Seasonal Adaptation in Gould’s
Long-eared Bat; *Nyctophilus gouldi* Tomes 1858
(Microchiroptera : Vespertilionidae)

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Gould's Long-eared Bat,
*Nyctophilus gouldi* Tomes, 1858
(Photograph taken by G.B. Baker)
Statement of Responsibility

This thesis is my own work except where specifically acknowledged

[Signature]

W.R. Phillips
Especially for my parents
SUMMARY

1. *Nyctophilus gouldi* is a non-migratory, insectivorous vespertilionid bat of 10 to 12 grams body weight which is found only on the subcoastal fringes of south-eastern and south-western Australia. The species is colonial, generally forming colonies of 10 to 20 individuals under the exfoliated bark of trees. Within its distribution, *N. gouldi* favours the areas of dense understorey near permanent water and lands on the ground to take prey or gleans insects off low shrubs.

2. In temperate Australia the activity of *N. gouldi* is highly seasonal with the period of least activity encompassing the colder months from April to September. During the hibernation season males appear to be more active than females. *Nyctophilus gouldi* deposit both brown and white body fats in late-summer and autumn. These fats are slowly depleted over the period of winter torpor.

3. *Nyctophilus gouldi* is monoestrous in south-eastern Australia. Females first become receptive to males in April and oestrus continues throughout the hibernation season until ovulation occurs in September or early October. Spermatogenesis takes place in summer following which spermatozoa
are stored in the epididymides until the following spring. Male libido continues throughout the hibernation season during which time females are inseminated a number of times. A copulatory plug forms in the vagina following mating and sperm are stored in the oviducts or uterine glands. A twinning rate of 54.4 per cent was recorded in captive *N. gouldi* (33 females) during this study.

By maintaining a captive colony of *N. gouldi* in an artificial outdoor roost and flight arena the thermal energetics of 'naturally' acclimatized, homeothermic bats were examined and compared in winter, spring and summer. *Nyctophilus gouldi* maintained on plentiful food were capable of regulating body temperature in the typical homeothermic manner in all seasons. Minimum metabolism occurred at ambient temperatures between 34 and 36°C and bats routinely had rectal temperatures of between 35 to 39°C. As ambient temperature dropped below 35°C, controlled cooling of the rectum occurred in homeothermic animals such that at temperatures near 10°C, rectal temperatures of 28 to 32°C were common.

The metabolic curves of winter-adapted male and female *N. gouldi* were depressed in a parallel fashion below that of summer- and spring-adapted animals. In addition to parallel translation, the metabolic curve of 'winter' males underwent a reduction in slope that was not
observed in females. The mean homeothermic rectal temperatures of male and female *N. gouldi* in winter were between two and three degrees Celsius less than in summer-adapted bats at all ambient temperatures.

The mean regulated temperatures of a number of other body regions were recorded in winter- and summer-acclimatized bats to confirm that the seasonal difference in mean rectal temperatures was present in other body regions. Thermocouples, subcutaneously implanted in the interscapular, chest and mid-dorsal regions were used in addition to thermocouples recording the surface temperature of the dorsal and ventral surfaces of the folded wing membrane. A thermocouple was also implanted in the peritoneal cavity. There was no consistent seasonal change in the temperatures of these body regions although the extreme lability of body temperatures is held to be responsible for this inconclusive finding. As ambient temperature fell below thermal-neutrality the temperature of all six regions, including the peritoneal cavity, underwent varying degrees of controlled cooling in bats continuing to maintain homeothermy.

The insulative value of the fur from summer- and winter-acclimatized bats was measured. For males, winter fur was 14.1 per cent more
insulative than in summer, however, there was no significant seasonal change in the insulative value of female pelts.

8. Using the recorded body temperatures, rates of oxygen consumption and known insulative values of the fur and naked membranes, heat loss was partitioned into thorax, abdomen and naked body part components. At thermal-neutrality only 10 to 11 per cent of body heat was lost through the furred body regions. At a temperature of 5°C the converse situation prevailed: as a consequence of regional heterothermy only 13 to 14 per cent of body heat passing to the environment via the naked membranes.

9. The seasonal changes in the absolute heat loss through the fur could not account for the reduced metabolic requirements of winter-adapted *N. gouldi*. Upon re-examination of the rectal temperature data it was found that the summer to winter drop in the mean rectal-ambient temperature differential of females was proportional to the reduction in the rate of oxygen consumption, indicating that the primary mechanism of winter adaptation in *N. gouldi* is a reduction in body temperature. For males, the reduction of body temperature in winter is accompanied by improved fur insulation resulting in heat retaining capacities superior to females at low ambient temperatures.
10. *Nyctophilus gouldi* were acclimated to 22°C in mid-winter in an attempt to prematurely stimulate the natural seasonal acclimatization process. Ambivalent results were obtained indicating thermal characteristics of both winter- and summer-adapted bats. Warm-acclimation had the effect of prematurely initiating the spring reproductive phases. Pregnancy commenced in warm-acclimated females and they gave birth 10 to 12 weeks before outdoor captive females. Spermatogenesis was advanced by several weeks in warm-acclimated males.

11. *Nyctophilus gouldi* which had access to *ad libitum* food on the evening prior to experimentation entered torpor only at ambient temperatures less than 18°C. Spring-adapted bats showed the greatest tendency to enter torpor apart from animals denied food for the 12 hours before experimental cold-exposure. In food-deprived bats, entry into torpor was less controlled and occurred at ambient temperatures as high as 25°C. Well-fed animals always aroused from torpor once chamber temperature exceeded 15 to 17°C whereas a temperature of 20°C or greater was needed to stimulate arousal in food-deprived bats.

12. Rates of oxygen consumption for torpid *N. gouldi* were routinely 14 to 20 fold less than homeothermic animals at the same ambient
temperature although values as much as 200 to 300 fold less were recorded from torpid bats given several hours to equilibrate.

13. The regional heterothermy present in homeothermic bats was also present in torpid animals with the thorax being maintained warmer than the abdomen and the abdomen warmer than the wings.

14. The metabolic cost of entry into torpor was about one third of that required for arousal. The mean time taken by winter-adapted *N. gouldi* to arouse from a body temperature of near 10°C was 37.5 minutes. The one summer recorded arousal took almost twice as long.

15. Prior to entering torpor, *N. gouldi* sometimes used 'test-drops' in body temperature, the function of which is not understood. Cold-exposed homeothermic bats were able to expand the thermal gradient from thorax to abdomen to wings through a series of stepped readjustments to body heat distribution. The entry into torpor was generally achieved by a series of stepped reductions in body temperature. The wings were used as heat 'radiators' during each step-down in body temperature. Arousal from torpor was marked by a rapid increase in thoracic temperatures with a slower rewarming by the abdomen and naked body parts.
In the final discussion the factors determining the form of seasonal adaptation in homeotherms are discussed and it is proposed that the temperate-zone bats are especially well adapted to winter conditions. Their small size and mobility allows them to more readily seek out and move between suitable microniches than larger homeotherms. Apart from providing the means of their mobility the wings provide an ideal avenue for the dissipation of excess body heat produced during flight activity.

The final discussion also addresses the question of male/female differences in winter adaptation and activity patterns. The hypothesis is proposed that males are seeking out females with which to copulate during the hibernation season and that the superior heat retaining capacities of winter-acclimatized males are therefore adaptive to greater activity at the coldest time of the year. An ambient temperature threshold is proposed as the primary cue determining the annual activity patterns of the temperate-zone bats and differences in male and female activity rhythms can therefore be explained in terms of dichotomous microclimate selection.

The thermoregulatory competence of the insectivorous bats is defended and the point made that relatively low or variable body temperature is not necessarily indicative of poor thermoregulatory
abilities. The minimum metabolic rate of *N. gouldi* is much higher than that reported for any other insectivorous bat and more aligned with values recorded from small frugivorous and nectarivorous species. The tree-dwelling habit is the explanation offered for the difference in the minimum metabolic rate of *N. gouldi* and the other insectivorous bats. Unlike the thermally stable cave environment, tree-dwelling species must cope with extreme variations in microenvironmental temperature and it is argued that exposure to high day-time temperatures has selected for higher metabolic rates and possibly greater thermoregulatory competence in the tree-dwelling microchiroptera.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontispiece</td>
<td>i</td>
</tr>
<tr>
<td>Statement of Responsibility</td>
<td>ii</td>
</tr>
<tr>
<td>Dedication</td>
<td>iii</td>
</tr>
<tr>
<td>Summary</td>
<td>iv</td>
</tr>
<tr>
<td>List of Plates</td>
<td>xix</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xxi</td>
</tr>
<tr>
<td>List of Tables</td>
<td>xxix</td>
</tr>
<tr>
<td>Definitions</td>
<td>xxxv</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>xxxviii</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>xxxx</td>
</tr>
</tbody>
</table>

**Chapter One: General Introduction**

**Chapter Two: The Description, Ecology and Reproductive Biology of *Nyctophilus gouldi***

2.1 Introduction 12  
2.2 Species Description 13  
2.3 Ecology of *Nyctophilus gouldi*  
2.3.1 Introduction 14  
2.3.2 Methods 15  
2.3.3 Results  
2.3.4 Annual Activity Pattern 17  
2.3.5 Annual Body Weight and Fat Cycles 21  
2.3.6 Discussion 23
<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4 Reproductive Biology of <em>Nyctophilus gouldi</em></td>
<td></td>
</tr>
<tr>
<td>2.4.1 Introduction</td>
<td>27</td>
</tr>
<tr>
<td>2.4.2 Methods</td>
<td>28</td>
</tr>
<tr>
<td>2.4.3 Results</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>30</td>
</tr>
<tr>
<td>Females</td>
<td>32</td>
</tr>
<tr>
<td>2.4.4 Discussion</td>
<td>35</td>
</tr>
<tr>
<td>Plates, Figures and Tables</td>
<td>43</td>
</tr>
</tbody>
</table>

Chapter Three: Oxygen Consumption and Body Temperature Regulation in Endothermic *Nyctophilus gouldi* in Winter, Spring and Summer

<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 Introduction</td>
<td>58</td>
</tr>
<tr>
<td>3.2 Methods</td>
<td></td>
</tr>
<tr>
<td>3.2.1 Maintenance in Captivity</td>
<td>61</td>
</tr>
<tr>
<td>3.2.2 General Experimental Procedures</td>
<td>63</td>
</tr>
<tr>
<td>3.2.3 Statistical Methods</td>
<td>66</td>
</tr>
<tr>
<td>3.3 Results</td>
<td></td>
</tr>
<tr>
<td>3.3.1 Males</td>
<td>70</td>
</tr>
<tr>
<td>3.3.2 Females</td>
<td>72</td>
</tr>
<tr>
<td>3.3.3 Comparison of Male and Female Patterns</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>74</td>
</tr>
<tr>
<td>Winter</td>
<td>75</td>
</tr>
<tr>
<td>Spring</td>
<td>76</td>
</tr>
<tr>
<td>3.4 Discussion</td>
<td>77</td>
</tr>
<tr>
<td>Plates, Figures and Tables</td>
<td>83</td>
</tr>
</tbody>
</table>
Chapter Four: Comparison of Body Tissue Temperatures in Summer- and Winter-Adapted *Nyctophilus gouldi*

4.1 Introduction 97
4.2 Methods 100
   4.2.1 Distribution of Adipose Tissue 100
   4.2.2 Measurement of Body Temperatures 103
   4.2.3 Statistical Methods 103
4.3 Results 105
   4.3.1 Adipose Tissue 106
   4.3.2 Body Temperatures 109
4.4 Discussion 111
   Plates, Figures and Tables 111

Chapter Five: The Insulative Value of *Nyctophilus gouldi* Fur in Summer and Winter

5.1 Introduction 126
5.2 Methods 127
   5.2.1 Experimental Procedures 127
5.3 Results 131
5.4 Discussion 132
   Plates, Figures and Tables 135
Chapter Six: The Partitioning and Seasonal Comparison of Body Insulation

6.1 Introduction

6.2 Methods

6.2.1 Measurement of Body Surface Areas

6.2.2 Methods of Calculating and Partitioning Body Insulation

6.3 Results

6.3.1 Surface Areas

6.3.2 Body Insulation

6.4 Discussion

Plates, Figures and Tables

Chapter Seven: Warm-Acclimation in Mid-Winter: Effects on Endothermic Temperature Regulation and Body Tissue Temperatures

7.1 Introduction

7.2 Methods

7.2.1 Animal Maintenance during Acclimation

7.2.2 Experimental Procedures

7.2.3 Statistical Methods

7.3 Results
Chapter Eight: Torpor: Incidence, Costs and Regional Control of Body Heat During the Entry and Arousal Phases

8.1 Introduction 221
8.2 Incidence of Torpor Entry and Arousal 225
8.2.1 Methods Incidence of Entry into Torpor 225
Effect of Food Deprivation on Entry into Torpor 226
Initiating the Arousal Process 226
8.2.2 Results Incidence of Entry into Torpor 227
Effect of Food Deprivation on Entry into Torpor 228
Initiating the Arousal Process 228
8.3 The Metabolic Costs of Regulating Torpor
8.3.1 Methods
8.3.2 Results

8.4 Regional Heterothermy During Torpor
8.4.1 Methods
8.4.2 Results

8.5 Partitioning of Body Insulation in Torpid
   N. gouldi
8.5.1 Methods

8.6 The Metabolic Costs of Torpor Entry and
   Arousal
8.6.1 Methods
8.6.2 Results

8.7 Heat Distribution During Entry, Arousal and
   Cold Exposure
8.7.1 Methods
8.7.2 Results

8.8 Discussion
Plates, Figures and Tables

Chapter Nine: Final Discussion

9.1 Factors Determining the Form of Seasonal
   Adaptation in Homeotherms

9.2 Differences in Male and Female Activity
   Patterns, Critical Arousal Temperature and
   Micro-habitat Selection

9.3 Nutritional Status and the Incidence of Entry
   into Torpor
9.4 The Thermoregulatory Competence of the Insectivorous Bats and the Evolution of Divergent Temperature Regulation Strategies in the Chiroptera

<table>
<thead>
<tr>
<th>Plates, Figures and Tables</th>
<th>286</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bibliography</td>
<td>299</td>
</tr>
</tbody>
</table>

Appendix I: The Total Surface Area of Vespertilionid Bats Compared to 'Wing-less' Mammals

Appendix II: Complete Form of Regression Equations Fitted in Chapters Three, Six, Seven and Eight
LIST OF PLATES

Frontispiece: Gould's Long-eared Bat, Nyctophilus gouldi Tomes, 1858

Plate 2.1 A typical trap-site used during the field study

Plate 2.2 The pond where most N. gouldi were trapped during the study

Plate 2.3 A Transverse section through the testis of a male N. gouldi in March

B Transverse section through the caudal portion of the epididymis from a male in August

C Longitudinal section through the copulatory plug in the vagina of a female in July

D The oviduct of a female N. gouldi in July showing stored spermatozoa

Plate 2.4 A female N. gouldi with seven day-old young attached to each nipple

Plate 3.1 The outdoor cages used to house the experimental population of N. gouldi

Plate 3.2 The timber nest boxes provided as roosts for captive N. gouldi
Plate 3.3

The metabolic chamber used for all measurements of oxygen consumption and body temperature in homeothermic and torpid *N. gouldi* 85
**LIST OF FIGURES**

| Figure 2.1 | The distribution of *N. gouldi* in Australia | 46 |
| Figure 2.2 | Map of the area used to study the ecology of *N. gouldi* | 47 |
| Figure 2.3 | Annual changes in the mean monthly minimum and maximum temperature, numbers of insects and male and female *N. gouldi* caught per trap-night | 48 |
| Figure 2.4 | The numbers of *N. gouldi* caught at sites within four elevation classes and at pond trap-sites | 49 |
| Figure 2.5 | The annual body weight cycles of captive and free-living *N. gouldi* | 50 |
| Figure 2.6 | The annual body fat cycle of captive *N. gouldi* | 51 |
| Figure 2.7 | The reproductive cycles of male and female *N. gouldi* | 52 |
| Figure 3.1 | The times when winter, spring and summer experimentation took place | 86 |
| Figure 3.2 | Flow diagram of the laboratory set-up | 87 |
| Figure 3.3 | Theoretical comparison of rectilinear and curvilinear lines-of-best-fit fitted to the same data | 88 |
Figure 3.4  Plots of rectal temperature and oxygen consumption responses to ambient temperature in winter-, spring- and summer-adapted, male *N. gouldi*

Figure 3.5  Plots of rectal temperature and oxygen consumption responses to ambient temperature in winter-, spring- and summer-adapted, female *N. gouldi*

Figure 3.6  Comparison of oxygen consumption and rectal temperature curves for male *N. gouldi* in winter, spring and summer

Figure 3.7  Comparison of oxygen consumption and rectal temperature curves for female *N. gouldi* in winter, spring and summer

Figure 3.8  Comparison of rectal temperature and oxygen consumption curves of male and female *N. gouldi* in winter, spring and summer

Figure 4.1  Diagrammatic representation of the body layers present in a bat

Figure 4.2  The locations of thermocouple recording sites in regional heterothermy experiments
| Figure 4.3 | The major brown fat deposits of a female *N. gouldi* in July | 113 |
| Figure 4.4 | Dorsal and ventral views of a *N. gouldi* in winter showing the distribution of subcutaneous white and brown adipose tissue | 114 |
| Figure 4.5 | The temperatures of six body regions in summer- and winter-adapted, male *N. gouldi* at ambient temperatures of 5, 15, 25, and 35°C | 115 |
| Figure 5.1 | An exploded view of the apparatus used to measure the insulative value of *N. gouldi* fur in winter and summer | 135 |
| Figure 6.1 | Surface areas of the naked and furred body regions of *N. gouldi* | 169 |
| Figure 6.2 | Linear regression lines and equations describing the mean temperatures of the thorax-shell, abdomen-shell, abdomen-core and naked membranes of *N. gouldi* | 170 |
| Figure 6.3 | Absolute heat loss from the thorax, abdomen and naked membranes of male *N. gouldi* in summer and winter | 171 |
| Figure 6.4 | Whole-animal rates of thermal conductance calculated in three different ways for *N. gouldi* in summer | 172 |
Figure 6.5  Whole-animal rates of thermal conductance calculated in Watts/°C for male *N. gouldi* in summer 173

Figure 6.6  A comparison of the theoretical effects on the metabolic curve of a drop in body temperature and an improvement to the animals insulation 174

Figure 6.7  Plots of oxygen consumption against rectal temperature for male and female *N. gouldi* in winter, spring and summer 175

Figure 6.8  Comparison of the curves describing the relationship between oxygen consumption and rectal temperature of male and female *N. gouldi* in winter, spring and summer 176

Figure 6.9  Plots of oxygen consumption against rectal-ambient temperature differential for male and female *N. gouldi* in winter, spring and summer 177

Figure 6.10  Comparison of the curves describing the relationship between oxygen consumption and the rectal-ambient temperature differential of male and female *N. gouldi* in winter, spring and summer 178

Figure 7.1  The time of warm-acclimation and subsequent experimentation relative to other seasonal testing 203
Figure 7.2  Plots of rectal temperature and oxygen consumption responses to ambient temperature in male and female N. gouldi warm-acclimated in mid-winter  

Figure 7.3  Comparison of oxygen consumption and rectal temperature curves for male N. gouldi in winter spring and summer and following mid-winter warm-acclimation  

Figure 7.4  Comparison of oxygen consumption and rectal temperature curves for female N. gouldi in winter, spring and summer and following mid-winter warm-acclimation  

Figure 7.5  Plots of oxygen consumption against rectal-ambient temperature differential for male N. gouldi warm-acclimated in mid-winter and the comparison of this curve with those of winter-, spring- and summer-adapted males  

Figure 7.6  Plots of oxygen consumption against rectal-ambient temperature differential for female N. gouldi warm-acclimated in mid-winter and the comparison of this curve with those of winter-, spring- and summer-adapted females
Figure 7.7  Comparison of rectal temperature and oxygen consumption curves of male and female *N. gouldi* after warm-acclimation in mid-winter 209

Figure 7.8  Comparison of the mean body weights of male and female *N. gouldi* maintained in the outdoor flyway with those of animals warm-acclimated in mid-winter 210

Figure 8.1  The effect of over-night food deprivation on the thermo-regulation of a cold-exposed, male *N. gouldi* compared with that of a well-fed animal 253

Figure 8.2  Comparison of the time arousal from torpor was initiated in well-fed and food-deprived *N. gouldi* exposed to a warming environment 254

Figure 8.3  Temperature-induced arousal from torpor by an *N. gouldi* male 255

Figure 8.4  Plots of rectal temperature and oxygen consumption response to ambient temperature in torpid, male *N. gouldi* in winter and summer 256
Figure 8.5  Plots of rectal temperature and oxygen consumption response to ambient temperature in torpid, female *N. gouldi* in winter and summer

Figure 8.6  Regression equations describing the mean temperature responses of the thorax, peritoneal cavity, mid-dorsal region and naked wing membranes of torpid, male *N. gouldi*

Figure 8.7  Entry into torpor by a female *N. gouldi* in winter

Figure 8.8  Arousal from torpor by a male *N. gouldi* in winter

Figure 8.9  Test-dropping followed by entry into torpor and immediate arousal by a male *N. gouldi* in spring

Figure 8.10  Readjustment to the heat distribution in a homeothermic *N. gouldi* resulting in an expansion of the thorax-abdomen-wing thermal gradient

Figure 8.11  A heat shunt in a male *N. gouldi* in autumn
Figure 9.1  Comparison of the number of *N. gouldi* caught per trap-night in each month with the mean number of days when maximum temperature was greater than or equal to the critical arousal threshold (18°C)  299

Figure 9.2  Mass-specific basal rates of metabolism for summer- and winter-adapted *N. gouldi* relative to those of other insectivorous bats and fruit and nectar-eating families  300

Figure 9.3  Mass-specific basal rates of metabolism for insectivorous, nectar-eating, fruit-eating and carnivorous bats relative to Kleiber's curve and the minimum boundary curve for continuous endothermy  301

Figure I  Regression of total surface area against body weight for members of the Family Vespertilionidae  329
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1</td>
<td>The numbers of adult and juvenile <em>N. gouldi</em> caught each month at the Bull's Head study site</td>
<td>53</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>The numbers of adult male and female <em>N. gouldi</em>, <em>N. geoffroyi</em>, <em>Chalinolobus morio</em>, <em>Eptesicus sagittula</em> and <em>E. regulus</em> caught during the active and hibernation seasons</td>
<td>54</td>
</tr>
<tr>
<td>Table 2.3</td>
<td>Numbers of each species banded and retrapped in the Bull's Head study area between March 1980 and March 1983</td>
<td>55</td>
</tr>
<tr>
<td>Table 2.4</td>
<td>The numbers of each species caught at the site of previous encounter and at distances up to and greater than one kilometre</td>
<td>56</td>
</tr>
<tr>
<td>Table 2.5</td>
<td>Breeding information for captive <em>N. gouldi</em></td>
<td>57</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>Seasonal changes in the mean body weight, minimum metabolism and areas under the $\dot{V}O_2$ and $T_R$ curves in male <em>N. gouldi</em></td>
<td>94</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>Seasonal changes in the mean body weight, minimum metabolism and areas under the $\dot{V}O_2$ and $T_R$ curves in female <em>N. gouldi</em></td>
<td>95</td>
</tr>
<tr>
<td>Table</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>-------</td>
<td>--------------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>3.3</td>
<td>Metabolic Q10's for <em>N. gouldi</em> in winter, spring and summer</td>
<td>96</td>
</tr>
<tr>
<td>4.1</td>
<td>The mean temperatures of body regions in endothermic, male <em>N. gouldi</em> at ambient temperatures of 35, 25, 15 and 5°C in summer and winter</td>
<td>116</td>
</tr>
<tr>
<td>4.2</td>
<td>Statistical comparison of the mean interscapular and chest temperatures in winter- and summer-adapted, male <em>N. gouldi</em></td>
<td>118</td>
</tr>
<tr>
<td>4.3</td>
<td>Statistical comparison of the mean chest and mid-dorsal temperatures in winter- and summer-adapted, male <em>N. gouldi</em></td>
<td>119</td>
</tr>
<tr>
<td>4.4</td>
<td>Statistical comparison of the mean internal and external wing-surface temperatures in winter- and summer-adapted, male <em>N. gouldi</em></td>
<td>120</td>
</tr>
<tr>
<td>4.5</td>
<td>Statistical comparison of the mean interscapular temperature in winter- and summer-adapted, male <em>N. gouldi</em></td>
<td>121</td>
</tr>
<tr>
<td>4.6</td>
<td>Statistical comparison of the mean chest temperature in winter- and summer-adapted male <em>N. gouldi</em></td>
<td>122</td>
</tr>
<tr>
<td>4.7</td>
<td>Statistical comparison of the mean mid-dorsal temperature in winter- and summer-adapted, male <em>N. gouldi</em></td>
<td>123</td>
</tr>
</tbody>
</table>
Table 4.8  Statistical comparison of the mean internal (ventral) wing-surface temperature in winter- and summer-adapted, male *N. gouldi*  124

Table 4.9  Statistical comparison of the mean external (dorsal) wing-surface temperature in winter- and summer-adapted, male *N. gouldi*  125

Table 5.1  Insulative values of male and female *N. gouldi* furs in summer and winter  136

Table 6.1  Body surface areas for male and female *N. gouldi*  179

Table 6.2  Heat loss from the three main body divisions: thorax, abdomen and naked membranes, compared for male *N. gouldi* in summer and winter  180

Table 6.3  'Effective' surface area of the naked membranes in summer- and winter-adapted, male, *N. gouldi*  181

Table 6.4  Comparison of heat loss through the furred body regions in *N. gouldi* males in summer and winter  182

Table 6.5  The equations used to calculate mean skin temperature based on the proportionality of 'effective' membrane surface area and actual furred surface area  183
Table 6.6  Comparison of mean skin temperatures and mean rectal temperatures of male *N. gouldi* in summer and winter  184

Table 6.7  Comparison of whole-animal rates of thermal conductance in summer and winter-adapted *N. gouldi*  185

Table 6.8  Whole-animal rates of thermal conductance for male and female *N. gouldi* in summer and winter calculated in surface-area specific terms  186

Table 7.1  Comparison of the mean body weight, minimum metabolism and areas under the $\dot{V}O_2$ and $T_R$ curves in male *N. gouldi* in spring and following mid-winter warm-acclimation  211

Table 7.2  Comparison of the mean body weight, minimum metabolism and areas under the $\dot{V}O_2$ and $T_R$ curves in female *N. gouldi* in spring and following mid-winter warm-acclimation  212

Table 7.3  Metabolic Q10's for *N. gouldi* in spring and following mid-winter warm-acclimation  213

Table 7.4  Statistical comparison of the mean interscapular temperature in winter, summer and mid-winter, warm-acclimated, male *N. gouldi*  215
Table 7.5  Statistical comparison of the mean chest temperature in winter, summer and mid-winter, warm-acclimated, male \textit{N. gouldi}  
216

Table 7.6  Statistical comparison of the mean mid-dorsal temperature in winter, summer and mid-winter, warm-acclimated male \textit{N. gouldi}  
217

Table 7.7  Statistical comparison of the mean internal wing-surface temperature in winter, summer and mid-winter, warm-acclimated, male \textit{N. gouldi}  
218

Table 7.8  Statistical comparison of the mean external wing-surface temperature in winter, summer and mid-winter, warm-acclimated, male \textit{N. gouldi}  
219

Table 7.9  The mean temperatures of body regions in endothermic, male \textit{N. gouldi} at ambient temperatures of 35, 25, 15 and 5°C following mid-winter, warm-acclimation  
220

Table 8.1  The incidence of torpor in well-fed \textit{N. gouldi} exposed to ambient temperatures less than 20°C  
264

Table 8.2  Details of torpor entry and arousal in male and female \textit{N. gouldi} in winter and summer  
265
Table 9.1  Whole-animal rates of thermal conductance for male and female *N. gouldi* in summer and winter  302

Table 9.2  The numbers of adult male and female *N. gouldi*, *N. geoffroyi*, *Chalinolobus morio*, *Eptesicus sagittula* and *E. regulus* caught during the active and hibernation seasons  303

Table 2.2  The body weight and total surface area of vespertilionids used in the regression presented graphically in Fig. I.  330
LIST OF DEFINITIONS

Acclimation*: A physiological change occurring within the lifetime of an organism, which reduces the strain caused by experimentally induced stressful changes in particular climatic factors.

Acclimatization*: A physiological change occurring within the lifetime of an organism which reduces the strain caused by stressful changes in the natural climate.

Adaptation*: A change which reduces the physiological strain produced by a stressful component of the total environment. This change may occur within the lifetime of an organism (phenotypic) or be the result of genetic selection in a species or subspecies (genotypic). Used here in the phenotypic sense and interchangeably with acclimatization.

Endothermy: Used interchangeably with homeothermy and normothermy; meaning the pattern of thermoregulation in which body core temperature varies little relative to environmental temperature and body heat is produced by endogenous sources.
Heterothermy:

see Endothermy.

Normothermy:

Minimum Metabolic Rate:
Can be considered equivalent to basal or standard metabolic rate in theory although in practice MMR may be less than the others because of the method of determination (refer Fig. 3.3). Minimum metabolic rate is the rate of heat production in an awake, resting endotherm which is post-absorptive and exposed to thermal-neutral temperature.

Lability:

Variability.

Hibernation:
A prolonged state of inactivity and reduced responsiveness to stimuli concomitant with a reduction in metabolism and body temperature. Used in this work to infer winter torpor as distinct from short-term diurnal or daily torpor in warmer seasons.

Minimum Metabolic Rate:
Used here only in the sense of the animal tolerating some body parts being maintained at less than core or deep body temperature.
Oxy-Caloric Equivalent: The amount of thermal energy (in calories) produced from the oxidative metabolism of a specified volume of oxygen.

Thermal-neutral Zone*: The range of ambient temperature within which metabolic rate is at a minimum, and within which temperature regulation is achieved by nonevaporative physical processes alone. The expression 'at thermal-neutrality' is used and means within the thermal-neutral zone.

Thermogenesis: Heat production; may be physical or mechanical when heat is produced by shivering or muscle activity associated with movement or can be chemical when aerobic metabolism yields heat.

Torpor: A state of inactivity and reduced responsiveness to stimuli concomitant with a reduction in metabolism and body temperature (see Hibernation for the distinction between winter torpor and daily torpor).

*Definitions taken from Bligh and Johnson (1973).
LIST OF ABBREVIATIONS

\( \delta \)  
- male

\( \varphi \)  
- female

1\(^\circ\), 2\(^\circ\), 3\(^\circ\)  
- primary, secondary, etc

\( T_A \)  
- ambient temperature

\( T_R \)  
- rectal temperature

\( T_{R-T_A} \)  
- difference between rectal and ambient temperature

\( T_B \)  
- body temperature

\( T_{Sk} \)  
- subcutaneous skin temperature

\( T_{Th} \)  
- subcutaneous thorax temperature

\( T_{Ab} \)  
- subcutaneous abdomen temperature

\( T_M \)  
- naked membrane temperature

\( \bar{T}_{Sk}, \bar{T}_{Th} \)  
- mean skin temperature, mean thorax temperature

\( \bar{T}_{Ab}, \bar{T}_M \)  
- temperature, etc

\( \dot{V}O_2 \)  
- instantaneous rate of oxygen consumption

\( O_2 \)  
- oxygen

\( STP \)  
- standard temperature and pressure

\( BMR \)  
- basal metabolic rate

\( MMR \)  
- minimum metabolic rate

\( Q_{10} \)  
- change in \( \dot{V}O_2 \) with a 10 degree shift in ambient temperature

\( RQ \)  
- ratio of oxygen consumed to carbon dioxide produced

\( TNZ \)  
- thermal-neutral zone

\( C \)  
- rate of thermal conductance

\( K \)  
- thermal conductivity constant

\( HS \)  
- rate of heat storage

\( I \)  
- insulation

\( Q/T \)  
- heat flow per unit time

\( EHL \)  
- evaporative heat loss
S  surface area of the insulating layer
L  thickness of the insulating layer
W  watts
°C  degrees Celcius
%  per cent
g  grams
µm  micrometres
mm  millimetres
cm  centimetres
m  metres
km  kilometres
mls  millilitres
hr  hours
N  number of animals
n  number of observations
±  plus or minus
s.d. or SD  standard deviation
ANOVA  analysis of variance
R²  correlation coefficient
F  F-value
P  probability
DF  degrees of freedom
χ²  chi-square value
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Chapter One

GENERAL INTRODUCTION
1.1 GENERAL INTRODUCTION

The selective pressures by which constant, regulated body temperature evolved are still hotly debated, but the success of the endothermic adaptation is beyond question. A critical component of this success has been the capacity of the endothermic system to adjust or adapt to suit changing environmental demands. Ecologists and physiologists have long been intrigued by the behavioural and physiological responses of endotherms to environmental stress, and most especially, by how they cope with the combined effects of cold environmental temperature and food shortage.

Although a successful adaptation to the thermal rigours of the environment, endothermy is energetically expensive and requires a relatively constant supply of food. For temperate-zone endotherms, and especially the specialist insectivores, the several colder months of the year which necessitate an increase in metabolized thermal energy are coincident with substantial reductions in the amount of available food. This energetic dilemma has been overcome by the temperate-zone species in a number of ways, employing a suite of both physiological and behavioural modifications to the summer patterns.

Many temperate-zone birds, and some mammals, migrate long distances to ensure the annual continuity of food resources in more equable environments. Equally, short-range migrations can serve to moderate the severity of the winter food scarcity if more suitable microclimates can be found. For those animals overwintering in cold climates, a food cache may serve to reduce
the time spent, outside the nest or burrow, exposed to the more thermally demanding environment. Accumulated insulative materials within the nest or burrow combined with the heat conserving effects of huddling with others may further reduce the thermal energy needs of the overwintering endotherm.

Physiological adaptation to winter may take the form of an enhanced capacity to produce metabolic heat to offset the greater rates of thermal loss or of an improved ability to retain body heat by improvements to the insulation of the peripheral body layers. Some homeotherms (= endotherms) hibernate to avoid the harsh winter conditions. The body's 'thermostat' is effectively turned down, the temperature falls to near that of the environment, and the animal survives on stored body fats.

The insectivorous bats are one group of small mammals which have successfully adapted to the environmental demands of the temperate climate. Despite their greater mobility than other small mammals, very few temperate-dwelling bats are migratory (Daan 1973; McNab 1982). Prolonged bouts of torpor are common during winter and in other seasons homeothermy is interspersed with shorter, diurnal torpor bouts.

The bats have invaded a wide spectrum of ecological situations since they evolved in the tropics some 60 million years ago. The result of this widespread radiation has been a diversity of morphological, behavioural and dietary variations far in advance of any other mammalian
order. McNab (1982) points out that the bats are ".... unparalleled subjects ...." for studies of physiological ecology because they offer an opportunity to examine the influence of food habits and body size on the energetics of endothermy. Apart from insects and fruit, bat species are known to consume nectar, pollen, small vertebrates, blood, fish and other bats. The smallest insectivorous bats weigh only two or three grams and the largest fruit bats have body weights in excess of one kilogram (McNab 1982).

Despite the widespread distribution and abundance of the Chiroptera comparatively little is known about the thermoregulatory adaptations which occurred to enable the radiation of the bats into the colder temperate zones and which today permit the extant species to overwinter there.

The subject of this dissertation is the thermal biology of Nyctophilus gouldi Tomes 1858 (frontispiece); a small, insectivorous, tree-dwelling, temperate-zone bat. The primary aim of the study was to describe how the thermal 'balance-sheet' of the species changed during an annual seasonal cycle. The study did not attempt to compile a complete thermal energy budget but rather to examine the way physiological and behavioural adaptations modify the rates of heat production and loss during the seasonal transition from winter to summer. It would be both naïve and narrow-minded to examine the question of thermal adaptation without giving consideration to the seasonal changes in the ecology as well as the physiology of the species. Consistent with this view, the study undertaken included both a field ecology
component and a laboratory examination of the seasonal changes in the species' thermoregulatory physiology.

Before proceeding to describe the methods used and results obtained, it is worth spending a few moments to consider what it means to be a small, insectivorous, tree-dwelling bat resident in a temperate-zone.

In homeotherms, the ratio of heat dissipating surface area to heat producing body mass increases with the reduction in body size. A direct consequence of this is that energy demands, relative to body weight, also increase as body size decreases. For the bats this thermal/energy 'problem' is exacerbated by the greater energy demands of aerial feeding (Bartholomew 1972) and the addition of the flight membranes to the surface area of the body. The wings, tail and sometimes the ear membranes are large, naked and highly vascularized, potentially offering very efficient heat dissipating surfaces.

Because of their flight membranes, bats have total surface areas approximately six times greater than 'wing-less' mammals of the same body weight (see Section 6.3.1 and Appendix I). A bat weighing 11 grams, such as Nyctophilus gouldi, has an actual (as opposed to effective) surface area equivalent to that of a 127.3 gram terrestrial mammal but has only 8.6 per cent of the body mass. Understandably, the bats have rates of thermal conductance which are greater than non-volant, homeotherms of equivalent
weight, although less than expected from surface area to volume ratio alone (Bradley and Deavers 1980). This thermal economy is reflected in the weight-specific basal metabolic rates (BMR) of the insectivorous bats which fall below the general mammalian Kleiber curve and seem to result from the regulation of body temperatures a few degrees less than the normal homeothermic level (Henshaw 1970; McNab 1982).

The thermoregulatory patterns of the bats are extremely varied (Stones and Wiebers 1965; McManus and Nellis 1967; Henshaw 1970; Lyman 1970; McNab 1982) and reflect differences in food habit, body size and latitude. At all latitudes the insectivorous bats maintain lower body temperatures, when subjected to a cooling environment, than do those species with other food habits. The temperate-zone insectivorous bats are generally considered to be poor thermoregulators; species showing little resistance to entry into torpor when inactive and exposed to moderately cool environments (i.e. less than 20°C) (Henshaw 1970; Dwyer 1971; McNab 1982). Many of the large, tropical fruit and nectar eaters are constant homeotherms although as body size decreases there is reduced ability to regulate constant body temperature in these groups also.

The thermoregulatory strategies of the bats are not simply defined. Within the literature there is considerable speculation and conflicting interpretation of the observed temperature regulation patterns. Behavioural factors can modify the thermal requirements of a bat irrespective of dietary preferences, body size or latitude.
The ability to fly enables the bats to rapidly alter their micro or macroenvironment. Precise microclimate selection through daily and seasonal movements within and between roosts can greatly reduce the energetic expense of temperature regulation (Pagels 1975). By selecting summer roosts, with temperatures near thermal-neutraliy, pregnant and lactating females can minimize their own requirements for thermal energy, permitting them to cope better with the demands placed upon them by the young (Studier and O'Farrell 1972).

Clustering behaviour can also greatly reduce the thermal burden placed upon the individual bat. Clustering has been observed in many bat species and has the effect of warming and humidifying the microenvironment, thus reducing the losses of body heat and water (Rasweiler 1973; Howell 1976). Animals clustered together are buffered from minor temperature fluctuations and are able to maintain higher body temperatures at reduced metabolic cost. Herreid (1967) found that metabolic rate was a function of cluster size: the larger the cluster, the lower the metabolic rate of each individual.

Careful consideration must be given to the bat's reproductive condition and nutritional status when interpreting the thermoregulatory pattern observed. The energy demands on the female during a particular phase of the reproductive cycle can determine the manner of temperature regulation. Studier and O'Farrell (1972) found that female *Myotis lucifugus* and *M. thysanodes* were more prone to enter torpor during the demanding lactation phase than at any other time. Equally, many workers have
shown that food deprivation induces torpor in bats (Herreid 1963a; Kulzer 1965; Arata and Jones 1967). McNab (1982) sounds a warning to those investigating the temperature regulation of bats, suggesting that consideration should be given to the nutritional state of the animal when interpreting laboratory obtained data. Not only can poor nutritional condition increase the incidence of torpor in bats but the provision of ample food, in captivity, may enhance the ability to maintain constant high body temperatures giving an equally distorted appreciation of the natural thermoregulatory pattern.

No previous studies of the type presented here have been published. A number of workers have reported a natural seasonal acclimatization in temperate-zone bats (Pohl 1961; Menaker 1962; Mejsnar and Jansky 1967; Studier and O'Farrell 1972). However, these studies have given little consideration to factors other than the incidence of torpor and the metabolic requirements of temperature regulation. The metabolic costs of temperature regulation have been reported to undergo a seasonal change in temperate-zone bats although conflicting results have been published. Menaker (1962) demonstrated that bats capable of spontaneous arousal from winter torpor were incapable of the same response in summer and further, that the effect could be mimiced by cold-acclimating 'summer' bats and warm-acclimating 'winter' bats. Holyoak and Stones (1971) like-wise concluded that winter adaptation took the form of improved thermogenic ability in the same species, Myotis
In contrast, Mejsnar and Jansky (1967) reported a seasonal insulative adaption in *M. myotis*. This finding was supported by Shump and Shump (1980) who found that the insulative value of the pelts of *M. lucifugus*, *M. keenii* and *Eptesicus fuscus* improves by 26 per cent in winter.

Most of the studies which have examined temperature regulation in temperate-zone bats have concentrated upon the more readily obtained and observed cave-dwelling species (Dwyer 1964; Studier and O'Farrell 1972; Funakoshi and Uchida 1978). "Little is known of the physiology and ecology of tree bats" (McNab 1982). It is not known whether the thermoregulatory abilities of the temperate-zone tree-dwelling bats are similar to those of the cave-dwellers. The tree-roost does not possess the thermal inertia of the cave and fluctuating microclimate within the tree hollow or bark roost may require more refined or proficient thermoregulatory skills than have been exhibited by the temperate-zone cave inhabitants. Equally, the tree-roost cannot accommodate the large aggregations of bats, commonly found in caves, effectively limiting the use of clustering as a heat conservation and environmental buffering mechanism.

This study therefore gives an opportunity not only to examine the questions of thermal adaptation in a temperate-zone bat but also to compare the thermoregulatory abilities of tree-dwelling and cave-dwelling species. The study reported here consisted of four parts: one field and three laboratory phases.
The primary objective of the field study (Chapter Two) was to establish whether *Nyctophilus gouldi* hibernated or migrated to avoid the winter conditions of temperate Australia. Given the very low incidence of migration in temperate-zone species (Daan 1973) hibernation was the anticipated finding. The corollary to the primary field aim was therefore to determine the duration and timing of the hibernation season. It was essential for subsequent laboratory work that the period of winter adaptation be accurately defined in a free-living population.

Giving consideration to the importance of reproductive condition to the thermoregulatory patterns of the microchiroptera, the field study also aimed to describe the breeding cycle of *N. gouldi*, specifically pinpointing the critical phases of pregnancy and lactation in females.

The accumulation of body fats in autumn precedes entry into winter torpor in most hibernators and may alter temperature regulation, and therefore activity patterns of the temperate-zone bats (Ewing, Studier and O'Farrell 1970). The final objective of the ecological phase of this project was to describe the annual body weight cycle of *N. gouldi* defining the periods of fat deposition and use.

Although it was considered desirable to locate and describe the typical roost selected by the species, such a pursuit was considered beyond the scope of this study.
The laboratory component of the study was divided into three stages. Stages one and two investigated the changes in metabolic heat production and loss (respectively) in *N. gouldi* undergoing the transition from winter to summer. These phases of the laboratory work were concerned only with the seasonal changes in the costs of endothermic regulation. The basic rationale for this approach was that any change in the rates of body heat production and loss would be more readily detected in the homeothermic rather than the torpid animal. Despite the fact that torpor may be the primary winter adaptation in the species, it would be a meaningless exercise to compare the cost of homeothermy in summer with the cost of torpor in winter if the intention is to detect forms of physiological adaptation other than hibernation.

Stage one of the laboratory work (Chapter Three), therefore, compared the metabolic costs of homeothermy in winter, spring and summer-adapted animals. Given that in an endotherm maintaining a constant body temperature, the rates of heat production and heat loss are equivalent, any seasonal change in the metabolic costs of homeothermy was taken as an indication of some form of thermal adaptation.

Stage two of the laboratory work aimed to determine the form of seasonal adaptation by comparing the insulative properties of the peripheral body layers in winter and summer. An insulative adaptation to winter may be the result of an improved capacity or tolerance for cooling the peripheral body tissues (Chapter Four), a thicker less-conductive fur (Chapter Five) or a combination of both these heat conserving factors. A winter escalation
in thermogenic capacity may be evidenced by warmer body extremities and greater rates of thermal transfer through the pelage.

In the third experimental stage (Chapter Seven) an attempt was made to mimic the natural seasonal transition from winter to summer by warm-acclimating animals in mid-winter. After three weeks of exposure to 22°C the metabolic costs of homeothermy and the regulated temperatures of the peripheral tissues were measured and compared with those of winter, spring and summer-adapted *N. gouldi*.

Throughout the laboratory investigation primary emphasis was placed upon the seasonal changes in the energetics of endothermic regulation. This is not to say that the importance of the torpid condition was ignored and Chapter Eight presents and considers the thermoregulatory ramifications of these findings.
Chapter Two

THE DESCRIPTION, ECOLOGY AND REPRODUCTIVE BIOLOGY OF NYCTOPHILUS GOULDII

2.1 Introduction

2.2 Species Description

2.3 Ecology of Nyctophilus gouldii
   2.3.1 Introduction
   2.3.2 Methods
   2.3.3 Results
      Annual Activity Pattern
      Annual Body Weight and Fat Cycles
   2.3.4 Discussion

2.4 Reproductive Biology of Nyctophilus gouldii
   2.4.1 Introduction
   2.4.2 Methods
   2.4.3 Results
      Males
      Females
   2.4.4 Discussion
2.1 INTRODUCTION

The ecology of Australian bats is generally not well described. The one notable exception to this is the work of Dwyer (1964 and 1968 for example) on the cave-dwelling *Miniopterus schreibersii*. In contrast, to the cave-dwellers, tree-dwelling species pose a much greater problem to the researcher. They are solitary or form relatively small groups and their roosts are generally difficult to locate. The advent of the collapsible, light-weight and very efficient harp trap (Plate 2.1) (Tidemann and Woodside 1978), has greatly improved the ability to trap forest-dwelling species, enabling detailed ecological studies to be undertaken.

Prior to this study, very little was known about the biology of *Nyctophilus gouldi*. The species was known to be restricted to the sub-coastal fringes of south-eastern (Hall and Richards 1979) and south-western Australia (Kitchener and Vicker 1981) (Fig. 2.1). A known tree-dweller, *N. gouldi* had been observed to glean insects from foliage close to the ground and even to land on the forest floor to take prey items (Tidemann, pers. comm.).

A more detailed understanding of the species' ecology and reproductive biology was necessary if meaningful conclusions were to be drawn from the laboratory findings. The field study undertaken with this intention is described in Sections 2.3 and 2.4.
2.2 SPECIES DESCRIPTION

The genus *Nyctophilus* belongs to the sub-family Nyctophilinae within the family Vespertilionidae. The vespertilionids are the largest and most cosmopolitan family within the sub-order Microchiroptera. There are 15 families in the Microchiroptera but only three of the insectivorous families: the Vespertilionidae, the Molossidae and the Rhinolophidae, have successfully adapted to the temperate zones.

The genus *Nyctophilus*, which contains six species, is considered to be "probably" endemic in Australasia (Hall 1981). Within eastern Australia, the four *Nyctophilus* species are *N. bifax* Thomas 1915, *N. geoffroyi* Leach 1821, *N. gouldi* Tomes 1858 and *N. timoriensis* Geoffroy 1806 (Hall and Richards 1979). The major distinguishing morphological features of bats in this genus are the very large, rounded ears and small simple nose-leaf (frontispiece). The large ears are joined "... by a band uniting their inner margins across the forehead ..." (Wood Jones 1923). The tragus is short and triangular in shape. The ventral pelage is light grey in colour whilst the dorsal fur is slightly darker.

*Nyctophilus gouldi* is intermediate in body size between *N. geoffroyi* and *N. timoriensis*. Adult males weigh between 10 and 13 grams. Females are one or two grams heavier. Forearm lengths for adult *N. gouldi* range from 39 to 45 mm. Females have forearms one to two millimetres longer than males.
2.3 ECOLOGY OF NYCTOPHILUS GOULDI

2.3.1 Introduction

A number of environmental and social factors can influence the activity patterns of the temperate-zone, insectivorous bats (Erkert 1982). The timing of emergence, the time spent foraging and whether emergence occurs at all can be affected by environmental temperature, light level, wind-strength and the intensity of rainfall. Of primary importance, and in part, controlled by these factors, is the amount of available insect food.

The foraging activity of near-term female bats may decline in response to the bulk and weight of the foetus (Ransome 1973) and then precipitously increase to meet the energetic demands of lactation. During late summer, when adipose tissue is accumulated in preparation for the hibernation season, the time spent foraging may decrease and the duration of torpor bouts increase (Twente 1955; Krzanowski 1961) so that the "... harvested energy ..." can be converted into body fats (McNab 1982).

Social factors can also affect the activity patterns of hibernating bats. In species which cluster tightly together, the arousal from torpor by one member of the group may sufficiently disturb the others to bring about their rewarming. A number of authors have reported that males are more active than females during the hibernation season (McNab 1974; Tuttle 1976; Tidemann 1982) although the precise reasons for this has not been conclusively established.
The primary aims of the field study described here were presented in detail in Chapter One (page nine). Briefly re-stated these aims were:

1. To define the annual activity pattern of *N. gouldi*. Did the species migrate or hibernate in winter? If hibernation occurred, what was the timing and duration of the inactivity phase?
2. To describe the breeding cycle of *N. gouldi* noting the timing of the pregnancy and lactation phases (Section 2.4) and
3. To determine the annual body weight cycle with special reference to the times of fat deposition and use.

2.3.2 Methods: Ecology of *Nyctophilus gouldi*

The forest-dwelling bat community monitored during this study is located near Bull's Head (35°24'S, 148°50'E) in the Australian Capital Territory, 40 kilometres from Canberra (Fig. 2.1). Between January 1979 and March 1983, bats were trapped every three to four weeks using harp traps (Tidemann and Woodside 1978). From March 1980 each individual *N. gouldi* was fore-arm banded prior to release with a size three aluminium bird band. Suitable locations along the firetrails (Plate 2.1) and over ponds (Plate 2.2) constituted 26 regular trap-sites. Seven to nine sites were trapped on any one night.
The study area ranged in elevation from 800 (trap-site 26, Fig. 2.2) to 1300 metres (trap-site 16) with slopes of predominantly south-eastern aspect. The forest is mature, wet sclerophyll with a number of deep, wet gullies. Snow was common on the higher slopes in winter.

To reduce bias in trap placement during trap sessions, the study area was divided into ten, one kilometre squares and no more than one trap placed within each square per night (Fig. 2.2). One of two pond trap-sites was used in every trap session. Bats were removed from traps after dawn and weighed using a Pesola 0 to 50 gram balance. The length of fore-arms were measured to the nearest 0.1 mm with dial calipers and adults were recognised by complete ossification of the digital epiphyses of the wing. Reproductive status was also assessed at this time (Section 2.4.2).

In order to detect the movements of banded bats out of the area under observation an additional one or two traps were placed across firetrails at distances ranging from 0.5 to 20 kilometres from the perimeter of the study area during each trap session.

Meteorological information was provided by CSIRO, Division of Forest Research, which maintains recording stations at Bull’s Head (elevation 1354 metres) and Lees Creek Nursery (elevation 750 metres). The Bull’s Head recording site is less than two kilometres north of the study area and the Lees Creek Nursery site, six kilometres north-east.
Monthly insect abundance data was provided by C.R. Dickman. To gain an index of insect availability, he exposed eight to ten "Aeroxon" sticky strips for each month over a three year period (1979 to 1981). These were hung from foliage approximately two metres above the ground. For each month a mean figure for insect numbers per strip was calculated. Dickman's study area was approximately five kilometres north-east of my study area at elevations of 750 to 800 metres.

2.3.3 Results: Ecology of *Nyctophilus gouldi*

*Annual Activity Pattern*

In four years and three months of trapping, 245 adult and 74 juvenile *N. gouldi* were trapped and released within the study area in 371 trap-nights. Table 2.1 summarizes these data.

The activity of *N. gouldi* is expressed as the number caught per trap-night for each month. The period of least activity, from April to September, coincided with the time of coldest ambient temperatures and reduced insect numbers (Fig. 2.3). Snow was common in winter and prevented trapping in July of each year, except in 1982. The snow rarely persisted for more than four weeks and was restricted to the slopes above 1000 metres.

The activity of *N. gouldi* was highly seasonal. In a total of 109 trap-nights for the months of April, May,
June, July, August and September, only 16 *N. gouldi* were trapped (ie. 0.15 per trap-night). In the remaining six months 262 trap-nights yielded 303 *N. gouldi* at a trap-rate of 1.16 per trap-night.

Because of the low capture rate of *N. gouldi* and the marked seasonality of the species activity, the monthly trap-success data for 1979, 1980, 1981, 1982 and January, February and March of 1983 were combined. A comparison of the annual pattern of adult male and female activity was done by contingency table analysis. To satisfy the conditions of the contingency table and avoid biasing the chi-square value, the data for the months of April, May, June, July, August and September were combined. Zar (1974) states that this is an acceptable practice when repetition of the experiment with larger sample sizes is not possible.

The annual activity patterns of adult males and females were different from one another $\chi^2[1,6] = 20.3; p<0.005)$. The peak in female activity (0.79 per trap-night) was in January (Fig. 2.3) when most females were lactating. The greatest number of males caught per trap-night was in October (0.64) at the start of the active season.

Males may be more active than females during the hibernation season. Ten adult males and five adult females were trapped in the six months from April to September (Table 2.2). For three of these months (June, July and August) no females were trapped. July was the only month when males were not trapped.
Seven vespertilionids other than *N. gouldi* were trapped in the study area. These were *Nyctophilus geoffroyi*, *Eptesicus sagittula*, *E. regulus*, *E. vulturinus*, *Chalinolobus morio*, *C. gouldii* and *Pipistrellus tasmaniensis*. The smallest of these was *Eptesicus vulturinus*, weighing only 3 to 4 grams and the largest *Pipistrellus tasmaniensis* at 19 to 20 grams. For the species trapped in greater numbers than *N. gouldi*, more males than females were also trapped during the six months of reduced activity (Table 2.2).

The numbers of each of the species banded and retrapped during the study is given in Table 2.3. Comparatively few of the *Nyctophilus* species were retrapped. For *Nyctophilus gouldi* and *N. geoffroyi* the number of banded animals retrapped on at least one occasion was 5.5 and 2.8 per cent, respectively. Except for *Pipistrellus tasmaniensis* and *Chalinobolus gouldii* (the two largest species) the rate of recapture in the other species was between ten and twenty per cent (Table 2.3). This difference in the recapture rate of *Nyctophilus* species and the others is considered in greater detail in section 2.3.4.

Of the eight *N. gouldi* retrapped, seven had been banded at trap-site 11; Moonlight Hollow pond (Plate 2.2) and one at trap-site 9 (Fig. 2.2). Six of those banded at trap-site 11 were recaptured there and the seventh animal was retrapped at trap-site 16, 0.9 kilometres away (Fig. 2.2). The *N. gouldi* banded at trap-site 9 was retrapped at trap-site 11, a distance of only 0.4 kilometres.
During the banding study (March 1980 to March 1983) most *N. gouldi* were trapped at the two permanent ponds (trap-sites 11 and 26). Forty-seven trap-nights at the two ponds caught 112 *N. gouldi* (ie. 2.38 per trap-night) or 67.9 per cent of the total number trapped. Fifty-three animals were trapped at the remaining 24 road-sites in 229 trap-nights (0.23 per trap-night). The discrepancy between this total of animals trapped during the banding study (165) and that reported in Table 2.3 (145 + 8 retraps = 153) is the result of removing animals from the study area to maintain the numbers in the captive colony (Section 3.2.1).

By grouping the data for trap-sites, other than the ponds, into 100 metre elevation classes it was possible to test for favoured foraging spaces within the study area (Fig. 2.4). There was no significant difference between the mean numbers caught per trap-night at each elevation class (one-way ANOVA, $F = 0.43, p = 0.26$) although more were caught at those sites below 1000 metres than anywhere else. The peripheral or drift trapping at sites below 1000 metres consistently caught more of the two *Nyctophilus* species than at the higher elevation sites.

No *N. gouldi* banded within the study area were caught during the 69 trap-nights of peripheral trapping, suggesting that the species is relatively sedentary. This observation is reinforced by the findings for the other species present within the study area. Of the 1320 banded, only two were re-trapped off the study grid. One *Chalinolobus morio* was re-trapped 0.5 kilometres away
and the other 12.5 kilometres distant. Most bats (78.1%) were re-trapped within one kilometre of where they were last handled (Table 2.4) and 32.9 per cent were re-trapped at the site where they were banded or recaught. The mean distance travelled between retraps for all species was less than one kilometre and for *N. gouldi* it was only 0.16 kilometres (+ 0.33 (SD), n = 8) (Table 2.4).

**Annual Body Weight and Fat Cycles**

The highly seasonal activity pattern of *N. gouldi* made it difficult to describe the annual body weight cycle from field animals. To overcome the problem of reduced animal trappability for six months of the year, it was necessary to maintain a colony of *N. gouldi* in captivity. These animals were provided with plentiful food year-round and kept in an outdoor flight enclosure (Section 3.2.1). This enabled clearer definition of the annual body weight and body fat cycles. The topography of seasonal fat deposits is described in Section 4.3.1 (page 105).

The body weights of wild and captive *N. gouldi* increased as a result of body fat deposition in February and March (Fig. 2.5). From February through to August subcutaneous white adipose tissue was present on the dorsal and ventral abdominal surfaces and surrounding the base of the skull. During the hibernation season, white fat was also found encapsulating the ovaries. Very little or no subcutaneous white fat was observed in *N. gouldi* from September to January. Brown fat was always present in the superficial interscapular and jugular regions (Section 4.3.1).
Females in captivity reached maximum body weights of 16 to 18 grams during the latter stages of pregnancy in October and November (Section 2.4.3). In the field population, near-term females of this body weight were never caught, resulting in a much lower mean body weight value during these months (Fig. 2.5). Captive animals were on average one or two grams heavier than their free-living counterparts although a similar annual cycle of body weights was observed in both populations.

Post-hibernation body weights in the captive colony never reached the low levels of the field animals. The provision of ad libitum food throughout the year was probably responsible for this. In captive *N. gouldi*, there was little cessation of nightly feeding activity, even in winter, unless the weather was wet or windy. Despite this, the body weight, and by inference body fat levels, of captive *N. gouldi* declined throughout the hibernation season as observed in the free-living population.

Inwards (1984, pers. comm.) described the annual fat cycle for a captive colony of *N. gouldi* and his findings reinforced the body weight cycle presented above. The investigation done by Inwards was carried out in a separate part of the same holding cage used to house my experimental animals and maintenance conditions were identical for both populations. Using tritiated water to label body water, Inwards found a good relationship between the fat content of the animal and the relative volume of the body water pool.
Using the derived relationship and monthly measurements of the body water volume, Inwards was able to indirectly record the annual body fat cycle of male and female *Nyctophilus gouldi* (Fig. 2.6). This technique indicated that *N. gouldi* are leanest in January with fat contributing only 5-7 per cent of body mass. From February until April, fat is deposited and reaches a maximum in the latter month of between 17 and 23 per cent. Females deposit more fat in autumn than males. Throughout the hibernation season these body fat reserves are slowly used and achieve a level similar to the January base level in September.

2.3.4 Discussion: Ecology of *Nyctophilus gouldi*

The reduced numbers of *Nyctophilus gouldi* trapped in the wild from April to September suggests that substantial periods of torpor or hibernation occur in this species during the colder months. In a captive colony of *N. gouldi*, Tandy (1979) found that activity during "... winter was much reduced ...". Although not conclusively shown by this study it seems unlikely that *N. gouldi* migrates to lower elevations to avoid the winter food restriction.

*Nyctophilus gouldi* may be capable of short-range migrations to lower elevations, however, the slow and indirect flight style of the species would not facilitate lengthy migratory flights (Inwards and Phillips, in press). The poor rate of recapture of banded *N. gouldi* did little to assist in detecting the movements of the species.
within and out of the study area. For the other vespertilionids present in the Bull's Head community there were much clearer indications that individuals were moving little distance from a regular foraging area. The rates of recapture for these species were much higher than for *N. gouldi*, yet only two of 1320 banded animals were trapped outside the study area. Relatively sedentary behaviour was also indicated by the finding that almost one third (32.9%) of recaptures occurred at the site of previous encounter. The most convincing evidence that *N. gouldi* remains in the locality year-round was that animals were trapped in the study area during every month of the year except July; not an expected finding for a migratory species.

The low recapture rate for *N. gouldi* may indicate that they are not restricted to the trails and tracks when moving within the forest. Although the majority were trapped at the two water sites (67.9%), the road sites where they were encountered were usually within dense vegetation closely associated with streams (Plate 2.1). *N. gouldi* shows a close affinity with free-water, and it seems likely that the species forages along the wet gullies thus avoiding road trap-sites and reducing the chances of recapture.

The observation that males of a number of species were trapped more frequently than females during the hibernation season is consistent with the findings of Tidemann (1982) for *Eptesicus vulturinus*. Tidemann (1982)
found that male *E. vulturinus* are more active than females throughout the winter and suggests they are seeking out females with which to copulate. In the captive colony of *N. gouldi* this would seem to have been the case also. Copulations were observed in winter and males were seen mating with torpid females. Inwards (1984) found indirect evidence that captive male *N. gouldi* were more active than females during the first half of the hibernation season. The water turnover of males (estimated using the tritiated water method) was significantly greater than females in April, May and June. Because so few *N. gouldi* were trapped during the hibernation season it was not possible to confirm that the sexual bias in the activity of the captive animals was also present in free-living bats. Although more adult males than females were caught during the hibernation season (10 males, 5 females) it is necessary to assume equal trappability of the sexes, equivalence of each trap-site and a sex ratio of one to one for this finding to be considered meaningful.

In the absence of an appropriate method for naturally simulating the seasonal fluctuations in food supply, captive bats were provided with *ad libitum* food year round. Although the activity of these animals was reduced during winter, in general, nightly feeding activity continued. As a result captive animals were always heavier than free-living bats. Presumably this is a reflection not only of the freely available food but also of the reduced foraging effort required by captive bats.
Despite the provision of plentiful food, the body weight cycles of captive and field populations followed a similar pattern. Late summer and autumn increases in body weight as a result of fat deposition were in synchrony and similar pre-hibernation body weights were achieved in both populations. An exception to this were two first-year males trapped in May. These animals were excluded from the field body weight cycle because they were unusually light and not considered representative. One possible explanation for the low body weight of these bats is that weaning may not have been completed sufficiently early in the summer to allow time for body fat accumulation to the level of the other animals. A similar suggestion was made by Humphrey, Richter and Cope (1977) for the tree-dwelling *Myotis sodalis*. These authors observed that an unusually cool summer resulted in a two week delay in the first flights of juveniles and proposed that fat deposition and winter survival may have been affected.
2.4 REPRODUCTIVE BIOLOGY OF NYCTOPHILUS GOULDI

2.4.1 Introduction

The reproductive cycles of the hibernating bats are greatly influenced by winter dormancy (Wimsatt 1969; Racey 1973; Gustafson 1979; Oxberry 1979). Monoestry and temporal asynchrony of copulation and ovulation have resulted from winter insect scarcity and associated hibernation.

Two reproductive patterns have been described in the hibernating bats: delayed fertilisation and delayed implantation (Gustafson 1979). In most temperate-zone vespertilionids and some rhinolophids, spermatogenesis and mating occur before winter although some copulation occurs during the hibernation season and early spring. Ovulation and pregnancy commence upon arousal from hibernation (Wimsatt 1969). Males or females or both may store spermatozoa over the winter period (Racey 1982). In European Miniopterinae, mating and ovulation occur in autumn and implantation of the blastocyst is delayed until spring (Wimsatt 1969). Miniopterus schreibersii in Australia differs from this pattern in that the blastocyst does not remain free throughout the winter but is implanted and commences slow development before arousal from winter torpor (Wallace 1978).

Monoestry is not the rule for the Vespertilionidae. Local environmental factors such as food supply, rainfall and ambient temperature can influence the timing and frequency of the reproductive effort. In neo-tropical
species, polyoestry and year-round breeding is common (Wilson and Findley 1970) although monoestry and sperm storage is sometimes observed (Gopalakrishna and Madhavan 1971; Medway 1972; Krishna and Dominic 1978).

Reported here is a brief review of the breeding cycle of *N. gouldi*. For a more detailed account of the reproductive events see Phillips and Inwards (in press). In this section only those observations relevant to the thesis topic are presented.

The reproductive status of the wild population (Section 2.3) was monitored and these findings supplemented with observations of the captive colony maintained throughout the study (Section 3.2). At key times in the reproductive cycle, animals were sacrificed to permit histological confirmation of overt reproductive condition.

2.4.2 Methods: Breeding cycle of *Nyctophilus gouldi*

The reproductive status of adult animals was classified in the field using a one to four numerical code. In females, the four reproductive classes were:

1. Quiescent, that is, not obviously pregnant, not lactating and not post-lactating;
2. Pregnant, as determined by palpation of the lower abdomen and increased body weight;
3. Lactating, when the nipples were enlarged and milk visible in the mammary glands;
4. Post-lactating, when regrowth of fur around the nipples had commenced and the nipples had decreased in size.
The degree of enlargement of the testes and epididymides was used as a comparative measure of the reproductive status of males. The testes and epididymides of *N. gouldi* are scrotal at all times of the year.

The categories used to describe male reproductive condition in the field were:

1. No obvious testicular hypertrophy or epididymal enlargement;
2. Minor hypertrophy of the testes;
3. Maximum (or near maximum) enlargement of the testes, no epididymal distension;
4. Regressed testes, distended epididymides.

The majority of animals used for histological examination of the reproductive tract were taken from the captive colony. For a detailed description of the methods used to maintain *N. gouldi* in captivity see Section 3.2.1 (page 61). Some additional animals were collected from areas immediately adjacent to the field study area. These specimens were transported to the laboratory alive and killed by cervical dislocation within 24-48 hours of capture. Specimens were also taken from the museum collection of CSIRO, Division of Wildlife and Rangelands Research, Canberra. Museum samples were selected from areas within a 200 kilometre radius of Canberra.

In total, 21 male and 13 female tracts were microscopically examined. Twelve of these (6 male, 6 female)
were from the museum collection, 18 (11 male, 7 female) were from the captive population and four (males) came from near the study area.

Reproductive tracts were fixed in aqueous Bouins solution for 48 hours, transferred through a series of alcohol washes and finally embedded in paraffin wax. Male organs were transversely sectioned (7µm) and every tenth section retained. Female tracts were serially sectioned along a longitudinal axis (8µm). Sections were stained with haematoxylin (Mayer's Acid haemalum) and counterstained with 0.5% alcoholic eosin. The presence or absence of spermatozoa in the male and female tracts was determined by microscopic examination. In the female case, the location of spermatozoa within the tract and whether antral follicles or corpora lutea were present in the ovaries was also noted.

2.4.3 Results: Breeding cycle of Nyctophilus gouldi

Between March 1980 and March 1983 the overt reproductive condition of 89 adult male and 131 adult female N. gouldi was assessed. Figure 2.7 presents these data. Males

The onset of testicular enlargement coincident with spermatogenic activity was not synchronized within the field population. Two of twenty-one males in October had minor enlargement of the testes. In the remaining 19 animals, no testicular hypertrophy was apparent (Fig. 2.7). Only six males were trapped in November: of these, the
testes were apparently quiescent in four and showed minor enlargement in two. December was the first month in which males were caught with near maximally enlarged testes ($n = 3$). The degree of testicular enlargement was not uniform within the population at this time: two males showed no signs of testis enlargement and a further five had only minor hypertrophy.

In January, 10 of 17 males had maximally enlarged testes and in the remaining seven animals the epididymides were distended (Fig. 2.7). From January onwards, the majority of males trapped had regressed testes and enlarged epididymides (February, 12 of 17 and March 8 out of 13). Although very few *N. gouldi* were trapped after March in each year, this was the last month when males with maximally enlarged testes were trapped.

Mature spermatozoa were present in the lumen of the seminiferous tubules (Plate 2.3A) and the caudal portion of the epididymides of two males in March. From observations of free-living animals, sperm were stored in the epididymides from January through to September, although only three males were trapped after April. Histologically examined epididymides of two males in May, four in June, three in July and two in August contained densely packed spermatozoa (Plate 2.3B). There were no sperm in the epididymides of two males in October, four in November and two in December. In November and December the initial stages of spermatogenic activity were observed in the testes of all animals.
Females

Visibly pregnant females (reproductive stage two) were first trapped in October (Fig. 2.7) \((n = 2)\). The remaining 17 females trapped in this month showed no overt signs of pregnancy. In November, nine females were trapped: seven of these were classified as pregnant. The first occurrence of lactating females in the population was in December (11 of 15). No pregnant \(N. gouldi\) were trapped in January; 29 were lactating; 25 post-lactating and two had apparently remained reproductively inactive for that breeding season. In February, only one of the 24 trapped was post-lactating and the remainder were reproductively quiescent.

Juvenile animals were first trapped in December, however, the greatest numbers were caught in January and February (Table 2.1). One female trapped in May did not have ossified wing epiphyses. No animals were classified as juvenile in spring indicating that the epiphyses are ossified in \(N. gouldi\) emerging from their first hibernation season. This was the case in captive animals also.

Male \(N. gouldi\) reach sexual maturity within 12 to 15 months of birth. If this were not the case, reproductively inactive animals should have been present in the population during the spring and summer. This was not generally the case; all males with ossified epiphyses caught after December were reproductively active (stage three or four). All females trapped in December were either pregnant or lactating indicating they are sexually
mature at seven to nine months of age. In January, two apparently reproductively inactive females were caught when all other (44) were lactating or post-lactating. These two females were classified as first year or yearling animals on the basis of lighter coloured ventral surface fur (Phillips and Inwards, unpublished findings). In the captive colony one first year female in 1979 and three in 1982 gave birth to young. Captive first year males routinely exhibited the cycle of testis enlargement seen in older males.

In the captive colony, the first copulations observed each year were in late April. Discarded vaginal plugs were found in the roosting enclosure from this time until August. Three matings were also observed in May and two in June. Males were seen to copulate with torpid females on two occasions.

In 1982, 27 vaginal plugs were collected from the roost during the six weeks from the beginning of June to the middle of July. Fifteen females and twelve males were present in the captive colony at this time. In May 1982, three first-year females, later to produce young, expelled copulatory plugs when hand-held and arousing from torpor. The plugs were not sperm storage sites as they contained only leucocytes and cellular detritus including decapitate spermatozoa (Plate 2.3C). Spermatozoa were present in both oviducts of one female in March, one in April, two in July (Plate 2.3D) and three in August. Except for the two July specimens, the female tracts microscopically examined during the hibernation season were from the CSIRO collection. One
of the July females also had spermatozoa within the uterine glands. The lumina of the uterine horns frequently contained spermatozoa surrounded by leucocytes. This was not the case in the oviducts where, in most cases, no leucocytes were present and the sperm were oriented with the head in close association with the epithelium.

Ovulation did not occur in *N. gouldi* females until September or October. Antral follicles were present in the ovaries of the seven females collected between March and August. The terminal phase of follicle growth was arrested until the spring. Two captive females in early October and one in early September had recently ovulated, although the fertilised ova had been lost during sectioning and could not be found in the reproductive tract. The ovaries of two females in late October each contained a large corpus luteum. The corpus luteum occupied most of the ovary and incipient parturition was indicated by the large foetuses (crown-rump lengths, 8-11 mm) present in both uterine horns of these animals.

Parturition occurred in the captive population in late October, or the first half of November (Table 2.5). In 1979 and 1981 some females did not give birth until early December. Of the 33 females which gave birth in captivity in four breeding seasons, 18 (54.5%) had twins. Four of these females were yearlings one of which had twins and the others single young. There were annual differences in the timing of the parturition period in the captive colony. In 1982, the first female gave birth on 25 October and in 1981 parturition did not occur until 20 days later on 13 November.
Parturition was not closely synchronised in females within the captive colony in each breeding season, even though all were maintained under identical conditions. Twenty-seven days separated the first and last birth dates in the colony of eight females in 1979. In 1981 and 1982 parturition within groups of eight and nine females spanned intervals of 17 and 12 days, respectively. The breeding season of 1980 was an exception when only four days separated the first and last birth dates in four females.

The young of *N. gouldi* are born naked. They attach to the nipples and remained so, at least during the day-light hours, for seven to ten days (Plate 2.4). Lactating females were never trapped in the wild with the young attached. In the captive population, the young were never carried during foraging flights, not even for the first two or three days. They were left in the roost or in the corner of the flight enclosure whilst their mothers fed and drank. The young formed a cluster with the older, larger individuals on the periphery covering the younger infants. Young animals undertook their first 'training' flights when four to five weeks of age and were fully weaned and independent at six weeks.

2.4.4 Discussion: Breeding cycle of *Nyctophilus gouldi*

In summary, *Nyctophilus gouldi* has many of the reproductive characteristics of the other temperate-zone vespertilionids in which delayed fertilisation occurs (Kitchener 1975; Kitchener and Coster 1981; Kitchener and Halse 1978; Gustafson 1979; Racey 1982). In south-eastern
Australia the species is monoestrous. Females first become receptive to males in April and oestrus continues throughout the hibernation season until ovulation occurs in September or early October. Large antral follicles are present within the ovaries during the prolonged oestrus. Spermatogenesis takes place during the summer; following which the seminiferous tubules regress and spermatozoa are stored in the epididymides until the following spring. Male libido continues throughout the hibernation season during which females may be inseminated a number of times. A copulatory plug forms in the vagina following, or as a result of, mating and sperm are stored in the oviducts or uterine glands. Female *N. gouldi* are sexually active within seven to nine months of birth. Males become sexually mature after their first hibernation season, at 12 to 15 months of age. This is not an unusual finding. Tuttle and Stevenson (1982) list 17 vespertilionids in which sexual maturity is reached at a similar age to *N. gouldi*.

In captive *N. gouldi* the twinning rate was 54.4 per cent (33 females). Racey (1982) suggests that litter size may increase in captive bats as a consequence of plentiful food. It is not known whether captivity enhanced twinning in *N. gouldi*. Free-living *Nyctophilus geoffroyi* commonly give birth to twins (Ryan 1963; Green 1966); however, no studies have reported the twinning frequency of wild *N. gouldi*. The natural litter-size within species can vary from year to year, from place to place and with the age of the mother (Tuttle and Stevenson 1982). In *Antrozous pallidus*, which frequently has twins, yearling females usually have only single young
(Davis 1969). The twinning rate of yearling *N. gouldi* was 25.0 per cent (of 4 females) compared with 58.6 per cent (of 29 females) in older bats.

In Nearctic bats, Humphrey (1975) showed that a significant inverse relationship exists between litter size and the population of the typical nursery colony. "Species normally having twins are solitary or form nurseries of moderate size in trees, rock crevices and buildings" (Humphrey 1975). Species producing single young commonly form larger colonies, especially in caves. Humphrey suggests that larger litter size in less colonial or solitary species has been selected to offset greater mortality as a result of greater predation pressure and exposure to more variable microclimate. Tidemann (pers. comm.) has recently located several *N. gouldi* roosts using radio-tracking techniques. He reports that they are usually under loose bark in groups of 10 to 20 individuals. If twinning is as frequent in free-living *N. gouldi* as it is in captive animals then this species clearly conforms to Humphrey's correlation of increased litter size with exposed roost and moderate colony size.

In captive *N. gouldi* there were annual differences in the timing of the birth period. In 1979, 1980 and 1981 the birth period commenced during the second and third weeks of November (Table 2.5). In 1982, first births were about three weeks earlier. The mean monthly minima and maxima in Canberra for September, October and November in these four years were very similar; however, August of 1982 was unusually warm. The mean monthly maximum
temperatures for August in 1979, 1980 and 1981 were 12.7, 14.0 and 11.7°C, respectively. In 1982 the mean maximum temperature in August was 17.4°C. *N. gouldi* ovulates at the end of the hibernation season in September or early October. In 1982 it seems likely that the onset of warmer spring environmental temperatures at the end of August advanced the time of ovulation resulting in earlier parturition than in other years. I can only conclude that captive females were primarily responding to temperature cues since photoperiod and food supply were unaltered from year to year.

In temperate-zone bats the period of embryonic growth is primarily controlled by environmental temperature and the availability of insects (Tuttle and Stevenson 1982). Racey and Swift (1981) showed that the mean gestation period of a free-living colony of *P. pipistrellus* was ten days greater in the second of successive years because of colder weather and reduced insect activity. If food is unlimited then warmer environmental temperature, during the second half of the hibernation season or early spring, may advance the commencement of the pregnancy and/or accelerate the rate of foetal development. Should winter end early, the pregnancy can commence immediately to take advantage of the favourable conditions. The young can then be born at the earliest possible time, thus maximising the period between weaning and the start of the next hibernation season. At the latitude and elevation of the study area used in this work, juvenile *N. gouldi* were volant and nearly weaned by mid-January. The young have only 8 to 10 weeks to become
efficient foragers and deposit sufficient body fat to survive the six months of the hibernation period.

The converse situation can also apply. Colder ambient temperatures during the gestation period increase the maintenance energy needs of the mother, if she remains homeothermic, and reduce the food supply. Pregnant *Myotis thysanodes* and *M. lucifugus* females show a greater tendency to enter torpor in the early and late phases rather than in the middle of gestation (Studier and O'Farrell 1972). Ransome (1973) also showed that the rate of embryonic growth was most likely to be retarded in pregnant *Rhinolophus ferrumequinum* during early and late pregnancy. He suggested that the food consumption of near term females was restricted because the bulk of the embryo in the abdomen meant the stomach could not be engorged to the usual extent. This factor, combined with the shorter night length of summer and the increased wing loading associated with greater body mass, made *R. ferrumequinum* more prone to torpor when in the latter stages of pregnancy.

*Nyctophilus gouldi* may behave in a similar manner to other temperate-zone vespertilionids during the latter stages of pregnancy. In the field population the activity of near-term females declined (section 2.3). Kunz and Anthony (1977) suggest that if harp traps should show any bias in adult trappability, then it will be towards less manoeuvrable females in late pregnancy. In four breeding seasons, only one female in the later stages of pregnancy
(body weight greater than 16 grams) was trapped. In the captive colony females weighed more than 16 grams for the last 10 to 14 days of pregnancy. It is most unlikely that in four years, my trapping of the population did not coincide with that particular phase of pregnancy on at least one occasion.

Births were not closely synchronised within the captive *N. gouldi* colony in each year, especially when it is considered that all females were provided with constant, plentiful food and experienced similar microclimate within the roost. Birth synchrony must be considered in relative terms. Parturition is relatively asynchronous within those tropical communities where breeding is virtually continual and one or two birth peaks occur each year coincident with rainfall, or an insect bloom. By contrast, Racey and Swift (1981) monitored a free-living colony of the temperate-zone species *P. pipistrellus* and found that in two successive years 80 per cent of about 300 females present gave birth within two days.

The microhabitat chosen by the female during the pregnancy may be critical to the ultimate timing of birth. One suggested effect of microhabitat selection is that the synchrony of births is greater in highly colonial species which select cooler roosts (Racey 1982; Tuttle and Stevenson 1982). A number of studies have found that the birth period is contracted if the weather is colder during the gestation period. Rakhmatulina (1972) found that the birth period in a colony of *P. pipistrellus* in 1969 was 10 days shorter than in 1967 and suggested it was because
the spring of 1969 was colder. Racey (1972 cited by Racey 1982) reported a similar finding for captive colonies of *P. pipistrellus* and *Nyctalus noctula*.

The greatest synchrony of births in captive *N. gouldi* was in 1980 and the least in 1979. Yearly differences in mean environmental temperature cannot explain this finding. Mean Canberra temperatures for August, September, October and November were slightly greater in 1980 (difference <2°C). The relative asynchrony of births in *N. gouldi* may be an artefact of captivity although there is evidence to suggest that extended birth periods are common in temperate-zone, tree-dwelling bat communities. Kitchener (1975) examined the uterine horns of nine *Chalinolobus gouldii* captured on the one day and found development ranging from early pregnancy to near term. From age-estimates of young *N. geoffroyi* found in a maternity colony in Victoria, Ryan (1963) calculated that the birth period was at least 13 days. He knew that three animals had already dispersed from the colony indicating that they were older than those present and that the birth period was even greater than 13 days.

During this study, vaginal plugs were frequently found in the roosting enclosure of *N. gouldi*. Multiple copulations occurred as an increasing number of these 'gelatinous' plugs accumulated as winter progressed. A number of authors have suggested that multiple copulations may be necessary in species where immediate fertilisation does not occur (Guthrie 1933; Hartman 1933; Krutzsch 1975). Oxberry (1979) suggests that "... the initial
spermatozoa may either lose their capacity to fertilize or be expelled from the females reproductive tract ..." in "... species that frequently arouse from hibernation and resume normal activity for short periods of time".

There was only limited cessation of nightly feeding activity in captive *N. gouldi* during the hibernation season although the bats were generally torpid during daylight hours. Three females were observed to expel the copulatory plug when arousing from torpor, however, it is not known whether the plug was expelled every time rewarming occurred. Equally, it is not known whether the spermatozoa were lost from the tract as a result of each arousal from torpor.

In *N. gouldi*, spermatozoa were stored throughout winter by males and females. The duration of sperm storage in males was eight to nine months. If the spermatozoa or their viability are lost each time the female rewarms and the vaginal plug is expelled, then continuing male libido and sperm storage throughout the hibernation period is essential if the species is to reproduce.
Plate 2.1 A typical trap-site used during the field study showing harp trap erected and in place.

Plate 2.2 The pond where most *N. gouldi* were trapped during the study (trap-site 11, Fig. 2.2). The harp trap was placed across the normal flight corridor of the bats and on this occasion two mist-nets were used to improve the rate of capture.
A. Transverse section through the testis of a male *N. gouldi* in March. The tails of the mature spermatozoa (S) can be seen trailing into the lumen of the seminiferous tubule. The heads of the spermatozoa appear to be closely associated with Sertoli cells (arrowed).

Scale bar: 20 µm

B. Transverse section through the caudal portion of the epididymis from a male in August. Spermatozoa (S) were present in the epididymides from March until August.

Scale bar: 20 µm

C. Longitudinal section through the copulatory plug (CP) in the vagina of a female in July. The plug is acellular, except for some leucocytes and cellular detritus including decapitate spermatozoa.

Scale bar: 1000 µm

D. The oviduct of a female *N. gouldi* in July showing spermatozoa (S) oriented with the head toward the epithelium. The tails are trailing into the lumen of the oviduct.

Scale bar: 20 µm
Plate 2.4  A female *N. gouldi* with seven day-old young attached to each nipple.
Figure 2.1 The distribution of *Nyctophilus gouldi* in Australia

(Hall & Richards 1979, Kitchener & Vicker 1981)
Bat trap sites
Streams
Roads or Firetrails

Scale

Contour interval 25 metres

Figure 2.2 Map of the area used to study the ecology of *N. gouldii*
Figure 2.3 Annual changes in the mean monthly minimum and maximum temperature (Lees Ck., 750 m.), numbers of insects and male and female N. gouldi caught per trap-night in the Bull's Head study area (Jan. 1979 to Mar. 1983). All means are ± one standard deviation. Field methods are described in section 2.3.2.
Figure 2.4 The numbers of *N. gouldi* caught at sites within four elevation classes and at pond trap-sites in the Bull's Head study area. Total trap-nights are subscripted and the number of trap-sites within each group is shown under the appropriate elevation class.
Figure 2.5 The annual body weight cycles of captive and free-living *N. gouldi*. Sample sizes for free-living bats are as shown in Table 2.1. For captive animals sample sizes are:


☆: two sub-adult males not considered representative of adult body weights in May
Figure 2.6 The annual body fat cycle of captive N. gouldii. Monthly determinations of total body water were used to predict the proportion of adipose tissue in the body (Inwards, 1984 unpublished findings). Refer section 2.3 for a more detailed description of the methods.
Figure 2.7 The reproductive cycles of male and female *N. gouldi* determined from histological and field collected data. In Section 2.4.2 the four categories used to classify overt reproductive status are described and the histological methods are detailed.
Table 2.1. The numbers of adult and juvenile *Nyctophilus gouldi* caught each month at the Bull’s Head study site. The combined results for January 1979 to and including March 1983 are presented.

<table>
<thead>
<tr>
<th>Month</th>
<th>Total Trap-nights</th>
<th>Adult Numbers Caught</th>
<th>Juvenile Numbers Caught</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adult</td>
<td>Juvenile</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>d</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>62</td>
<td>18</td>
<td>17</td>
<td>49</td>
</tr>
<tr>
<td>F</td>
<td>44</td>
<td>21</td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td>M</td>
<td>54</td>
<td>16</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>A</td>
<td>14</td>
<td>2</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>M</td>
<td>24</td>
<td>4</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>J</td>
<td>20</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A</td>
<td>17</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S</td>
<td>25</td>
<td>1</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>O</td>
<td>33</td>
<td>21</td>
<td>-</td>
<td>19</td>
</tr>
<tr>
<td>N</td>
<td>24</td>
<td>6</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>D</td>
<td>45</td>
<td>11</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>Totals</td>
<td>371</td>
<td>103</td>
<td>33</td>
<td>142</td>
</tr>
</tbody>
</table>
Table 2.2. The numbers of adult male and female *N. gouldi*, *N. geoffroyi*, Chalinolobus morio, *Stereocaulus sagittula* and *E. repulue* caught during the active and hibernation seasons in the Bull's Head study area. January 1979 to March 1983.

<table>
<thead>
<tr>
<th>Species</th>
<th>Active Season (Oct, Nov, Dec, Jan, Feb, Mar)</th>
<th>Hibernation Season (Apr, May, Jun, Jul, Aug, Sep)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Trap-nights = 262</td>
<td>Total Trap-nights = 109</td>
</tr>
<tr>
<td><strong>Number Caught</strong></td>
<td><strong>Total</strong></td>
<td><strong>Total</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Trap-nights</strong></td>
<td><strong>Trap-nights</strong></td>
</tr>
<tr>
<td><em>N. gouldi</em></td>
<td>93</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>137</td>
<td>5</td>
</tr>
<tr>
<td>Ratio ♂:♀</td>
<td>0.68</td>
<td>2.0</td>
</tr>
<tr>
<td><em>N. geoffroyi</em></td>
<td>43</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>206</td>
<td>4</td>
</tr>
<tr>
<td>Ratio ♂:♀</td>
<td>0.21</td>
<td>1.25</td>
</tr>
<tr>
<td><em>C. morio</em>:</td>
<td>227</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>367</td>
<td>18</td>
</tr>
<tr>
<td>Ratio ♂:♀</td>
<td>0.62</td>
<td>2.44</td>
</tr>
<tr>
<td><em>S. sagittula</em></td>
<td>266</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>156</td>
<td>4</td>
</tr>
<tr>
<td>Ratio ♂:♀</td>
<td>1.04</td>
<td>7.25</td>
</tr>
<tr>
<td><em>E. repulue</em></td>
<td>231</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>189</td>
<td>14</td>
</tr>
<tr>
<td>Ratio ♂:♀</td>
<td>1.22</td>
<td>1.57</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>860</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>1055</td>
<td>45</td>
</tr>
</tbody>
</table>
Table 2.3. Numbers of each species banded and retrapped in the Bull's Head study area between March 1980 and March 1983.

("1" = retrapped once, "2" = retrapped twice etc).

<table>
<thead>
<tr>
<th>Species</th>
<th>Number Banded</th>
<th>Number Retrapped</th>
<th>Percent Retrapped</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1&quot;   2&quot;   3&quot;   4&quot;   5&quot;   6&quot;   7&quot;</td>
<td></td>
</tr>
<tr>
<td>N. gouldi</td>
<td>145</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>N. Geoffroyi</td>
<td>209</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>C. morio</td>
<td>439</td>
<td>46</td>
<td>7     4     1</td>
</tr>
<tr>
<td>C. gouldii</td>
<td>36</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>E. agitella</td>
<td>302</td>
<td>35</td>
<td>3     3     1</td>
</tr>
<tr>
<td>E. regulus</td>
<td>295</td>
<td>48</td>
<td>12    4     1</td>
</tr>
<tr>
<td>E. vulturine</td>
<td>6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>P. tasmaniensis</td>
<td>33</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>1465</td>
<td>145</td>
<td>22    11    2</td>
</tr>
</tbody>
</table>

("1" = retrapped once, "2" = retrapped twice etc).
Table 2.4. The numbers of each species caught at the site of previous encounter and at distances up to and greater than one kilometre in the Bull’s Head study area, March 1980 to March 1983 inclusive.

<table>
<thead>
<tr>
<th>Species</th>
<th>At site of previous encounter</th>
<th>Number Caught</th>
<th>Mean distance between release and retrap (x + SD) km</th>
<th>Mean distance between release and retrap</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Between 0.1 - 0.5 km</td>
<td>Between 0.6 - 1.0 km</td>
<td>At distances &gt; 1 km</td>
</tr>
<tr>
<td>Pithecus gouldi</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pithecus geoffroyi</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>C. morio</td>
<td>17</td>
<td>8</td>
<td>28</td>
<td>23*</td>
</tr>
<tr>
<td>P. gouldii</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P. vittatus</td>
<td>30</td>
<td>22</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>P. regulus</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P. vulturineae</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P. tamarineda</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Totals 78 50 57 52

*one animal retrapped 12.5 kms from study area: not included in mean distance between retrace calculation
Table 2.5. Breeding information for captive *N. gouldi*.

<table>
<thead>
<tr>
<th>Breeding season</th>
<th>Number of females to give birth to:</th>
<th>Dates of first and last birth</th>
<th>Number of days between first and last birth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single young</td>
<td>Twins</td>
<td></td>
</tr>
<tr>
<td>1979</td>
<td>4</td>
<td>4</td>
<td>6.11.79, 4.12.79</td>
</tr>
<tr>
<td>1980</td>
<td>1</td>
<td>3</td>
<td>17.11.80, 20.11.80</td>
</tr>
<tr>
<td>1981</td>
<td>4</td>
<td>4</td>
<td>13.11.81, 1.12.81</td>
</tr>
<tr>
<td>1982</td>
<td>3</td>
<td>5</td>
<td>25.10.82, 8.11.82</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>
## Chapter Three

**ENDOTHERMIC METABOLIC AND BODY TEMPERATURE REGULATION IN WINTER, SPRING AND SUMMER—ACCLIMATIZED *NYCTOPHILUS GOULDI***

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Introduction</td>
<td>58</td>
</tr>
<tr>
<td>3.2</td>
<td>Methods</td>
<td>61</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Maintenance in Captivity</td>
<td>61</td>
</tr>
<tr>
<td>3.2.2</td>
<td>General Experimental Procedures</td>
<td>63</td>
</tr>
<tr>
<td>3.2.3</td>
<td>Statistical Methods</td>
<td>66</td>
</tr>
<tr>
<td>3.3</td>
<td>Results</td>
<td>70</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Males</td>
<td>70</td>
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<td>3.3.2</td>
<td>Females</td>
<td>72</td>
</tr>
<tr>
<td>3.3.3</td>
<td>Comparison of Male and Female Patterns</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>76</td>
</tr>
<tr>
<td>3.4</td>
<td>Discussion</td>
<td>77</td>
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</table>
3.1 INTRODUCTION

Seasonal adaptations which are manifested in physical or physiological changes within the animal, can be detected by comparing the metabolic costs of temperature regulation in summer and winter-adapted representatives of the species. Improvements in the heat producing capability or insulative properties of a winter acclimatized animal are recognizable by seasonal changes in the metabolic curve.

In temperate-dwelling bats natural seasonal acclimatization has been reported or inferred by a number of workers (Pohl 1961; Menaker 1962; Mejsnar and Jansky 1967; Holyoak and Stones 1971). Rather than attempting to describe the processes of seasonal acclimatization, the early workers used acclimated bats to simulate and compare summer and winter thermoregulatory capacities (Pohl 1961; Menaker 1962; Holyoak and Stones 1971). They found that cold exposure enhanced the thermogenic abilities of hibernating bats. Holyoak and Stones (1971) acclimated *Myotis lucifugus* to 10, 20 and 30°C in winter and summer and concluded that the observed seasonal adaptation was a metabolic rather than an insulative adjustment. Their conclusions are unconvincing, however, as no consideration was given to the possible role of photoperiod as a cue in seasonal acclimatization and all bats were maintained in captivity under a 14 hour light:10 hour dark regime, irrespective of season.
Mejsnar and Jansky's (1967) study of *Myotis myotis* has many features in common with the study reported here. Male *M. myotis* were kept in captivity for up to three months. 'Summer' bats were held at room temperature and fed every second day. 'Winter' bats were fed after each experimental day and maintained at the temperature of their natural winter roost, 7°C. No indication was given by these authors of the frequency of winter experimentation or the lighting regime to which captive animals were exposed. Consequently their findings must also be considered cautiously. In contrast to the metabolic adjustments reported by Holyoak and Stones (1971) for *M. lucifugus*, *M. myotis* underwent a seasonal insulative adaptation indicated by lower body temperatures and metabolic maintenance costs in winter.

Support for the findings of Mejsnar and Jansky (1967) is provided by the recent work of Shump and Shump (1980) who have shown that the insulative value of the pelts of *M. lucifugus*, *M. keenii* and *Eptesicus fuscus* improves by 26 per cent in winter. Shump and Shump's (1980) findings for *Myotis lucifugus* are in direct conflict with those of Holyoak and Stones (1971).

The primary aim of my experimental work was to determine the pattern of seasonal change in total heat flux in homeothermic *Nyctophilus gouldi*. Discussion of the incidence and specific details of torpor encountered during this work has been reserved for Chapter Eight.
The metabolic and body temperature responses of male and female *N. gouldi* were measured across a wide range of ambient temperatures (5 to 38°C) in summer, winter and spring (Fig. 3.1). Females in spring were in the middle to late stages of pregnancy (Section 2.4) and the investigation aimed to compare metabolic levels at these reproductive stages with those of non-pregnant bats in other seasons.

A captive colony of *N. gouldi* was established to permit laboratory measurements to be made at regular intervals across the seasons. The difficulties of locating *N. gouldi* roosts and the very low activity levels of the species for six months of the year (Section 2.3) made this an essential step in comparing the costs of thermoregulation to the species in winter, spring and summer. The outdoor cage (Section 3.2.1) exposed experimental animals to natural ambient temperatures and photoperiod. In the absence of an appropriate method for simulating the seasonal fluctuations in insects, the captive bats were provided with *ad libitum* food year-round. Although the constant food supply was 'unnatural' in winter, this approach was essential if the homeothermic metabolic requirements of thermoregulation were to be legitimately compared in each season.
3.2 METHODS

3.2.1 Maintenance of Animals in Captivity

*Nyctophilus gouldii* were trapped within the Uriarra State Forest of the Brindabella Ranges, Australian Capital Territory, from areas adjacent to or within the field study site (Section 2.3). Only adults were selected for the experimental work and transferred to the captive colony. Permits to take live animals were issued by the Department of the Capital Territory, Conservation and Agriculture (Permit No. AS 79/44).

The colony of male and female *N. gouldii* was kept within an outdoor enclosure (Plate 3.1) on the campus of the Australian National University, Canberra. The three flight arenas were six metres long, three metres wide and two metres high and enclosed with fibreglass mesh. Bats were housed in timber nestboxes with perspex backs to enable observations of the colony (Plate 3.2). Mealworms (*Tenebrio sp.*) and water were provided *ad libitum*. During periods of mealworm shortage, cockroaches and blowfly pupae were substituted. On a diet of blowfly pupae the bats maintained constant body weight but were unable to deposit body fats in late summer and autumn. Experimental data were not taken from bats on diets other than mealworms.

The colony bred successfully each year (Section 2.4). No fur loss was shown by the bats and vitamin and lactate supplements were not required. The body weights of captive bats were generally one gram greater than free-
living animals and followed the same seasonal cycle (Fig. 2.5). The provision of *ad libitum* food and confinement to a relatively small flight space appeared to alter none of the observable, natural physiological rhythms in the species. Autumn fat deposition commenced at a similar time in both field and captive populations. Parturition and spermatogenesis in the two groups were also in synchrony (Section 2.3).

Loss of fur and erratic fluctuations of body weight were evident in bats which were confined to small cages not permitting flight. These animals became obese and died. The mortality rate within the outdoor colony during the study was about ten per cent. Most deaths were the result of misadventure, that is, drowning. The success in maintaining *N. gouldi* in captivity is undoubtedly related to the aerial agility and natural ground-feeding habits of the species. Animals brought in from the wild learnt within two or three days to land in the ground-level feeding tray to take mealworms.

Individuals were initially wing-tattooed to enable recognition. This proved unsatisfactory as re-tattooing was essential every four weeks. Thereafter individuals were uniquely banded on the forearm with coloured, plastic bird bands.
3.2.2 General Experimental Procedures

Metabolic chambers were designed after Studier and O'Farrell (1972) (Plate 3.3). An inner stainless steel mesh cage was added to promote mixing of the air and to ensure the central restraint of the bat within the chamber. Tracy (1972) illustrated the effects of "wind speed" (air flowrate) on the thermoregulatory performance of small homeotherms within metabolic chambers. The mesh cage obviates the formation of heat-sinks and an airflow of 200 to 300 millilitres per minute (Standard Temperature and Pressure) was maintained throughout all comparable experiments.

Room air was pumped through a control valve (Fig. 3.2) and carbon dioxide and water absorbent chambers to a one metre, coiled, copper tube immersed in a temperature-controlled water bath. The temperature equilibrated air passed into the lower end of the metabolic chamber which was also immersed in the water bath to within one centimetre of the top (Plate 3.3). The exhalant air from the top of the chamber passed through a Fischer and Porter flowmeter and to a Beckman OMI4 oxygen analyzer (accuracy ± 0.5% full scale). The response time of the oxygen analyzer to transient levels was between five and ten seconds. Stable recording was established at 60 seconds.

Continuous recordings of rectal temperature were made with plastic sheathed, 38 gauge copper-constantan thermocouples linked to a Leeds and Northrup 15-channel recorder. Rectal thermocouples were inserted to a depth of
one centimetre and glued to the perianal fur with tissue
cement. Thermocouples were calibrated against a Leeds and
Northrup 8078 Precision Temperature Bridge (calibrated at
the National Standards Laboratory to an accuracy of \( \pm 0.05^\circ C \))
and then comparatively calibrated in ice water to determine
any differences between them. These differences amounted to
less than \( \pm 0.2^\circ C \).

Bats were weighed to the nearest 0.1 g before and
after testing and the average used in calculations of weight
specific oxygen consumption. All testing was done during
day-light hours in a well lit room. Six metabolic chambers
were employed. Five chambers were loaded with a single bat
each for simultaneous measurements. The sixth chamber was
used as a control: non-respired air, free of carbon dioxide
and water was passed through the chamber to continually
check the calibration of the oxygen analyzer.

Experimental animals were given 90 minutes to
equilibrate at each experimental ambient temperature (\( T_A \)).
Individuals were exposed to three or four different
temperatures on each experimental day. Measurements were
not taken from bats unless they were inactive and the
experimental results of the first day for each individual
were discarded to reduce the effects of induced stress.

It was considered unwise to test animals at \( T_A \)'s
in excess of 38°C as the importance of evaporative cooling
to the thermoregulation of the species was unknown. Within
the restraining stainless steel cage, bats could not initiate
cooling mechanisms such as the wing unfolding observed on
hot summer afternoons in the artificial roosts of the outdoor holding facility (Section 3.2.1). Care was taken at all times to minimize the stress placed upon individuals during experimentation as colony animals were difficult to replace, especially during the hibernation season from April to September.

Bats were tested singly at all times. The thermoregulatory 'savings' afforded to clustering bats are well documented (Studier 1970; Howell 1976). For this reason, solitary testing can be criticized as unnatural, however, the adaptive physiology rather than the behaviour of the individual was the major consideration in this work.

In order to make oxygen consumption and rectal temperature ($T_R$) measurements it was essential that the bats be restrained by the stainless steel cage within the metabolic chamber. Microchiroptera are commonly found in narrow, confining roosts and the similar nature of the experimental chamber should not have added greatly to the stress placed upon the animal.

In all seasons, bats were brought into the laboratory and provided with *ad libitum* food and water on the evening preceding the day of the experiment. Experimentation usually commenced around 0800 hours with first recordings being taken at 1000 hours. Given the rapid food passage time of bats, such as *N. gouldi* (Buchler 1975) all animals could be considered post-absorptive during experimentation.
The metabolic and body temperature testing of male and female *N. gouldi* was carried out in winter, spring and summer at the times indicated on Figure 3.1. Instantaneous oxygen consumption was calculated using the method of *Withers* (1977) and is presented in mls. $O_2/g/hr$ (STP) and in some cases Watts/kg. An oxy-caloric equivalent of 4.8 calories per millilitre of oxygen consumed was used in this conversion and the respiratory quotient assumed to be equal to one.

3.2.3 *Statistical Methods*

A direct result of the application of Newton's Law of Cooling to metabolic data has been the tendency for experimenters to adopt rectilinear rather than curvilinear lines of best fit (Studier 1980). Curvilinear or polynomial lines of fit permit a more accurate description of metabolic responses over the entire range of test temperatures. The concept of the thermal-neutral zone (TNZ) has been misused in the past by attempts to fit rectilinear models to naturally curvilinear data.

The upper and lower critical temperatures of the TNZ have gained importance as indicators of climatic and seasonal adaptation and are supposedly apparent as sudden elevations of thermoregulatory metabolic requirements above a basal level; the basal metabolic rate (BMR). However, the metabolic curve of a typical homeotherm is best described by a curvilinear model with two points of inflection (Fig. 3.3): one at the level of minimum metabolism and the other at the point where physical thermoregulatory mechanisms such
as piloerection of fur or feathers and peripheral vasoconstriction reduce the rates of thermal conductance and the curve plateaus.

The use of rectilinear models requires the subjective grouping of the data by the researcher to allow three lines to be fitted. The curvilinear method uses all of the data in the calculation of the regression line and avoids the inaccuracies resulting from somewhat arbitrary grouping of the data. The concept of a basal or standard metabolic rate should be considered as distinct from the minimum metabolic rate (MMR) determined by the curvilinear model, as the BMR will generally be higher than the MMR because of the inclusion of points toward the extremity of the thermal-neutral zone (Fig. 3.3)

The traditional method of determining the average rate of thermal conductance for a species from the slope of the metabolic curve has recently received considerable criticism because of the inherent assumption that the rate of heat loss to the environment is constant at all ambient temperatures less than the lower critical temperature of the TNZ (Tracy 1972; McNab 1980). The estimates of thermal conductive rate determined in this manner generally underestimate "mean minimal conductance calculated from individual measurements of metabolism below thermo-neutrality" (McNab 1980) because of the combination of physical and chemical thermoregulation present in most homeotherms, especially at cold ambient temperatures. The thermoregulatory responses of an endotherm at any $T_A$ should be considered as unique and treated as such.
The averaging of oxygen consumption that has occurred in the past and undoubtedly yielded innaccurate results is the unfortunate by-product of the relative ease with which rectilinear lines-of-best-fit are calculated. The abundance and simplicity of statistical packages enabling curvilinear models to be fitted will, hopefully, see the end of previous less satisfactory methods of describing metabolic data (Studier 1980).

A Hewlett-Packard 85 calculator was used to fit curves to the oxygen consumption and rectal temperature data of *Nycophillus gouldi*. The Basic Statistics and Data Manipulation and Regression Analysis packs were used in these computations.

Polynomial curves of degree three (i.e., cubic curves) were fitted to all oxygen consumption data. Third-degree polynomial regression gave the best fit to the data as indicated by the value of the regression coefficient ($R^2$). In some instances the cubic component of the regression did not significantly improve the $R^2$ and has been omitted from the equation. The relevant F values indicated such cases and these regressions have been presented as quadratic equations.

In all but one case the responses of rectal temperature to ambient temperature were best described by polynomial equations of degree two. The addition of the cubic component to the regression improved the $R^2$ value of the equation in only one instance.
The inflection points of the metabolic curves were determined by calculating the roots of the first derivative of the regression equation; that is, the point where the slope of the line equals zero.

Comparisons of oxygen consumption and rectal temperature curves were done by analysis of co-variance on the UNIVAC 1100 computer of the Australian National University using the GLIM statistical package. In situations where lines of best fit did not intersect, the areas under the curves were calculated to enable a quantitative means of comparing differences in vertical displacement. The areas under the curves were estimated using Simpson's Rule by the "Roots" program of the HP85's Standard pack. For consistency, areas under the curve were calculated over the standard interval of \( T_A = 10-30^\circ C \).

When using the equation of the curve for predictive purposes the maximum number of significant figures must be used to gain an accurate result. Considerable errors will result if rounding off occurs. The equations given in Figures 3.4 and 3.5 have been simplified for ease of graphical presentation and should not be used in predictive work. The more complete equations are given in Appendix II.
3.3 RESULTS

_Nyctophilus gouldi_ maintained on plentiful food were capable of regulating body temperature in the typical homeothermic manner in all seasons (Figs. 3.4 and 3.5). As ambient temperature was reduced oxygen consumption increased to maintain homeothermic body temperature. Rectal temperature ($T_R$) was regulated but not constant over the range of $T_A$'s tested. Minimum metabolism occurred, in all cases, at ambient temperatures between 34 and 36°C (Tables 3.1 and 3.2) and bats routinely had $T_R$'s of 35 to 39°C. At $T_A$'s between 35 and 10°C, mean rectal temperatures dropped uniformly with declining ambient temperature and were maintained between three and five degrees lower at an ambient temperature of 10°C. Rectal temperatures were more variable than oxygen consumption rates: this observation is reinforced by the respective correlation coefficients (Figs. 3.4 and 3.5).

Torpor occurred in all seasons and in both sexes. In well-fed _N. gouldi_, the incidence of torpor was only at $T_A$'s less than 18°C. If _N. gouldi_ were denied food for the 24 hours preceding experimentation then torpor was induced at much higher ambient temperatures (25-27°C) (Section 8.3.1). These data are considered in detail in Chapter Eight.

3.3.1 Males

The oxygen consumption and rectal temperature curves of male _N. gouldi_ in winter and summer indicated significantly different responses to ambient temperature (Fig. 3.6). The slope and vertical displacement of the
oxygen consumption curves were significantly different by analysis of co-variance (slopes: $F[1,136] = 13.68, p<0.001$; vertical displacement: $F[1,137] = 98.73, p<0.001$). The summer to winter transition resulted in an overall depression of the metabolic curve with a reduction in the slope. The shift in the slope of the curve was also illustrated by the metabolic $Q_{10}$'s which showed a reduction in winter tested male $N$. gouldi of between 16 and 28 per cent (Table 3.3).

The pattern of rectal temperature regulation in response to changing ambient temperature remained unchanged with the seasons (slopes: $F[1,161] = 2.08, p>0.20$) however, the mean $T_R$'s of males in winter were between two and three degrees less than in summer (vertical displacement; $F[1,162] = 84.48, p<0.001$) (Fig. 3.6).

The seasonal change in the cost of thermoregulation was shown also in the levels of minimum metabolic rate (MMR) and the corresponding areas under the curves. Minimum metabolic rate in winter males was 21.4 per cent less than in summer (Table 3.1) and the area under the oxygen consumption ($\dot{V}O_2$) curve at ambient temperatures between 10 and 20°C, was 27.4 per cent less in winter. The area under the rectal temperature curve was 5.7 per cent less in winter males (Table 3.1).

Males in spring regulated body temperature at a similar metabolic cost to males in summer. The slopes $F[1,103] = 0.65, p>0.50$ and vertical positions ($F[1,104] = 0.24, p>0.50$) of the $\dot{V}O_2$ curves were not significantly
different in spring and summer. The mean rectal temperatures regulated by males in spring were different from those in males in summer and winter (Fig. 3.6). The spring and winter TR curves were skewed; spring males maintaining lower mean rectal temperatures at $T_A$'s less than 15°C and higher TR's at warmer temperatures (slopes; $F[1,138] = 7.41, p<0.02$; vertical displacement; $F[1,139] = 0.46, p>0.50$). Spring males maintained significantly lower TR's than summer males at all ambient temperatures (vertical displacement: $F[1,153] = 50.27, p<0.001$) although the slopes of the two curves did not differ ($F[1,152] = 1.86, p>0.20$).

3.3.2 Females

The seasonal changes observed in the metabolic and rectal temperature regulation of male *N. gouldi* were also seen in the females. The $\dot{V}O_2$ and TR curves underwent a similar transition from summer to winter resulting in lower mean oxygen consumption rates and rectal temperatures at all ambient temperatures (Fig. 3.7). Unlike the males, the winter adaptation to the oxygen consumption curve did not include a reduction in the slope (slopes; $F[1,140] = 0.51, p>0.50$; vertical displacement; $F[1,141] = 8.98, p<0.01$), that is, there was no rotation of the metabolic curve in winter-adapted females. Metabolic $Q_{10}$'s remained seasonally unchanged and confirmed the parallel nature of the summer to winter metabolic curve translation (Table 3.3).

Minimum metabolism was 40.6 per cent less in winter females and the area under the $\dot{V}O_2$ curve was 11.1 per cent less than in summer females. The area under the rectal
temperature curve was 5.0 per cent less in winter females (vertical displacement; $F[1,155] = 34.91$, $p<0.001$) although the slopes of the summer and winter $T_R$ curves were not significantly different ($F[1,154] = 0.16$, $p>0.50$).

The oxygen consumption curve of spring females was similar to that of summer females (slopes; $F[1,104] = 0.18$, $p>0.50$; vertical displacement, $F[1,105] = 0.45$, $p>0.50$). The metabolic curves of spring and winter-adapted females had a similar slope ($F[1,90] = 0.73$, $p>0.50$) but the cost of thermoregulation in spring females was, on average, greater at all ambient temperatures tested ($F[1,91] = 25.19$, $p<0.001$). Minimal metabolic rate in spring females was 27.9 per cent greater than in winter females and 17.3 per cent less than in summer females (Table 3.1).

No oxygen consumption values were obtained for homeothermic spring females at $T_A$'s less than 15°C (Fig. 3.5). When exposed to ambient temperatures less than 15°C, all but one female entered torpor (Section 8.3.1). For technical reasons it was not possible to record the rates of oxygen consumption for the one sub-15°C homeothermic female although $T_R$'s were still taken. The overall pattern of rectal temperature regulation observed in spring females was similar to that of male *N. gouldi* at the same time.

The rectal temperature curves of spring and summer females were significantly different (slopes; $F[1,127] = 13.43$, $p<0.001$; vertical displacement; $F[1,128] = 5.33$, $p<0.05$) however, the curves intersected at an ambient temperature of 23.0°C and it was only at $T_A$'s less than this
that the differences in mean $T_R$ were of significance. Analysis of co-variance indicated that winter and spring rectal temperatures in females followed a similar pattern in response to changing ambient temperature (slope; $F[1,106] = 0.20, p>0.50$) but the mean regulated rectal temperatures were significantly different (vertical displacement; $F[1,107] = 10.50, p<0.005$). Close examination of the curves (Fig. 3.7) showed that only near ambient temperatures of $15^\circ C$ were the mean $T_R$'s of winter and spring females similar: the curves being skewed.

3.3.3 Comparison of Male and Female Patterns

Summer

The pattern of rectal temperature regulation was the same in male and female *N. gouldi* in summer (Fig. 3.8) although the level of minimum metabolism was 12.5 per cent higher in females. The slopes of the male and female $T_R$ curves were significantly different ($F[1,175] = 5.70, p<0.05$) although the mean rectal temperatures at any $T_A$ were not separable (vertical displacement; $F[1,176] = 1.68, p>0.20$). This apparently contradictory statement illustrates the cautious attitude which should be adopted when equating biological significance with the statistically significant findings of analysis of co-variance.

The male and female rectal temperature curves in summer were skewed and intersected at an ambient temperature of around $20^\circ C$. Near the intersection point the mean $T_R$'s of males and females were clearly not different. Over the
complete range of test $T_A$'s the rate of drop in mean rectal
temperature in response to environmental cooling was
significantly different by the statistical test. Only with a
large data set, such as that used here, would such a finding
have resulted, especially given the lability of $T_R$'s in
$N. gouldi$. Whether the finding has biological
significance remains to be established.

There was no significant difference in the metabolic
costs of temperature regulation in summer-adapted male and
female $N. gouldi$ (slopes; $F[1,151] = 2.67, p>0.20$: vertical displacement; $F[1,152] = 3.79, p>0.10$).

Winter

In winter-adapted $N. gouldi$ there was no
difference between the sexes in the mean $T_R$'s or their rates
of cooling with diminishing ambient temperature (Fig. 3.8)
(slopes; $F[1,142] = 1.61, p>0.20$: vertical displacement;
$F[1,143] = 3.40, p>0.10$). The oxygen consumption curves of
males and females in winter had significantly different
slopes ($F[1,125] = 15.87, p<0.001$); with the male curve
below that of the females. The curves converged towards the
thermal-neutral zone, and it was only at ambient temperatures
less than 15°C that males required less metabolic energy
than females for the maintenance of homeothermy. At an
ambient temperature of 10°C males required 7.24 mls. $O_2$/g/hr
(STP) to maintain a rectal temperature of 31.0°C. At the
same $T_A$ females required 8.80 mls. $O_2$/g/hr (STP) and
maintained the same mean $T_R$. The area under the $VO_2$ curves was 11.3 per cent less in winter-adapted males over the ambient temperature range of 10 to 30°C.

**Spring**

Spring females maintained significantly higher mean rectal temperatures than males (Fig. 3.8) (vertical displacement; $F[1,105] = 5.47$; $p < 0.05$) although the difference amounted to less than 2°C. The tendency of spring bats to enter torpor at ambient temperatures less than 15°C prevented the comparison of male and female $VO_2$ values across the normal range. Minimum metabolism was 16.1 per cent higher in spring males than in spring females. At all ambient temperatures tested, $VO_2$ was significantly higher in males (vertical displacement; $F[1,58] = 9.32$; $p < 0.01$) but the slopes of the metabolic curves for males and females were the same ($F[1,57] = 0.13$; $p > 0.25$).
3.4 DISCUSSION

Assuming no seasonal change in the amount of heat produced from oxygen consumed, winter-adapted *Nyctophilus gouldi* required between 10 and 20 percent less thermal energy than summer and spring-adapted bats to regulate constant, homeothermic body temperature at ambient temperatures between 5 and 38°C. In female *N. gouldi*, the winter metabolic curve paralleled but was depressed below that of summer females indicating equivalent reductions in the metabolic cost of thermoregulation across the entire range of tested environmental temperatures. In winter males a similar parallel translation and depression of the oxygen consumption curve to that noted in females occurred, however, improved insulative properties were indicated by a reduction in the slope of the curve.

The seasonal adaptation noted in both sexes of *N. gouldi* included a winter drop in the level of minimum metabolic rate. McNab (1968) suggested, from work with two desert rodents, that body fat levels of 20 per cent of body mass and greater could significantly depress weight-specific metabolic rates, if the adipose tissue was metabolically inactive. Despite the autumn accumulation of body fat in *N. gouldi* (Section 2.3.3) McNab's findings cannot be considered applicable here as at the time of winter experimentation, namely July, the per cent body fat levels of male and female *N. gouldi* were 11.4 and 15.5 per cent respectively (Section 2.3.2, Fig. 2.6). It should be remembered that this measure of total body lipids includes not only white fat but also brown adipose tissue; the
thermogenic capacities of which are well established (Hayward and Lyman 1966; Himms-Hagen 1970). The relative proportions of brown and white fat are not known and although it would be possible to calculate fat-free body mass for *N. gouldi* throughout the year, such an action would disregard the undoubted importance of the brown fat as a thermogenic site in the bat.

*Nyctophilus gouldi* were at their leanest in January when body fat accounted for only five to seven per cent of body mass (Fig. 2.6). At this time, there were no obvious white fat deposits in animals dissected to describe the distribution of adipose tissue, and only brown fat was observed (Section 4.3.1). Allowing for a seasonal hypertrophy in brown fat, it is unlikely that white fat constituted much in excess of ten per cent of total body weight at any time of the year. It therefore seems improbable that the seasonal change to the oxygen consumption curves of *N. gouldi* was the result of accumulated body fats in winter animals. Male *N. gouldi* in which parallel translation and slope reduction of the winter curve occurred had similar body fat levels during winter (July 11.4%) and summer (February 11.0%) experimentation although the ratio of metabolically inactive to active adipose tissue may have been different at these times.

Female *N. gouldi* provided the strongest evidence that seasonal changes in body fat level did not influence metabolic rates. Although there was a summer to winter drop in minimum metabolic rate, there was no significant difference in the body weights of experimental females in these seasons. The total body lipids of females in February
was 11.9 per cent of body mass compared with 15.5 per cent in July.

Homeothermic *Nyctophilus gouldi* did not maintain a constant rectal temperature across the range of experimental ambient temperatures to which they were exposed. Controlled and gradual cooling of the rectal region occurred as the ambient temperature was cooled from 35 to 5°C. At an ambient temperature of 5°C, rectal temperature was typically four to five degrees less than at thermal-neutrality. As is characteristic of small mammals, the zone of thermal-neutrality was very narrow, and as noted by Holyoak and Stones (1971) for *Myotis lucifugus*, can only be considered a thermal-neutral point. In winter, spring and summer, thermal-neutrality for *N. gouldi* was at an ambient temperature within one degree of 35°C. Rectal temperatures were labile, ranging up to two degrees about the mean described by the curvilinear model. Similar variability in rectal temperatures was reported by Studier (1980) for *M. lucifugus* and *M. thysanodes*. Studier was able to show that the maintenance of a slightly lower rectal temperature reduced the thermal requirements of the bat in both the homeothermic and the torpid state. The inference (mine) being that rectal temperature is a good measure of 'effective body temperature'.

A distinction is commonly made in the literature between actual and effective surface area and how these values differ appreciably in cold exposed animals which are capable of cooling the peripheral body layers. In animals with such a capacity for regional heterothermy, rectal
temperature may not indicate the mean or 'effective' body temperature; that is, the average temperature of the body determining the rate of thermal conductance to the environment. If rectal temperature is a measure of 'effective' body temperature then the observation that mean rectal temperatures were lower in winter-adapted *N. gouldi* is consistent with the findings for oxygen consumption. Like *Nyctophilus gouldi*, *Myotis myotis* also maintains lower rectal temperatures in winter than in summer, coincident with a depression of the metabolic curve (Mejsnar and Jansky 1967). One plausible explanation for the seasonal adaptation found in both species is that the winter reduction in regulated homeothermic rectal temperature is representative of a generalised drop in the body to environment thermal gradient resulting in a reduction in the rate of thermal loss.

The precise relationship between rectal temperature and oxygen consumption in *N. gouldi* must be questioned however. The rectal temperature curves of winter males and females were completely superimposed (Fig. 3.8) yet the oxygen consumption curves had significantly different slopes. At ambient temperatures less than 15°C, winter males maintained the same mean rectal temperatures as females but in the process required 10 to 15 per cent less thermal energy. The mean rectal temperatures of spring bats were dissimilar to those of summer *N. gouldi* yet the oxygen consumption curves were no different. As stated previously, the rectal temperatures of endothermic *N. gouldi* were extremely labile in all seasons and it may be that the rectal region does not offer the best
measure of 'effective' body temperature. In the chapter which follows, a seasonal comparison of the temperatures of different body regions is reported. These data enabled the regulated temperatures of the rectal region to be placed within a whole animal context.

Single *Nyctophilus gouldi*, within metabolic chambers, were capable of competent homeothermic regulation for prolonged periods at ambient temperatures as low as 5°C in both winter and summer. Similar findings have been reported for cold exposed *Myotis lucifugus* provided with *ad libitum* food (Stones and Wiebers 1967). If denied food, for the 24 hours preceding an experiment, *N. gouldi* entered a temperature-conforming state unlike the torpor seen in well-fed bats (Section 8.3.1). Torpor in well-fed *N. gouldi* was only at ambient temperatures less than 18°C whereas the body temperature of food-deprived bats closely conformed with ambient temperature, even at temperatures as high as 25°C. Well-fed *N. gouldi* in torpor maintained rectal temperatures which were generally 2 to 5°C greater than ambient temperature; the difference between rectal and ambient temperature in torpid, food-denied bats was always less than one degree and usually less than 0.5°C.

*Nyctophilus gouldi* provided with *ad libitum* food showed the greatest propensity to enter torpor in spring. The females at this time were entering the last third of pregnancy and although there was no escalation of metabolic levels the greater incidence of torpor is similar to that reported by Studier and O'Farrell (1972) for pregnant
Myotis thysanodes and M. lucifugus and Ransome (1973) for Rhinolophus ferrumequinum. Why males should be equally prone to enter torpor in spring is not known but will be considered in greater detail in Chapters Eight and Nine. On the few occasions when spring animals remained homeothermic at ambient temperatures less than 18°C, they regulated lower rectal temperatures than in either winter or summer. The reason for this is unclear but may be related to the onset of spring moult.
Plate 3.1 The outdoor cages used to house the experimental population of *N. gouldi*. *Ad libitum* food and water was provided in the ground-level trays and bats roosted in nest boxes (see below) within the hut at the rear of the flight arena.
Plate 3.2  The timber nest boxes provided as roosts for captive *N. gouldi*. The clear perspex back enabled observations of winter activity, breeding behaviour and mother-young interactions without disturbing the bats. The activity recording apparatus (bottom left) was not used in this study (see Inwards 1984).
Plate 3.3  The metabolic chamber used for all measurements of oxygen consumption and body temperature in homeothermic and torpid *N. gouldi*. The bat was restrained within the stainless steel mesh cage; the chamber was almost completely submerged in a temperature-controlled water bath and air passed through the copper coil to be respired by the experimental subject. The internal diameter of the chamber was 4.4 cms. Respired air passed out through one of the ports in the top rubber bung to the flowmeter and oxygen analyzer. The additional ports in the bung were used to pass thermocouples into the chamber for measuring body temperatures.

(Photograph taken by R.E. Barwick)
Figure 3.1  The times when summer, winter and spring experimentation took place. Also shown is the time when females gave birth in the year spring testing occurred. Females normally ovulated in October or early September.
Figure 3.2 Flow diagram of the laboratory set-up used for measuring rates of oxygen consumption and body temperature responses of N. gouldi to changes in ambient temperature.
Figure 3.3 A theoretical comparison of rectilinear and curvilinear lines-of-best-fit fitted to the same data. In general the rectilinear model will overestimate minimum metabolic rate because values from the outer limits of the thermal-neutral point or zone are included in the regression.
Figure 3.4 Plots of rectal temperature and oxygen consumption responses to ambient temperature in (A) Winter-, (B) Spring- and (C) Summer-acclimatized male N. gouldi. The method of curve fitting is described in Section 3.2.3, and a more detailed form of the regression equations is given in Appendix II. N = number of animals and the Tr = Ta line is indicated (----).
Figure 3.5 Plots of rectal temperature and oxygen consumption responses to ambient temperature in (A) Winter-, (B) Spring- and (C) Summer-acclimatized female N. gouldi. Section 3.2.3. describes the method of curve fitting and a more detailed form of the regression equations is given in Appendix II. N = number of animals and the $Tr - Ta$ line is indicated (---).
Comparison of oxygen consumption and rectal temperature curves for male *N. gouldi* in winter, spring and summer. The results of the statistical comparison of these curves are given in Section 3.3.1. (p. 70).

Tr = Ta line (---).
Figure 3.7 Comparison of oxygen consumption and rectal temperature curves for female N. gouldi in winter, spring and summer. The statistical comparison of these curves is described in Section 3.3.2. (p. 72). Tr = Ta line (---).
Figure 3.8 Comparison of the rectal temperature (Tr in °C) and oxygen consumption (VO2 in mlO2/g/hr.) curves of male and female N.gouldi in winter, spring and summer. The results of the statistical comparison of these curves are given in Section 3.3.3. (summer p.74, winter p.75, spring p.76). Tr = Ta line (-).
Table 3.1. Seasonal changes in the mean body weight, minimum metabolism and areas under the $V_o_2$ and $T_R$ curves in male *Nyctophilus gouldii*. Winter, spring and summer testing occurred at the times shown in Fig. 3.1. The units for the areas under the curves are given by the multiplication of the units from the respective axes. Males used in experimentation weighed significantly more in winter than in either summer ($F[1,40] = 21.86$, $p<0.001$) or spring ($F[1,37] = 39.07$, $p<0.001$). The mean body weight of males in summer was significantly greater than spring-tested males ($F[1,35] = 5.67$, $p<0.05$).

<table>
<thead>
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<th></th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean Body Weight</strong></td>
<td>12.1 ± 1.2</td>
<td>10.1 ± 0.6</td>
<td>10.6 ± 0.7</td>
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<td>(± sd, no. of bats, no. of observations</td>
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<td>(6, 17)</td>
<td>(9, 20)</td>
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<tr>
<td><strong>Minimum metabolism</strong></td>
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<td>3.1</td>
<td>2.8</td>
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<td>(mls. $O_2$/g/hr, STP)</td>
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<td>35.9</td>
<td>36.1</td>
<td>-</td>
</tr>
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<td>at $T_A (°C)$</td>
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<td></td>
<td></td>
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<tr>
<td>with a $T_R (°C)$ of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35.1</td>
<td>36.7</td>
<td>38.3</td>
<td></td>
</tr>
<tr>
<td><strong>Areas under curves</strong></td>
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<tr>
<td>($T_A = 10-30°C$)</td>
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<tr>
<td>$V_o_2$ vs. $T_A$</td>
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<td>141.9</td>
<td>139.5</td>
<td>-27.4</td>
</tr>
<tr>
<td>$T_R$ vs. $T_A$</td>
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<td>637.4</td>
<td>674.6</td>
<td>-5.7</td>
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Table 3.2. Seasonal changes in the mean body weight, minimum metabolism and areas under the $V_O_2$ and $T_R$ curves in female N. gouldii.

There was no significant difference in the mean body mass of females used in experimentation in winter and spring ($F[1,37] = 0.03, p>0.5$), winter and summer ($F[1,40] = 1.09, p>0.5$) or spring and summer ($F[1,31] = 1.01, p>0.5$). The units for the areas under the curves are given by the multiplication of the units from the respective axes.

*Estimate required extrapolation of the curve beyond the lowest recorded values.

<table>
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<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Summer-Winter</th>
<th>% Change</th>
</tr>
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<td>$11.7 \pm 1.3$</td>
<td>+ 4.9</td>
<td></td>
</tr>
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<td>(± sd, no. of bats, no. of observations)</td>
<td>(13, 24)</td>
<td>(6, 15)</td>
<td>(10, 18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum Metabolism</td>
<td>$1.9$</td>
<td>$2.6$</td>
<td>$3.2$</td>
<td>-40.6</td>
<td></td>
</tr>
<tr>
<td>(mls. $O_2$/g/hr, STP)</td>
<td>at $T_A$ (0°C)</td>
<td>$34.73$</td>
<td>$4.8^*$</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>with a $T_R$ (0°C) of</td>
<td>$35.2$</td>
<td>$37.5$</td>
<td>$36.9$</td>
<td>- 4.6</td>
<td></td>
</tr>
<tr>
<td>Areas under curves</td>
<td>($T_A = 10-30^\circ C$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_O_2$ vs. $T_A$</td>
<td>$114.1$</td>
<td>$124.5^*$</td>
<td>$128.5$</td>
<td>-11.1</td>
<td></td>
</tr>
<tr>
<td>$T_R$ vs. $T_A$</td>
<td>$639.9$</td>
<td>$651.5^*$</td>
<td>$673.6$</td>
<td>- 5.0</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.3. Metabolic Q₁₀'s for *Nyctophilus gouldi* in winter, spring and summer. Two Q₁₀'s are given (Tₐ = 30-20°C and Tₐ = 20-10°C) because of the curvilinear models fitted to the data. All values are in mls. O₂/g/hr (STP). *Indicates where oxygen consumption recordings were not taken over the entire range of ambient temperature.

<table>
<thead>
<tr>
<th></th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tₐ (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-20</td>
<td>2.5</td>
<td>3.4</td>
<td>3.5</td>
</tr>
<tr>
<td>20-10</td>
<td>2.1</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-20</td>
<td>3.3</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>20-10</td>
<td>3.0</td>
<td></td>
<td>2.9</td>
</tr>
</tbody>
</table>
Chapter Four

COMPARISON OF BODY TISSUE TEMPERATURES IN SUMMER- AND WINTER-ADAPTED NYCTOPHILUS GOULDI

4.1 Introduction 97

4.2 Methods 100
4.2.1 Distribution of Adipose Tissue 100
4.2.2 Measurement of Body Tissue Temperatures 100
4.2.3 Statistical Methods 103

4.3 Results 105
4.3.1 Adipose Tissue 105
4.3.2 Body Temperatures 106

4.4 Discussion 109
4.1 INTRODUCTION

In an endotherm regulating a constant body temperature, the rate of heat transfer from the body core to the environment is dependent upon the sum total of the thermal gradients established across the body layers (Tracy 1972). The skin is the primary heat exchange site and the temperature differential maintained between the peripheral body surfaces and the environment largely determines the rate of whole animal thermal conductance (Strunk 1971; McNab 1980).

Many factors may influence the rate of heat transfer from the body core across the shell layers to the environment:

i. The presence or absence of adipose tissue and its distribution and abundance within the body;

ii. The capacity to vasoconstrict the peripheral body circulation and subsequently tolerate the cooling of the surface tissue layers; and

iii. The insulative properties of the fur or feathers as primarily determined by the configuration and density of the covering elements.

In a resting bat, the heat lost from the naked surfaces of the folded wings, tail and ear membranes must also be considered. Many bat species are known to wrap their ventral surface with the wings and tail membrane
(Shump and Shump 1980), effectively forming an additional still air insulating barrier between the body and the environment.

The core-shell concept is a useful way of systematically defining the thermal exchange interfaces within an animal and between the animal and its environment. In the presence of a subcutaneous fat layer there are six conceptual heat transfer surfaces in a simplified cross-section through a bat (Fig. 4.1): body core/subcutaneous fat layer; fat layer/skin surface; skin surface/fur; fur/environment; inner (or ventral) wing surface/environment; and outer (or dorsal) wing surface/environment.

The principle objective of this series of experiments was to determine the 'effective' body temperature of endothermic *N. gouldi* in winter and summer. The lower rectal temperatures of winter-adapted bats (Section 3.3) suggested that reduced levels of heat storage may have been responsible for the metabolic savings in that season. To test this hypothesis, the precise relationship between rectal temperature and 'effective body' temperature had to be determined. As a preliminary step the temperatures of a number of body regions were measured at ambient temperatures of 5, 15, 25 and 35°C in homeothermic summer- and winter-adapted *N. gouldi*.

This chapter aims to compare the tissue temperatures in summer and winter acclimatized animals and determine whether or not there was a seasonal change in the patterns of body heat distribution indicative of a reduction
in the level of regulated body temperature. The precise relationship between the 'effective' body temperature and rectal temperature of *N. gouldi* is further considered in Chapter Six.

*Nyctophilus gouldi* undergoes a seasonal cycle of body fat accumulation and depletion (Section 2.3.2). Given the potential of white adipose tissue to affect the rate of thermal transfer across the body shell and the thermogenic capacity of brown fat, the distribution of these deposits was examined in *N. gouldi* at a number of times during the year and is described in Section 4.3.1.
4.2 METHODS

4.2.1 Distribution of Adipose Tissue

The distribution of subcutaneous brown and white fat was described from male and female *N. gouldi* specimens taken throughout the year. The pelts were removed leaving the fat intact and sketches made of the dorsal and ventral surfaces.

The interscapular brown fat deposit was removed to enable description of the subscapular and associated fat deposits. Only the brown adipose tissue deposits in the interscapular-jugular net were described as they account for the majority of brown fat in the body (Rauch and Hayward 1969a and b) and are frequently used as an indicator of the seasonal cycle (O'Farrell and Schreiweis 1978; Tidemann 1982).

No attempt was made to quantify the volumes of adipose tissue in any season as this had been done for *N. gouldi* (Inwards 1984, pers. comm., Section 2.3.3).

4.2.2 Measurements of Body Temperatures

The apparatus used in experiments to record the patterns of regional heat distribution was the same as that described for standard determinations of metabolic and rectal temperature responses to ambient temperature (Section 3.2.2).
In addition to a rectal thermocouple, up to six other thermocouples were used to simultaneously monitor the temperatures of body regions (Fig. 4.2). Naked, 38-gauge, copper-constantan thermocouples were subcutaneously implanted in the interscapular, chest and mid-dorsal regions. Small incisions were made in the skin (< two mm long) about one centimetre from the intended point of recording (Fig. 4.2(a)). Bats were lightly anaesthetised, with anaesthetic ether, before thermocouple attachment.

Thermocouples were positioned and secured to the fur near the incision with tissue cement. Naked thermocouples were glued to the dorsal and ventral surfaces of the wing membrane and then covered with adhesive tape. The recording site chosen was the centre of the inner wing membrane; the patagium (Fig. 4.2). This location was chosen so as to permit the bat to fold the wing in a normal manner with the thermocouple in position. All wing recordings were taken from animals with their wings folded. Care was taken to accurately locate the recording site at each measurement occasion.

Body core temperature could not be recorded within the thoracic cavity or central nervous system because of the size of the experimental subject and technical limitations. Instead, the core temperature was measured using a naked, 40 or 42 gauge thermocouple implanted within the peritoneal cavity. This thermocouple was positioned through the ventral body wall at the midpoint of the abdomen and slightly to the left of the midline (Fig. 4.2). A fine gauge silk suture was used to hold the thermocouple firmly in place.
Continuous recordings of body temperatures were taken on a 15-channel Leeds and Northrup multipoint recorder. This device registered the temperature of each body region once per minute. In most cases, only four thermocouples were used in recording from the body: rectal and mid-dorsal with either chest or interscapular and internal or external wing surface. At times, both wing and thoracic sites were monitored simultaneously to examine the patterns of heat distribution within the body more closely (Chapter Eight). At all test temperatures, the rate of oxygen consumption was recorded by the methods described in Section 3.2.2.

Bats were kept indoors and provided with *ad libitum* food and water for the 12 hours preceding an experiment. All experimental animals were otherwise maintained within the outdoor captive colony (Section 3.2.1). Only male *N. gouldi* were used in these experiments. At the time of summer testing females were lactating and therefore disturbed as little as possible. In addition, the seasonal metabolic adaptation was more pronounced in males; especially at ambient temperatures of less than 15°C.

Bats were tested in winter (June and July) and summer (December, January and February). Individuals were placed within the metabolic chamber at an initial ambient temperature of 35°C. Following 90 minutes equilibration, body temperatures and rate of oxygen consumption were recorded for a further 30 minutes. Ambient temperature within the chamber was then reduced by 10°C; a procedure taking 30 to 45 minutes. After another 90 minute
stabilization period, 30 minute recordings were taken before again reducing the chamber temperature by 10°C. This procedure was repeated until stable 30 minute recordings were made at ambient temperatures of 35, 25, 15 and 5°C. Only one bat was tested per day, and provided that the animal remained homeothermic and inactive when cold-exposed, it was possible to test at all four ambient temperatures within a 10 hour daylight period. Instances of torpor are reported in Chapter Eight.

4.2.3 Statistical Methods

Stable, 25 minute recordings were taken from experimental animals at each ambient temperature. The temperature of each body region at five minute intervals within the recording period was used for statistical comparison. The first and last (at time zero and at 25 minutes) temperature observations were discarded leaving four recordings of each body temperature for each experimental animal in summer and winter.

Two-level nested analysis of variance (Biometry Statistical Package) was used to compare the body temperature data from summer- and winter-adapted *N. gouldi*. The test was also used to compare the thermal responses of different body regions within each season. Computations were made on the Univac 1100 of the Australian National University.

The two-level nested ANOVA allows the one-step statistical comparison of data collected in the manner described above. The test compares variances within and
between experimental treatments. Analysis is carried out in an hierarchical manner: testing firstly within each treatment to determine whether the responses of each animal varied significantly more than the mean responses of the experimental group, before proceeding to compare the mean response in each season.
4.3 RESULTS

4.3.1 Distribution of Adipose Tissue

The only brown adipose tissue deposits considered were those of the thoracic and cervical regions. This included five of the 13 brown fat deposits described by Rauch and Hayward (1969a) for the hibernating bat, *Myotis lucifugus*: interscapular, subscapular, clavoscapular, jugular and carotid. Figure 4.3 illustrates these major brown fat deposits in a female *N. gouldi* in July.

To confirm the presence or absence of brown fat throughout the year, 15 males and 18 females were examined. Brown fat was always present in the interscapular and jugular regions, although a marked seasonal cycle was apparent. By visual assessment, lactating females (December n = 6 and January n = 2) had the least brown adipose tissue in these body regions.

Little or no subcutaneous white fat was observed in *N. gouldi* in September (n = 2), October (n = 2), November (n = 5), December (n = 7) or January (n = 3). From February through to August (n = 14 in total) white adipose tissue was present on the dorsal and ventral body surfaces and surrounding the base of the skull (Fig. 4.4).

These findings are consistent with the body fat cycle for this species reported in Section 2.3.3 (Fig. 2.6). Females and males were leanest in January; lipids constituting 5.7 and 6.3 per cent of body mass, respectively. In February when white subcutaneous fat was first deposited,
total body lipids almost doubled to 11.9 per cent of body mass in females and 11.0 per cent in males. The maximum level of body fats was achieved in April (22.8% in females and 17.2% in males) and thereafter the fat reserves were gradually depleted throughout the hibernation season and attained the summer basal level in September.

4.3.2 The Temperatures of Body Regions in Winter and Summer

In homeothermic, male *N. gouldi* there was a consistent pattern of regional heterothermy or peripheral body cooling as the environmental temperature within the metabolic chamber was reduced. The mean temperatures of each body region monitored, in winter and summer, are given in full in Table 4.1 and presented graphically in Fig. 4.5. The statistical comparison of body temperature data has been tabulated and is presented in Tables 4.2 to 4.9.

The sample sizes at an ambient temperature of 5°C were generally less than at warmer *T_A*’s (Table 4.1) because of the propensity of bats to enter torpor when *T_A* was cooled below 15°C. It was not possible to compare the intraperitoneal temperature in different seasons as the thickness and texture of the abdominal subcutaneous white fat (Section 4.3.1) prevented the implantation of the thermocouple into the abdominal cavity in winter.

The regional heterothermy observed in *N. gouldi* involved all of the body parts monitored, including the abdominal cavity (Fig. 4.5). Because the abdominal cavity is
not maintained at a constant temperature it cannot be considered part of the body core except at or near thermal-neutrality ($T_A = 35°C$, Section 3.3). At the thermal-neutral temperature, all body regions, including the wings, had mean temperatures within the range 34.5-38.0°C. A body core or deep body temperature of 37-38°C is indicated, given that at thermal-neutrality the mean shell temperature of the thoracic zone was 36.8°C and within the abdominal cavity it was 37.8°C (Table 4.1).

At the four ambient temperatures tested, the mean subcutaneous interscapular and chest temperatures of male *N. gouldi* were not significantly different in winter and summer (Table 4.2). At temperatures below the thermal-neutral point, a thorax to abdomen temperature gradient was present. The abdominal shell temperatures, as indicated by the recordings from the subcutaneous mid-dorsal region, were significantly less than those of the chest (and by inference the interscapular) region (Table 4.3). One exception to this was at an ambient temperature of 25°C in summer bats where the mean mid-dorsal and chest temperatures were not significantly different (Table 4.3). Small sample sizes, and in some cases variable data, combined to yield such anomalous results. The approach adopted to overcome this problem was that unless the mean temperatures of a body region were significantly different for at least two of the four ambient temperatures, then no seasonal change was considered to have occurred.

As ambient temperature was reduced from 35°C to 5°C the thoracic-abdominal thermal gradient expanded (Fig. 4.5).
from less than one degree to six or seven degrees. The external surface of the wing membrane was the coolest body region at all $T_A$'s less than 35°C (Fig. 4.5). The internal or ventral surface of the wing was generally warmer than the external; however, there was no significant difference in the mean temperature of either wing surface (Table 4.4). Both wing temperatures were extremely variable and precipitous rises and falls were common (Section 8.7.2).

The temperatures of body regions monitored in normothermic *N. gouldi* remained generally unchanged from summer to winter. In no instance was the mean temperature of a body region significantly different at all ambient temperatures in winter and summer. Despite the greater volume of interscapular brown fat in winter *N. gouldi* there was no seasonal change in the temperature of this body region (Table 4.5). Equally, the autumn deposition of an abdominal white fat layer (Section 4.3.1) did not alter the subcutaneous temperature of the mid-dorsal recording site (Table 4.7).

The seasonal comparison of body temperatures presented here does not support the findings of Chapter Three where winter-adapted *N. gouldi* regulated mean rectal temperatures two or three degrees less than in summer. None of the other body temperatures measured in male *N. gouldi* indicated a winter reduction in generalized body temperature.
Regional heterothermy is a well documented heat conservation mechanism in aquatic, semi-aquatic and polar homeotherms (Scholander, Hock, Walters, Johnson and Irving 1950; Irving and Krog 1955; Hammel 1968; Henshaw 1972; Baust and Brown 1980). By vasoconstricting and therefore cooling the peripheral body layers, the thermal gradient between the environment and the primary site of heat loss is reduced, effectively conserving thermal energy.

Regional heterothermy occurred in endothermic *Nyctophilus gouldi* at all ambient temperatures below thermal-neutrality. Once ambient temperature fell below 35°C, a thorax-abdomen-wing thermal gradient was established. The thermal separation between these three major body divisions increased as cooling of the environment occurred. At an ambient temperature of 5°C, the subcutaneous thoracic region was generally 6 to 7°C warmer than the abdominal shell and 20°C warmer than the wings. Rectal temperature was always intermediate between the shell temperatures of the thorax and abdomen.

The primary aim of the experimental work described here was to compare the temperatures of a number of body regions in summer and winter-adapted *N. gouldi*. In winter, *N. gouldi* regulated mean rectal temperatures 2 to 3°C lower and required 10 to 20 per cent less thermal energy for temperature regulation than in summer indicating that winter adaptation was in the form of lower generalized body temperature or at least an expansion of the thermal
gradient between thorax and abdomen. The seasonal comparison of body temperatures presented here does not support either hypothesis. Apart from the rectum, no body region showed any significant seasonal change in regulated temperature suggesting that winter adaptation was not manifested by a reduction in the mean body temperature. Despite the lower mean rectal temperatures of winter acclimatized *N. gouldi* there was no seasonal change in the abdominal shell (mid-dorsal) temperature, indicating that the thermal gradient between thorax and abdomen did not change.

The temperature records made for other body regions in winter and summer do not support the rectal temperature findings; however, throughout this series of experiments the lability of all body temperatures was at times extraordinary (Section 8.3.5) and indicative of a precisely controlled and versatile system of body temperature regulation. Although the winter reduction in rectal temperature was not concomitant with a reduction in the temperatures of other body regions, considerably more rectal temperature measurements were made, engendering more confidence in these results. A seasonal change in the regulated body temperature does not preclude a summer to winter adjustment to the rate of heat transfer through the skin and fur layer. In Chapter Five the insulative properties of *N. gouldi* fur in winter and summer are considered.
Figure 4.1 Diagrammatic representation of the body layers present in a bat
(modified from Tracy 1972).

* indicate sites of temperature recording in regional heterothermy experiments.
Figure 4.2  The locations of thermocouple recording sites used in regional heterothermy experiments. In Section 4.2.2 the methods of thermocouple attachment are described.

(a) shows a schematic of a sub-cutaneously implanted thermocouple.
Figure 4.3  The major brown fat deposits of a female _N.gouldi_ in winter (July). In the dorsal view the left-hand interscapular deposit has been removed to show the dorsal portion of the jugular deposit.
Figure 4.4  Dorsal and ventral views of an *N. gouldi* in winter showing the distribution of subcutaneous white and brown adipose tissue.
Figure 4.5 The temperatures of six body regions in summer- (○) and winter-adapted (●), male N. gouldi at ambient temperatures of 5, 15, 25 and 35 °C. All values are means ± one s.d. The recording sites are diagrammatically represented in Fig.4.2. Actual values and sample sizes are given in Table 4.1 and the results of the statistical comparisons of summer and winter means are presented in Tables 4.5 to 4.9.
Table 4.1. The mean temperatures of body regions in endothermic, male, *N. gouldii* at ambient temperatures of 35, 25, 15 and 5°C in summer and winter. The recording sites are shown in Figure 4.1 and the experimental methods described in Section 4.2.

All values are given as mean ± one standard deviation, number of bats used. The number of observations used to calculate the mean is four times the number of bats (Section 4.2.3), enabling standard deviations to be presented when only one or two animals were tested.

Rectal temperature values do not show standard deviations or sample sizes as they were calculated by substitution into the TR curves described in Section 3.3.

<table>
<thead>
<tr>
<th>Body Region</th>
<th>Season</th>
<th>Ambient Temperature (°C)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>35</td>
<td>25</td>
<td>15</td>
<td>5</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peritoneal</td>
<td>Summer</td>
<td>37.7 ± 0.5, 4</td>
<td>35.0 ± 0.9, 5</td>
<td>33.5 ± 2.1, 3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>38.0 ± 0.2, 1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Interscapular</td>
<td>Summer</td>
<td>36.6 ± 0.8, 4</td>
<td>34.4 ± 1.7, 6</td>
<td>33.3 ± 2.2, 5</td>
<td>31.2 ± 0.4, 1</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>36.8 ± 0.5, 5</td>
<td>34.6 ± 1.7, 6</td>
<td>35.7 ± 2.2, 3</td>
<td>34.3 ± 2.8, 4</td>
</tr>
<tr>
<td>Chest</td>
<td>Summer</td>
<td>37.3 ± 0.7, 3</td>
<td>34.4 ± 0.6, 5</td>
<td>34.0 ± 1.7, 4</td>
<td>31.5 ± 0.9, 3</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>36.6 ± 0.9, 5</td>
<td>34.1 ± 1.1, 6</td>
<td>34.7 ± 0.6, 4</td>
<td>34.2 ± 0.6, 3</td>
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<td>Rectal*</td>
<td>Summer</td>
<td>37.8</td>
<td>34.5</td>
<td>32.8</td>
<td>32.8</td>
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<td></td>
<td>Winter</td>
<td>35.5</td>
<td>32.4</td>
<td>31.1</td>
<td>31.6</td>
</tr>
<tr>
<td>Mid-dorsal</td>
<td>Summer</td>
<td>36.3 ± 1.4, 4</td>
<td>32.3 ± 2.2, 6</td>
<td>27.9 ± 3.6, 5</td>
<td>24.8 ± 0.2, 1</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>36.0 ± 0.8, 4</td>
<td>31.5 ± 1.7, 5</td>
<td>28.6 ± 1.9, 4</td>
<td>28.6 ± 2.0, 3</td>
</tr>
<tr>
<td>Internal wing-surface</td>
<td>Summer</td>
<td>36.4 ± 0.9, 4</td>
<td>30.9 ± 1.3, 5</td>
<td>27.0 ± 2.5, 4</td>
<td>20.2 ± 3.2, 2</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>34.6 ± 0.8, 4</td>
<td>30.2 ± 0.6, 5</td>
<td>26.6 ± 1.1, 4</td>
<td>22.4 ± 1.8, 3</td>
</tr>
<tr>
<td>External wing-surface</td>
<td>Summer</td>
<td>35.7 ± 0.9, 5</td>
<td>28.6 ± 1.2, 6</td>
<td>23.8 ± 1.6, 4</td>
<td>17.4 ± 5.1, 2</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>35.2 ± 1.0, 6</td>
<td>30.0 ± 0.5, 7</td>
<td>25.1 ± 1.1, 5</td>
<td>20.3 ± 1.4, 4</td>
</tr>
</tbody>
</table>
The following tables: 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8 and 4.9 are the results of the two-level nested ANOVA's (section 4.2.3) carried out to compare the mean temperatures of five body regions in male *N. gouldi* in winter and summer. In all cases, the information has been presented in a standard manner.

**Among:** refers to the comparison between treatments; for example summer versus winter.

**Within:** refers to the comparison of the variation of the data within the group of replicates constituting a treatment.

The details of recording sites and experimental protocol are given in Section 4.2. Sample sizes and mean temperatures for each body region are presented in Table 4.3.

Table 4.2 compares the subcutaneous interscapular and chest temperatures in winter and summer *N. gouldi*.

Table 4.3 compares chest and mid-dorsal temperatures in winter and summer animals and Table 4.4 compares the mean temperatures of the internal and external wing-surfaces in both seasons.

Tables 4.5, 4.6, 4.7, 4.8 and 4.9 present the results of the winter/summer comparison of the mean temperatures in each of the five body regions: interscapular, chest, mid-dorsal, internal wing-surface and external wing-surface, respectively.
Table 4.2 Statistical comparison of the mean interscapular and chest temperatures in winter and summer-adapted, male, *N. goulii*.

<table>
<thead>
<tr>
<th>Ambient Temperature (°C)</th>
<th>Level</th>
<th>Degrees of Freedom</th>
<th>F-value</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Winter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Among</td>
<td>1</td>
<td>0.003</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>5</td>
<td>165.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Among</td>
<td>1</td>
<td>1.47</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>5</td>
<td>62.84</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Among</td>
<td>1</td>
<td>0.31</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>10</td>
<td>298.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Among</td>
<td>1</td>
<td>0.82</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>9</td>
<td>1.02</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Summer</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
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Table 4.3 Statistical comparison of the mean chest and mid-dorsal temperature in winter and summer-adapted, male, *N. gouldi*.

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<th>Probability</th>
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Table 4.4  Statistical comparison of the mean internal and external wing surface temperatures in winter and summer-adapted, male, N. gouldi.

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<th>Probability</th>
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Table 4.5. Statistical comparison of the mean interscapular temperature in winter and summer-adapted, male *N. gouldi*.

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<th>Probability</th>
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</tr>
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<td></td>
</tr>
<tr>
<td>25</td>
<td>Among</td>
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<td>Among</td>
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Table 4.6. Statistical comparison of the chest temperature in winter and summer-adapted, male *N. Gouldi*.

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Table 4.7. Statistical comparison of the mean mid-dorsal temperature in winter and summer-adapted, male *N. gouldi*.

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Table 4.8. Statistical comparison of the mean internal (ventral) wing-surface temperature in winter and summer-adapted, male *N. gouldi*.

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</tr>
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<td></td>
</tr>
<tr>
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<td>Among</td>
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<td>1.79</td>
<td>&gt;0.2</td>
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<td>&lt;0.001</td>
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</tr>
<tr>
<td>35</td>
<td>Among</td>
<td>1</td>
<td>0.51</td>
<td>&gt;0.5</td>
</tr>
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<td></td>
<td>Within</td>
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<td>&lt;0.001</td>
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Chapter Five

THE INSULATIVE VALUE OF *NYCTOPHILUS GOULDI* FUR
IN SUMMER AND WINTER

<table>
<thead>
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<th>Section</th>
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<tbody>
<tr>
<td>5.1 Introduction</td>
<td>126</td>
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<tr>
<td>5.2 Methods</td>
<td>127</td>
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<td>131</td>
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<td>5.4 Discussion</td>
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5.1 INTRODUCTION

The insulative effectiveness of fur improves with thickness, however, small mammals must compromise the insulative value of the outer covering against the cumbersome nature of very thick fur (Hart 1956). Hart (1956) points out that as a result of small body size, the small mammals have potentially "... the greatest metabolic response to temperature change ..." yet "... only limited possibilities for seasonal insulative change".

Apart from Hart's 1956 paper where he compared the seasonal insulative changes in the pelts of a number of Arctic and northern temperate animals, very few studies have attempted to measure the effective insulation of mammal fur (Dawson and Webster 1967; Hulbert and Dawson 1974a). In a departure from this trend, Shump and Shump (1980) compared the insulative properties of summer and winter furs from five North American vespertilionid bats and related their findings to differences in the species' roosting habits.

In winter-adapted Nyctophilus gouldi, homeothermic temperature regulation required 10 to 20 per cent less thermal energy than in summer (Chapter Three). Despite lower mean rectal temperatures of winter bats there was no significant change in the peripheral tissue temperatures regulated by N. gouldi, suggesting that the thermal economy in winter animals may not have resulted from increased regional heterothermy. The next logical step to take was to compare the insulation of summer and winter N. gouldi pelts.
5.2 METHODS

5.2.1 Experimental Procedures

Pelts were prepared from freshly killed *N. gouldi* in winter (June and July, 5 males, 2 females) and summer (December, 5 males and 5 females). The pelts were pinned flat and their outlines immediately traced to allow estimates of surface area (Section 6.2.1). Following this procedure, the furs were air dried for seven to ten days, passed through a standard series of chloroform washes to remove fat and stored in a dessicating chamber containing silica gel.

Figure 5.1 illustrates the equipment used to measure the rates of thermal transfer through the furs. Three circular heat-flow transducers (Thermonetics Co-operation), 13 millimetres in diameter, embedded in thin brass plate, were used to measure the rate of heat loss from an underlying water cell through the pelt to the environment.

Water circulated through the brass cell at 38°C (± 0.5°C). Calibrated thermocouples recorded the temperature of the inlet and outlet water and the rate of water circulation was adjusted to maintain temperature equivalence between the two recording points. The water cell was totally encased in 5 cm thick polystyrene foam except for the surface carrying the fur under test. The foam encasing the sides and base of the apparatus was specifically intended to direct heat loss from the circulating water solely through the surface carrying the heat-flow cells and test fur.
The device employed was similar to that used by Grant and Dawson (1978), Hulbert and Dawson (1974a) and Dawson and Fanning (1981) to measure the insulative properties of pelts from much larger animals such as the platypus (*Ornithorhynchus anatinus*) and the water rat (*Hydromys chrysogaster*). The small size of the bat pelts prevented the use of the conventional guard ring to clamp the fur to the perimeter of the recording surface (Fig. 5.1). A perspex guard ring with three apertures of the same diameter as the heat flow discs was used instead. The larger brass guard ring was used to apply uniform pressure to the outer edge of the perspex ring. Tests for edge loss, as previously described by Dawson and Webster (1967), were carried out and indicated an insignificant error resulting from this factor. The thickness and composition of the perspex guard ring was the same as that used to calibrate the heat flow discs (see below).

All pelts were "fluffed", using an air brush, prior to attachment to the equipment and the process was repeated following positioning of the perspex guard ring. To reduce the effects of air turbulence above and within the fur, a cylindrical, perspex cover (10 cms. high with a diameter of 10 cms.) closed at the top was placed over the recording surface. Numerous small holes in the cover permitted rapid equilibration to each ambient temperature.

The complete unit was placed within an environmental cabinet. Heating elements and injected carbon dioxide controlled the temperature within the chamber. An
additional aluminium sheet, 30 cms. square, was placed on top of the perspex turbulence cover to act as a baffle to the carbon dioxide injected during cooling.

A digital thermometer with 38 gauge copper-constantan thermocouples was used to simultaneously monitor the temperature of:

i. the inlet and outlet water;

ii. the environment, two cms. above the fur; and

iii. under the pelt, between the skin and the heat flow disc.

The thermocouples were calibrated in the manner described in Section 3.2.2.

The three heat flow discs were connected in series and the output signal amplified 100-fold by a Grass P18 amplifier (accuracy ± 5% full scale). Continual recordings of these signals were made on a Brush recorder. The stated accuracy of the heat flow transducers was ± 10 per cent.

A calibration curve relating heat flow in Watts/m²/°C against the millivolt output from the amplifier was calculated using a 3.16 mm (+ 0.02 mm, SD, n = 5) thick perspex sheet with a known thermal conductivity constant (K) of $5.0 \times 10^{-4}$ calories/cm/sec/°C. The regression coefficient for the calibration curve was 0.97.
Thermal insulation was calculated using the formula:

\[ I = \frac{T_{Sk} - T_A}{Q/T} \]

where:  
- \( I \) = insulation in W\(^{-1}\)m\(^2\).°C  
- \( T_{Sk} \) = subcutaneous skin temperature in °C  
- \( T_A \) = ambient temperature in °C and  
- \( Q/T \) = heat flow per unit time in W/m\(^2\)/hr/°C

To gain measurements of the thermal loss through the fur, each fur was tested at an ambient temperature of 15°C. Equilibration normally took 30 minutes. The rate of heat flow was measured at the single ambient temperature of 15°C (+ 1°C) to avoid problems associated with the non-linear responses of the transducers at different environmental temperatures.

The thermal properties of dorsal and ventral surface fur could not be separately tested because of the small size of the bat pelts. The rate of heat flow through wing membrane samples was also measured using the same apparatus and experimental procedures. The wing membranes of four males killed in summer (January) were pinned out and dried flat at room temperature for 21 days before testing.
5.3 RESULTS

Male *N. gouldi* pelts were, on average, 14.1 per cent more insulative in winter than summer (Table 5.1). This represented an insulative improvement of $0.029 \text{ W}^{-1}\text{.m}^2\text{.}{^\circ}\text{C}$ in the winter fur. The summer to winter change in male fur insulative value was significant at the 0.2 per cent confidence level ($F[1,8] = 22.74$).

There was no significant seasonal change in the insulative value of female *N. gouldi* furs ($F[1,5] = 0.79$, $P = 0.42$) although only two female pelts were obtained in winter and the measured rates of thermal transfer through summer furs were extremely variable. In summer females the mean insulative value of the fur was $0.230 \text{ W}^{-1}\text{.m}^2\text{.}{^\circ}\text{C}$ ($\pm 0.016$, SD; $n = 5$) although values ranged from 0.219 to 0.256 $\text{ W}^{-1}\text{.m}^2\text{.}{^\circ}\text{C}$ (Table 5.1). Summer-adapted females obtained significantly more insulation from their pelts than males ($F[1,8] = 9.19$, $P = 0.02$). Four of the five females sacrificed for their furs in summer were lactating and the fifth did not breed in that season. The insulative value of the non-lactating female's fur was $0.220 \text{ W}^{-1}\text{.m}^2\text{.}{^\circ}\text{C}$ compared with a mean of $0.232 \text{ W}^{-1}\text{.m}^2\text{.}{^\circ}\text{C}$ ($\pm 0.017$, SD; $n = 4$) for lactating animals.

Wing membrane had about one third the insulative value of fur. The mean insulative value of wing membrane taken from male *N. gouldi* in summer was $0.084 \text{ W}^{-1}\text{.m}^2\text{.}{^\circ}\text{C}$ ($\pm 0.004$, SD; $n = 4$).
5.4 DISCUSSION

Large homeotherms show the greatest relative seasonal change in the insulative properties of their pelts. Hart (1956) compared the summer and winter insulative values of the pelts from nine Arctic and northern temperate-zone mammals including the deer mouse (*Peromyscus maniculatus*), the brown lemming (*Dicrostonyx groenlandicus*), the red squirrel (*Sciurus hudsonicus*), the muskrat (*Ondatra zibethica*), the varying hare (*Lepus americanus*), the red fox (*Vulpes fulva*), the wolverine (*Gulo luscus*), the wolf (*Canis lupus*), the black bear (*Euarctos americanus*) and the polar bear (*Thalarctos maritimus*). The maximum winter improvement to fur insulation was 51.7 per cent in the black bear; whilst in the deer mouse it was only 21.4 per cent.

Shump and Shump (1980) compared the insulative properties of summer and winter furs from five North American vespertilionid bats. In three colonial, cave-dwelling species, *Myotis lucifugus*, *M. keenii* and *Eptesicus fuscus*, the insulative effectiveness of the fur improved in winter by 26 per cent. For the two solitary, tree-dwelling species *Lasiurus borealis* and *L. cinereus*, winter samples were not obtained preventing a complete seasonal comparison, however, the insulative values of the *Lasiurus* species furs in summer were similar to those of the three cave-dwelling species in winter.
In *Nyctophilus gouldi*, there was no seasonal change in the fur insulation of females whilst in males, winter pelts were on average 14.1 per cent more insulative than in summer. For *M. lucifugus*, *M. keenii* and *E. fuscus*, Shump and Shump (1980) found no difference in the insulation of male and female pelts and pooled the data for both sexes in their analysis. It was only in summer that the sexual difference in pelt insulation was present in *N. gouldi*, the insulative value of male pelts in winter and female pelts in summer and winter being the same. The more insulative fur of females in summer may be related to lactation and the rearing of young. *Myotis velifer* males moult in spring around the time that females give birth whereas females do not moult until after lactation is completed (Kunz 1974). Four of the five female *N. gouldi* pelts tested in summer were from lactating animals. A more insulative fur, at this time, may help to reduce the thermoregulatory requirements of both the mother and the young. Thermal energy savings during the lactation phase could serve to reduce the greater energy demands placed upon the female by the suckling of young.

The more insulative fur of male *N. gouldi* in winter is consistent with the seasonal metabolic adaptation observed (Section 3.3.1). The 10 to 20 per cent reduction in the thermal energy needs of winter males is readily accounted for by the 14.1 per cent increase in the insulative value of the pelt, although this remains to be established in absolute thermal conductance terms. In female *N. gouldi* the situation is not as clear; the summer to winter metabolic adaptation resulted in reduced thermoregulatory energy...
demands yet fur insulative value did not change seasonally. It must be remembered that the summer metabolic curve was derived from pregnant females (Section 3.2.2) and the majority of females used in the determination of fur insulation were lactating. Clearly, the direct correlation of these two data sets may not be legitimate given the differences in reproductive condition and associated energy needs (Studier and O'Farrell 1972; Inwards 1984 pers. comm.).

In Chapter Six the results which have been presented in the preceding three chapters are used to calculate and compare whole body thermal conductance (or insulation) in summer and winter-adapted *Nyctophilus gouldi*. 
Figure 5.1 An exploded view of the apparatus used to measure the insulative value of *N. gouldi* fur in winter and summer.
Table 5.1. Insulative values of male and female *N. gouldi* furs in summer and winter. All values are given as mean ± one standard deviation in watts·m⁻¹·m²·°C.

The methods used in determining these insulative values are described in section 5.2.1. The results of between-sex and between-season comparisons of these data by one-way ANOVA are shown in the appropriate places in the table. Sample sizes are given below each mean.

<table>
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<th></th>
<th>Summer</th>
<th>Winter</th>
<th>Summer-Winter % Change</th>
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<tbody>
<tr>
<td><strong>Males</strong></td>
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<td></td>
<td>0.206 ± 0.007</td>
<td>0.235 ± 0.011</td>
<td>+14.1</td>
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<td></td>
<td>n = 5</td>
<td>n = 5</td>
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<td></td>
<td>$F(1,8) = 9.19$</td>
<td>$F(1,5) = 3.51$</td>
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<tr>
<td></td>
<td>$p = 0.02$</td>
<td>$p = 0.12$</td>
<td></td>
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<tr>
<td><strong>Females</strong></td>
<td></td>
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<tr>
<td></td>
<td>0.230 ± 0.016</td>
<td>0.220 ± 0.001</td>
<td>-4.3</td>
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<tr>
<td></td>
<td>n = 5</td>
<td>n = 2</td>
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<td>$F(1,5) = 0.79$</td>
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Chapter Six

THE PARTITIONING AND SEASONAL COMPARISON OF BODY INSULATION

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1</td>
<td>Introduction</td>
<td>137</td>
</tr>
<tr>
<td>6.2</td>
<td>Methods</td>
<td></td>
</tr>
<tr>
<td>6.2.1</td>
<td>Measurement of Body Surface Areas</td>
<td>143</td>
</tr>
<tr>
<td>6.2.2</td>
<td>Methods of Calculating and Partitioning Body Insulation</td>
<td>143</td>
</tr>
<tr>
<td>6.3</td>
<td>Results</td>
<td></td>
</tr>
<tr>
<td>6.3.1</td>
<td>Surface Areas</td>
<td>148</td>
</tr>
<tr>
<td>6.3.2</td>
<td>Body Insulation</td>
<td>148</td>
</tr>
<tr>
<td>6.4</td>
<td>Discussion</td>
<td>156</td>
</tr>
</tbody>
</table>
6.1 INTRODUCTION

The principal aim of this Chapter is to bring together the information presented in Chapters Three, Four and Five by partitioning body insulation into tissue and fur components and comparing these findings for summer- and winter-adapted *N. gouldi*. The estimation of thermal conductance is central to the performance of this analysis. Either Newton's Law of Cooling or Fourier's Law of Heat Flow can be used to calculate the rate of thermal conductance from a body to the environment.

Newton's Law of Cooling has been used more extensively in the past than Fourier's method to describe heat loss from endothermic organisms. Claims that the Newtonian equation is too simplistic to accommodate situations where physical heat conserving mechanisms, such as regional heterothermy, reduce the rate of heat loss from the body, have raised doubts about the applicability of the model to the homeothermic system (Kleiber 1972). Strunk (1971) states that "... the conditions necessary for true Newtonian cooling to occur are unrealistic for an animal ...". The use of Newton's Law assumes that the body of the animal in question has uniform temperature throughout and "... that virtually all of the resistance to heat flow is confined to the boundary layer ..." (Strunk 1971). The same considerations were stated by Kleiber (1972) but in another way: the "... newtonian animal ..." is "... a body with a material of sufficiently high heat conductivity that the difference between the temperature at the centre and at the surface is negligible in comparison with the temperature
drop from surface to ambient temperature ...". This condition holds for very few cold-exposed homeotherms.

In most homeotherms, Newton's Law can only validly describe heat transfer across the boundary layer from body surface to environment (Strunk 1971). McNab (1980) recognised the same limitations to the use of Newton's Law by suggesting that the most appropriate body temperatures to use in the calculation of thermal conductance were "... skin surface temperature or fur surface temperature ...". In most studies, the collection of such data is impractical and rectal or colonic temperature is substituted into the Newtonian equation (see below). This practice is generally unacceptable because the assumption that the temperature of the major heat exchange site, the skin, is the same as the rectal region does not always hold.

The form of Newton's Law of Cooling commonly used by biologists and presented in the literature is:

\[
M = C \left( T_B - T_A \right) + EHL
\]

where

- \( M \) = rate of heat production or loss
- \( C \) = the proportionality constant, thermal conductance
- \( T_B \) = body temperature (usually measured rectally or colonically)
- \( T_A \) = ambient temperature
- \( EHL \) = evaporative heat loss
The heat flow from an endothermic organism to the environment is more appropriately described by Fourier's Law of Heat Flow (Strunk 1971; Kleiber 1972). In 1932, Kleiber used a simplified form of Fourier's equation to describe the heat flow from a homeotherm.

\[ \frac{dQ}{dt} = \frac{S(T_B - T_S) + EHL}{L} \]

where \( \frac{dQ}{dt} \) = rate of heat production = thermal conductivity constant

\( S \) = surface area of the insulating layer surrounding the body core

\( L \) = the thickness of the insulating layer surrounding the body core

\( T_B \) = body core temperature

\( T_S \) = temperature of the body surface

\( EHL \) = evaporative heat loss.

Although more appropriate for homeotherms than Newton's Law, the simplified Fourier model is difficult to apply because in addition to the body core temperature, measurements of the surface area, thickness and external temperature of the insulating layers are required. By strict definition, rectal or colonic temperatures should only be used in calculations of thermal conductance using the Fourier method and when it is known that they closely approximate or equal body core temperature.

Whole body thermal conductances calculated using the Newtonian method frequently include a surface area component (for example, Hulbert and Dawson 1974a),
presumably in recognition that most thermal exchange with the environment occurs through the skin. Dawson and Fanning (1981) added a heat storage parameter in their calculations of thermal conductance (see below) for the water rat, *Hydromys chrysogaster*, to accommodate situations when body temperature did not remain constant:

\[
C = \frac{HP - EHL + HS}{(T_B - T_A)SA}
\]

where: \(C, EHL, T_B, T_A\) and \(SA\) are given above and

- \(HP\) = rate of heat production
- \(HS\) = rate of heat storage

McNab (1980) criticizes the calculation of surface area specific values of thermal conductance, primarily on the grounds that the units become "... dimensionally inconsistent ..."; that is, the product of thermal conductance and the temperature differential does not equal heat production. McNab (1980) also rightly states that area-specific values of thermal conductance should be calculated using effective not actual surface area and that the former is "... nearly impossible ..." to measure.

For a bat at rest, McNab's comments about actual and effective surface area are especially pertinent. The total surface area of *N. gouldi*, for example, is 85 to 90 per cent naked membrane (Fig. 6.1; Table 6.1) yet when inactive, the wings, tail and ears are folded and it would be naive to suggest that actual surface area even closely
approximated effective surface area. However, the inclusion of surface area in the calculation of thermal conductance is essential if the intention is to partition the avenues of heat loss into specific body regions. In such cases, heat loss from a nominated body region is determined by the thermal constant of the fur or tissue, the thermal gradient established across the layer and the surface area effectively engaged in thermal exchange with the environment.

Two fundamental problems must be considered when attempting to partition the pathways of heat loss and thereby calculate the rate of whole body thermal conductance of a bat.

1. Unlike most endotherms, the outer body layer does not have a uniform coefficient for thermal exchange. The wing membrane of *N. gouldi* transfers heat 2.5 to 3.0 times faster than the skin and fur layer (Section 5.3).

2. The portion of the bat's external surface with the greatest potential to lose heat, namely the naked membranes, constitutes 85 to 90 per cent of the total surface area. To assume equivalence between effective and actual surface area in the calculation of area-specific thermal conductance could potentially introduce large scale errors and biased conclusions.

As the partitioning of body insulation is primarily concerned with the boundary layers of the animal,
Newton's Law of Cooling was used to calculate the rates of thermal conductance for *N. gouldi* in summer and winter. The thermal gradients established across these interfaces in summer- and winter-adapted males were reported in Chapter Four and the thermal constants of fur and wing membrane were presented in Chapter Five. To enable the partitioning of heat loss pathways it was necessary to determine the relative surface areas of furred and naked body regions (Section 6.2.1). Provided with this information, the final question to be considered was, could the improved insulative properties of winter pelts account for the reduction in the metabolic costs of homeothermic temperature regulation in winter-adapted *N. gouldi*?
6.2 METHODS

6.2.1 Measurement of Body Surface Areas

The total surface areas of male and female *N. gouldi* were measured from tracings of the naked membranes and furred body parts. Bats killed for the description of fat distribution (Section 4.2.1) or removal of reproductive tracts (Section 2.4) were pinned to polystyrene foam and paper, with wings expanded, and the outlines of the wings, tail and ears traced. The pelts were removed after cutting around the perimeter of the furred body portion and pinned out in a similar manner to the wings, ears and tail. Tracings were taken from freshly removed pelts to avoid errors associated with drying and shrinkage. Care was taken to avoid excess stretching of the furs and consequent over-estimation of the furred surface area.

The areas of the tracings were estimated using a digitiser and Intecolor 8001 (Intelligent System Corporation) desk-top computer. The stated accuracy of the measuring system was ± 5 per cent.

6.2.2 Methods of Calculating and Partitioning Body Insulation

Thermal conductance (C) values were calculated for *N. gouldi* using the same equation as Dawson and Fanning (1981):
\[ C = \frac{\text{Heat Production} - \text{EHL} \pm \text{Heat Storage}}{(T_B - T_A)\text{Surface Area}} \]

No attempt was made to measure evaporative heat loss in this study and as such all calculations of thermal conductance must be considered as 'wet'. As ambient temperature falls below thermal-neutrality, evaporative heat loss becomes relatively insignificant and usually accounts for between 5 and 15 per cent of total heat losses (McNab 1980 cites a number of cases).

The heat storage parameter was not considered in any of the calculations that follow. Nadel (1977) points out that when the body temperature of an animal achieves a "thermal steady state", the rate of heat storage is zero and therefore does not influence the rate of thermal conductance. All recordings of homeothermic body temperature made in this study were from inactive animals in thermal steady state.

For *Nyctophilus gouldi*, separate thermal conductance values were calculated for the furred body regions of the thorax and abdomen and the tissue of the naked membranes. The distinction between the furred regions of the abdomen and thorax was made because of the thorax to abdomen thermal gradient observed in all homeothermic *N. gouldi* (Section 4.3.2). At all ambient temperatures below thermal-neutrality, the rectal and subcutaneous mid-dorsal regions were cooler than the subcutaneous thoracic zones (Table 4.3).
The head was considered part of the thorax in all calculations of thermal conductance because no measurements of subcutaneous head temperature were taken. The assumption was also made that the surface area of the abdomen and the thorax (including the head) equalled half of the furred surface area (Table 6.1). The additional assumption was made that the thermal properties and regulated temperatures of the uropatagium and ear membranes were the same as those recorded for the wing.

Females were not considered in the calculations of thermal conductance as they had been excluded from the description of regional body temperatures (Chapter Four); information that was central to performing the analysis. Although it is not unreasonable to assume that female subcutaneous and wing membrane temperatures were the same as those of males at all times of the year, only the male data were used to describe the seasonal changes in body insulation. Given conclusive findings as to the form of the seasonal adaptation in male *N. gouldi*, the extrapolation to the female case was considered more appropriate than assuming equivalent regulated subcutaneous temperatures.

The mean temperatures of each body region at ambient temperatures of 5, 15, 25 and 35°C were obtained from linear regression equations calculated from the data presented in Chapter Four. Summer and winter means for each animal were combined as there were no significant seasonal changes in the temperatures of the five body regions.
monitored (Section 4.3.2); namely, subcutaneous chest, interscapular and mid-dorsal regions and the internal and external wing-surfaces. As there was no significant difference in the temperature of the chest and interscapular regions in summer or winter (Table 4.2) these data were combined to yield a regression equation describing mean subcutaneous thoracic temperature. The mid-dorsal recordings were used as a measure of the abdominal shell temperature. A linear regression of mean wing membrane temperature against ambient temperature was calculated from the combination of the measured internal and external wing responses. There was no difference in the temperatures of the inner and outer wing-surfaces in winter or summer. A linear regression equation was also calculated for peritoneal temperature against ambient temperature. This was for animals at ambient temperatures of 15, 25 and 35°C only and with one exception (a male at a \( T_A \) of 35°C in winter) the data came from animals in summer.

The best indication of deep body temperature was given by the temperature of the abdominal cavity at the point of thermal-neutrality (\( T_A = 35°C \)). In summer the mean temperature within the peritoneal cavity was 37.7°C (± 0.5°C, SD, \( n = 4 \)) and in one male in winter a recording of 38.0°C was registered. In all calculations which follow the assumption has been made that the deep body temperature of \( N. gouldi \) was maintained at a constant 38.0°C in homeothermic animals, at all ambient temperatures and at all times of the year. Apart from the convergence of all body temperatures towards 38.0°C at or near thermal-neutrality
(Section 4.3.2) little justification exists for this assumption beyond the expectation that at thermal-neutrality the body core should include the abdominal viscera.

The total body surface area of selected males and females of similar weight and skin temperature. Data were from adults. The venous blood volume was average 33% per cent. 0.2 per cent. The total body water volume was significantly larger in males (0.01) vs. females (0.001), and significant at (0.001) and (0.001) than in males (0.01). Although there is no significant difference in the venous (0.001) vs. females (0.001) and total water (0.001) of males and females.
6.3 RESULTS

6.3.1 Surface Area

The total body surface areas of male and female *N. gouldi* are presented in Table 6.1. Primarily because of their wing and tail membranes, bats have total surface areas approximately six times greater than similar body weight 'wing-less' mammals (Appendix I). The wing and tail membranes of male *N. gouldi* account for 76.9 per cent and 7.3 per cent, respectively, of the total body surface area (Fig. 6.1). Female *N. gouldi* have significantly larger wings ($F[1,50] = 9.60$, $p = 0.003$) and uropatagium ($F[1,50] = 4.51$, $p = 0.036$) than males (Table 6.1; Fig. 6.1), although there is no significant difference in the ear ($F[1,16] = 0.63$, $p = 0.45$) and furred surface areas ($F[1,16] = 0.77$, $p = 0.40$) of males and females.

6.3.2 Body Insulation

The regression equations describing the mean temperature of the thoracic and abdominal shell, the wing membrane and the peritoneal cavity are presented in Figure 6.2. Rectilinear models were fitted to these data although it is likely that curvilinear responses, such as those recorded for the rectal temperature (Section 3.3), would have been noted if measurements had been taken at ambient temperatures intermediate to 5, 15, 25 and 35°C. The slope of the wing membrane curve (0.51) was greater than the other three body regions (thoracic shell, 0.10; peritoneum or abdominal core, 0.22; abdominal shell, 0.33) indicating the greatest dependence upon ambient temperature.
These regression equations enabled the calculation of mean subcutaneous and surface temperatures for the three body surface components: furred thorax, furred abdomen and and naked membrane, at ambient temperatures of 5, 15, 25 and 35°C. The rate of thermal conductance across each of these body surfaces was calculated using Newton's Law of Cooling. A sample calculation is shown below:

Example: Partitioning of Heat Loss

At an ambient temperature of 15°C in winter, male *N. gouldi* weighed, on average, 12.1 grams and produced 35.99 Watts/kg. to maintain constant body temperature.

That is, the heat produced = \(3.599 \times 10^{-2}\) Watts/g. and therefore, the whole animal produced 0.4355 Watts of thermal energy.

Assuming thermal steady state, heat produced equalled heat lost, therefore, heat lost to the environment = 0.4355 Watts also.

In the thoracic region, mean shell temperature \(\bar{T}_{Sk}\) was 34.0°C at an ambient temperature of 15°C (Fig. 6.2), that is \((\bar{T}_{Sk} - T_A) = 19\)°C. The surface area of the thorax = \(
\frac{\text{total furred surface area}}{2}\)

\[= \frac{39.6}{2} = 19.8 \text{ cm}^2\]
The insulative value of male fur in winter was 0.235 W m$^{-1}$.°C, that is, the rate of thermal conductance through the fur was \( \frac{1}{0.235} = 4.255 \text{ W/m}^2/°\text{C} \)

or \( 4.255 \times 10^{-4} \text{ W/cm}^2/°\text{C} \)

now substituting in \( C = \frac{\text{HL}}{(\overline{T}_{sk} - T_A)\text{SA}} \)

which rearranges to \( \text{HL} = C(\overline{T}_{sk} - T_A)\text{SA} \) (1)

At this point the assumption was made that by comparison with the folded membranes, the actual surface area of the furred body parts was an acceptable (or at least more acceptable) estimate of effective surface area. Thus, actual furred surface area was substituted in (1) to yield an estimate of thermal loss from the thoracic shell. The validity and implications of this assumption are discussed in Section 6.4.

Substituting in (1)

Heat loss thorax = \( 4.25 \times 10^{-4} \times (19.0) \times 19.8 \)

= 0.160 W.

Similar logic was applied to calculate the heat loss through the abdominal shell. In this case, mean abdominal shell temperature was 29.2°C (Fig. 6.2) and therefore

\( (\overline{T}_{sk} - T_A) \text{ abdomen} = 14.2°C \)

again substituting in (1)

Heat loss abdomen = \( 4.25 \times 10^{-4} \times (14.2) \times 19.8 \)

= 0.119 W.
By addition, $0.160 + 0.119 = 0.279$ W out of a whole body total of 0.4355 W were lost through the furred body region of male *N. gouldi* at a $T_A = 15^\circ$C in winter. By inference, the remaining 0.156 W passed from the body to the environment via the naked membranes.

For the membranes \((\bar{T}_{Sk} - T_A)_{membrane} = 10.4^\circ$C \)

and \(C = 12.05$ W/m$^2$/°C \) (Section 5.3.1) \(= 1.205 \times 10^{-3}$ W/cm$^2$/°C

by substituting in (1)

\[0.156 = 1.205 \times 10^{-3}(0.4).SA\text{ effective}\]

ie. \(SA\text{ effective} = 12.4$ cm$^2$ compared with an actual surface area of 282.8 cm$^2$.

Table 6.2 presents heat loss partitioned in the manner shown above for males in summer and winter and at $T_A$'s of 5, 15, 25 and 35°C. These data are presented graphically in Figure 6.3.

There was little seasonal variation in the proportion of heat lost from each of the three body regions; thorax, abdomen and naked membranes (Table 6.2), although, the pathways of heat loss were considerably different at the four ambient temperatures. The poorer insulative properties of the naked membrane were offset by the greater tendency of the tissue temperature to conform with $T_A$ (Fig. 6.2). At an ambient temperature of 5°C, heat loss via the naked body parts constituted only 13-14 per cent of the total heat loss. The converse was also the case; at thermal-neutrality ($T_A = 35^\circ$C) the superior insulative properties of the fur resulted in only 10-11 per cent of body heat being lost to the environment from the thorax and abdomen (Table 6.2).
In response to environmental cooling, the changes in the 'effective' surface area of the naked body parts followed a similar pattern to that of heat loss (Table 6.3). At thermal-neutrality, the 'effective' surface area of the membranes was 78.6 per cent of actual surface area in winter and 88.6 per cent in summer. Below thermal-neutrality, the 'effective' surface area of the naked body regions fell precipitously and at $T_A$'s of 25°C and less, 'effective' surface area never accounted for more than 8 per cent of actual surface area. At an ambient temperature of 5°C, the 'effective' surface area of the membrane component was only 1.4 and 1.3 per cent of actual surface area in summer- and winter-adapted *N. gouldi*, respectively.

Unlike the furred body regions, absolute heat loss from the membranes did not decline in a linear manner with increasing ambient temperature (Fig. 6.3). Heat loss via the naked body parts was at a minimum when $T_A$ equalled 5°C, rose to a maximum at 15°C and fell again to an intermediate level at ambient temperatures of 25 and 35°C.

To determine whether or not seasonal changes in the absolute heat loss through the furred body regions could account for the reduced metabolic requirements of winter-adapted *N. gouldi*, the overall seasonal differences in whole animal heat loss were compared with the differences in absolute heat loss through the fur (Table 6.4). At the coldest $T_A$ (5°C), the winter improvement in fur insulation accounted for 93.5 per cent of the whole animals reduction in thermal losses. At all warmer ambient temperatures, the
improved insulative properties of winter furs accounted for much less of the observed thermal economy. At thermal-neutrality, only 15 per cent of the winter thermal savings could be accounted for by improved pelt insulation.

Newton's Law of Cooling was also used to calculate whole-body conductance values for summer and winter bats. This procedure required the estimation of mean skin temperature. Mean skin temperature was calculated from the proportion of total surface area represented by each body region (Stitt and Hardy 1971; Hulbert and Dawson 1974a; Dawson and Fanning 1981). For *N. gouldi* the estimates of 'effective' surface area calculated earlier in this section (Table 6.3) were used in place of actual surface area. A sample calculation of whole-body thermal conductance is shown below:

Example: Calculation of Whole-Body Thermal Conductance

At an ambient temperature of 15°C in winter the heat produced by a male *N. gouldi* was 35.99 W/kg or 0.4355 W. (see earlier example calculation).

The surface areas were

- thorax (actual) = 19.8 cm²
- abdomen (actual) = 19.8 cm²
- naked membranes ('effective') = 12.4 cm²

that is, a total surface area of 52.0 cm².

Mean temperature differentials were

\[ T_{Sk} - T_A \text{ thorax} = 19.0°C \]
\[ T_{Sk} - T_A \text{ abdomen} = 14.2°C \]
\[ T_{Sk} - T_A \text{ membrane} = 10.4°C \]
Therefore, mean skin temperature differential is

\[
(T_{sk} - T_A) = \left(\frac{SA_{\text{thorax}}}{SA_{\text{total}}} \times [T_{sk} - T_A]_{\text{thorax}}\right) + \text{etc.}
\]

\[
= \left(\frac{19.8}{52.0} \times 19.0\right) + \left(\frac{19.8}{52.0} \times 14.2\right) + \left(\frac{12.4}{52.0} \times 10.4\right)
\]

\[= 15.1^\circ C\]

Substituting in C = \[
\frac{HL}{(T_{sk} - T_A) \text{'effective'} \times SA}
\]

\[= 0.4355 \left(\frac{15.1}{52.0}\right)
\]

\[= 5.55 \times 10^{-4} \text{ W/cm}^2/\text{°C}
\]

or \[5.55 \text{ W/m}^2/\text{°C}\]

The equations used to calculate mean skin temperature at each ambient temperature are given in Table 6.5. Figure 6.4 presents a comparison of whole-body thermal conductances calculated in the manner shown above with values obtained when rectal and core temperature (38°C) were substituted for mean skin temperature and actual surface area was used in place of 'effective' surface area.

The estimates of whole-body thermal conductance calculated using mean skin temperature and 'effective' surface area were eight to ten fold greater than those determined using either rectal or deep body temperature with actual surface area (Fig. 6.4). The surface area component of the equation had a greater effect on the calculated
thermal conductance value than did the respective body temperatures used. When the surface area parameter was removed from the equation, and thermal conductance calculated in Watts/°C, the difference in the estimates was greatly reduced, although rates of thermal conductance calculated using mean skin temperature remained up to 40 per cent greater than the other two (Fig. 6.5). This is consistent with the finding that mean skin temperatures were always up to four degrees less than rectal temperatures and as much as 10 degrees less than deep body temperature (Table 6.6).

Irrespective of the method of calculation, rate of thermal conductance increased only slightly as ambient temperature rose from 5 to 25°C and then precipitously increased at thermal-neutrality indicating effective vasomotor control (Figs. 6.4 and 6.5).
6.4 DISCUSSION

Acclimatization to winter cold can involve either improved heat producing ability, improved heat conserving capacity or a compromise between the two (Carlson 1963; Hart 1963; Barnett and Mount 1967). In Nyctophilus gouldi winter adaptation results in a ten to twenty per cent reduction in the thermal energy needed to maintain homeothermy at ambient temperatures from 5 to 38°C. However, seasonal adaptation in this species cannot be considered solely an adjustment to the insulative properties of the fur covered body parts. The 14 per cent increase in the insulative value of male N. gouldi fur only accounts for the winter metabolic savings at cold ambient temperatures (5°C). At ambient temperatures of 15°C and above, less than 50 per cent of the winter thermal economy results from the more insulative fur.

The parallel nature of the summer to winter transition in male and female metabolic curves strongly suggests a lowering of 'effective' body temperature as the winter adaptive mechanism in N. gouldi. To briefly recapitulate, the concept of 'effective' body temperatures was introduced in Chapter Three (page 79); the rationale being that just as cooling of the peripheral body tissues alters the effective surface area of a cold-exposed homeotherm, recognition should also be given to simultaneous changes in the 'effective' body temperature or the temperature determining the rate of thermal transfer to the environment.
The fact that winter-adapted *N. gouldi* regulate rectal temperatures 2 to 3°C lower than in summer supports the hypothesis that winter adaptation is primarily manifested through a reduction in 'effective' body temperature. However, the rectal temperature results were not supported by the seasonal comparison of temperatures maintained in five other body regions (Section 4.3.2). Given the reservations previously expressed (page 110) about the validity of the temperature data from other body regions and the inability of improved fur insulation to account for the winter metabolic savings in *N. gouldi*, a closer examination of the rectal temperature findings is warranted.

A winter reduction in body temperature is more compatible with the observed seasonal change in the metabolic curves of *N. gouldi* than is improved fur insulation (Carlson 1963). The primary distinction between these two forms of winter adaptation lies in the thermal energy savings afforded the animal and specifically how these change with falling ambient temperature (Fig. 6.6). Because a more insulative fur becomes more effective as ambient temperature drops, and the thermal gradient between skin surface and fur tip is expanded, savings in thermal energy will equally increase as the environmental temperature drops. Theoretically, the net result of improved fur insulation is a reduction in the slope of the oxygen consumption curve indicating an increase in thermal conservation as ambient temperature declines (Barnett and Mount 1967). In contrast to the fur insulative adaptation, a constant reduction in the level of winter regulated body
temperatures will result in equivalent savings of thermal energy at all ambient temperatures including at or near thermal-neutrality. In such a case, the rate of thermal conductance and therefore the slope of the metabolic curve should not alter from summer to winter, but the amount of body heat lost to the environment, in absolute terms, will be less.

In female *N. gouldi*, the latter situation prevailed; there was no seasonal change in the slope of the metabolic curve and the cost of maintaining homeothermy in winter was consistently reduced by 10 to 20 per cent at environmental temperatures from 5 to 38°C. The winter furs of *N. gouldi* females were no more or less insulative than summer furs although only two winter furs were tested and summer pelts were taken from animals at a different time to when endothermic responses were recorded (Section 5.4). Unlike the females, summer to winter transition in the metabolic curve of male *N. gouldi* indicated a combination of improved insulation and reduced body temperature. Slope reduction occurred and this effect was added to the parallel curve translation observed in females. The summer to winter improvement in male fur insulation is compatible with this finding as is the 2 to 3°C reduction in mean rectal temperature.

On the basis of theory alone then, the observed summer to winter transition in the metabolic curves of *Nyctophilus gouldi* can be readily explained if rectal temperature is accepted as a measure of 'effective' body temperature. To do this, the temperature data for other
body regions must be considered anomalous, or at least less robust than those measured from the rectal region. The rectal temperature data does inspire greater confidence than that recorded from other body regions as more bats were used in these experiments, the recording site was more repeatable and a considerably larger data set spread across a wider range of ambient temperatures was used.

If winter thermal saving in homeothermic _N. gouldi_ primarily results from a reduction in 'effective' body temperature (as measured by rectal temperature) then the relationship between the rate of oxygen consumption and rectal temperature should remain unchanged with the seasons. In Figure 6.7 this relationship is plotted for male and female bats in winter, spring and summer. There is a seasonal shift in the curves of both male and female _N. gouldi_, indicating that the cost of regulating a specific rectal temperature does not remain constant with the seasons (Fig. 6.8). Bats in winter require less thermal energy to regulate the same rectal temperature as animals in summer. This does not infer, however, that bats in winter and summer are maintaining the same rectal temperature at the same ambient temperature. The curves describing the relationship between oxygen consumption and rectal temperature in spring-adapted bats lie intermediate to those of winter and summer animals indicating the transitional physiological state of _N. gouldi_ at this time.

Given that rectal temperature is indicating the 'effective' body temperature of _N. gouldi_, then the
relationship between rate of oxygen consumption and the differential between rectal and ambient temperature is of far greater significance than the relationship between oxygen consumption and rectal temperature (Fig. 6.9). Consider Newton's Law of Cooling again but omit the surface area component which has no relevance at this point:

\[
\text{Rate of Thermal Conductance} = \frac{\text{Heat production} = \text{Heat loss}}{(T_{\text{Rectal}} - T_{\text{Ambient}})}
\]

As stated earlier in this discussion, if seasonal adaptation is solely manifested through a change in 'effective' body temperature then the rate of thermal conductance will not alter with the time of year. Assuming that rectal temperature equals 'effective' body temperature for *N. gouldi* then the relationship between heat production (as measured by the rate of oxygen consumption) and the rectal-ambient temperature differential will remain constant irrespective of the season. Any change to the rectal-ambient temperature differential should be matched by a proportional change in the rate of heat production.

In female *N. gouldi* this was the case. The curves describing the relationship between oxygen consumption and the rectal-ambient temperature differential for animals in winter, spring and summer were virtually superimposed (Fig. 6.10). Despite their similarity, the winter and summer curves of females were significantly different in both slope \((F[1,132] = 7.20, \ p<0.05)\) and vertical position \((F[1,133] = 8.81, \ p<0.05)\). The greatest difference in the curves was at \(T_R - T_A\)'s less than 2.0°C. When these values were excluded
there was no significant difference between the curves (slopes: F[1,117] = 3.45, p>0.1; vertical displacement: F[1,118] = 4.71, p >0.5). Only when oxygen consumption values corresponding to rectal-ambient temperature differentials less than 1.0°C were included in the comparison did the significant difference between the curves exist (slopes: F[1,128] = 5.72, p<0.05; vertical displacement, F[1,129] = 7.62, p<0.02).

Unlike female *N. gouldi*, the relationship between rate of oxygen consumption and the rectal-ambient temperature differential in males changed from summer to winter (Fig. 6.10). Whilst the spring and summer curves were very similar the winter curve had a significantly reduced slope (F[1,114] = 13.21, p<0.0025) and was significantly vertically displaced below the summer relationship (F[1,115] = 25.09, p<0.001). This is not an unexpected finding given that the fur of male *N. gouldi* in winter was more insulative than in summer. In such a case, the rate of thermal conductance decreases as environmental temperature drops and the proportional relationship between rate of heat loss and the rectal-ambient temperature differential alters: in effect, rectal temperature no longer represents a true measure of 'effective' body temperature.

Whole-animal thermal conductance values calculated using rectal temperature (Table 6.7) clearly illustrate the male/female differences in winter adaptation in *Nyctophilus gouldi*. At ambient temperatures of 5 and 25°C, the rate of thermal conductance in female *N. gouldi* did not
change from summer to winter; that is, the winter reduction in the differential between rectal and ambient temperature was proportional to the drop in oxygen consumption. At an ambient temperature of 15°C this was not the case, and thermal conductance was 9.4 per cent greater in winter females. It is not known whether this finding represents a seasonal difference of real significance or is the result of natural variation in the data. The summer and winter oxygen consumption curves of *N. gouldi* females were slightly convergent at or near ambient temperatures of 15°C (Fig. 3.7) resulting in the elevated thermal conductance value in winter bats.

The rates of thermal conductance were less for winter males than summer males at ambient temperatures of 5, 15 and 25°C (Table 6.7). This is consistent with the seasonal differences in the relationship between oxygen consumption and the rectal-ambient temperature differential observed previously (Fig. 6.10) and the improved insulative properties of male fur in winter. The summer to winter difference in whole-animal thermal conductance in *N. gouldi* males increased from 3.0 per cent at an ambient temperature of 25°C to 6.9 per cent at 15°C and 10.0 per cent at 5°C. Again this finding is compatible with the observed 14 per cent improvement to the insulative properties of winter pelts and further illustrates the increasing effectiveness of fur insulation with a decline in ambient temperature.

Given the findings presented above, the form of winter adaptation in *N. gouldi* can be confidently
identified. In both males and females the primary energy
saving mechanism in winter-adapted animals is a 2 to 3°C
reduction in the 'effective' body temperature. Rectal
temperature is a good measure of effective body temperature
except where changes to the insulative properties of the
peripheral body layers occur. In female Nyctophilus
gouldi, a reduction in the 'effective' body temperature is
the sole form of winter adaptation in the homeothermic
animal. In males this is not the case; added to the winter
drop in body temperature is a 14 per cent improvement to the
insulative properties of the fur layer. The ecological
significance of this male/female difference in winter
adaptation will be discussed in Chapter Nine.

What are the ramifications of these findings in
terms of the partitioning of heat loss pathways and the
'effective' surface areas calculated earlier in this Chapter?
(Section 6.3.2). The partitioning of heat loss and
calculations of 'effective' surface area were based upon the
body tissue temperatures recorded in Chapter Four. Unlike
rectal temperature, these body temperatures showed no
seasonal change. The accountability of seasonally changing
rectal temperatures for the metabolic savings of winter
acclimatized animals indicates that the mean temperatures
recorded from other body regions were not a true indication
of seasonal adaptation in N. gouldi. Although it is
clearly invalid to make seasonal comparisons of heat loss
pathways using these data, they do provide an opportunity to
examine the thermoregulatory role played by the naked body
surfaces and therefore to comment on the differences between
'effective' and actual surface area in the cold-exposed bat.
The partitioning of heat loss pathways and subsequent determinations of 'effective' surface area were based upon a number of assumptions and as such should be considered as only estimates. To assume that the head was maintained at the same temperature as the thorax may not be valid and could have resulted in underestimation of the thermal losses from the furred body portions. Equally, overestimation of heat loss from the naked body surfaces may have resulted from the assumption that the uropatagium was maintained at the same temperature as the wings. On the few occasions when the surface temperature of the uropatagium was measured (Chapter Eight), it was always cooler than the wings.

The primary assumption made to enable the partitioning of heat loss was that by comparison with the naked membranes the actual surface area of the furred body regions was an acceptable estimate of effective surface area. Given the considerable regional heterothermy present in cold-exposed \textit{N. gouldi} (Chapter Four) this assumption becomes less valid as ambient temperature drops and would unquestionably result in overestimation of the heat lost through the furred body regions. When calculating surface area-specific values of thermal conductance the assumption that actual surface area equals effective surface area, irrespective of environmental temperature, is quite common in the literature. In animals capable of sustaining regional heterothermy this assumption is clearly invalid. The more theoretically acceptable method of calculating surface area-specific rates of thermal conductance is to use a sliding scale of effective surface areas, such as that
employed in this study, thus accommodating situations where regional heterothermy becomes more pronounced as ambient temperature falls.

In *Nyctophilus gouldi* the heat loss pathways altered markedly with changes in environmental temperature. At thermal-neutrality only 10 to 11 per cent of body heat was lost through the furred regions, the remainder passing out through the wings, ears and tail. The naked membranes cannot be considered a thermoregulatory 'burden' to *N. gouldi*, however. Once ambient temperature dropped below thermal-neutrality, the 'effective' surface area of the naked body parts was always less than eight per cent of actual surface area and at an ambient temperature of 5°C only 13 to 14 per cent of body heat was lost via this pathway. Unlike the thermal transfer through the furred body parts, heat loss from the membranes did not decline in a linear manner with falling ambient temperature. Similar amounts of body heat were lost through the membranes at ambient temperatures of 25 and 35°C. At 5°C, membrane heat loss was at its lowest value; however, at the intermediate ambient temperature of 15°C more heat passed out through the wings, ears and tail than at any of the other three test temperatures.

The environmental temperature of 15°C would appear to have some physiological and possibly ecological significance to *N. gouldi* (discussed further in Chapter Nine). During endothermic regulation experiments bats never entered torpor unless ambient temperature dropped below 18°C. If animals remained homeothermic at ambient temperatures
below 15°C, plateauing of the oxygen consumption curve invariably commenced. In either case, savings of thermal energy resulted and in homeothermic animals it would seem that passing this threshold temperature triggers the further vasoconstriction and cooling of the naked body regions.

When calculating the rate of thermal conductance for any animal considerable errors can result from matching metabolic rate with the inappropriate body temperature or body surface area estimate. For *N. gouldi* a comparison was made of the whole-body thermal conductances calculated using deep body temperature with actual surface area, rectal temperature with actual surface area, and mean skin temperature, with 'effective' surface area (Fig. 6.4). The combination of mean skin temperature with 'effective' surface area yielded an estimate of thermal conductance which was 8 to 10 times greater than either of the other two methods of calculation. The surface area component within the equation was primarily responsible for this disparity. When surface area was removed from the calculation the mean skin temperature-determined thermal conductance values remained up to 40 per cent higher than the rectal and deep body temperature-determined estimates (Fig. 6.5).

Given the findings of this study, rectal temperature would seem the most appropriate body temperature to use when calculating the rates of thermal conductance for *N. gouldi*. If deep body or core temperature is used and this is greater than the 'effective' body temperature (as it generally would be), thermal conductance will be underestimated
because body heat is not being lost at a rate proportional to the thermal gradient between deep body temperature and ambient temperature. Equally, to use the mean skin temperature may be inappropriate and result in overestimation of the rate of thermal conductance if, as is the case in *N. gouldi*, 'effective' body temperature lies between core temperature and mean skin temperature.

To blindly assume that actual surface area equals effective surface area is not justified for any animal and most especially for a bat. When calculating surface area-specific rates of thermal conductance large scale errors can result from this assumption and because of the inordinately larger surface areas of the Chiroptera these errors are further amplified. In most cold-exposed homeotherms, effective surface area is less than actual surface area meaning that estimates of thermal conductance calculated using actual surface area will be underestimates.

I hesitate to recalculate the thermal conductance values for *N. gouldi* using rectal ('effective' body) temperature and in surface area-specific terms because the estimates of 'effective' surface area calculated earlier in this chapter were based upon the mean skin temperatures of the thorax, abdomen and wing surface; values which have proven to be an unreliable indicator of the seasonal body temperature adaptation in this species. However, Table 6.8 (and Table 9.2) present whole animal conductances calculated in this way primarily for the purposes of comparison with other species (Chapter Nine). Despite the numerous
assumptions made and the obvious limitations to their use, the estimates of 'effective' surface area made here will yield thermal conductance values much closer to reality than if actual surface areas were used. For the purpose of these calculations an average was taken of the summer and winter 'effective' surface areas of male *N. gouldi* and it was assumed that the 'effective' surface areas of males and females were equivalent at all ambient temperatures. Although this method of calculating whole-animal thermal conductances in surface area-specific terms has a number of shortcomings and can be readily criticized, I consider that given the available data this is the best estimate possible at this time.
Furred Surface Area 39.6 ± 4.1 (10) 12.3%

Ears 11.2 ± 1.8 (10) 3.5%

Uropatagium 23.5 ± 5.2 (26) 7.3%

Wings 248.1 ± 17.2 (26) 76.9%

Male/Female Comparison:
- Females , Wings 6.7% larger (p=0.003)
- Females , Uropatagium 12.3% larger (p=0.04)
- No significant difference in the areas of the ears or furred body parts (p=0.45 and 0.40 respectively)

Figure 6.1 Surface areas of the naked and furred body regions of male N. gouldi. All values are given as mean± one s.d. (sample size)

and female data are presented in Table 6.1
Figure 6.2 Linear regression lines and equations calculated from the mean responses of each body region at ambient temperatures of 5, 15, 25 and 35°C in winter and summer. See page 145 for a description of the assumptions made in calculating these regressions.

Equations:

**Peritoneal cavity:**

\[ T_b = 30.09 + 0.22T_a \quad R^2 = 0.67 \]

\[ F(1,11) = 22.8 \quad p < 0.001 \]

**Thorax-shell:**

\[ T_b = 32.51 + 0.10T_a \quad R^2 = 0.31 \]

\[ F(1,65) = 28.7 \quad p < 0.001 \]

**Abdomen-shell:**

\[ T_b = 24.21 + 0.33T_a \quad R^2 = 0.66 \]

\[ F(1,30) = 57.7 \quad p < 0.001 \]

**Naked membranes:**

\[ T_b = 17.75 + 0.51T_a \quad R^2 = 0.88 \]

\[ F(1,68) = 517.4 \quad p < 0.001 \]
Figure 6.3 Absolute heat loss from the thorax, abdomen and naked membranes of male N. gouldi in winter and summer. A sample calculation showing the method used to calculate these values is shown in section 6.3.2 (page 149).
Figure 6.4 Whole-body rates of thermal conductance for summer-adapted male *N. gouldi* calculated using Newton’s Law of Cooling. In case (A) mean skin-ambient temperature differential ($Ts-Ta$) and ‘effective’ surface area ($SAe$) were used. For case (B) rectal temperature ($Tr$) and actual surface area ($SAa$) were substituted in the equation and for case (C) core temperature ($Tc$) and actual surface area.
Figure 6.5  Whole-animal rates of thermal conductance for male N. gouldi in summer calculated in the same way as described in Fig. 6.4 except the surface area parameter has been omitted to show the effect on the resultant values.
Figure 6.6 A comparison of the theoretical effects on the metabolic curve of a drop in "effective" body temperature and an improvement to the animals insulation.

(Based on Carlson 1963)
Figure 6.7 Plots of oxygen consumption against rectal temperature for male and female *N. gouldi* in (A) winter, (B) spring, and (C) summer. A more detailed form of the regression equations is given in Appendix II.
Figure 6.8 Comparison of the curves describing the relationship between oxygen consumption and rectal temperature of male and female *N. gouldi* in winter, spring, and summer.
Figure 6.9 Plots of oxygen consumption (VO2) against rectal-ambient temperature differential (Tr - Ta) for male and female N. gouldi in (A) Winter, (B) Spring and (C) Summer. The regression equations are given in Appendix II.
Figure 6.10 Comparison of the curves describing the relationship between oxygen consumption and the rectal-ambient temperature differential of male and female *N. gouldi* in winter, spring and summer. The statistical comparisons of these curves are discussed on pages 160-1.
Table 6.1  Body surface areas for male and female Nyctophilus geoffidi. All values are given as mean ± one standard deviation in cm². The methods used in obtaining these measurements are given in section 6.2.1. The results of male-female comparisons of surface area by one-way ANOVA are presented in Figure 6.1.

<table>
<thead>
<tr>
<th>Body Surface</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wings</td>
<td>248.1 ± 17.2</td>
<td>265.8 ± 23.5</td>
</tr>
<tr>
<td></td>
<td>n = 26</td>
<td>n = 26</td>
</tr>
<tr>
<td>Uropatagium</td>
<td>23.5 ± 5.2</td>
<td>26.8 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>n = 26</td>
<td>n = 26</td>
</tr>
<tr>
<td>Ears</td>
<td>11.2 ± 1.8</td>
<td>11.9 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>n = 10</td>
<td>n = 8</td>
</tr>
<tr>
<td>Total Naked Membrane</td>
<td>282.8</td>
<td>304.5</td>
</tr>
<tr>
<td>Total Furred Body Region</td>
<td>39.6 ± 4.1</td>
<td>40.5 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>n = 10</td>
<td>n = 8</td>
</tr>
<tr>
<td>Total Body Surface Area</td>
<td>322.4</td>
<td>345.0</td>
</tr>
</tbody>
</table>
Table 6.2. Heat loss from the three main body divisions: thorax, abdomen and naked membranes, compared for male *N. gouldi* in summer and winter at ambient temperatures of 5, 15, 25 and 35°C. Section 6.3.2 describes the method of calculation.

<table>
<thead>
<tr>
<th>Ambient Temperature (°C)</th>
<th>Body Division</th>
<th>Summer</th>
<th></th>
<th></th>
<th>Winter</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heat Loss (Watts)</td>
<td>% of Total Heat Loss</td>
<td></td>
<td></td>
<td>Heat Loss (Watts)</td>
<td>% of Total Heat Loss</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Thorax</td>
<td>0.269</td>
<td>49.6</td>
<td></td>
<td>Thorax</td>
<td>0.236</td>
<td>49.2</td>
</tr>
<tr>
<td></td>
<td>Abdomen</td>
<td>0.201</td>
<td>37.1</td>
<td></td>
<td>Abdomen</td>
<td>0.176</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>Membrane</td>
<td>0.072</td>
<td>13.3</td>
<td></td>
<td>Membrane</td>
<td>0.068</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.542</td>
<td></td>
<td></td>
<td>Total</td>
<td>0.480</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Thorax</td>
<td>0.182</td>
<td>35.1</td>
<td></td>
<td>Thorax</td>
<td>0.160</td>
<td>36.8</td>
</tr>
<tr>
<td></td>
<td>Abdomen</td>
<td>0.136</td>
<td>26.3</td>
<td></td>
<td>Abdomen</td>
<td>0.119</td>
<td>27.3</td>
</tr>
<tr>
<td></td>
<td>Membrane</td>
<td>0.200</td>
<td>38.6</td>
<td></td>
<td>Membrane</td>
<td>0.156</td>
<td>35.9</td>
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<td>Total</td>
<td>0.518</td>
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<td></td>
<td>Total</td>
<td>0.435</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Thorax</td>
<td>0.097</td>
<td>31.1</td>
<td></td>
<td>Thorax</td>
<td>0.085</td>
<td>33.9</td>
</tr>
<tr>
<td></td>
<td>Abdomen</td>
<td>0.072</td>
<td>23.1</td>
<td></td>
<td>Abdomen</td>
<td>0.063</td>
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<tr>
<td></td>
<td>Membrane</td>
<td>0.143</td>
<td>45.8</td>
<td></td>
<td>Membrane</td>
<td>0.103</td>
<td>41.0</td>
</tr>
<tr>
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<td>Total</td>
<td>0.312</td>
<td></td>
<td></td>
<td>Total</td>
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<td>35</td>
<td>Thorax</td>
<td>0.011</td>
<td>6.5</td>
<td></td>
<td>Thorax</td>
<td>0.009</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Abdomen</td>
<td>0.007</td>
<td>4.1</td>
<td></td>
<td>Abdomen</td>
<td>0.006</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Membrane</td>
<td>0.151</td>
<td>89.4</td>
<td></td>
<td>Membrane</td>
<td>0.134</td>
<td>90.0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.169</td>
<td></td>
<td></td>
<td>Total</td>
<td>0.149</td>
<td></td>
</tr>
</tbody>
</table>
Table 6.3. 'Effective' surface area of the naked membranes in summer and winter-adapted, male, *N. gouldi*. The method of calculation is shown in (Section 6.3.2).

*Actual surface area = 282.8 cm² (Table 6.1).

To gain total 'effective' surface area, the area of the furred body region (39.6 cm²) was considered constant at all ambient temperatures and added to 'effective' membrane surface area.

<table>
<thead>
<tr>
<th>Ambient Temperature (°C)</th>
<th>Summer 'Effective' Membrane Surface Area (cm²)</th>
<th>% of 'Actual Membrane Surface Area</th>
<th>Winter 'Effective' Membrane Surface Area (cm²)</th>
<th>% of 'Actual Membrane Surface Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3.9</td>
<td>1.4</td>
<td>3.7</td>
<td>1.3</td>
</tr>
<tr>
<td>15</td>
<td>16.0</td>
<td>5.7</td>
<td>12.4</td>
<td>4.4</td>
</tr>
<tr>
<td>25</td>
<td>21.6</td>
<td>7.6</td>
<td>15.5</td>
<td>5.5</td>
</tr>
<tr>
<td>35</td>
<td>250.6</td>
<td>88.6</td>
<td>24.4</td>
<td>78.6</td>
</tr>
</tbody>
</table>
Table 6.4. Comparison of heat loss through the furred body regions in *N. gouldi* males in summer and winter. Method of calculation is shown in section 6.3.2.

*The proportion of the total seasonal change in heat flux accounted for by the improved insulative value of winter fur.

| Ambient Temperature (°C) | Heat loss through the furred body regions (Watts) | Summer/Winter Difference in heat loss through the furred body regions | Overall difference in summer/winter whole-animal heat loss | *Per cent of whole-animal heat loss accounted for by summer/winter change in fur insulation*
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Summer</td>
<td>Winter</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.470</td>
<td>0.412</td>
<td>0.058</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.318</td>
<td>0.279</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.169</td>
<td>0.148</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>0.018</td>
<td>0.015</td>
<td>0.003</td>
</tr>
</tbody>
</table>
Table 6.5. The equations used to calculate mean skin temperature based on the proportionality of 'effective' membrane surface area and actual furred surface area. Section 6.3.2 describes the methods.

<table>
<thead>
<tr>
<th>Ambient Temperature (°C)</th>
<th>Summer</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$T_{Sk} - T_A = 0.45 (T_{Th} - T_A) + 0.45 (T_{Ab} - T_A)$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$+ 0.10 (T_M - T_A)$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$= 23.5 ^\circ C$</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$T_{Sk} - T_A = 0.36 (T_{Th} - T_A) + 0.36 (T_{Ab} - T_A)$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$+ 0.28 (T_M - T_A)$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$= 14.9 ^\circ C$</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$T_{Sk} - T_A = 0.32 (T_{Th} - T_A) + 0.32 (T_{Ab} - T_A)$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$+ 0.36 (T_M - T_A)$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$= 7.6 ^\circ C$</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$T_{Sk} - T_A = 0.07 (T_{Th} - T_A) + 0.07 (T_{Ab} - T_A)$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$+ 0.86 (T_M - T_A)$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$= 0.6 ^\circ C$</td>
<td></td>
</tr>
</tbody>
</table>
Table 6.6. Comparison of mean skin temperatures and mean rectal temperatures of male *N. gouldi* in summer and winter. The method of calculating mean skin temperature is described in section 6.3.2 and demonstrated in Table 6.5. Mean rectal temperatures come from the regression equations presented in Chapter 3.

<table>
<thead>
<tr>
<th>Ambient Temperature (°C)</th>
<th>Summer</th>
<th></th>
<th>Summer</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_{\text{Skin}}$</td>
<td>$T_{R}$</td>
<td>$T_{\text{Skin}}$</td>
<td>$T_{R}$</td>
</tr>
<tr>
<td>5</td>
<td>28.5</td>
<td>32.0</td>
<td>28.7</td>
<td>31.6</td>
</tr>
<tr>
<td>15</td>
<td>29.9</td>
<td>32.8</td>
<td>30.1</td>
<td>31.1</td>
</tr>
<tr>
<td>25</td>
<td>32.6</td>
<td>34.5</td>
<td>32.9</td>
<td>32.4</td>
</tr>
<tr>
<td>35</td>
<td>35.6</td>
<td>37.8</td>
<td>35.6</td>
<td>35.5</td>
</tr>
</tbody>
</table>
Table 6.7  Comparison of whole-animal rates of thermal conductance in summer and winter-adapted *Nyctophilus gouldi*. All values are given in Watts/°C. Newton's Law of Cooling was used to calculate these values and rectal temperature substituted as the estimate of 'effective' body temperature. Section 6.4 discusses these data.

<table>
<thead>
<tr>
<th>Ambient Temperature (°C)</th>
<th>Males Summer</th>
<th>Winter</th>
<th>Summer-Winter % Difference</th>
<th>Females Summer</th>
<th>Winter</th>
<th>Summer-Winter % Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.020</td>
<td>0.018</td>
<td>-10.0</td>
<td>0.023</td>
<td>0.023</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>0.029</td>
<td>0.027</td>
<td>-6.9</td>
<td>0.029</td>
<td>0.032</td>
<td>+9.4</td>
</tr>
<tr>
<td>25</td>
<td>0.033</td>
<td>0.034</td>
<td>-3.0</td>
<td>0.035</td>
<td>0.035</td>
<td>0</td>
</tr>
<tr>
<td>35</td>
<td>0.059</td>
<td>0.30</td>
<td>+80.3</td>
<td>0.109</td>
<td>0.433</td>
<td>+74.8</td>
</tr>
</tbody>
</table>
Table 6.8 Whole-animal rates of thermal conductance for male and female *N. gouldi* in summer and winter calculated in surface-area specific terms. All values are in Watts/m²/°C. Mean rectal temperatures (Chapter Three) and ‘effective’ surface areas (Section 6.3) were used in calculating these values and the assumptions made are described and discussed in Section 6.4 (page 167). Table 9.1 presents whole-animal rates of thermal conductance in ml's O₂/g/hr./°C.

<table>
<thead>
<tr>
<th>Ambient Temperature (°C)</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Summer Winter</td>
<td>Summer Winter</td>
</tr>
<tr>
<td>5</td>
<td>4.61 4.15</td>
<td>5.30 5.30</td>
</tr>
<tr>
<td>15</td>
<td>5.39 5.02</td>
<td>5.39 5.95</td>
</tr>
<tr>
<td>25</td>
<td>5.68 5.85</td>
<td>6.02 6.02</td>
</tr>
<tr>
<td>35</td>
<td>2.13 10.83</td>
<td>1.93 15.63</td>
</tr>
</tbody>
</table>
Chapter Seven

WARM-ACCLIMATION IN MID-WINTER: EFFECTS ON
ENDOTHERMIC TEMPERATURE REGULATION AND
BODY TISSUE TEMPERATURES

7.1 Introduction 187

7.2 Methods
7.2.1 Animal Maintenance during Acclimation 191
7.2.2 Experimental Procedures 191
7.2.3 Statistical Methods 192

7.3 Results
7.3.1 Oxygen Consumption and Rectal Temperature Regulation 193
Males 194
Females 195
Comparison of Male and Female Patterns 196

7.3.2 Regional Body Temperatures 196

7.3.3 Effects of Warm-acclimation on the Reproductive Cycle 197

7.4 Discussion 199
7.1 INTRODUCTION

The laboratory simulation of winter cold or summer heat has long been a popular method of examining the mechanisms of physiological adaptation in homeotherms (Hart 1953a,b,c; Cassuto and Chaffee 1966; Jansky and Hart 1968). Laboratory animals such as rats, hamsters and mice are the most frequently used experimental subjects and in these animals acclimation to cold or heat normally produces opposite physiological responses (Chaffee and Roberts 1971). As a result of cold acclimation, the heat producing abilities or cold resistance of the animal are generally enhanced (Hart 1953c). The basal or standard metabolic rate increases and the amount of shivering declines commensurate with an improved capacity for non-shivering or chemical thermogenesis. In cold-acclimated rats there are also changes in blood flow; cardiac output is increased and the brown and white fat deposits, pancreas, kidney, intestine and liver all receive more blood than in control animals (Jansky and Hart 1968).

Acclimation of mammals to a hot environmental temperature (>30°C) normally results in a reduction in the rate of heat production and/or an increase in the rate of thermal conductance. In heat-acclimated hamsters, Cassuto and Chaffee (1966) found that heat tolerance was increased and cold tolerance decreased. The metabolic rate was significantly less than in control animals and the relative mass of the heat producing organs, such as liver and brown fat, was significantly reduced.
Laboratory simulation of different thermal environments also allows the experimenter to examine the rate with which physiological adaptation occurs. Guernsey and Whittow (1981) recorded the rate of cold-acclimation and deacclimation in laboratory rats. They found that after six days of acclimation at 5°C, the basal metabolic rates of the experimental animals had not altered from those of the controls held at room temperature. By 15 days, cold-acclimated rats had basal metabolic rates 18.9 per cent greater than controls. When the cold-acclimated rats were returned to the room temperature environment it took between 7 and 21 days for their basal metabolic rates to return to control levels. Bartunkova, Jansky and Mejsnar (1971) reported a similar time for deacclimation to occur in laboratory rats. In white mice, Hart (1953c) found that it was during the initial stages of cold-acclimation and deacclimation that cold resistance was gained or lost fastest. In Peromyscus leucopus novaboracensis the development of cold resistance did not require constant exposure to cold but was acquired through short periods (two or three hours daily) of cold exposure over an eight week period (Hart 1953c).

Bats show similar metabolic and insulative adjustments in response to hot or cold acclimation as do other homeotherms. Holyoak and Stones (1971) acclimated summer- and winter-caught Myotis lucifugus to ambient temperatures of 10, 20 and 30°C. They found that irrespective of season, bats acclimated to 30°C had the lowest overall metabolic rates at ambient temperatures from zero to 35°C. These authors concluded that "... an
increasing degree of thermoregulatory ability in *Myotis lucifugus* appeared inversely related to the level of acclimation temperature independent of the season of capture ..." and that seasonal acclimatization to cold was manifested through increased thermogenic capacity. These findings are in contrast to those of Mejsnar and Jansky (1967) who found that in winter-captured *Myotis myotis* both metabolic rate and body temperature were reduced by comparison with summer-captured bats, indicating an insulative adaptation to winter cold.

In *Nyctophilus gouldi* the 'natural' seasonal acclimatization, in animals held captive but within an outdoor holding cage, indicated a similar insulative adaptation to that in *M. myotis* (Mejsnar and Jansky 1967). In winter-adapted *N. gouldi*, metabolic rates and rectal temperatures were lower than in spring- and summer-adapted bats.

The aim of the acclimation experiment described here was to simulate the onset of spring in mid-winter and test the plasticity of the winter-spring-summer seasonal transition observed in 'naturally' acclimatized animals. *Nyctophilus gouldi* were taken from the outdoor colony in mid-winter and acclimated to an ambient temperature of 22°C. During this time they were given *ad libitum* food and water and exposed to natural day-length. The rates of oxygen consumption and levels of regulated body temperature were recorded after three weeks and compared with those of winter, spring and summer-adapted bats previously described (Chapter Three). Figure 7.1 shows the timing of the...
warm-acclimation and subsequent experimentation relative to the times when winter-, spring- and summer-adapted bats were tested.
7.2 METHODS

7.2.1 Animal Maintenance during Acclimation

On the 26th June, five days after the winter solstice, five male and five female *N. gouldi* were transferred from the outdoor holding cage (Section 3.2.1) to a room of the Zoology Department's animal house. All were adult animals and had been maintained within the outdoor captive colony for six to eight months prior to the commencement of acclimation. The room measured two metres square and three metres high and therefore permitted the experimental animals some flight activity. They were provided with *ad libitum* food (mealworms, *Tenebrio* sp.) and water. Acclimation was for three weeks and the mean temperature within the room during this period was $21.8 \pm 0.8$ (SD)$^\circ$C. Relative humidity was always between 40 and 50 per cent. The south-facing wall of the room contained a window approximately one metre square and thus the animals were exposed to natural daylength throughout the period of acclimation. The bats chose to roost together in a piece of hessian hanging from the door of the room rather than in the timber roosts provided.

7.2.2 Experimental Procedures

The methodologies and equipment used to describe the endothermic metabolic and rectal temperature curves of warm-acclimated *N. gouldi* were the same as those described in Section 3.2.2. The pattern of homeothermic regional body cooling was described for male *N. gouldi*
using the techniques given in Section 4.2.2. In warm-acclimated males the regulated temperatures of the interscapular, chest, mid-dorsal, ventral wing-surface and dorsal wing-surface were monitored at ambient temperatures of 5, 15, 25 and 35°C. The precise recording points are shown in Fig. 4.2.

7.2.3 Statistical Methods

Polynomial curves-of-best-fit were applied to the oxygen consumption and rectal temperature responses of warm-acclimated *N. gouldi* (Section 3.2.3). The comparison of these curves with those recorded from spring-adapted bats (section 3.3) was by analysis of co-variance. Curves were also fitted to the plots of $T_R - T_A$ against oxygen consumption and compared with those derived in Section 6.4 for winter, spring and summer-adapted *N. gouldi*. Body temperatures measured in regional heterothermy experiments were compared with those data from summer and winter-adapted *N. gouldi* (Section 4.3) by two-level nested analysis of variance (Section 4.2.3).
7.3 RESULTS

7.3.1 Oxygen Consumption and Rectal Temperature Regulation

In general, warm-acclimated *N. gouldi* regulated similar rectal temperatures to spring animals but required 20-30 per cent less metabolic energy to do so. The oxygen consumption curve of warm-acclimated males was most similar to that of the winter-adapted males, although rectal temperatures were regulated at levels similar to those of spring and summer bats (Fig. 7.3). The \( \dot{V}O_2 \) curve for warm-acclimated females was unlike that at any other time of the year (Fig. 7.4) although \( T_R \) regulation followed the same pattern as that observed in warm-acclimated males (Fig. 7.7).

As was the case with summer-, winter- and spring-adapted *N. gouldi*, the rectal region cooled as \( T_A \) was reduced from 35 to 5°C. In warm-acclimated males and females, the rectal temperature curves were mirror images of the spring pattern.

Torpor occurred in warm-acclimated bats exposed to ambient temperatures less than 18°C, although less frequently than in spring-adapted *N. gouldi* (Section 3.3). These data are considered in Chapter Eight. Apart from altering the thermal energetics of *N. gouldi*, warm-acclimation had the additional effect of prematurely initiating the spring phase of the reproductive cycle. Pregnancy was initiated in females and the cycle of testicular enlargement
indicative of spermatogenesis in males was advanced by at least two months. A more detailed description of these results is given in Section 7.3.3.

**Males**

The oxygen consumption curves of spring-adapted and warm-acclimated male *N. gouldi* had similar slopes ($F_{[1,81]} = 2.75, \ p>0.2$) but the weight-specific metabolic cost of thermoregulation was significantly less at all ambient temperatures in warm-acclimated animals ($F_{[1,82]} = 45.45, \ p<0.001$) (Fig. 7.3). Reinforcing this finding was the observation that minimum metabolic rate was 45.2 per cent less and the area under the oxygen consumption curve 21.1 per cent less in acclimated males than in spring males (Table 7.1).

The rectal temperature curves of acclimated and spring males were significantly different in the vertical plane ($F_{[1,137]} = 9.74, \ p<0.005$) but the slopes of the curves did not differ ($F_{[1,136]} = 0.25, \ p>0.50$). The curves were mirror images, converging at the coldest and warmest $T_A$'s tested. Greatest difference in the mean level of $T_R$ was at ambient temperatures between 15 and 25°C. The area under the rectal temperature curve was 5.5 per cent greater for the warm-acclimated males (Table 7.1).

The metabolic cost of regulating a specific $T_R-T_A$ was the same in warm-acclimated and winter-adapted males (Fig. 7.5: slopes, $F_{[1,97]} = 4.67, \ p>0.05$; vertical displacement $F_{[1,98]} = 0.05, \ p>0.5$). In contrast, spring-
and summer-adapted males required 20-30 per cent more thermal energy than acclimated animals to maintain the same rectal temperature.

**Females**

In warm-acclimated females, the minimum metabolic rate was 23.5 per cent greater than in spring females (Table 7.2). The metabolic curves of spring and acclimated females intersected at $T_A = 30^\circ C$ (Fig. 7.4). At all ambient temperatures less than $30^\circ C$, warm-acclimated females used significantly less oxygen per gram body mass per hour than did spring females (vertical displacement: $F[1,82] = 21.67$, $p<0.001$). The slopes of the curves were not significantly different ($F[1,81] = 0.18$, $p>0.50$) possibly because of the variation within the data. The metabolic curve of acclimated females was a slight anti-clockwise rotation of the spring female curve. Unlike all previous seasons where MMR was at $T_A$’s within one degree of $35^\circ C$ (Section 3.3), in warm-acclimated females thermal-neutrality was at an ambient temperature of $31.6^\circ C$. Spring females were at a later stage of pregnancy than acclimated animals (Section 7.3.3) yet weighed 17.0 per cent less ($F[1,33] = 17.87$, $p<0.001$) (Table 7.2).

The metabolic cost of regulating a specific rectal-ambient temperature differential was between 10 and 30 per cent less in warm-acclimated females than in winter- (vertical displacement $F[1,94] = 15.21$, $p<0.001$), spring- ($F[1,63] = 14.71$, $p<0.001$) and summer-adapted females ($F[1,116] = 30.23$, $p<0.001$) (Fig. 7.6).
A Comparison of Male and Female Patterns

There was no difference between the sexes in the rectal temperature curves of warm-acclimated *N. gouldi* (slopes: $F[1,115] = 0.45, p>0.50$; vertical displacement: $F[1,116] = 0.04, p>0.25$) (Fig. 7.7). The major difference in the thermal regulation of warm-acclimated male and female *N. gouldi* was in the oxygen requirements of maintaining homeothermy. Minimum metabolic rate was 50 per cent greater in females and occurred at an ambient temperature 5°C less than in males (Tables 7.1 and 7.2). The slopes of the male and female VO$_2$ curves did not differ significantly ($F[1,106] = 1.62, p>0.20$) although because of the differences in high $T_A$ metabolic costs, the curves were significantly displaced along the oxygen consumption axis ($F[1,107] = 4.05, p<0.05$).

7.3.2 Regional Body Temperatures

The temperatures of the five body regions measured in warm-acclimated *N. gouldi* decreased as ambient temperature fell from 35 to 5°C. As was the case in summer and winter-adapted bats (Section 4.3) there was a thorax-abdomen-wing thermal gradient established once ambient temperatures dropped below thermal-neutrality. This thermal gradient expanded as ambient temperature declined. There was no significant difference in the temperatures of the body regions maintained by warm-acclimated, summer-adapted and winter-adapted *N. gouldi*. Tables 7.4, 7.5, 7.6, 7.7 and 7.8 summarize these findings. The mean temperatures of the body regions in acclimated males are presented in Table 7.9. Because of the similarity of these data with those
collected from summer and winter animals (and presented in Fig. 4.6) no graphical presentation of this information has been made.

7.3.3 Effects of Warm-acclimation on the Reproductive Cycle

Mid-winter warm-acclimation advanced the onset of the spring phase of the reproductive cycle; namely pregnancy and spermatogenesis. The body weights of acclimated females began to increase rapidly after three weeks of exposure to 22°C and the first female to give birth did so 64 days after warm-acclimation commenced (Fig. 7.8). This was 67 days before the first female in the outdoor colony produced young. Only four of the five warm-acclimated females gave birth; three had single young and one twins. Fifteen days separated the first and last parturition within the group of warm-acclimated females.

The onset of testicular hypertrophy, indicating spermatogenesis, was earlier in the acclimated males than in those held captive outdoors and in free-living animals. On 26 October, four months after acclimation commenced, the testes of the four remaining warm-acclimated males (one had died) were maximally enlarged (reproductive stage three, Section 2.4.2). At the same time, the testes of three males in the captive colony and six from the Bull's Head population (Section 2.4. showed little or only minor enlargement (reproductive stages one or two). By 21 November, the testes of warm-acclimated males were regressing and the epididymides distended with spermatozoa.
Captive, outdoor males had just reached maximum testes dimensions. No field males were trapped at this time.
7.4 DISCUSSION

The aim of warm-acclimatizing *N. gouldi* in mid-winter was to simulate the onset of spring conditions and initiate the seasonal adaptation observed in acclimatized animals (Chapter Three). One of the limitations to acclimation experiments is that the chronic exposure to a hot or cold environment, at an 'unnatural' time of year, does not always elicit the same physiological adaptations occurring naturally in the free-living homeotherm (Chaffee and Roberts 1971). This was the case in *Nyctophilus gouldi*. The metabolic curves of warm-acclimated animals were not the same as those of spring-adapted bats and the rectal temperature curves were unlike those of *N. gouldi* from any season.

Male and female thermoregulatory patterns were not affected in the same way by warm-acclimation. Although both sexes regulated similar rectal temperatures following acclimation, the metabolic cost to females was between 10 and 20 per cent less, at least at ambient temperatures from 10 to 25°C. Warm-acclimated females had a minimum metabolic rate 50 per cent greater than males and this occurred at an ambient temperature almost five degrees lower. However, the lack of intensive experimentation at high ambient temperatures places some doubts over the validity of this finding. The heat tolerating capacity of pregnant females was not known at the time of this experimentation and consequently exposure to ambient temperatures above 30°C was kept to a minimum.
The difference in male and female responses to warm-acclimation may be due to the initiation of pregnancy in females, although a reduction in the metabolic costs of homeothermy was not observed in pregnant spring females. Warm-acclimated and spring-adapted females were not at the same stage of gestation and this may account for the differences in the costs of endothermy. It could be significant that although warm-acclimated females were in the middle third and spring-acclimatized animals in the last third of pregnancy, the former group were significantly heavier (by 17 per cent). The most likely explanation for this body weight anomaly is that acclimated females retained their remaining winter fat deposits throughout the period of exposure to 22°C and subsequent experimentation.

Warm-acclimated and spring-adapted bats showed dissimilar propensities for torpor when exposed to ambient temperatures less than 20°C (Section 8.2). The frequency of torpor entry in warm-acclimated *N. gouldi* was about half that of spring-adapted bats. Studier and O'Farrell (1972) found a similar shift in the tendency to regulate homeothermy or enter torpor in pregnant *Myotis lucifugus* and *M. thysanodes*. In the early and late stages of pregnancy these females entered torpor more frequently than during the intermediate phase. The fact that male *N. gouldi* showed a similar shift in the tendency to enter torpor is curious and has not been previously reported. Although warm-acclimation initiated the spring reproductive phase in male *N. gouldi*, spermatogenesis has not been shown to be energetically costly and so any correlation between tendency to enter torpor and reproductive status
is unlikely. Until clearer definition is given to the factors determining torpor entry, especially within the confines of a metabolic chamber, it is premature to place too great an emphasis on these results.

Warm-acclimating *N. gouldi* in mid-winter did not advance the seasonal adaptations to oxygen demands and regulated rectal temperatures seen in acclimatized bats. Spring-adapted *N. gouldi* were in a transitional state; maintaining rectal temperatures at an intermediate level to winter and summer animals and requiring intermediate rates of oxygen consumption to achieve this level. Likewise, the thermoregulatory pattern of warm-acclimated *N. gouldi* was transitional between winter and spring, suggesting that acclimation should have been conducted at a higher temperature or for a longer period.

Exposure to 22°C did have some effect on the thermal energetics of *N. gouldi*. In male bats the change in regulated rectal temperatures was proportional to the shift in the metabolic requirements of thermoregulation. This was shown by the similarity of the curves describing the relationship between the rectal-ambient temperature differential and oxygen consumption for winter-adapted and warm-acclimated males. The effect of warm-acclimation on the energetics of body temperature control in female *N. gouldi* cannot be described as simply. The metabolic cost of regulating a specific differential between rectal and ambient temperature did not change in female bats during the transition from winter to summer yet warm-acclimated females required 10 to 20 per cent less thermal energy to
regulate similar rectal temperatures. I can offer no explanation for this anomaly, except to suggest that the initiation of pregnancy during the warm-acclimation procedure was in some way responsible.

Although warm-acclimation did not result in complete thermal adaptation to the spring condition, the reproductive cycles of both males and females were prematurely initiated. A number of previous workers have reported this effect, and also shown that spermatogenesis cannot be prematurely initiated by environmental factors in the first half of the hibernation season (Racey and Tam 1974; Gustafson 1979). In female hibernating bats the terminal stages of follicle maturation leading to ovulation can only be stimulated in the first half of winter by administering exogenous gonadotrophins (Guthrie and Jeffers 1938; Racey 1982). In the second half of winter, the simulation of spring conditions induces ovulation. Racey (1982) claims that these findings show a "... refractory period ... when the pituitary-gonad axis cannot be stimulated by environmental factors" rather than any proximate influence of increasing day length.
The time of warm-acclimation and subsequent experimentation relative to the times when summer, winter, and spring testing occurred in the outdoor maintained colony. Shown also are the periods when acclimated and outdoor females gave birth.
Figure 7.2 Plots of rectal temperature and oxygen consumption responses to ambient temperature in (A) Male and (B) Female N. gouldi warm-acclimated in mid-winter. A detailed form of the regression equations is given in Appendix II. N = number of animals and the Tr = Ta line is indicated (---).
Figure 7.3. Comparison of oxygen consumption and rectal temperature curves for male *N. gouldi* in winter, spring and summer and following mid-winter warm-acclimation. The results of the statistical comparison of these curves are given in Section 7.3.1 (p.194). Tr-Ta line (—).
Figure 7.4 Comparison of oxygen consumption and rectal temperature curves for female *N. gouldi* in winter, spring and summer and following mid-winter warm-acclimation. The results of the statistical comparison of these curves are given in Section 7.3.1 (p.195).  Tr = Ta line (---)
Figure 7.5 Plot of oxygen consumption against rectal-ambient temperature differential for male *N. gouldi* warm-acclimated in mid-winter and a comparison of this curve with those of winter-, spring- and summer-adapted males. The statistical comparisons of these curves are discussed on page 194 and the regression equations are presented in Appendix II.
Figure 7.6  Plot of oxygen consumption against rectal-ambient temperature differential for female N. gouldi warm-acclimated in mid-winter and a comparison of this curve with those of winter-, spring- and summer-acclimatized females. The statistical comparisons of these curves are discussed on page 195 and the regression equations are given in Appendix II.
Figure 7.7 Comparison of rectal and oxygen consumption curves of male and female *N. gouldii* after warm-acclimation in mid-winter. The statistical comparison of these curves is discussed on page 196.
Figure 7.8 Comparison of the mean body weights of N. gouldi maintained in the outdoor flyway with those warm-acclimated in mid-winter. Shown also are the differences in reproductive cycle which resulted from this division of the population.
Table 7.1  Comparison of the mean body weight, minimum metabolism and areas under the VO₂ and TR curves in male *Nyctophilus gouldi* in spring and following mid-winter warm-acclimation. The times of spring and warm-acclimated testing are shown in Fig. 7.1. *Warm-acclimated males weighed significantly more than spring males during the period of experimentation (F[1,16] = 25.42, p<0.001).

<table>
<thead>
<tr>
<th></th>
<th>Spring</th>
<th>Warm-acclimated</th>
<th>Spring/Warm-acclimated % Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean Body Weight</strong></td>
<td>10.1 ± 0.6</td>
<td>11.6 ± 1.1</td>
<td>+12.9</td>
</tr>
<tr>
<td>(+ SD, no. of bats, no. of observations)</td>
<td>(6, 17)</td>
<td>(5, 21)</td>
<td></td>
</tr>
<tr>
<td><strong>Minimum Metabolism</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mls. O₂/g/hr, STP)</td>
<td>3.1</td>
<td>1.7</td>
<td>+ 9.2</td>
</tr>
<tr>
<td>at Tₐ (°C)</td>
<td>35.9</td>
<td>36.7</td>
<td></td>
</tr>
<tr>
<td>with a TR (°C) of</td>
<td>36.7</td>
<td>38.3</td>
<td>+ 4.2</td>
</tr>
<tr>
<td><strong>Areas under curves</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Tₐ = 10 -30°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO₂ vs. Tₐ</td>
<td>141.9</td>
<td>111.9</td>
<td>-21.1</td>
</tr>
<tr>
<td>TR vs. Tₐ</td>
<td>637.4</td>
<td>674.6</td>
<td>+ 5.5</td>
</tr>
</tbody>
</table>
Table 7.2  Comparison of the mean body weight, minimum metabolism and areas under the VO2 and TR curves in female Nyctophilus gouldi in spring and following mid-winter, warm-acclimation. The times of spring and warm-acclimated testing are shown in Fig. 7.1.

*1 Warm-acclimated females weighed significantly more than spring females during the period of experimentation (F[1,33] = 17.87, p<0.001).

*2 Estimate required extrapolation of the curve beyond the lowest recorded values.

<table>
<thead>
<tr>
<th></th>
<th>Spring</th>
<th>Warm-acclimated</th>
<th>Spring/Warm-acclimated % Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Body Weight</td>
<td>12.2 ± 1.7</td>
<td>*1 14.7 ± 1.7</td>
<td>+17.0</td>
</tr>
<tr>
<td>(+ SD, no. of bats, no. of observations)</td>
<td>(6, 15)</td>
<td>(5, 20)</td>
<td></td>
</tr>
<tr>
<td>Minimum Metabolism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mLs. O2/g/hr, STP)</td>
<td>2.6</td>
<td>3.4</td>
<td>+23.5</td>
</tr>
<tr>
<td>at TA (°C)</td>
<td>34.8</td>
<td>31.6</td>
<td></td>
</tr>
<tr>
<td>with a TR (°C) of</td>
<td>37.5</td>
<td>36.2</td>
<td>- 3.5</td>
</tr>
<tr>
<td>Areas under Curves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(TA = 10-30°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO2 vs. TA</td>
<td>*2 124.5</td>
<td>100.9</td>
<td>-19.0</td>
</tr>
<tr>
<td>TR vs. TA</td>
<td>*2 651.5</td>
<td>654.8</td>
<td>+ 0.5</td>
</tr>
</tbody>
</table>
Table 7.3 Metabolic Q_{10}'s for *Nyctophilus gouldi* in spring and following mid-winter warm-acclimation. Two Q_{10}'s are given (T_A = 30-20°C and T_A = 20-10°C) because of the curvilinear models fitted to the data. All values are in ml. O_2/g/hr (STP).

*Indicates where oxygen consumption recordings were not taken over the entire range of ambient temperature.

<table>
<thead>
<tr>
<th>T_A (°C)</th>
<th>Spring</th>
<th>Warm-acclimated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-20</td>
<td>3.4</td>
<td>3.1</td>
</tr>
<tr>
<td>20-10</td>
<td>2.0</td>
<td>2.2</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-20</td>
<td>2.7</td>
<td>1.5</td>
</tr>
<tr>
<td>20-10</td>
<td>*</td>
<td>2.8</td>
</tr>
</tbody>
</table>
The following tables: 7.4, 7.5, 7.6, 7.7 and 7.8 are the results of the two-level nested ANOVA's (Section 4.2.3) carried out to compare the mean temperatures of five body regions in male *N. gouldi* in winter, summer and following mid-winter warm-acclimation. In all cases, the information has been presented in a standard manner.

Among: refers to the comparison between treatments; for example summer versus winter versus warm-acclimated.

Within: refers to the comparison of the variation of the data within the group of replicates constituting a treatment.

The details of recording sites and experimental protocol are given in Section 4.2. Sample sizes and mean temperatures for each body region are presented in Table 7.9.

Table 7.4 compares the subcutaneous interscapular temperatures in 'summer', 'winter' and warm-acclimated *N. gouldi*.

Table 7.5 compares chest temperatures in 'summer', 'winter', and warm-acclimated animals and Table 7.6 compares the mean temperatures of the mid-dorsal region.

Tables 7.7 and 7.8 present the results of the 'summer', 'winter', and warm-acclimated comparison of the mean temperatures of the internal wing-surface and external wing-surface, respectively.
Table 7.4  Statistical comparison of the mean interscapular temperature in winter, summer and mid-winter, warm-acclimated, male *N. gouldi*.

<table>
<thead>
<tr>
<th>Ambient Temperature (°C)</th>
<th>Level</th>
<th>Degrees of Freedom</th>
<th>F-value</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Among</td>
<td>2</td>
<td>0.49</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>5</td>
<td>288.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Among</td>
<td>2</td>
<td>2.45</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>11</td>
<td>61.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Among</td>
<td>2</td>
<td>1.41</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>14</td>
<td>289.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Among</td>
<td>2</td>
<td>0.58</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>11</td>
<td>42.36</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>Total</td>
<td>42</td>
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</table>
Table 7.5  Statistical comparison of the mean chest temperature in winter, summer and mid-winter, warm-acclimated, male *N. gouldi.*

<table>
<thead>
<tr>
<th>Ambient Temperature (°C)</th>
<th>Level</th>
<th>Degrees of Freedom</th>
<th>F-value</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Among</td>
<td>1.60</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Within</td>
<td>170.73</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td></td>
<td>Total</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Among</td>
<td>1.21</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Within</td>
<td>130.63</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td></td>
<td>Total</td>
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<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>Among</td>
<td>1.03</td>
<td>&gt;0.2</td>
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<tr>
<td></td>
<td></td>
<td>Within</td>
<td>122.46</td>
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</tr>
<tr>
<td>25</td>
<td></td>
<td>Among</td>
<td>2.51</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Within</td>
<td>64.81</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td></td>
<td>Total</td>
<td>39</td>
<td></td>
</tr>
</tbody>
</table>
Table 7.6  Statistical comparison of the mean mid-dorsal temperature in winter, summer and mid-winter, warm-acclimated, male *N. gouldi*.

<table>
<thead>
<tr>
<th>Ambient Temperature (°C)</th>
<th>Level</th>
<th>Degrees of Freedom</th>
<th>F-value</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Among</td>
<td>2</td>
<td>2.49</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>2</td>
<td>284.09</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>Total</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Among</td>
<td>2</td>
<td>0.12</td>
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</tr>
<tr>
<td></td>
<td>Within</td>
<td>8</td>
<td>481.14</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>Total</td>
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<td></td>
</tr>
<tr>
<td>25</td>
<td>Among</td>
<td>2</td>
<td>0.56</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>10</td>
<td>137.34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Among</td>
<td>2</td>
<td>0.96</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>7</td>
<td>513.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total</td>
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<td></td>
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</tr>
</tbody>
</table>
Table 7.7  Statistical comparison of the mean internal wing-surface temperature in winter, summer and mid-winter, warm-acclimated, male *N. gouldi*.

<table>
<thead>
<tr>
<th>Ambient Temperature (°C)</th>
<th>Level</th>
<th>Degrees of Freedom</th>
<th>F-value</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Among</td>
<td>2</td>
<td>1.94</td>
<td>&gt;0.2</td>
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<tr>
<td></td>
<td>Within</td>
<td>5</td>
<td>235.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>15</td>
<td>Among</td>
<td>2</td>
<td>0.92</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>10</td>
<td>92.57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Among</td>
<td>2</td>
<td>0.48</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>11</td>
<td>357.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
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<td>5.80</td>
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</tr>
<tr>
<td></td>
<td>Within</td>
<td>9</td>
<td>154.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>32</td>
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</tr>
</tbody>
</table>
Table 7.8  Statistical comparison of the mean external wing-surface temperature in winter, summer and mid-winter, warm-acclimated, male *N. gouldi*.

<table>
<thead>
<tr>
<th>Ambient Temperature (°C)</th>
<th>Level</th>
<th>Degrees of Freedom</th>
<th>F-value</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Among</td>
<td>2</td>
<td>0.53</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>6</td>
<td>555.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>27</td>
<td></td>
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</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Among</td>
<td>2</td>
<td>1.71</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>11</td>
<td>157.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Among</td>
<td>2</td>
<td>2.89</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>14</td>
<td>172.85</td>
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<tr>
<td></td>
<td>Total</td>
<td>51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Among</td>
<td>2</td>
<td>1.01</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>12</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>39</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The mean temperatures of body regions in endothermic, male *N. gouldi* at ambient temperatures of 35, 25, 15 and 5°C following mid-winter warm-acclimation. The recording sites are shown in Fig. 4.1 and the experimental methods described in Sections 4.2 and 7.2.

All values are given as mean ± one standard deviation, number of bats used. The number of observations used to calculate the mean is four times the number of bats (Section 4.2.3) enabling standard deviations to be presented when only one or two animals were tested.

*Rectal temperature values do not show standard deviations or sample sizes as they were calculated by substitution into the TR curve presented in Fig. 7.2.

<table>
<thead>
<tr>
<th>Body region</th>
<th>Ambient Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35</td>
</tr>
<tr>
<td>Interscapular</td>
<td>36.2 ± 0.3,5</td>
</tr>
<tr>
<td></td>
<td>35.6 ± 0.9,5</td>
</tr>
<tr>
<td></td>
<td>35.3 ± 1.2,6</td>
</tr>
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<td></td>
<td>33.4 ± 1.9,3</td>
</tr>
<tr>
<td>Chest</td>
<td>36.1 ± 0.3,5</td>
</tr>
<tr>
<td></td>
<td>35.4 ± 1.4,5</td>
</tr>
<tr>
<td></td>
<td>35.4 ± 1.2,6</td>
</tr>
<tr>
<td></td>
<td>32.8 ± 2.5,3</td>
</tr>
<tr>
<td><em>Rectal</em></td>
<td>36.2</td>
</tr>
<tr>
<td></td>
<td>34.5</td>
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<tr>
<td></td>
<td>31.9</td>
</tr>
<tr>
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<td>Mid-dorsal</td>
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<td>23.0 ± 0.4,1</td>
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<tr>
<td>Internal Wing-surface</td>
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<td>30.1 ± 2.0,5</td>
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<td>External Wing-surface</td>
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<td>25.4 ± 1.2,5</td>
</tr>
<tr>
<td></td>
<td>18.8 ± 2.1,3</td>
</tr>
</tbody>
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Table 7.9

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*TR = Thermal Regulator curve.*

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220
## Chapter Eight

**TORPOR: INCIDENCE, COSTS AND REGIONAL CONTROL OF BODY HEAT DURING THE ENTRY AND AROUSAL PHASES**

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1</td>
<td>Introduction</td>
<td>221</td>
</tr>
<tr>
<td>8.2</td>
<td>Incidence of Torpor Entry and Arousal</td>
<td>225</td>
</tr>
<tr>
<td>8.2.1</td>
<td>Methods</td>
<td>226</td>
</tr>
<tr>
<td></td>
<td>Incidence of Entry into Torpor</td>
<td>225</td>
</tr>
<tr>
<td></td>
<td>Effect of Food Deprivation on Entry into Torpor</td>
<td>226</td>
</tr>
<tr>
<td></td>
<td>Initiating the Arousal Process</td>
<td>226</td>
</tr>
<tr>
<td>8.2.2</td>
<td>Results</td>
<td>227</td>
</tr>
<tr>
<td></td>
<td>Incidence of Entry into Torpor</td>
<td>227</td>
</tr>
<tr>
<td></td>
<td>Effect of Food Deprivation on Entry into Torpor</td>
<td>228</td>
</tr>
<tr>
<td></td>
<td>Initiating the Arousal Process</td>
<td>228</td>
</tr>
<tr>
<td>8.3</td>
<td>The Metabolic Costs of Regulating Torpor</td>
<td>229</td>
</tr>
<tr>
<td>8.3.1</td>
<td>Methods</td>
<td>229</td>
</tr>
<tr>
<td>8.3.2</td>
<td>Results</td>
<td>229</td>
</tr>
<tr>
<td>8.4</td>
<td>Regional Heterothermy During Torpor</td>
<td>232</td>
</tr>
<tr>
<td>8.4.1</td>
<td>Methods</td>
<td>232</td>
</tr>
<tr>
<td>8.4.2</td>
<td>Results</td>
<td>233</td>
</tr>
</tbody>
</table>
8.5 Partitioning of Body Insulation in Torpid *N. gouldi*
8.5.1 Methods

8.6 The Metabolic Costs of Torpor Entry and Arousal
8.6.1 Methods
8.6.2 Results

8.7 Heat Distribution During Entry, Arousal and Cold Exposure
8.7.1 Methods
8.7.2 Results

8.8 Discussion
Torpor is one of the most physiologically sophisticated forms of thermoregulatory adaptation. Setting aside the special case of aestivation, torpor is the ultimate form of heat conservation by regional heterothermy. Although there is now a vast literature on the subject, the study of the torpid state is not simple and many fundamental questions remain unanswered. The recently published review of hibernation and torpor by Lyman, Willis, Malan and Wang (1982) clearly describes the areas requiring specific attention. Controversy still surrounds the relationship between metabolism and body temperature in the animal entering torpor and whether the body temperature of the torpid animal is controlled in the same way as in the homeotherm. Torpor can be induced in many birds and mammals by food-deprivation but "the ecological significance of this .... has not been clearly established" (Lyman et al. 1982). The stimuli responsible for periodic arousal during hibernation continue to be debated as do the precise and relative contributions of shivering and non-shivering thermogenesis to the rewarming process.

The difficulties associated with keeping some species in captivity and in using them as experimental subjects has led to a concentration of research effort on the more manageable rodents. The bats are one group which have been largely overlooked in torpor studies. As Lyman et al. (1982) point out, bats are usually difficult to maintain in captivity and generally not good experimental
subjects. Most bats are small: a disadvantage if cannulation or other invasive experimental procedures are required.

The temperate-zone microchiroptera are of primary importance in the further understanding of torpor physiology. They are probably the most efficient and frequent exponents of torpor with the longest, continuous periods of inactivity (Menaker 1964). Some bats can rewarmin less than 40 minutes (Hayward 1968; Kulzer, Nelson, McKean and Mohres 1970) and may undergo periods of daily torpor in seasons other than winter.

The major features of torpor shown by the homeotherms for which it is the primary winter survival adaptation are:

1. The entry process is a controlled and sometimes step-wise readjustment of the thermoregulatory control mechanism to a lower level (Strumwasser 1959; Heldmaier 1970; Heller, Colliver and Beard 1977; Lyman et al. 1982);

2. Animals in torpor generally maintain a body temperature a few degrees above environmental temperature (Lyman et al. 1982);

3. There is usually an optimum ambient temperature for hibernation, below which metabolic rate increases to off-set further cooling and potential freezing (Davis and Reite 1967; Wang and Hudson 1971; Lyman et al. 1982);
4. Torpor bouts are shortest at the beginning and end of the hibernation season and longest in mid-winter (Twente and Twente 1967; Wang 1973);

5. The arousal process is marked by an anterio-posterior temperature differential with the 'vital' organs such as the brain and heart being warmed first (Rauch and Hayward 1970; Rauch 1973; Studier 1974);

6. Both shivering and non-shivering thermogenesis play a major role in the rewarming from torpor. Brown fat is an important but not essential source of heat during arousal (Hayward and Ball 1966; Rauch 1973; Lyman et al. 1982).

The literature contains many different uses of the words lethargy, dormancy, hibernation and torpor, primarily because there are so many birds and mammals now known to use some form of body cooling and reduced activity as an energy conserving adaptation to winter. The difficulty lies in finding definitions that encompass the great variety of torpor patterns ranging from the lethargy of the bears to the prolonged inactivity of the temperate-zone bats. Lyman et al. (1982) favour a distinction between "deep hibernators" and those which enter an "intermediate" type of hibernation they call "torpor". Whilst they recognise that torpor "... covers a very broad field ....", Lyman et al. (1982) also state that in "... the microchiropteran bats, daily torpor and deep hibernation are physiologically almost indistinguishable except for the duration of the dormant state".
I consider Lyman's distinction between torpor and deep hibernation to be misleading. Torpor is a physiological state not a type of hibernation. Deep hibernation is a form of torpor which in direct response to colder winter temperatures is characterized by long bouts of inactivity and low body temperatures. The word hibernation has been greatly misused in the past and for this reason I prefer the term 'winter torpor'. A distinction can be made between shallow and deep winter torpor and daily torpor bouts in other seasons. In the presentation of results and discussion which follows, the term hibernation has been used only to refer to winter torpor.

The experimental results assembled here describe a number of aspects of the torpor observed in *Nyctophilus gouldi* during the course of this study. The seasonal changes in the incidence of torpor during experimental runs are described, as are the patterns of body heat distribution in bats preparing for, entering, maintaining and arousing from torpor. The metabolic costs of the transition from homeothermy to torpor, of maintaining torpor, and the return to homeothermy were measured and the effect of food-deprivation on the propensity to enter torpor was also tested. Because of the diversity of methods used to collect these data the organisation of this chapter is different to that encountered in previous chapters. Each discrete set of methods and results are presented together in the relevant subsection and a general discussion concludes the chapter.
8.2 INCIDENCE OF TORPOR ENTRY AND AROUSAL

8.2.1 Methods

The Incidence of Entry into Torpor

During measurements of endothermic oxygen consumption and rectal temperature regulation (Chapter Three), *N. gouldi* frequently entered torpor when exposed to ambient temperatures of less than 20°C. This section includes a seasonal comparison of the frequency of entry into torpor by male and female bats during these experiments. The experimental set-up and procedures followed are described in detail in Section 3.2.2.

Irrespective of the season, animals were brought into the laboratory from the outdoor holding cage on the afternoon before experimentation and provided with plentiful food and water. The temperature within the room was always between 18 and 22°C. This pre-experimental procedure was followed to ensure the short-term nutritional equivalence of experimental animals in all seasons (Section 3.2.2). Single bats were placed in metabolic chambers at approximately 0800 hours the following day. The initial ambient temperature within the chamber was usually 20°C. Following equilibration of the bat to the initial ambient temperature, three or four degree step-wise reductions in temperature, with intervening stabilization periods, followed. By 1600 hours of the experimental day the temperature within the chamber was normally between five and eight degrees Celsius. Experiments were concluded between 1700 and 1800 hours.
The Effect of Food Deprivation on Entry into Torpor

To test the effect of food deprivation on the incidence of entry into torpor, two *N. gouldi*, of the same sex, were brought into the laboratory from the captive colony on the afternoon before experimentation; one was provided with *ad libitum* food and water and the other with only water. During the following experimental day, both bats were subjected, in separate metabolic chambers, to a range of ambient temperatures from 30 down to five degrees Celcius and their oxygen consumption and body temperature responses noted. This experimental procedure was followed on three occasions, twice in July and once in August.

Initiating the Arousal Process

In the experiments intended to measure the metabolic costs of arousal from torpor, pain stimulus or physical disturbance was used to initiate the rewarming process (Section 8.6). In the experiments described in this section, torpid animals were not disturbed and the ambient temperature within the chamber was slowly increased to determine the point at which the return to homeothermy was initiated by environmental temperature alone.
8.2.2 Results

Incidence of Entry into Torpor

Bats which had access to *ad libitum* food on the evening prior to experimentation entered torpor only at ambient temperatures less than 18°C. The highest $T_A$ at which torpor was recorded in well-fed *N. gouldi* was 17.2°C in a spring-adapted female (Fig. 8.5).

The greatest frequency of torpor entry during general experimentation was in spring-adapted *N. gouldi*. In males and females in spring, torpor resulted from seven of the eight exposures of bats to ambient temperatures less than 20°C (Table 8.1). This almost obligate entry into torpor by spring bats was not apparent in animals warm-acclimated in mid-winter (Chapter Seven) despite the attempt to mimic the spring adaptation in these animals. The four warm-acclimated males were exposed to sub-20°C ambient temperatures on 16 separate occasions; only five entries into torpor resulted. Warm-acclimated females ($n = 5$) entered torpor five times from 12 separate exposures to $T_A$'s less than 20°C (Table 8.1).

Overall, summer-adapted bats showed the least tendency to enter torpor at cold ambient temperatures (females, two entries from nine exposures and males, three entries from nine exposures). The incidence of torpor induction in winter-adapted *N. gouldi* was intermediate to that of spring and summer bats (Table 8.1).
The Effects of Food Deprivation on Entry into Torpor

On the three occasions _N. gouldi_ were intentionally denied food for the 12 hours preceding an experiment, entry into torpor was induced (Fig. 8.1). Food-deprived animals were unable to 'resist' entry into torpor once ambient temperature fell below 25°C. The entry process in these bats was noticeably passive by comparison with that shown by well-fed animals (Fig. 8.9). When in torpor, starved animals maintained body temperatures much closer to environmental temperature than well-fed torpid bats, however, they were not hypothermic. When ambient temperature was increased to greater than 20°C, food-deprived bats were capable of endogenous heat production of sufficient intensity to restore their body temperature to homeothermic levels. No pain stimulus initiated arousals were attempted with food-deprived bats so it is not known whether they are capable of rewarming from colder ambient temperatures using only endogeneous heat.

Initiating the Arousal Process

Well-fed animals routinely aroused from torpor once ambient temperature exceeded 15-17°C (Fig. 8.2). At lower temperatures, body temperatures were regulated two to five degrees above the environment (Section 8.4). Warming of the chamber environment by one or two degrees was not sufficient to initiate the arousal process unless the critical thermal barrier of 15-17°C was passed. At this point the rapid arousal phase was initiated (Fig. 8.3).
8.3 THE METABOLIC COSTS OF REGULATING TORPOR

8.3.1 Methods

Bats which entered torpor during the course of an experimental day were given one to two hours to stabilize before recordings of body temperature and oxygen consumption were taken. Section 3.2.2 describes the recording instruments and methods in detail.

The torpor results from spring and summer animals were combined to calculate the lines of best-fit for rectal temperature and oxygen consumption data. For simplicity this group of data has been called 'Summer'. The similarity of the summer and spring oxygen consumption curves of homeothermic *N. gouldi* (Section 3.3) provides justification for this action. Data from torpid, warm-acclimated bats was not used in this analysis; nor was that information collected from food-deprived bats (Section 8.2). Lines of best-fit were calculated by the Hewlett-Packard 85 and associated Regressions Pac. The polynomial model most suited to the data was determined from the respective F-values of each additional component in the equation up to a degree of three (Section 3.2.3).

8.3.2 Results

Torpid, well-fed *N. gouldi* regulated rectal temperatures at levels between two and five degrees greater than ambient temperature (Figs. 8.4 and 8.5). The rectal temperature responses of torpid animals to changes in
ambient temperature were best described by rectilinear models. The addition of quadratic or cubic components to these equations did not improve the ability of the line to describe the average response. In males in summer and females in winter, the slopes of the $T_R$ lines were 1.07 and 0.90, respectively, indicating virtual parallelism with the $T_R = T_A$ line (Fig. 8.4 and 8.5). The rectal temperature regression lines for males in winter and females in summer had slopes of less than one (0.74 and 0.72) and these intersected with the $T_R = T_A$ line at ambient temperatures of 18.8°C and 22.9°C, respectively.

The oxygen consumption ($\dot{V}O_2$) data for torpid $N. gouldi$ was highly erratic. There was no significant relationship between $T_A$ and $\dot{V}O_2$ in males in summer and females in summer and winter (Figs. 8.4 and 8.5). Only in winter-adapted males was there a significant relationship between oxygen consumption and ambient temperature ($F[1,11] = 4.89, p<0.05$). The data were best described by a quadratic or polynomial equation of degree two. The inflection point at $T_A = 9.4^\circ C$ indicates the environmental temperature where minimum oxygen consumption occurred in torpid males in winter. At ambient temperatures above and below 9.4°C increased oxygen consumption was necessary to regulate body temperature a few degrees above ambient.

As a result of the generally erratic oxygen consumption data no seasonal or between-sex comparisons of torpor costs could be attempted. The variation present within the $\dot{V}O_2$ data may be a legacy of the experimental protocol used. The timing of entry into torpor was
unpredictable and the amount of stabilization time given each animal was not always the same (although in all cases it was more than one hour). An animal which entered torpor early in the experimental day had many more hours to equilibrate than did one entering torpor in late afternoon. Greater consistency would have been present in the VO2 data had it been possible to allow all animals at least three to four hours to stabilize in the torpid state.

Typical rates of oxygen consumption for torpid animals were less than 1.0 mls. O2/g/hr (STP). In animals given more than two hours to equilibrate at ambient temperatures near 10°C, rates of VO2 were usually less than 0.5 mls. O2/g/hr (STP) and rates as low as 0.05 and 0.03 mls. O2/g/hr (STP) were also recorded. Given that homeothermic N. gouldi had rates of oxygen consumption between seven and 10 mls. O2/g/hr (STP) at TA's of 10°C (Section 3.3), torpor results in at least a 14-20 fold saving in metabolic energy and possibly as much as 200-300 fold.
8.4 REGIONAL HETEROTHERMY DURING TORPOR

8.4.1 Methods

Male *Nyctophilus gouldi* which had been prepared for the recording of homeothermic regional heterothermy occasionally entered torpor during experiments, permitting measurements of the temperature of a number of body regions in the torpid animal. There was no significant seasonal change in the temperature of any body region in homeothermic bats (Section 4.3.2) and in accordance with this the summer and winter data for torpid animals was combined and linear regression models fitted.

The regression equations were calculated using the mean temperature of each body region for each experimental animal at ambient temperatures of 5 and 15°C. As for homeothermic animals, subcutaneous chest and interscapular measurements were combined to yield the thoracic shell regression; abdominal shell temperature was represented by the subcutaneous mid-dorsal measurements and the internal and external wing surface temperatures were included in the general membrane regression. The recording sites are shown in Figure 4.2 and the methods of thermocouple attachment are described in Section 4.2.2. A few recordings of intraperitoneal temperature were also made, enabling a regression of abdominal core temperature against ambient temperature to be derived for torpid *N. gouldi*. Lines-of-best-fit were compared by analysis of co-variance to test for homogeneity in the distribution of body heat in torpid animals (Section 3.2.3).
8.4.2 Results

The regional heterothermy observed in endothermic *N. gouldi* was also present in torpid bats although there were minor differences in the thermal gradients present within the body. In torpid *N. gouldi* all body regions were regulated a few degrees above ambient temperature (Fig. 8.6). There was no significant difference in the temperature of the peritoneal cavity and the subcutaneous mid-dorsal region (slopes: $F[1,10] = 0.09$, $p > 0.5$; vertical displacement: $F[1,10] = 0.07$, $p > 0.5$). The data for these two body regions were combined to test for a thorax-abdomen temperature difference in the torpid animal. The slopes of the thoracic shell and abdominal temperature curves did not differ significantly ($F[1,21] = 0.57$, $p > 0.5$); however, the thoracic shell was one or two degrees warmer at all $T_A$'s from 5 to 15°C (vertical displacement: $F[1,22] = 7.71$, $p < 0.025$). As in homeothermic *N. gouldi* the wings of the torpid animal were the coldest part of the body and conformed more closely with ambient temperature than any other body region (Fig. 8.6). The wings were significantly colder than the abdominal region (vertical displacement: $F[1,23] = 5.93$, $p < 0.025$) and, by inference, the thoracic shell, although the slopes of the wing and abdominal curves were not significantly different ($F[1,22] = 1.49$, $p > 0.25$).
8.5 PARTITIONING OF BODY INSULATION IN TORPID BATS

8.5.1 Methods

Body insulation was partitioned into furred and naked body surface components for homeothermic *N. gouldi* to permit a seasonal comparison of the heat loss pathways (Section 6.3.2). The primary assumptions made when partitioning the heat loss of homeothermic animals were that the actual surface area of the furred body parts was an acceptable estimate of effective furred surface area and that effective (actual) surface area remained constant across ambient temperatures from five to 35°C. The limitations and implications of these assumptions have been discussed previously (Section 6.4, page 164), however, the end result was a more realistic estimate of the effective surface area of the naked body parts.

The assumption that the actual furred surface area equalled the effective furred surface area could not be made for torpid *N. gouldi* and consequently no partitioning of body insulation was possible. The thermal gradient maintained between the body and the environment in torpid *N. gouldi* was always less than five degrees, compared with 15 to 25°C in the homeothermic animal (Table 4.1). By assuming the equivalence of actual and effective surface area in the torpid animal massive over-estimation of the heat loss through the fur would be massively overestimated, precluding any meaningful conclusions to be drawn about the thermal role played by the wings, ears and tail membranes. The
variable oxygen consumption values recorded from torpid animals also made attempts to partition body insulation meaningless and, therefore best left for future studies.
8.6 THE METABOLIC COSTS OF TORPOR ENTRY AND AROUSAL

8.6.1 Methods

The metabolic costs of torpor entry and arousal were measured whenever the opportunity existed for the complete transitional phases to be monitored. When animals entered torpor during an experimental day they were given at least one hour, and sometimes as long as four hours, to stabilize before administering pain stimulus to initiate the arousal process. A pair of forceps were used to squeeze the foot of the bat and thereafter minute by minute readings of oxygen consumption were made until the rewarming to homeothermic body temperatures was complete and oxygen consumption had again stabilized. At 10 to 15 minute intervals during the arousal period, the calibration of the oxygen analyzer was confirmed using nonrespired room air (Section 3.2.2). Rectal and a number of other body temperatures were continually recorded during the entry and arousal phases (Section 8.7 below).

The entry period was defined as the time from the last homeothermic level oxygen consumption recording to the first stable torpor value. Arousal from torpor was taken as the time from administering the pain stimulus to the attainment of the first stable homeothermic oxygen consumption value. The metabolic cost of the entry and arousal phases were estimated from the area under the oxygen consumption plot. This area was measured using a digitizer and Intecolor 8001 table top computer (Section 6.2.1).
8.6.2 Results

In total 41 entries into torpor were recorded for *N. gouldi* during the course of general experimental work (Chapter Three and Table 8.1). However, due to the irritability of bats during the final stages of the entry process only one case was fully recorded with oxygen consumption and body temperature data (Fig. 8.7). Animals generally commenced the entry into torpor whilst I was absent from the laboratory. On the few occasions when I was present at the commencement of the entry phase, manipulation of the experimental equipment was sufficient disturbance to cause the animal to terminate the body cooling process and resume homeothermy. Consequently, the entry into torpor was usually recorded with only body temperature information.

From the one entry recorded in full, a female in winter (Fig. 8.7), the metabolic cost was about one third of an arousal from torpor in the same season (Table 8.2). The entry into torpor was generally well controlled, marked by minor 'blips' in descending temperature and oxygen consumption (Fig. 8.7, A and B) or by major stepped reductions (Fig. 8.9). In the entry shown in Fig. 8.7, the initial drop in oxygen consumption preceded that of interscapular temperature by four to five minutes. Oxygen consumption stabilized at torpor levels after 29 minutes and a further 48 minutes elapsed before the interscapular region attained stable torpor temperature.
Although susceptible to disturbance during the entry phase, once torpid, _N. gouldi_ were remarkably unaffected by manipulations to the experimental equipment and sometimes required repeated pain stimulus before they would arouse. The normal protocol in these experiments was to initiate the arousal process when the animal was stable at an ambient temperature of between 5 and 10°C. During arousal the bat warmed the chamber by two or three degrees but apart from this no attempt was made to provide exogenous heat (Fig. 8.8). Four complete arousals were monitored in this way from animals in winter (Table 8.2).

There was little variation in the total metabolic cost or time taken for the arousal process. The mean metabolic cost of arousal was $268.8 \pm 17.6$ (SD) mls.0₂/g/hr (STP) or 1049.9 W/kg and the mean time taken for the complete arousal was $37.5 \pm 5.4$ (SD) minutes. The only additional arousal recorded with the inclusion of oxygen consumption measurements in winter was accompanied by an increase in chamber temperature from 13.2 to 22.0°C. The warming environment had no effect on the time taken (37 minutes) or the metabolic cost (289.4 mls.0₂/g/hr) of arousal. In the only arousal recorded with oxygen consumption data in summer, the animal was similarly subjected to an increase in chamber temperature (10.0 to 20.5°C); however, in this case, the rewarming took almost twice as long as in winter bats (65 minutes) and the metabolic cost was 38.0 per cent greater (Table 8.2).
The time taken for the interscapular or rectal temperature to reach 28.0°C in the arousing animal is presented in Table 8.2. Homeothermic *N. gouldi* exposed to ambient temperatures of 5 to 10°C, commonly had rectal temperatures of about 28°C (Section 3.3). The fastest arousal to a body temperature of 28°C was made by a male in winter, when interscapular temperature increased from 18.8 to 28.0°C in 13 minutes. In contrast, rectal temperature took 52 minutes to increase from 13.5 to 28.0°C in the summer-adapted *N. gouldi* in which the complete arousal phase took almost twice as long as in winter animals.

Given the thorax to abdomen thermal gradient present in homeothermic (Section 4.3.2) and torpid (Section 8.4.2) *N. gouldi*, it is probably inappropriate to compare the times taken by the interscapular and rectal regions to rewarm to 28.0°C. The interscapular region was always first to rewarm (Figs. 8.8 and 8.9, see Section 8.7), because of the large brown fat pad present there (Section 4.3.1; Fig. 4.3). During the winter arousal when chamber ambient temperature was increased, the rectal region rewarmed to 28°C in 24 minutes. A similar rewarming time to that of the interscapular region in the four other winter bats where chamber temperature remained relatively constant (mean = 22.7 ± 6.8 (SD) minutes). This is surprising, especially given that there was no difference in the time taken for the complete arousal in these two instances. The effect of supplying exogenous heat during the rewarming phase seemingly reduced the intrinsic thorax to abdomen temperature differential, resulting in a similar rate of abdominal to thoracic heating.
8.7 HEAT DISTRIBUTION DURING ENTRY, AROUSAL AND COLD EXPOSURE

8.7.1 Methods

When recording the pattern of regional heterothermy in endothermic *N. gouldi*, entry into torpor occasionally followed exposure to chamber temperatures less than 20°C. Since these bats were equipped with thermocouples to monitor the temperatures of a number of body regions, the changing patterns of heat distribution during the transitional phases from homeothermy to torpor and vice versa could be examined. In addition to a rectal thermocouple, as many as six other thermocouples were used to simultaneously monitor the temperatures of body regions (Fig. 4.2). The recording sites and methods of attachment were described in Section 4.2.2. To briefly reiterate, naked thermocouples were subcutaneously implanted in the interscapular, chest and mid-dorsal regions. Additional thermocouples were glued to the dorsal and ventral wing surfaces and then covered with adhesive tape. The wings could be folded with the thermocouples attached. In some cases a thermocouple was also implanted within the peritoneal cavity (Fig. 4.2). Two further thermocouples were occasionally used to record the temperatures of:

1. The ventral surface of the uropatagium overlying the femoral vein and
2. The subcutaneous mid-ventral region, immediately overlying the liver.
The complete range of recording sites were never used on a single bat during any one experiment. Generally four or five body regions were monitored, in which case the Leeds and Northrup multipoint temperature recorder registered each channel at 25 to 30 second intervals.

8.7.2 Results

Entry into torpor by *N. gouldi* was normally achieved by a series of stepped reductions in oxygen consumption and body temperature. Figure 8.9 shows a male *N. gouldi* entering and then arousing from torpor. At time zero the relative temperatures of the interscapular, chest, mid-dorsal, rectal and external wing regions were typical of those reported in the regional heterothermy experiments (Chapter Four). It was common in *N. gouldi* exposed to ambient temperatures of less than 25°C for occasional readjustments to the heat distribution pattern to occur (Fig. 8.9 A, and Fig. 8.10 A,B,C and D). These events were marked by a sudden drop in the thoracic temperatures by as much as 5°C, with a simultaneous or slightly delayed increase in the abdominal temperatures (Figs. 8.10 and 8.11). Abdominal temperatures never increased as much as thoracic temperatures decreased during these heat shunts.

There were minor differences in the pattern of temperature change recorded in the interscapular and chest sites probably as a result of differences in thermocouple location between experiments. For example, in the experiments shown in Figs. 8.10 and 8.11, the interscapular
region was nearly four degrees cooler than the chest and during the heat shunt interscapular temperature changed relatively little. In contrast, the interscapular region was warmer than the chest in most animals (Fig. 8.9) and underwent temperature fluctuations of a similar magnitude.

During heat shunts the temperature of the wings normally increased a few degrees before cooling to a level below that maintained prior to the heat shunt. After each cooling of the thorax, the wings most noticeably, and the abdominal temperatures to a lesser extent, remained at a lower temperature (Fig. 8.10). Note that throughout this experiment (Fig. 8.10), the ambient temperature was declining. In Fig. 8.11, $T_A$ was constant at 6.5°C and after the redistribution of body heat only the wing temperature remained lower than the pre-heat shunt level. The drops in body temperature associated with heat shunts were not always as great as those presented in Figs. 8.9, 8.10 and 8.11 and sometimes amounted to only a few points of a degree. Equally, the pattern of heat redistribution was not always the same as that figured and at times it was difficult to define the directions of heat flow (for example, Fig. 8.9 B). On most occasions, ambient temperature showed a transient increase during a heat shunt, indicating that heat was leaving the body (Fig. 8.11 A). The uropatagium was always cooler than the wings and its temperature rarely changed during redistributions of body heat.
It was possible to predict the occurrence of a heat shunt by observing the oxygen level shown on the analyzer. Five to ten seconds before the drop in body temperatures, oxygen consumption would also drop by five to ten per cent (Fig. 8.11). Allowing for the 15 second lag in the flow through system (Section 3.2.2) the drop in oxygen consumption occurred 20 to 25 seconds before body temperatures altered.

During entry into torpor each stabilization period between downward steps of thoracic temperature was marked by a sudden increase in wing temperature above abdominal temperature (Fig. 8.9, C,E and F). At the end of each reduction in thorax temperature, wing temperature was equivalent to or less than abdominal temperature, typical of the situation in homeothermic animals (Section 4.3.2). The abdominal temperatures followed a more passive path into torpor than either the chest, interscapular or wing temperatures.

In the experiment presented in Fig. 8.9, the bat was not allowed to stabilize in torpor; instead ambient temperature was immediately increased to observe the effect on the thermal role played by the wings. The onset of arousal was indicated by a rapid increase in interscapular and chest temperatures (Fig. 8.9 H). The wing and rectal temperatures became closely allied and began to increase at a much slower rate than the thoracic temperatures. The rewarming process was not always as uniform as displayed in Fig. 8.8, brief stabilization periods were common throughout arousals (Fig. 8.9 I,J and K). These stabilization periods
were represented by drops in the interscapular, rectal and wing temperatures, slowing of the rewarming in the chest region and brief increases in the mid-dorsal temperature.
Entry into torpor in response to food-deprivation has been recorded in birds (Kruger, Prinzinger and Schuchmann 1982) and many small mammals including bats (Bartholomew and Cade 1957; Ambid and Agid 1972; Dawson and Wolfers 1978; Frey and Vogel 1979; Racey and Swift 1981). The ecological significance of this response is held to represent an energy conservation adaptation to a proximate food shortage (Lyman et al. 1982). Twenty-four hours of starvation is sufficient to induce torpor in the two predominantly frugivorous phyllastomatids, Carolia perspicillata and Glossophaga soricina (Rasweiler 1973). Arata and Jones (1967) found that the body temperature of C. perspicillata, which fell to 24°C following fasting, returned to the normal homeothermic level within two hours of feeding.

The body temperature regulation of starved Nyctophilus gouldi was very different to that of well-fed animals and clearly geared towards the conservation of thermal energy. Nyctophilus gouldi provided with ad libitum food year-round entered torpor only at ambient temperatures of less than 18°C. When these same bats were denied food for the 12 hours preceding experimentation, torpor was induced at the higher ambient temperature of 25°C. In addition, the entry and arousal phases of food-deprived bats were more passive than in those animals given ad libitum food and the thermal gradients established within the body and maintained between the body and the environment were also greatly reduced in starved bats. An
ambient temperature of 20°C was required to initiate the rewarming process in starved *N. gouldi*, whereas in well-fed animals this critical thermal threshold was between 15 and 17°C (see Section 9.3 for further discussion of this point).

There was a seasonal change in the propensity of well-fed *N. gouldi* to enter torpor when exposed to ambient temperatures less than 18°C. Males and females were most prone to enter torpor in spring when the females were entering the last third of pregnancy (Section 2.4.3). Ransome (1973) showed that *Rhinolophus ferrumequinum* females were more likely to enter torpor when in the final stages of pregnancy. He suggested that the food consumption of near term females was restricted because the bulk of the embryo in the abdominal cavity precluded the engorgement of the stomach to the usual extent. The incidence of torpor in pregnant *N. gouldi* from the mid-winter warm-acclimation experiment was about half that of spring-adapted females (Table 8.1). Warm-acclimated females were tested during the middle third of pregnancy (Section 7.3.3), a time when Studier and O'Farrell (1972) found that female *Myotis lucifugus* and *M. thysanodes* were also less likely to enter torpor. The observation that *N. gouldi* males were equally prone to enter torpor in spring as were females cannot be explained at this time. Consideration should be given to the level of stored body fats in these post-hibernation animals and how this may influence their preparedness to maintain homeothermy when cold exposed.
Entry into torpor by many mammalian hibernators is preceded by a series of successively deeper drops in body temperature called 'test-drops' (Strumwasser 1960; Scott et al. 1974; Pivorun 1977; Hudson and Scott 1979). Test-drops are considered to be a physiological preparation for the transition from homeothermy to torpor. Periodic drops in body temperature occurred in *N. gouldi* exposed to ambient temperatures of less than 25°C. Entry into torpor did not always follow these test-drops and the patterns of heat redistribution or shunting were not always the same. In homeothermic bats exposed to declining ambient temperature, heat is shunted from the thorax to the other parts of the body generally resulting in sequential cooling of the abdomen and wings. The reduction of the thermal gradient between the abdomen and wings and the environment serves an obvious heat conservation function whilst maintaining the 'vital' organs of the anterior body regions at normothermic temperatures. A saving of thermal energy was indicated by the reduced rates of oxygen consumption maintained after each heat shunt.

Test-drops in *N. gouldi* sometimes showed no evidence of heat flow to the wings or abdomen and no expansion of the thorax-abdomen-wing thermal gradient was apparent. The function of these heat shunts is not understood. For thoracic cooling to occur heat was clearly leaving the body in some undefined way from a body surface not being monitored.
Heldmaier (1970) recorded test-drops in *Myotis myotis* and noted that the rewarming which followed a drop in body temperature was marked by a twin peak in oxygen consumption. A respiratory quotient near 0.75 showed that a short burst of non-shivering thermogenesis was responsible for the heat production. This is in contrast to Strumwasser's (1960) findings for *Citellus beecheyi* where recovery from a test-drop was accomplished by shivering thermogenesis.

Major test-drops in *N. gouldi* were always coincident with a drop in oxygen consumption, followed by a short single or twin peak, another drop and then stabilization at or below the pre-test-drop level. It is not known whether shivering played any role in the rewarming from test-drops in *N. gouldi* as electromyographs were not made. Although Heldmaier (1970) did not record drops in oxygen consumption preceding and following the peak in *M. myotis* it is interesting that *N. gouldi* also showed a double surge in oxygen requirements.

The drop in oxygen intake during the first phase of the test-drop possibly represents a shut-down of nonshivering heat production in the primary thoracic thermogenic sites (such as the brown fat and pectoral muscles) coincident with the shunting of warmed blood to the abdomen and wings and the replacement this blood warmed in the thorax with cooler blood from these body regions. The surge in oxygen consumption during the test-drop period clearly represents the initiation of the rewarming process but why this should again drop before stabilizing is not
known. It may be that minor thermogenic over-shoot, as is commonly observed in animals arousing from torpor, also occurs during the rewarming from a test-drop in *N. gouldi*.

Entry into torpor in most mammalian hibernators is a controlled, stepped, readjustment of the thermoregulatory system to a cooler level (Lyman *et al.* 1982). During entry, oxygen consumption declines and stabilizes before body temperature reaches stable levels. This was the pattern in *N. gouldi* also. The wings were used as heat radiators during each step-down in the body temperature. The plateaus or steps were occasionally only minor and evident by momentary reductions in the rate of body cooling. Lyman (1958) found that the stepped entry into torpor was not always present in woodchucks and suggested that the decline in body temperature was more uniform in those animals which had undergone entry a number of times. There was no evidence to suggest that this was the case in *N. gouldi*, as stepped entry into torpor occurred at all times of the year. The only instances of passive or uniform torpor entry were shown by those bats deprived of food prior to cold exposure.

Torpid *N. gouldi* did not maintain all body regions at the same temperature. The thermal gradient from thorax to abdomen to wing membrane observed in homeothermic *N. gouldi* (Section 4.3.2) was also present in torpid animals. Such differences in body temperature have not been previously detected in torpid bats although "... in hibernators which curl into a ball, the heart region is the
warmest ..." (Lyman et al. 1982). In *Eptesicus fuscus*, the patterns of blood distribution throughout the animal are very similar in the torpid and post-arousal states (Rauch 1973). Following arousal only the brown fat and kidneys receive significantly greater fractions of the circulating blood. The regional differences in body temperature, which amounted to two or three degrees in well-fed *N. gouldi*, gave preferential heating to the 'vital' organs within the anterior body parts. This thermal gradient was reduced to less than 0.5°C by starving bats for only 12 hours, illustrating the importance of immediate nutritional status to not only the induction of torpor but also the pattern of temperature regulation (discussed further in Section 9.3).

The arousal from torpor by most hibernators is marked by an anterior to posterior temperature differential (Wells 1971; Rauch 1973; Lyman et al. 1982). In rewarming *M. lucifugus* there is a significant increase in the blood flow to the skin of the dorsal, anterior region which overlies the interscapular brown fat deposit (Rauch and Hayward 1970). The muscles of the anterior limbs also receive greatly increased blood flow as do the brown fat deposits. The interscapular brown fat receives a blood flow 11.7 times greater during arousal than when the animal is torpid. Conversely, the blood flow to the stomach and intestines drops significantly during rewarming.

In arousing *N. gouldi* there was a rapid increase in the temperature of the interscapular and chest regions at the beginning of the rewarming phase. Hayward
and Ball (1966) found that the brown fat deposits receive maximum blood supply during early arousal when they are the major source of heat. The return to homeothermic temperatures by the abdomen and wings of *N. gouldi* was much slower than the thorax. At times the thermal gradient established between the interscapular and rectal regions was as much as 15°C. A maximum anterior-posterior temperature differential of 18°C has been recorded in *Eptesicus fuscus* during arousal (Rauch 1973). For rewarming *N. gouldi*, the wings were commonly 15 to 20°C cooler than the thorax at the time interscapular and chest temperatures had begun to plateau.

The importance of shivering thermogenesis to the arousal process in *N. gouldi* is not known. *Myotis lucifugus* uses shivering only during the final stages of arousal (Henshaw 1970). Likewise, hand-held *N. gouldi* shivered violently during rewarming, but only for the final few minutes. The arousal from torpor in *N. gouldi* was generally achieved by a rapid, constant increase in body temperature, although, plateauing occasionally occurred. These rewarming steps were marked by drops in thoracic temperature, coincident with increases in abdominal and wing temperature. Oxygen consumption during such heat shunts followed a similar but reversed pattern to that described previously for test-drops. The function of plateauing during arousal is not clear. The bat may be, in effect, sampling or checking the environmental temperature to determine whether complete arousal is essential or if a brief pulse of higher ambient temperatures has passed and torpor can continue. When increasing ambient temperature
was used to initiate the arousal from torpor, body temperature tracked that of the environment until a critical ambient temperature of 15 to 17°C was reached. At this critical thermal threshold rapid arousal commenced. If the critical temperature threshold was not exceeded, then torpor continued. The ecological significance of the critical arousal temperature is discussed in Section 9.2.
Figure 8.1  The effect of overnight food deprivation on the thermoregulation of a cold-exposed male  N. gouldi compared with that of a well-fed animal.
Figure 8.2 Comparison of the temperature-induced arousal time of a starved and well-fed *N. gouldi* exposed to a warming environment.
Figure 8.3  Temperature–induced arousal from torpor in a male *N. gouldi*. The active phase of rewarming was initiated once ambient temperature exceeded the critical arousal threshold of 15–17°C.
Figure 8.4 Plots of rectal temperature and oxygen consumption response to ambient temperature in torpid male *N. gouldi* in winter and summer. 'Summer' includes spring data also. The regression equations and associated information are given in Appendix II and these data are discussed on pages 229-30.
Figure 8.5 Plots of rectal temperature and oxygen consumption response to ambient temperature in torpid female *N.gouldi* in winter and summer. 'Summer' includes spring data also. The regression equations and associated information are given in Appendix II. These data are discussed on pages 229-30.
Figure 8.6  Linear regression lines and equations describing the mean temperature responses of the thorax, abdomen, peritoneal cavity and wing membranes of torpid male _N. gouldi_. The assumptions made and methods used to calculate these regressions are described on page 232 and statistical comparisons of them are discussed on page 233.

Equations:

- **Thorax-shell**: $T_b = 4.62 + 0.80T_a$  \( R^2 = 0.91 \), \( F(1,10) = 107.8 \), \( p < 0.001 \)
- **Peritoneal Cavity**: $T_b = 3.21 + 0.82T_a$  \( R^2 = 0.89 \), \( F(1,4) = 33.2 \), \( p < 0.001 \)
- **Abdomen-shell**: $T_b = 3.06 + 0.83T_a$  \( R^2 = 0.97 \), \( F(1,5) = 174.0 \), \( p < 0.001 \)
- **Wing Membrane**: $T_b = 1.63 + 0.91T_a$  \( R^2 = 0.98 \), \( F(1,11) = 725.2 \), \( p < 0.001 \)
Figure 8.7  Entry into torpor by a female *N. gouldi* in winter.
Figure 8.8 Arousal from torpor by a male N. gouldi in winter.
Figure 8.9 Test-dropping followed by entry into torpor and immediate arousal by a male *N.gouldi* in spring.
Figure 8.10  Readjustments to the heat distribution in a homeothermic Nagoldi resulting in an expansion of the thorax-abdomen-wing membrane thermal gradient.
Figure 8.11 A heat shunt in a male <i>N. gouldi</i> in autumn.
Table 8.1  The incidence of torpor in well-fed *N. gouldi* exposed to ambient temperatures less than 20°C during experimentation to describe homeothermic oxygen consumption and rectal temperature patterns (Chapter Three).

<table>
<thead>
<tr>
<th>Season</th>
<th>Sex</th>
<th>Number of bats</th>
<th>Instances of Exposure to $T_A$'s less than 20°C</th>
<th>Total Number of Entries into Torpor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>♂</td>
<td>10</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>12</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Spring</td>
<td>♂</td>
<td>5</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>4</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Summer</td>
<td>♂</td>
<td>8</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>7</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Mid-winter</td>
<td>♂</td>
<td>4</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Warm-acclimated</td>
<td>♀</td>
<td>5</td>
<td>12</td>
<td>5</td>
</tr>
</tbody>
</table>
Table 8.2  Details of torpor entry and arousal for male and female *N. gouldi* in winter and summer. "Duration" refers to the time interval from stable homeothermy to stable torpor or vice versa. Section 8.6.1 describes the methods in detail.

*1 indicates where the chamber temperature was increased during the arousal phase.

*2 the expression of metabolic cost in Watts/kg assumes a respiratory quotient of 0.7.

<table>
<thead>
<tr>
<th>Season</th>
<th>Sex</th>
<th>Body Weight (g)</th>
<th>Duration (minutes)</th>
<th>Time to reach a TR or T1 of 28°C (minutes)</th>
<th>Initial and final chamber temperatures (°C)</th>
<th>Initial and final chamber temperatures (°C)</th>
<th>Metabolic Cost ml O2/g/hr (STP) W/kg*2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Entry into Torpor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>♀</td>
<td>12.2</td>
<td>29</td>
<td>13 (T1*28°C)</td>
<td>9.0, 6.8</td>
<td>91.0</td>
<td>355.5</td>
</tr>
<tr>
<td><strong>Arousal from Torpor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>♂</td>
<td>11.0</td>
<td>65</td>
<td>52 (T1)</td>
<td>10.0, 20.5*1</td>
<td>432.9</td>
<td>1,690.9</td>
</tr>
<tr>
<td>Winter</td>
<td>♀</td>
<td>12.2</td>
<td>33</td>
<td>23 (T1)</td>
<td>7.0, 9.0</td>
<td>268.0</td>
<td>1,046.8</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>12.2</td>
<td>40</td>
<td>28 (T1)</td>
<td>6.8, 9.2</td>
<td>255.0</td>
<td>996.0</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>10.5</td>
<td>44</td>
<td>27 (T1)</td>
<td>9.8, 10.2</td>
<td>293.9</td>
<td>1,148.0</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>12.3</td>
<td>33</td>
<td>13 (T1)</td>
<td>10.0, 12.8</td>
<td>258.2</td>
<td>1,008.5</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>11.4</td>
<td>37</td>
<td>24 (T1)</td>
<td>13.2, 22.0*1</td>
<td>289.4</td>
<td>1,130.4</td>
</tr>
</tbody>
</table>
CHAPTER NINE

FINAL DISCUSSION

9.1 Factors Determining the Form of Seasonal Adaptation in Homeotherms 266

9.2 Differences in Male and Female Activity Patterns, Critical Arousal Temperature and Micro-habitat Selection 273

9.3 Nutritional Status and the Incidence of Entry into Torpor 282

9.4 The Thermoregulatory Competence of the Insectivorous Bats and the Evolution of Divergent Temperature Regulation Strategies in the Chiroptera 286
9.1 FACTORS DETERMINING THE FORM OF SEASONAL ADAPTATION IN HOMEOTHERMS

*Nyctophilus gouldi* conserves body heat and reduces the need for regular food intake in winter, principally through entering into torpor. During the colder months from April to September, when the insect food supply is reduced in the temperate climate of the Canberra region, resident *N. gouldi* enter torpor and rely on stored body fats as their major energy source. *Nyctophilus gouldi* also conserves thermal energy in winter through a reduction in endothermic body temperature and in males the insulative value of the fur improves.

Very few previous workers have considered winter adaptation in temperate-zone bats. The published reports give contradictory views as to whether improved metabolic heat production or insulative enhancement occurs. Holyoak and Stones (1971) reported that cold-acclimated and winter-adapted *Myotis lucifugus* had greater thermogenic capacity than either warm-acclimated or summer-acclimatized bats. With the same species, Zervanos and Henshaw (1970) found that the ability to produce metabolic heat was inversely related to the temperature of acclimation. Summer-acclimatized *M. lucifugus* were found to be the most thermolabile by Hurst and Wiebers (1967). However, these authors could distinguish no seasonal change in the heart rate, oxygen consumption and body temperature of bats rewarming from torpor.
In contrast to the findings of Holyoak and Stones (1971) and Zervanos and Henshaw (1970), the regulated body to ambient temperature differentials of torpid *M. lucifugus* reduce during the hibernation season; the minimum temperature differential being maintained in mid-winter (Henshaw and Folk 1966). Henshaw and Folk (1966) concluded that "... acclimatization by reduction of the temperature differential would make for greater economy in a very limited energy budget." Defining the winter adaptive strategy of *M. lucifugus* is further complicated by the 26 per cent increase in the insulative properties of the winter fur reported by Shump and Shump (1980). Unlike *M. gouldi*, homeothermic *M. lucifugus* have never been shown to regulate lower body temperatures in winter although it seems likely that no one has ever looked.

Many of the conflicting results published for *M. lucifugus* are of doubtful validity as acclimation procedures were used to simulate summer or winter conditions. In contrast to cold-acclimation, winter acclimatization involves the interplay of several environmental factors other than temperature and for this reason can elicit different responses from the same animal (Chaffee and Roberts 1971). A comparison of the physiological responses of white rats to cold-acclimation and winter acclimatization clearly illustrates this point. In cold-acclimated rats there is an increase in mean skin temperature and oxygen consumption with no changes in pelage insulation (Heroux, Depocas and Hart 1959). Rats maintained outdoors in winter show a reduction in skin temperature,
lower rates of oxygen consumption and the insulative value of the fur improves over that of summer animals (Hart 1957). In short, the cold-acclimation of white rats elicits a thermogenic enhancement and winter acclimatization results in improved insulative properties and consequently conservation of body heat.

Winter acclimatization involves insulative improvements in a number of temperate-zone bats. *Myotis myotis* conserves body heat in winter by a reduction in body temperature (Mejsnar and Jansky 1967) similar to that recorded for *N. gouldi*. Apart from the improved insulative value of winter acclimatized *M. lucifugus*, Shump and Shump (1980) observed similar reductions in the thermal losses through the fur of winter-adapted *Myotis keenii* and *Eptesicus fuscus*. O'Farrell and Bradley (1977) describe a "... seasonal acclimatization of body temperature ..." in some active bats. The rectal temperatures recorded from *Pipistrellus hesperus* and *Myotis californicus*, freshly caught and removed from mistnets, were significantly lower in winter than summer. The seasonal shift in rectal temperatures was 8 to 10°C in *P. hesperus* and 3 to 4°C in *M. californicus*. The slope of the curve describing the relationship between body and ambient temperatures did not change from winter to summer in these species, however, the curve for the spring transition period had a steeper slope and crossed over the winter and summer curves. There is an uncanny similarity in the seasonal transition of the rectal temperature curves of *P. hesperus* and *M. californicus* to that of *N. gouldi*. 
The 'hibernation-hypothermia annual cycle' proposed by Menaker (1962) was not evident in *N. gouldi*. Menaker (1962) showed that *M. lucifugus*, though capable of spontaneous arousal from winter torpor were incapable of the same response in summer. Subsequently, he observed, as had Pohl (1961) working with *M. myotis*, that 'summer' bats maintained in a refrigerator for three weeks gained sufficient capacity to augment heat production to arouse spontaneously from torpor. If bats with the ability to spontaneously arouse were maintained at room temperature for three weeks they lost the rewarming capacity.

Irrespective of season, *N. gouldi* from the outdoor colony were capable of rewarming from torpor although arousal in summer took longer than in winter (Section 8.6). Likewise, the warm-acclimation of *N. gouldi* in mid-winter did not remove the ability to rewarm from torpor. The depletion of the brown fat deposits is one suggested reason for the inability of summer-adapted or warm-acclimated animals to rewarm (Menaker 1962) although the precise thermal contribution of brown fat to the arousal process has not been determined (Hayward and Ball 1966; Rauch and Beatty 1975). *Nyctophilus gouldi* had brown fat at all times of the year which may account for the year-round ability of this species to rewarm from torpor.

The factors governing the form of winter adaptation, be it metabolic or insulative, are not well understood. Body size constraints and modifications to behaviour patterns seem to have played an important role
in selecting the form of seasonal adaptation. It has always been considered that very small mammals are precluded from significant winter fur thickening because of the consequent restrictions to movement and activity (Scholander et al. 1950; Hart 1956). Clearly a minimum body size exists, above which the cumbersome nature of thicker fur is of no consequence to the mobility of the animal and improved winter insulation is determined by physiological need rather than by physical constraints. Below this critical body size it is less clear what determines the form of seasonal adaptation.

Roberts, Hock and Smith (1966) suggest that for any homeotherm there is a point where insulation reaches an absolute maximum and any further demands for thermal energy must be answered by escalated metabolic heat production. These authors base their argument on a series of experiments with Peromyscus maniculatus sonorensis. In populations from sites at sea level and 1220 metres elevation, winter adaptation was marked by a significant drop in body temperature and metabolic rate. A population of P. m. sonorensis from an elevation of 3800 metres showed the opposite response to winter cold. Roberts et al. (1966) took this to indicate that the higher elevation group were operating on the maximum insulation compatible with activity in summer, and winter-cold necessitated elevated metabolic levels.
The amount of activity required of a small homeotherm during winter in order to maintain a balanced energy budget appears to be the main factor that determines the form of seasonal adaptation. A species required to forage for long periods in winter faces the thermoregulatory 'problem' of overheating and may have to compromise a more insulative fur for more ready maintenance of a constant body temperature when active. An energetic 'trade-off' must occur in small, active, over-wintering homeotherms: if insulative properties are relinquished to avoid overheating then a greater amount of time may have to be spent searching for food. Should an endotherm possess specialized methods of dumping body heat whilst active, then a more insulative outer covering can be tolerated and aid thermal retention when the animal is inactive.

The form of seasonal adaptation may also affect microhabitat selection during winter. The Alaskan red squirrel (Tamiasciurus hudsonicus preblei) reduces its level of activity in winter and relies upon the insulative properties of the nest to counter any escalation in thermal energy needs. In the red squirrel there is little seasonal change in the insulative value of the fur. In contrast, the Arctic red fox (Vulpes vulpes alascensis) and the porcupine (Erethizon dorsatum myops) are active and exposed to the harsh Alaskan winter for many hours of the day. The winter fur of these animals is much thicker in winter (Irving, Krog and Monson 1955).

The temperate-zone bats are especially well adapted to winter. They effectively avoid the harsh winter
conditions by extended periods of torpor and their small size allows them to more readily locate suitable microclimates than larger homeotherms. Should the chosen micro-habitat become unsuitable they can rapidly move to a more favourable site. Because they are inactive for most of the winter, temperate-zone bats can afford to have a thicker more insulative fur during the colder months. The naked and highly vascularized wings and tail membranes provide a ready avenue for the rapid dissipation of excess body heat produced during occasional bouts of activity (Reeder and Cowles 1951; this study). The naked body surfaces are an asset to the body temperature regulation of the bat rather than a thermoregulatory burden. Viewed in this way the wing and tail membranes of a bat can be considered functionally analogous to the beaver's tail (Steen and Steen 1965), the jackrabbit's ears (Hill and Veghte 1976) and the fur seal's flippers (Irving, Peyton, Bahn and Peterson 1962).
9.2 DIFFERENCES IN MALE AND FEMALE Activity Patterns, Critical Arousal Temperature and Microhabitat Selection

Homeothermic male and female *N. gouldi* conserve body heat in winter through a reduction in the 'effective' body temperature. Improved fur insulation in males results in an additional saving of thermal energy at low ambient temperatures. The rate of thermal conductance from male *N. gouldi* to the environment in winter varied from 26 per cent less than in females at an ambient temperature of 5°C, to 9 per cent less at 20°C. At an ambient temperature of 25°C, the rates of heat loss from males and females were equivalent (Table 9.1).

The superior heat retaining properties of males at low environmental temperatures may be a specific adaptation to greater activity during the colder months. In the captive colony *N. gouldi* males had significantly greater water turnover than females in April, May and June (Inwards pers. comm.). This sexual bias in activity was also present in the free-living population. Of 15 adult *N. gouldi* trapped during the four hibernation seasons monitored only five were females. The ratio of males to females trapped in the active season was 0.68 (93:137) whereas during the hibernation season it was almost three times greater at 2.0 (10:5). This trend was also observed in the four other main species present in the study community (Table 9.2). The active to hibernation season shift in the male to female sex...
ratio for *N. geoffroyi* was 0.21 to 1.25, for *Chalinolobus morio* 0.61 to 2.44, for *Eptesicus regulus* 1.22 to 1.57 and for *E. sagittula* 1.04 to 7.24. A seasonally changing sex ratio may indicate not only differences in activity but also differences in trappability (Cockrum and Cross 1964; O'Farrell and Bradley 1977; Kunz and Anthony 1977) and should be interpreted cautiously. The numbers of males and females caught during the active season for all of these species is possibly not a reflection of the true sex ratio in the population as mid-summer catches are biased toward lactating females.

In all bat species which delay fertilization, multiple copulations throughout the period of hibernation appear to be the norm (Racey and Tam 1974; Racey 1982). In a number of these species, males have been found to be more active than females during the winter. Tidemann (1982) showed that male *Eptesicus vulturinus* were more active than females throughout the winter and argues that they are searching for uninseminated females with which to copulate. The body weights and deposits of brown fat remain relatively constant throughout the winter in male *E. vulturinus* whereas in females they decline. Male *Rhinolophus ferrumequinum* arouse more frequently than females and also lose less body weight during the hibernation season (Ransome 1968). O'Farrell and Bradley (1977) reported catching more male than female *Pipistrellus hesperus* during the winter although it has been subsequently shown that there is no difference in the rates of brown fat use by the sexes (O'Farrell and Schreiweis 1978).
Like *N. gouldi* the four other vespertilionids from the Bull's Head study population, also delay ovulation over the hibernation season (Phillips, Tidemann, unpublished findings). Although it is not known whether multiple copulation occurs in these species, the greater activity of males during the colder months strongly suggests that this is the case.

In the captive colony of *N. gouldi*, vaginal plugs formed following copulation. These plugs were shed by the females at regular intervals conceivably enabling further inseminations by males. Male *N. gouldi* were occasionally observed copulating with torpid females during the winter, however, there was no discernible difference in the rates of body weight decline or fat utilisation by males and females over this period. This is not surprising given that food was freely available and captive animals rarely indulged in lengthy torpor bouts. If free-living male *N. gouldi* are active more often than females during the hibernation season then their better heat conserving abilities may serve to offset the greater energy demands placed upon them.

The occasional activity of temperate-zone bats during the colder months raises some interesting questions concerning the stimulus for arousal from torpor. Dubois (1896) was perhaps the first to consider this question and he suggested that the need to void the bladder was the primary arousal signal. He subsequently dismissed this hypothesis when he found that if the afferent pathways from the bladder were destroyed then the frequency of arousal did
not alter. In later work, Pengelley and Fisher (1961) and Fisher (1964) found there was no correlation between the length of the torpor bout and the volume of the distended bladder.

*Lyman et al.* (1982) suggest that arousal from winter torpor is simply a continuation of the natural circadian rhythm of body temperature: whether the frequency of arousal is governed by ambient temperature or some other factor is not known. In torpid *Myotis lucifugus* and *Eptesicus fuscus*, kept in total darkness, at ambient temperatures between three and 10°C, the endogenous rhythm continues and increases in rectal temperature occur every 24 hours (Menaker 1959; Stones and Wiebers 1965). Ransome (1971) found that *Rhinolophus ferrumequinum* arouse daily when kept at 7°C in summer but during winter they remain torpid. Despite these observations there is considerable evidence that the duration of the torpor bout in bats and other hibernators is primarily determined by ambient temperature.

Bouts of winter torpor are usually longest when environmental temperatures are lowest (Twente and Twente 1967; Wang 1973). In many hibernators there is a critical threshold temperature which causes arousal or at least initiates the rapid phase of rewarming. This critical arousal temperature is between 15 and 17°C in *N. gouldi*. Davis and Reite (1967) exposed torpid *E. fuscus*, *P. subflavus*, *M. sodalis* and *M. lucifugus* to stepwise increases in ambient temperature and found that arousal occurred only after the temperature of the environment
exceeded 10 to 15°C. If the critical temperature is not reached, *Myotis lucifugus* will not rewarm (Zervanos and Henshaw 1970). McNab (1974) found a slightly higher threshold temperature for *P. subflavus* than Davis and Reite (1967) at 18°C. *Lasiusus borealis* rewarms once ambient temperature passes 19°C (Davis and Lidicker 1956; Davis and Reite 1967) and *L. seminolus* arouses at 21°C (Constantine 1958). In rewarming *M. myotis*, shivering peaks at a rectal temperature of 17°C and then slowly falls away (Mejsnar and Jansky 1967). Threshold arousal temperatures are not only present in bats: *Mus musculus* arouses once rectal temperature passes 16 to 19°C (Hudson and Scott 1979) and a body temperature between 8 and 12°C is sufficient to initiate rewarming in *Perognathus hispidus* (Wang and Hudson 1970). Anderson *et al.* (1960) found that in the arousing birchmouse, a body temperature of 17 to 18°C marked the initiation of shivering and the rapid phase of body warming.

There is good ecological evidence that the critical arousal temperature is not simply an artefact of laboratory testing. Emergence by colonies of *Pipistrellus abramus* takes longer and involves fewer individuals once ambient temperature falls below 20°C. If environmental temperature falls below 15°C then emergence does not occur (Funakoshi and Uchida 1978). The critical arousal temperature of *Lasiusus borealis* in the laboratory is 19°C (Davis and Reite 1967) and in the wild this species is not active unless this temperature is exceeded during the day (Davis and Lidicker 1956).
*Nyctophilus gouldi* in a slowly warming environment remain in torpor until chamber temperature exceeds 15 to 17°C and then rewarm to homeothermic body temperatures. To examine the role played by this critical threshold temperature in the determination of the annual activity cycle of *N. gouldi*, the mean number of days per month when the daily maximum on the field study site was equal to or greater than 18°C was calculated and this pattern compared with the number of *N. gouldi* caught per trap-night for each month throughout the year (Fig. 9.1). A temperature of 18°C was chosen because 17.2°C was the highest ambient temperature at which torpor was recorded in *N. gouldi* within experimental chambers.

The laboratory results suggest that following a daily maximum of 18°C, all *N. gouldi* should arouse from torpor and emerge to forage on that night unless other weather factors preclude activity. There is a good correlation between the number of *N. gouldi* caught per trap-night in each month and the number of days with maximum temperature exceeding 18°C. The period of greatest *N. gouldi* activity (October to March) corresponds with the months in which the majority of days have maximum temperatures greater than the critical temperature. In the colder months, daily maximums of 18°C and over are rare on the study site and the numbers of *N. gouldi* caught are equally low.

This is a simplistic appreciation of the factors determining the annual activity pattern of a hibernating bat, however, a primarily temperature-determined arousal cue
readily accommodates a number of facets of temperate-zone bat ecology. For example, differences in the duration of the hibernation period, in the same species, at different latitudes and elevations, can be explained on the basis of the number of days per year or month which exceed the arousal threshold temperature.

The annual differences in the timing of the birth period observed in captive *N. gouldi* (Table 2.5) can also be explained in terms of daily maximum temperatures and the critical arousal temperature. Parturition was about three weeks earlier in 1982 than in the three previous years. It was argued previously (Section 2.4.4, page 37) that an unusually warm August in 1982 caused the advancement of ovulation and subsequently resulted in the earlier birth period. The mean monthly maximum temperature for August in 1982 was 17.4°C compared with 12.7°C in 1979, 14.0°C in 1980 and 11.7°C in 1981. Thirteen of the 31 days of August 1982 had maximum temperatures of 18°C and above. In the three previous years there were one, two and zero days, respectively, when daily maxima reached or exceeded the critical arousal threshold of *N. gouldi*. Under experimental conditions the spring reproductive phases of male as well as female *N. gouldi* were initiated in mid-winter by constant exposure to a temperature above the arousal threshold (22°C).

Differences in the activity patterns of individual bats or males and females may result from diverse micro-habitat selection. A number of previous workers have recognized the influence of micro-climate selection on
arousal frequency (McNab 1974; O' Shea and Vaughan 1977; Funakoshi and Uchida 1978). Hall (1982) found that in early autumn, *Miniopterus schreibersii blepotis* select cave sites with a mean ambient temperature of 19.5°C. During this time nightly feeding activity continues and bats are alert throughout the day. As body fats accumulate in autumn, the bats move to a cooler part of the cave (11.0°C) and roost activity declines. For the coldest months of winter, *M. s. blepotis* selects a hibernation site with a mean temperature of 9.5°C and prolonged bouts of torpor result.

Differences in microhabitat selection provide an explanation for the apparently different activity patterns of male and female vespertilionids during the hibernation season. The lower level of activity by females may be the consequence of hibernating in colder micro-climates (Beer and Richards 1956), as singletons, or in smaller clusters (Davis and Hitchcock 1964; Fenton 1970, 1972). Conversely, the greater arousal frequency of males may result from the selection of warmer hibernacula than females. *Myotis grisescens* females migrate north to the colder areas of northern Alabama, McNab (1974) suggests, to reduce their frequency of arousal during winter.

The specific differences in arousal threshold temperature suggests that they may be adaptive to the energy requirements of the species. Ransome (1971) states that the "... temperature selected (for hibernation) ..." by *R. ferrum-equinum* "... varies and is positively related to the maximum external air temperature on the day
of arousal". Further, Ransome (1971) states that the "... arousal frequency is adjusted in accordance with insect availability". For most of the bat species examined, the arousal temperature is between 15 and 20°C; temperatures at which many air-borne insects also become active and the cost of rewarming is almost certain to be offset by foraging success. Overnight food deprivation in captive *N. gouldi* shifted the arousal temperature from 15-17°C to 20°C effectively ensuring that, in the animal of poorer nutritional status, temperature induced arousal from torpor would not be without reward.

In the tree-dwelling *Lasiurus borealis* the arousal threshold is 19°C whereas in the cave hibernators *E. fuscus, P. subflavus, M. sodalis* and *M. lucifugus* an ambient temperature of 10 to 15°C is normally sufficient to initiate rewarming (Davis and Reite 1967). Should *L. borealis* inadvertently enter a cave and be unable to find the exit they will enter torpor and ultimately perish (Myers 1960), presumably because the arousal threshold is never passed. Although many cave species select microhabitats where ambient temperatures fluctuate to some extent (Dwyer 1971), in general, tree-dwelling species are exposed to much greater microclimatic variation (discussed below) and probably require larger energy stores. The higher arousal temperatures of tree-dwellers may be a compensatory adaptation which ensures the energy balance of bats required to cope with changeable hibernaculum temperatures.
9.3 NUTRITIONAL STATUS AND THE INCIDENCE OF ENTRY INTO TORPOR

The list of species which will enter torpor when deprived of food is constantly enlarging and presently includes the mammals *Perognathus longimembris* (Bartholomew and Cade 1957), *P. amplus* (Reichman and Brown 1979), *Peromyscus leucopus* (Morhardt 1970), *Mus musculus* (Hudson and Scott 1979), *Eliomys quercinus* (Ambid and Agid 1972), *Suncus etruscus* (Frey and Vogel 1979), *Microdipodops pallidus* (Bartholomew and Macmillen 1961), *Planigale gilesi* (Dawson and Wolfers 1978), *Tadarida mexicana* (Herreid 1963b), *Pipistrellus pipistrellus* (Racey and Swift 1981), *Glossophaga soricina* (Rasweiler 1973), *Carollia perspicillata* (Arata and Jones 1967) and *N. gouldi* (this study). It seems reasonable to predict that future studies will find that most animals which naturally employ some form of body temperature reduction as an energy conservation measure will also react to reduced food intake by entry into torpor.

The precise ecological and physiological significance of food-deprivation torpor has not been clearly established although there are obvious adaptive advantages in conserving thermal energy when faced by an immediate food shortage (Lyman et al. 1982). *Microdipodops pallidus* (Brown and Bartholomew 1969) and *Perognathus amplus* (Reichman and Brown 1979) will enter torpor if they are unable to forage, even if their food caches are quite large. In these species torpor seems to operate as a "... bioassay
for those environmental conditions under which foraging yields a net energy deficit." (Reichman and Brown 1979).

With the garden mouse, *Eliomys quercinus*, Montoya, Ambid and Agid (1979) found that it is quality not quantity of food which primarily influences entry into torpor. From a previous study (Ambid and Agid 1972) the caloric requirements of this species were known; if these needs were met but the diet deficient in protein then torpor resulted. The onset of torpor in the garden mouse was hastened by caloric shortage.

These results clearly illustrate the difficulties associated with testing the seasonally changing incidence of torpor and general thermoregulatory responses in free-living as well as captive animals. Even animals brought freshly into the laboratory for testing may yield spurious results due to one isolated unsuccessful foraging bout. Equally, studies, such as the one reported here, which maintain animals in captivity and provide them with unlimited food can be critized on the grounds that foraging effort is too readily rewarded and the diet provided may significantly alter the thermoregulatory patterns. It was considered more important in this study to standardize the nutritional status of the experimental animals for the comparison of endothermic energetics rather than to simulate seasonally changing food availability and gain a better appreciation of the changing patterns of activity and torpor in the species.
Although the seasonally changing incidence of entry into torpor by *N. gouldi* within experimental chambers has been reported (Section 8.2), I have placed little emphasis on these findings because of the 'unnatural' feeding regime used and because of the effect food deprivation had on the propensity to enter torpor. In all seasons, *N. gouldi* of presumed nutritional equivalence entered torpor only at ambient temperatures less than 18°C. Following food deprivation for just one night this torpor induction threshold was shifted to 25°C. The critical arousal threshold of food deprived *N. gouldi* was also moved to a higher temperature (from 15-17°C to 20°C). The body temperatures of bats which entered torpor following overnight food deprivation were regulated nearer to environmental temperature than in well-fed animals with a consequent reduction in the cost of maintaining torpor.

Considerable ecological significance could be placed on these results, although it is questionable whether this is justified. Low-cost torpor at higher ambient temperatures has obvious adaptive merit in times of food shortage, however it is not known whether starvation equivalent to that of experimentally food-deprived bats ever occurs in wild populations of *N. gouldi*. Food-deprived bats were kept overnight at room temperature, 2 to 3°C above the normal torpor induction temperature of well-fed bats. Such a situation is unlikely to arise in free-living *N. gouldi*. Even in mid-summer overnight minimums greater than the torpor induction temperature of 18°C are rare in this region (and presumably most temperate zones!). In the event that inclement weather prevents feeding activity,
entry into torpor should preserve the sound nutritional status of the animal. At all other times of the year the arousal threshold temperature should ensure that rewarming from torpor does not occur unless insects are available and an energy surplus is assured.

In the event of food shortage, the thermal costs of foraging activity may also be reduced. O'Farrell and Bradley (1977) report that the minimum rectal temperature necessary to initiate flight in *Plecotus townsendii* falls from 31.6°C to 19.6°C after eight days of food and water deprivation indicating an escalation of regional heterothermy in response to nutritional stress.

It is conceivable that fat-depleted bats which are forced to emerge prematurely from hibernation to forage could be confronted by low environmental temperatures, few insects and a net energy deficit for activity. In such 'emergency' situations, torpor at low-cost and high temperatures would greatly improve the survival chances of the individual. Equally, continual inclement weather in summer, when females are burdened with the additional energetic cost of lactation, may result in torpor at higher temperatures. Females commonly select roosts for their maternity colonies with day-time temperatures near thermal-neutrality: an action which should serve to delay the onset of food-deprived torpor.
9.4 THE THERMOREGULATORY COMPETENCE OF THE INSECTIVOROUS BATS AND THE EVOLUTION OF DIVERGENT TEMPERATURE REGULATION STRATEGIES IN THE CHIROPTERA

The insectivorous bats are considered to have only "... limited capacities for temperature regulation" (McNab 1982). In contrast, the fruit and nectar-eating bats are regarded as relatively competent homeotherms, showing a more precise control of body temperature. Irrespective of food habits, however, as body size decreases in the bats, so too does the tendency to rigorously maintain body temperature at a constant level (Henshaw 1970; McNab 1982).

"Variable body temperature has frequently been cited as a primitive character for mammals ...", however, even "a sophisticated regulator can operate with a broad or narrow band width and its setting may be altered as occasion demands" (Heller 1980). The variable body temperatures of endothermic *Myotis lucifugus*, for example, can result in substantial savings of thermal energy. A drop in body (rectal) temperature from 37 to 33°C (at an ambient temperature of 20°C) results in a 40 per cent reduction in the rate of oxygen consumption (Studier 1980).

McNab (1982) states that temperate-zone bats "... give up temperature regulation ..." when they "... enter seasonal torpor ..." and interprets thermolability in the homeothermic animal as an indication of imprecise body temperature control. Despite the variability in regulated body temperatures and the year-round tendency to enter
torpor when cold-exposed, the thermoregulatory abilities of *N. gouldi* are far from imprecise and in many ways show greater sophistication than reputedly competent homeotherms. The controlled manner in which *N. gouldi* changes the distribution of body heat, making use of the wings as heat 'radiators' during the entry into torpor is not indicative of a poor thermoregulator. Equally, the ability of *N. gouldi* to increase body temperature by 20 to 25°C in less than 40 minutes is similar to the time taken by other temperate-zone bats to arouse from torpor (Hayward, Lyman and Taylor 1965; Hayward and Ball 1966; Kulzer et al. 1970) and further reinforces the metabolic competence of these species. The regional heterothermy that is present in *N. gouldi* reduces the energetic costs of controlling body temperature in the torpid as well as the endothermic bat. The changing patterns of regional heterothermy exhibited by bats such as *N. gouldi* have been wrongly interpreted in the past as an inability to control body temperature (McNab 1982) rather than as an adaptation for heat retention.

The mass-specific rates of thermal conductance for *N. gouldi* illustrate the effectiveness of regional heterothermy in reducing heat loss from the homeothermic animal. Averaging the rates of thermal conductance calculated for five degree intervals of ambient temperature between 5 and 25°C in summer and winter yields values between 0.39 and 0.46 mls.\(\text{O}_2\)/g/hr/°C (Table 9.1). These values are within ten per cent of the levels predicted by the Bradley and Deavers (1980) equation which is based on thermal conductance values from seven families and 30
(mostly tropical) species of Chiroptera (where thermal conductance \( C = 1.54 \text{ B.Wt.}(g)^{-0.54} \)). The total surface area of \( N. gouldi \) is approximately six times that of an equivalent sized non-volant mammal (Appendix I) yet the thermal conductance value is only 37 per cent greater than that predicted for a theoretical 'wing-less' mammal of the same body mass \( (0.27 \text{ mlO}_2/\text{g/ hr/}^\circ\text{C}) \). The equation used to calculate this hypothetical thermal conductance value \( (C = 0.76 \text{ B.Wt.}(g)^{-0.426}) \) is based on data for 192 mammals from 41 families (Bradley and Deavers 1980).

The rate of thermal conductance for \( N. gouldi \) does not remain constant as ambient temperature drops and the effect of increasing regional heterothermy can be seen in declining rates of thermal conductance (Table 9.1). Although the average rate of thermal conductance in winter-adapted male \( N. gouldi \) was \( 0.39 \text{ mlO}_2/\text{g/hr/}^\circ\text{C} \) (for less than thermal-neutral temperatures) at an ambient temperature of \( 5^\circ\text{C} \) the rate of heat loss to the environment fell to the level of the theoretical 'wing-less' mammal of similar body weight, further emphasizing the heat conserving capacities and thermoregulatory abilities of cold-exposed \( N. gouldi \).

As a direct consequence of regional heterothermy, the rectal temperature of homeothermic \( N. gouldi \) is not a true indication of the temperature maintained in the more central body parts, especially in cold-exposed bats. Even at thermal-neutral temperatures, the rectum is as much as 2 to \( 3^\circ\text{C} \) cooler than the peritoneal cavity and by inference the thoracic cavity and brain. The body core or zone
maintained at constant temperature contracts in homeothermic bats exposed to cold and excludes the abdominal cavity at ambient temperatures less than thermal-neutrality. The rectal temperature of *N. gouldi* is a good measure of average or 'effective' body temperature; a situation which may prevail in other temperate-zone bats reported to regulate lower rectal temperatures.

Low rectal (or colonic) temperatures similar to those recorded in *N. gouldi* and many other temperate-zone bats (McNab 1969, 1982, 1983) are also found in the 'primitive' mammals; the monotremes, marsupials and members of the eutherian orders, the insectivora and edentata (Hulbert and Dawson 1974a). It is unfortunate that the word primitive carries with it the implication of simple or underdeveloped as these groups are, in general, competent homeotherms despite their low body temperatures and consequent low rates of metabolism (Dawson and Grant 1980; Shkolnik 1980). As Dawson (1973) points out, "... it may be the furnace that is primitive not the thermostat".

The 'primitive' mammals possibly conserve thermal energy through regional heterothermy, similar to that observed in *N. gouldi*. The body core may be kept at the temperature of the more 'advanced' mammals and the less vital tissues and peripheral body regions subjected to differential cooling dependent on the temperature of the environment. In this sense, the thermoregulatory system of the primitive mammals could be considered ".... 'superior' to that of advanced mammals" (Taylor 1980) and low body
temperatures should be viewed as an adaptation for heat conservation rather than as an inability to regulate higher body temperatures.

A direct result of low body temperature is an increase in tissue insulation and a reduction in the level of basal or minimum metabolic rate (MMR). Marsupials, for example, have mass-specific MMR's which are approximately 70 per cent of the normal eutherian level described by the Kleiber curve (MMR \( \propto \text{B.Wt.}^{-0.25} \)). Similarly, the MMR's of all insectivorous bats are reputed to fall below the Kleiber curve (McNab 1982, 1983). This generalized grouping of the insectivorous bats may not be warranted, however, especially when the MMR's of *N. gouldi* are considered. In both summer and winter, the MMR's of *N. gouldi* are greater than those reported by McNab (1982 and 1983) for other insectivorous bats. The minimum metabolic rate of winter-adapted *N. gouldi* lies on the Kleiber curve and is above the curve in summer (Fig. 9.2). These MMR's are more comparable with the values for the fruit and nectar feeding sub-families, the Glossophagininae and Carollinae rather than the family Vespertilionidae.

It is not possible to compare McNab's findings for insectivorous bats with those of *N. gouldi* in specific detail as the sources for many of the values given in his 1982 paper are unclear. McNab (1982) cites his 1969 paper as the source for insectivorous bat data yet in this work he quotes MMR's from only *Noctilio labialis, Histiotus velotus, Molossus molossus, Myotis austroriparius, M. myotis* (Mejsnar and Jansky 1967), *Antrozous pallidus, Tadarida*
brasiliensis, Myotis yumanensis (Licht and Leitner 1967) and Eumops perotis (Leitner 1966). The 1982 paper presents MMR's for 15 insectivorous species, only four of which (A. pallidus, M. yumanensis, Tadarida brasiliensis, E. perotis) correspond to the values quoted in the earlier work. It is not possible to place N. gouldi in context with the other insectivorous bats from McNab's (1982) paper without species information to indicate whether the data was collected from tropical or temperate-zone bats; what season the information was recorded; the nutritional status of the experimental subjects, or whether they were tree or cave-dwellers. Factors such as these may be primarily responsible for the variation in the data presented by McNab (1982) where the MMR's of some vespertilionids are as much as three times greater than other vespertilionids.

The tree-dwelling habits of N. gouldi may be responsible for the much higher MMR's of the species. By comparison with the temperate-zone cave-dwellers, species such as N. gouldi, which roost year-round in tree hollows and under exfoliated bark are exposed to a much greater range of environmental temperature. In response to the more variable microclimatic conditions, the tree-dwelling bats should be more adept at regulating their body temperature. Differences in experimental methods make it difficult to compare the thermoregulatory performance of N. gouldi with any other species which has been studied; however, N. gouldi is certainly not a poor thermoregulator and the much higher MMR may serve to establish this further.
At large body masses, the MMR's of mammals scale proportional to the Kleiber relationship (McNab 1983). At smaller body weights, the change in MMR with body mass follows a steeper curve (slope $a$ B.Wt$^{-0.67}$) that "... represents the limit to continuous endothermy ..." (Fig. 9.3). Animals which fall below this boundary curve, McNab (1983) maintains, will be obliged to use daily torpor to balance their energy budgets, at least when food is scarce. This group includes the desert-dwelling heteromyid rodents, most of the cricetine rodents, marsupials of less than 100 grams and the insectivorous bats. The winter MMR of *N. gouldi*, although conforming with the Kleiber relationship, falls below the boundary curve indicating the need for daily torpor at this time (Fig. 9.3). The MMR of summer-adapted *N. gouldi* lies very close to the boundary curve suggesting that in this season the species should be able to cope with moderate food shortage without resorting to the energy conserving measure of torpor.

McNab (1983) contends that below some critical mass of around 80 grams there are two options for homeotherms; facultative daily torpor or an escalation of metabolic rate in keeping with the boundary curve. The further below the boundary curve the MMR of an animal lies the more likely and frequent will be its need to use daily torpor (McNab 1983). In this regard the higher MMR's of *N. gouldi* would appear to indicate a thermoregulatory ability superior to that of other insectivorous bats. Whether this is true and the precise role played by the tree-dwelling habit in selecting for higher MMR is yet to be determined.
The smallest mammals, the shrews, are an ideal group in which to examine the influence of environment on body temperature regulation and metabolic rate. The shrews of the sub-family Crocidurinae have MMR's below the boundary curve (Fig. 9.3) and routinely use facultative daily torpor (Nagel 1977; Frey 1979). The soricine shrews, in contrast, have MMR's above the boundary curve, higher body temperatures than the crocidurines and have never been shown to enter torpor (Vogel 1980). An exception to this is the desert-dwelling soricine Notiosorex crawfordi which has an MMR below the boundary curve and enters torpor (Lindstedt 1980). Desert-dwelling marsupials (Hulbert 1980) and rodents (Hart 1971) also have lower MMR's than their non-desert-dwelling counterparts; a supposed secondary adaptation for greater heat tolerance and water economy (Hulbert 1980; Lindstedt 1980).

The different metabolic rates of the shrew sub-families are also reflected in their activity rhythms (Crowcroft 1957). The soricines are active both day and night whereas European crocidurines, in warmer seasons, and the tropical species need to forage only at night (Vogel 1980). The price paid by the soricine shrews for maintaining a constantly high body temperature is virtually continuous foraging activity.

Because of their dependance on constantly available food the distribution of the Soricinae, especially in temperate regions, is considerably restricted by comparison with the Crocidurinae. In this sense the high
body temperatures and metabolic rates of the soricine shrews impose greater limitations on their capacities to exploit the environment and the attributes of low body temperature, low metabolic rate and facultative daily torpor seem more adaptive to the vicissitudes of the temperate climate.

The anomaly of the divergent energetic strategies of the shrew sub-families is that the Crocidurinae had a tropical origin (palaeotropical region) and the Soricinae a temperate origin (holarctic region). Vogel (1980) points out that the difficulty in determining which of the shrew sub-families is the more primitive or evolved is fundamentally compounded by the co-existence of both conservative and evolved characteristics in each group. He concludes that the Crocidurinae were a secondary adaptation from the more soricine-like common ancestor. However, Vogel's (1980) argument may not convince adherents of the view that torpor evolved in the tropics and that higher more constantly regulated body temperatures were a subsequent development (McNab 1969, 1982; Dwyer 1971).

The first mammals were probably small, insectivorous, nocturnal and scansorial (Eisenberg 1980). Crompton, Taylor and Jagger (1978) argue that homeothermy evolved in two major steps. The first of these allowed the early mammals to exploit the nocturnal niche. Because this meant exposure to cooler temperatures when active, the low body temperatures and low metabolic rates inherited from their reptilian ancestors remained adaptive to their needs and have persisted in the extant nocturnal insectivores. The invasion of the diurnal niche by the early mammals
required an elevation in body temperature and metabolic rate, Crompton et al. (1978) argue, so that body temperature could be controlled mainly through insulative changes rather than by water-costly evaporative means.

I suggest that the development of higher body temperatures and metabolic rates in the soricine shrews was a consequence of invading cooler climates. If we accept that the early tropical crocidurines were nocturnal and had low body temperatures, low metabolic rates and the capacity for daily torpor then at a particular time during the movement into cooler environments a point was reached where the energetic constraints of small body size meant the shrews needed to extend their foraging time into daylight hours. The exposure to higher environmental temperatures when active was sufficient selective pressure to bring about an elevation in the body temperatures and metabolic rates of the divergent soricines so that insulative rather than evaporative means of thermoregulation could be exploited and water requirements reduced.

A similar line of thought can be used to examine the evolution of the different thermoregulatory strategies present within the Chiroptera. The first mammals possessed energetics closely resembling their immediate reptilian ancestor. This included both low body temperatures and metabolic rates as a consequence of an ability to tolerate the cooling of body tissues. These first mammals probably possessed the capacity for facultative heterothermy or simple torpor in response to food shortage. The diversification of dietary habits in the first bats resulted
in the major split between the megachiroptera and the microchiroptera. There may be some merit in the suggestion (McNab 1982) that the 'prebat' (Dwyer 1971) was omnivorous, thus giving rise to the megachiroptera and microchiroptera more readily. The relative constancy of the fruit and nectar food resource promoted not only greater stability of body temperature but also a tendency towards more crepuscular foraging habits. As tree-dwellers the early bats gained little protection from the hot day-time temperatures and this in combination with activity during warmer times selected for an increase in body temperature and metabolic rate.

The lower energetic costs of temperature regulation due to regional heterothermy and the capacity for diurnal torpor enabled the early insectivorous bats to radiate into temperate zones. Some species opted to continue the tree-dwelling habit whilst others exploited the more thermally stable and predictable cave niche. These species were exposed to cool daily temperatures when inactive and cool night-time temperatures whilst foraging and there has therefore been no selective pressure to alter their thermal energetics from those of very early bats.

The first temperate-zone, tree-dwelling species, in contrast to the cave-dwellers, were exposed to a much greater range of ambient temperatures, including very high afternoon temperatures in summer. Such exposure to high ambient temperatures, I have argued previously for the soricine shrews and frugivorous bats, should have resulted in the evolution of higher body temperatures and metabolic rates in the tree-dwelling temperate-zone bats.
Unfortunately, there is so little reliable body temperature and metabolic rate data available for temperate-zone bats that a comparison of cave and tree-dwelling species is not possible at this time. The uncertainty of the effects of acclimation procedures precludes the use of most data. Of equal consideration is the wide diversity of roosting sites chosen by bats and the compromises in energetic patterns which may have resulted. For example, what effect would overwintering in a cave but roosting in rooftops, crevices or trees for the rest of the year have on the level of body temperature and metabolic rate? Undoubtedly, the most useful comparison to make is that of a specialist tree-dweller with a specialist cave-dweller.

This study has repeatedly highlighted the need for more consistent laboratory and field observations of the thermal biology of bats and especially the temperate-zone cave and tree-dwellers. Much of the foregoing discussion has taken a simplistic stance in attempting to place _N. gouldi_ within an ecological and evolutionary context. The lack of comparable data has meant that much of this final discussion can be labelled speculative, however, speculation when tempered with moderation and based upon the available information, hopefully serves to stimulate the thought processes of others even if only in disagreement.

There are a number of questions concerning the evolution of bats which, I believe, can be answered by a comparison of the thermoregulatory abilities and energetics of the temperate-zone tree and cave-dwelling species. Until
recently, technological limitations prevented or hindered such research. With the advent of miniaturized radio-transmitters, the roosts of tree-dwelling species can now be routinely located enabling a comparison of the thermal demands placed upon the tree and cave-dwellers when at rest. This information, together with annual cycles of water requirements and energy expenditure, obtained using radioactive tracers in free-living bats, will permit clearer definition of the factors which determined the evolution of endothermy in bats and other homeotherms.
Figure 9.1 Comparison of the number of *N. gouldi* caught per trap-night in each month with the mean number of days when maximum temperature was greater than or equal to the critical arousal threshold (18°C). Trap and temperature data is for the years 1979, 1980, 1981, 1982 and January, February, March of 1983. Temperature data comes from the Lee's Creek recording site (750m) near the Bull's Head study site (Section 2.3.2).
Figure 9.2 Mass-specific basal rates of metabolism for summer and winter-adapted *N. gouldi* relative to those of other insectivorous bats and fruit and nectar-eating families. Kleiber's curve is also shown. Redrawn from McNab (1982) with the addition of *N. gouldi* data and the exclusion of information for blood and meat-eating species.
Figure 9.3 Mass-specific basal rates of metabolism for insectivorous, nectar-eating, fruit-eating and carnivorous bats relative to Kleiber's curve and the minimum boundary curve for continuous endothermy. Shown also are BMR’s of the soricine and crocidurine shrews. Redrawn from McNab (1983) with the inclusion of values for N. gouldi in summer and winter.
Table 9.1 Whole-animal rates of thermal conductance for male and female *N. gouldi* in summer and winter. Mean rectal temperatures were used in calculating these values (Chapter Three) and mean body weights are the same as those given in Tables 3.1 and 3.2.

The Bradley and Deavers (1980) equation was used to predict rates of thermal conductance in summer and winter using the mean body weights \([C = 1.54 \text{ B.Wt. (g)^{-0.54}}]\).

<table>
<thead>
<tr>
<th>Ambient Temperature (°C)</th>
<th>Summer</th>
<th>Winter</th>
<th>Summer</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.33</td>
<td>0.27</td>
<td>0.35</td>
<td>0.34</td>
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<tr>
<td>10</td>
<td>0.42</td>
<td>0.34</td>
<td>0.40</td>
<td>0.42</td>
</tr>
<tr>
<td>15</td>
<td>0.49</td>
<td>0.40</td>
<td>0.44</td>
<td>0.46</td>
</tr>
<tr>
<td>20</td>
<td>0.53</td>
<td>0.45</td>
<td>0.48</td>
<td>0.49</td>
</tr>
<tr>
<td>25</td>
<td>0.55</td>
<td>0.51</td>
<td>0.53</td>
<td>0.51</td>
</tr>
<tr>
<td>30</td>
<td>0.61</td>
<td>0.70</td>
<td>0.69</td>
<td>0.64</td>
</tr>
<tr>
<td>35</td>
<td>1.0</td>
<td>4.40</td>
<td>1.70</td>
<td>7.31</td>
</tr>
</tbody>
</table>

Mean Rate of Thermal Conductance \([T_A 5-25°C]\)

| Mean Body Weight (g) | 10.1   | 12.1   | 11.70  | 12.3   |

*Predicted Rate of Thermal Conductance* 0.44 0.40 0.41 0.40

Actual C/Predicted C (%) 104.5 97.5 107 110
Table 9.2. The numbers of adult male and female *N. gouldi*, *N. geoffroyi*, *Chalinolobus morio*, *Eptesicus sagittula* and *E. regulus* caught during the active and hibernation seasons in the Bull’s Head study area. January 1979 to March 1983.

<table>
<thead>
<tr>
<th>Species</th>
<th>Active Season (Oct, Nov, Dec, Jan, Feb, Mar)</th>
<th>Hibernation Season (Apr, May, Jun, Jul, Aug, Sep)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Trap-nights = 262</td>
<td>Total Trap-nights = 109</td>
</tr>
<tr>
<td>N. gouldi</td>
<td>d: 9</td>
<td>d: 9</td>
</tr>
<tr>
<td>Ratio d:9</td>
<td></td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>93:137</td>
<td>10:5</td>
</tr>
<tr>
<td>N. geoffroyi</td>
<td>43:206</td>
<td>5:4</td>
</tr>
<tr>
<td>Ratio d:9</td>
<td>0.21</td>
<td>1.25</td>
</tr>
<tr>
<td>C. morio</td>
<td>227:367</td>
<td>44:18</td>
</tr>
<tr>
<td>Ratio d:9</td>
<td>0.62</td>
<td>2.44</td>
</tr>
<tr>
<td>E. sagittula</td>
<td>266:156</td>
<td>29:4</td>
</tr>
<tr>
<td>Ratio d:9</td>
<td>1.04</td>
<td>7.25</td>
</tr>
<tr>
<td>E. regulus</td>
<td>231:189</td>
<td>22:14</td>
</tr>
<tr>
<td>Ratio d:9</td>
<td>1.22</td>
<td>1.57</td>
</tr>
<tr>
<td>Totals</td>
<td>860:1055</td>
<td>110:45</td>
</tr>
</tbody>
</table>
BIBLIOGRAPHY


Appendix I: The total surface area of vespertilionid bats compared to 'wing-less' mammals.
Although it is readily apparent that bats have a much greater body surface area than non-volant or 'wing-less' mammals, the difference between the two groups has not been quantified despite the relevance to thermal energetics such a comparison holds.

In the course of this study total surface area measurements were made for *N. gouldi* and six other Australian vespertilionids: *N. geoffroyi, Chalinolobus morio, C. gouldii, Eptesicus sagittula, E. regulus* and *Pipistrellus tasmaniensis* (Table I). The methods used in measuring these surface areas are described in Section 6.2.1 (page 143).

Norberg (1981) presented wing area data for 37 vespertilionids taken from a number of other authors. Because of the diversity of definitions and methods used to measure or estimate wing area it was necessary for Norberg (1981) to make a number of assumptions about and corrections to these data in order to obtain a strictly comparable data set. Norberg's definition of wing area included all of the ventral body surface except for the head. To obtain total surface area estimates from Norberg's data, I doubled wing area, having first added three per cent of the value to account for the excluded head area. Three per cent was the standard estimate of ventral head area used by Norberg in her manipulations to other authors data.

Table I presents the estimated total surface area data for all of these vespertilionids with their respective mean body weights. A linear regression model was fitted to
these data following their conversion to logarithms (base 10). The line so described is presented in Fig. I and compared with the Meeh equation for 'wing-less' mammals spanning the same body weight range.

The two equations are:

Meeh formula,
Total Surface Area (cm$^2$) = 9.7 x B.Wt.(g)$^{0.67}$

Vespertilionid formula,
Total Surface Area (cm$^2$) = 58.21 x B.Wt.(g)$^{0.61}$

For the body weight range 3.7 to 27.0 grams vespertilionid bats have total surface areas which are approximately six times greater than non-volant mammals. The thermoregulatory significance of this difference in total surface area is discussed in Chapter One (page 4) and further considered in Chapter Nine (page 288).
Figure I  Regression of total surface area against body weight for members of the Family Vespertilionidae. Also shown is the Meeh equation for 'wing-less' mammals. The raw data for the vespertilionid regression is presented in Table I.
Table 1. The body weight and total surface area of vespertilionids used in the regression presented graphically in Fig. 1. All data comes from Norberg (1981) except for those species marked (*) which were recorded during this study. The sample sizes for each mean are given in parentheses.

<table>
<thead>
<tr>
<th>Species</th>
<th>Body Weight (g)</th>
<th>Total Surface Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Myotophilus gouldi</td>
<td>11.2 (39)</td>
<td>287.6 (10)</td>
</tr>
<tr>
<td>*N. geoffroyi</td>
<td>8.0 (39)</td>
<td>222.2 (9)</td>
</tr>
<tr>
<td>*Chalinolobus mortio</td>
<td>9.9 (65)</td>
<td>232.0 (11)</td>
</tr>
<tr>
<td>*C. gouldii</td>
<td>15.3 (13)</td>
<td>320.7 (7)</td>
</tr>
<tr>
<td>*Eptesicus sagittula</td>
<td>6.9 (47)</td>
<td>193.6 (10)</td>
</tr>
<tr>
<td>*E. regulus</td>
<td>5.6 (38)</td>
<td>173.2 (10)</td>
</tr>
<tr>
<td>*Pipistrellus tasmaniensis</td>
<td>22.3 (8)</td>
<td>444.0 (8)</td>
</tr>
<tr>
<td>Barbastella barbastellus</td>
<td>10.3 (10)</td>
<td>228.7 (10)</td>
</tr>
<tr>
<td>Eptesicus fuscus</td>
<td>16.6 (3)</td>
<td>342.0 (56)</td>
</tr>
<tr>
<td>E. millesoni</td>
<td>9.2 (1)</td>
<td>230.8 (1)</td>
</tr>
<tr>
<td>E. serotinus</td>
<td>22.3 (6)</td>
<td>370.8 (6)</td>
</tr>
<tr>
<td>Lasionycteris noctivagans</td>
<td>10.6 (35)</td>
<td>261.6 (35)</td>
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<tr>
<td>Lasiusus borealis</td>
<td>13.1 (1)</td>
<td>302.8 (2)</td>
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<td>L. cinereus</td>
<td>27.0 (32)</td>
<td>428.5 (51)</td>
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<td>Miniopterus schreibersii</td>
<td>14.2 (10)</td>
<td>282.2 (10)</td>
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<td>Myotis bechsteinii</td>
<td>10.4 (2)</td>
<td>230.7 (2)</td>
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<td>M. daubentoni</td>
<td>7.0 (10)</td>
<td>201.9 (10)</td>
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<tr>
<td>M. emarginatus</td>
<td>6.7 (2)</td>
<td>191.6 (2)</td>
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<td>M. evotius</td>
<td>6.2 (5)</td>
<td>170.9 (5)</td>
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<td>M. grisescens</td>
<td>7.5 (22)</td>
<td>253.4 (38)</td>
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<td>M. kealii</td>
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<td>M. lechii</td>
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<td>M. lucifugus</td>
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<td>197.8 (1)</td>
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<td>228.7 (10)</td>
</tr>
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Appendix II: Complete Form of Regression Equations -
Presented in Chapters Three, Six and Seven
CHAPTER THREE

\[ N = \text{number of animals} \]

*indicates that the variable in the equation does not significantly \((p = 0.05)\) improve the ability of the curve to describe the data

MALES (Fig. 3.4)

Winter: \[ T_R = 32.543796 - 0.233938 T_A + 0.009090 T_A^2 \]
\[ N = 17 \]
\[ F[2,74] = 38.99 \quad p<0.001 \]
\[ \dot{V}_{O2} = 5.633383 + 0.456585 T_A - 0.035015 T_A^2 + 0.000547 T_A^3 \]
\[ F[3,64] = 78.58 \quad p<0.001 \]

Spring: \[ T_R = 29.649685 - 0.018886 T_A + 0.005998 T_A^2 \]
\[ N = 8 \]
\[ F[2,61] = 49.81 \quad p<0.001 \]
\[ \dot{V}_{O2} = 5.449452 + 0.833149 T_A - 0.051986 T_A^2 + 0.000751 T_A^3 \]
\[ F[3,30] = 116.94 \quad p<0.001 \]

Summer: \[ T_R = 33.356184 - 0.157623 T_A + 0.008186 T_A^2 \]
\[ N = 9 \]
\[ F[2,89] = 74.73 \quad p<0.001 \]
\[ \dot{V}_{O2} = 6.938780 + 0.660229 T_A - 0.046187 T_A^2 + 0.000684 T_A^3 \]
\[ F[3,77] = 217.88 \quad p<0.001 \]
Chapter Three (contd)

FEMALES (Fig. 3.5)

Winter:  \( T_R = 31.518201 - 0.110588 \, T_A + 0.006201 \, T_A^2 \)
\( N = 13 \)
\( F[2,63] = 16.63 \quad p<0.001 \)
\( \dot{V}O_2 = 7.413445 + 0.488070 \, T_A - 0.041956 \, T_A^2^* + 0.000672 \, T_A^3 \)
\( F[3,65] = 147.81 \quad p<0.001 \)

Spring:  \( T_R = 29.239004 + 0.050808 \, T_A + 0.005350 \, T_A^2^* \)
\( N = 6 \)
\( F[2,40] = 32.16 \quad p<0.001 \)
\( \dot{V}O_2 = -6.510855 + 2.174428 \, T_A - 0.041956 \, T_A^2^* + 0.001367 \, T_A^3 \)
\( F[3,29] = 62.80 \quad p<0.001 \)

Summer:  \( T_R = 34.675310 - 0.232567 \, T_A + 0.008439 \, T_A^2 \)
\( N = 10 \)
\( F[2,89] = 36.86 \quad p<0.001 \)
\( \dot{V}O_2 = 9.630238 + 0.183790 \, T_A - 0.026250 \, T_A^2 + 0.000449 \, T_A^3 \)
\( F[3,79] = 130.38 \quad p<0.00 \)
CHAPTER SIX

MALES (Fig. 6.7) Sample sizes as for Chapter Three

Winter: $\bar{V}_O_2 = 21.40 - 0.51 \, T_R \quad F[1,46] = 10.91 \quad p<0.001$

Spring: $\bar{V}_O_2 = 27.69 - 0.65 \, T_R \quad F[1,27] = 22.69 \quad p<0.001$

Summer: $\bar{V}_O_2 = 32.92 - 0.78 \, T_R \quad F[1,71] = 98.82 \quad p<0.001$

FEMALES (Fig. 6.7)

Winter: $\bar{V}_O_2 = 19.47 - 0.45 \, T_R \quad F[1,55] = 9.06 \quad p<0.001$

Spring: $\bar{V}_O_2 = 26.15 - 0.62 \, T_R \quad F[1,26] = 39.42 \quad p<0.001$

Summer: $\bar{V}_O_2 = 24.0 - 0.53 \, T_R \quad F[1,79] = 21.01 \quad p<0.001$
Chapter Six (cont'd)

*indicates that the variable in the equation does not significantly (p = 0.05) improve the ability of the curve to describe the data.

MALES (Fig. 6.9) Sample sizes as for Chapter Three

Winter: \[ \dot{V}O_2 = 1.444522 + 0.402810 \ (TR-TA) - 0.009217 \ (TR-TA)^2 + 0.000142 \ (TR-TA)^3 \]
\[ F[3,46] = 74.42 \quad p<0.001 \]

Spring: \[ \dot{V}O_2 = 3.136693 + 0.100926 \ (TR-TA) + 0.031302 \ (TR-TA)^2 - 0.001044 \ (TR-TA)^3 \]
\[ F[3,27] = 41.91 \quad p<0.001 \]

Summer: \[ \dot{V}O_2 = 2.324602 + 0.106114 \ (TR-TA) + 0.029303 \ (TR-TA)^2 - 0.000853 \ (TR-TA)^3 \]
\[ F[3,72] = 122.52 \quad p<0.001 \]

FEMALES (Fig. 6.9)

Winter: \[ \dot{V}O_2 = 0.549804 + 0.524203 \ (TR-TA) - 0.004991 \ (TR-TA)^2 - 0.000890 \ (TR-TA)^3 \]
\[ F[3,55] = 141.57 \quad p<0.001 \]

Spring: \[ \dot{V}O_2 = 1.538901 + 0.410557 \ (TR-TA) - 0.008567 \ (TR-TA)^2 + 0.000618 \ (TR-TA)^3 \]
\[ F[3,26] = 20.50 \quad p<0.001 \]

Summer: \[ \dot{V}O_2 = 3.118952 + 0.088124 \ (TR-TA) + 0.013264 \ (TR-TA)^2 - 0.000244 \ (TR-TA)^3 \]
\[ F[3,79] = 109.43 \quad p<0.001 \]
CHAPTER SEVEN

*indicates that the variable in the equation does not significantly (p = 0.05) improve the ability of the curve to describe the data

MALES (Fig. 7.2)

Warm-acclimation: \[ T_R = 27.900655 + 0.205394 \, T_A - 0.001409 \, T_A^2 \]
N = 4
\[ F[2,70] = 30.18 \quad p<0.001 \]
\[ \dot{V}O_2 = 5.688303 + 0.562931 \, T_A - 0.039587 \, T_A^2 + 0.000580 \, T_A^3 \]
\[ F[3,53] = 81.46 \quad p<0.001 \]

FEMALES (Fig. 7.2)

Warm-acclimation: \[ T_R = 27.633697 + 0.271619 \, T_A - 0.000755 \, T_A^2 \]
N = 5
\[ F[2,50] = 11.99 \quad p<0.001 \]
\[ \dot{V}O_2 = 9.972225 + 0.154742 \, T_A - 0.010977 \, T_A^2 + 0.000294 \, T_A^3 \]
\[ F[3,53] = 64.55 \quad p<0.001 \]

MALES (Fig. 7.5)

Warm-acclimation: \[ \dot{V}O_2 = 1.668233 + 0.061138 \, (T_R-T_A) + 0.030858 \, (T_R-T_A)^2 - 0.000925 \, (T_R-T_A)^3 \]
\[ F[3,55] = 70.26 \quad p<0.001 \]

FEMALES (Fig. 7.5)

Warm-acclimation: \[ \dot{V}O_2 = 4.695341 - 0.554172 \, (T_R-T_A) + 0.058631 \, (T_R-T_A)^2 - 0.001363 \, (T_R-T_A)^3 \]
\[ F[3,36] = 12.09 \quad p<0.001 \]
MALES (Fig. 8.4)

Winter:  \[ TR = 4.86 + 0.74 TA \quad R^2 = 0.82 \]
\[ N = 5 \quad F[1,15] = 68.48 \quad p<0.001 \]
\[ VO_2 = 2.05 - 0.38TA + 0.02 TA^2 \quad R^2 = 0.47 \]
\[ F[1,11] = 4.89 \quad p<0.05 \]

'Summer':  \[ TR = 2.46 + 1.07 TA \quad R^2 = 0.78 \]
\[ N = 6 \quad F[1,19] = 132.67 \quad p<0.001 \]
\[ VO_2 = 0.35 + 0.005 TA \quad R^2 = 0.01 \]
\[ F[1,14] = 0.1 \quad p>0.5 \]

FEMALES (Fig. 8.5)

Winter:  \[ TR = 2.58 + 0.90 TA \quad R^2 = 0.91 \]
\[ N = 7 \quad F[1,12] = 128.0 \quad p<0.001 \]
\[ VO_2 = 0.45 + 0.003 TA \quad R^2 = 0.001 \]
\[ F[1,11] = 0.01 \quad p>0.5 \]

'Summer':  \[ TR = 6.44 + 0.72 TA \quad R^2 = 0.84 \]
\[ N = 7 \quad F[1,18] = 97.50 \quad p<0.001 \]
\[ VO_2 = 0.61 + 0.05 TA \quad R^2 = 0.11 \]
\[ F[1,18] = 2.33 \quad p>0.25 \]
ERRATA

Omitted from Bibliography:

References out of order:
Fisher K.C. (1964)
Guthrie M.J. (1933)
Hall (1981)
Mejnsar J. and Jansky L. (1967)

References incorrectly sited:
should read
The ecology of temperate-zone bat community: I. Wing shapes and flight styles. Aust. J. Zool.