h, 1100-1300 h and 1300-1730 h, with the last sample interval increased to enable a statistically robust sample size to be gained. Mass was included as a covariate in these analyses since expressing physiological data as a ratio (i.e. dividing by mass) does not always adequately remove the confounding effects of body size (Packard and Boardman, 1988). To allow for repeated measures, the linear mixed-effects function in *R* was employed (Pinheiro and Bates, 2000). In addition, a simpler model with trial-type at only two levels (1 for trial 1 (first trial) and 2 for trials 2-5 (subsequent trials)), was tested for comparison with five levels of trials using a likelihood ratio test. The model with trial-type at two levels (1 vs. 2) was confirmed as an acceptable simplification ($\chi^2 = 3.604$, 3 d.f., $P = 0.308$). I also explored the probability of individuals reaching a steady-state during the 80 min time frame over the five trials by using binomial models that included an "unsettled" category.

The time to steady-state (TSS) is another useful measure for deciding whether conditioning is important. Since this is similar to survival time data (time to an event), it is likely to have a skewed distribution with occasional large values. The reciprocal transformation $\frac{1}{x}$ is recommended as a means to achieve normally distributed data and for minimising the effects of right-censoring (Armitage, 1971), e.g., when an individual does not settle in the allocated 80 min. Settling rate (reciprocal of the TSS) was

analysed using analyses of variance, where the time spent in the chamber was censored data for those animals that did not settle. Confidence intervals (95%) were also calculated to estimate an upper bound for the TSS, as if the animals had been given longer to reach a steady-state and the data had not been right-censored. Diagnostic graphs confirmed normality and constant variance for the settling rate data used for the analyses.

The consistency of individual variation of the geckos was explored by classifying the metabolic responses into three categories: reaching a steady-state, reaching a higher than average metabolic rate, and reaching a lower than average metabolic rate. A three-by-three contingency table of initial state by subsequent state (at the next trial) used a Pearson's chi-squared statistic (χ^2) to test transition rates among the three categories.

2.4 Results

The mean mass of the 22 *N. manukanus* in this study was 7.3 ± 0.5 g (range = 5.1 to 10.1 g). Metabolic rate was significantly affected by mass ($P = 0.002$) and trial, with $\dot{V}O_2$ significantly lower after one trial ($P < 0.001$; Figure 2.1). Neither sex ($P = 0.718$) nor time of day ($P = 0.216$) influenced $\dot{V}O_2$. The least squares estimate of $\dot{V}O_2$ for trial 1 (for the average mass of all geckos; $n = 5$) was 0.888 ± 0.064 ml O_2 h^{-1}, and that for combined trials 2-5 ($n = 64$) was 0.672 ± 0.047 ml O_2 h^{-1} (Figure 2.2). The probability of reaching a steady-state by 80 min was significantly lower at trial 1 (24%) than subsequent trials (range = 60-82%, mean = 74%; $P < 0.001$), with no significant difference among subsequent trials (mean = 26%; $P = 0.413$; Figure 2.3). Only 5 of the 22 animals tested reached a steady-state in the first trial; whereas 13-16 animals reached steady-state in trials 2-5.

Settling rates were significantly longer for trial 1 (66.0 ± 7.5 min) than combined trials 2-5 (47.4 ± 2.6 min; $P < 0.001$), with no significant effect of sex ($P = 0.141$), mass ($P = 0.149$) or time of day ($P = 0.440$). The (asymmetric) 95% confidence intervals resulting

from the back-transformation were (55.0, 82.4) min for trial 1 and (43.9, 51.6) min for trials 2-5 with no overlap between intervals.

Individuals were more likely to return to the same behavioural state than to change behaviour among trials (χ^2 = 9.898, d.f = 4, P = 0.042; Table 2.1). However, individuals that did not reach a steady-state in a previous trial were also likely to have a higher than average metabolic rate on a subsequent trial. After allowing for mass and trial effect, 72% of the remaining variance occurs among individuals and 28% within individuals.

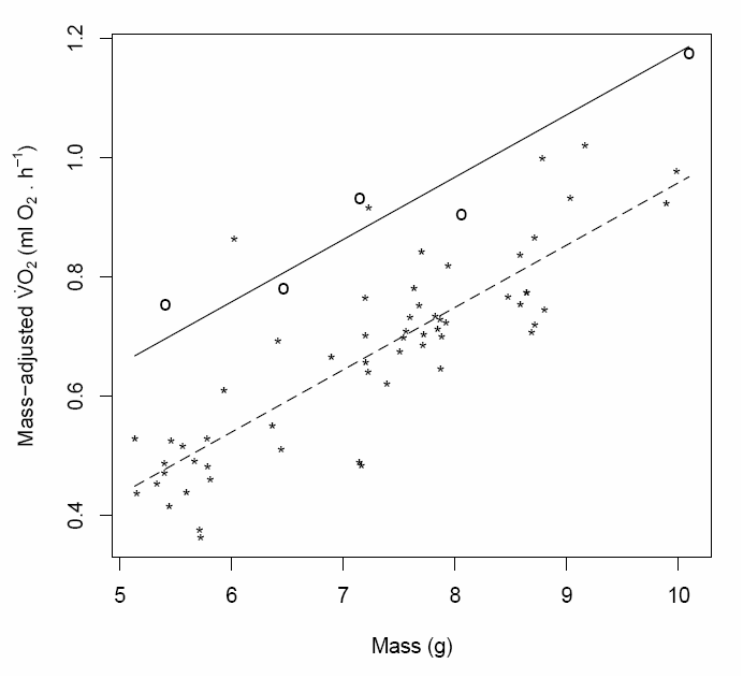

Figure 2.1: $\dot{V}O_2$ of *N. manukanus* at 24 °C for first trial (open circles) and subsequent trials (trials 2-5; stars), adjusted for individual effects by subtracting the estimated individual coefficients. ANCOVA lines are fitted (first trial = solid line; n = 5 individuals, r^2 = 0.969; subsequent trials = dashed line, n = 64 data points from 13-18 individuals per trial, r^2 = 0.862). Each trial was on a consecutive day, and each lizard spent between 9 and 11 h within the chamber each day. Mean mass = 7.3 ± 0.5 g.

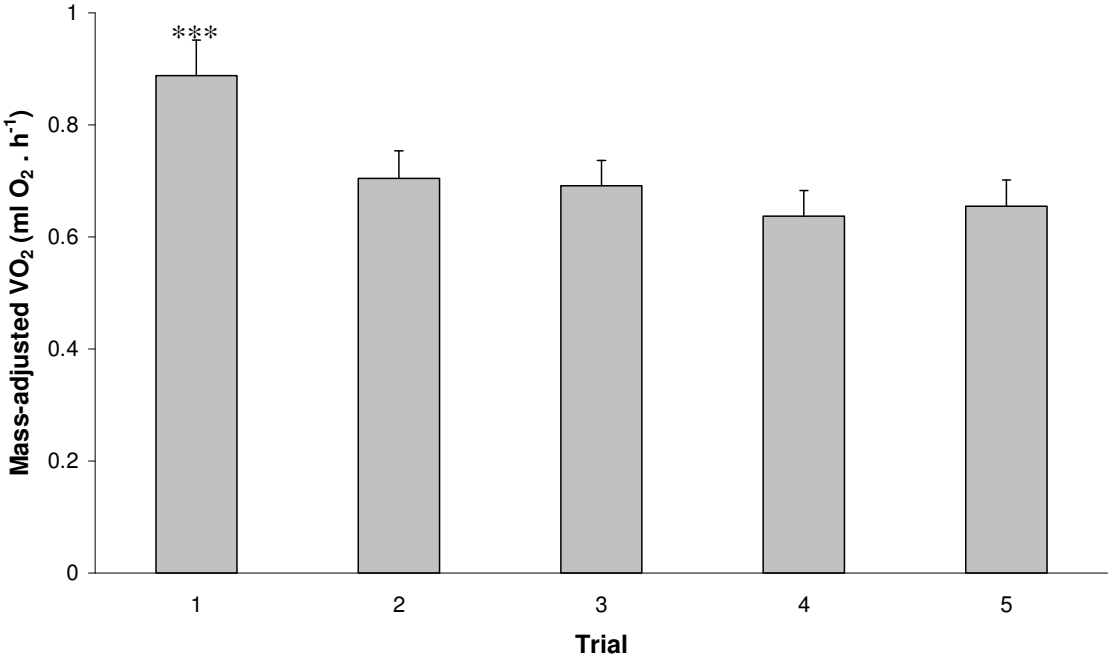

Figure 2.2: $\dot{V}O_2$ residuals corrected for body mass of *N. manukanus* over five trials at 24 °C. Each trial was on a consecutive day, and each lizard spent between 9 h and 11 h within the chamber each day. Mean mass = 7.3 ± 0.5 g; mass range = 5.1 to 10.1 g; n = 5, 13, 18, 17, 16 (for individuals that reached a steady-state of $\dot{V}O_2$ for trials 1 to 5, respectively; error bars are 1 SE; *** = $P < 0.001$.

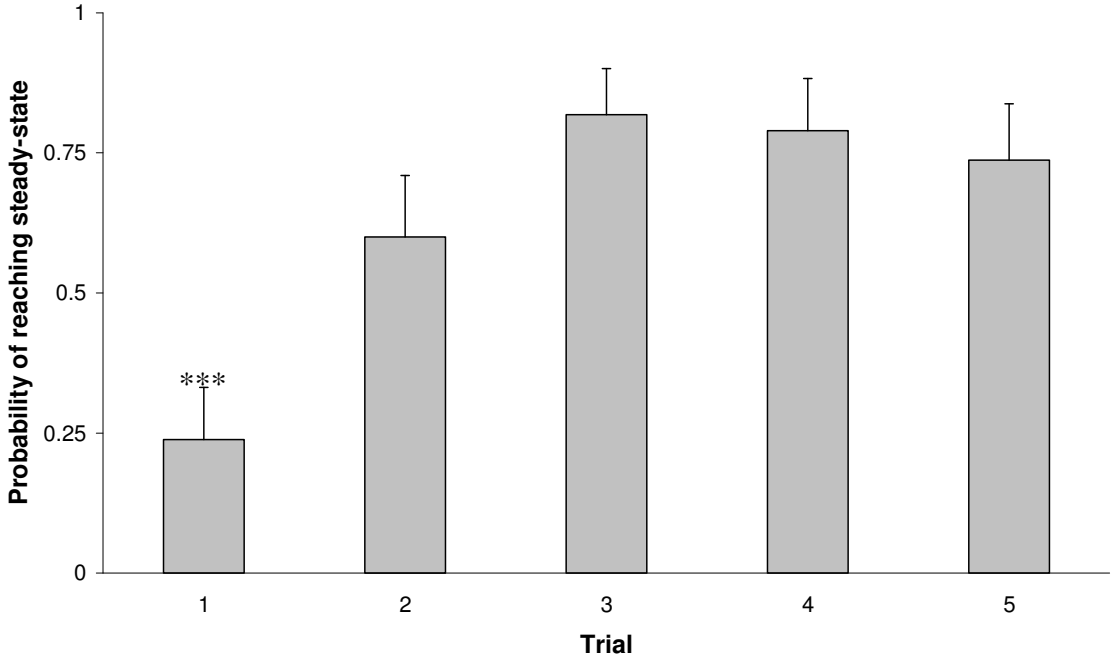

Figure 2.3: Probability of reaching a steady-state $\dot{V}O_2$ (as indicated by the lowest horizontal steady value on the recorded trace) by 80 min for 22 *N. manukanus* over five trials at 24 °C. Each trial was on a consecutive day, and each lizard spent between 9 h and 11 h within the chamber each day. Error bars are 1 SE; *** = $P < 0.001$.

Table 2.1: Contingency table of initial behavioural state by subsequent behavioural state using a Pearson's chi-squared statistic test of observed and expected transition rates among the three categories: 1) No steady-state = no steady-state of $\dot{V}O_2$ by 80 min (as indicated by the lowest horizontal steady value on the recorded trace); 2) High $\dot{V}O_2$ = a higher than average $\dot{V}O_2$; 3) Low $\dot{V}O_2$ = a lower than average $\dot{V}O_2$. Positive Pearson's residuals indicate positive associations, i.e. an increased likelihood of one behavioural state following another.

First behaviour	Subsequent behaviour: No steady-state		High $\dot{V}O_2$		Low $\dot{V}O_2$	
	Observed (Expected)	Pearson's residual	Observed (Expected)	Pearson's residual	Observed (Expected)	Pearson's residual
No steady-state	10 (8.5)	0.509	12 (10.1)	0.586	8 (11.3)	-0.995
High $\dot{V}O_2$	6 (6.2)	-0.097	10 (7.4)	0.942	6 (8.3)	-0.806
Low $\dot{V}O_2$	5 (6.2)	-0.497	3 (7.4)	-1.626	14 (8.3)	1.967

2.5 Discussion

In this study, novel experimental procedures increased $\dot{V}O_2$ in the first, but not subsequent, measurements of the diurnal gecko *N. manukanus*. A single conditioning trial also decreased the time to reach a steady-state. There were no effects of sex or time of day on any of the response variables. Many studies use arbitrary conditioning periods (for a few days, overnight, or for a couple of hours) to equilibrate animals within chambers to enable the specified body temperature to be achieved (e.g., Earll, 1982; Fusari, 1984; Beaupre et al., 1993; Thompson and Daugherty, 1997; Wang et al., 2003). The differing conditioning periods are likely to generate unwanted variation in the $\dot{V}O_2$ measurements. Minor disturbances may also elevate the $\dot{V}O_2$ of some animals for long periods of time (Bennett, 1978).

Some researchers have considered the influence of novel environments on measures of metabolic rate. For example, the gecko *H. maculatus* apparently does not require experimental conditioning, with no significant difference in metabolic rate over 24 h periods after 2 h of equilibration (Tocher and Davison, 1996). However, some data were discarded from that and other studies, due to some individuals not reaching a steady-

state of oxygen consumption over the measurement time-frame (e.g., Coulson and Hernandez, 1980; Tocher and Davison, 1996).

Other researchers that have discussed or tried to account for the possibility of novel environments influencing metabolic rate, have either randomised temperatures during the first measurement (and thus swamp possible novel environmental effects and probably increase overall variation in their measurements), assumed that a state of rest has been reached without making $\dot{V}O_2$ measurements over the conditioning period, or provided conditions that are assumed to be less 'stressful' (e.g., Tsuji, 1988; Beaupre et al., 1993; Hopkins et al., 2004). Overall, however, the data from previous research and ours suggests that either the result of being in a novel environment is different among species, or that the time to settle may differ among species. It also appears that either 24 h, or a single, lengthy conditioning trial, may be the minimum requirement for metabolic rate measures that are truly representative of relaxed animals, at least for squamates (e.g., Feder and Feder, 1981; Tsuji, 1988).

The time taken to condition animals to experimental procedures is minimal compared with the benefit of obtaining metabolic results from conditioned animals that can be compared with confidence across studies. Conditioning also has the added benefit that a steady-state of $\dot{V}O_2$ will be achieved in a significantly shorter period of time, and less data will be discarded as more animals will reach a steady-state during the measurement timeframe. Therefore, at least one conditioning trial is recommended to decrease the time required to reach a steady-state, and increase the probability of animals reaching a steady-state during measurements, and subsequently increase the overall sample size.

There is substantial individual variation among measures of metabolic rate, or reaching a steady-state of oxygen consumption, in *N. manukanus.* Other researchers have also demonstrated consistency of variation in metabolic rates, indicating good repeatability of measures of metabolic rate in a single individual (e.g., Garland and Else, 1987; Garland and Bennett, 1990; Marais and Chown, 2003; Nespolo et al., 2003). Thus, each

individual has a characteristic $\dot{V}O_2$ response. This variation in metabolic rates among individuals may be due to inherent variations in metabolic processes (e.g., Bennett and Dawson, 1976; Baldwin et al., 1995); however, other factors, such as technical and statistical artefacts, or sporadic movement of individuals within the experimental apparatus may play a role (Feder and Feder, 1981; Hopkins et al., 2004). It is also likely that some animals have an intrinsically active phenotype and may never settle to experimental procedures. This conclusion is supported by the fact that, in our study, animals that did not reach a steady-state of $\dot{V}O_2$ were more likely to either not reach a steady-state, or have a generally higher than average metabolic rate, in subsequent trials.

The elevated metabolic rate at trial 1 is not due to the effects of specific dynamic action (SDA) due to digestion (e.g., Coulson and Hernandez, 1980; Hopkins et al., 2004) since the geckos had at least 72 h to reach a post-absorptive state, and there has been no reported effect of SDA in other small lizards after 72 h of fasting (Coulson and Hernandez, 1980; Robert and Thompson, 2000b). In addition, the gut passage time of a similar sized gecko from New Zealand (*H. maculatus*), when held at 17°C, is never longer than 48 h (Lawrence, 1997), and our lizards were kept at warmer temperatures. A gradual decrease in metabolic rate over time, as seen in other squamates, would also be expected (e.g., Robert and Thompson, 2000b), not the sudden drop in metabolic rate seen in our experiments. Elevated $\dot{V}O_2$ at the first exposure to experimental conditions may be related to an increase in corticosterone, as seen in some captive reptiles (Tyrrell and Cree, 1998). Thus, the elevated metabolic rates observed during trial 1 reflect non-conditioned animals.

Andrews and Pough (1985) asked "why do metabolic rates (of squamates) during inactivity vary?" and concluded that ecological category (day-active predator, herbivore, reclusive predator or fossorial predator), as well as season, temperature and metabolic state (resting metabolic rate vs. standard metabolic rate) played a large part in the metabolic variation seen among species. This information is what researchers are seeking. However, support for the conclusion that some of the variation of metabolic

rates obtained by different researchers is confounded by effects of novel environments as well as the variable lengths of conditioning to experimental procedures is provided. The results of this investigation have implications for studies of metabolic rate, especially those that make repeated measurements of the same animals.

It is clear that researchers should take care to confirm that animals are conditioned to experimental procedures prior to initial measurement of $\dot{V}O_2$, which can be done in two ways: 1) measure the $\dot{V}O_2$ of a sample of animals at each trial until there is no significant difference in oxygen consumption among trials, or 2) use lengthy (24 h +) acclimation times, or at least one conditioning trial within the experimental apparatus prior to initial measurement. However, as length of conditioning appears to differ among species, I recommend that more than one conditioning trial is used if option 2 (above) is chosen. Data from animals that do not settle should be discarded as accurate calculations are impossible without a horizontal (steady state) $\dot{V}O_2$. It may be useful, however, to keep the factor 'no steady-state' in tables of data to ascertain whether the proportion of individuals settling is species-specific. As the $\dot{V}O_2$ of animals is highly variable within a population, I also express caution when small samples are used, since by chance the animals selected may all be those that are, for example, intrinsically active.

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CHAPTER 3

Daily patterns of metabolic rate among New Zealand lizards (Reptilia: Lacertilia: Diplodactylidae & Scincidae)[1]

3.1 Abstract

In addition to the effects of temperature fluctuations on metabolic rate, entrained endogenous rhythms in metabolism, which are independent of temperature fluctuations, may be important in overall energy metabolism in ectotherms. Daily entrained endogenous rhythms may serve as energy conserving mechanisms during an animal's active or inactive phase. However, as nocturnal lizards often take advantage of thermal opportunities during the photophase (light), their daily metabolic rhythms may be less pronounced than those of diurnal species. The rate of oxygen consumption ($\dot{V}O_2$) was measured as an index of metabolic rate of eight temperate lizard species (four nocturnal, three diurnal and one crepuscular/diurnal; n = 7 to 14) over 24 h at 13 °C and in constant darkness to test whether daily patterns (including amplitude, magnitude and time of peak $\dot{V}O_2$) of metabolic rate in lizards differ with activity period. I also tested for phylogenetic differences between skinks and geckos. Three daily patterns were evident: 24 h cycle, 12 h cycle or no daily cycle. The skink *Cyclodina aenea* has a crepuscular pattern of $\dot{V}O_2$. In four other species, $\dot{V}O_2$ increased with, or in anticipation of, the active part of the day, but three species had rhythms offset from their active phase. Although not correlated with activity period or phylogeny, amplitude of daily variation in $\dot{V}O_2$ may be correlated with whether a species is temperate or tropical. In conclusion, the metabolic rate of many species does not always correlate with the recorded activity period. The dichotomy of ecology and physiology may be clarified by more in-depth studies of species behaviours and activity periods.

Based on: Hare, K. M., Pledger, S., Thompson, M. B., Miller, J. H., and Daugherty, C. H. Daily patterns of metabolic rate among New Zealand lizards (Reptilia: Lacertilia: Diplodactylidae & Scincidae). *Physiological and Biochemical Zoology* (accepted with revisions)

3.2 Introduction

Many biochemical, physiological and behavioural parameters exhibited by animals have daily fluctuations (Sheeba et al., 1999; Wagner-Smith and Kay, 2000). Most of these fluctuations persist when animals are maintained under constant environmental conditions, indicating that the patterns are driven by endogenous factors (Tosini et al., 2001). Reptiles are ectotherms, and thus ambient temperature influences their physiological, behavioural and ecological characteristics, including activity patterns (e.g., Bennett and Dawson, 1976; Bennett, 1982; Huey, 1982). A disadvantage of ectothermy is that individuals cannot always achieve their optimal temperature (e.g., for activity, digestion etc). Temperature-dependent metabolic support systems constrain the activity and behavioural ability of lizards at sub-optimal body temperatures (Bennett, 1980). For example, nocturnal and diurnal species do not differ in optimal temperatures for sprinting, implying that performance in nocturnal lizards may be suboptimal at low night temperatures (Huey et al., 1989). Also, nocturnal lizards in the laboratory will often select body temperatures higher than those achievable during their active period (e.g., Huey and Bennett, 1987). Thus, physiological mechanisms that enable activity at low temperatures may have evolved in nocturnal lizards.

Studies of metabolism are important in physiological ecology as they identify potential energetic constraints that operate on individual organisms, and provide mechanistic explanations for large scale ecological and evolutionary patterns (Zaiden, 2003). Daily fluctuation in the rate of oxygen consumption ($\dot{V}O_2$) may, among other functions, serve as an energy conserving mechanism during the inactive part of the day (reviewed by Bennett and Dawson (1976)). However, some nocturnal species are active throughout both the night and day. For example, the nocturnally foraging geckos *Hoplodactylus maculatus* and *H.* aff. *maculatus* "Canterbury" (as *H. maculatus* in Tocher (1992)) bask 'indirectly' (under substrate) in the field and actively seek warm sites during the day (e.g., Werner and Whitaker, 1978; Tocher, 1992; Rock et al., 2002). Diurnal thermoregulation by nocturnal species is thought to facilitate physiological processes

such as digestion and reproduction (e.g., Beaupre et al., 1993; Rock et al., 2000). Thus, nocturnal species may have less pronounced patterns of magnitude and amplitude of $\dot{V}O_2$ than diurnal species, as nocturnal species may thermoregulate at high temperatures throughout the day.

The New Zealand lizard fauna, which consists of two families (Scincidae and Diplodactylidae; Gill and Whitaker, 2001; Han et al., 2004), provides an ideal model system to study adaptations for a nocturnal lifestyle. Each family consists of two genera, one predominantly nocturnal and the other predominantly diurnal. The two families have different evolutionary histories in relation to nocturnality. Geckos are ancestrally nocturnal (Vitt et al., 2003), which means the diurnal geckos in New Zealand are secondarily diurnal. Conversely, skinks are ancestrally diurnal (Vitt et al., 2003), but some species in New Zealand have evolved nocturnality or are crepuscular. Also, New Zealand has a temperate climate with relatively cool summers and mild winters compared with other locations inhabited by reptiles (Cree, 1994; NIWA 2005). Some New Zealand lizards remain active at body temperatures as low as 10 °C (Werner and Whitaker, 1978). Thus, amplitudes and magnitudes of $\dot{V}O_2$ of New Zealand lizards may be more pronounced when compared with lizards from relatively warm, stable climates, such as the tropics.

The hypothesis that nocturnal lizards have less pronounced daily patterns of $\dot{V}O_2$ than diurnal and crepuscular lizards was tested, by investigating the daily patterns of $\dot{V}O_2$ in eight lizard species with differing activity periods (nocturnal, diurnal and diurnal/crepuscular). Specifically, I asked: 1) What are the daily patterns of $\dot{V}O_2$ in nocturnal, diurnal and diurnal/crepuscular lizards? 2) Do the peaks of $\dot{V}O_2$ correspond to predicted peak activity times of the lizards? 3) Does amplitude and magnitude of $\dot{V}O_2$ differ with activity period or family? 4) Do temperate species differ in amplitude and magnitude of $\dot{V}O_2$ from tropical species?

3.3 Materials and methods

I measured $\dot{V}O_2$ of eight lizard species over 24 h: three nocturnal gecko species (*Hoplodactylus maculatus*, *H. chrysosireticus* and *H. stephensi*), one diurnal gecko species (*Naultinus manukanus*), two diurnal skink species (*Oligosoma nigriplantare polychroma* and *O. zelandicum*), one crepuscular/diurnal skink species (*Cyclodina aenea*) and one nocturnal skink species (*C. macgregori*). The activity period of each species was based on a published field guide (Gill and Whitaker, 2001). Because *H. maculatus* is a species complex (Hitchmough, 1997), I ensured that the populations were a single species (R. A. Hitchmough pers. comm.; New Zealand Department of Conservation).

3.3.1 Animal collection and husbandry

All animals were collected within the latitudinal range 40° 50' 35" to 41° 20' 83" in the Cook Strait region of New Zealand. *Hoplodactylus maculatus*, *H. stephensi* and *N. manukanus* were captured on Stephens Island (Takapourewa) in November 2002 and *H. chrysosireticus* and *C. macgregori* on Mana Island in November 2003. *Cyclodina aenea*, *H. maculatus*, *O. n. polychroma* and *O. zelandicum* were captured on the mainland in the greater Wellington region between January and April 2004. Only adult males and non-pregnant females were tested as $\dot{V}O_2$ may be elevated in pregnant individuals (e.g., DeMarco, 1993; Robert and Thompson, 2000a). Adult males were distinguished from females by inspection of the ventral tail base for protruding hemipenal sacs in geckos and hemipene eversion in male skinks (Gill and Whitaker, 2001; Harlow, 1996). Reproductive status of females was determined by abdominal palpation (see Cree and Guillette (1995) and Wilson and Cree (2003) for information on accuracy of this procedure in New Zealand geckos).

All animals collected on islands had their $\dot{V}O_2$ measured at field stations, to minimise stress of transportation and captivity, thus there were some differences in holding conditions between islands and mainland New Zealand. To assess the potential effects

of seasonal timing of measurement between lizards caught on the mainland and islands the widespread and locally abundant species *H. maculatus* was used as a control group (Hitchmough, 1997; Gill and Whitaker, 2001).

All island lizards were housed individually in 2 L plastic containers with a square of 1 x 1 mm wire mesh (50 x 50 mm) in the lids for ventilation and small pieces of vegetation (*Coprosma repens*) as cover. Wet paper towels provided water *ad libitum*. All individuals were kept for a maximum of ten days except for three *H. stephensi* that, due to the rarity and elusiveness of the species (Cree, 1992; Hare and Cree, 2005), were kept for three weeks until the entire sample was collected. The three *H. stephensi* readily ate moths (Lepidoptera) during this time. Room temperature ranged from 11-25 °C and photoperiod during November in both years was 14:10 light:dark (sunrise at ~0600 h). Times are recorded in New Zealand daylight-savings time (GMT + 13 h).

All mainland lizards were held in captivity at Victoria University of Wellington (VUW) in the same room to acclimate them to identical light and temperature regimes (3 to 4 weeks). Room temperature ranged from 16-25 °C, and photoperiod was on a 12:12 light:dark cycle (on at 0600 h). Lizards were kept individually in transparent plastic boxes (215 x 330 x 110 mm) with 1 x 1 mm wire mesh (165 x 120 mm) in the lid for ventilation until experiments were undertaken (3 to 4 weeks after capture). Each transparent plastic enclosure had 30 mm depth of leaf litter provided as cover. Food (mealworm larvae (*Tenebrio molitor*) and/or canned, pureed pear (Watties$^{TM)}$)) and water were supplied *ad libitum.*

3.3.2 Metabolic experiments

To reduce the possible effects of stress of novel environments, I conditioned all lizards to the experimental procedures at least three times prior to the first $\dot{V}O_2$ measurements (Hare et al., 2004). Lizards were fasted for at least 72 h prior to measurements, except for the larger skink *C. macgregori* which was fasted for at least 96 h to ensure a post-

absorptive state (Coulson and Hernandez, 1980; Robert and Thompson, 2000b; Hopkins et al., 2004). All lizards defecated within this time period and did not defecate during or after the respirometry trials. During the fasting period, lizards were housed individually in 2 L plastic containers with a square of 1 x 1 mm wire mesh (50 x 50 mm) in the lids for ventilation. Saturated paper towels provided water *ad libitum,* and food was withheld during the experimental period.

The slight difference in experimental design on mainland and islands reflects the availability of electricity at the different sites. All $\dot{V}O_2$ in mainland lizards were measured in single individuals continuously over the scotophase (dark). Each lizard was placed individually into the experimental apparatus (Appendix 1A) in the afternoon, measured overnight, and removed the following morning. All other measures of $\dot{V}O_2$ (mainland photophase and all island measures) were taken periodically (once every 4-6 h) during the day or night. During this sampling, seven or eight lizards were placed individually into respirometry chambers within the experimental apparatus on the morning of the day of $\dot{V}O_2$ measurement, and removed after the last measurement was taken either that evening or the following morning. Individuals were sampled between three and six times a day.

For at least 1 hr prior to measurements (range = 1-4 h), lizards were thermally equilibrated to the experimental temperature (mean = 13.1 ± 0.1 °C) within the experimental apparatus (chamber and water bath). One hour acclimation is sufficient after animals have been conditioned to experimental procedures (Hare et al., 2004). The temperature chosen for experiments is an ecologically relevant nocturnal temperature that New Zealand lizards are likely to be exposed to during the scotophase, and at which nocturnal species are able to actively forage (Werner and Whitaker, 1978; Rock et al., 2002). Temperature within the incubator was measured at 15 min intervals using data loggers accurate to ± 0.3 °C (StowAway® TidbiT®, Onset™ Computer Corporation, Massachusetts, USA).

Sable Systems (UI2) and MS Windows software recorded output from the oxygen analyser. The animals were weighed immediately after removal from the chamber on a Sartørius™ top-loading balance. Barometric pressure was recorded at the beginning and end of each measurement series and the average pressure used in $\dot{V}O_2$ calculations. A steady-state $\dot{V}O_2$ was calculated with DATACAN (Sable Systems Inc. USA) using equations of Withers (1977).

Lizards were individually contained in clear Perspex™ respirometry chambers (84 ml for all geckos, 227 ml for *C. macgregori*, and 29 ml for all other skinks) within a water bath incubator for measurements of $\dot{V}O_2$. The incubator was completely enclosed with opaque sides, and a quiet room was used so that lizards were not disturbed by the presence of the researcher or by background noise. A reference chamber (no lizard present) was also included within the incubator to obtain baseline oxygen concentrations. Oxygen concentration in the reference chamber was measured at the beginning and end of each lizard $\dot{V}O_2$ measurement. Metabolic measurements were recorded until a steady-state was obtained (as indicated by the lowest horizontal steady-state value on the recorded trace) for at least 5 min.

A flow controller and pump (Sable Systems International Inc., Las Vegas, Gas Analyzer Sub-sampler) drew air from outside the building, through a long tube in the 13 °C water bath, and through the respiratory and reference chambers at flow rates of 9 (Stephens Island), 11 (Mana Island) and 12 (laboratory) ml min^{-1}. Differences in air flow rate were corrected using Sable Systems software and equations by Withers (1977). A soap-bubble flow-through system was used to calibrate the flow controller (Long and Ireland, 1985). Air was not dried prior to being passed over the lizards since some New Zealand lizards have relatively high rates of water loss for their size (Cree and Daugherty, 1991; Neilson, 2002). However, the excurrent air from the chamber passed through a column of self-indication Drierite®, soda lime and then Drierite® again before entering the oxygen analyser (a two-channel Sable Systems FC-2). The scrubbed chamber air was

continuously compared with the scrubbed air from outside the building to ensure that atmospheric oxygen concentration (20.94%) was used for the experiments.

3.3.3 Statistical analysis

Statistical analyses were performed using the statistics package *R* (Gentleman et al. 2003; Version 1.5.1). Statistical significance was assumed at $P < 0.05$. Data are expressed as means ± 1 SE unless otherwise stated.

In longitudinal data sets, such as daily rhythms, the set of observations on one subject is intercorrelated as data are collected from one individual over a long period of time (Diggle et al., 2003). Therefore, I created models (24 h and 12 h) of daily rhythms with the parameters amplitude (height of $\dot{V}O_2$ differences) and phase (length of cycle), including sex as a factor for each species (Appendix 1B). Mass was included as a covariate to account for the effects of body size (Packard and Boardman, 1988). To allow for repeated measures over time individual was also included as a random grouping variable in the non-linear mixed effects function in *R* (Pinheiro and Bates, 2000). Akaike Information Criteria (AIC; Burnham and Anderson, 1998; McCallum, 2000) were used to select the model(s) that best explained the data.

The ubiquitous species *H. maculatus* was used to test for differences in laboratory vs. island research (e.g., light regime, spermatogenesis etc). A mass-adjusted non-linear mixed-effects model was fitted to all *H. maculatus* data using maximum likelihoods. Individual was included as a random effect. For all species, the lower upper quartile values of $\dot{V}O_2$ were estimated using model curves fitted to the data from all individuals of a species (e.g., Figure 3.1; Appendix 1B). Upper quartile values are representative of resting metabolic rate (RMR) and lower quartile values of standard metabolic rate (SMR). Use of upper and lower quartile values removes measurements influenced by effects such as intrinsic activity, and has successfully given best estimates of metabolic rate in other species (e.g., Litzgus and Hopkins, 2003; Hopkins et al., 2004). Also,

taking the lowest and highest values over 24 h can introduce error by anomalous readings from possible bouts of activity or sleep.

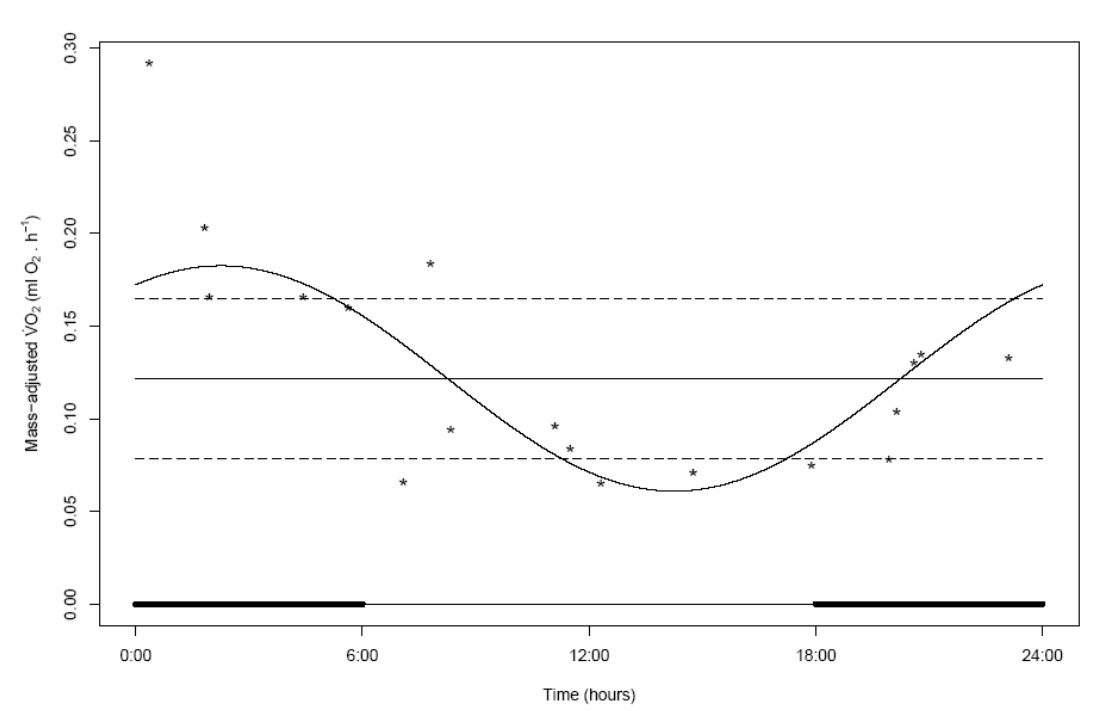

Figure 3.1: $\dot{V}O_2$ of the diurnal skink *Oligosoma nigriplantare polychroma* at 13 °C and in darkness, adjusted for individual effects by subtracting the estimated individual coefficients. The curve is the fitted model of $\dot{V}O_2$ rhythm over 24 h; the solid horizontal line indicates mean $\dot{V}O_2$ for all data points; the dashed lines indicate the upper and lower quartile values. Mean mass = 3.30 ± 0.21 g; n = 7. Scotophase (dark) normally experienced by the animals is indicated with black bars. N. B. the points on the graph indicate the mid-point of a measurement period. For clarity the figure is an oversimplification as there are no connecting lines showing repeated measures and within-individual daily variation.

Both magnitude (fractional change) and amplitude (actual change) provide measures that try to explain the change in overall metabolic rate over a 24 h period. Which measure is used depends on the researcher's preference and the type of analyses used. In squamates, the magnitude of change of $\dot{V}O_2$ (highest value divided by lowest value) is around 1.5 to 5 times higher during the active phase than during the inactive phase (Waldschmidt et al., 1987), and is strongly dependent on absolute $\dot{V}O_2$ values. Conversely, amplitude provides a more robust comparison of metabolic rate over 24 h as it is instead related to the overall differences in metabolic rate (1/2 the height

difference of lowest and highest $\dot{V}O_2$). When data include mass-adjusted $\dot{V}O_2$, amplitude becomes the natural measure of variation due to the corresponding strong differences in absolute $\dot{V}O_2$ levels. Both are reported here for completeness.

Randomisation tests were employed to compare whether amplitudes of $\dot{V}O_2$ were statistically as well as phylogenetically different between families (skinks and geckos) or with activity periods (nocturnal or diurnal) (Harvey and Pagel, 1991). The randomisation tests for this data used 10,000 permutations of the sample. Test statistics were calculated for each analysis (as above), and the relative ranking reported as a *P* value.

3.4 Results

Overall, un-adjusted $\dot{V}O_2$ was greatest in *C. macgregori* (daily mean = 0.619 ml O_2 h^{-1}) and least in *O. n. polychroma* (daily mean = 0.122 ml O_2 h^{-1}). $\dot{V}O_2$ lower quartile values (SMR) ranged from 0.079 to 0.547 ml O_2 h^{-1} and upper quartile values (RMR) from 0.157 to 0.692 ml O_2 h^{-1} among all the species (Table 3.1).

Mass-adjusted $\dot{V}O_2$ did not differ between the sexes for the five species where both sexes were measured: *O. n. polychroma* ($P = 0.962$), *O. zelandicum* ($P = 0.771$), *H. maculatus* (Wellington only; $P = 0.262$), *H. stephensi* ($P = 0.310$), and *C. aenea* ($P = 0.539$). There was no significant difference in mass-adjusted $\dot{V}O_2$ of populations of *H. maculatus* from Stephens Island or the mainland ($t_6 = 0.732$, $P = 0.470$).

Except for *H. maculatus*, all species showed significant changes in $\dot{V}O_2$ over the 24 h period (Table 3.2). For all species with a daily rhythm of $\dot{V}O_2$, the 24 h cycle was the best fit (AIC lower by 1.4 to 11.4), except for *C. aenea,* where the best fit was a 12 h cycle (AIC lower by 4.4). The mean time estimates of peak $\dot{V}O_2$ ranged from 0215 h to 1035 h in diurnal species and 2047 h to 0819 h in nocturnal species (Table 3.2). Amplitudes of mass-adjusted $\dot{V}O_2$ ranged from 0.034 ml O_2 h^{-1} to 0.119 ml O_2 h^{-1}, with

Table 3.1: Lower and upper quartile values of mass-adjusted $\dot{V}O_2$ over 24 h periods among eight species of lizard from the genera *Cyclodina, Hoplodactylus, Naultinus* and *Oligosoma*.

Activity	Family	Species	*n*	Mass (g)		$\dot{V}O_2$ (ml O_2 h^{-1})			Magnitude
				Mean	Range	Mean	Lower	Upper	
C/Dl	S	*C. aenea*	11	2.47 ± 0.18	1.36 - 3.36	0.126	0.094	0.157	1.7
Dl	D	*N. manukanus*	7	6.47 ± 0.56	4.39-8.12	0.187	0.116	0.258	2.2
Dl	S	*O. nigriplantare polychroma*	7	3.30 ± 0.21	2.61-4.10	0.122	0.079	0.165	2.1
Dl	S	*O. zelandicum*	11	3.75 ± 0.18	2.74-4.61	0.150	0.126	0.174	1.4
N	S	*C. macgregori*	8	17.59 ± 1.04	14.50-22.50	0.619	0.547	0.692	1.3
N	D	*H. chrysosireticus*	8	6.87 ± 0.55	5.25-9.75	0.379	0.295	0.463	1.6
N	D	*H. maculatus* (SI)	7	6.83 ± 0.58	7.04-12.20	0.286	-	-	-
N	D	*H. maculatus* (Wgtn)	7	8.99 ± 0.66	4.95-9.82	0.184	-	-	-
N	D	*H. stephensi*	7	7.43 ± 1.04	4.11-11.53	0.262	0.224	0.299	1.3

Values are from fitted models and refer to $\dot{V}O_2$ from an animal of mean mass; *Hoplodactylus maculatus* has only one value since no daily rhythm was established; mean $\dot{V}O_2$ expresses average $\dot{V}O_2$ over the whole 24 h period; C/D = crepuscular/diurnal; Dl = diurnal; D = Diplodactylidae; S = Scinicidae; N = nocturnal; SI = from Stephens Island; Wgtn = from mainland Wellington; Magnitude = change in $\dot{V}O_2$ gained by dividing upper $\dot{V}O_2$ by lower $\dot{V}O_2$ values; Values are all ± 1 SE.

no statistical difference in amplitudes among species, whether grouped by family (P = 0.423) or activity period (P = 0.546; Table 3.2; Figure 3.2).

Although *H. maculatus* has an amplitude similar to the overall mean of all the species studied here, there was no pattern to the data, indicating the absence of a daily rhythm. This is mainly due to large scatter in the data. The magnitudes of increase of $\dot{V}O_2$ during the active phase compared to the inactive phase ranged from 1.3 in *C. macgregori* and *H. stephensi* to 2.2 in *N. manukanus* (Table 3.1).

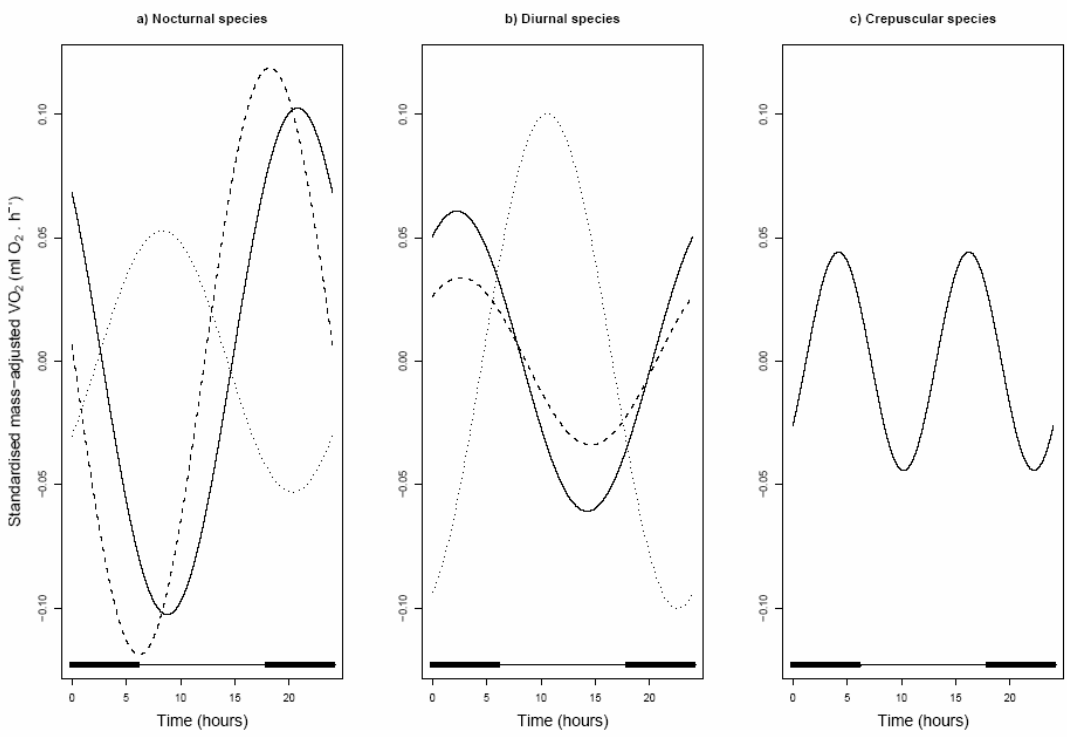

Figure 3.2: Standardised $\dot{V}O_2$ of daily rhythm of six lizard species at 13 °C. Individual mass effects are adjusted by subtracting the estimated individual coefficient. $\dot{V}O_2$ is standardised to zero to clarify comparisons of amplitude and phase. Actual $\dot{V}O_2$ values are expressed in Table 3.2. Masses and sample sizes are given in Table 3.1. a) solid line is *Cyclodina macgregori*, dashed line is *Hoplodactylus chrysosireticus*, dotted line is *H. stephensi*; b) solid line is *Oligosoma nigriplantare polychroma*, dashed line is *O. zelandicum*, dotted line is *Naultinus manukanus*; c) solid line is *C. aenea*. Scotophase (dark) normally experienced by the animals is indicated with black bars.

Table 3.2: Mean statistical estimates for amplitude (ml O_2 h^{-1}) of $\dot{V}O_2$ and times of peak activity of metabolic daily rhythms among eight species of lizard from the genera *Cyclodina, Hoplodactylus, Naultinus* and *Oligosoma*.

Activity	Family	Species	*n*	Amplitude estimate		Time estimate		*P* value
				Mean	95% CI	Mean	95% CI	
C/Dl	S	**C. aenea*	11	0.044 ± 0.015	(0.015, 0.074)	0412 & 1612 ± 48	(0215, 0609)(1515, 1809)	**0.009**
Dl	D	*N. manukanus*	7	0.100 ± 0.024	(0.053, 0.148)	1035 ± 53	(0851, 1219)	**0.001**
Dl	S	*O. nigriplantare polychroma*	7	0.061 ± 0.015	(0 .031, 0.091)	0215 ± 48	(0041, 0349)	**0.003**
Dl	S	*O. zelandicum*	11	0.034 ±0.014	(0.007, 0.061)	0236 ± 78	(0004, 0508)	**0.024**
N	S	*C. macgregori*	8	0.103 ± 0.025	(0.053, 0.152)	2047 ± 67	(0218, 2257)	**0.002**
N	D	*H. chrysosireticus*	8	0.119 ± 0.029	(0.043, 0.195)	1813 ± 70	(1554, 2031)	**0.011**
N	D	*H. maculatus* (SI)	7	0.045 ± 0.026	(-0.006, 0.095)	-	-	0.111
N	D	*H. maculatus* (Wgtn)	7	0.058 ± 0.030	(-0.001, 0.117)	-	-	0.069
N	D	*H. stephensi*	7	0.053 ± 0.019	(0.016, 0.090)	0819 ± 68	(0606, 1032)	**0.016**

Hoplodactylus maculatus has no time of peak activity as no daily rhythm was established; Time is in 24 h clock; SE for time estimates are in minutes; C/Dl = crepuscular/diurnal; Dl = diurnal; N = nocturnal; D = Diplodactylidae; S = Scinicidae; SI = from Stephens Island; Wgtn = from mainland Wellington; CI = confidence interval; * = model with 12 h cycle is best fit; all other species (except *H.* maculatus) have a 24 h cycle; *P* values in bold are significant. Values are ± 1 SE.

3.5 Discussion

The daily patterns of $\dot{V}O_2$ followed expected daily trends in only four of the seven species measured. The highest $\dot{V}O_2$ values were recorded during four species' active phase, or just prior to the active phase. However, the nocturnal gecko *H. stephensi* had its highest $\dot{V}O_2$ in the early morning during the photophase, and both diurnal *Oligosoma* skinks in the early morning during the scotophase. The daily patterns included a 24 h cycle for most diurnal species and nocturnal species, a 12 h cycle in the crepuscular/diurnal species *C. aenea*, and no pattern of $\dot{V}O_2$ over 24 h in the ubiquitous nocturnal species *H. maculatus.*

3.5.1 Daily patterns of $\dot{V}O_2$

Although daily oscillations of $\dot{V}O_2$ are common in reptiles (e.g., Bennett and Dawson, 1976), some species of reptiles, such as the diamondback rattlesnake *Crotalus adamanteus* (Dorcas et al., 2004), have no daily cycle of $\dot{V}O_2$. Most reptiles that show no daily pattern of $\dot{V}O_2$ tend to be nocturnal species (e.g., *Coleonyx switaki* (as *Anarbylus switaki* in Putnam and Murphy (1982)) and *Cosymbotus platyurus* (Feder and Feder, 1981)), or species that live in dark areas (e.g., burrowers such as *Tryphlosaurus cregoi bicolour* and *Acontias meleagris meleagris* (Brownlie and Loveridge, 1983) and cave dwellers such as *Xantusia smithii* (Mautz, 1979)). Thus, the lack of a daily $\dot{V}O_2$ pattern in *H. maculatus* is not unusual, even though other species in the genus exhibit a daily pattern, including *H. chrysosireticus* and *H. stephensi*. Also, the existence of sun compass orientation is based on circadian time keeping (Reebs, 2002) The diurnal skink *O. n. polychroma* (as *Leiolopisma nigriplantare* in Marshall (1983)) uses the sun for orientation; whereas, the nocturnal gecko *H. maculatus* does not (Marshall, 1983). Instead, it is likely that a species-specific factor is responsible for the lack of a cycle in *H. maculatus*, especially since two separate measures of $\dot{V}O_2$ for *H. maculatus* from different locations show the same result. It may be that the ubiquitous, broadly-adapted life style of *H. maculatus* has resulted in less variation in metabolic rate.

The 12 h cycle of $\dot{V}O_2$ displayed by *C. aenea* (Figure 2c) is indicative of a crepuscular species. However, daily activity traces of an Auckland population of *C. aenea* point to diurnal activity (Porter, 1987). Most *Cyclodina* species prefer shaded habitats and are nocturnal or crepuscular (Gill and Whitaker, 2001). Thus, the population of *C. aenea* in this study is crepuscular.

3.5.2 Does peak $\dot{V}O_2$ correspond to peak activity time?

Peak $\dot{V}O_2$ corresponded with the animals recorded activity phase in only four of the seven species (Figure 2). While having peak $\dot{V}O_2$ during or prior to the active phase is usual for animals, and is postulated to enable species to anticipate and prepare for their active phase (Moore-Ede 1986), it is unusual for a species to have its peak $\dot{V}O_2$ during the inactive phase, like *H. stephensi* (nocturnal), *O. n. polychroma* and *O. zelandicum* (both diurnal). The only other study I am aware of that demonstrates higher $\dot{V}O_2$ during the inactive phase is for the night lizard *Xantusia henshawi* (Mautz 1979). However, although the xantusiids are active at night, some are also active beneath substrates during the day (Lee 1974). As such, xantusiids may be active throughout 24 h (Pianka and Vitt 2003). Confusion over strict categorisation of species as nocturnal, diurnal or even crepuscular is thus apparent. Similarly, while searching for the nocturnal gecko *H. stephensi* for this study, more emerged between midnight and dawn than directly after sunset. *Hoplodactylus stephensi* also bask high on branches during the day (D. Keall pers. comm). The results for $\dot{V}O_2$, together with these observations on capture times, suggest that a behavioural study of *H. stephensi* is warranted.

Other taxa also show plasticity in their activity periods, either altering activity periods completely, or being active during their usual inactive phase. For example, many fish species alter their activity period within a lifetime; whereas, other species have some individuals that are nocturnal when other individuals are diurnal (Reebs, 2002). Also, nocturnal rats are able to modify their resting $\dot{V}O_2$ through 24 h independently of changes in activity or state of arousal, with periods of sleep in the active hours and

bouts of activity intermingled with sleep during the inactive hours, and still have a higher overall metabolic rate at night (Mortola, 2004). Thus, as in the nocturnal rat, nocturnal lizards may be able to maintain 'activity' during both the day and night.

The peaks of $\dot{V}O_2$ reported here indicate that there is a large portion of the day when $\dot{V}O_2$ does not differ significantly among individuals of a species. For example, the diurnal gecko *N. manukanus* has a peak $\dot{V}O_2$ around 1030 h, but $\dot{V}O_2$ does not vary significantly during the active period (0700 h to 1730 h) (Hare et al., 2004). Nevertheless, $\dot{V}O_2$ of *N. manukanus* is significantly higher in the active than the inactive parts of the day (Figure 2).

Both diurnal *Oligosoma* skinks have an unexpected time of peak $\dot{V}O_2$ of around 0200 h. Some other diurnal *Oligsoma* species also have peaks of $\dot{V}O_2$ during the night (Preest, 1985). Southern populations of *O. n. polychroma* are most active 5-10 h after sunrise (Patterson, 1992; Freeman, 1997), and *O. zelandicum* are most active between 1200 h and 1700 h (Neilson et al., 2004). However, Neilson et al. (2004) only observed activity of skinks above the substrate, and 47% were recorded as emergent between 2100 h and 0600 h. Also, even though 24 h temperature cycles can entrain the circadian clock(s) of ectothermic vertebrates, this does not preclude species from responding immediately to changes in the environment. For example, the diurnal skink *Tiliqua rugosa* will surface after a drought at night temperatures as low as 8.5 °C to rehydrate in rain (Kerr and Bull, 2004). Thus, it could be that time of peak $\dot{V}O_2$ is a trade-off enabling the species to emerge at night if conditions permit, as well as early in the morning when temperatures are low. An unknown physiological mechanism apart from activity period may also be driving the daily rhythm of $\dot{V}O_2$ in *H. stephensi* (nocturnal), *O. n. polychroma* and *O. zelandicum* (both diurnal). Nonetheless, it is important to know the natural history and activity of species when making inferences from physiological studies.

3.5.3 Do amplitudes or magnitudes of $\dot{V}O_2$ differ with activity period or family?

Amplitudes and magnitudes both provide a means of measuring the overall change of metabolic rate over 24 h. There were no differences in amplitude or magnitude of $\dot{V}O_2$ at 13 °C over 24 h among the nocturnal, diurnal and crepuscular species measured. There were also no differences in amplitude of $\dot{V}O_2$ between families. The magnitudes of $\dot{V}O_2$ change are generally slightly lower than the expected range (1.5 to 5) for squamates (Waldschmidt et al., 1987). Even though amplitudes are a more robust method of comparison than magnitude of increase of metabolic rate (due to not being influenced by absolute measures of $\dot{V}O_2$), it is still difficult to adequately compare amplitudes from this study with others as the temperature of experiments may influence amplitude (Bennett and Dawson, 1976). As such, until more is known about the direction of these patterns I caution against direct comparisons and interpretation, and instead make general discussions on the overall patterns of a few temperate and tropical species below.

3.5.4 Do amplitudes and magnitudes of $\dot{V}O_2$ differ between temperate and tropical species?

The data on amplitude and magnitude of $\dot{V}O_2$ of eight temperate lizards provides a source to compare with published accounts of other temperate species as well as tropical species. However, note that comparisons made here are not due to differences in how mass is incorporated in calculations of $\dot{V}O_2$ by different researchers. The seasonal amplitudes of $\dot{V}O_2$ for the temperate diurnal lizard *Lacerta viridis* at 30 °C are within the range obtained for the temperate lizards in this study at 13 °C. The amplitudes of *L. viridis* are 0.05 in winter and 0.06 ml O_2 g^{-1} h^{-1} in spring with an overall daily magnitude of 3.3 and 1.8 times higher $\dot{V}O_2$ during the day than at night respectively (Rismiller and Heldmaier, 1991). This suggests that amplitudes of $\dot{V}O_2$ are similar among temperate species. Also, although the amplitude is slightly lower in winter in *L. viridis*, the magnitude during winter is much greater due to an overall decrease in $\dot{V}O_2$

in winter. Therefore, as mentioned earlier, magnitudes are less robust than amplitudes in comparing differences of daily patterns of $\dot{V}O_2$ among species.

Conversely, the amplitudes of some nocturnal tropical Philippine geckos measured at 27 °C are much lower than the values obtained in this study; *Cosymbotus platyurus, Hemidactylus frenatus* and *Lepidodactylus lugubris* have $\dot{V}O_2$ amplitudes of around 0.01, 0.02 and 0.03 ml O_2 g^{-1} h^{-1} respectively, and magnitudes of change of 1.2, 1.4 and 1.4 respectively (Feder and Feder, 1981). Although small, the amplitudes of $\dot{V}O_2$ in the Philippine geckos are still large enough to show significant patterns of $\dot{V}O_2$ over 24 h in *H. frenatus* and *L. lugubris*, but not in *C. platyurus*. However, the magnitudes are smilar to those previously reported for squamates in general (e.g., Waldschmidt et al., 1987; Andrews and Pough, 1985). It appears, therefore, that the amplitudes of $\dot{V}O_2$ in tropical lizards may be lower than those for temperate lizards. Lower amplitudes of daily $\dot{V}O_2$ in nocturnal tropical geckos may be due to there being less variation in environmental temperature between day and night, reducing the need for high $\dot{V}O_2$ at low temperatures. However, this is conjecture until more research is undertaken to gain comparable metabolic data across species and families from both temperate and tropical locales (i.e., at similar temperatures).

3.5.5 Conclusions

There are three daily $\dot{V}O_2$ patterns reported for lizards: 24 h cycle, 12 h cycle or no daily cycle. Although amplitude of $\dot{V}O_2$ was not correlated with activity period, it appears that it may be correlated with whether a species is temperate or tropical. Care must be taken when comparing $\dot{V}O_2$ among species with differing activity periods. First, the $\dot{V}O_2$ is often significantly different at different times of the day, so standard and resting metabolic rates must be stipulated. Second, the time when the highest $\dot{V}O_2$ is attained may not correspond to the known activity period of the species, or may be shifted to one end of the activity period. Therefore, I recommend that the timing of measures of $\dot{V}O_2$

be fully described. Also, when undertaking studies of $\dot{V}O_2$, measures should also be taken over 24 h periods to ascertain the times of lowest and highest $\dot{V}O_2$.

Although many nocturnal species are emergent (either foraging or thermoregulating) during most parts of the day, their $\dot{V}O_2$ is usually only elevated during the active (foraging) phase. Thus, many nocturnal lizards are active at suboptimal temperatures, and emergent and thermoregulating when their metabolic rate is low. However, as metabolic rate of nocturnal species will increase with the corresponding increase in temperature from diurnal thermoregulation, the lower metabolic rate of nocturnal species during the day may not be highly significant for their physiology.

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CHAPTER 4
Thermal sensitivity of metabolic rate is lower in nocturnal lizards than in diurnal lizards[1]

4.1 Abstract

Physiological processes, such as rate of oxygen consumption ($\dot{V}O_2$), generally increase with temperature in ectothermic vertebrates. Physiological acclimation and acclimatisation to low temperatures result in an increase of $\dot{V}O_2$. I tested the hypothesis that nocturnal lizards have higher $\dot{V}O_2$ than diurnal and crepuscular lizards at the same temperatures by measuring the $\dot{V}O_2$ of eight temperate species of lizard (four nocturnal, three diurnal, and one crepuscular) at 13 °C and 26 °C. All measures were made during day hours. Published $\dot{V}O_2$ data of temperate lizards and tropical lizards (Scincomorpha and Gekkota) at low and high temperatures were also compared. Mass-specific $\dot{V}O_2$ is positively correlated with temperature in all species. Differences in $\dot{V}O_2$ at 13 °C are a result of differences among species, not differences in temperatures usually experienced during activity. At 26 °C, diurnal and crepuscular skinks have higher $\dot{V}O_2$ than nocturnal skinks and all geckos (including the secondarily diurnal gecko *Naultinus manukanus*). Temperate and tropical lizards do not appear to differ in $\dot{V}O_2$ at the same temperature, but more research on tropical species at low temperatures is required before this can be confirmed. Nocturnal lizards do not have higher $\dot{V}O_2$ than diurnal species at low body temperatures, but nocturnal lizards (and *N. manukanus*) have lower energy requirements (lower $\dot{V}O_2$) at high temperatures than diurnal and crepuscular skinks. Nocturnal lizards have lower thermal sensitivity and greater stability of metabolism than diurnal and crepuscular species (Q_{10} = 1.7-2.4 and 2.9-4.0 respectively; $P < 0.001$). Consequently, diurnal lizards can quickly take advantage of changes in

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environmental temperature, but nocturnal lizards are less influenced by changes in environmental temperature.

4.2 Introduction

Reptiles are ectotherms, and their physiology and activity are positively correlated with temperature within the species' biological limits (e.g., Avery, 1982; Bennett, 1982; Huey, 1982). Ectothermic animals have the advantage of lower energy requirements than endothermic animals (Pough, 1980), but it comes at a cost as individuals are often unable to achieve optimal temperatures for biological processes. For example, optimal temperatures for sprinting in diurnal and nocturnal lizards do not differ, implying that performance in nocturnal lizards is suboptimal at low night temperatures (Huey et al., 1989). However, nocturnal geckos have substantially lower energetic costs of locomotion than diurnal lizards, offsetting most of the reduced aerobic capacity brought about by low temperatures (e.g., Autumn et al., 1994; Farley and Emshwiller, 1996; Autumn et al., 1997; Autumn et al., 1999; Chapter 5). Nocturnal lizards may also have other adaptations to low temperatures, such as cold-adapted enzymes and higher metabolic rate at low temperatures.

Measures of metabolism in physiological ecology can identify potential energetic constraints that operate on individual organisms, as well as providing mechanistic explanations for large scale ecological and evolutionary patterns (Zaiden, 2003). Physiological processes, such as rate of oxygen consumption ($\dot{V}O_2$), generally increase with temperature (e.g., Bennett and Dawson, 1976; Karasov and Anderson, 1998; McConnachie and Alexander, 2004). In some lizards metabolism has a temperature-independent plateau through metabolic regulation, which increases the range of temperatures over which activity can occur (e.g., Aleksiuk, 1971; Grimmond and Evetts, 1980; Waldschmidt et al., 1986). For example, the temperate subspecies of garter snake *Thamnophis sirtalis parietalis* has a higher $\dot{V}O_2$ than the tropical subspecies (*T. s. sirtalis*) at the same body temperature. Temperate *T. s. parietalis* also has a plateau of temperature-independent metabolism between 10 °C and 15 °C

(Aleksiuk, 1971). Nocturnal lizards may also have a wide range of temperatures over which metabolic activity can take place, with a lower thermal sensitivity of $\dot{V}O_2$ than diurnal lizards, which would result in overall greater metabolic stability and less dependence of $\dot{V}O_2$ on body temperature.

The lizard families in New Zealand (Scincidae and Diplodactylidae) (Gill and Whitaker, 2001; Han et al., 2004) provide an ideal model system to study nocturnality. Each family consists of two genera, one predominantly nocturnal and the other predominantly diurnal. Each family has a different evolutionary history in relation to nocturnality. As geckos are ancestrally nocturnal (Vitt et al., 2003) diurnal geckos are secondarily diurnal. Conversely, the skinks are ancestrally diurnal (Vitt et al., 2003), but nocturnality or a crepuscular habit (active in the twilight) has evolved in some species. Also, New Zealand has a cool-temperate climate with relatively cool summers and mild winters (Cree, 1994; NIWA, 2005), but some local lizards remain active at body temperatures as low as 10 °C (Werner and Whitaker, 1978). Thus, $\dot{V}O_2$ of New Zealand lizards may be higher than that of lizards from relatively warm, stable climates, such as the tropics, when measured at the same temperatures.

The hypothesis that at the same temperatures nocturnal lizards have higher metabolic rates than diurnal lizards, and temperate lizards have higher metabolic rates than tropical lizards was tested. Specifically, I asked the following questions: 1) Do nocturnal lizards have a higher $\dot{V}O_2$ than diurnal and crepuscular lizards? 2) Is the thermal sensitivity of $\dot{V}O_2$ higher in diurnal and crepuscular lizards than nocturnal lizards? 3) Does overall $\dot{V}O_2$ differ with evolutionary history? (i.e., do skinks and geckos differ?) 4) Do temperate lizards have a higher $\dot{V}O_2$ than tropical species?

4.3 Materials and methods

4.3.1 Animal collection and husbandry

Animal collection and husbandry follow the same procedures as described in Chapter 3. Individuals from eight lizard species were collected over a two year study period: three nocturnal gecko species (*Hoplodactylus maculatus*, *H. chrysosireticus* and *H. stephensi*), one diurnal gecko species (*Naultinus manukanus*), two diurnal skink species (*Oligosoma nigriplantare polychroma* and *O. zelandicum*), one nocturnal skink species (*Cyclodina macgregori*), and one crepuscular/diurnal skink species (*C. aenea*) (Table 4.1). The activity period of each species was based on a published field guide (Gill and Whitaker, 2001). As *H. maculatus* is a species complex (Hitchmough, 1997), I ensured that the populations in this study are a single species (R. A. Hitchmough pers. comm.).

All animals were collected within the latitudinal range 40° 50' 35" to 41° 20' 83" in the Cook Strait region of New Zealand. *Hoplodactylus maculatus*, *H. stephensi* and *N. manukanus* were captured on Stephens Island (Takapourewa) in November 2002, and *H. chrysosireticus* and *C. macgregori* on Mana Island in November 2003. *Cyclodina aenea*, *H. maculatus*, *O. n. polychroma* and *O. zelandicum* were captured on the mainland in the Wellington region from January to April 2004. Only adult males or non-pregnant adult females were examined. Adult males were distinguished from females by inspection of the ventral tail base for protruding hemipenal sacs in geckos and hemipene eversion in skinks (Gill and Whitaker, 2001; Harlow, 1996). Reproductive status of females was determined by abdominal palpation (see Cree and Guillette (1995) and Wilson and Cree (2003) for information on accuracy of this procedure in New Zealand geckos). To manage the discrepancies in season (see below) between lizards caught on the mainland and on islands, the cosmopolitan species *H. maculatus* was used as a control group since this species is very widespread and locally abundant on both the islands and mainland New Zealand (Hitchmough, 1997; Gill and Whitaker, 2001).

Table 4.1: Mass, number of individuals that reached a steady-state after five 80 min trials, and Q_{10} values (temperature coefficient) for $\dot{V}O_2$ between 13 °C and 26 °C for eight lizard species from the genera *Cyclodina, Hoplodactylus, Naultinus* and *Oligosoma*.

Active	Family	Species	Mass (g)		n_t	n_{13} (%)	n_{26} (%)	Q_{10}
			Mean	Range				
Crepuscular	Scincidae	*C. aenea*	2.6 ± 0.2	1.4 - 3.4	14	12 (86)	12 (86)	3.5 ± 0.3
Diurnal	Diplodactylidae	*N. manukanus*	6.0 ± 0.2	4.4 - 8.4	30	29 (97)	29 (97)	3.1 ± 0.3
Diurnal	Scincidae	*O. nigriplantare polychroma*	3.3 ± 0.1	2.2 - 4.4	30	25 (83)	25 (83)	4.0 ± 0.3
Diurnal	Scincidae	*O. zelandicum*	3.8 ± 0.1	2.7 - 4.7	18	17 (94)	18 (100)	2.9 ± 0.2
Nocturnal	Scincidae	*C. macgregori*	19.0 ± 0.6	14.0 - 27.8	30	29 (97)	26 (87)	1.8 ± 0.1
Nocturnal	Diplodactylidae	*H. chrysosireticus*	5.9 ± 0.3	4.0 - 9.8	30	26 (85)	27 (90)	1.7 ± 0.1
Nocturnal	Diplodactylidae	*H. maculatus* (Wgtn)	6.7 ± 0.3	4.8 - 10.1	29	29 (100)	28 (97)	2.5 ± 0.2
Nocturnal	Diplodactylidae	*H. maculatus* (MI)	9.5 ± 0.6	6.0 - 15.3	17	16 (94)	15 (88)	2.2 ± 0.3
Nocturnal	Diplodactylidae	*H. maculatus* (SI)	8.1 ± 0.4	5.5 - 12.4	23	22 (96)	23 (100)	2.3 ± 0.1
Nocturnal	Diplodactylidae	*H. maculatus* (comb.)	7.8 ± 0.3	4.8 – 15.3	69	67 (97)	66 (96)	2.4 ± 0.1
Nocturnal	Diplodactylidae	*H. stephensi*	8.2 ± 0.6	4.4 - 11.7	15	13 (87)	15 (100)	2.3 ± 0.1

comb. = all *H. maculatus* populations; MI = from Mana Island; SI = from Stephens Island; Wgtn = from mainland Wellington; n_t = number of individuals trialled at each temperature; n_{13} = number of individuals that reached a steady-state of $\dot{V}O_2$ at 13 °C; n_{26} = number of individuals that reached a steady-state of $\dot{V}O_2$ at 26 °C; Numbers in parentheses are percentages of total number of individuals to reach a steady-state at that temperature; Values are means ± 1 SE.

Ambient temperature on islands ranged from 11-25 °C. Photoperiod during November in both years was 14:10 light:dark (sunrise at ~0600 h). On the mainland (Victoria University of Wellington (VUW)) all lizards were held in the same room to acclimate them to identical light and temperature regimes (3 to 4 weeks). Ambient temperature on the mainland ranged from 16-25 °C, and photoperiod was on a 12:12 light:dark cycle (on at 0600 h).

4.3.2 Metabolic experiments

Resting metabolic rate (RMR; highest $\dot{V}O_2$ values) is defined as metabolism during the period of normal activity, and standard metabolic rate (SMR; lowest $\dot{V}O_2$ values) as metabolism during the inactive period (Andrews and Pough, 1985). Thus, RMR is greater than SMR when measured at the same temperature. Metabolic experiments followed the same procedure as described in Chapter 3. To minimise stress of transportation and captivity, all animals collected on islands had their $\dot{V}O_2$ measured at field stations. Measures of $\dot{V}O_2$ took place during the photophase between 0700 h and 1730 h. Measures were only taken during the photophase because electricity was only available during the day. Therefore, $\dot{V}O_2$ measures were RMR for diurnal species and SMR for all nocturnal and crepuscular species. The RMR of *H. stephensi* was taken during the day since it has highest $\dot{V}O_2$ during the early hours of the photophase (Chapter 3). *Hoplodactylus maculatus* were measured on Mana Island, Stephens Island and mainland Wellington. To ensure a post-absorptive state, lizards were fasted for at least 72 h prior to measurements, except for the larger skink *C. macgregori*, which was fasted for at least 96 h (Coulson and Hernandez, 1980; Robert and Thompson, 2000; Hopkins et al., 2004).

Seven or eight lizards were placed in individual respirometry chambers within the experimental apparatus (Appendix 1A) on the morning of the day of $\dot{V}O_2$ measurement. Measurements were taken consecutively throughout the day. No lizards were removed until the last measurement was made at the end of the day. The time of day and temperature that each lizard's $\dot{V}O_2$ was measured were randomised. All lizards were

thermally equilibrated to the experimental temperatures (mean = 13.1 ± 0.1 °C or 26.0 ± 0.1 °C) within the experimental apparatus (chamber and water bath) for at least 1 h prior to measurements. Most diurnal New Zealand lizards are active at body temperatures between 14 °C and 33 °C (e.g., Werner and Whitaker, 1978; Morris, 1981). Conversely, many nocturnal New Zealand lizard species are commonly active at body temperatures between 10 °C and 18 °C (Werner and Whitaker, 1978; Thomas, 1981). Therefore ecologically relevant temperatures within the upper and lower limits that New Zealand lizards may experience daily while still remaining active were chosen. I chose not to measure diurnal species at lower temperatures, which may induce a state of torpor or apnoea (Morris, 1981; Morris, 1984). Animals were conditioned to the experimental procedure three times prior to the first $\dot{V}O_2$ measurement (Hare et al., 2004). If no steady-state was reached for an individual after the fifth 80 min trial, the animal was excluded from the analyses (Table 4.1).

4.3.3 Statistical analysis

Data were analysed using the statistical programme *R* (Gentleman et al., 2003; R-Development-Core-Team, 2004; Version 1.5.1). Statistical significance was assumed at $P < 0.05$. Data are expressed as mean ± 1 SE unless otherwise stated. Thermal sensitivity of $\dot{V}O_2$ is expressed as a temperature coefficient (Q_{10} value), which was calculated as:

$$Q_{10} = (k_2 / k_1)^{10 / (T2-T1)}$$

where k = reaction rate at temperatures 1 and 2 in Kelvin.

The probability of individuals reaching a steady-state during an 80 min time-frame over the 5 trials at 13 °C and 26 °C using binomial models that included an "unsettled" category was explored. Analyses of covariance (ANCOVA; adjusted for individual effects by subtracting the estimated individual coefficients) were used to investigate the categorical variables of temperature, time of day (at four levels) and sex on the dependent variable $\dot{V}O_2$. The levels used for time of day were 0700-0900 h, 0900-1100 h, 1100-1300 h and 1300-1730 h, with the last sample interval increased to gain a

statistically robust sample size. Mass was included as a covariate to correct for the effects of body size on $\dot{V}O_2$ (Packard and Boardman, 1988). To allow for repeated measures, the linear mixed-effects function in *R* was used (Pinheiro and Bates, 2000).

As many studies use different experimental temperatures, I tested for differences in metabolic rate with small (2 °C) differences in temperature. Data from *N. manukanus* measured *in situ* on Stephens Island at 26 °C (this study) with *N. manukanus* measured in the laboratory at 24 °C (Hare et al., 2004) were compared. The linear mixed-effects function was used to allow for repeated measures, with location as a factor, $\dot{V}O_2$ as the dependent variable and mass as a covariate. Similarly, RMR data from all eight species (data from Chapter 3 and this study) were used to enable comparisons of RMR among all species at 13 °C. I compared Q_{10} values within species using ANCOVA, with Q_{10} as the dependent variable, sex as a factor and mass as a covariate.

As data may be dependent on phylogenetic relationships among species, randomisation (permutation) tests were employed. The randomisation tests for the data used 10,000 permutations of the sample (Harvey and Pagel, 1991). The relative ranking of the observed test statistic is reported as a *P* value. Randomisation tests were employed to compare whether the variable ($\dot{V}O_2$) was statistically and phylogenetically significant among species, activity period (nocturnal, diurnal or crepuscular) and family (Scincidae and Diplodactylidae).

I also compared $\dot{V}O_2$ of temperate and tropical lizards from published data sources. Metabolism is influenced by many factors, including body temperature, mass, reproductive condition, sex, time of day, season, ecological category and activation energy of key metabolic enzymes (e.g., Brownlie and Loveridge, 1983; Andrews and Pough, 1985; Rismiller and Heldmaier, 1987; Christian et al., 1999). I chose to compare the results gained here only with published studies that met strict guidelines to limit these confounding effects. Only data from the Gekkota and Scincomorpha were used, and experimental temperatures within 2 °C of the experimental temperatures used here.

The study had to use only quiescent, non-gravid or non-pregnant adult individuals in a post-absorptive state. In addition, the studies needed to include species that were of a similar ecological category (see Andrews and Pough (1985) for definitions of ecological category) (Appendix 1C). Standard and phylogenetic allometric contrasts were used to evaluate the interspecific variation in $\dot{V}O_2$ among tropical and temperate species. The complete model included simultaneous tests of equal slopes and intercepts and was compared with a reduced model that assumed the factor being tested (latitudinal range) was irrelevant for the response variable ($\dot{V}O_2$).

4.4 Results

Mass-specific $\dot{V}O_2$ was 2-4 times higher at 26 °C than at 13 °C in all species ($F_{1,223}$ = 3199.650, $P < 0.001$; Figure 4.1). At both temperatures, $\dot{V}O_2$ was significantly different among species ($F_{9,223}$ = 44.796, $P < 0.001$), even after using randomisation tests for effects of species ($P = 0.009$). At 13 °C, mass-specific $\dot{V}O_2$ was highest in *H. chrysosireticus* (0.06 ml O_2 g^{-1} h^{-1}) and lowest in *C. macgregori* (0.03 ml O_2 g^{-1} h^{-1}). At 26 °C, mass-specific $\dot{V}O_2$ was highest in *O. n. polychroma* (0.16 ml O_2 g^{-1} h^{-1}), and lowest in *C. macgregori* (0.06 ml O_2 g^{-1} h^{-1}).

Mass-specific $\dot{V}O_2$ was not significantly different between skinks and geckos at either 13 °C or 26 °C ($P = 0.199$ and 0.543 respectively). At 26 °C, $\dot{V}O_2$ was significantly higher in crepuscular and diurnal lizards than nocturnal lizards ($P = 0.0243$). Among geckos, *H. stephensi* had a significantly higher mass-specific $\dot{V}O_2$ at 26 °C than the other geckos measured in this study ($P = 0.028$). Among skinks, *C. macgregori* had a significantly lower mass-specific $\dot{V}O_2$ at 26 °C than the other skinks measured in this study ($P = 0.038$). At 13 °C, $\dot{V}O_2$ was highest in nocturnal species ($P = 0.008$). The appearance of an elevated $\dot{V}O_2$ in nocturnal species at 13 °C is largely a result of the extremely high value for *H. chrysosireticus*. When *H. chrysosireticus* was removed from the analysis there were no differences in $\dot{V}O_2$ among species with differing activity periods at 13 °C ($P = 0.124$).

$\dot{V}O_2$ was influenced by mass for all species ($F_{1,206}$ = 234.555, P < 0.001), but not sex ($F_{1,223}$ = 3.615, P = 0.059), nor time of day ($F_{1,204}$ = 2.594, P = 0.109). The probability of reaching a steady-state $\dot{V}O_2$ by 80 min within 5 trials was not significantly different among species, activity periods or between skinks and geckos at 13 °C or 26 °C (range = 83-100%; P > 0.05 for all tests). At most, five individuals of any one species did not reach a steady-state $\dot{V}O_2$ after 5 trials (Table 4.1).

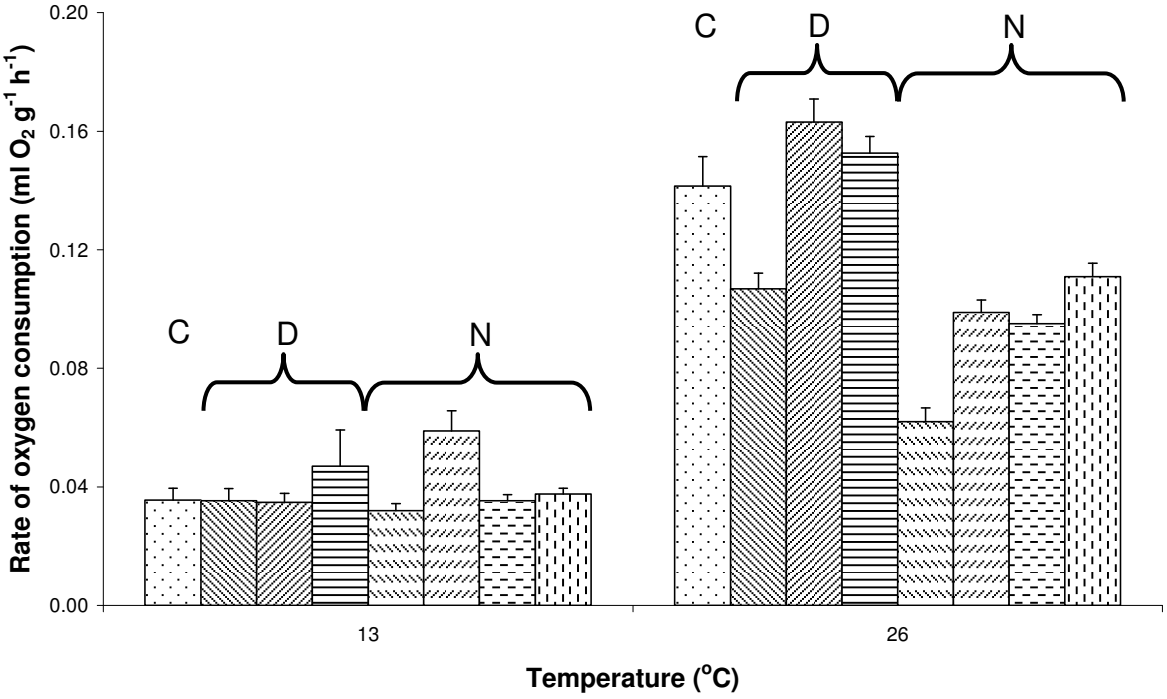

Figure 4.1: Mass-specific $\dot{V}O_2$ of eight lizard species measured during the photophase (light) at 13 °C and 26 °C. Sample size, mass and Q_{10} values are presented in Table 4.1. From left, the shading in bars indicates the following species; = *Cyclodina aenea*; = *Naultinus manukanus*; = *Oligosoma nigriplantare polychroma*; = *O. zelandicum*; = *C. macgregori*; = *Hoplodactylus chrysosireticus*; = *H. maculatus* (combined data for populations from mainland Wellington, Mana Island and Stephens Island); = *H. stephensi*. *Cyclodina* and *Oligosoma* genera are in the family Scincidae. *Naultinus* and *Hoplodactylus* genera are in the family Diplodactylidae. C = crepuscular; D = diurnal; N = nocturnal. Note that nocturnal and crepuscular species are measured during the inactive phase and diurnal species during the active phase. Error bars are 1 SE.

Mass-specific $\dot{V}O_2$ did not differ among the three populations of *H. maculatus* at either temperature ($F_{2,65}$ = 1.228, P = 0.298). Similarly, $\dot{V}O_2$ was not significantly different for *N. manukanus* measured in captive animals at 24 °C (Hare et al., 2004) compared with

field-caught animals on Stephens Island at 26 °C ($F_{1,44}$ = 0.005, P = 0.942). Resting metabolic rate at 13 °C (comparing RMR data from Chapter 3 and this study) did not differ significantly among species (P = 0.999) or between skinks and geckos (P = 0.064; 4.2). However, RMR of mass-specific $\dot{V}O_2$ at 13 °C was significantly higher in the crepuscular species *C. aenea* (0.06 ± 0.01 ml O_2 h^{-1}; P = 0.004) compared with all other species (mean = 0.04 ± 0.01 ml O_2 h^{-1}; Table 4.2). When *C. aenea* was removed from the analyses, there was no significant difference with activity period, i.e., between nocturnal and diurnal species (P = 0.999).

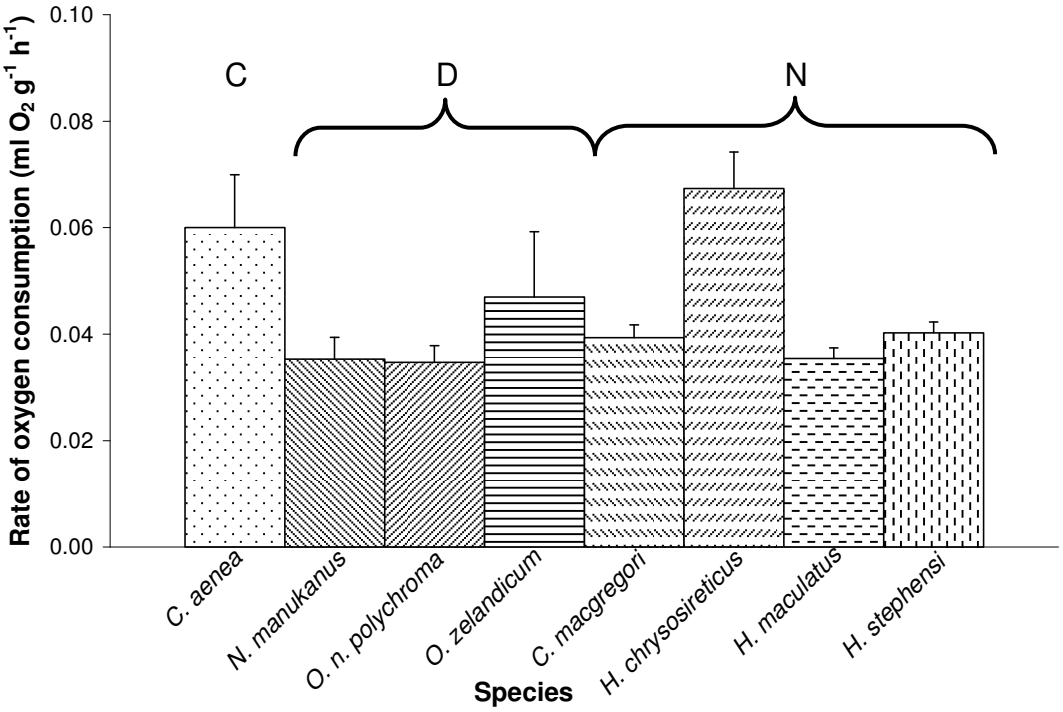

Figure 4.2: Mass-specific $\dot{V}O_2$ of eight lizard species measured at rest during their active phase at 13 °C (some data are from Chapter 3). Sample sizes and mass are presented in Table 4.2. *Cyclodina* and *Oligosoma* genera are in the family Scincidae. *Naultinus* and *Hoplodactylus* genera are in the family Diplodactylidae. C = crepuscular; D = diurnal; N = nocturnal. Active phase data for nocturnal and crepuscular species are from Chapter 3; *Hoplodactylus maculatus* includes data from mainland Wellington, Mana Island and Stephens Island populations. NB. *H. stephensi* has its highest $\dot{V}O_2$ during the inactive phase (Chapter 3), and this measure is used here. Error bars are 1 SE.

Table 4.2a. Rate of oxygen consumption ($\dot{V}O_2$) of New Zealand lizards measured at 13-15 °C from this study and others.

Active	Family	Species	State	T history	T	*n*	Mass	$\dot{V}O_2$	Citation
C	S	*Cyclodina aenea*	RMR	Variable	13	11	2.5	0.06	Chapter 3
C	S	*C. aenea*	SMR	Variable	13	12	2.5	0.04	This chapter
N	S	*C. macgregori*	RMR	Spring	13	8	17.6	0.03	Chapter 3
N	S	*C. macgregori*	SMR	Spring	13	29	19.0	0.04	This chapter
N	D	*Hoplodactylus chrysosireticus*	RMR	Spring	13	8	6.9	0.09	Chapter 3
N	D	*H. chrysosireticus*	SMR	Spring	13	25	5.9	0.06	This chapter
N	D	*H. maculatus*	-	Variable	13	67	7.9	0.04	This chapter
N	D	[1]*H.* aff. *maculatus* "Canterbury"	SMR	5 °C	15	10	8.2	0.04	Tocher and Davison, 1996
N	D	[1]*H.* aff. *maculatus* "Canterbury"	SMR	25 °C	15	10	8.3	0.03	Tocher and Davison, 1996
N	D	[1]*H.* aff. *maculatus* "Southern Alps"	SMR	5 °C	15	9	7.9	0.05	Tocher and Davison, 1996
N	D	[1]*H.* aff. *maculatus* "Southern Alps"	SMR	25 °C	15	10	8.4	0.03	Tocher and Davison, 1996
N	D	*H. stephensi*	RMR	Spring	13	15	8.2	0.04	This chapter
N	D	*H. stephensi*	SMR	Spring	13	7	7.4	0.03	Chapter 3
Dl	D	*Naultinus manukanus*	RMR	Spring	13	29	6.0	0.04	This chapter
Dl	D	*N. manukanus*	SMR	Spring	13	7	6.5	0.02	Chapter 3
Dl	S	[2]*Oligosoma maccanni*	RMR	Variable	15	5	2.9	0.08	Evetts and Grimmond, 1982
Dl	S	*O. n. polychroma*	RMR	Variable	13	25	3.3	0.03	This chapter
Dl	S	*O. n. polychroma*	SMR	Variable	13	7	3.3	0.02	Chapter 3
Dl	S	*O. zelandicum*	RMR	Variable	13	17	3.8	0.05	This chapter
Dl	S	*O. zelandicum*	SMR	Variable	13	11	3.8	0.03	Chapter 3

[1] as *H. maculatus* in Tocher and Davison (1996); [2] as *Leiolopisma n. maccanni* in Evetts and Grimmond (1982), and Grimmond and Evetts (1980), N.B. most likely *O. maccanni*, but could also contain *O. n. polychroma* (Freeman, 1997); $\dot{V}O_2$ is in ml O_2 g^{-1} h^{-1}; C = crepuscular; Dl = diurnal; N = nocturnal; D = Diplodactylidae; S = Scincidae; RMR = resting metabolic rate; SMR = standard metabolic rate; T history = acclimation or acclimatisation history; Variable = includes a range of temperatures; T = temperature (°C); *n* = sample size; Average mass of individuals is in g; *Hoplodactylus maculatus* includes data for mainland Wellington, Mana Island and Stephens Island populations.

Table 4.2b. Rate of oxygen consumption ($\dot{V}O_2$) of New Zealand lizards measured at 24-26 °C from this study and others.

Active	Family	Species	State	T history	T	*n*	Mass	$\dot{V}O_2$	Citation
C	S	*Cyclodina aenea*	SMR	Variable	26	14	2.6	0.14	This chapter
N	S	*C. macgregori*	SMR	Variable	26	26	19.2	0.06	This chapter
N	D	*Hoplodactylus chrysosireticus*	SMR	Spring	26	27	6.0	0.10	This chapter
N	D	*H. maculatus*	-	Variable	26	66	7.8	0.10	This chapter
N	D	[1]*H.* aff. *maculatus* "Canterbury"	SMR	5 °C	25	10	8.2	0.08	Tocher and Davison, 1996
N	D	[1]*H.* aff. *maculatus* "Canterbury"	SMR	25 °C	25	10	8.3	0.07	Tocher and Davison, 1996
N	D	[1]*H.* aff. *maculatus* "Southern Alps"	SMR	5 °C	25	10	7.9	0.11	Tocher and Davison, 1996
N	D	[1]*H.* aff. *maculatus* "Southern Alps"	SMR	25 °C	25	10	8.4	0.07	Tocher and Davison, 1996
N	D	[2]*H.* aff. *maculatus* "Otago/Southland large"	RMR	4 °C	25	5	5.1	0.09	Grimmond and Evetts, 1980; Evetts and Grimmond, 1982
N	D	[2]*H.* aff. *maculatus* "Otago/Southland large"	RMR	25 °C	25	5	5.6	0.10	Grimmond and Evetts, 1980; Evetts and Grimmond, 1982
N	D	*H. stephensi*	RMR	Spring	26	15	8.1	0.11	This chapter
Dl	D	*Naultinus manukanus*	RMR	Spring	24	22	7.3	0.09	Hare et al., 2004
Dl	D	*N. manukanus*	RMR	Spring	26	29	6.1	0.11	This chapter
Dl	S	[3]*O. maccanni*	RMR	Variable	25	5	3.0	0.20	Grimmond and Evetts, 1980; Evetts and Grimmond, 1982
Dl	S	[3]*O. maccanni*	RMR	4 °C	25	5	2.9	0.27	Grimmond and Evetts, 1980; Evetts and Grimmond, 1982
Dl	S	[3]*O. maccanni*	RMR	25 °C	25	5	2.9	0.16	Grimmond and Evetts, 1980; Evetts and Grimmond, 1982
Dl	S	*O. n. polychroma*	RMR	Variable	26	25	3.2	0.16	This chapter
Dl	S	*O. zelandicum*	RMR	Variable	26	18	3.6	0.15	This chapter

[1] as *H. maculatus* in Tocher and Davison (1996); [2] as *H. maculatus* in Evetts and Grimmond (1982), and Grimmond and Evetts (1980); [3] as *Leiolopisma n. maccanni* in Evetts and Grimmond (1982), and Grimmond and Evetts (1980), N.B. most likely *O. maccanni*, but could also contain *O. n. polychroma* (Freeman, 1997); $\dot{V}O_2$ is in ml O_2 g^{-1} h^{-1}; C = crepuscular; Dl = diurnal; N = nocturnal; D = Diplodactylidae; S = Scincidae; RMR = resting metabolic rate; SMR = standard metabolic rate; T history = acclimation or acclimatisation history; Variable = includes a range of temperatures; T = temperature (°C); *n* = sample size; Average mass of individuals is in g; *Hoplodactylus maculatus* includes data for mainland Wellington, Mana Island and Stephens Island populations.

Thermal sensitivity of $\dot{V}O_2$ (calculated from Q_{10} models for data at 13 °C and 26 °C) was significantly different among species ($F_{1,9}$ = 10.187, P < 0.001), being highest in *O. zelandicum* (4.0) and lowest in *H. chrysosireticus* (1.7) (Table 4.1). Q_{10} values were significantly higher in diurnal and crepuscular species than nocturnal species (P < 0.001), but were not significantly different between skinks and geckos (P = 0.194). Q_{10} values were not significantly influenced by mass for any species ($F_{1,9}$ = 0.046, P = 0.830), but were influenced by sex of individuals (F_1 = 5.161, P = 0.030) in *C. macgregori* and *H. chrysosireticus*. *Cyclodina macgregori* had significantly lower Q_{10} values in females than males (Q_{10} = 1.5 ± 0.2 and 2.1 ± 0.2 respectively; $F_{1,25}$ = 1.671, P = 0.044), and *H. chrysosireticus* had significantly higher Q_{10} values in females than males (Q_{10} = 1.8 ± 0.02 and 1.6 ± 0.01 respectively; $F_{1,23}$ = 4.541, P = 0.044). No other species had differences in thermal sensitivity of $\dot{V}O_2$ between the sexes ($F_{1,7}$ = 3.746, P = 0.055).

Using allometric analyses there was no significant differences among $\dot{V}O_2$ of tropical and temperate Scincomorpha and Gekkota at 25 °C (P = 0.329) (Figure 4.3). Mass-specific $\dot{V}O_2$ of all Gekkota and Scincomorpha also did not differ at 15 °C ($F_{1,28}$ = 0.155, P = 0.697) or 25 °C ($F_{1,50}$ = 3.850, P = 0.055). Mass was significantly and positively correlated with $\dot{V}O_2$ ($F_{1,34}$ = 97.12, P < 0.001). There were no significant differences among slopes of the allometric lines relating $\dot{V}O_2$ and mass for temperate and tropical species at 25 °C ($F_{1,34}$ = 1.338, P = 0.256). Little data were available on $\dot{V}O_2$ of tropical lizards at 15 °C (Appendix 1C). The two tropical species measured at 15 °C (*Lepidophyma gaigeae* and *L. smithii*; Mautz, 1979) had $\dot{V}O_2$ values near, or below the 95% confidence limits for $\dot{V}O_2$ of temperate species at 15 °C (Figure 4.3). The $\dot{V}O_2$ values for all New Zealand lizards fell within the 95% confidence limits of temperate species (Figure 4.3).

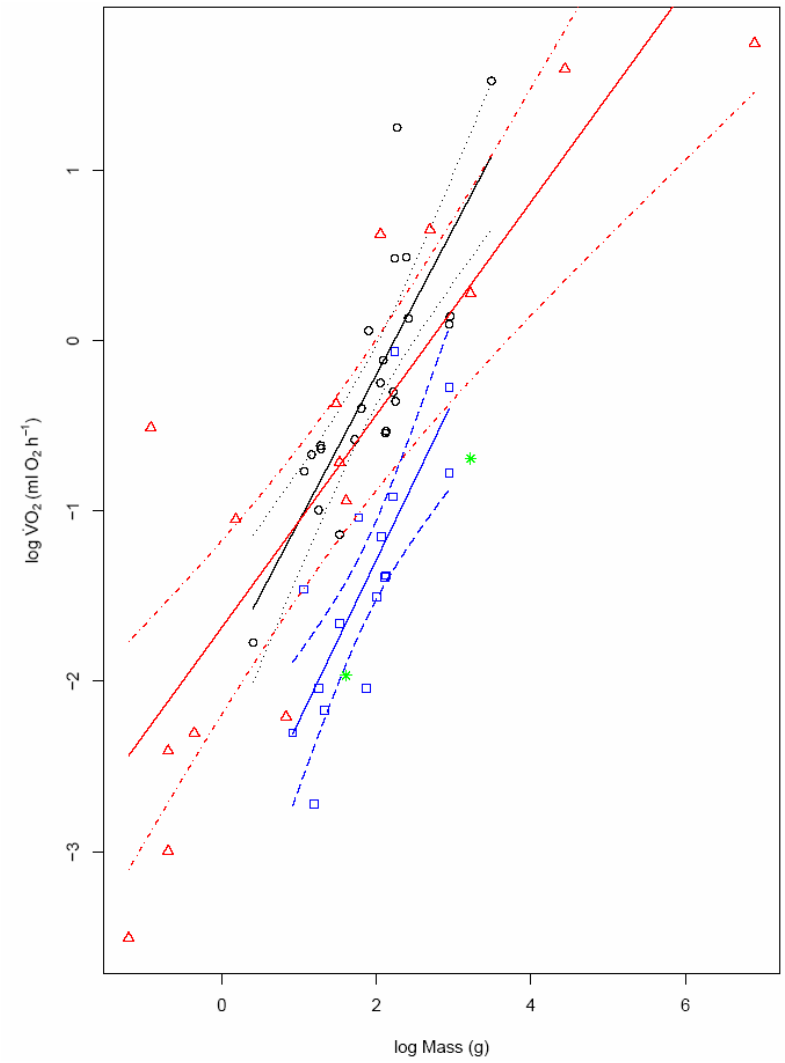

Figure 4.3: Log rate of oxygen consumption ($\dot{V}O_2$) versus log body mass of lizards from Scincomorpha and Gekkota. Data and allometric relationships for temperate species at 15 °C (blue squares; log $\dot{V}O_2$ = -3.18 + 0.94 log Mass; r^2 = 0.63), tropical species at 15 °C (green stars), temperate species at 25 °C (black circles; log $\dot{V}O_2$ = -1.92 + 0.86 log Mass; r^2 = 0.67) and tropical species at 25 °C (red triangles; log $\dot{V}O_2$ = -1.68 + 0.62 log Mass; r^2 = 0.75) are from this study (Table 4.2) and literature values (Appendix 1C). No regression line is available for tropical lizards at 15 °C as there are only two data points. To increase sample size, standard metabolic rate is used where data are available, otherwise resting metabolic rate is used. Thus, there is high variation within data sets. The solid lines represent least-squared regressions with 95% confidence limits (dashed and dotted lines) in the same colours described above.

4.5 Discussion

I found no difference in mass-specific $\dot{V}O_2$ of nocturnal and diurnal lizards at low temperatures. At high temperatures, diurnal and crepuscular skinks have much higher $\dot{V}O_2$ than nocturnal skinks and all geckos, including the secondarily diurnal species *N. manukanus*. However, the thermal sensitivity of $\dot{V}O_2$ is much higher in diurnal and crepuscular lizards than in nocturnal lizards. Consequently, nocturnal species are less influenced by changes in body temperatures. The $\dot{V}O_2$ of New Zealand lizards does not differ significantly from other temperate species. Mass-specific $\dot{V}O_2$ of temperate and tropical lizards do not differ at high temperatures, but at low temperatures the data are not conclusive.

The $\dot{V}O_2$ is significantly influenced by mass but not sex for all lizards in this study. Body mass is an important source of variation in metabolic parameters (Bennett and Dawson, 1976; Andrews and Pough, 1985), but the influence of sex is less clear. Metabolic rate of ectothermic vertebrates may be influenced by the sex of individuals (e.g., Niewiarowski and Waldschmidt, 1992; Ryan and Hopkins, 2000). However, in this study and elsewhere (e.g., Beaupre et al., 1993; Zaiden, 2003), there is no significant difference in $\dot{V}O_2$ between the sexes. Since thermal sensitivity of $\dot{V}O_2$ differs between males and females of two species (*H. chrysosireticus* and *C. macgregori*), the sexes may have different thermal preferences, but the thermal biology of these species is yet to be investigated.

4.5.1 Does $\dot{V}O_2$ differ with activity period or between skinks and geckos?

Neither the RMR nor SMR of nocturnal species differs significantly from that of diurnal or crepuscular species at 13 °C. Differences in $\dot{V}O_2$ at 13 °C are species-specific responses. As some nocturnal species experience large daily variations in body temperature through diurnal thermoregulation (e.g., Tocher, 1992), their metabolic rates may be selected to function over a broad range of temperatures rather than specifically at low temperatures. Shifts in patterns of metabolism at low body temperatures may allow species in cold climates to maintain comparatively high rates of metabolism at

cold temperatures (e.g., Aleksiuk, 1971). It may be that 13 °C is not cold enough to show a difference among species with differing activity periods. However, a nocturnal *Hoplodactylus* gecko does not have an elevated $\dot{V}O_2$ when acclimated to low temperatures (5 °C), whereas a diurnal *Oligosoma* skink does have an elevated $\dot{V}O_2$ when acclimated to 5 °C (Grimmond and Evetts, 1980; Evetts and Grimmond, 1982). Thus, a higher $\dot{V}O_2$ in nocturnal species, compared to diurnal species, appears not to have evolved.

The results indicate that the evolutionary history of skinks and geckos is not related to mass-specific $\dot{V}O_2$. Mass-specific $\dot{V}O_2$ is not significantly different among species at low temperatures, but at high temperatures diurnal and crepuscular skinks have significantly higher $\dot{V}O_2$ than secondarily diurnal geckos and nocturnal lizards. The differences in $\dot{V}O_2$ among nocturnal and diurnal species at 26 °C could be due to the timing of the metabolic measurements as, in general, SMR of nocturnal species was compared with RMR of diurnal species. However, there were some exceptions where RMR nocturnal species were compared with RMR of diurnal species, such as nocturnal gecko *H. stephensi* (Table 4.2b). Both *H. stephensi* and *N. manukanus* have significantly lower $\dot{V}O_2$ compared with diurnal skinks. Also, RMR data from other nocturnal *Hoplodactylus* species at 25 °C are also lower than diurnal skink species (Table 4.2b).

Ecological category explains 45% of variation in metabolic rate among species, with generally higher metabolic rate in day-active predators (all diurnal species) than reclusive predators (all nocturnal species; Andrews and Pough, 1985). However, this pattern is not supported by the data. Instead, the secondarily diurnal gecko *N. manukanus* may have retained nocturnal traits for low $\dot{V}O_2$. Conversely, as *N. manukanus* is a sit-and-wait/ambush predator (Gill and Whitaker, 2001), a low $\dot{V}O_2$ may enable them to conserve energy while still remaining vigilant, as seen in some Australian pythons (Bedford and Christian, 1998), and pygopodid lizards (Wall et al., unpub.). Further research, including data on more species of diurnal sit-and-

wait/ambush predators, may help to show whether *N. manukanus* has low metabolic rates from its ecology, or nocturnal ancestry.

4.5.2 Does thermal sensitivity (Q_{10}) of $\dot{V}O_2$ differ with activity period?

The diurnal and crepuscular lizards in this study have a significantly higher thermal sensitivity of metabolism than the nocturnal lizards. Normally, a Q_{10} value of 2 is expected for a species studied within its normal range of body temperatures (Hochachka and Somero, 2002). At relatively low body temperatures, Q_{10} values may exceed 2, indicating a change in the properties of the underlying biochemical systems (Hochachka and Somero, 2002). High Q_{10} values indicate a rapid increase in metabolic rate with an increase in temperature. As a result, energy is conserved at low temperatures when the lizards are inactive. The high thermal sensitivity of metabolic rate of diurnal species also indicates that optimal metabolic rates can be rapidly attained. Thus, an increase in temperature after morning emergence or a period of cold weather could induce an effective shift between torpid and active states in *N. manukanus*, *Oligosoma* spp. and *C. aenea*. Consequently, *N. manukanus,* which has lower $\dot{V}O_2$ than other diurnal species, has lower metabolic expenditure at high temperatures than other diurnal lizards. This is further supported by the lower energetic cost of locomotion in *N. manukanus* than any other diurnal lizard species (Chapter 6).

Although nocturnal lizards may elevate their body temperature during the day by 'indirect' basking (e.g., Werner and Whitaker, 1978; Tocher, 1992; Kearney and Predavec, 2000), they do not reach high temperatures while foraging at night. The low thermal sensitivity of $\dot{V}O_2$ of nocturnal lizards, compared with diurnal lizards over the same temperature range, indicates that nocturnal lizards have higher metabolic stability and low thermal dependence of metabolism on temperature. Thus, nocturnal lizards may remain active over a wider range of body temperatures than diurnal lizards, which may be further explored by more research integrating thermal preferences, activity temperatures and metabolic rates of nocturnal and diurnal species.

4.5.3 Temperate species vs. tropical species

The $\dot{V}O_2$ of temperate and tropical lizards are similar among the Gekkota and Scincomorpha at 25 °C. However, it is unclear whether there are differences among tropical and temperate lizards at 15 °C as there is a lack of metabolic data on tropical species at low temperatures. Garter snakes (*Thamnophis sirtalis parietalis*) have higher $\dot{V}O_2$ at all temperatures in temperate sub-species compared with tropical sub-species (Aleksiuk, 1971). Cool-temperate lizards have partially compensated for low activity temperatures by having lower energetic costs of locomotion than warm-temperate and tropical lizards (Chapter 6). However, a low cost of locomotion does not fully offset the thermal handicap of activity at low temperatures (Autumn, 1999; Chapter 6). Although measuring the $\dot{V}O_2$ of tropical lizards at low temperatures is not ecologically relevant for the species, more data on wider temperature ranges would help determine whether temperate lizards have partly adapted to cold temperatures by elevating their metabolic rate.

4.5.4 Conclusions

In New Zealand, nocturnal lizards do not offset the thermal handicap of activity at low body temperatures by elevating $\dot{V}O_2$, but nocturnal lizards (and *N. manukanus*) have lower energy requirements at high temperatures than diurnal and crepuscular skinks. Mass-specific $\dot{V}O_2$ does not appear to differ among temperate and tropical species of lizard, but more research on tropical species at low temperatures is required before this can be confirmed. Diurnal lizards are able to quickly take advantage of changes in environmental temperature, reaching high metabolic rates at body temperatures not experienced by nocturnal species during their activity periods. The lower thermal sensitivity of nocturnal lizards indicates that they probably operate over a wider temperature range than diurnal lizards.

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CHAPTER 5
Low cost of locomotion in lizards that are active at low temperatures[1]

5.1 Abstract

The physiology and activity of ectothermic taxa such as reptiles are dependent on temperature. A low energetic cost of locomotion (C_{min}) has evolved in nocturnal geckos, which increases maximum aerobic speed and partially offsets the decrease in maximum oxygen consumption caused by activity at low nocturnal temperatures. This is termed the nocturnality hypothesis. The generality of the nocturnality hypothesis is tested by comparing the C_{min} values of four lizard species (two nocturnal and two diurnal; n = 5 to 9 individuals per species), including a nocturnal scincid lizard. C_{min} is calculated as the energy required to move a gram of body mass over a kilometre and is measured during steady exercise on a treadmill respirometer. I accept the hypothesis that nocturnal lizards in general have a low C_{min}. Evidence is also provided that low C_{min} is present in high-latitude diurnal lizards that experience low temperatures during their activity periods. The C_{min} values of the four lizard species measured in this study (range = 0.21-2.00 ml O_2 g^{-1} km^{-1}) are lower than diurnal lizards from elsewhere, and within or below the 95% confidence limits of published C_{min} values of nocturnal geckos. A low C_{min} increases the range of locomotor speeds possible at low temperatures and provides an advantage to species that are active at these temperatures. I conclude that there may be a reduced C_{min} in all nocturnal lizards as well as lizards from high latitudes. The low C_{min} in lizards living at high latitudes may enable extension of their latitudinal range into thermally sub-optimal habitats.

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5.2 Introduction

Environmental temperature and activity of reptiles are positively correlated (Bennett, 1982) and the thermal optima for sprinting in diurnal and nocturnal lizard species do not differ (Huey et al., 1989). Consequently, the locomotor performance of nocturnal lizard species is suboptimal at low night temperatures (e.g., Huey et al., 1989; Autumn et al., 1994; Autumn et al., 1999). However, at low temperatures nocturnal geckos can sustain speeds up to three times those of diurnal lizards at the same temperatures, with performance approaching that of diurnal lizards at higher optimal temperatures (Autumn et al., 1994; Autumn et al., 1997; Autumn et al., 1999). This occurs despite a decrease in aerobic capacity by a factor of about two for each 10 °C decrease in temperature (Bennett, 1982). The mechanism proposed to enable activity of nocturnal geckos is a low minimum cost of locomotion (C_{min}; energy required to move a gram of body mass over one km; Autumn et al., 1994; Autumn et al., 1997; Autumn, 1999; Autumn et al., 1999).

Research on nocturnality has mainly focused on the locomotor efficiency of species. Locomotor efficiency is an ideal performance variable for three reasons: 1) it is quantifiable (Huey and Dunham, 1987), 2) it is a good indicator of an animal's ability to function effectively (Garland and Bennett, 1990), and 3) the relationships between temperature (environmental level), aerobic metabolism (physiological level), and endurance capacity (performance level) are well known (see Bennett, 1982 for review; Autumn et al., 1999). A low C_{min} has evolved in nocturnal geckos, which increases maximum aerobic speed and partially offsets the decrease in maximum oxygen consumption caused by activity at low nocturnal temperatures. (Autumn et al., 1994; Farley and Emshwiller, 1996; Autumn et al., 1997; Autumn, 1999; Autumn et al., 1999). It is unknown whether C_{min} is characteristic of nocturnal lizards in general or is a trait specific to nocturnal geckos.

Lizards are ancestrally diurnal, and nocturnality has arisen independently within three squamates lineages; geckos, snakes, and skinks (Vitt et al., 2003). Therefore, if

nocturnal snakes and skinks have a low C_{min}, as reported for nocturnal geckos, it is likely that a low C_{min} is required for adaptation to a cold environment in squamates (Autumn et al., 1997). Alternatively, the low C_{min} observed in geckos could be an evolutionary artefact and may not be present in all nocturnal lizard groups, or may even appear in diurnal species from cool temperate regions. The nocturnal rattlesnake *Crotalus cerastes* also has a low C_{min} (Secor et al., 1992). However, snakes use a different mode of locomotion than lizards, and a more relevant comparison would be obtained from studying another nocturnal lizard (Autumn et al., 1997). Further study of other secondarily diurnal geckos as well as diurnal lizards active at low temperatures will provide a robust test of the generality of the nocturnality hypothesis (Autumn et al., 1994).

The lizard families in New Zealand (Scincidae and Diplodactylidae) (Gill and Whitaker, 2001; Han et al., 2004) provide an ideal model system in which to study nocturnality. Each family consists of two genera, one predominantly nocturnal and the other predominantly diurnal. Each family has a different evolutionary history in relation to nocturnality. Geckos are ancestrally nocturnal (Vitt et al., 2003), which means diurnal geckos are secondarily diurnal. Conversely, skinks are ancestrally diurnal (Vitt et al., 2003), and some species in New Zealand have evolved nocturnality or are crepuscular (active in the twilight). New Zealand has a temperate maritime climate with relatively cool summers and mild winters (NIWA, 2005). Mean annual temperatures in New Zealand range from 10 °C in the south to 18 °C in the far north (NIWA, 2005). In the sub-tropical far north, summer temperatures usually range from 22 to 26 °C and seldom exceed 30 °C (NIWA, 2005). Some New Zealand lizards remain active at body temperatures as low as 10 °C (Werner and Whitaker, 1978). Thus, the Cmin of New Zealand lizards may be generally low compared with lizards from relatively warm, stable climates, such as in the tropics.

I tested whether the low C_{min} of nocturnal geckos is unique to geckos, or whether it represents a more general pattern of convergent evolution among lizards to enable

nocturnality. The C_{min} of four species of lizard (two nocturnal and two diurnal) was measured, asking the following questions: 1) Do nocturnal lizards other than geckos have lower C_{min} than diurnal lizards? 2) Do geckos have lower C_{min} than other lizard taxa? 3) Do lizards active at low temperatures have a lower C_{min} than those active at warm temperatures?

5.3 Materials and methods

I measured the C_{min} of four lizard species: the nocturnal gecko *Hoplodactylus maculatus* (n = 17), the diurnal gecko *Naultinus manukanus* (n = 23), the diurnal skink *Oligosoma nigriplantare polychroma* (n = 23) and the nocturnal skink *Cyclodina macgregori* (n = 24). The activity period assigned to each species is based on a published field guide (Gill and Whitaker, 2001).

5.3.1 Animal collection and husbandry

Animal collection and husbandry follows the same procedures as described in Hare et al. (2004b), Chapter 3 and Chapter 4. All animals were collected within the latitudinal range 40° 50' 35" to 41° 20' 83" in the Cook Strait region of New Zealand. *Naultinus manukanus* were collected from Stephens Island (Takapourewa) between 9-14 March 2003 and transferred to Victoria University of Wellington (VUW). *Hoplodactylus maculatus* and *O. n. polychroma* were captured in the greater Wellington region between 25 January and 23 April 2004 and measured at VUW. *Cyclodina macgregori* were captured on Mana Island and measured at a field station between 27 October and 18 November 2004. Only adult males and non-pregnant females were examined since $\dot{V}O_2$ may be elevated in pregnant individuals (e.g., DeMarco, 1993; Robert and Thompson, 2000a), and extra costs are associated with locomotion in some pregnant lizards (Shine, 2003). Adult males were distinguished from females by inspection of the ventral tail base for protruding hemipenal sacs in geckos and hemipene eversion in skinks (Gill and Whitaker, 2001; Harlow, 1996). Reproductive status of females was

determined by abdominal palpation (see Cree and Guillette (1995) and Wilson and Cree (2003) for information on accuracy of this procedure in other New Zealand geckos).

Ambient temperature on Mana Island ranged from 8-23 °C, and photoperiod during November was 14:10 light:dark (sunrise at ~0600 h). On the mainland (VUW), all lizards were held in the same room to acclimate them to identical light and temperature regimes (3-4 weeks for *O. n. polychroma* and *H. maculatus*; 6 months for *N. manukanus* due to the delayed treadmill construction). Room temperature on the mainland ranged from 16-25 °C, and photoperiod was on a 12:12 light:dark cycle (on at 0600 h).

5.3.2 Treadmill experiments

Lizards were fasted for at least 72 h prior to the first measurement, except for *C. macgregori* which were fasted for at least 96 h because of their larger size. The fasting time ensures a post-absorptive state in all lizards (Coulson and Hernandez, 1980; Robert and Thompson, 2000b; Hopkins et al., 2004). All lizards defecated within this time period and did not defecate during or after the respirometry trials. During the fasting period, the lizards were housed individually in 2 L plastic containers with a 50 x 50 mm square of 1 x 1 mm wire mesh in the lids for ventilation. Saturated paper towels provided water *ad libitum*. Food was withheld during the experimental period.

Ten trials were conducted following the methodology of Hare et al. (2004b) to measure $\dot{V}O_2$ of restrained (wearing a respirometry mask), resting *N. manukanus*. The respirometry masks were fashioned from the finger of a transparent surgical glove, with respirometry tubing attached for air flow. The lightweight mask was loosely attached with surgical tape to the nape of the neck and the sternum. The masks fitted entirely over the head but did not impede locomotion or respiration. $\dot{V}O_2$ was measured for each lizard once a day for five consecutive days over two periods (five trials in period A, and five trials in period B), with a rest period of five days between periods A and B. Food was withheld during both periods. Animals were fed on the fifth day of period A after the fifth trial, and then fasted again prior to period B. Two periods were conducted to

test the 'memory' of animals with regard to experimental conditioning. After three trials $\dot{V}O_2$ did not differ significantly among trials ($P = 0.250$), even after a five-day rest period ($F_{1,112} = 0.512$, $P = 0.475$; Appendix 1D). Thus, all lizards were conditioned to the experimental conditions, including walking on a treadmill, at least three times prior to taking the first measurement.

The lizards' ability to walk on a treadmill improved over the course of the conditioning trials. If a lizard did not learn to walk on the treadmill during trials or struggled for more than 15 s, they were excluded from the experiments. Thus, sample sizes were lower than anticipated because of exclusion of 70% of the individuals. Measures of $\dot{V}O_2$ for all speeds were gained for 9 of 20 (45%) *C. macgregori*, 5 of 24 (21%) *H. maculatus*, 6 of 17 (35%) *N. manukanus*, and 5 of 23 (22%) *O. n. polychroma*.

All experiments were undertaken from 0730 h to 1730 h. One hour prior to an experiment, a lizard was fitted with a respirometry mask. Air flowed past the lizard's head at 12 ml min^{-1} (laboratory) or 10 ml min^{-1} (Mana Island) from outside the building using a flow controller and pump (Sable Systems International Inc., Las Vegas, Gas Analyzer Sub-sampler). Analyses were conducted to correct for differences in air flow rate using Sable systems software and the equations of Withers (1977). A soap bubble flow-through system was used to calibrate the flow controller (Long and Ireland, 1985). Air was not dried prior to being passed over the lizards since some New Zealand lizards have relatively high rates of water loss for their size (Cree and Daugherty, 1991; Neilson, 2002). However, the excurrent air from the mask passed through a column of self-indication Drierite®, soda lime and then Drierite® again before entering the oxygen analyser (a two-channel Sable Systems FC-2). The scrubbed mask air was continually compared with scrubbed air from outside the building to ensure that atmospheric oxygen (20.94%) was being delivered to the lizards in all experiments.

All lizards were thermally equilibrated to the experimental temperature (25 ± 0.2 °C) within the treadmill with respirometry masks in place for at least 1 h prior to

measurements. I chose 25 °C as it is an ecologically relevant temperature that some New Zealand lizards are able to achieve by thermoregulation (Werner and Whitaker, 1978; Rock et al., 2002). Temperature within the treadmill was measured at 15 min intervals using thermal data loggers accurate to 0.3 °C (StowAway® TidbiT®, Onset™ Computer Corporation, Massachusetts, USA).

The lizards were exercised in a miniature temperature-controlled treadmill-respirometer (Herreid et al., 1981; Autumn et al., 1994; Autumn et al., 1997; Appendix 1A) on five consecutive days. Treadmill speeds ranged from 0.054 km h^{-1} to 0.254 km h^{-1}, in increments of 0.040 km h^{-1}. Some species were able to run at all speeds (e.g., *C. macgregori*), whereas others were not (e.g., *O. n. polychroma*). On each day, lizards ran at no more than two treadmill speeds, the latter speed always one increment faster than the first speed. Lizards were encouraged to walk or run by gently tapping the hind legs and tail with a thin plastic rod. Cold fibre optic lighting at high intensity directed at the sides of the chamber helped to encourage steady runs in the nocturnal lizards and were also used for diurnal lizards to ensure consistency of experimental procedures.

Output from the oxygen analyser was recorded using Sable Systems (UI2) and MS Windows software. The animals were weighed immediately after removal from the treadmill on a Sartørius™ top-loading balance. Barometric pressure was recorded at the beginning and end of each measurement series, and the average pressure was used in $\dot{V}O_2$ calculations. The steady-state $\dot{V}O_2$ was calculated with DATACAN (Sable Systems Inc. USA) using equations of Withers (1977). A reference line (no lizard present) was also included to obtain baseline oxygen concentrations. Measurement of oxygen concentration in the reference line was taken at the beginning and end of each $\dot{V}O_2$ measurement.

Aerobically submaximal $\dot{V}O_2$ for an individual at a single speed was calculated from the mean of a steady-state $\dot{V}O_2$ during the last 3 min of at least 7 min of continuous locomotion. Most trials did not exceed 12 min of continuous locomotion. At speeds

above maximum sustained aerobic speed (MAS), some individuals were not able to continue locomotion for >2 mins, and these speeds were not included in the calculation of C_{min}.

5.3.3 Statistical analysis

Data were analysed using the statistical programme *R* (Gentleman et al., 2003; Version 1.5.1). Statistical significance was assumed at $P < 0.05$. Data are expressed as means ± 1 SE unless otherwise stated.

To determine whether $\dot{V}O_2$ is similar in restrained (wearing a respirometry mask) and unrestrained (free within respirometry chamber) individuals, the $\dot{V}O_2$ values of restrained lizards was compared with data of unrestrained individuals from the results of Hare et al. (2004b) at 24 °C and data from Chapter 3 at 26 °C. I also compared $\dot{V}O_2$ data from restrained individuals of *C. macgregori*, *H. maculatus* and *O. n. polychroma* with data obtained in Chapter 3 at 26 °C. The linear mixed effects function and likelihood ratio tests in *R* were used for all analyses (Pinheiro and Bates, 2000; Hare et al., 2004b).

The maximum aerobic speed (MAS) is a function of an animal's maximum rate of oxygen consumption during exercise ($\dot{V}O_{2max}$) and C_{min} (Autumn, 1999), as described by the equation:

$$MAS = (\dot{V}O_{2max} - y_0) / C_{min} \qquad \text{(Equation 1)}$$

where $\dot{V}O_{2max}$ is the maximum rate of oxygen consumption during exercise (aerobic capacity), and y_0 is the intercept of the $\dot{V}O_{2max}$ versus speed regression (idling cost; see Gatten et al., 1992 for review). To identify the MAS for each individual, the linear regressions of $\dot{V}O_2$ against speed for the three lowest speeds were first calculated, and then by sequentially including higher speeds the fit of the data was compared with the regressions. The MAS was defined as the speed above which the r^2 of the regression decreased. This method closely agreed with a visual analysis of the data.

To evaluate interspecific variation in C_{min} on MAS, standard and phylogenetic allometric contrasts were used to compare the values measured in this study with values from previous studies (as reported in Autumn et al. (1999) and Autumn (1999); Table 5.1). I followed the statistical and experimental methodology of Autumn et al. (1999), except that varanid lizards were included in all calculations for a complete data set. Therefore, some calculations and interpretations differ from Autumn et al. (1999). Species were classified as nocturnal or diurnal, as well as by the taxonomic categories: Anguimorpha, Gekkota, Iguania and Scincomorpha (described in Pianka and Vitt (2003)) and the latitudinal ranges: tropical, subtropical and temperate. I defined tropical as between the tropics of Cancer (23.5 ° N) and Capricorn (23.5 ° S), temperate as latitudes higher than the tropics of Cancer or Capricorn, and subtropical as the populations found at the edge of the tropical/temperate border (within 2 ° of the 23.5 ° N or S), or where the populations were not defined and the species' range included both tropical and temperate areas (Table 5.1).

The C_{min} values measured at different temperatures can be compared because C_{min} is not thermally sensitive in lizards (e.g., John-Alder and Bennett, 1981; Autumn et al., 1994). $\dot{V}O_{2max}$ is influenced by temperature, and therefore the methodology employed by Autumn et al. (1999) was followed, using a Q_{10} model to adjust $\dot{V}O_{2max}$ in all species to reflect a body temperature of 35 °C. In lizards, Q_{10} for $\dot{V}O_{2max}$ varies with body mass (Bennett, 1982). Following Bennett (1982), I used a Q_{10} of 2.5 for lizards < 10 g, a Q_{10} of 2.25 for lizards between 10-100g, and a Q_{10} of 2.0 for lizards > 100 g. It follows from the thermal insensitivity of C_{min} that y_0 (see Equation 1) has the same thermal sensitivity as $\dot{V}O_{2max}$; therefore, the same Q_{10} relationship described above to adjust y_0 to reflect body temperature of 35 °C was used. Using measured values of C_{min} and the Q_{10}-adjusted values of $\dot{V}O_{2max}$ and y_0 in equation (1), I calculated a predicted value for MAS at 35 °C for each species in order to evaluate the effect of change in C_{min} on MAS at a typical diurnal temperature of most lizards worldwide.

Table 5.1: Reported energetic cost of treadmill locomotion at aerobically submaximal speeds in lizards.

Active	Taxa	Species	Lat.	Mass	T_b	$\dot{V}O_{2rest}$	$\dot{V}O_{2max}$	y_0	C_{min}	MAS	Citation
D	A	*Heloderma horridum*	Te	803.0	31	-	1.010	-	-	-	Beck et al., 1995
D	A	*Heloderma suspectum*	Te	396.0	31	-	1.130	-	-	-	Beck et al., 1995
D	A	*Heloderma suspectum*	Te	463.9	35	-	0.896	0.258	0.617	1.030	John-Alder et al., 1983
D	A	*Varanus acanthurus*	Tr	73.6 (53.4)	35	- (0.117)	2.310 -	-	-	-	Thompson and Withers, 1997
D	A	*Varanus brevicauda*	ST	17.5	35	0.156	3.230	-	-	-	Thompson and Withers, 1997
D	A	*Varanus caudolineatus*	Tr	14.9 (13.1)	35	- (0.173)	6.290 -	-	-	-	Thompson and Withers, 1997
D	A	*Varanus eremius*	ST	40.0 (35.9)	35	- (0.172)	2.300 -	-	-	-	Thompson and Withers, 1997
D	A	*Varanus exanthematicus*	Te	1025.0	35	0.189	1.260	-	0.620	1.200	Gleeson et al., 1980
D	A	*Varanus gilleni*	Te	8.4 (20.0)	35	- (0.179)	5.179 -	-	-	-	Thompson and Withers, 1997
D	A	*Varanus gouldii*	Tr	1086.0	35	0.090	1.354	-	-	-	Christian and Conley, 1994
D	A	*Varanus mertensi*	Tr	904.0	35	0.078	0.909	-	-	-	Christian and Conley, 1994
D	A	*Varanus panoptes*	Tr	931.0	35	0.132	1.340	-	-	-	Christian and Conley, 1994

D = diurnal; A = Anguimorpha; Classifications of taxa are from Pianka and Vitt (2003). Lat. = latitudinal range; Te = temperate; Tr = tropical; ST = sub-tropical (populations found within 2 ° of tropical/temperate border or populations not defined and species range covers tropical and temperate areas; T_b = body temperature (°C) during the experiment; C_{min} = cost of locomotion (ml O_2 g^{-1} km^{-1}) as measured by calculating the slope of the regression rate of the rate of oxygen consumption ($\dot{V}O_2$) against speed; MAS = maximum aerobic speed (km h^{-1}) calculated statistically as the speed above which there is no significant increase in $\dot{V}O_2$; Values of body mass (g) in parentheses are measurements of $\dot{V}O_2$ at rest in parentheses; y_0 = y-intercept of $\dot{V}O_2$ versus speed (idling cost); $\dot{V}O_{2max}$ = maximum rate of $\dot{V}O_2$ during exercise (aerobic capacity); $\dot{V}O_{2rest}$ = resting rate of $\dot{V}O_2$; Data to 1999 are from Autumn et al. (1999).

Table 5.1 cont.

Active	Taxa	Species	Lat.	Mass	T_b	$\dot{V}O_{2rest}$	$\dot{V}O_{2max}$	y_0	C_{min}	MAS	Citation
D	A	*Varanus rosenbergi*	Tr	1287.0	35	0.132	1.133	-	-	-	Christian and Conley, 1994
D	A	*Varanus tristis*	ST	103.2 (99.0)	35	- (0.159)	3.000 -	-	-	-	Thompson and Withers, 1997
D	G	*Naultinus manukanus*	Te	6.6	25	0.025	0.245	.0125	0.812	0.14	This study
D	G	*Phelsuma madagascarensis*	Tr	23.9	25	0.149	0.475	0.151	1.389	0.224	Autumn, 1999
D	G	*Rhoptropus bradfieldi*	Tr	4.7	25	0.091	0.425	0.188	2.468	0.094	Autumn, 1999
D	I	*Amblyrynchus cristatus*	Tr.	2885.0	35	0.100	0.665	0.292	0.373	1.000	Gleeson, 1979
D	I	*Conolophus subcristatus*	Tr	3885.3	35	0.100	0.623	0.310	0.361	0.867	Gleeson, 1979
D	I	*Cyclura nubila*	ST	1136.0	35	-	0.893	-	-	-	Christian and Conley, 1994
D	I	*Dipsosaurus dorsalis*	Te	51.3	40	0.319	2.002	0.62	1.442	0.800	John-Alder and Bennett, 1981
D	I	*Iguana iguana*	Te	850.0	35	0.183	0.832	-	-	0.500	Gleeson et al., 1980
D	I	*Moloch horridus*	Te	27.7	35	0.114	0.990	-	-	-	Clemente et al., 2004
D	I	*Phrynosoma douglassi*[1]	Te	4.5	35	-	1.100	0.425	2.550	0.265	Autumn et al., 1997
D	S	*Eumeces skiltonianus*[1]	Te	4.8	35	-	1.650	0.35	2.550	0.510	Farley and Emshwiller, 1996
D	S	*Oligosoma nigriplantare polychroma*	Te	3.2	25	0.159	0.701	0.410	1.999	0.16	This study

D = diurnal; A = Anguimorpha; G = Gekkota; I = Iguania; S = Scincomorpha; Classifications of taxon are from Pianka and Vitt (2003). Lat. = latitudinal range; Te = temperate; Tr = tropical; ST = sub-tropical (populations found within 2 ° of tropical/temperate border or populations not defined and species range covers tropical and temperate areas; T_b = body temperature (°C) during the experiment; C_{min} = cost of locomotion (ml O_2 g^{-1} km^{-1}) as measured by calculating the slope of the regression rate of the rate of oxygen consumption ($\dot{V}O_2$) against speed; MAS = maximum aerobic speed (km h^{-1}) calculated statistically as the speed above which there is no significant increase in $\dot{V}O_2$; Values of body mass (g) in parentheses are measurements of $\dot{V}O_2$ at rest in parentheses; y_0 = y-intercept of $\dot{V}O_2$ versus speed (idling cost); $\dot{V}O_{2max}$ = maximum rate of $\dot{V}O_2$ during exercise (aerobic capacity); $\dot{V}O_{2rest}$ = resting rate of $\dot{V}O_2$; Data to 1999 are from Autumn et al. (1999). [1] = measured at 25 °C and adjusted to 35 °C, assuming a Q_{10} of 2.5 (Bennett, 1982).

Table 5.1 cont.

Active	Taxa	Species	Lat.	Mass	T_b	$\dot{V}O_{2rest}$	$\dot{V}O_{2max}$	y_0	C_{min}	MAS	Citation
D	S	*Tiliqua rugosa*	Te	548.0	35	0.144	0.600	-	-	-	Christian and Conley, 1994
D	S	*Tiliqua rugosa*[2]	Te	474.0	35	0.730	0.722	0.107	0.921	0.670	John-Alder et al., 1986
D	S	*Tupinambis nigropunctatus*	Tr	865.0	35	0.136	0.672	0.348	0.521	0.840	Bennett and John-Alder, 1984
N	G	*Coleonyx variegatus*	Te	4.2	25	-	0.500	0.160	1.490	0.230	Autumn et al., 1997
N	G	*Diplodactylus galeatus*	ST	4.3	20	0.112	0.377	0.001	1.689	0.220	Autumn et al., 1999
N	G	*Diplodactylus intermedius*	ST	4.9	20	0.107	0.466	0.093	1.434	0.260	Autumn et al., 1999
N	G	*Eublepharis macularius*	Te	32.8	25	0.140	0.505	0.273	1.090	0.213	Autumn et al., 1999
N	G	*Hoplodactylus maculatus*	Te	6.0	25	0.106	0.250	0.149	0.755	0.13	This study
N	G	*Nephrurus asper*	ST	25.1	20	0.069	0.312	0.124	0.762	0.24	Autumn et al., 1999
N	G	*Nephrurus levis*	ST	12.7	20	0.050	0.268	0.091	0.970	0.183	Autumn et al., 1999
N	G	*Pachydactylus bibroni*	Tr	14.8	25	0.130	0.532	0.225	1.194	0.24	Autumn et al., 1999
N	G	*Teratoscincus przewalski*	Te	11.2	25	0.100	0.518	0.165	1.060	0.330	Autumn et al., 1994
N	S	*Cyclodina macgregori*	Te	20.2	25	0.049	0.104	0.072	0.211	0.16	This study

D = diurnal; N = nocturnal; G = Gekkota; S = Scincomorpha; Classifications of taxon are from Pianka and Vitt (2003). Lat. = latitudinal range; Te = temperate; Tr = tropical; ST = sub-tropical (populations found within 2 ° of tropical/temperate border or populations not defined and species range covers tropical and temperate areas; T_b = body temperature (°C) during the experiment; C_{min} = cost of locomotion (ml O_2 g^{-1} km^{-1}) as measured by calculating the slope of the regression rate of the rate of oxygen consumption ($\dot{V}O_2$) against speed; MAS = maximum aerobic speed (km h^{-1}) calculated statistically as the speed above which there is no significant increase in $\dot{V}O_2$; Values of body mass (g) in parentheses are measurements of $\dot{V}O_2$ at rest in parentheses; y_0 = y-intercept of $\dot{V}O_2$ versus speed (idling cost); $\dot{V}O_{2max}$ = maximum rate of $\dot{V}O_2$ during exercise (aerobic capacity); $\dot{V}O_{2rest}$ = resting rate of $\dot{V}O_2$; Data to 1999 are from Autumn et al. (1999). [2] as *Trachydosaurus rugosus* in John-Alder et al. (1986).

Randomisation (permutation) tests were used to account for possible dependence of data due to phylogenetic relationships among species. The randomisation tests for the data used 10,000 permutations of the sample (Harvey and Pagel, 1991). The relative ranking of the observed test statistic is reported as a *P* value. The complete model included simultaneous tests of equal slopes and equal intercepts and was compared with a reduced model that assumed the factor being tested (activity period, latitudinal range or taxonomic position) was irrelevant for the response variable ($\dot{V}O_{2max}$, C_{min} or MAS). Randomisation tests were employed to determine whether the variable was statistically and phylogenetically significant among species, activity periods (nocturnal and diurnal), taxonomic level and latitudinal range (tropical, subtropical and temperate).

5.4 Results

Only *Naultinus manukanus* had a significantly lower mass-specific $\dot{V}O_2$ with the masks on at 25 °C than unrestrained and at rest within a chamber at 26 °C ($F_{2,5} = 29.434$, $P = 0.002$). All other species had a similar $\dot{V}O_2$ with respirometry masks on at 25 °C, and unrestrained and at rest within a chamber at 26 °C (Table 5.2). Compared with previous studies (Chapter 3; Hare et al., 2004b) *N. manukanus* had an unrestrained $\dot{V}O_2$ 73% lower than expected.

5.4.1 Maximum aerobic speed, $\dot{V}O_{2max}$ and C_{min}

Maximum aerobic speeds for the species in this study were similar, ranging from 0.13 km h^{-1} in *H. maculatus* to 0.16 km h^{-1} in *C. macgregori* and *O. n. polychroma* (Table 5.3). Strong positive trends between $\dot{V}O_2$ and speed were apparent in all but one individual ($r^2 = 0.8$-1.0, *C. macgregori* individual number 3 where $r^2 = 0.7$). $\dot{V}O_{2max}$ ranged from 0.10 ml O_2 g^{-1} h^{-1} in *C. macgregori* to 0.70 ml O_2 g^{-1} h^{-1} in *O. n. polychroma*. The average C_{min} values for all lizards ranged from 0.21 ml O_2 g^{-1} km^{-1} in *C. macgregori* to 2.00 ml O_2 g^{-1} km^{-1} in *O. n. polychroma* (Table 5.3). *Hoplodactylus maculatus* individuals had less variable $\dot{V}O_2$ at different speeds than other species, with *C. macgregori* the most variable (Figure 5.1). For all species but *N. manukanus*, the

ratio of y_0 to $\dot{V}O_{2rest}$ (cost of maintaining posture while running) was comparable to that of other vertebrates (range = 0.64 to 2.9 ml O_2 g^{-1} h^{-1}; Paladino and King, 1979; Table 5.3). The higher cost of maintaining posture in *N. manukanus* (7.2 ml O_2 g^{-1} h^{-1}) was due to a very low $\dot{V}O_{2rest}$ compared with previous studies (e.g., Chapter 3; Hare et al., 2004b).

Table 5.2: Comparison of rate of oxygen consumption ($\dot{V}O_2$; ml O_2 g^{-1} h^{-1}) of lizards at rest wearing a respirometry mask (25 °C) or sitting quietly, unrestrained within a respirometry chamber (26 °C) after at least three conditioning trials of experimental procedures. Species are from the genera *Cyclodina*, *Hoplodactylus*, *Naultinus* and *Oligosoma*.

Species	Mask			Chamber*			*P*-value
	Mass (g)	*n*	$\dot{V}O_2$	Mass (g)	*n*	$\dot{V}O_2$	
C. macgregori	20.0	10	0.05	19.2	26	0.06	0.103
H. maculatus	6.5	7	0.11	6.7	28	0.10	0.992
N. manukanus	6.8	7	0.03	6.1	29	0.11	**0.002**
O. n. polychroma	3.4	5	0.16	3.2	25	0.16	0.539

* data are from Chapter 4; All $\dot{V}O_2$ data are ± 0.01 SE; The *P*-values in bold is significant.

5.4.2 Standard and phylogenetic allometric contrast analysis

Phylogenetic allometric contrasts (PAC) did not alter the results from the standard allometric contrast analysis (Appendix 1D). Thus, both analyses may not be required in studies comparing among species, instead phylogenetic allometric analyses are adequate. Using predicted $\dot{V}O_{2max}$ for lizards measured at 35 °C and excluding the species from this study, the allometric slopes relating mass and $\dot{V}O_{2max}$ were similar in diurnal lizards and geckos, although the intercepts differ significantly (Autumn et al., 1999; Figure 5.2). The linear regression lines of PAC relating mass and $\dot{V}O_{2max}$ were significantly different among nocturnal and diurnal lizards ($P = 0.027$). I did not include data from this study for regression analyses as all the species studied here have substantially lower $\dot{V}O_{2max}$ than predicted for any other lizard species to date ($P < 0.001$).

Table 5.3a: Energetic cost of treadmill locomotion at aerobically submaximal speeds in two species of nocturnal lizard from the families Scincidae (genus *Cyclodina*) and Diplodactylidae (genus *Hoplodactylus*).

Species & animal no.	Mean mass (g)	$\dot{V}O_{2rest}$ (ml O_2 g^{-1} h^{-1})	y_0 (ml O_2 g^{-1} h^{-1})	C_{min} (ml O_2 g^{-1} km^{-1})	r^2	$\frac{y_0}{\dot{V}O_{2rest}}$	$\dot{V}O_{2max}$ (ml O_2 g^{-1} h^{-1})	$\frac{\dot{V}O_{2max}}{\dot{V}O_{2rest}}$	$\dot{V}O_{2max}$ - $\dot{V}O_{2rest}$	MAS (km h^{-1})
Cyclodina macgregori										
1	21.5	0.042	0.061	0.349	1.00	1.4	0.105	2.5	0.063	0.13
2	23.2	0.039	0.041	0.187	0.88	1.1	0.065	1.7	0.027	0.13
3	16.5	0.059	0.073	0.364	0.70	1.2	0.132	2.2	0.073	0.16
4	19.8	0.049	0.067	0.122	1.00	1.4	0.081	1.7	0.032	0.12
5	20.8	0.049	0.062	0.288	0.99	1.3	0.099	2.0	0.049	0.13
6	18.3	0.070	0.107	0.156	1.00	1.5	0.127	1.8	0.056	0.13
7	18.3	0.059	0.084	0.188	0.90	1.4	0.122	2.1	0.063	0.20
8	21.9	0.040	0.093	0.062	1.00	2.3	0.105	2.6	0.065	0.19
9	21.8	0.037	0.056	0.179	0.83	1.5	0.098	2.7	0.061	0.24
Mean	**20.2**	**0.049**	**0.072**	**0.211**	**-**	**1.5**	**0.104**	**2.1**	**0.054**	**0.16**
Hoplodactylus maculatus										
1	7.2	0.100	0.173	0.501	0.85	1.7	0.236	2.4	0.136	0.13
2	5.5	0.106	0.192	0.411	0.86	1.8	0.247	2.3	0.141	0.13
3	6.0	0.103	0.163	0.590	0.88	1.6	0.236	2.3	0.133	0.13
4	5.5	0.112	0.081	1.509	1.00	0.7	0.301	2.7	0.189	0.15
5	5.6	0.108	0.137	0.763	0.99	1.3	0.230	2.1	0.122	0.12
Mean	**6.0**	**0.106**	**0.149**	**0.755**	**-**	**1.4**	**0.250**	**2.4**	**0.144**	**0.13**

C_{min} = minimum cost of locomotion; MAS = maximum aerobic speed; $\dot{V}O_{2max}$ = maximum rate of oxygen consumption during exercise; $\dot{V}O_{2rest}$ = rate of oxygen consumption during rest; y_0 = y-intercept of VO_2 versus speed function.

Table 5.3b: Energetic cost of treadmill locomotion at aerobically submaximal speeds in two species of diurnal lizard from the families Scincidae (genus *Oligosoma*) and Diplodactylidae (genus *Naultinus*).

Species & animal no.	Mean mass (g)	$\dot{V}O_{2rest}$ (ml O_2 g^{-1} h^{-1})	y_0 (ml O_2 g^{-1} h^{-1})	C_{min} (ml O_2 g^{-1} km^{-1})	r^2	$\frac{y_0}{\dot{V}O_{2rest}}$	$\dot{V}O_{2max}$ (ml O_2 g^{-1} h^{-1})	$\frac{\dot{V}O_{2max}}{\dot{V}O_{2rest}}$	$\dot{V}O_{2max}$ - $\dot{V}O_{2rest}$	MAS (km h^{-1})
Naultinus manukanus										
1	7.4	0.018	0.114	0.756	0.96	6.2	0.205	11.2	0.187	0.12
2	6.5	0.008	0.176	0.377	0.93	20.7	0.235	27.7	0.227	0.16
3	6.3	0.027	0.204	0.370	1.00	7.5	0.249	9.1	0.222	0.12
4	5.7	0.027	0.148	1.103	1.00	5.4	0.293	10.7	0.266	0.13
5	8.4	0.031	0.104	0.788	1.00	3.3	0.203	6.5	0.172	0.13
6	5.4	0.041	0.004	1.478	1.00	0.1	0.282	6.9	0.241	0.19
Mean	**6.6**	**0.025**	**0.125**	**0.812**	**-**	**7.2**	**0.245**	**12.0**	**0.219**	**0.14**
Oligosoma nigriplantare polychroma										
1	3.2	0.128	0.312	3.533	0.81	2.4	0.813	6.3	0.685	0.14
2	4.0	0.178	0.566	1.416	0.99	3.2	0.835	4.7	0.657	0.19
3	2.6	-	0.303	2.176	0.98	-	0.567	-	-	0.12
4	2.8	0.169	0.548	0.172	0.82	3.2	0.583	3.4	0.414	0.20
5	3.2	0.162	0.323	2.699	0.81	2.0	0.709	4.4	0.547	0.14
Mean	**3.2**	**0.159**	**0.410**	**1.999**	**-**	**2.7**	**0.701**	**4.7**	**0.576**	**0.16**

C_{min} = minimum cost of locomotion; MAS = maximum aerobic speed; $\dot{V}O_{2max}$ = maximum rate of oxygen consumption during exercise; $\dot{V}O_{2rest}$ = rate of oxygen consumption during rest; y_0 = y-intercept of $\dot{V}O_2$ versus speed function.

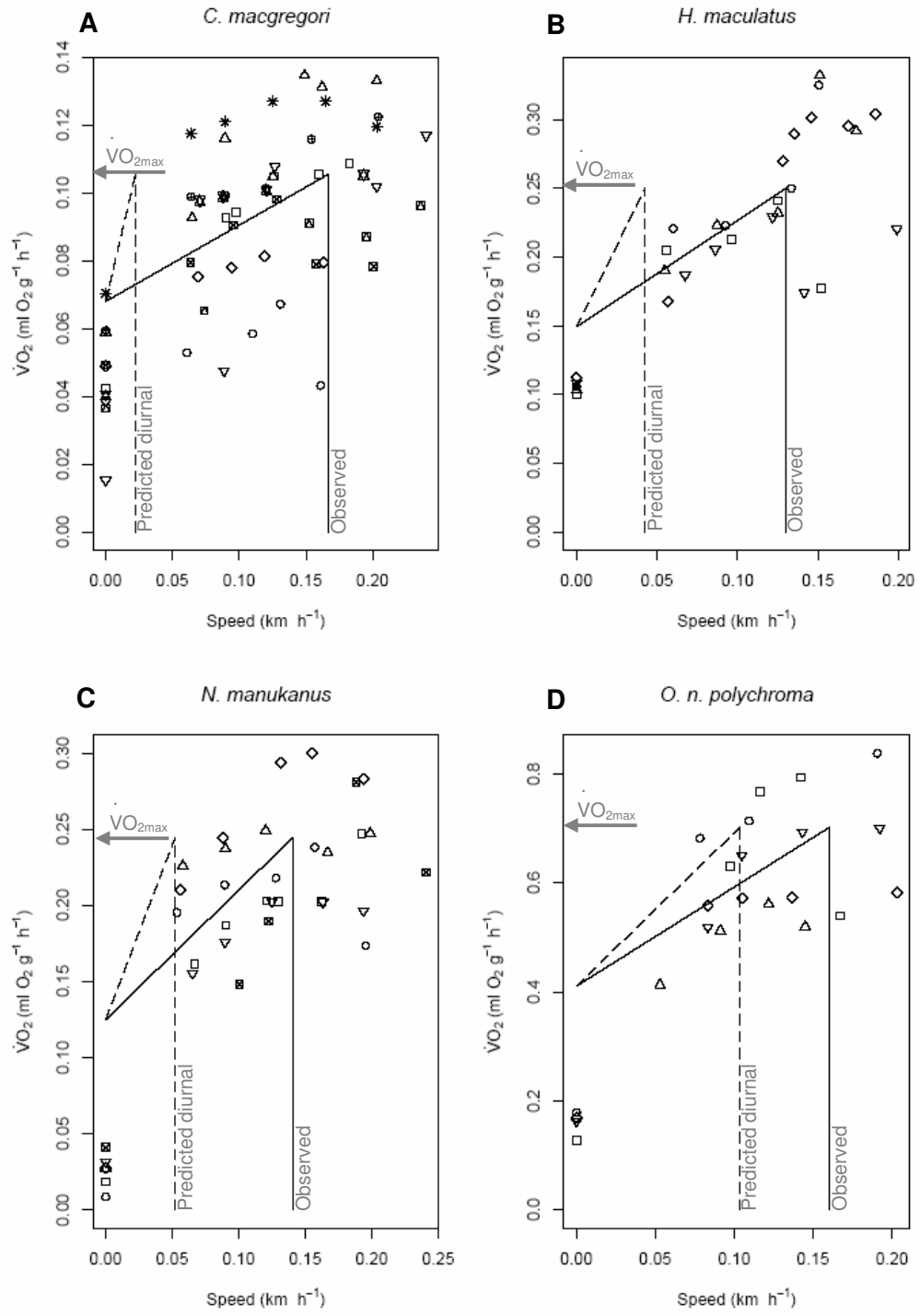

Figure 5.1: Mass-specific steady-state rate of oxygen consumption ($\dot{V}O_2$) during treadmill exercise at 25 °C in the nocturnal skink *Cyclodina macgregori*, nocturnal gecko *Hoplodactylus maculatus*, diurnal skink *Oligosoma nigriplantare polychroma*, and secondarily diurnal gecko *Naultinus manukanus*. Symbols represent different individuals. The slope of the solid line relates aerobically suboptimal $\dot{V}O_2$ and speed and represents the minimum cost of locomotion (C_{min}) calculated from individual animals (see Table 5.3). The slope of the dashed line represents C_{min} predicted for diurnal lizards of similar mass (See Figure 5.2). The predicted values for maximum aerobic speed (dashed vertical lines) are based on observed mean values of y_0 (y-intercept) and maximum aerobic speed ($\dot{V}O_{2max}$; horizontal arrows), and allometrically predicted C_{min} for each species. Because I calculated C_{min} first for each individual and then calculated mean C_{min}, the observed MAS does not coincide with any one treadmill speed used in the experiment.

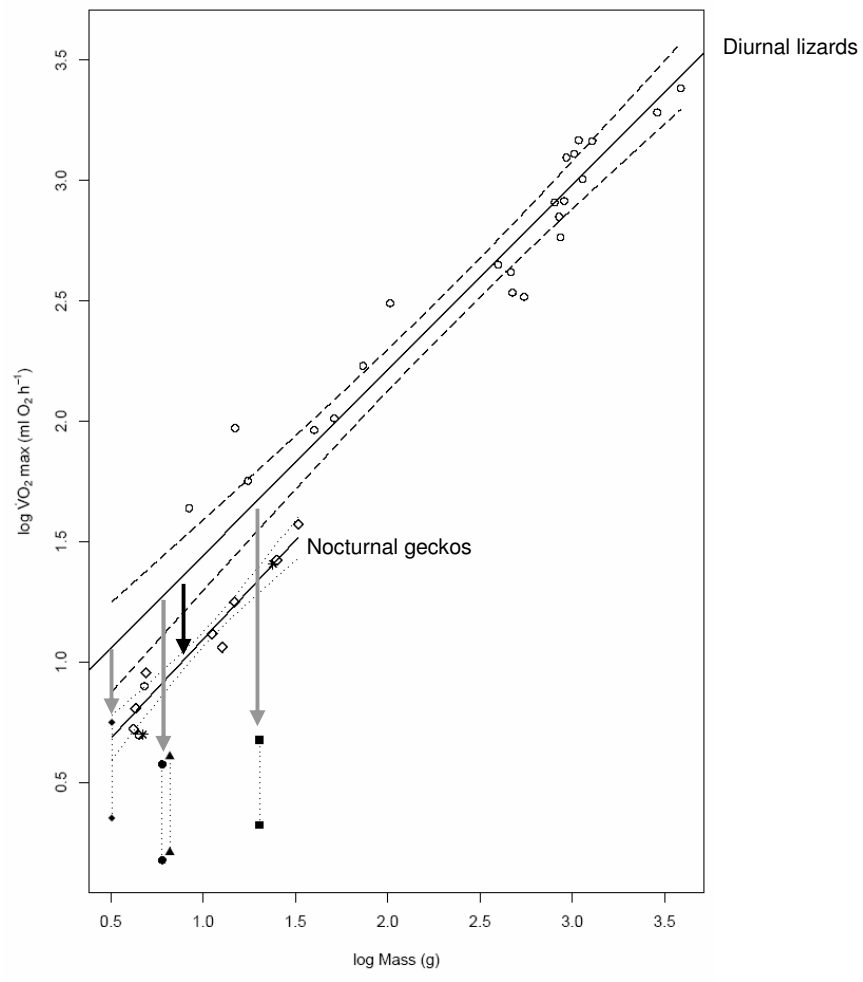

Figure 5.2: Log maximum rate of oxygen consumption ($\dot{V}O_{2max}$; adjusted to 35 °C using Q_{10} models) versus log body mass in lizards. Data and allometric relationships for ancestrally diurnal lizards (open circles), nocturnal geckos (open diamonds), and secondarily diurnal geckos (stars) are from literature values (see Table 5.1). Data from the present study are shown as solid geometric shapes; *Cyclodina macgregori* = solid square (nocturnal skink); *Hoplodactylus maculatus* = solid circle (nocturnal gecko); *Naultinus manukanus* = solid triangle (secondarily diurnal gecko); *Oligosoma nigriplantare polychroma* = small solid diamond (diurnal skink). The vertical dotted lines join measures for species in this study at 25 °C (lower shape) and adjusted to 35 °C (upper shape). Solid lines represent least squares regressions with 95% confidence limits for diurnal lizards (dashed lines) and nocturnal geckos (dotted lines). Diurnal line = log $\dot{V}O_{2max}$ = 0.67 + 0.77 log Mass; r^2 = 0.93. Nocturnal line = log $\dot{V}O_{2max}$ = 0.27 + 0.82 log Mass; r^2 = 0.94. The grey arrows represent the thermal handicap of activity on $\dot{V}O_{2max}$ for lizards in this study; the black arrow represents the thermal handicap of activity on $\dot{V}O_{2max}$ in nocturnal geckos.

Nocturnal geckos were outside the 95% confidence limits (CL) of the standard $\dot{V}O_{2max}$ allometry for diurnal lizards at 35 °C (Figure 5.2). Five diurnal lizard species had $\dot{V}O_{2max}$ within the 95% CL of nocturnal geckos. These included the secondarily diurnal geckos *Phelsuma madagascarensis* and *Rhoptropus bradfieldi* (Autumn, 1999), as well as the diurnal lizards *Eumeces skiltonianus*, *Phrynosoma douglassi* and *O. n. polychroma*. In general, Anguimorpha had significantly higher $\dot{V}O_{2max}$ than all other taxa ($P < 0.001$), and New Zealand lizards had lower than predicted $\dot{V}O_{2max}$ than other lizards measured at 35 °C ($P < 0.001$; Figure 5.2).

The linear regression lines of the PAC relating mass and C_{min} values differed significantly among nocturnal and diurnal lizards ($P = 0.007$). In general, nocturnal lizards had lower C_{min} values than all diurnal lizards, including most secondarily diurnal geckos ($P = 0.027$; Figure 5.2). The C_{min} values among Anguimorpha, Gekkota, Iguania and Scincomorpha did not differ, nor did C_{min} values differ with latitudinal range ($P > 0.05$; Appendix 1D). New Zealand lizards had significantly lower C_{min} values than all other lizards ($P < 0.001$; Figure 5.3). The C_{min} values of the two nocturnal species in this study were 68-87% lower than predicted by standard allometry for diurnal lizards of similar body mass (Figure 5.1a,b). The C_{min} values of the two diurnal species were 65-85% lower than predicted by standard allometry for all other diurnal lizards of similar body mass (Figure 5.1c,d). The C_{min} values of *N. manukanus* were 36% lower than predicted for a nocturnal gecko of similar body mass (Figure 5.1c), partly because the nocturnal value should be obtained from a regression line from nocturnal New Zealand lizards, since New Zealand lizards differ substantially from all other lizards measured. Nocturnal lizards have lower C_{min} values than diurnal lizards ($P = 0.025$). The C_{min} value of the diurnal skink *O. n. polychroma* was between the confidence limits of the nocturnal geckos and diurnal lizards (Figure 5.3). The C_{min} values of C. *macgregori* and *N. manukanus* were well below those of nocturnal geckos, with the nocturnal skink *C. macgregori* having the lowest C_{min} value of any other lizard (Table 5.2).

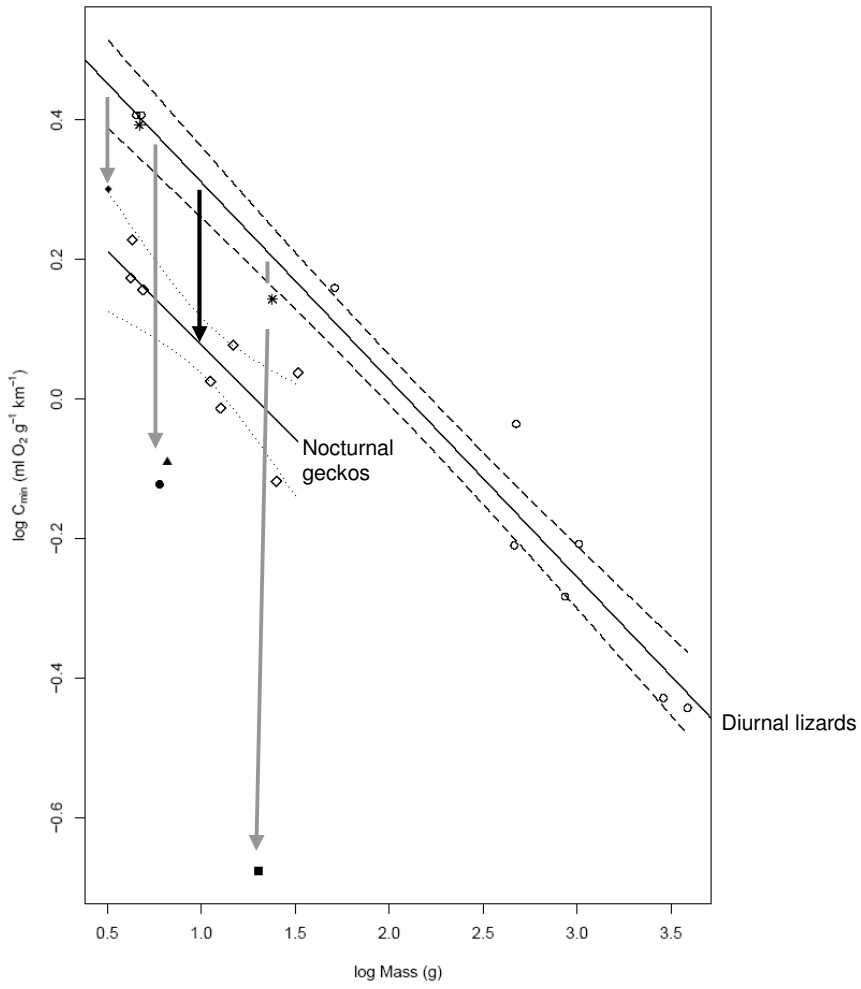

Figure 5.3: Log minimum cost of locomotion (C_{min}) versus log body mass in lizards. Data and allometric relationships for ancestrally diurnal lizards (open circles), nocturnal geckos (open diamonds), and secondarily diurnal geckos (stars) are from literature values (see Table 5.1). Data from the present study are shown as solid geometric shapes; *Cyclodina macgregori* = solid square (nocturnal skink); *Hoplodactylus maculatus* = solid circle (nocturnal gecko); *Naultinus manukanus* = solid triangle (secondarily diurnal gecko); *Oligosoma nigriplantare polychroma* = small solid diamond (diurnal skink). Solid lines represent least squares regressions with 95% confidence limits for diurnal lizards (log C_{min} = 0.59 - 0.28 log Mass; r^2 = 0.97; dashed lines) and nocturnal geckos (log C_{min} = 0.35 – 0.27 log Mass; r^2 = 0.69; dotted lines). Arrows indicate the decrease in C_{min} for nocturnal geckos reported in the literature (black arrow) and the lizards in this study (grey arrows). Nocturnal geckos have C_{min} values one third to one half lower than diurnal lizards (Autumn et al., 1999), and New Zealand lizards have C_{min} values 65-87% lower than diurnal lizards.

The effect of decreased $\dot{V}O_{2max}$ (Figure 5.2) combined with the effect of low C_{min} (Figure 5.3), gave the nocturnal lizards in this study an average MAS 39-80% of the values predicted for lizards of the same size with a typical nocturnal C_{min} (Figure 5.4). The diurnal lizards in this study had an average MAS 28-36% of the values predicted for lizards of the same size with typical diurnal C_{min} (Figure 5.4). The MAS did not differ among species for any variables (activity period, latitudinal range and taxon), except when secondarily diurnal geckos were excluded from the analysis (Appendix 1D). When secondarily diurnal geckos were excluded, nocturnal lizards had significantly lower MAS than diurnal lizards ($P = 0.015$). Thus, the low C_{min} values in secondarily diurnal geckos and nocturnal lizards were not sufficient to fully offset the effects of low temperature. Since New Zealand lizards were not significantly different in MAS from other lizards ($P = 0.117$), their low C_{min} is sufficient to offset the effects of low temperature on $\dot{V}O_{2max}$. This is despite New Zealand lizards having a MAS 28-88% lower than predicted for diurnal lizards of the same size (Figure 5.4).

5.5 Discussion

Nocturnal lizards in general have a low C_{min}, enabling activity at low temperatures that is comparable to diurnal lizards at higher temperatures. Diurnal lizards from New Zealand also have low C_{min} values. This implies that New Zealand lizards, and perhaps most lizards at high latitudes, live in thermally suboptimal habitats.

Mass-specific $\dot{V}O_2$ values are similar among conditioned, restrained animals and conditioned, unrestrained animals in all species measured here, except *N. manukanus*. Both $\dot{V}O_2$ and $\dot{V}O_{2max}$ decrease in the lizard *Amphibolurus nuchalis* when kept in a sedentary state in captivity (Garland et al., 1987). Thus, the low $\dot{V}O_{2rest}$ in *N. manukanus* is likely to have arisen from the extended time in captivity without exercise. Similarly, the $\dot{V}O_{2max}$ and MAS values of *N. manukanus* should probably be higher than reported here (Table 5.3), approaching that of other secondarily diurnal geckos (Autumn, 1999). Therefore, $\dot{V}O_2$ results from *N. manukanus* should be interpreted with care. However, it is likely that C_{min} of *N. manukanus* is unaltered as the slope of the line

should not be influenced by an overall reduction in $\dot{V}O_2$ at each speed (as indicated by the thermal insensitivity of C_{min}) (e.g., John-Alder and Bennett, 1981; Autumn et al., 1994).

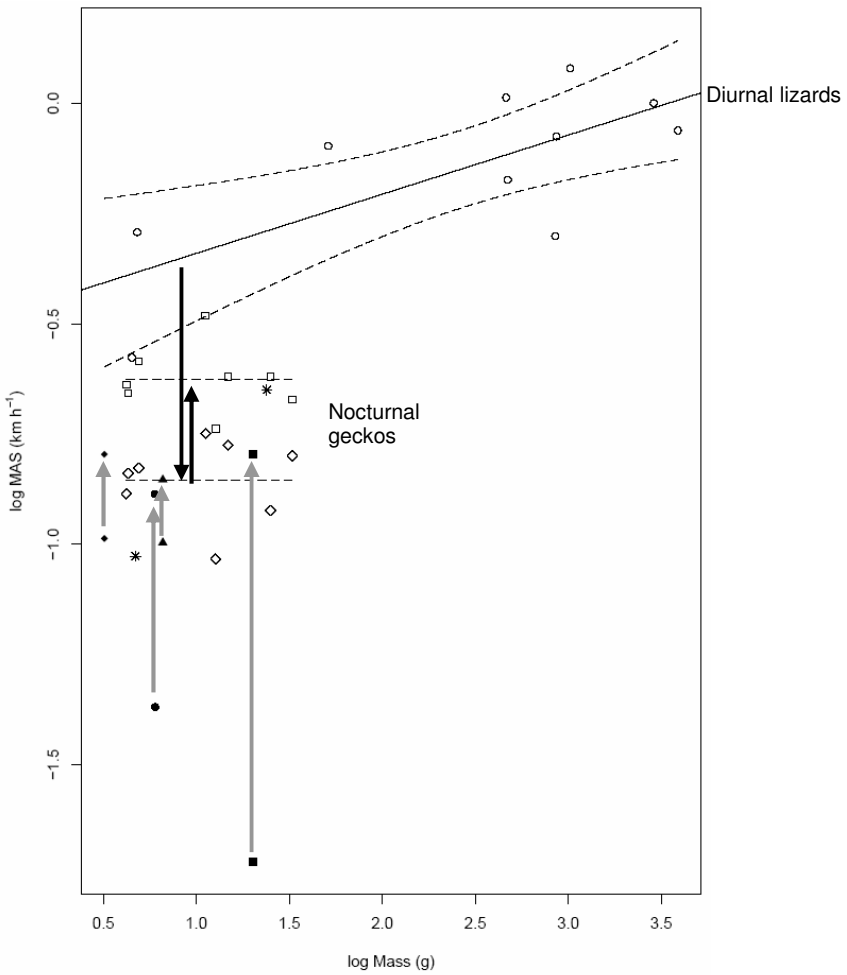

Figure 5.4: Log maximum aerobic speed (MAS) versus log body mass in lizards. Data and allometric relationships for ancestrally diurnal lizards (open circles), nocturnal geckos (open diamonds), and secondarily diurnal geckos (stars) are from literature values (see Table 5.1). The open diamonds indicate predicted MAS of geckos given a diurnal C_{min}. Data from the present study are shown as solid geometric shapes; *Cyclodina macgregori* = solid square (nocturnal skink); *Hoplodactylus maculatus* = solid circle (nocturnal gecko); Naultinus *manukanus* = solid triangle (secondarily diurnal gecko); *Oligosoma nigriplantare polychroma* = small solid diamond (diurnal skink). The solid line represents a least squares regression with 95% confidence limits for diurnal lizards (log MAS = 0.47 + 0.13 log Mass; $r^2 = 0.53$; dashed lines). Horizontal dashed lines indicate the mean observed nocturnal gecko MAS (upper) and predicted MAS of geckos given a diurnal C_{min} (lower). Downward arrows indicate the effect of the thermal handicap (Figure 5.2) on MAS for nocturnal geckos. The upward arrows indicate the adaptive effect of a low C_{min} on nocturnal geckos (black arrow) and lizards in the present study (grey arrows).

Low $\dot{V}O_2$ in *N. manukanus* also has implications for the costs of maintaining posture while running (y_0:$\dot{V}O_{2max}$), costs that are very high in *N. manukanus* (Table 5.3). In quadruped vertebrates, the cost of maintaining posture ranges from 0.64 ml O_2 g^{-1} h^{-1} to 2.9 ml O_2 g^{-1} h^{-1} (Paladino and King, 1979), but some species are outside this range. For example, the Galápagos land iguana *Conolophus subcristatus* has a cost of maintaining posture of ~ 3.1 ml O_2 g^{-1} h^{-1} (Gleeson, 1979). If *N. manukanus* had the expected $\dot{V}O_{2rest}$, then the cost of maintaining posture would be within the expected range for quadruped vertebrates.

5.5.1 Do nocturnal lizards other than geckos have lower C_{min} than diurnal lizards?

Nocturnal skinks and geckos have a lower C_{min} than diurnal lizards (Autumn et al., 1994; Farley and Emshwiller, 1996; Autumn et al., 1997; Autumn et al., 1999; this study). A low C_{min} enables nocturnal lizards to partially compensate for the effect of activity at low temperatures, reducing the overall $\dot{V}O_{2max}$. The C_{min} determines how quickly $\dot{V}O_{2max}$ is gained, meaning that a low C_{min} increases the range of speeds over which activity can occur (Autumn et al., 1999). Thus, the low C_{min} of nocturnal lizards enables them to reach speeds at low temperatures, which diurnal lizards can only attain at high temperatures during the day.

For any quiescent individual, $\dot{V}O_2$ generally differs when measured during the active part of the day than when measured during the inactive part of the day (e.g., Bennett and Dawson, 1976; Chapter 3). The daily cycle in $\dot{V}O_2$ is likely to influence the $\dot{V}O_{2max}$ and MAS obtained here and in other studies. The differences in $\dot{V}O_{2max}$ may be less pronounced if all experiments are undertaken during an animal's active phase. This would mean that the influence of C_{min} would still be significant, but that the differences in MAS among nocturnal and diurnal species would not be as high.

5.5.2 Do geckos have lower C_{min} than other lizard taxa?

The C_{min} values do not differ significantly among Anguimorpha, Gekkota, Iguania and Scincomorpha, despite some Anguimorpha (specifically the family Varanidae) having a higher $\dot{V}O_{2max}$ (e.g., Christian and Conley, 1994). C_{min} values are not correlated specifically with the lizard taxon; instead, they are related to the thermal restraints acting on a species during activity. For example, some secondarily diurnal geckos have C_{min} values similar to diurnal lizards, which suggests an evolutionary trade-off that outweighs the performance advantage of a low C_{min} in a diurnal environment (Autumn, 1999). Also, the nocturnal skink *C. macgregori* has a lower C_{min} than diurnal lizards, indicating that low C_{min} values are not restricted to geckos.

The higher $\dot{V}O_{2max}$ of Anguimorpha is likely to be due to some species of Varanidae not having mechanical constraints upon their ventilation by locomotion. Despite lateral bending of the trunk, the mechanics of lizard locomotion is similar to the mechanics of locomotion of other tetrapod animals (Farley and Ko, 1997). However, ventilation is mechanically constrained by locomotion for some reptiles, but not others (Boggs, 2002). For example, *Iguana iguana* cannot increase ventilation to match the increase in oxygen demand with an increase in speed at moderate and high speeds (Wang et al., 1997). Conversely, *Varanus exanthematicus* can sustain elevated ventilation at higher speeds because they also employ gular pumping during ventilation (Wang et al., 1997).

5.5.3 Do lizards active at low temperatures have a lower C_{min}?

Nocturnal lizards have low C_{min} values compared with diurnal lizards. All New Zealand lizards have low C_{min} values when compared with diurnal lizards from elsewhere, and nocturnal New Zealand lizards have low C_{min} values when compared with nocturnal geckos from outside New Zealand. Not all temperate species are similarly affected. The lizards from this study are from the highest latitude that lizard energetics have been measured, with the continental nocturnal gecko *Teratoscincus przewalskii* (from 40 °10’N, 94°50’E) closest to the latitudinal range of species studied here (Autumn et al., 1994). The relatively cool summers and mild winters (with small temperature variation)

experienced by New Zealand lizards (NIWA, 2005) may have resulted in adaptation of locomotor energetics to low temperature in all species.

In the Cook Strait region (where species in this study were obtained) summers and winters are both relatively cool. Mean summer air temperatures range from 19-26 °C and rarely rise above 31 °C, and mean winter air temperatures range from 10-15 °C (NIWA, 2005). In southern Australia, a comparable temperate climate, lizards experience warm summers and cool winters, with mean annual temperatures ranging from 6-32 °C (AGBOM, 2005). At low temperatures, most lizards world-wide go into torpor rather than attempting to remain active (e.g., *Lacerta vivipara*; Grenot et al., 2000). New Zealand lizards are often forced to function at low temperatures, as they have little opportunity to thermoregulate to body temperatures commonly obtained by reptiles overseas.

Many New Zealand lizards are able to be active at cool temperatures. For example, the nocturnal geckos *H. maculatus* and *H. duvaucelii* are active at body temperatures ranging from 10-13 °C and 9.5-18.2 °C, respectively (Werner and Whitaker, 1978; Thompson et al., 1992). The diurnal lizards *O. maccanni* (as *Leiolopisma zelandica* in Morris (1981)) and *N. manukanus* are active at body temperatures ranging from 13.6-32.5 °C and 16.5-31.1 °C, respectively, with active body temperatures usually around 25 °C (Werner and Whitaker, 1978; Morris, 1981). New Zealand lizards are strongly influenced by the low temperatures of the environment. All but one species of the 80+ proposed lizard species found in New Zealand are viviparous (Gill and Whitaker, 2001; Hitchmough, (in press)), and many have a low reproductive output compared to species elsewhere (Cree, 1994). Some also have biennial reproduction (Cree and Guillette, 1995) and are possibly constrained latitudinally by thermal requirements for successful reproduction (Hare et al., 2002; Hare et al., 2004a).

A low C_{min} should be an advantage at high temperatures as well as at low temperatures, so there must be an evolutionary trade-off for some factor other than locomotor costs,

which outweighs performance advantage of low C_{min} at high activity temperatures (Autumn, 1999). The secondarily diurnal geckos *Phelsuma madagascarensis* and *Rhoptropus bradfieldii* are from a tropical environment and have C_{min} values comparable to diurnal lizards from outside New Zealand (Autumn, 1999). It is unlikely that New Zealand lizards are alone in having low C_{min} values. Research to date has mainly been on tropical or warm-temperate lizards, rather than lizards from cool-temperate climes. More research on lizards from cool-temperate climates (such as Tasmania, Australia), as well as other lizards from New Zealand may help elucidate whether a low C_{min} is present in all cool-active lizards, or just New Zealand lizards. A low C_{min} may also be present in other vertebrates that commonly experience low temperatures during activity, such as tuatara (*Sphenodon* sp.), temperate turtles and polar fish.

A low C_{min} does not fully offset the thermal handicap of activity at low temperatures (Figure 5.4), and more factors may be at play. Lizards rarely move continuously, with locomotion in many species characterised by brief activity followed by pauses (Hertz et al., 1988). Short and intense activity is more energetically expensive than steady-state locomotion, but intermittent activity of short duration can be more economical relative to single bouts of the same activity (Weinstein and Full, 1999; Gleeson and Hancock, 2002). Endurance in the laboratory is positively correlated with both the percentage of time spent moving and the daily distance moved in the field (Garland, 1999), which suggests that endurance capacities of lizards are co-adapted with typical locomotor behaviour (Garland, 1999). Even though a species may be classed as an active forager, its activity is unlikely to be continuous. More research into the type of activity of each species may help elucidate whether lizards that are active at low temperatures employ activity patterns that use less energy than those that are typically active at higher temperatures.

Seasonal acclimatisation may also influence C_{min} values. Seasonal temperatures influence many aspects of reptile physiology, including $\dot{V}O_2$ (e.g., Tsuji, 1988;

Christian et al., 1996; Christian et al., 2003), metabolic enzyme function (e.g., Olson and Crawford, 1989; Seebacher et al., 2003) and selected body temperatures (e.g., Firth and Belan, 1998; Rock et al., 2000). Winter-acclimatised lizards may have lower C_{min} values than summer-acclimatised lizards due to winter lizards experiencing overall lower temperatures. Future research should include possible effects of acclimation and acclimatisation on C_{min} values.

5.5.4 Conclusions

The greatest changes in MAS, C_{min} and $\dot{V}O_{2max}$ (at activity temperatures) in the evolutionary history of lizards all coincided with the evolution of nocturnality in geckos (Autumn et al., 1999). However, I provide evidence that low C_{min} values are also present in other nocturnal taxa as well as diurnal lizards that are active at low temperatures. A low C_{min} enables lizards to reach speeds at low temperatures normally only achievable by lizards active at much higher temperatures. The results indicate that the development of nocturnality is not a requirement for obligatory acquisition of a low cost of locomotion.

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CHAPTER 6

Total lactate dehydrogenase activity of tail muscle is not cold-adapted in nocturnal lizards from cool-temperate habitats[1]

6.1 Abstract

The dependence of metabolic processes on temperature constrains the behavior, physiology and ecology of many ectothermic animals. The evolution of nocturnality in lizards, especially in temperate regions, requires adaptations for activity at low temperatures when optimal body temperatures are unlikely to be obtained. I examined whether nocturnal lizards have cold-adapted lactate dehydrogenase (LDH). LDH was chosen as a representative metabolic enzyme. LDH activity of tail muscle was measured in six temperate lizard species (n=123: three nocturnal, two diurnal and one crepuscular) between 5 °C and 35 °C and found no differences in LDH-specific activity or thermal sensitivity among the species. Similarly, the specific activity and thermal sensitivity of LDH were similar between skinks and geckos. Similar enzyme activities among nocturnal and diurnal lizards indicate that there is no selection of temperature specific LDH enzyme activity at any temperature. As many nocturnal lizards actively thermoregulate during the day, LDH may be adapted for a broad range of temperatures rather than adapted specifically for the low temperatures encountered when the animals are active. Comparison of published records of total activity of LDH in tropical and temperate lizards indicate that total activity of LDH is not cold-adapted. More data are required on biochemical adaptations and whole animal thermal preferences before trends can be established.

[1]**Based on:** Hare, K. M., Miller, J. H., Clark, A. G., and Daugherty, C. H. 2005. Total lactate dehydrogenase activity of tail muscle is not cold-adapted in nocturnal lizards from cool-temperate habitats. *Comparative Biochemistry and Physiology, Part B* (*in press*).

6.2 Introduction

Ectotherms experience variation in body temperatures daily, seasonally and over their habitat range. Reptiles are ectotherms, and their physiology and activity are positively correlated with body temperature, within the biological limits of the species (e.g., Bennett and Dawson, 1976; Bennett, 1982; Huey, 1982). The metabolic system in particular is influenced by temperature changes, with general enzyme catalysis halving for every 10 °C drop in temperature (Q_{10} = 2; Hochachka and Somero, 2002), and reduced enzyme activity affecting overall metabolic processes (Pierce and Crawford, 1997). Ectothermic animals show a variety of strategies from biochemical to behavioural, to manage effects of temperature on metabolic processes (e.g., Huey and Slatkin, 1976; Fields and Somero, 1998; Kearney and Predavec, 2000; Hochachka and Somero, 2002). Biochemical adaptation, as opposed to behavioural responses, may be especially important if animals are unable to achieve optimal body temperatures during activity.

Nocturnality often involves activity at low temperatures, especially in temperate regions where ambient night temperatures can be extremely low. However, optimal temperatures for sprinting in diurnal and nocturnal lizards do not differ, implying that performance of nocturnal lizards is maintained at suboptimal temperatures at night (Huey et al., 1989). Despite suboptimal temperatures, nocturnal lizards are able to sustain speeds of up to three times those of diurnal lizards at low temperatures by having a low energetic cost of locomotion (Autumn et al., 1994; Farley and Emshwiller, 1996; Autumn et al., 1999; Chapter 5). However, a low cost of locomotion does not fully offset the thermal handicap of activity at low body temperatures (Autumn et al., 1999; Chapter 5). The energetic cost of movement depends at least partly on the efficiency with which the animal's skeletal muscles can convert chemical energy to mechanical work during locomotion (Farley and Emshwiller, 1996). Therefore, there may be an underlying biochemical mechanism that enhances the performance of lizards at low temperatures. One way this might occur is by an adaptive increase in the activity of enzymes at low temperatures in nocturnal lizards compared with diurnal lizards.

Lizards cannot attain or sustain high levels of oxygen consumption (Bennett, 1980), especially at low temperatures. The rate of oxygen consumption decreases with decreasing temperature, limiting available oxygen to the cells (Bennett and Dawson, 1976). Consequently, any activity greater than a walk must be fuelled by non-sustainable anaerobic metabolism (Bennett and Licht, 1972; Bennett and Dawson, 1976; Bennett, 1980). If the rate of oxygen consumption is limited at low temperatures, then glycolysis may play a greater role in ATP production. Lactate dehydrogenase (LDH) is a key metabolic enzyme involved in the glycolytic pathway, and is correlated with endurance (Guderley, 2004). Multiple LDH isozymes exist, each with different temperature profiles (Conn et al., 1987). Therefore, LDH has some capacity to function over a wide range of temperatures.

Temperature adaptation of muscle LDH (A_4-LDH) enzymes in cold-adapted polar fish have been well studied (e.g., Fields and Somero, 1998; Tschantz et al., 2002). The general trend is that fish adapted to polar conditions have higher total metabolic enzyme activity of A_4-LDH than those adapted to tropical conditions (Hochachka and Somero, 2002; Kawall et al., 2002). Temperature adaptation of LDH in reptiles does not appear to follow a general trend. For example, the American alligator (*Alligator mississippiensis*) has elevated LDH activity in winter compared with summer months (Seebacher et al., 2003), whereas the turtle *Chrysemys picta marginata* has its highest LDH activity in the autumn (Olson and Crawford, 1989), and the snake *Thamnophis sirtalis* has elevated LDH activity in tropical forms (*T. s. sirtalis*) compared with temperate forms (*T. s. parietalis*; Aleksiuk, 1971). Nonetheless, performance in reptiles may be less dependent on attaining a ‘preferred’ or ‘optimal’ body temperature range than previously thought (Seebacher et al., 2003). Enzyme acclimatisation may be the mechanism by which nocturnal lizards enhance their performance at low temperatures.

The New Zealand lizard fauna, which consists of two families, the Scincidae and Diplodactylidae (Gill and Whitaker, 2001; Han et al., 2004), provides an ideal model system to study nocturnality. Each family consists of two genera, one predominantly

nocturnal and the other predominantly diurnal. The two families have different evolutionary histories in relation to nocturnality (Vitt et al., 2003). Geckos are ancestrally nocturnal (Vitt et al., 2003), which means that diurnal geckos in New Zealand are secondarily diurnal. Conversely, skinks are ancestrally diurnal (Vitt et al., 2003), but some species in New Zealand have evolved nocturnality or are crepuscular (active in the twilight). Also, New Zealand has a temperate climate with relatively cool summers and mild winters compared with other locations inhabited by reptiles (Cree, 1994; NIWA 2005). Some New Zealand lizards remain active at body temperatures as low as 10 °C (Werner and Whitaker, 1978). Thus, at least some New Zealand lizards may, in general, be more cold-adapted than lizards from relatively warm, stable climates, such as the tropics.

I tested the hypothesis that nocturnal lizards have cold-adapted muscle LDH by comparing six lizard species with differing activity periods (nocturnal, diurnal and crepuscular). In particular I asked: 1) Does LDH activity differ with mass, sex or tail regeneration? 2) Is the activity of LDH greater in nocturnal lizards compared with diurnal and crepuscular lizards? 3) Does activity of LDH differ with phylogenetic history of the species? 4) Does activity of LDH, from published data sources) differ among temperate and tropical lizards?

6.3 Materials and methods

6.3.1 Animal collection and husbandry

The specific activity of LDH was measured in six lizard species: two nocturnal gecko species (*Hoplodactylus maculatus* and *H. chrysosireticus*), two diurnal skink species (*Oligosoma nigriplantare polychroma* and *O. zelandicum*), one nocturnal skink species (*Cyclodina macgregori*), and one crepuscular/diurnal skink species (*C. aenea*). See explanation in section 6.3.2 for why no diurnal geckos were examined. Activity period of each species was based on a published field guide (Gill and Whitaker, 2001). As *H.*

maculatus is a species complex (Hitchmough, 1997), I ensured that the populations were a single species (R. A. Hitchmough pers. comm.).

All animals were collected within the latitudinal range 40° 50' 35" to 41° 20' 83" in the Cook Strait region of New Zealand. Only adult males or non-pregnant females were examined. Adult males were distinguished from females by inspection of the ventral tail base for protruding hemipenal sacs in geckos and hemipene eversion in skinks (Gill and Whitaker, 2001; Harlow, 1996). Reproductive status of females was determined by abdominal palpation (see Cree and Guillette (1995) and Wilson and Cree (2003) for information on accuracy of this procedure in New Zealand geckos). *Cyclodina aenea* (*n* = 7), *H. maculatus* (*n* = 30), *O. n. polychroma* (*n* = 19) and *O. zelandicu*m (*n* = 10) were captured from the mainland in the Wellington region from January to April 2004. *Cyclodina macgregori* (*n* = 19)*, H. chrysosireticus* (*n* = 25) and *H. maculatus* (*n* = 13) were captured on Mana Island in November 2004. To control for the discrepancies in the timing of collection, *H. maculatus* was used as a control group as this species is very widespread and locally abundant in New Zealand (Hitchmough, 1997; Gill and Whitaker, 2001).

Lizards captured from mainland Wellington were held in captivity at Victoria University of Wellington (VUW) to acclimate them to identical light and temperature regimes (4-5 weeks). Ambient temperatures ranged from 16-25 °C, and photoperiod was on a 12:12 light:dark cycle (on at 0600 h). Lizards were kept individually in transparent plastic boxes (215 x 330 x 110 mm) with 1 x 1 mm wire mesh (165 x 120 mm) in the lid for ventilation. Lizards were exercised during their time in captivity (see Chapter 5). Each transparent plastic enclosure had 30 mm depth of leaf litter provided as cover. Food (mealworm larvae (*Tenebrio molitor*) and/or canned, pureed pear (Watties^{TM})) and water were supplied *ad libitum.*

Lizards from Mana Island were housed individually in 2 L plastic containers with a 50 x 50 mm square of 1 x 1 mm wire mesh in the lids for ventilation and small pieces of

vegetation (*Coprosma repens*) as cover. Water was provided *ad libitum,* and animals were not fed during the three days they were held. Tails tips were removed on the day of capture. Room temperature ranged from 8-23 °C, and photoperiod was 14:10 light:dark (sunrise at ~0600 h).

6.3.2 Tissue collection

The non-lethal sampling method of tail muscle collection was employed (e.g., Hopkins et al., 2001; Jackson et al., 2003), assuming that tail muscle tissue is indicative of locomotor muscle in lizards. This is likely as tails are important in the locomotion of squamates (e.g., Chapple and Swain, 2002; Chapple et al., 2004), particularly in some geckos (Bauer and Russell, 1994). No samples from diurnal geckos (*Naultinus* spp.) were obtained. Lizards in the genus *Naultinus* are arboreal and have prehensile tails, which are rarely autotomised due to a partial reduction in autotomy planes (Bauer and Russell, 1994; Gill and Whitaker, 2001). Removal of tail parts of *Naultinus* spp. could severely restrict their arboreal locomotor ability.

Tail samples were collected by inducing tail release (caudal autotomy). Autotomy was induced at approximately 20 mm from the tail tip by grasping the tail with forceps and allowing the animal to hang over a bucket and break free naturally. Tail tissue was frozen at -80 °C immediately following autotomy. One to two months after collection, muscle tissue was dissected while frozen, and isolated from the skin and caudal bones. The dissection was undertaken on a glass plate on ice to limit thawing of the tissue.

6.3.3 LDH assay

The activity of LDH was measured following the methodology of Seebacher et al. (2003). Enzyme activity was determined with a spectrophotometer (Philips PU8630 UV/VIS/NIR kinetics spectrophotometer) equipped with a temperature-controlled cuvette holder. Assays were carried out in triplicate at 5, 15, 25, and 35 °C (± 0.1 °C). Assay temperatures were chosen for their ecological relevance as indicated by body temperature measurements of some of the species or their close relatives (e.g., Werner,

1978; Tocher, 1992; Rock et al., 2000, 2002). Calculations of the reaction rates were based on the linear portions of the progress curves. Enzyme activity was expressed as specific activity μmol min^{-1} g $protein^{-1}$. Saturating substrate concentrations were determined in preliminary tests to ensure that substrates were not limiting the reaction rate, i.e., doubling homogenate concentration in the assays doubled activity, but doubling substrate concentrations did not alter reaction rates (data not presented).

Muscle tissue (0.13 to 2.91 g) was homogenised in nine volumes of extraction buffer (pH 7.5; 50 mM imidazole/HCl, 2 mM $MgCl_2$, 5 mM ethylene diamine tetra-acetic acid, 1 mM reduced glutathione and 1% Triton X-100). Tissue was kept on ice during homogenisation. Tissue homogenates were further diluted with extraction buffer by a factor of 50. LDH activity was determined using the decrease in absorbance of NADH at 340 nm. The millimolar extinction coefficient of NADH with 10 mm path length is 6.22. The assay medium included 0.1 mM potassium phosphate (KH_2PO_4/K_2PO_4) buffer (pH 7.0), 0.16 mM NADH and 0.4 mM pyruvate.

6.3.4 Protein assay

I corrected LDH activity for protein concentration of each sample as there may have been differences in protein concentration between original and regenerated tails (e.g., Meyer et al., 2002), and also because the efficiency of homogenisation can vary. Protein concentrations were measured in duplicate using a modification of the Lowry protein assay (Appendix 1E) adapted to 96-well plates (Lowry et al., 1951). Homogenate buffer was used in blanks, and bovine serum albumin standards were used to generate a standard curve. Absorbance was read at 570 nm.

6.3.5 Kinetics

To ensure that changes in kinetic processes (K_m and V_{max}) were not occurring, a few samples of homogenate were selected from species with different activity periods. Apparent K_m^{PYR} (Michaelis-Menten constant; affinity constant of pyruvate to enzyme; Hochachka and Somero, 2002) and V_{max}^{PYR} (maximal velocity) of LDH were measured

for *C. macgregori*, *H. chrysosireticus*, *O. n. polychroma*, and *O. zelandicum* at 25 °C. Assays were performed in a Cary 1E UV-visible spectrophotometer. Five pyruvate concentrations (0.400, 0.133, 0.100, 0.067 and 0.040 mM) were used with duplicate measurements to determine K_m^{PYR} and V_{max}^{PYR}. The computer programme LucenzIII (version 1.01; Clark, 2000) calculated K_m^{PYR}, V_{max}^{PYR}, and coefficients of variation were determined using weighted linear regressions.

6.3.6 Statistical analysis

Data were analysed using the statisticical programme *R* (Gentleman et al., 2003; R-Development-Core-Team, 2004; Version 2.0.1). Statistical significance was assumed at $P < 0.05$. Data are expressed as mean ± 1 SE unless otherwise stated. Thermal sensitivities of enzymes are expressed as temperature coefficients (Q_{10} values; thermal sensitivity) and were calculated as:

$$Q_{10} = (k_2 / k_1)^{10 / (T2-T1)}$$

where k = reaction rate at temperatures 1 and 2, and T = temperature in Kelvin.

A linear-mixed effects model was used to test whether assay temperature, species, mass, sex or tail regeneration had an influence on LDH specific activity or Q_{10}. To allow for repeated measures the factor 'individual' was included as a grouping variable (Pinheiro and Bates, 2000). I used *H. maculatus* to test for differences in laboratory vs. island research (e.g., light regime). A model was fitted to all *H. maculatus* data using maximum likelihoods, and individual was included as a random effect. Sample sizes were too small ($n = 2$ for each species) to carry out robust statistical analyses on K_m^{PYR} and V_{max}^{PYR}, so my comparisons are qualitative.

Randomisation tests were used to compare whether differences in specific activity and thermal sensitivity of LDH were statistically as well as phylogenetically significant between families (skinks and geckos) or with activity period (nocturnal, diurnal or crepuscular) (Harvey and Pagel, 1991). The randomisation tests for the data used 10,000

permutations of the sample. Test statistics were calculated for each analysis (as above), and the relative ranking reported as a *P* value.

6.4 Results

Specific activity of LDH was significantly greater at higher temperatures for all species ($F_{3,372}$ = 988.797, $P < 0.001$; Figure 6.1). The averages of all species ranged from 48.9 ± 4.7 μmol min^{-1} g $protein^{-1}$ at 5 °C to 257.2 ± 4.7 μmol min^{-1} g $protein^{-1}$ at 35 °C. There was no significant difference in enzyme specific activity among species ($F_{7,116}$ = 1.452, P = 0.191), and the lack of difference was still apparent using randomisation tests for effects of species (P = 0.186), activity period (P = 0.260) or between skinks and geckos (P = 0.681). There was no significant difference in enzyme activity or Q_{10} values for Mana Island or mainland Wellington populations of *H. maculatus* ($F_{1,43}$ = 0.353, P = 0.556 and $F_{1,43}$ = 0.317, P = 0.576 respectively). Specific activity of enzymes did not vary with mass ($F_{1,116}$ = 0.584, P = 0.446), between sexes ($F_{1,116}$ = 0.600, P = 0.440), or whether the tail tissue sample was from regenerated or original tail tissue ($F_{1,116}$ = 0.700, P = 0.405).

Q_{10} values for specific activity of LDH between 5-15 °C, 15-25 °C and 25-35 °C also differed with temperature ($F_{2,245}$ = 64.805, $P < 0.001$; Table 6.1). The highest Q_{10} values were recorded in the temperature range 5-15 °C (average of all species = 2.3 ± 0.1, range = 2.1-2.4). Over all the temperatures (5-35 °C), the mean Q_{10} value for all species was 1.8 ± 0.1. There was no significant difference in the Q_{10} value among species ($F_{7,115}$ = 1.254, P = 0.280). This was still apparent after using randomisation tests for effects of phylogeny on species (P = 0.280), activity period (P = 0.080) or testing between skinks and geckos (P = 0.857). There was also no significant difference in Q_{10} value with mass ($F_{1,115}$ = 3.056, P = 0.083), sex ($F_{1,115}$ = 0.134, P = 0.715) or whether the tail tissue sample was from regenerated or original tail tissue ($F_{1,115}$ = 0.097, P = 0.756).

Table 6.1: Q_{10}, V_{max}^{PYR} (µmol min^{-1} g $protein^{-1}$) and K_m^{PYR} (mM) values of lactate dehydrogenase from tail tissue muscle of six lizard species from the genera *Cyclodina, Hoplodactylus* and *Oligosoma*.

Activity	Family	Species	*n*	Mean mass of lizard (g)	Q_{10} values				Kinetics	
					5-15 °C	15-25 °C	25-35 °C	5-35 °C	V_{max}	K_m
C	S	*C. aenea*	7	2.62 ± 0.17	2.22 ± 0.18	1.77 ± 0.18	1.41 ± 0.18	1.75 ± 0.05	-	-
Dl	S	*O. nigriplantare polychroma*	19	3.42 ± 0.13	2.42 ± 0.09	1.41 ± 0.09	1.70 ± 0.09	1.79 ± 0.03	188.38 ± 0.66	0.09 ± 0.02
Dl	S	*O. zelandicum*	10	3.60 ± 0.14	2.18 ± 0.09	1.73 ± 0.08	1.52 ± 0.08	1.79 ± 0.04	292.89 ± 9.06	0.13 ± 0.01
N	S	*C. macgregori*	19	22.71 ± 0.73	2.44 ± 0.22	1.97 ± 0.21	1.66 ± 0.21	1.86 ± 0.06	254.56 ± 25.36	0.14 ± 0.01
N	D	*H. chrysosireticus*	25	6.97 ± 0.30	2.29 ± 0.07	1.40 ± 0.07	1.76 ± 0.07	1.76 ± 0.02	135.08 ± 14.08	0.09 ± 0.02
N	D	*H. maculatus* (MI)	13	8.91 ± 0.28	2.05 ± 0.05	1.41 ± 0.05	1.84 ± 0.05	1.74 ± 0.02	-	-
N	D	*H. maculatus* (Wgtn)	30	6.78 ± 0.24	2.30 ± 0.09	1.53 ± 0.09	1.61 ± 0.09	1.73 ± 0.03	-	-
N	D	*H. maculatus* (comb.)	43	7.94 ± 0.05	2.09 ± 0.08	1.44 ± 0.03	1.78 ± 0.03	1.73 ± 0.02	-	-

C = crepuscular; Dl = diurnal; N = nocturnal; D = Diplodactylidae; S = Scincidae; All samples are from adults; Data for *H. maculatus* includes individuals from Mana Island (MI), mainland Wellington (Wgtn), and both populations (comb.); Values are mean ± 1 SE.

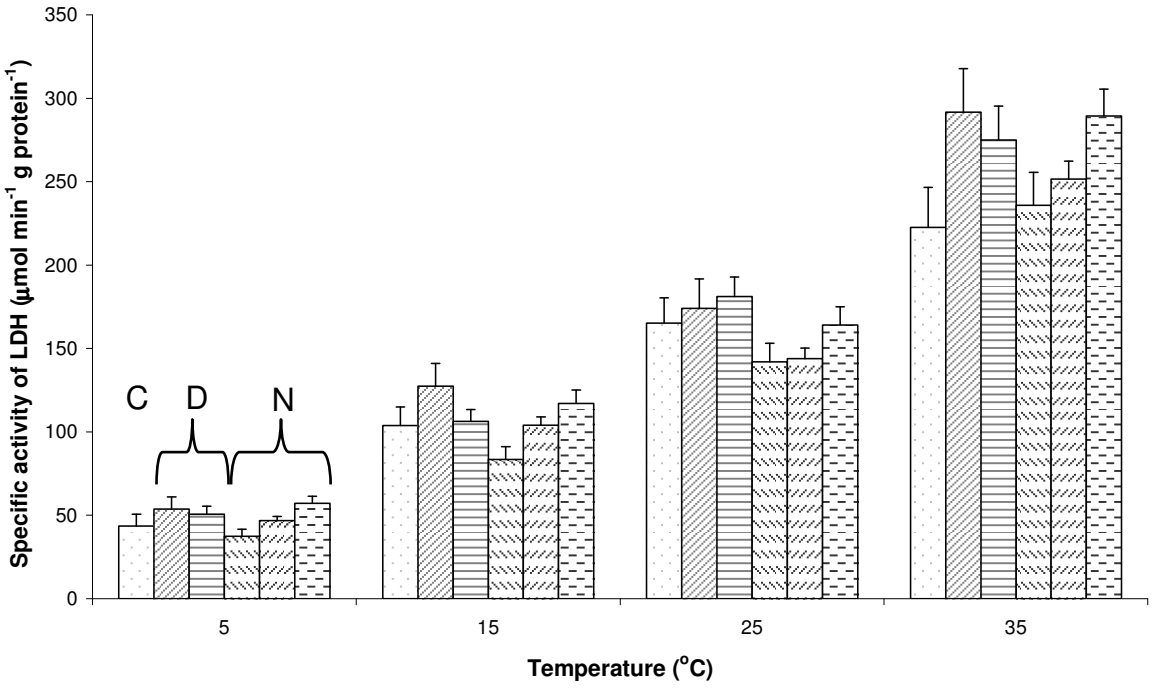

Figure 6.1: Effect of temperature on specific activity of muscle lactate dehydrogenase (LDH) from tail muscle tissue of six lizard species. Sample sizes are indicated in Table 6.1. D = diurnal species; N = nocturnal species; C = crepuscular species. From left the shading in bars indicates the following species; = *Cyclodina aenea*; = *Oligosoma nigriplantare polychroma*; = *O. zelandicum*; = *C. macgregori*; = *Hoplodactylus chrysosireticus*; = *H. maculatus* (includes data from mainland Wellington and Mana Island populations); *Cyclodina* and *Oligosoma* genera are in the family Scincidae; *Hoplodactylus* is in the family Diplodactylidae; Error bars are 1 SE.

The K_m^{PYR} ranged from 0.09-0.14 mM over all species (Table 6.1). The V_{max}^{PYR} ranged from 135-355 µmol min^{-1} g $protein^{-1}$ over all species (Table 6.1). Sample sizes were too small to show significance, but it appeared that K_m^{PYR} and V_{max}^{PYR} were similar among species, between skinks and geckos and with activity period

6.5 Discussion

The range of temperatures that nocturnal and diurnal lizards are emerged and active differ substantially. In general, nocturnal lizards from New Zealand are emerged and active at body temperatures ranging from 10 °C to 15 °C, whereas diurnal lizards are emerged and active at temperatures ranging from 13 °C to 33 °C (e.g., Werner and Whitaker, 1978; Morris, 1981; Tocher, 1992; Rock et al., 2000, 2002). In fish,

differences in average or maximal habitat temperature of only a few degrees Celsius are sufficient to favour selection for adaptively different homologs of A_4-LDH among species (e.g., barracuda fish *Sphyraena* spp.; Graves and Somero, 1982). Nonetheless, no difference in either specific activity or thermal sensitivity of LDH among species, between skinks and geckos or with activity period was found.

6.5.1 Does LDH activity differ with mass, sex or tail regeneration?

Specific activity and thermal sensitivity of LDH are similar in original and regenerated tails of the lizards studied here. Enzyme activity may vary between regenerated and original lizard tails (e.g., Magon, 1975; Shah and Ramachandran, 1976). Where enzyme activity does not vary with tail regeneration (e.g., Meyer et al., 2002), tails may have regenerated sufficiently for LDH levels to return to those of original tails. I chose individuals with long tails (either original or near full regeneration) to limit any possibility of resource limitation on individuals from tail tip removal. Thus, the lizards in this study may have sufficiently regenerated tails for LDH levels to return to those of original tails.

Mass and sex of individuals do not influence specific activity or thermal sensitivity of LDH in the lizards in this study. Enzyme activity is mass-specific (as in scaling) in some reptiles, but not others. For example, specific activity of heart muscle of the lizard *Ctenophorus nuchalis* is mass dependent, but not related to ontogeny, with larger individuals having higher LDH activity (Garland and Else, 1987). However, specific activity of LDH does not differ with mass in muscle tissue of *Alligator mississippiensis* (Seebacher et al., 2003). The lack of mass-specificity of LDH in this study may relate to the relatively narrow body mass ranges within the species measured. LDH activity also does not vary between the sexes for either *C. nuchalis* or *A. mississippiensis* (Garland and Else, 1987; Seebacher et al., 2003). Thus, for many reptiles both sexes may have similar anaerobic metabolic needs.

6.5.2 Does LDH activity differ with activity period or between skinks and geckos?

The specific activity of LDH does not differ among species, with activity period or between skinks and geckos for the species studied here. As specific activity of LDH does not differ among species (and hence overall for the complex of LDH isozymes measured), it is likely that the single isozymes of LDH also do not vary. In snakes, overall specific activity of isozyme complexes varies when there are differences in temperature specificity of different LDH isozymes (Aleksiuk, 1971). Acclimatisation of enzymes to cold temperatures occurs in response to long-term changes in environmental conditions, such as seasonal or latitudinal variation (Scheiner, 1993; Wilson and Franklin, 2000). Nocturnal lizards may experience large daily variation in temperature through diurnal thermoregulation (e.g., Tocher, 1992; Rock et al., 2002), reaching temperatures close to those experienced by diurnal lizards (e.g., Werner and Whitaker, 1978). Selection may decrease the thermal sensitivity of biochemical traits in species with broad temperature ranges (Wilson and Franklin, 2000). Thus, acclimatisation of LDH may not be apparent in lizards, as LDH may be selected to function over a broad range of temperatures rather than specifically at low (or high) temperatures.

Thermal sensitivity and specific activity of LDH are similar among the lizards in this study. Therefore it is unlikely that the kinetic properties of LDH differ among the species. The temperature sensitivities of LDH were carried out at saturating substrate concentrations, which means that the magnitude of the activation enthalpy of the reaction is indicated, more or less, by the size of the Q_{10} values (Hochachka and Somero, 2002). The pilot study on the kinetic properties of LDH suggests that they do not differ among species.

Many ectothermic vertebrates show changes in kinetic properties with temperature adaptation of a species (e.g., Aleksiuk, 1971; Holland et al., 1997; Hochachka and Somero, 2002), whereby organisms have adapted to different thermal environments by adjusting the kinetic parameters of their enzymatic reactions (Hochachka and Somero, 2002). At any given temperature of measurement, K_m is lowest for the most warm-

adapted species and highest (lowest affinity) for the most cold-adapted species (Hochachka and Somero, 2002). The K_m^{PYR} values (Table 6.1) are low when compared with other ectothermic taxa at 25 °C, including the goby fish *Gillichthys seta* and *G. mirabilis* and the barracuda fish *Sphyraena idiastes* (0.14 mM, 0.22 mM and 0.30 mM, respectively; Holland et al., 1997; Fields and Somero, 1998; Hochachka and Somero, 2002). However, lizards are not always more warm-adapted than fish, even though most lizards have opportunities to elevate their body temperatures on a daily basis whereas fish are constrained by ambient water temperatures. For example, the lizard *Elgaria multicarinata* (as *Gerrhonotus multicarinatus* in Hochachka and Somero, 1984) has lower K_m^{PYR} than some Amazon catfish (Siluriformes; Hochachka and Somero, 1984). The thermal evolutionary history of an animal is likely more important to enzyme adaptation than its phylogenetic placement.

The overall thermal sensitivity of LDH in this study is close to the expected value of 2 for species studied within their normal range of body temperatures (Hochachka and Somero, 2002). Thus, behavioural and physiological processes of lizards may be able to function (albeit less efficiently) over a wide temperature range, allowing lizards to react immediately to favourable changes in the environment. For example, the diurnal skink *Tiliqua rugosa* will surface after a drought at night temperatures as low as 8.5 °C to rehydrate in rain (Kerr and Bull, 2004).

6.5.3 Do tropical and temperate forms differ?

All lizards in this study are from a similar latitude and actively forage at body temperatures ranging from 10 °C to 33 °C (Werner and Whitaker, 1978; Tocher, 1992; Rock et al., 2000, 2002). Conversely, tropical lizards are active at much narrower and higher temperature ranges, around 18-36 °C (e.g., Christian and Weavers, 1994; Vitt et al., 2001). Some reptiles have differences in LDH activity with latitude (e.g., snakes *Thamnophis sirtalis*; Aleksiuk, 1971), or season (e.g., turtles *Chrysemys picta marginata*; Olson and Crawford, 1989).

Lizards have no clear pattern of LDH activity or kinetic properties (specifically K_m^{PYR}) among species with differing (or similar) thermal histories. For example, two species of lizards from temperate California have different K_m^{PYR} activities: *Dipsosaurus dorsalis* (Iguania) have lower K_m^{PYR} than *Elgaria multicarinata* (Anguimorpha; Hochachka and Somero, 1984). Similarly, the nocturnal tropical gecko *Hemidactylus mabouia* from Florida, USA, has a mean LDH activity of tail muscle of 175-250 (± 75-200 SD) μmol min^{-1} g^{-1} at 25 °C (Meyer et al., 2002). This appears to be slightly higher than the combined LDH specific activity for temperate lizards at 25 °C in this study (157 ± 4 μmol min^{-1} g $protein^{-1}$). However, the data fall within the large SD range of *Hemidactylus mabouia*. Conversely, three tropical diurnal lizard species (*Tropidurus* spp.; Iguania) have much higher LDH specific activity at 35 °C than species in this study (675 ± 45 to 710 ± 50 μmol min^{-1} g $tissue^{-1}$) (Kohlsdorf et al., 2004). The lack of data on activity and kinetics of lizard enzymes makes it difficult to determine whether the clear differences between polar and tropical fish may also occur in lizards. Since lizards can actively alter their body temperature from ambient, they may have species-specific differences related to individual species' thermal preferences. More biochemical research on lizards is required before a comprehensive comparative assessment can be undertaken.

6.5.4 Conclusions

The temperatures that nocturnal and diurnal lizards are emerged and active, as well as tropical and temperate lizards, differ. However, selection for temperature specific total LDH enzyme activity in nocturnal or diurnal lizards is not evident. As many nocturnal lizards emerge during the day to thermoregulate, LDH may be selected to function over a broad range of temperatures rather than specifically at low temperatures. Whether differences in LDH activity or enzyme kinetic properties exist between tropical and temperate lizards is also unclear. Although it is likely that LDH activity of lizards is related to their thermal evolutionary history, more data are required on enzyme activities and whole animal thermal preferences to ascertain whether the clear thermal patterns seen in fish also exist among lizards. The underlying biochemical mechanism

behind enhanced performance of nocturnal lizards at low temperatures remains to be determined.

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CHAPTER 7
Unravelling the nocturnality paradox

7.1 Introduction

Nocturnality in lizards is a paradox. Nocturnality often involves activity at low temperatures, especially in temperate regions where ambient night temperatures can be extremely low, but nocturnal lizards prefer substantially higher body temperatures than are achievable at night (e.g., Licht et al., 1966; Huey and Bennett, 1987; Arad et al., 1989). The evidence collected in this thesis is reviewed in this chapter, as well as previous reports in the literature, to describe the currently known physiological mechanisms that enable nocturnal lizards to be active at low temperatures. Recommendations for future studies are made based on this review.

The four genera of endemic New Zealand lizards were used to help determine what physiological mechanism or mechanisms might explain the nocturnality paradox. This included lizards from the families Diplodactylidae and Scincidae. In general, nocturnal skinks are from the genus *Cyclodina*, nocturnal geckos are from the genus *Hoplodactylus*, diurnal geckos are from the genera *Naultinus*, and diurnal skinks are from the genus *Oligosoma* (Gill and Whitaker, 2001). Four aspects of reptile physiology in nocturnal and diurnal lizards were compared. A multi-species approach was used to allow separation of evolutionary history from potential causative links between physiology and activity period. The physiological measures targeted for investigation included daily rhythms of metabolic rate, levels of metabolic rate at low and high temperatures, locomotor energetics, and biochemical adaptation.

7.2 Results and implications

7.2.1 Conditioning to experimental procedures

In Chapters 2 and 5 the diurnal gecko *Naultinus manukanus* was used as a general indicator of lizard behaviour, to test whether the rate of oxygen consumption ($\dot{V}O_2$) is influenced by first and subsequent exposures to novel environments in both unrestrained (loose within a respirometry chamber; Chapter 2) and restrained (wearing a respirometry mask; Chapter 5) individuals. The results indicate that experimental procedures may influence $\dot{V}O_2$ and the time to reach a steady-state of $\dot{V}O_2$. When lizards are unrestrained, only one exposure to experimental procedures is required to gain a steady-state $\dot{V}O_2$ from most animals. However, when lizards are restrained, at least two exposures to experimental conditions are required before a steady-state can be reached. Restrained and unrestrained lizards have comparable $\dot{V}O_2$ values after conditioning. Consequently, for all $\dot{V}O_2$ experiments, lizards were conditioned to the experimental procedures before taking measurements.

7.2.2 Daily rhythms of metabolic rate

Daily rhythms of $\dot{V}O_2$ were measured among eight lizard species with differing activity periods (Chapter 3). The patterns and amplitudes of $\dot{V}O_2$ were compared among the species. Three daily patterns of $\dot{V}O_2$ were apparent: 24 h cycle, 12 h cycle, and no daily cycle. The daily patterns of $\dot{V}O_2$ and peak $\dot{V}O_2$ do not always coincide with the time when the species is active during the day. The diurnal/crepuscular skink *Cyclodina aenea* has a 12 h cycle of $\dot{V}O_2$, peaking at dawn and dusk, which suggests that this species should be categorised as crepuscular. The nocturnal gecko *Hoplodactylus maculatus* has no rhythm of $\dot{V}O_2$ over 24 h. All other lizards have a 24 h rhythm of $\dot{V}O_2$ that mainly peaks during the animal's active part of the day, or in anticipation of the active part of the day. However, the nocturnal gecko *H. stephensi* has its greatest $\dot{V}O_2$ during the first quarter of the photophase (light), and the two diurnal *Oligosoma* skinks tested had highest $\dot{V}O_2$ during the second half of the scotophase (dark). Amplitudes of $\dot{V}O_2$ did not differ among species with differing activity periods and (including published literature) were typically lower in tropical lizards than temperate lizards. This

suggests that amplitudes of $\dot{V}O_2$ are correlated with large scale latitudinal differences in temperature. The study of daily rhythms showed that the definitions of metabolic rate usually employed (resting and standard metabolic rate) are not always directly linked to the animal's active and inactive parts of the day. The definitions used to describe daily activity patterns may need revising to more adequately reflect lizard behavioural patterns.

7.2.3 Absolute levels of metabolic rate

Rates of oxygen consumption were measured in eight lizard species at a low (13 °C) and high temperature (26 °C). Using published literature comparisons were made among lizards that are generally active at low body temperatures (nocturnal and temperate species) or high body temperatures (diurnal and tropical species). Temperate and tropical lizards have similar $\dot{V}O_2$ at high temperatures. However, data for tropical species at low temperatures are limited, and comparisons with temperate species are equivocal. Nocturnal lizards and *Naultinus manukanus* (a secondarily diurnal gecko) have a low $\dot{V}O_2$ at high temperatures, indicating overall lower energy requirements. Nocturnal lizards have lower thermal sensitivity (low Q_{10} values) and greater metabolic stability than diurnal and crepuscular species. Consequently, diurnal lizards can quickly take advantage of changes in environmental temperature, but nocturnal lizards are less influenced by changes in environmental temperature.

7.2.4 Locomotor energetics

The energetic cost of locomotion (C_{min}) was calculated by measuring the $\dot{V}O_2$ of four lizard species during steady exercise on a treadmill. Comparisons were made among these four species and reported values for other lizard species, including their activity periods, latitudinal ranges and evolutionary histories. Nocturnal lizards have a lower C_{min} than diurnal lizards, and a low C_{min} is also characteristic of diurnal lizards that experience low temperatures during their active period. The low C_{min} of all lizards from cool-temperate locales may be the mechanism that permitted range extension of lizards into cool-temperate regions. A low C_{min} enables species that are active at low

temperatures to reach speeds that are normally only achievable by species active at much higher temperatures. However, a low C_{min} does not fully compensate for the thermal handicap of activity at low temperatures.

7.2.5 Biochemical adaptation

The specific activity and thermal sensitivity of the glycolytic enzyme lactate dehydrogenase (LDH) was compared among six lizard species. Specific activity of LDH was comparable among nocturnal and diurnal lizards at all temperatures tested. Evolutionary histories and latitudinal location (tropical vs. temperate) of lizards were also not correlated with differences in LDH activity at different temperatures. As many nocturnal lizards actively thermoregulate during the day, LDH may have adapted to function over a broad range of temperatures rather than specifically at low temperatures when the animals are active.

7.3 Discussion & synopsis

The definitions of 'nocturnal', 'diurnal' and 'crepuscular' are restrictive. Some species are active when environmental conditions are beneficial regardless of the time of day (e.g., *Oligosoma striatum* and *O. zelandicum*; Neilson et al., 2004), and some species are active beneath the substrate during the day, as well as active above substrate at night (e.g., *Xantusia henshawi*; Lee, 1974). However, in general, foraging activity is confined to one part of the daily cycle. A trend of intermittent activity and sleep may be the mechanism by which nocturnal lizards are able to maintain what appears to be a 24 h 'activity' pattern (Chapter 3).

In New Zealand, nocturnal and diurnal lizards have slightly different ranges of temperatures at which they are active. However, many nocturnal lizards emerge to regulate their body temperatures during the day, achieving similar temperatures to active diurnal lizards (e.g., Werner and Whitaker, 1978; Tocher, 1992; Kearney and Predavec, 2000; Rock 2000). Diurnal thermoregulation by nocturnal species is the likely

explanation for the similarity of many physiological processes among species with differing activity periods. For example, values of metabolic rate over 24 h (Chapter 3), metabolic rate at low temperatures (Chapter 4), and specific activity of the glycolytic enzyme LDH (Chapter 6) are similar among nocturnal and diurnal lizards. Physiological trends are more easily recognised when there are large scale differences in thermal biology (e.g., with latitude) than when there are small-scale differences (e.g., activity temperature). However, the scarcity of information on $\dot{V}O_2$ of tropical lizards at low temperatures makes large-scale patterns difficult to predict and interpret at present.

Some differences in the physiology of nocturnal and diurnal lizards are apparent, including higher thermal sensitivity of $\dot{V}O_2$ in diurnal than nocturnal lizards (Chapter 4). This indicates that diurnal lizards are able to quickly take advantage of changes in environmental temperature (e.g., at sunrise), whereas nocturnal lizards are less influenced by changes in environmental temperature. In addition, at high temperatures nocturnal lizards (and the secondarily diurnal gecko *Naultinus manukanus*) appear to have lower energy requirements than diurnal lizards. This is consistent with these species also having a lower energetic cost of locomotion (C_{min}) than diurnal lizards (Chapter 5).

Nocturnal lizards have a lower C_{min} than diurnal lizards (Autumn et al., 1994; Farley and Emshwiller, 1996; Autumn et al., 1997; Autumn et al., 1999; Chapter 5), and all lizards from high latitudes (higher than 40 °) have a lower C_{min} than lizards from lower latitudes (Chapter 5). However, a low C_{min} does not fully offset the thermal handicap of being active at low temperatures. It is likely that other physiological processes are involved. A low C_{min} is not apparent in all secondarily diurnal geckos (Autumn, 1999; Chapter 5). Therefore, it is also likely that an evolutionary trade-off outweighs the performance advantage of low C_{min} at high activity temperatures (Autumn, 1999).

A low energetic cost of locomotion partially offsets the thermal handicap imposed on lizards that are active at low temperatures. Other physiological mechanisms may be

integrated with a low energetic cost of locomotion to help further compensate the thermal handicap, including having a low thermal sensitivity of metabolic rate. If no other physiological adaptations are used, then nocturnal lizards must not be as efficient at low temperatures as diurnal lizards are at high temperatures. Much scope is available for further studies in nocturnality, and in cold-adaptation of lizards and ectotherms in general.

7.4 Recommendations for future research

1. *Are published activity periods of nocturnal and diurnal lizards strictly correct?*

From metabolic daily rhythms and anecdotal evidence (Chapter 3), it appears that the published daily activity periods of some species may not be strictly correct. More robust data on activity periods, including time-budget data, are necessary to allow informed conclusions to be made when integrating the physiology and biology of different species.

2. *Do nocturnal species employ activity strategies that use less energy*?

Short and intense activity is more energetically expensive than steady-state locomotion, but intermittent activity of short duration can be more economical than single bouts of the same activity (Weinstein and Full, 1999; Gleeson and Hancock, 2002). Although a species may be classed as an active forager, its activity is unlikely to be continuous. More research into the type of activity of each species may help elucidate whether nocturnal lizards employ activity patterns that use less energy than diurnal lizards.

3. *Do nocturnal species have lower thermal limits of emergence and activity than diurnal species?*

Nocturnal lizards will emerge during day hours to thermoregulate (e.g., Werner and Whitaker, 1978; Rock et al., 2000), but they do not move far from their retreat site while thermoregulating (J. M. Hoare unpubl. data). Similarly, diurnal lizards will emerge and move short distances at low body temperatures (e.g., at dawn) to obtain

optimal body temperatures for activity (e.g., Coddington and Cree, 1998). However, nocturnal lizards can, and do, move hundreds of meters at low ambient temperatures at night (J. M. Hoare unpubl. data). From these data it appears that both nocturnal and diurnal lizards may have similar emergence body temperatures even though they are mainly active at differing body temperatures.

4. *What are the thermal preferences of New Zealand lizards?*

Data on thermal preferences and activity temperatures of New Zealand lizards are scarce. More thermal data for lizards would enable species-specific differences of physiology to be integrated with other physiological data. This would allow finer scale determinations of thermal adaptation, as well as help tease apart large-scale (latitudinal) patterns of thermal restraints.

5. *Are nocturnal lizards able to operate over a wider range of body temperatures than diurnal lizards?*

Comparison of sprint speed rates as a function of temperature in nocturnal and diurnal lizards would provide data to clarify whether nocturnal species have greater locomotor ability at a wider range of body temperatures than diurnal species.

6. *Do the specific activity and/or the kinetic properties (specifically K_m and K_{cat}) of aerobic enzymes differ among nocturnal and diurnal species?*

From data presented here (Chapter 6) it is likely that the glycolytic metabolic pathway does not differ between nocturnal and diurnal lizards. However, similar studies using enzymes involved in aerobic metabolism (e.g., citrate synthase and cytochrome oxidase) may show marked differences in activity and kinetic properties of enzymes with temperature.

7. *Do nocturnal species have a higher concentration of mitochondria in their muscle cells?*

Mitochondrial responses to cold-acclimation include increases in abundance and oxidative capacities of oxidative enzymes, and adjustments of ADP affinities for the enzymes (Guderley and St-Pierre, 2002). For example, at 37 °C the $\dot{V}O_2$ of mitochondria in mice is 4-11 times higher than that of lizard mitochondria (Berner, 1999). However, the thermal sensitivity of $\dot{V}O_2$ of mitochondria is lower in lizards than in mice (Berner, 1999). Thus, the overall concentration of mitochondria within muscle cells may have increased in nocturnal lizards to help offset the thermal handicap associated with activity at low temperatures.

8. *Do all lizards that are active at low temperatures have a low C_{min}?*

Research on the locomotor energetics of lizards from other cool-temperate locations, such as Tasmania, Australia, may help to elucidate whether all lizards active at low-temperatures have a low energetic cost of locomotion (C_{min}), or whether New Zealand lizards are especially cold-adapted. At present there is not enough information available on the energetics of other cool-temperate species found at latitudes higher than 40°.

9. *Do nocturnal and cold-adapted ectotherms, other than lizards, also have low C_{min}?*

Research on C_{min} of other cold-adapted ectotherms such as polar fish, nocturnal insects (e.g., weta, which are Orthoptera in the families Stenopelmatidae and Rhapidophoridae), and other cold-adapted reptiles (e.g., tuatara, *Sphenodon* spp.), would help to determine whether a low C_{min} is restricted to squamates, reptiles and/or vertebrates, or is a physiological adaptation of all cold-adapted ectotherms.

10. *Does seasonal acclimatisation alter values of C_{min}?*

As seasonal temperatures influence many aspects of reptile physiology, including selection of body temperatures (e.g., Rock et al., 2000), C_{min} values may be lower in winter-acclimatised lizards than summer-acclimatised lizards.

7.5 Overall conclusion

This thesis provides evidence that nocturnal skinks and geckos have a lower energetic cost of locomotion (C_{min}) than diurnal lizards. Diurnal lizards from high latitudes also have low C_{min} values. Thus, a low C_{min} appears to be related not specifically to nocturnality but to activity at low temperatures. Also, nocturnal lizards have less thermally sensitive metabolic rates than diurnal lizards, indicating that nocturnal lizards are less influenced by changes in environmental temperature.

7.6 Literature cited

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APPENDIX 1
Methodological and statistical information referred to in thesis chapters

A *Chapter 2, 3, 4 & 5* - Experimental set-up

For experiments in Chapters 2, 3 and 4 all lizards were kept in individual clear Perspex^{TM} respirometry chambers (Figure A1) within a water bath incubator (Figure A2) during measures of $\dot{V}O_2$. The incubator had a sleeve of water heated by an aquarium heater and circulated with an aquarium pump, which kept temperature constant. The incubator was completely enclosed with an opaque sides and lid made of 2.5 cm thick polystyrene. Output from the oxygen analyser was recorded using Sable Systems (UI2) and MS Windows software (Figure A3). For the treadmill experiments in Chapter 5, all lizards were thermally equilibrated to the experimental temperature (25 ± 0.2 °C) within the treadmill with respirometry masks in place (Figure A4). The treadmill had temperature regulated by circulating heated water from the incubator. All other equipment used for measuring oxygen was the same as in Chapters 2, 3 and 4 (Figure A3).

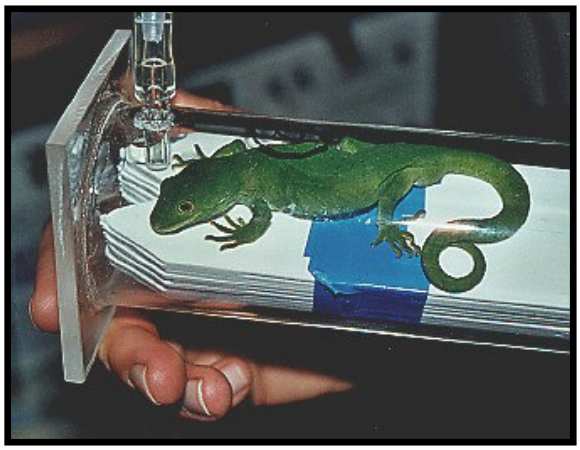

Figure A1: A male gecko (*Naultinus manukanus*) within a clear respirometry chamber used for measures of rate of oxygen consumption. The white pegs were used to reduce 'dead-air-space' for smaller individuals.

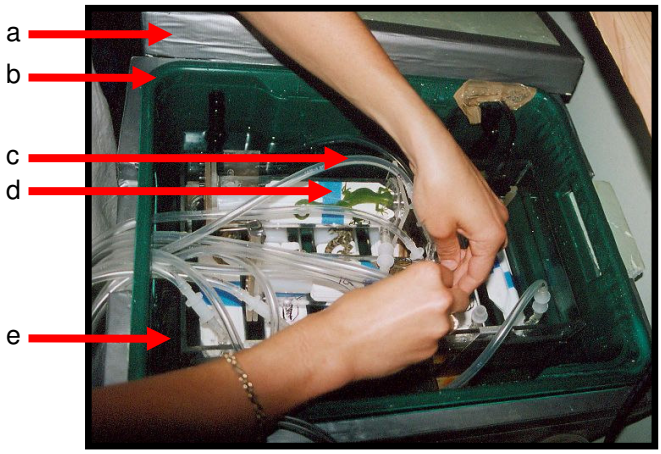

Figure A2: Respirometry chambers within the water-bath incubator. a = opaque insulated lid, b = opaque insulated sides, c = respirometry tubing, d = gecko (*Naultinus manukanus*) within a clear respirometry chamber, e = water sleeve, which was kept to temperature with an aquarium heater (heater not shown).

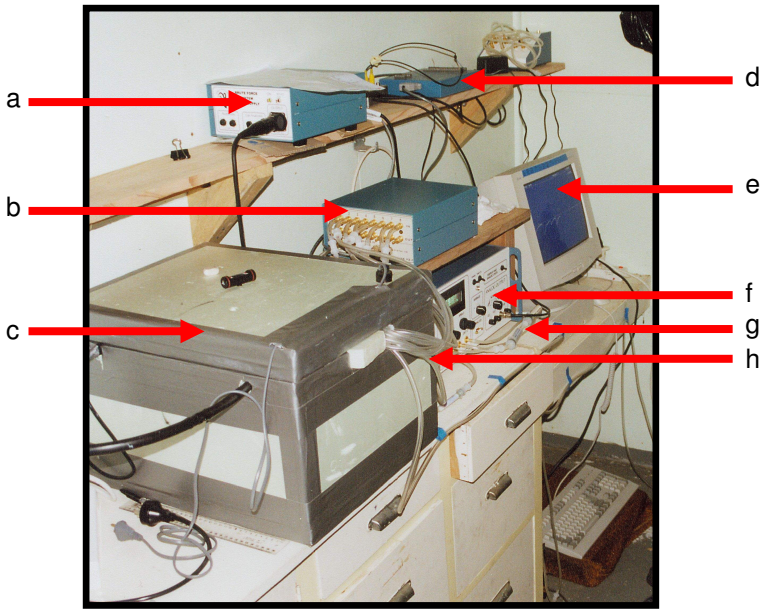

Figure A3: Respirometry equipment set-up. Air flowed past the lizard's head from outside the building using a flow controller and pump (Sable Systems International Inc., Las Vegas, Gas Analyzer Sub-sampler). The excurrent air from the chamber passed through a column of self-indication Drierite®, soda lime and then Drierite® again before entering the oxygen analyser (a two-channel Sable Systems FC-2). a = oxygen analyser power converter, b = multiplexer (automatically switches from one respirometry chamber to another), c = water bath incubator, d = UI2 software used to interface between the oxygen analyser and computer, e = instant read-out from oxygen analyser on a computer screen, enabling instantaneous steady-state $\dot{V}O_2$ to be determined, f = oxygen analyser, g = columns of Drierite® and soda lime, h = respirometry tubing.

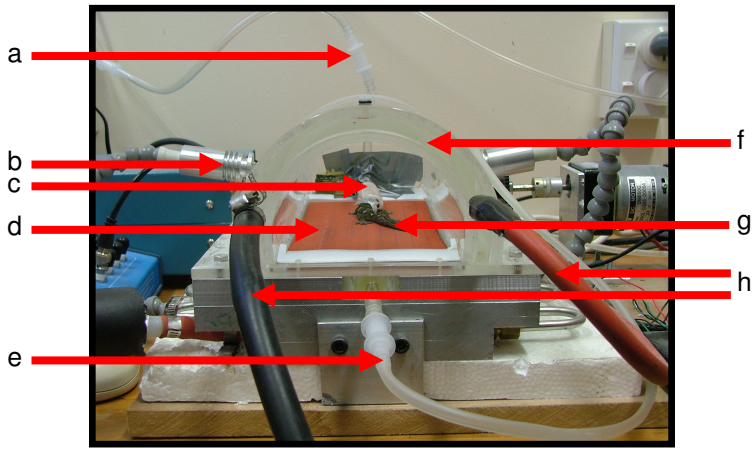

Figure A4: Treadmill equipment set-up. Air flowed past the lizard's head from outside the building using a flow controller and pump (Sable Systems International Inc., Las Vegas, Gas Analyzer Sub-sampler). The excurrent air from the lizard mask passed through a column of self-indication Drierite®, soda lime and then Drierite® again before entering the oxygen analyser (a two-channel Sable Systems FC-2). a = excurrent air tubing, b = bright fibre optic lighting, c = respirometry mask, d = rubber band (moving part of treadmill), e = air pumped in to treadmill from outside the building (warmed by first passing through the water sleeve of the incubator), f = water sleeve used to keep the temperature constant within the treadmill, g = gecko at rest (*Hoplodactylus maculatus*), h = tubing that supplies warmed water to the treadmill (from the incubator).

B *Chapter 3* - Daily $\dot{V}O_2$ patterns in lizards

Statistical model describing $\dot{V}O_2$ and time of day

The statistical model describing the relationship between rate of oxygen consumption ($\dot{V}O_2$) and time of day including both mass and sex effect is:

$$\dot{V}O_2 = \mu + A_i + \beta \cdot \text{mass} + \gamma \cdot \text{sex} + \text{amplitude x cos}((\text{time} - \text{phase})\,\pi/12) + E_{ij}$$

where the factors include mass (g), time (hours after midnight), and sex (indicator variable for sex; 0 = female, 1 = male). The term A_i is a random effect for individual (with $N(0, \sigma^2_A)$ distribution), and E_{ij} is the error term for observation j on individual (with $N(0, \sigma^2)$ distribution). The parameters estimated are the μ (average $\dot{V}O_2$ level), β (slope for mass covariate), γ (sex difference), amplitude, phase, σ^2_A (the variance between individuals) and σ^2 (the residual variance). The model assumes a 24 h cycle, and is modified for a 12 h cycle by using $\pi/6$.

Figures of daily $\dot{V}O_2$

We created models (24 h and 12 h) of daily rhythms of $\dot{V}O_2$ for all species (see previous page for models). The lower and upper quartile values of $\dot{V}O_2$ were estimated using model curves fitted to the data from all individuals. Daily rhythms of metabolic rate included: 24 h rhythms for the nocturnal species *Cyclodina macgregori* (Figure B1), *Hoplodactylus chrysosireticus* (Figure B2), and *H. stephensi* (Figure B3); 24 h rhythms for the diurnal species *Naultinus manukanus* (Figure B4), *Oligosoma nigriplantare polychroma* (Figure B5), and *O. zelandicum* (Figure B6); a 12 h rhythm for the crepuscular/diurnal species *C. aenea* (Figure B7) and no daily rhythm for the nocturnal species *H. maculatus* (Figures B8 and B9).

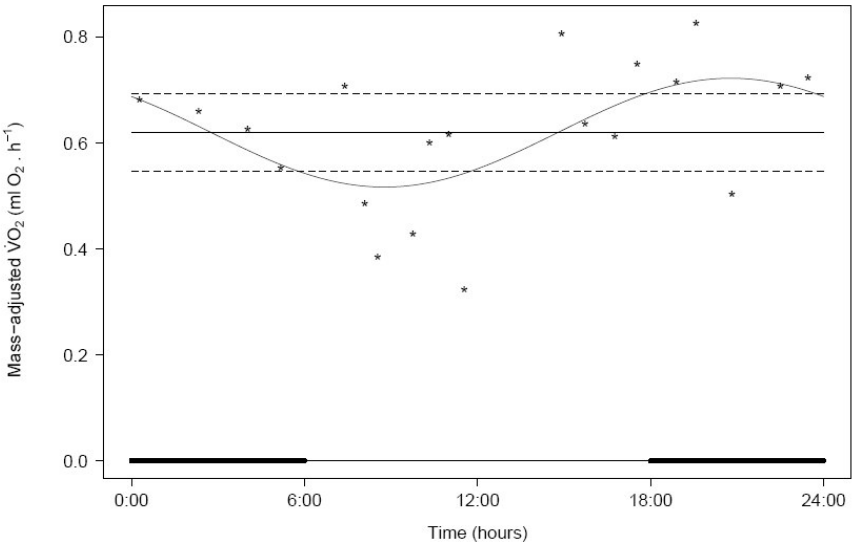

Figure B1: Daily $\dot{V}O_2$ of the nocturnal skink *Cyclodina macgregori* at 13 °C and in darkness, adjusted for individual effects by subtracting the estimated individual coefficients. The curve is the fitted model of $\dot{V}O_2$ rhythm over 24 h; the solid horizontal line indicates mean $\dot{V}O_2$ for all data points; the dashed lines indicate the upper and lower quartile values. Mean mass = 17.6 ± 1.0 g; n = 8. Scotophase (dark) normally experienced by the animals is indicated with black bars. N. B. the points on the graph indicate the mid-point of a measurement period. For clarity the figure is an oversimplification as there are no connecting lines showing repeated measures and within-individual daily variation.

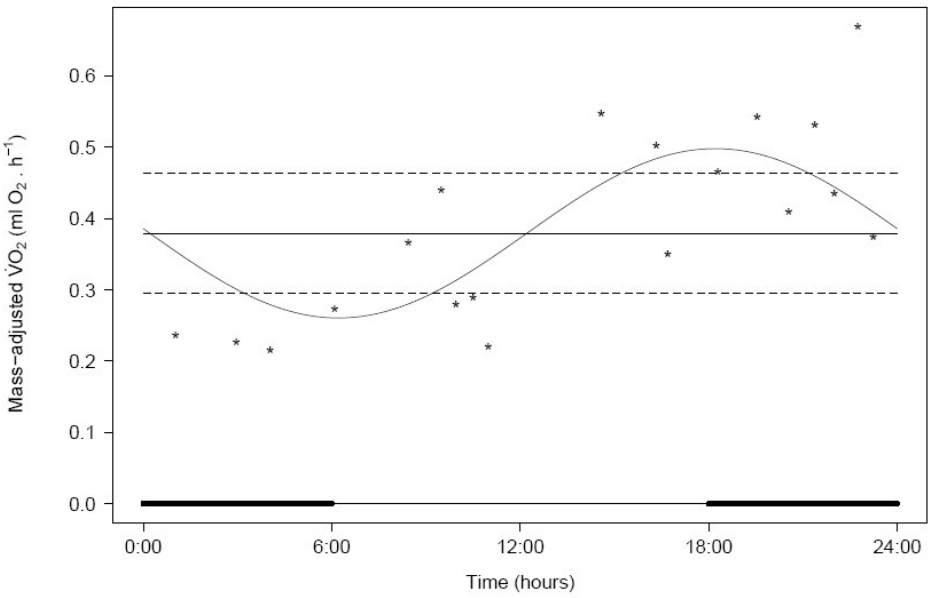

Figure B2: Daily $\dot{V}O_2$ of the nocturnal gecko *Hoplodactylus chrysosireticus* at 13 °C and in darkness, adjusted for individual effects by subtracting the estimated individual coefficients. The curve is the fitted model of $\dot{V}O_2$ rhythm over 24 h; the solid horizontal line indicates mean $\dot{V}O_2$ for all data points; the dashed lines indicate the upper and lower quartile values. Mean mass = 6.9 ± 0.6 g; n = 8. Scotophase (dark) normally experienced by the animals is indicated with black bars. N. B. the points on the graph indicate the mid-point of a measurement period. For clarity the figure is an oversimplification as there are no connecting lines showing repeated measures and within-individual daily variation.

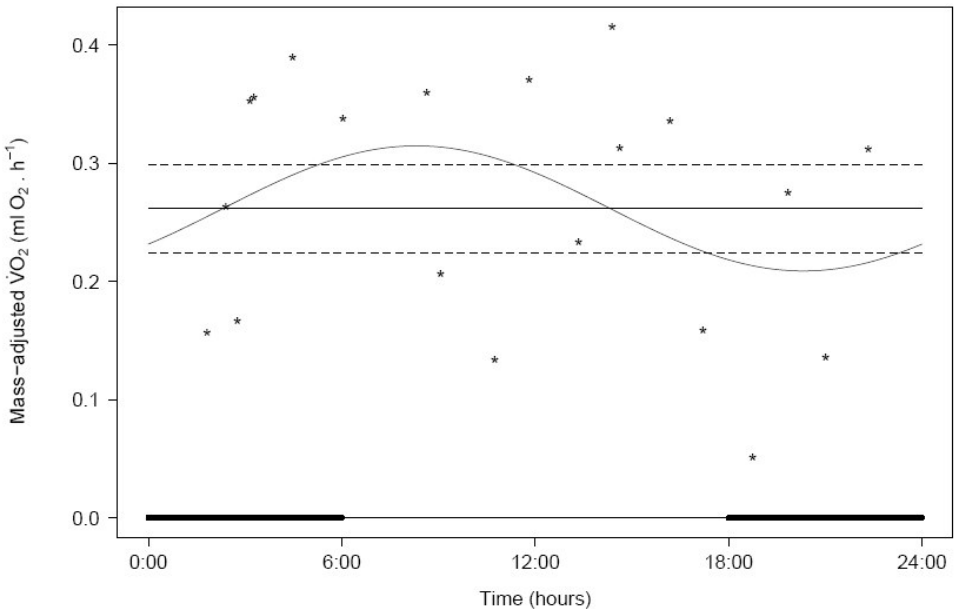

Figure B3: Daily $\dot{V}O_2$ of the nocturnal gecko *Hoplodactylus stephensi* at 13 °C and in darkness, adjusted for individual effects by subtracting the estimated individual coefficients. The curve is the fitted model of $\dot{V}O_2$ rhythm over 24 h; the solid horizontal line indicates mean $\dot{V}O_2$ for all data points; the dashed lines indicate the upper and lower quartile values. Mean mass = 7.4 ± 1.0 g; n = 7. Scotophase (dark) normally experienced by the animals is indicated with black bars. N. B. the points on the graph indicate the mid-point of a measurement period. For clarity the figure is an oversimplification as there are no connecting lines showing repeated measures and within-individual daily variation.

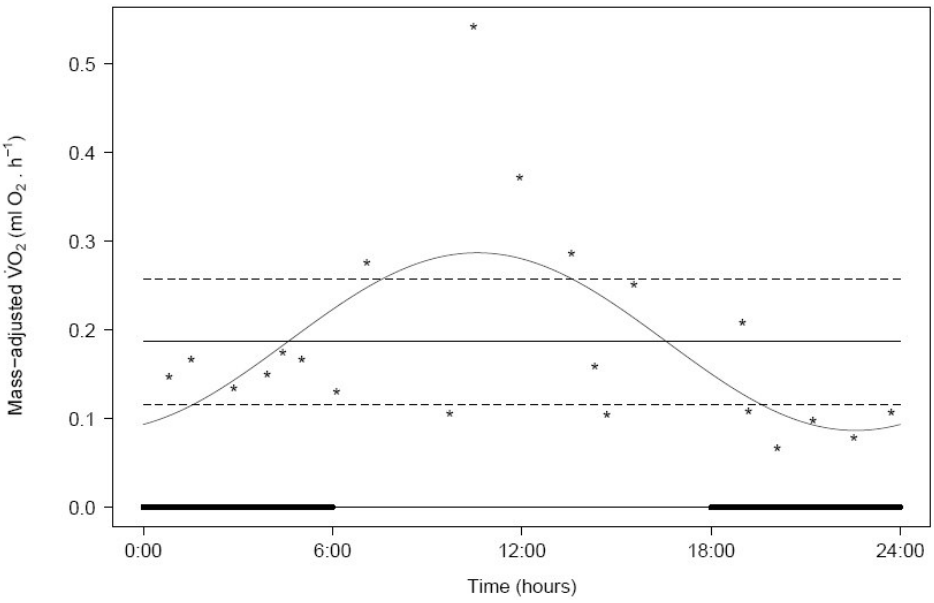

Figure B4: Daily $\dot{V}O_2$ of the diurnal gecko *Naultinus manukanus* at 13 °C and in darkness, adjusted for individual effects by subtracting the estimated individual coefficients. The curve is the fitted model of $\dot{V}O_2$ rhythm over 24 h; the solid horizontal line indicates mean $\dot{V}O_2$ for all data points; the dashed lines indicate the upper and lower quartile values. Mean mass = 6.5 ± 0.6 g; n = 7. Scotophase (dark) normally experienced by the animals is indicated with black bars. N. B. the points on the graph indicate the mid-point of a measurement period. For clarity the figure is an oversimplification as there are no connecting lines showing repeated measures and within-individual daily variation.

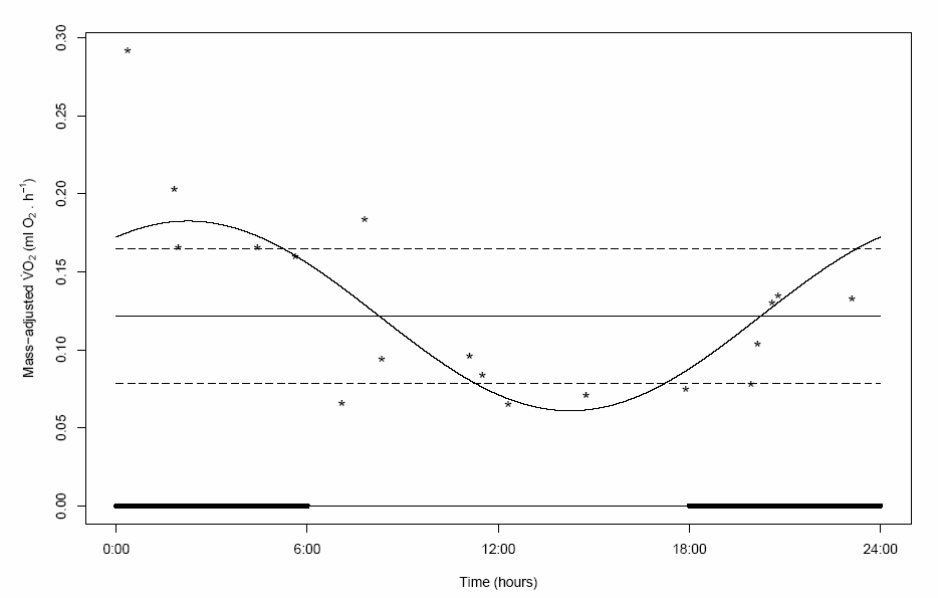

Figure B5: Daily $\dot{V}O_2$ of the diurnal skink *Oligosoma nigriplantare polychroma* at 13 °C and in darkness, adjusted for individual effects by subtracting the estimated individual coefficients. The curve is the fitted model of $\dot{V}O_2$ rhythm over 24 h; the solid horizontal line indicates mean $\dot{V}O_2$ for all data points; the dashed lines indicate the upper and lower quartile values. Mean mass = 3.3 ± 0.2 g; n = 7. Scotophase (dark) normally experienced by the animals is indicated with black bars. N. B. the points on the graph indicate the mid-point of a measurement period. For clarity the figure is an oversimplification as there are no connecting lines showing repeated measures and within-individual daily variation.

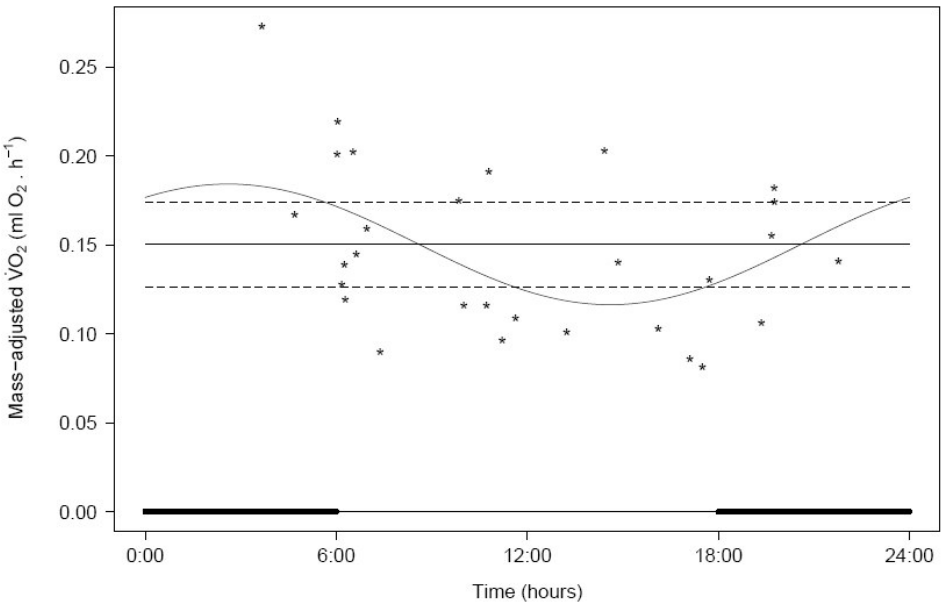

Figure B6: Daily $\dot{V}O_2$ of the diurnal skink *Oligosoma zelandicum* at 13 °C and in darkness, adjusted for individual effects by subtracting the estimated individual coefficients. The curve is the fitted model of $\dot{V}O_2$ rhythm over 24 h; the solid horizontal line indicates mean $\dot{V}O_2$ for all data points; the dashed lines indicate the upper and lower quartile values. Mean mass = 3.8 ± 0.2 g; n = 11. Scotophase (dark) normally experienced by the animals is indicated with black bars. N. B. the points on the graph indicate the mid-point of a measurement period. For clarity the figure is an oversimplification as there are no connecting lines showing repeated measures and within-individual daily variation.

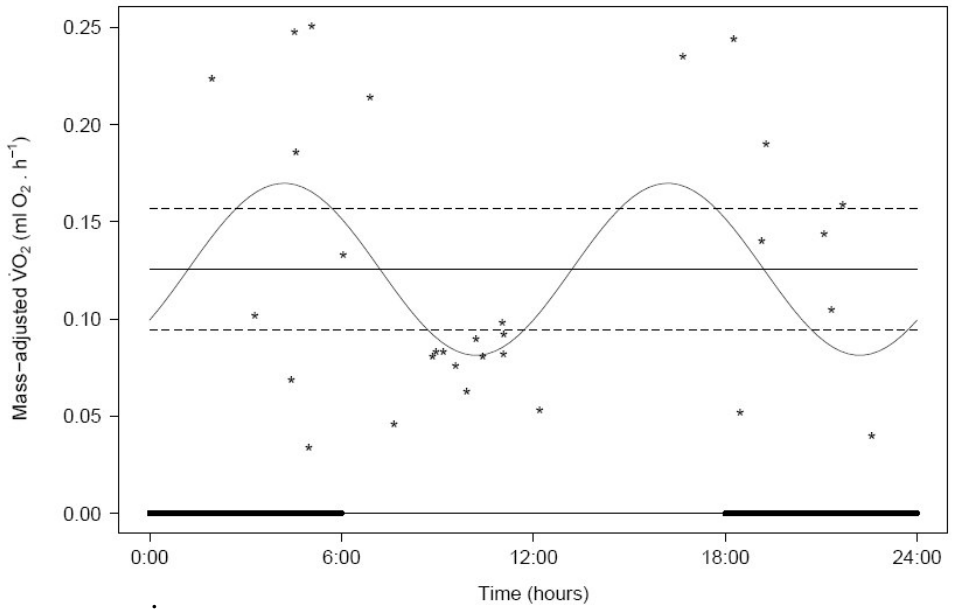

Figure B7: Daily $\dot{V}O_2$ of the crepuscular/diurnal skink *Cyclodina aenea* at 13 °C and in darkness, adjusted for individual effects by subtracting the estimated individual coefficients. The curve is the fitted model of $\dot{V}O_2$ rhythm over 24 h; the solid horizontal line indicates mean $\dot{V}O_2$ for all data points; the dashed lines indicate the upper and lower quartile values. Mean mass = 2.5 ± 0.2 g; n = 11. Scotophase (dark) normally experienced by the animals is indicated with black bars. N. B. the points on the graph indicate the mid-point of a measurement period. For clarity the figure is an oversimplification as there are no connecting lines showing repeated measures and within-individual daily variation.

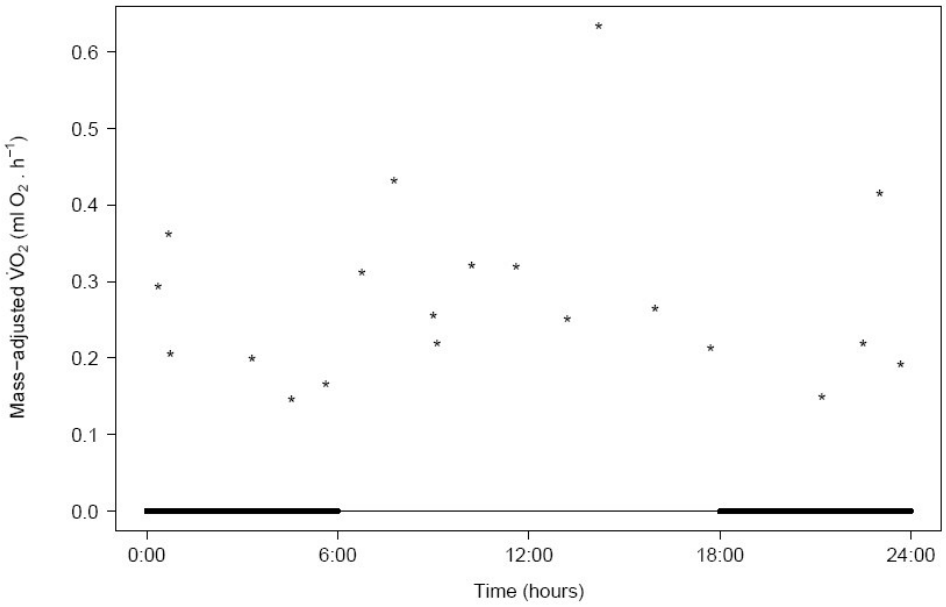

Figure B8: Daily $\dot{V}O_2$ of the nocturnal gecko *Hoplodactylus maculatus* from Stephens Island at 13 °C and in darkness, adjusted for individual effects by subtracting the estimated individual coefficients. Mean mass = 6.8 ± 0.6 g; n = 7. Scotophase (dark) normally experienced by the animals is indicated with black bars. N. B. the points on the graph indicate the mid-point of a measurement period. For clarity the figure is an oversimplification as there are no connecting lines showing repeated measures and within-individual daily variation.

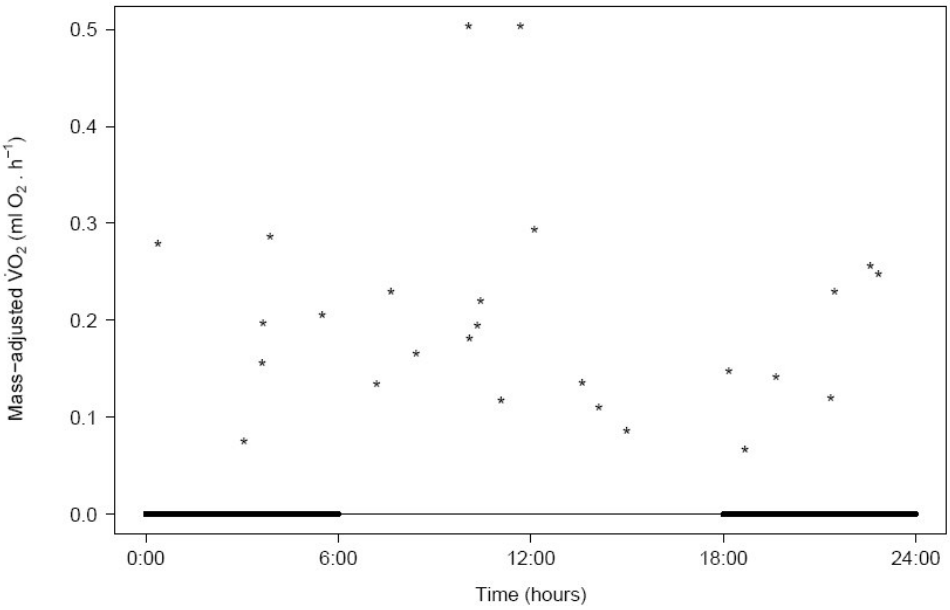

Figure B9: Daily $\dot{V}O_2$ of the nocturnal gecko *Hoplodactylus maculatus* from mainland Wellington, measured in the laboratory at 13 °C and in darkness, adjusted for individual effects by subtracting the estimated individual coefficients. Mean mass = 9.0 ± 0.7 g; n = 7. Scotophase (dark) normally experienced by the animals is indicated with black bars. N. B. the points on the graph indicate the mid-point of a measurement period. For clarity the figure is an oversimplification as there are no connecting lines showing repeated measures and within-individual daily variation.

C *Chapter 4* - Metabolic rate of lizards

Species used in metabolic rate comparisons

Mass-specific $\dot{V}O_2$ of temperate and tropical lizards were compared from published studies (Tables C1a,b and c) that met strict guidelines to limit many confounding factors on metabolism. We only used data from the Gekkota and Scincomorpha, and experimental temperatures within 2 °C of our experimental temperatures. The study had to use only quiescent, non-gravid or non-pregnant adult individuals in a post-absorptive state. In addition, the studies needed to include species that were of a similar ecological category (see Andrews and Pough (1985) for definitions of ecological category).

Table C1a: Rate of oxygen consumption (ml O_2 g^{-1} h^{-1}) at 15 °C in some temperate lizards.

Taxon	Species	State	Mass	$\dot{V}O_2$	Citation
G	*Hoplodactylus maculatus*	SMR	7.9	0.32	Chapter 4
G	[1]*H. aff. maculatus "Canterbury"*	SMR	8.3	0.25	Tocher and Davison, 1996
G	[1]*H. aff. maculatus "Southern Alps"*	SMR	8.4	0.25	Tocher and Davison, 1996
G	*H. chrysosireticus*	SMR	5.9	0.35	Chapter 4
G	*H. stephensi*	SMR	7.4	0.22	Chapter 3
G	*Naultinus manukanus*	SMR	6.5	0.13	Chapter 3
S	*Acanthodactylus erythrurus*	SMR	9.4	0.94	Pough and Busack, 1978
S	*Cyclodina aenea*	SMR	2.5	0.1	Chapter 4
S	*C. macgregori*	SMR	19	0.76	Chapter 4
S	*Mabuya capensis*	SMR	9.2	0.4	Brownlie and Loveridge, 1983
S	[2]*Oligosoma maccanni*	RMR	2.9	0.23	Grimmond and Evetts, 1980; Evetts and Grimmond, 1982
S	*O. nigriplantare polychroma*	SMR	3.3	0.07	Chapter 3
S	*O. zelandicum*	SMR	3.8	0.11	Chapter 3
S	[3]*Proscelotes arnoldi*	SMR	4.6	0.19	Brownlie and Loveridge, 1983
S	*Xantusia henshawi*	SMR	3.5	0.13	Mautz, 1979
S	[4]*Xantusia riversiana*	SMR	19.0	0.46	Mautz, 1979

G = Gekkota; S = Scincomorpha; SMR = standard metabolic rate; RMR = resting metabolic rate; Mass is in g; $\dot{V}O_2$ is in ml O_2 g^{-1} h^{-1}; [1] as *H. maculatus* in Tocher and Davison (1996); [2] as *Leiolopisma n. maccanni* in Evetts and Grimmond (1982), and Grimmond and Evetts (1980), N.B. most likely *O. maccanni*, but could also contain *O. n. polychroma* (Freeman, 1997); [3] as *Proscelotes arnoldi arnoldi* in Brownlie and Loveridge (1983); [4] as *Klauberina riversiana* in Mautz (1979).

Table C1b: Rate of oxygen consumption (ml O_2 g^{-1} h^{-1}) at 25 °C in some temperate lizards.

Taxon	Species	State	Mass	$\dot{V}O_2$	Citation
G	[1]*Coleonyx switaki*	SMR	9.5	0.70	Putnam and Murphy, 1982
G	*Coleonyx variegatus*	SMR	3.6	0.53	Putnam and Murphy, 1982
G	*Eublepharis macularius*	SMR	32.8	4.59	Autumn et al., 1999
G	*H. maculatus*	SMR	7.8	0.78	Chapter 4
G	[2]*H.* aff. *maculatus* "Canterbury"	SMR	8.3	0.58	Tocher and Davison, 1996
G	[3]*H.* aff. *maculatus* "Otago/Southland large"	RMR	5.6	0.56	Grimmond and Evetts, 1980; Evetts and Grimmond, 1982
G	[2]*H.* aff. *maculatus* "Southern Alps"	SMR	8.4	0.59	Tocher and Davison, 1996
G	*H. stephensi*	RMR	8.1	0.89	Chapter 4
G	*Naultinus manukanus*	RMR	6.1	0.67	Chapter 4
G	*Teratoscincus przewalskii*	SMR	11.2	1.14	Autumn et al., 1994
S	*Acanthodactylus erythrurus*	SMR	9.4	1.62	Pough and Busack, 1978
S	*A. schreiberi*	RMR	10.9	1.63	Duvdevani and Borut, 1974
S	*A. scutellatus*	RMR	6.7	1.06	Duvdevani and Borut, 1974
S	*Cyclodina aenea*	SMR	2.6	0.36	Chapter 4
S	*C. macgregori*	SMR	19.2	1.15	Chapter 4
S	*Mabuya capensis*	SMR	9.2	0.74	Brownlie and Loveridge, 1983
S	[4]*Oligosoma maccanni*	RMR	2.9	0.46	Grimmond and Evetts, 1980; Evetts and Grimmond, 1982
S	*O. nigriplantare polychroma*	RMR	3.2	0.51	Chapter 4
S	*O. zelandicum*	RMR	3.6	0.54	Chapter 4
S	[5]*Procelotes arnoldi*	SMR	4.6	0.32	Brownlie and Loveridge, 1983
S	*Xantusia henshawi*	SMR	3.5	0.37	Mautz, 1979
S	[6]*Xantusia riversiana*	SMR	19.0	1.10	Mautz, 1979
S	*Xantusia vigilis*	SMR	1.5	0.17	Mautz, 1979

G = Gekkota; S = Scincomorpha; SMR = standard metabolic rate; RMR = resting metabolic rate; Mass is in g; $\dot{V}O_2$ is in ml O_2 g^{-1} h^{-1}; [1] as *Anarbylus switaki* in Putnam and Murphy (1982); [2] as *H. maculatus* in Tocher and Davison (1996); [3] as *H. maculatus* in Grimmond and Evetts (1980) and Evetts and Grimmond (1982); [4] as *Leiolopisma n. maccanni* in Evetts and Grimmond (1982) and Grimmond and Evetts (1980), N.B. most likely *O. maccanni*, but could also contain *O. n. polychroma* (Freeman, 1997); [5] as *Proscelotes arnoldi arnoldi* in Brownlie and Loveridge (1983); [6] as *Klauberina riversiana* in Mautz (1979).

Table C1c: Rate of oxygen consumption (ml O_2 g^{-1} h^{-1}) at 25 °C in some tropical lizards.

Taxon	Species	State	Mass	$\dot{V}O_2$	Citation
G	*Cosymbotus platyurus*	SMR	1.2	0.35	Feder and Feder, 1981
G	*Hemidactylus flaviviridis*	RMR	4.2	0.45	Zari, 1997
G	*H. frenatus*	SMR	4.4	0.69	Snyder and Weathers, 1976
G	*Lepidodactylus lugubris*	SMR	0.7	0.10	Feder and Feder, 1981
S	*Lepidophyma gaigeae**	SMR	5.0	0.14	Mautz, 1979
S	*Lepidophyma gaigeae*	SMR	5.0	0.39	Mautz, 1979
S	*Lepidophyma smithii**	SMR	25.0	0.50	Mautz, 1979
S	*Lepidophyma smithi*	SMR	25.0	1.32	Mautz, 1979
G	*Pachydactylus bibroni*	SMR	14.8	1.92	Autumn et al., 1999
G	*Rhoptropus afer*	SMR	2.3	0.11	Peterson, 1990
G	*Sphaerodactylus beattyi*	RMR	0.4	0.60	Snyder, 1979
G	*S. cinereus*	SMR	0.5	0.05	Dunson and Bramham, 1981
G	*S. notatus*	SMR	0.3	0.03	Dunson and Bramham, 1981
G	*S. macrolepis*	RMR	0.5	0.09	Snyder, 1975
S	*Acanthodactylus boskianus*	RMR	7.8	1.87	Duvdevani and Borut, 1974
S	*Cnemidophorus murinus*	SMR	85.0	4.93	Bennett and Gorman, 1979
S	*Tupinambis teguixin*	RMR	988.0	5.73	Bennett and John-Alder, 1984

G = Gekkota; S = Scincomorpha; SMR = standard metabolic rate; RMR = resting metabolic rate; Mass is in g; $\dot{V}O_2$ is in ml O_2 g^{-1} h^{-1}; * = measures taken at 15 °C.

D CHAPTER 5 - Energetics of locomotion

Conditioning trials with respirometry masks

Ten trials were conducted to measure the $\dot{V}O_2$ of restrained (wearing a respirometry mask), resting *Naultinus manukanus*. The $\dot{V}O_2$ was measured for each lizard once a day for five consecutive days over two periods (five trials in period A, and five trials in period B), with a rest period of five days between periods A and B. Food was withheld during both periods. Animals were fed on the fifth day of period A, and then fasted again prior to period B. After three trials $\dot{V}O_2$ did not differ significantly among trials (P = 0.475), even after a five day rest period (Figure D1).

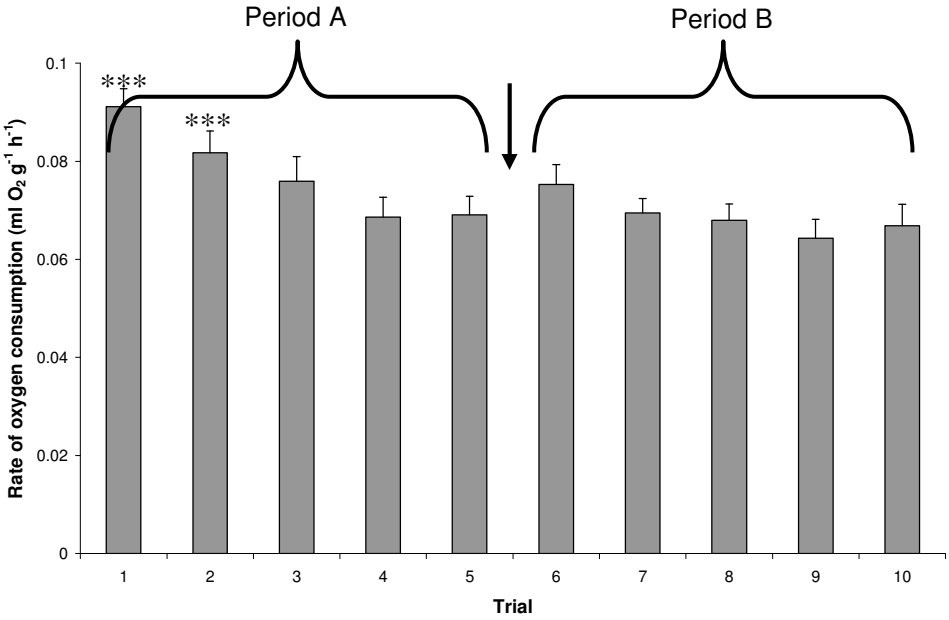

Figure D1: Rate of oxygen consumption at 25 °C is greatest in the first 2 trials compared with subsequent trials for post-absorptive *Naultinus manukanus* wearing respirometry masks. Trials 1 to 5 (Period A) and 6 to 10 (Period B) were on consecutive days with five days of no experiments (arrow) between periods A and B. Mean mass = 7.6 ± 0.1 g; n = 17; Error bars are 1 SE; $^{***}P < 0.001$.

Statistical values from allometric analyses

Standard and phylogenetic allometric contrasts were employed to evaluate interspecific variation in three measures of locomotor efficiency among lizards (Table D1). Species were classified as nocturnal or diurnal, as well as to the taxonomic levels Anguimorpha, Gekkota, Iguania and Scincomorpha and the latitudinal ranges tropical, subtropical and temperate. We defined tropical as between the tropics of Cancer (23.5 ° N) and Capricorn (23.5 ° S), temperate as latitudes higher than the tropics of Cancer or Capricorn, and subtropical where the populations are from the edge of the tropical/temperate border (within 2 ° of 23.5 ° N or S), or the populations are not defined and the species' range includes both tropical and temperate areas.

Table D1: Comparisons of three measures of locomotion in lizard species as grouped by activity period, phylogenetic taxon and latitudinal location. Comparisons give *P*-values from standard allometric analysis and phylogenetic allometric analyses (using permutation tests; Harvey and Pagel, 1991). Differences between the two statistical methods (standard vs. phylogenetic analysis) are negligible.

Comparison	Standard allometric analysis			Phylogenetic allometric analysis		
	$\dot{V}O_{2max}$	C_{min}	MAS	$\dot{V}O_{2max}$	C_{min}	MAS
N vs. D	**0.028**	**0.025**	0.248	**0.027**	**0.007**	0.199
N vs. D(-°2D)	**0.002**	**0.014**	**0.015**	**0.002**	**0.001**	**0.006**
All four taxa	**<0.001**	0.889	0.324	**<0.001**	0.710	0.275
A vs. G	**<0.001**	0.921	0.300	**<0.001**	0.867	0.252
A vs. I	**<0.001**	0.343	0.487	**0.004**	0.134	0.617
A vs. S	**<0.001**	0.985	0.778	**0.001**	0.859	0.523
G vs. I	0.814	0.095	0.097	0.754	0.077	0.072
G vs. S	0.829	0.993	0.432	0.730	0.983	0.409
I vs. S	0.618	0.708	0.780	0.598	0.622	0.667
Tr vs. Te	0.528	0.481	0.298	0.414	0.322	0.257
Tr vs. ST vs. Te	0.073	0.733	0.481	>0.050	0.534	0.414
NZ vs. All lizards	**<0.001**	**<0.001**	0.117	**<0.001**	**<0.001**	0.098

D = diurnal; N = nocturnal; -°2D = minus secondarily diurnal species; A = Anguimorpha; G = Gekkota; I = Iguania; S = Scincomorpha; Classifications of taxon are from Pianka and Vitt (2003). Te = temperate; Tr = tropical; ST = sub-tropical (populations found on edge of tropical/temperate border or populations not defined and species range covers tropical and temperate areas); Where Tr vs. Te is compared ST is included in Te in most cases; NZ = New Zealand; $\dot{V}O_{2max}$ = maximum rate of oxygen consumption ($\dot{V}O_2$) during exercise (aerobic capacity) adjusted by Q_{10} models to 35 °C; C_{min} = cost of locomotion (ml O_2 g^{-1} km^{-1}) as measured by calculating the slope of the regression $\dot{V}O_2$ on speed; MAS = maximal aerobic speed (km h^{-1}) calculated statistically as the speed above which there is no significant increase in $\dot{V}O_2$; P-values in bold are significant at the 0.05 level.

E *Chapter 6* - Specific activity of LDH

Modification of the lowry assay

The lowry protein assay (Lowry et al., 1951) was modified by the following:

Fifty µl of each tissue homogenate was added to individual wells of a 96-well plate, after which 100 µl of ABC reagent was added. ABC reagent is made from Reagent A: 20 g L^{-1} NaOH, 85.5 g L^{-1} Na_2CO_3 anhydrous; Reagent B: 24 g L^{-1} NaK tartrate $4H_2O$, and Reagent C: 10 g L^{-1} $CuSO_4$ $5H_2O$. The sample and ABC reagent were left for approximately 15 min at room temperature, and then 50 µl of Folin Reagent (diluted 1/5) was added. The final product was incubated for 1 h at room temperature.

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APPENDIX 2

Natural history of *Hoplodactylus stephensi* (Reptilia: Gekkonidae) on Stephens Island, Cook Strait, New Zealand[1]

Abstract

The striped gecko (*Hoplodactylus stephensi*) is one of the rarest and most elusive of New Zealand's lizards. It is currently known from just three locations; Stephens Island (Takapourewa) in Cook Strait, Maud Island in Pelorus Sound, and the Coromandel Peninsula. The striped gecko is a relatively poorly studied species with little data available on many aspects of its biology. We report on the first estimate of longevity in *H. stephensi* (a minimum of 16 years) and provide baseline data on population structure, habitat use, morphometrics and pregnancy rate. Our data show the value of permanently marked populations of reptiles available for long-term study by different researchers.

Introduction

Poor knowledge of species biology can hinder species conservation management. For example, in New Zealand conservation management of the critically endangered kakapo (*Strigops habroptilus*) for many years included intensive supplementary feeding to encourage successful nesting. However, recent research has shown that female kakapo fed a supplemented diet produce more male than female offspring, further biasing an already male-biased sex ratio and threatening the species recovery (Clout et al., 2002).

Ecologically, the reptile fauna of New Zealand is characterised by extended life histories and low reproductive rates in general, which make many species vulnerable to human disturbance and mammalian predation (Daugherty et al., 1993; Cree, 1994). The

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striped gecko (*Hoplodactylus stephensi*) is one of New Zealand's rarest geckos. It is listed in the New Zealand threat classification system as range-restricted (Cook Strait island populations) and data deficient (Coromandel Peninsula population; Hitchmough, 2002), and as vulnerable in the IUCN Red List (IUCN, 2002). Conservation management for *H. stephensi* is hampered by the elusive behaviour of this species and the limited information available on its general biology. This species is of moderate size (up to 85 mm snout-vent length) and is nocturnal and arboreal. It is known only from forest remnants and shrublands on Stephens Island, Maud Island and the Coromandel Peninsula, although it possibly once inhabited lowland forest throughout the North Island (Whitaker, 1991; Whitaker et al., 1999; Gill and Whitaker, 2001; Whitaker, 2001). The population of *H. stephensi* in 1 ha of *Muehlenbeckia australis* vine land beside Ruston Bush on Stephens Island has been estimated to number approximately 600 individuals (Cree, 1990). Data for captive specimens are also limited, with a maximum life span in captivity recorded at 10 years after capture as an adult (I. Borich, Ti Point Reptile Zoo, Warkworth, New Zealand, pers. comm.).

Knowledge of a species' longevity is important for conservation management, particularly when planning post-translocation surveys and estimating population recovery times after predator removal (Bannock et al., 1999; Towns and Ferreira, 2001). However, few studies in New Zealand have followed individually marked lizards in the wild for the decades required to trace individuals throughout their natural life span. Among those that have, considerable longevity is evident. For example, longevity of one individual *H. duvaucelii* on North Brother Island was estimated at 36 years (Thompson et al., 1992). Some *H. maculatus* were estimated to reach a minimum age of 27 years at Turakirae Head, Wellington (Anastasiadis and Whitaker, 1987; Green, 2001), and *H. maculatus* 36 years on Motunau Island, Canterbury (Bannock et al., 1999). Compared to other small, free-living lizard species worldwide, New Zealand geckos show extreme longevity. For example, the life span of the Australian geckos *Diplodactylus stenodactylus* and *D. conspicillatus* in the wild is 3 years, and of the gecko *Rhynchoedura ornata* is 2 years (Read, 1999)).

The longevity of some New Zealand reptile species has been linked with their late maturity and low reproductive capacity (Whitaker, 1982; Cree, 1994). All gecko species in New Zealand have a maximum clutch size of two. Females typically give birth during late summer to early autumn, but some do not produce young each year (Cree, 1994; Cree and Guillette, 1995; Gill and Whitaker, 2001). Our study provides an estimate of longevity, and baseline data on population structure, habitat use, morphometrics and reproduction of *H. stephensi*.

Materials and methods

Stephens Island (40°35 S 173°55 E) is a 150 ha Nature Reserve located at the northern tip of the Marlborough Sounds, Cook Strait. Two surveys were carried out for *H. stephensi* on Stephens Island. The first survey was conducted by AC from 01 February to 11 March 1990. The second, by KMH, was from 29 October to 8 December 2002. The 1990 study was undertaken to determine whether *H. stephensi* preferentially used the invasive weed *Tradescantia fluminensis* as habitat, prior to control of the weed (Cree, 1990; Cree, 1992).

During the 1990 study, the distribution of, and range of habitats used by *H. stephensi* were estimated by 1) repeated spotlight searching over several nights in study plots of 10 x 10 m in three habitat types (mature forest, *T. fluminensis* and vinelands), 2) timed spotlight searches at night in areas outside the study plots (Table 1), and 3) daytime searches under debris and *T. fluminensis* within mature forest (Keepers Bush).

Some habitat types searched in 1990 were unavailable for searching in 2002. For example, access was not permitted to the southern end of the island including inner Ruston Bush or the Frog Bank. Also, *T. fluminensis* was greatly reduced after the 1990 study (very few *H. stephensi* were present in this vegetation). During the 2002 study, animals were located by spotlighting along forest margins or forest tracks at night, or searching under debris within mature forest during the day (Keepers Bush). Only forest margins and tracks were searched at night as the 2002 search season was during spring

when sea birds (e.g., *Pachyptila turtur*) nest in the forest, and it is very difficult to move under the canopy at night without breaking burrows and possibly burying chicks alive.

Table 1. Search intensity (number of person-hours) and capture rate (number of geckos observed/person-hour) of *H. stephensi* during night searches of some habitat types on Stephens Island during summer 1990 and spring 2002. Value ± 1 SE.

Habitat type		1990		2002	
		Person-hours	**Geckos per hour**	**Person-hours**	**Geckos per hour**
Forest	Frog Bank	1.5	0	-	-
	Keepers (tracks)	-	-	10.0	0.2
Forest	Southern Ruston	8.0	0.9	8.0	0.9
margins	Eastern Keepers	-	-	1.0	0
(fence	Northern Keepers	-	-	9.0	0.3
lines)	Regenerated (Ruston)	-	-	4.5	0.9
Plots	Forest[1] (n = 3)	9.9	0	-	-
	M. australis[2] (n = 3)	15.6	0.6 ± 0.4	-	-
	T. fluminensis[3] (n = 2)	5.6	0.1 ± 0.1	-	-
Vineland	*M. australis*[4]	4.0	0.5	-	-
	M. complexa[5]	4.0	0	1.0	0
	Mixture of two[6]	5.0	0	-	-
Wooden shed and overgrown gardens in Keepers[7]		3.0	0	1.5	0

[1]two plots in Ruston and one in Keepers; [2]*Muehlenbeckia australis* in Southern Ruston; [3]*Tradescantia fluminensis* in Keepers Bush; [4]North east of radar station; [5]track to radar station; [6]above Queens and Landing Beaches; [7]includes only overgrown gardens for 2002 searches.

To minimise habitat disturbance during spotlighting, only geckos readily seen on exposed surfaces were recorded. Captured animals were given permanent, individual marks by clipping three toes (1990), or temporarily marked with a number on the ventral surface (2002). Sex and size measurements (snout-vent length (SVL), vent-tail length (VTL), and regeneration (R) ± 1 mm, and mass ± 0.1 g) were recorded. Reproductive status of females was also recorded, including pregnancy status (yes/no) and number of embryos felt by abdominal palpation (Cree, 1990; see Cree and Guillette, 1995 and Wilson and Cree, 2003 for information on accuracy of this procedure in other New Zealand geckos).

Morphometric data from 1990 and 2002 were pooled and analysed using the statistics programme SAS (Version 6.21). All data were tested for normality, and statistical significance was assumed at $P < 0.05$. Data are expressed as mean ± 1 SE.

Longevity was estimated using recapture data of permanently marked individuals over the two trips. We analysed whether size is sexually dimorphic in *H. stephensi* by assessing the differences in SVL between all adult males and females using a general linear model (GLM) with sex as the independent factor and SVL as the dependent variable. Mass was also compared between sexes, with SVL as a covariate. Similarly we tested whether reproduction is size-related in females by determining whether pregnant females were larger (SVL) than non-pregnant females using a GLM, with reproductive status as the independent factor and SVL as the dependent variable. We analysed whether tail loss as an adult is significantly different between the sexes using a Chi-Squared (χ^2) analysis.

Results

The overall capture rate of *H. stephensi* at night was 0.52 geckos/person hour in 1990 and 0.46 geckos/person hour in 2002. A total of 40 *H. stephensi* were seen during the 1990 search, and 33 captured. In 2002, 19 individuals were seen and captured. Recruitment into the population was established in both studies by capture of juveniles (Table 2), with juveniles accounting for 18% of the total captured population in 1990 and 5% in 2002.

In 1990 and 2002 all animals were found between 0.1 and 3.5 m above the ground. The maximum height of vegetation varied in different habitats: vineland about 2.5 m, *T. fluminensis* 1 m and mature bush 6 m. The slopes of Stephens Island between the cliffs and summit were once covered by dense native forest, but lighthouse operations since 1892 led to the loss of around 90% of the original forest (Dieffenbach, 1843; Walls, 1983). In 1990 vegetation cover was estimated using a planimeter from an aerial

photograph of Stephens Island taken in 1989; mature forest covered 5.5 ha with Ruston Bush 3 ha, Keepers Bush 2 ha and the Frog Bank Bush 0.2 ha (Cree, 1990). In 2002 vegetation cover was estimated using the computer programme AutoCad. Intensive regeneration efforts since 1990 meant that mature and 10+ year old regenerating forest covered approximately 21.6 ha with Ruston Bush extending to 4.8 ha and Keepers Bush 2.5 ha. Search effort (person hours) was not equal over all the available habitat types due to access restrictions. More time was spent searching the edges of forests than the interior. Therefore, capture rate (geckos/person hour) was estimated for each vegetation type (Table 1).

Table 2. Number of *H. stephensi* individuals captured on Stephens Island during summer 1990 and spring 2002.

		1990	**2002**	**Total**
Female	p	4	3	7
	np	7	7	14
Male		16	8*	23
Immature		6	1	7

np = not pregnant, p = pregnant, * = including one recaptured individual, toe-clipped in 1990.

In 1990, *H. stephensi* were predominantly located on *Muehlenbeckia australis* (*n* = 22), or on mature trees or low vegetation that were partly overgrown by, or within 2 m of, *M. australis* (*n* = 15). A few animals (*n* = 3) were found on *T. fluminensis* (Table 1). No animals were found under debris during day searches, on *M. complexa,* or in mixed vine vegetation along the eastern cliff edges. In 2002, most individuals were found on *M. australis* or on mature trees or low vegetation associated with *M. australis* (*n* = 12). However, four individuals were found in regenerating forest on flaxes (*Phormium cookianum*) over 10 m from *M. australis* (Table 1). Three animals were found under large piles of nikau palm (*Rhopalostylis sapida*) leaves during day searches (capture rate = 0.5 geckos/person hour). No animals were found under any other type of debris, or on *M. complexa.*

Mature geckos ranged in size from 60 mm SVL to 81 mm SVL (Table 3). There was no difference in SVL between pregnant and non-pregnant females ($F_{1, 19} = 3.72$, $P = 0.07$) or between the sexes ($F_{1, 42} = 1.00$, $P = 0.32$). Pregnant females were significantly heavier for their length than non-pregnant females or males ($F_{2, 50} = 12.94$, $P < 0.01$). Males were significantly heavier for their length than non-pregnant females ($F_{2, 34} = 68.59$, $P < 0.01$). The frequency of tail loss for this species was 43% of the captured population. Tail loss was not significantly different for adult males and females ($\chi^2_1 = 0.77$, $P < 0.01$). The sex ratio of the adult population is approximately 1:1 male:female, with males making up 52% of the adult population (Table 3). Of the 11 adult females caught in summer 1990 only four were pregnant (36%), and by palpation it was determined that three carried two embryos and one probably carried one embryo. Of the 10 adult females caught in spring 2002 only three were pregnant (30%) with two embryos each. Thus, the annual reproductive output for *H. stephensi* is 0.62 offspring/female/yr.

Table 3. Mean (± 1 SE) snout-vent length (SVL) and body mass of *H. stephensi* captured on Stephens Island during summer 1990 and spring 2002.

			SVL (mm)		Mass (g)	
		n	**Mean**	**Range**	**Mean**	**Range**
Female	p	7	75.4 ± 1.1	72 – 81	11.4 ± 0.6	9.6 - 13.5
	np	14	71.3 ± 1.4	60 – 78	7.9 ± 0.5	4.4 - 11
Male		23	74.0 ± 2.0	60 – 80	9.6 ± 1.2	5.8 - 12.3
Immature		7	54.0 ± 2.3	44 – 60	4.2 ± 0.5	2.0 - 5.0

np = not pregnant, p = pregnant.

One male marked as an adult in 1990 was recaptured in 2002 within 5 m of the original capture site. Age in relation to SVL could not be estimated directly for *H. stephensi* due to the low number of recaptures. However, assuming that *H. stephensi* has a life history comparable to the similar sized *H. maculatus* (Anastasiadis and Whitaker, 1987), conservatively the male was at least 4 years old at first capture, and thus a minimum of 16 years old when recaptured. The recaptured animal had lost its tail between captures,

but the SVL (76 mm) was only 1 mm different from the original measurement (75 mm). Three other individuals captured in 2002 had some missing toes (maximum of two missing), but these were from natural toe loss.

Discussion

Habitat use by *H. stephensi* in 1990 and 2002 was similar, with the highest capture rates from the forest margins of Ruston Bush. Most captures were also made on vegetation associated with *M. australis* and during night searches. However, it does appear that *H. stephensi* is occupying more habitat as it becomes available in the form of regenerating forest. Perhaps the concentration of *H. stephensi* in vegetation associated with *M. australis* is simply a reflection of the fact that, for many years, the largest continuous area of vegetation on Stephens Island was Ruston Bush plus the surrounding vinelands.

The higher capture rate of *H. stephensi* in vegetation associated with *M. australis* and Ruston Bush margins could also be due to the physical structure of the habitats. Most trees in Ruston Bush have associated *M. australis* vines. Also, *H. stephensi* may be underestimated in the canopy. Forest margins are easier to search than the tree canopy, and the vinelands reached a maximum of 2.5 m in height, which enabled most vineland areas to be effectively searched. The forest canopy is substantially higher and more difficult to search effectively. However, the edges of Keepers Bush were also searched, and had relatively low capture rates of *H. stephensi* compared with Ruston Bush forest margins. Keepers Bush margins have fewer vines associated with the vegetation.

The sex ratio of *H. stephensi* on Stephens Island is 1:1 male:female and the minimum size at maturity is likely to be 60 mm SVL, as the smallest sexually mature individuals of both sexes captured were this size. Several other *Hoplodactylus* species or populations are also sexually mature at around 60 mm SVL, for example, *H. maculatus* from Motunau Island is sexually mature at 61.5 mm SVL (Bannock et al., 1999) and *H. chrysosireticus* at 60 mm SVL (Flannagan, 2000). There was no apparent difference

between the sexes of *H. stephensi* for SVL at maturity. In other species of *Hoplodactylus* differences in SVL between the sexes at sexual maturity are minor. For example, *H. duvaucelii* females mature at 95 mm SVL, whereas the males mature at 98 mm SVL (Barwick, 1982).

There is no sexual size dimorphism in SVL in *H. stephensi*, the mean adult SVL being 74.2 mm. However, there is sexual dimorphism in shape, with mass relative to SVL significantly greater in adult males than in non-pregnant females. This trend is apparent in another New Zealand reptile, *Sphenodon guntheri*, where females (gravid and non-gravid combined) also have a much lower mass relative to SVL than males (Hoare, 2002). Differences in shape could be due to females expending more energy on reproduction than males. Males also have a bulkier body shape, and a proportionally wider head than females (Cree, 1990).

Clutch size in *H. stephensi* is the same as in other New Zealand viviparous geckos, i.e., one or two offspring (Cree, 1994). The low pregnancy rate of female *H. stephensi* captured in 1990 and 2002 suggests a low reproductive output for this species of around 0.62 offspring/female/yr, which is a trait found in other New Zealand geckos. For example, the annual reproductive output of *H. maculatus* at Macraes is 0.85 offspring/female/yr (Cree, 1994; Cree and Guillette, 1995), and of *Naultinus manukanus* on Stephens Island is 1.28 offspring/female/yr (Hitchmough, 1978; Cree, 1994). This is very low when compared with the annual reproductive output of (oviparous) geckos worldwide, which range from 2 to ≥ 4 offspring/female/yr (Cree, 1994).
Our research demonstrates the value of having marked populations available for long-term study by different researchers and adds another species to a growing list of New Zealand *Hoplodactylus* with extreme longevity in the wild. The recaptured male was found at virtually the same location at which it was first marked, suggesting strong site fidelity, comparable to *H. chrysosireticus*, *H. duvaucelii* and *H. maculatus* (Whitaker, 1982; Thompson et al., 1992; Bannock et al., 1999; Flannagan, 2000).

Toe clipping has been a valuable permanent identification tool for long-term studies of New Zealand geckos (Anastasiadis and Whitaker, 1987; Thompson et al., 1992; Cree, 1994; Bannock et al., 1999; Green, 2001) and does not appear to affect locomotor performance or survival in several lizard species elsewhere (Petren and Case, 1996; Paulissen and Meyer, 2000; Borges-Landaez and Shine, 2003). For example, toe clipping of the arboreal gecko *Hemidactylus turcicus* did not lessen individual climbing ability compared with non-toe clipped individuals (Paulissen and Meyer, 2000). However, natural toe loss can occur, so to minimise misidentification of individuals we recommend that at least three toes be marked.

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APPENDIX 3
Hoplodactylus maculatus (common gecko) aggregations[1]

Aggregative behaviour has been observed in many squamate lineages (e.g., Brattstrom, 1974; Cooper and Garstka, 1987; Gregory, 2004). However, lizard aggregations in diurnal retreat sites are rarely documented (Shah et al., 2003). We report here on the demographic structure of an unusually large (n = 94), diurnal aggregation of *Hoplodactylus maculatus* on a New Zealand island.

On 17 May 2004 (autumn), we surveyed the *H. maculatus* occupying wooden, pest-control bait boxes as diurnal retreat sites. The bait boxes, deployed on the shoreline of Mana Island (40°40'S, 174°00'E) to control accidental rodent (e.g., *Rattus* sp.) incursions, ranged in size from 8.3 L to 12.4 L, each with an internal central 0.5 L bait partition where the geckos commonly congregated beneath the bait holder. Fourteen bait boxes were surveyed between 1000 h and 1500 h.

A total of 183 *H. maculatus* were found within the 14 bait boxes, with a mean of 13 ± 6 SE geckos per box. However, one bait box contained about half (n = 94) of all geckos captured, and two lacked geckos entirely. The aggregation of 94 geckos (39 juveniles, 11 males, 44 females) was very densely packed within the 0.5 L partition of the bait box, filling the entire area to capacity. Snout-vent length of adult males was slightly larger than adult females (72.7 ± 0.7 and 69.0 ± 0.7 SE mm respectively; F_1 = 10.330, P = 0.002) and did not differ between the large and smaller aggregations (F_1 = 2.015, P = 0.160). Overall, 36% of geckos in bait boxes were juvenile or sub-adult, 16% adult males and 48% adult females. The adult sex ratio varied substantially among bait box aggregations (range = 1:7 to 2:0 m:f). The only other lizard species found within the

[1] **Based on**: Hare, K. M., and Hoare, J. M. 2005. *Hoplodactylus maculatus* (common gecko) aggregations. *Herpetological review 36*: 179.

bait boxes was the skink *Oligosoma lineoocellatum*, one of which was found in a bait box with six *H. maculatus*, but not within the bait partition containing geckos.

Hoplodactylus maculatus, a moderate-sized (up to 82 mm snout-vent length) widespread, endemic, nocturnal gecko (Gill and Whitaker, 2001), is frequently observed in diurnal aggregations. However, few aggregations have been documented, and details and measurements of aggregations have not been reported. For example, on Stephens Island, Cook Strait, up to 200 individuals were found beneath a corrugated iron sheet (Bauer, 1990) and on Mana Island mixed age and sex groups of 10-15 individuals are common, with occasional large aggregations of >60 individuals (Whitaker, 1993).

The aggregation of *H. maculatus* in the bait box is unlikely to be due to a lack of suitable natural retreat sites as the shore platform provides a complex habitat of rocks and logs that could be readily used. Aggregation, despite an abundance of retreat sites, could imply that the benefit may derive from social groups (Shah et al., 2003). However, the high variance in adult sex ratios suggests that aggregations may not always represent family groups or harems. Aggregative behaviour in the nocturnal gecko *Nephrurus milii* may have evolved to provide facultative control over rates of thermal exchange (Shah et al., 2003), which provides a possible explanation for aggregations of *H. maculatus*. Other *Hoplodactylus* species, including *H. duvaucelii* (Robb, 1980) and *H.* aff. *maculatus* 'Otago-Southland large' (as *H. maculatus* in Southey, 1986)), have also been observed to aggregate in mixed size and sex class groups. Aggregation may be widespread and frequent in the genus *Hoplodactylus,* but its purpose needs clarification.

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