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Energy metabolism and thyroid function of Antechinus stuartii

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Energy metabolism and thyroid function

of Antechinus stuartii

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CONTENTS

Title page
Declaration i
Acknowledgements ii
Contents iii
List of tables vi
List of figures viii

Chapter one Literature Review

1.1 Metabolism
Marsupial and eutherian basal metabolism 1
Non-shivering thermogenesis 2
Cold acclimation 3
Torpor 7

1.2 The thyroid
Thyroid hormones and their effects 8
Assessment of thyroid activity 9
Response of the thyroid to cold 12
Seasonality of thyroid secretion rate 14
Food consumption and thyroid activity 14
Marsupial thyroid activity 16

1.3 Life History of Antechinus stuartii
Seasonal pattern 18
Male die-off 20
A. stuartii Metabolism 20
Cold acclimation and acclimatization 23
Nutrition of A. stuartii 25
Torpor 26
Chapter two  Introduction to thesis  

Chapter three  Animals and Methods  

3.1 Animals  
3.2 The cold acclimation study  
3.3 The seasonal study  
3.4 Body mass  
3.5 Food energy intake  
3.6 Measurement of daily oxygen consumption  
3.7 Measurement of thyroid activity  
3.8 Statistical analysis  

Chapter four  Results and Discussion of the Cold Acclimation Study  

4.1 Body mass  
4.2 Food energy intake  
4.3 Thyroid activity  
4.4 Metabolic rate  
4.5 Discussion  

Chapter five  Results and Discussion of the Seasonal Study  

5.1 Body mass  
5.2 Food energy intake  
5.3 Thyroid activity in captivity of freshly-caught *A. stuartii*  

### List of tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Effects of cold and warm acclimation on body mass of <em>A. stuartii</em> and <em>M. musculus</em>.</td>
<td>38a</td>
</tr>
<tr>
<td>4.2</td>
<td>Effects of cold and warm acclimation on thyroidal radioiodine release rate, iodine content and thyroid secretion rate of <em>A. stuartii</em> and <em>M. musculus</em>.</td>
<td>38b</td>
</tr>
<tr>
<td>4.3</td>
<td>Effects of cold and warm acclimation on metabolism and food energy intake of <em>A. stuartii</em> at 5°C and 25°C.</td>
<td>38c</td>
</tr>
<tr>
<td>5.1</td>
<td>Seasonal variations in food energy intake and body mass of fresh-caught <em>A. stuartii</em>.</td>
<td>48a</td>
</tr>
<tr>
<td>5.2</td>
<td>Seasonal variations in thyroidal radioiodine release rate of fresh-caught <em>A. stuartii</em> in captivity and free-ranging.</td>
<td>49a</td>
</tr>
<tr>
<td>5.3</td>
<td>Seasonal variations in calculated thyroid secretion rate of fresh-caught <em>A. stuartii</em> in captivity and free-ranging <em>A. stuartii</em>.</td>
<td>50a</td>
</tr>
<tr>
<td>5.4</td>
<td>Metabolism and food energy intake at Ambient temperature cycle of <em>A. stuartii</em> freshly-caught at various times of the year.</td>
<td>51a</td>
</tr>
<tr>
<td>5.5</td>
<td>Metabolism and food energy intake at 25°C of <em>A. stuartii</em> freshly-caught at various times of the year.</td>
<td>51d</td>
</tr>
</tbody>
</table>
5.6 Metabolism and food energy intake at 5°C of *A. stuartii* freshly-caught at various times of the year.

6.1 A comparison of thyroid radiodine release rates in eutherians, marsupials and monotremes.

6.2 Summary of captive and free-ranging metabolic rates of *A. stuartii*.

6.3 Daily food energy intake of several *Antechinus* species in captivity.
### List of figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Cold acclimation study protocol</td>
<td>30a</td>
</tr>
<tr>
<td>3.2</td>
<td>Seasonal study protocol</td>
<td>31a</td>
</tr>
<tr>
<td>3.3</td>
<td>Calculation of thyroid radioiodine release rate of free-ranging <em>A. stuartii</em></td>
<td>31b</td>
</tr>
<tr>
<td>4.1</td>
<td>Effects of cold and warm acclimation on thyroid radioiodine release rate of <em>A. stuartii</em> and <em>M. musculus</em>.</td>
<td>38d</td>
</tr>
<tr>
<td>4.2</td>
<td>Effects of cold and warm acclimation on thyroid iodine content of <em>A. stuartii</em> and <em>M. musculus</em>.</td>
<td>39a</td>
</tr>
<tr>
<td>4.3</td>
<td>Effects of cold and warm acclimation on thyroid iodine secretion rate of <em>A. stuartii</em> and <em>M. musculus</em>.</td>
<td>39b</td>
</tr>
<tr>
<td>4.4</td>
<td>Effects of cold and warm acclimation on daily metabolic rate of <em>A. stuartii</em> at 5°C and 25°C.</td>
<td>40a</td>
</tr>
<tr>
<td>4.5</td>
<td>Daily variations in metabolic rate at 5°C and 25°C of cold and warm acclimated <em>A. stuartii</em>.</td>
<td>40b</td>
</tr>
<tr>
<td>5.1</td>
<td>Seasonal variations in thyroid radioiodine release rate of captive <em>A. stuartii</em> and the same animals while free-ranging</td>
<td>49b</td>
</tr>
<tr>
<td>5.2</td>
<td>Seasonal variations in thyroid iodine content of <em>A. stuartii</em></td>
<td>49c</td>
</tr>
<tr>
<td>5.3</td>
<td>Daily metabolic rate at ambient temperature cycle, 25°C and 5°C of <em>A. stuartii</em> fresh-caught at various times of the year.</td>
<td>51b</td>
</tr>
</tbody>
</table>
5.4 Mean metabolic rate over 24 hours at ambient temperature cycle, 25°C and 5°C of *A. stuartii* fresh-caught at various times of the year.

5.5 Daily variations in metabolic rate at ambient temperature cycle of representative male and female *A. stuartii* individuals fresh-caught during July and August.

5.6 Daily variations in metabolic rate at 25°C of representative male and female *A. stuartii* individuals fresh-caught during July and August.

5.7 Daily variations in metabolic rate at 5°C of representative male and female *A. stuartii* individuals fresh-caught during July and August.

5.8 Daily variations in metabolic rate at ambient temperature cycle of representative female and female plus pouch young *A. stuartii* fresh-caught during October, November and December.

5.9 Daily variations in metabolic rate at 25°C of representative female and female plus pouch young *A. stuartii* fresh-caught during October, November and December.
5.10 Daily variations in metabolic rate at 5°C of representative female and female plus pouch young
*A. stuartii*fresh-caught during October, November and December.

5.11 Daily variation in metabolic rate at 5°C of a male *A. stuartii*fresh-caught during July.
1.1 Metabolism

Marsupial and eutherian basal metabolism

Australian marsupials have been distinct from eutherians for about 130 million years (McKenna, 1969). Adaptive radiation during this time has resulted in considerable variety in ecological niche and anatomical features of marsupials. The relationship of the urinary and genital tracts to each other and the relatively undeveloped state of young, (born after a short gestation period) are among the major features distinguishing marsupials from eutherians (Tyndale-Biscoe, 1973).

Early physiological studies revealed that marsupials had lower body temperatures (Sutherland, 1897) and lower minimum metabolic rates (Martin, 1903). More comprehensive surveys (MacMillen & Nelson, 1969; Dawson & Hulbert, 1970) have confirmed these early findings and quantified these differences. Marsupial body temperatures and metabolic rates are, on average, approximately 2.5°C and 30% lower respectively than eutherian values (Dawson & Hulbert, 1970). Because of these lower body temperatures and metabolic rates, marsupials and monotremes were for some time regarded as primitive mammals. However they have been found to display thermoregulatory capabilities that are as advanced as those of eutherians (Dawson, 1973).

Controversy exists as to whether the differences in body temperature and metabolic rate are phylogenetic or environmental in origin. McNab (1978, 1986) has suggested that
these differences are a consequence of the differences in diets of the animals surveyed. It has also been suggested that most of these differences stem from comparisons of homeothermic eutherians with homeothermic and heterothermic marsupials (Kinnear & Shield, 1975) although Hume (1982) has refuted this argument. If indeed there are phylogenetic differences then what is their physiological cause? Hulbert (1980, 1985) has suggested that the low metabolism of marsupials is the result of their low body temperatures.

Since the marsupials and eutherians separated in the early Cretaceous (McKenna, 1969), the earth's climate is believed to have cooled (Nairn, 1961). The eutherian and marsupial evolutionary responses to this environmental change appear to have been different (Hulbert, 1980). A new thermogenic mechanism has evolved within the eutherians. Termed non-shivering thermogenesis, it may be influential in any of the following situations:

(i) neonatal thermogenesis
(ii) acclimation to cold
(iii) arousal from hibernation or torpor

**Non-shivering thermogenesis**

Non-shivering thermogenesis consists of heat-production mechanisms which do not involve muscular contractions (Jansky, 1973). Heat production under conditions of basal metabolism is mostly non-shivering thermogenesis and is referred to as obligatory or basal non-shivering thermogenesis. Regulatory non-shivering (NST) is the additional heat production which occurs at ambient temperatures below the thermoneutral zone.
Heat production due to the specific dynamic action of food may also be included in regulatory NST. Brown adipose tissue (BAT) and skeletal muscle are significant sites of NST (Jansky, 1973). NST is additional to shivering and occurs at mildly lowered temperatures, whilst a heavier cooling load is required for the induction of shivering (Jansky, 1973).

The amount of BAT and incidence of NST in mammals is inversely related to body mass (Heldmaier, 1971; Jansky, 1973). Many mammals possess BAT and utilize NST early in life. However, in most of these, BAT and regulatory NST are reduced to much lower levels within a few weeks (Chaffee & Roberts, 1971). Neonates have large surface area to body volume ratios which render them more susceptible to heat loss. They have relatively undeveloped pelage. Their small size precludes reliance on pelage to any great extent (Schmidt-Neilsen, 1975). Furthermore, their muscles are underdeveloped, thus, thermogenic mechanisms such as exercise and shivering would be unsuitable for a large proportion of neonatal heat production. Thus, it is not surprising that BAT deposits and NST should play such a significant role in neonatal thermoregulation in eutherians.

Cold acclimation

Acclimation is the adjustment of an organism to an artificial or laboratory environment. Acclimatization is adjustment to a natural environment (Schmidt-Nielsen, 1975). In general, exposure of a mammal to reduced ambient temperature for a period of several weeks or months imbues it with increased ability for sustained heat production and homeothermy at low
environmental temperatures. Frequently, increased heat production also occurs at temperatures in the thermoneutral zone, but this is not a characteristic of cold acclimation and does not occur in all species. Cold acclimatization is primarily concerned with heat conservation, whereas cold acclimation is generally more concerned with enhanced thermogenesis (Hart, 1971).

Some of the possible adjustments of mammals during cold acclimation and cold acclimatization listed by Adams (1971) are:

* Increased shivering thermogenesis (ST) ability
* Increased shivering efficiency
* Increased fur insulation
* Increased tissue thermal insulation
* Increased peripheral vascularization
* Alteration of nerve conduction characteristics
* Development of heat production mechanisms not related to shivering
* Modified thresholds to hormone and metabolite levels
* Increased total blood volume

Small mammals, because of their large surface area to volume ratio are limited in their ability to reduce heat loss when exposed to low ambient temperatures. The core to skin surface component of insulation has only a very small effect on total insulation because the rate of heat transfer through skin is about 10 times that through fur and very small distances are involved (Dawson & Dawson, 1982). It is fur depth that largely determines
total insulation, and small mammals are very limited in their ability to carry thick fur (Dawson & Dawson, 1982). Thus, to remain homeothermic when ambient temperatures are low, small mammals must possess a large metabolic scope (Dawson & Dawson, 1982). Winter pelage insulation has been found to increase in white rats kept outdoors during winter, but remain unchanged in controls kept in the laboratory at 6°C (Heroux, Depocas & Hart, 1959). Cold exposure has been reported to result in increased hair growth compared to controls in mice, pigs and cats, but only after several months (Precht et al., 1973).

Cold acclimation results in the replacement of shivering thermogenesis (ST) by NST in rats (Himms-Hagen, 1972). Cold acclimation in many small eutherians involves an increase in NST (Hart, 1971; Abbots & Wang, 1980) and an increase in deposits of BAT (Sundin, 1980; Fellenz et al., 1982). This increased growth of BAT involves more cells, more mitochondria per cell and more cristae per mitochondrion (Himms-Hagen, 1972). The maximum capacity for NST develops within 2-3 weeks of exposure to cold (Bruck, 1980). Even short daily exposure to cold induces BAT deposition in some species (Heldmaier, 1975). The amount of BAT is higher in winter than in summer in meadow voles captured in the wild and also in rats maintained outdoors (Chaffee & Roberts, 1971). The maximum rate of heat production is also greater in cold acclimated or winter acclimatized animals than in warm acclimated or summer acclimatized animals (Hart, 1971; Abbots & Wang, 1980).

Because of the involvement of the thyroid in responding to reduced temperatures, studies of NST have frequently been concerned with thyroid hormones. However, the relationship
between thyroid hormones, BAT and NST is not clearly understood at present. Confusion over data obtained from young mammals probably results from the involvement of thyroid hormones in development. In 1850, Curling described symmetrical swellings of fat on the sides of the necks of hypothyroid babies. This tissue was later recognised as BAT. Similar deposits have been found in hypothyroid rats, but this BAT is unlike normal BAT in that it does not respond to noradrenaline (Hemon, Ricquier & Mory, 1976). It appears that thyroid hormones are necessary for the normal utilization of lipids by BAT in young rats and results from adult rats also indicate that thyroid hormones play a permissive role in the response of BAT to cold (Hemon et al., 1976).

There is controversy over whether BAT and NST are present in marsupials or monotremes. An extensive study by Rowlatt, Mrosovsky & English (1971) has failed to find BAT in marsupial or monotreme neonates and a similar study in adult marsupials failed to detect BAT (Green, 1963). If indeed, marsupials do lack the capacity for effective NST, then it is possible that increased ST and/or thyroid calorogenesis are involved in cold acclimation. The maximum sustained rate of heat production in cold thermogenesis is approximately 8-9 times SMR in dasyurids (Dawson & Dawson, 1982). These results were obtained under winter conditions which would be expected to elicit NST if indeed dasyurids possessed the capacity for NST. The reported lack of capacity of marsupials for NST combined with the finding that the maximum sustained rate of heat production during exercise in dasyurids is also 8-9 times standard metabolic rate (SMR) has prompted Dawson & Dawson
(1982) to suggest that dasyurids rely primarily on shivering for regulatory heat production.

Torpor

Torpor is a state of reduced body temperature ($T_b$) in which an animal is capable of spontaneously increasing its $T_b$ to the level routinely observed in thermoneutrality. The capacity for spontaneous arousal and the regulation of $T_b$ during torpor in many species indicates that torpor is an advanced adaptation, rather than an indication of primitiveness and poor thermoregulation (Wallis, 1982). Reduced $T_b$ with its concomitant reduction in metabolism gives a small mammal significant energy savings when ambient temperature ($T_a$) is low and the energetic costs of homeothermy are high, particularly if food availability is low.

Thermogenesis during arousal from torpor is derived from BAT and shivering, the relative contribution varying between species (Hudson, 1973). It has been suggested that arousal rates from torpor may be lower in marsupials than in eutherians (Wallis, 1976). However, Geiser (1985) found no significant difference in arousal rate between marsupials and eutherians, and suggested that any apparent difference was based on studies from too few species. Fleming (1980) has suggested that during arousal from torpor, marsupials may use ST and anaerobic metabolism to a greater extent than eutherians.
1.2. The thyroid.  

Thyroid hormones and their effects

The thyroid gland in most mammalian species consists of a small bilobed structure situated on either side of the trachea. The lobes are connected by an isthmus lying ventral to the trachea. The thyroid possesses a relatively large vascular supply and has the capacity to accumulate and concentrate iodide to levels up to 300 times greater than those of plasma (Frye, 1967).

Iodide ions are sequestered from the circulatory system into the thyroid follicular cells and combined with tyrosine residues in thyroglobulin to form iodotyrosines. Two hormones, triiodothyronine (T₃) and tetraiodothyronine or thyroxine (T₄) are synthesised from iodotyrosines, stored in and secreted from the thyroid gland.

Thyroid-stimulating hormone (TSH), produced in the anterior pituitary and secreted into the circulatory system stimulates the secretion of T₃ and T₄ from the thyroid gland. Thyrotropin-releasing hormone (TRH) produced in the hypothalamus is conveyed to the anterior pituitary via hypothalamic-pituitary portal circulation. TRH stimulates and T₃ and T₄ inhibit the synthesis and secretion of TSH. Thyroid control lies mainly in excess circulating T₃ and T₄ reducing TSH secretion and insufficient circulating T₃ and T₄ stimulating the secretion of TRH (Williams, 1974).

Thyroid hormones exert a wide range of effects including elevation of metabolism (the calorigenic effect),
alteration of membrane fatty acid composition and stimulation of protein synthesis, growth and development (Hulbert, 1978).

**Assessment of thyroid activity.**

A number of techniques which purport to measure thyroid activity have been reported in the literature. It is possible that some of the confusion over thyroid activity and the effects of thyroid hormones result from inappropriate application of these techniques and/or incorrect interpretation of results. Hulbert & Williams (1987) have reviewed five of the parameters which may be used in the assessment of thyroid activity.

(a) Histological analyses are unreliable in that sometimes there is no correlation between histology and other parameters of thyroid activity. For example, Kracht (1954), on the basis of histological analysis reported increased thyroid activity due to stress in rabbits, freshly-captured and maintained in captivity. However, using thyroidal radioiodine release rate, Brown-Grant *et al.*, (1954 a) could not confirm this finding.

(b) Radioiodide uptake studies reveal whether the thyroid is concentrating iodide from the circulatory system. However, they do not reveal anything about the rate of secretion by the gland (Hulbert & Hudson, 1976). The uptake of radioiodide is only indirectly related to the rate of hormone secretion (Brown-Grant *et al.*, 1954 b). Uptake of iodide is probably less dependent on pituitary stimulation than is release (Brown-Grant & Gibson, 1955) and there is a lack of correlation between radioiodide uptake and either release of radioiodine
(Brown-Grant et al., 1954 b) or secretion of iodine (Withers & Hulbert, 1987). An extreme example of the above situation is the continued uptake of iodide by the thyroid during hibernation when secretion rate is virtually nil (Hulbert & Hudson, 1976). Furthermore there is a lack of correlation between radiiodide uptake and metabolic rate in desert rodents (Yousef & Johnson, 1975).

(c) Determination of the concentration of $T_3$ or $T_4$ in serum is really a static measurement of a parameter that is in dynamic equilibrium. Thus, a high secretion rate combined with a high tissue disposal or clearance may result in the same total serum level of $T_3$ and $T_4$ as a combination of low secretion rate and low tissue disposal or clearance. Furthermore, only a small percentage of the total serum $T_3$ or $T_4$ is in the unbound or physiologically active form. High total serum $T_3$ or $T_4$ levels can be misleading when considered in isolation (Hulbert & Williams, 1987).

Much of the literature concerning thyroid activity, since the discovery of thyroxine in 1916 by Kendall, has been concerned with plasma levels of $T_4$ and is thus open to question. Scott, Yousef & Johnson (1976), reported significant relationships between plasma $T_4$ levels and metabolic rate in rodents. However, Hulbert, Hinds & MacMillen (1985) found no correlation of total plasma $T_4$ levels with either minimum metabolic rate or summit metabolism.
(d) Measurements of the rate of disappearance of injected thyroid hormone provide an indication of the rate at which the hormone is metabolised and excreted but not necessarily an indication of the rate of hormone secretion by the gland. The method relies upon the assumption that in the steady state the rate of hormone secretion equals the rate of hormone loss (Kaethner & Good, 1975; Wills & Schindler, 1970).

(e) Radioiodide release rate is expressed as a percentage of the thyroidal iodine content released per day. The loss of radioiodine from the thyroid is directly related to thyroid hormone secretion in that radioiodide is incorporated into the hormones secreted by the thyroid gland (Brown-Grant et al., 1954 b). Organic binding of accumulated iodide by the thyroid is very rapid (Brown-Grant & Gibson, 1955). The area of radioiodide accumulation is assumed to represent the thyroid (Hulbert & Williams, 1987), and a plot of neck radioactivity versus thyroid radioiodine content indicates that measuring neck radioactivity gives a good indication of the amount of radioiodine in the thyroid (Brown-Grant et al., 1954 b). Changes in the slope of the release rate plot are related to changes in thyroid secretion rate, provided, that the amount of hormone in the gland remains constant (Brown-Grant et al., 1954 b).

Recycling of radioiodide occurs unless an inhibitor of iodide uptake is used, the higher the radioiodine release rate, the greater will be the underestimate due to recycling (Hulbert & Williams, 1987). In the rabbit, radioiodide recycling resulted in an underestimate of radioiodine release rate of approximately 10% (Brown-Grant et al., 1954 b).
The secretion rate of iodine from the gland equals the product of radioiodide release rate and thyroid iodine content (Hulbert & Williams, 1987). T_4 is the major secretory product of the thyroid gland (Ingbar & Woeber, 1974). Thus a good indication of T_4 secretion rate (T_4SR) can be obtained using a combination of radioiodine release rate and thyroid iodine content. This process would of course unfortunately require the removal of part or all of the thyroid gland to measure thyroidal iodine content.

Response of the thyroid to cold.

Exposure to cold for less than 24 hrs is usually termed acute cold exposure and exposure to cold for longer periods is usually termed chronic cold exposure.

It is widely accepted that the thyroid activity of small mammals held in the laboratory increases during exposure to ambient temperatures below their thermoneutral zone and decreases when ambient temperatures are above it (Brown-Grant et al., 1954b; Ingram & Kaciuba-Uscilko, 1977).

When rats are exposed to temperatures ranging from 3°C to 5°C, (TRF) release from the hypothalamus occurs within 5 minutes (Guillemin, Burgus & Vale, 1969) and increased plasma concentration of (TSH) within 30 minutes after exposure (Fortier et al., 1970; Hershman et al., 1970). Increased thyroidal ^131I release rate occurs in guinea pigs within 2 hours of cold exposure (Yamada et al., 1965). Increased ^131I release rate occurs in rats within 4 hours and in rabbits within 6 hrs of their removal from warm environments (approximately 27-29°C)
and exposure to moderately cold environments (approximately 6.5-11°C). Exposure to temperatures of approximately 0-2°C results in a reduction in $^{131}$I release rate. The former results were interpreted as cold exposure increasing thyroid secretion rate and the latter results as intense cold acting as a stress and inhibiting thyroid secretion rate (Brown-Grant et al., 1954b).

Thus, there is a general consensus that acute exposure to a moderately cold environment results in an increased thyroid secretion rate within a few hours.

The role of the thyroid during chronic cold exposure is controversial (Rhodes, 1980). Some authors have reported that chronic cold exposure results in increased thyroid activity. Dempsey & Astwood (1943) and Heroux & Brauer (1965) showed that the amount of $T_4$ required to prevent goitre was higher at lower environmental temperatures. Increased $T_4$SR in guinea pigs at 0°C based on the disappearance of exogenous $T_4$ has been reported by Stevens et al., (1955). $^{131}$I uptake in rats exposed to 4°C for six weeks was 300% higher than those at 22°C, without any change in thyroid iodine content. All rats were fed ad libitum (Galton & Nisula, 1969). These results were interpreted as indicating an increase in $T_4$SR. However, the amount of exogenous $T_4$ metabolised in the peripheral tissues was slightly lower at 4°C than at 22°C.

Other authors have reported no change in thyroid activity with chronic cold exposure. Bakke & Lawrence (1971) found no change in TSH secretion rate and 24hr $^{131}$I uptake of rats after 6 weeks at 5°C. Fortier et al., (1970) found no difference in $T_4$...
secretion rates of rats at 5°C and 25°C after 32 days, although the rate of disappearance of plasma $^{131}$I labelled $T_4$ was faster in animals at 5°C. Thyroid activity, estimated using total iodine turnover in the thyroid was not found to be greater in cold than warm acclimated rats (Hart, 1971).

**Seasonality of thyroid secretion rate.**

Increased thyroid activity is not essential for increased thermogenesis without shivering in white rats kept outdoors in groups during winter. The thyroid activity of freshly-caught rodents, evaluated by histophysiological and radioiodine incorporation studies is minimal in winter and maximal during the summer-autumn period. It appears that rodents acclimatized to winter display reduced thyroid activity compared to summer animals (Hart, 1971). The lack of environmental cues such as temperature and photoperiod regimes may be a significant factor in the difference in thyroidal responses to reduced temperature between mammals in the wild and those in the laboratory (Rhodes, 1980).

**Food consumption and thyroid activity.**

It is well documented that cold exposure will result in increased food intake if food is available (Hart, 1971). It has been suggested that increased thyroid activity of cold exposed animals may be due to increased faecal loss of thyroid hormones rather than to cold per se (Galton & Nisula, 1969). However, many of the experiments on which these claims are based measured relative amounts of radioiodine excreted, rather than amounts of
thyroid hormone excreted.

Cold acclimated rats exhibit greater biliary clearance of thyroid hormones (Chaffee & Roberts, 1971) and greater faecal excretion of radioiodine than warm acclimated rats (Cottle & Veress, 1964). However, although enterohepatic loss of $T_4$ may be reduced in baboons, $T_4$ production rate is not lowered as a result of a high calorie low residue diet (Gale, 1975). If faecal bulk is kept constant by varying roughage content in rats exposed to 22°C and 4°C, the faecal loss of $T_4$ in both groups is the same (Hillier, 1968). However, even when faecal loss of radioiodide is the same in cold acclimated and control rats, thyroidal radioiodide release is increased in the cold (Chaffee & Roberts, 1971). Ingram & Kaciuba-Uscilko, (1977) found that increased bulk of food consumed had no effect on the rate of disappearance of labelled $T_4$ in young pigs.

Thus, it appears that the increased food consumption associated with chronic cold exposure will result in increased faecal loss of thyroid hormones. However, there is controversy over whether this significantly influences thyroidal hormone secretion rates.

Some of the confusion regarding cold exposure and thyroid gland activity may arise from:

(i) different methods used to assess thyroid activity
(ii) the assumption that the rate of radioiodine excretion via urine and faeces is representative of the rate of thyroid hormone excretion.
(iii) different temperatures involved
(iv) variations in the duration of cold exposure
Marsupial thyroid activity.

It was originally suggested that the body temperature and metabolism of marsupials may be lower than eutherian levels because of reduced thyroid activity (Katsh & Windsor, 1955). However, virtually no studies on thyroid function have been carried out at this time on marsupials and monotremes.

In a marsupial, monotreme and eutherian comparison, Hulbert & Augee (1982) found that thyroidectomy resulted in a reduction in standard metabolic rate of marsupials and eutherians. Furthermore, thyroid hormones were responsible for an equal proportion of standard metabolic rate in both marsupials and eutherians. However, thyroidectomy in the echidna, results in no significant change in standard metabolic rate (Dawson & Grant, 1980; Hulbert & Augee, 1982). The echidna also has a relatively low thyroid radiiodine release rate and plasma T₄ concentration. These findings suggest that the relatively low metabolic rate of the echidna is due at least in part to a reduced thyroid activity (Hulbert, 1985). The platypus, after correction for low body temperature has a higher metabolic rate than the echidna. However, although, it also has a higher plasma T₄ concentration than the echidna, it is not known whether the higher metabolism of the platypus is due to increased thyroid activity (Hulbert, 1985).

Plasma T₄ levels of marsupials are about one-third of those of eutherians (Hulbert & Augee, 1982). However, free T₄ (FT₄) values of marsupials are similar to those reported for eutherians (Setchell, 1974; Hulbert & Augee, 1982). Hulbert &
Augee (1982) have suggested that plasma $\text{FT}_4$ levels may be the same in all mammalian groups, even though total plasma $T_4$ levels may be low in marsupials. Setchell (1974) has suggested that marsupials produce the same total amount of $T_4$ as eutherians of a similar size to modulate their metabolism at a level 30% lower than that of eutherians. He concluded that the lower metabolism of marsupials was genetically determined and was independent of thyroid activity (Setchell, 1974). The unusually low basal metabolic rate (BMR) of the rabbit-eared bandicoot *Macrotis lagotis* (Hulbert & Dawson, 1974) may be due to reduced thyroid activity superimposed on the low metabolism of this desert dwelling marsupial (Hulbert & Augee, 1982). Hulbert (1980) has proposed that there are both "phylogenetic" and "ecological" levels of metabolism, and low plasma $T_4$ levels have been found in desert rodents which generally have low BMR (Scott, Yousef & Johnson, 1976). Thus, it is generally considered that one role of the thyroid is to modulate metabolic rate about a predetermined set point as temperature and food availability vary seasonally.

Very few studies have been done concerning the effects of cold exposure on thyroid activity in marsupials. Setchell (1974) found that $T_4\text{SR}$ was increased in *M. eugenii* during acute cold exposure and also after cold acclimation. Increases in thyroid secretion rates during colder months of the year and decreases during warmer months have been reported in *Didelphis virginianus* (Bauman & Turner, 1966) and *M. eugenii* (Ralph, 1972; Kaethner & Good, 1975). In a similar experiment in
the same study area, Setcheli (1974) failed to detect this effect in M. eugenii. However, he pointed out that competition among M. eugenii for food and shelter within the study area was probably greater during the period when Ralph conducted his experiments. Setcheli (1974) did however, find that thyroid activity was modulated in part by the change in temperature range from one measurement to the next.

1.3 Life History of Antechinus stuartii.

Seasonal pattern.

The brown antechinus, Antechinus stuartii is a dasyurid marsupial of body mass 17-36 g (females) and 29-71 g (males) inhabiting forest areas of eastern Australia. A. stuartii is predominantly nocturnal but may be active during the day (Wood 1970).

A. stuartii has an unusual life history. Females are monoestrous (Marlow, 1961; Woolley, 1966) with a highly synchronised breeding period in winter or early spring of about 3 weeks duration (Woolley 1966; Wood, 1970; Selwood, 1982). Mating generally occurs later at lower latitudes and higher altitudes (Dickman, 1982). The timing of mating is controlled by an endogenous, circannual rhythm, synchronised by photoperiod (Dickman, 1985). Mating is followed by total male mortality (Woollard, 1971). Females are invariably pregnant after the mating season (Lee & Cockburn, 1985). The gestation period is about 27 days and newborn young have a crown-rump length of approximately 5mm (Selwood, 1980). The number of nipples (3-5
pairs) varies between areas, and most females trapped with pouch young carry complete litters (Woolley, 1966). The young attach to a teat soon after birth and remain attached for approximately 35 days (Wood, 1970; Woolley, 1966). Thereafter, they are suckled in a nest and weaned at approximately 90 days after birth (Marlow, 1961). Braithwaite (1979) reported that juveniles may continue to share a nest until the onset of territorial behaviour, which is approximately two months before mating. Evidence now suggests that males disperse at weaning (Cockburn, Scott & Scotts, 1984), but females may continue to share a nest with their mother (Dickman 1982; Cockburn et al., 1984). Scotts (1983) reported nest sharing by groups composed of both sexes of *A. stuartii* until the male die off.

Male *A. stuartii* may survive longer if captured and maintained in the laboratory before the start of the mating season. However, the probability of surviving longer than free-ranging animals is nil if captured after the mating season has commenced (Barker, Beveridge, Bradley & Lee, 1978). Castrated males are reported to survive in the field beyond the period of natural mortality (Lee & Cockburn, 1985). Females may survive in the wild for up to approximately 3 years and have been known to breed a second time (Woolley, 1966; Wood, 1970; Woollard, 1971). However, most females produce only one litter in a lifetime (Lee & Cockburn, 1985). The adaptive significance of the total male die-off in the wild is believed to reside in the removal of competition between mature males and mature females (and their young) for food. Litters of *Antechinus* species may weigh three times as much as the mother (Lee & Cockburn, 1985).
Male die-off.

The cause of male mortality in *A. stuartii* has been the subject of much research. Barker *et al.*, (1978) found haemorrhages associated with gastric and duodenal ulcers and infections of a *Babesia* species. An increase in plasma corticosteroids coincides with the period of male mortality, indicating that stress is a factor involved in the die-off (Barnett, 1973; Lee, Bradley & Braithwaite, 1977). Male *A. stuartii*, but not females, show increasing aggressiveness leading up to the mating period (Braithwaite, 1974). Reduction in haemoglobin concentration and haemotocrit at this time correlates with an increase in resting metabolic rate (RMR) and a reduction in aerobic scope. These changes occur at a time when males expend considerable energy and this may contribute to their demise (Cheal, Lee & Barnett, 1976).

*A. stuartii* Metabolism.

The BMR of *A. stuartii* is 50.1 kcal/kg^{0.75}/day and body temperature is 34.4°C (Dawson & Hulbert, 1970). At T_{a} 9-20°C, the T_{b} of normothermic inactive *A. stuartii* remains fairly constant at approximately 34°C. However, T_{b} during torpor at T_{a} 10.5-20°C ranges between 19.9 and 28.7°C (Geiser, 1985). Resting oxygen consumption rate (OCR) of *A. stuartii* measured at T_{a} 30.0-32.0°C increases significantly in males from May to June and during August and in females from July to August. Maximum OCR at T_{a} 30.0-32.0°C measured during
exercise on a treadmill increases significantly in males from May to July. These changes were not solely attributable to increases in body mass, as they were weight-corrected values. As maximum OCR did not change during August when minimum OCR increased, it was reported that the aerobic scope or capacity of aerobic metabolism to supply energy for work (the difference between resting and maximum oxygen consumption rate) decreases in August (Cheal et al., 1976). Haematocrit and blood haemoglobin concentration were also measured. These did not change significantly between late February and mid-August. However, both of these parameters decreased significantly during late August, with the greatest decrease occurring in males in both cases. A significant negative correlation was found between haematocrit and resting OCR and also between haemoglobin concentration and resting OCR where these data were obtained from the same animal. Mating in this particular A. stuartii population occurs during the first fortnight in August. Thus, this energetically demanding activity occurs at a time when resting metabolism is increased and aerobic scope reduced (Cheal et al., 1976).

Nagy, Seymour, Lee & Braithwaite (1978) and Lee (personal communication) used the doubly-labelled water technique (Lifson & McClintock, 1966) to determine metabolic rates of A. stuartii in the field. They found no significant difference in field metabolic rate of A. stuartii between the August mating period and the pre-mating period in July. They also found no significant differences between males and females during or between these periods. There was no significant relationship between body size of A. stuartii and field
metabolic rate. The lack of a substantial increase in field metabolic rate in male *A. stuartii* at the time of mating (Nagy et al., 1978, Lee, personal communication), combined with a 17% increase in RMR at this time (Cheal et al., 1976) suggests that energy expenditure during mating substitutes for other avenues of energy expenditure prior to mating (Lee & Cockburn, 1985). Reduced chiton content and increased wateriness of faeces during mating are possibly indicative of a reduction in feeding during this time (Lee & Cockburn, 1985). Stress-increased gluconeogenesis may provide an alternative source of energy to that derived directly from digestion of food and allow substitution of mating behaviour for feeding (Lee & Cockburn, 1985).

Nagy et al., (1978) have averaged values available in the literature for the standard metabolic rate of *A. stuartii* (resting, post absorptive in the thermoneutral zone) and converted this value to yield 145 kcal.kg\(^{-1}\).day\(^{-1}\). Using Figure 2 of Wallis (1976), they have determined that the metabolic rate of resting normothermic *A. stuartii* at 8°C (the mean field air temperature during their study) was 5 ml O\(_2\).g\(^{-1}\).hr\(^{-1}\) i.e., 575 kcal.kg\(^{-1}\).day\(^{-1}\). Thus, assuming that their field animals remained normothermic and exposed to open air conditions continuously and that heat produced by activity and assimilation does not substitute for heat required for temperature regulation they suggested that approximately 85% of energy expenditure was allocated to body temperature regulation and basal maintenance. This leaves approximately 100 kcal.kg\(^{-1}\).day\(^{-1}\) for activity and other costs.

In another species of *Antechinus, A.swainsonii*,
lactating females show a 76% increase in field metabolic rate, as assessed using doubly labelled water (Lee, personal communication). It has been suggested that late lactation would be the most energetically demanding time for suckling females, as they are caring for the metabolic needs of litters with a combined weight that is several times their own (Lee & Cockburn, 1985).

Cold Acclimation and Acclimatization.

Reports of BAT or NST in marsupials are few. However, Wallis (1977) recorded a small amount of NST in A. stuartii (0-15% increase in metabolic rate), after injections of relatively large amounts of noradrenaline. A large increase in oxygen consumption rate (300-400%) would have been expected for a eutherian of similar size (Wallis, 1977). He also reported NST after measuring the difference in heat production between resting and exercising animals. Mammals with poorly developed NST possess similar metabolic rates when resting and exercising (Wallis, 1982). However, the data points from which the lines denoting these two parameters have been drawn, are noticeably absent (Wallis, 1977). Gotts (1975) measured the difference in heat production between resting and exercising A. swainsonii and reported NST in males captured during winter and in cold acclimated juveniles. Noradrenaline mediated NST has also been reported in a third marsupial, the potoroo, Potorous tridactylus (Nicol, 1978). However, the small response observed and the large dose of noradrenaline administered cast doubt on the physiological significance of the response (Reynolds & Hulbert, 1982). A 48% rise in metabolic rate in response to a
subcutaneous injection of noradrenaline (40ug/100g body weight) has been reported in Bennett's wallaby (*Macropus rufogriseus rufogriseus*) above 250g in body mass (Loudon, Rothwell & Stock, 1985). Handling and injection of noradrenaline is likely to induce considerable stress in these animals. The lack of control animals (sham injected) in all of the above experiments involving injections of noradrenaline in marsupials casts doubt on their validity. However, Loudon *et al.*, (1985) also report the finding of BAT in their study.

In a comparative study of cold acclimation in *A. stuartii* and the eutherian *M. musculus*, Reynolds & Hulbert (1982) demonstrated the absence of noradrenaline-mediated NST in *A. stuartii*. The body temperature of the cold acclimated animals remained high when they were exposed to 5°C, however, that of the animals acclimated to 25°C was reduced when exposed to 5°C. This indicated that both species had indeed acclimated to 5°C after 6-8 weeks exposure. Oxygen consumption rates at 5°C of cold acclimated animals were higher than those in warm acclimated animals. A thermogenic response to noradrenaline confirming the presence of noradrenaline-mediated NST was evident in *M. musculus*, but not in *A. stuartii*. This thermogenic response was greater in the cold acclimated *M. musculus* than in the warm acclimated *M. musculus*.

Oxygen consumption rate at 30°C of *A. stuartii* was significantly higher in the cold acclimated than in the warm acclimated animals. However, there was no significant difference in oxygen consumption rate at 30°C between cold and warm acclimated *M. musculus* (Reynolds & Hulbert, 1982).
These results indicate that cold acclimated *A. stuartii* are capable of utilising a different thermogenic mechanism than that of *M. musculus*. Cold acclimation in another small marsupial, *Dasyuroides byrnei*, produced similar results to those in *A. stuartii*. Heat production in unrestrained animals at 15°C and at 25°C was higher after cold acclimation than warm acclimation (Smith & Dawson 1984).

Wallis (1979) has observed and recorded shivering at ambient temperatures less than 17°C, but not at higher temperatures. He suggested that resting *A. stuartii* were not shivering at their maximum capacity when measured at 0°C (Wallis, 1982). That increased thermogenesis persists at 30°C in cold acclimated *A. stuartii* (Reynolds & Hulbert, 1982), indicates it is unlikely to result from shivering alone.

*A. stuartii* and *M. musculus* exhibited no change in weight-specific thermal conductance (C), the inverse of insulation, after cold acclimation (Reynolds & Hulbert, 1982). However, winter-captured *A. stuartii* have significantly lower C and thicker fur than summer-captured *A. stuartii*. Torpid *A. stuartii* display even lower C values (Wallis, 1977). Posture, regional heterothermy and superficial hypothermia are likely to contribute to these low C values during torpor (Wallis, 1982). Gotts (1975) has reported lower C values in *A. swainsonii* in winter than in summer.

**Nutrition of *A. stuartii***

*A. stuartii* is an opportunistic feeder, with a diet consisting mainly of arthropods (Hall, 1980a). In the field it may consume 60% of its body mass in arthropods per day (Nagy et
al., 1978). However, during the colder months of the year when arthropods are less abundant, plant material (especially *epacrid* flowers in one area studied) may constitute up to 41% of the diet of *A. stuartii* (Fletcher, 1977). Statham (1982) examined the food availability of *A. stuartii* in different forest habitats at Petroi, New South Wales over a two year period. She found that adequate food was available for *A. stuartii* during summer and autumn but if a food shortage occurred then it would most likely be during winter when arthropod numbers and activity were lowest. She suggested that it was improbable that an energetically expensive event such as the 20% increase in body mass (5-10g) between July and August observed in males in all habitats studied would occur in a food limited situation. Thus, she concluded that there was no shortage of food for *A. stuartii* in any of the habitats during her study (Statham, 1982).

**Torpor.**

Torpor in *A. stuartii* appears to be an emergency measure rather than a daily event. *A. stuartii* enters torpor under conditions of reduced food availability and reduced ambient temperature (Wallis, 1977; Geiser 1985). Wallis (1977) found that *A. stuartii* entered torpor during autumn and winter but not during summer. Geiser (1985) found that torpor can be induced in *A. stuartii* during all seasons of the year if food supply and ambient temperature are reduced. The tendency to enter torpor, which is greatest in autumn and winter, is reduced as body mass increases, especially in the males. Torpor during spring was induced only in females without pouch young.
A. stuartii show only a weak tendency to enter spontaneous torpor if food is available ad libitum. Spontaneous torpor appears to be restricted to females during winter (Geiser, 1985). The significance of torpor in the life of A. stuartii is also complicated by development changes (Geiser, 1985).
Chapter 2
Introduction to thesis

As pointed out in the previous chapter, the involvement of the thyroid gland in responses to reduced ambient temperatures in eutherian mammals is well documented. It is possible that because of their apparent lack of BAT, *A. stuartii* maintain increased thyroid secretion during chronic cold exposure rather than reducing it to "normal" levels as some eutherians appear to do. If so, then this may result in alterations in the energy requirements of *A. stuartii*. If cold acclimation occurs in the field, then similar changes may be found in free-ranging *A. stuartii*.

Aims

The present study of *A. stuartii* was undertaken to answer the following questions:

Metabolism

What are the metabolic costs and food requirements of the seasonal life history pattern of *A. stuartii*? Are there any sex differences? What are the costs of maintaining pouch young? Are there any seasonal differences? What are the effects of laboratory acclimation to cold?
The Thyroid

Does cold acclimation alter thyroid activity?
Does thyroid activity vary seasonally?
Is thyroid activity different in captive and free-ranging animals?
Are the effects of cold acclimation on thyroid activity in Antechinus similar to those in a similar-sized eutherian – Mus musculus?
Chapter 3 Animals and Methods

3.1 Animals

The *A. stuartii* were captured in forest areas near Wollongong N.S.W. Elliott traps baited with bread and peanut butter were laid late in the afternoon and retrieved early the following morning. The *M. musculus*, CBA strain, were obtained from The Australian National University. *A. stuartii* and *M. musculus* were individually housed in plastic containers (40x25x15 cm) containing sawdust and woodwool. The *A. stuartii* were fed "Dine Beef and Heart" cat food (Uncle Ben's Australia) and the *M. musculus* were fed "Rat and Mouse Cubes" (Allied Feeds). Food and water were supplied ad. libitum.

3.2 The cold acclimation study

The cold acclimation study was carried out between April and June 1986, according to the protocol outlined in Figure 3.1. Adult animals were randomly divided into two groups. One group was maintained at 5±3°C and the other at 25±3°C for 6-8 weeks. All animals were kept under a 12 hr light : 12 hr dark photoperiod. After 6-8 weeks, the daily oxygen consumption of some *A. stuartii* randomly selected from each group was determined at 5°C and 25°C. The thyroidal radiiodine release rate and iodine content of animals, randomly selected, from each group was also determined.
Figure 3.1. Cold acclimation study protocol.

A. stuartii and M. musculus

random division

cold (5°C) \rightarrow warm (25°C)

6-8 weeks

O₂ consumption measured at

and then at

125I release

Thyroid iodine content
3.3 The seasonal study

The seasonal study was carried out according to the protocol in Figure 3.2. *A. stuartii* were fresh-caught at different times of the year and caged externally, under natural photoperiod and temperature regimes. Twenty four hours later, they were randomly divided into two groups. In one group, daily rate of oxygen consumption was measured at the ambient temperature cycle, then at 25°C and 5°C. These *A. stuartii* were then released. In the other group, radioiodine release rate, body mass and rate of food consumption was measured during a week in captivity. These *A. stuartii* were then tagged by a system of ear notches and re-injected with $^{125}$I. Body mass and maximum neck radioactivity were re-measured 24hrs later. The *A. stuartii* were then released at their sites of capture. Traps were laid at these sites approximately one week later and some of these *A. stuartii* were recaptured, enabling field radioiodine release rate to be calculated. Figure 3.3. indicates how this was done. It is assumed that the slope of the line drawn between the two points plotted is representative of the average thyroidal radioiodine release rate in the field during the period between release and capture.

Another group of *A. stuartii* was fresh-caught in an adjoining habitat at the same time as the release rate animals. These were killed within two days of capture and their thyroid iodine content determined. These *A. stuartii* had not been ear-notched and the radioactivity of their thyroid, when removed, was not above background. Radioiodine release rate
Figure 3.2. Seasonal study protocol

Fresh-caught *A. stuartii*

- Oxygen consumption
  - Ambient
    - 25°C
    - 5°C
    - Released

- Random division

- Thyroid activity
  - Release (1 week) (captivity)
  - 125I
  - 1 week
  - 1-2 days
  - Thyroid iodine content

- 125I release (1 week) free-ranging then re-capture & re-measure
Figure 3.3. Calculation of thyroid radioiodine release rate of free-ranging and captive A. stuartii

Radioiodine release rate = slope of \( \ln (M/BG) / \ln (S/BG) \)

- \( M \) Maximum neck radioactivity
- \( BG \) Background radioactivity
- \( S \) Radioactivity of injectate standard

- 19.1%/day
- 33.6%/day

- Captive
- Free-ranging
and thyroid iodine content was determined in different animals for two reasons. First, $^{125}$I was used to determine the percentage recovery of iodine in the thyroid iodine assay and second, $^{125}$I-injected animals were required for the comparison of free-ranging with captive animals. Several fresh-caught *A. stuartii* were killed after a week in captivity to determine whether there was a significant change in thyroid iodine content between fresh-caught and captive animals.

### 3.4 Body mass

Body mass was measured to ±0.1 g using a Sartorius 1265 MP balance.

### 3.5 Food energy intake

The rate of food consumption of *A. stuartii* was measured over several days. New food was added and old food removed at approximately 4 pm each day. The food was weighed to ±0.1 g using a Sartorius 1265 MP balance. Tests carried out at ambient temperature, 25°C and 5°C indicated that daily evaporative water loss ranged from 3 to 5% of the mass of the food. This, along with the average energy content of the food (supplied by Uncle Ben's Australia) was used to calculate the daily food energy intake of the *A. stuartii*.

### 3.6 Measurement of daily oxygen consumption

Each animal was weighed before and after each measurement period of approximately 24 hrs. It was placed inside an airtight metabolism chamber (44 x 30.5 x 12cm), with
wood shavings as floor covering. Inside each chamber was a small nest tube (3.8cm diameter x 12cm long). Four chambers were kept simultaneously in a temperature controlled cabinet.

Daily oxygen consumption was determined using an open circuit technique for each chamber and calculated using equation A of Hill (1972). Air was dried with silica gel and CO₂ removed (Vivalyme, C.I.G.). The airflow through each chamber was measured and regulated at 40 l.h⁻¹ with Brooks flowmeters. The air leaving each chamber was dried, CO₂ removed and oxygen content measured by a Taylor Servomex type OA 272 Oxygen Analyser and recorded by a National Pen Recorder model VP-6521A. A solenoid valve system with timer was used to sample air leaving each chamber for six minutes every half hour. The oxygen content of incoming air was also measured and recorded every half hour using the solenoid valve system. The oxygen analyser was checked and calibrated manometrically three times per day. Little change in calibration was observed. The temperature of inflow air was measured with a 38 gauge copper-constantan thermocouple and recorded on a Leeds and Northrup Speedomax W Recording Potentiometer.

Daily oxygen consumption was measured under slightly different conditions in the cold acclimation study and seasonal study.

In the cold acclimation study daily oxygen consumption was measured at both 25±1°C and 5±1°C. Warm acclimated animals were first measured at 25°C then 5°C whilst cold acclimated animals were first measured at 5°C then at 25°C. All animals were kept and measured at 12h : 12h photoperiod.
In the seasonal study, animals freshly-caught at various times of the year were subjected to the following regimen. Daily oxygen consumption was measured for two days at ambient temperature followed by one day at $25 \pm 1^\circ C$ and another day at $5 \pm 1^\circ C$. The photoperiod used was that occurring naturally at that time of year. Ambient temperature conditions were obtained by not regulating cabinet temperature, and a flow of air from outside the building kept the chambers at close to ambient temperature. The average minimum and maximum temperatures for each measurement period are given in Table 5.4. Body temperature was not measured during oxygen consumption experiments in order to minimise disturbance and stress, which may have inhibited entry into torpor.

### 3.7 Measurement of thyroid activity

**Thyroid radiiodine release rate**

Each animal was given an intraperitoneal injection of 0.3 ml of 0.9% NaCl solution containing 3 $\mu$Ci $^{125}$I as sodium iodide. Maximum neck radioactivity and injectate radioactivity was measured 24 hours later and at the same time each day for approximately a week. A Bicron P-14 sodium iodide thallium-activated crystal connected to an Eberline Mini Scaler model MS-2 was used to measure $^{125}$I radioactivity. The ventral surface of the animal was held against a horizontal perspex platform approximately 700 mm x 180 mm x 10 mm on which two perpendicular axes were marked. The crystal was housed in a 15mm diameter circular hole in a lead container below the intersection of the axes. The animal was moved along the long
axis and the midline radioactivity measured. A repeatable maximum neck radioactivity was recorded after several midline body scans of the animal. Thyroid radioiodine release rate was determined using linear regression analysis after correcting for background radiation and radioactive decay. This was calculated using values obtained after the period of maximum neck radioactivity was achieved. Preliminary tests had shown that maximum neck radioactivity occurred in *A. stuartii* within 6 hours and in *M. musculus* usually between 24 and 36 hours post injection.

**Thyroidal iodine content**

Thyroidal iodine content was measured by the method of Riesco, Taurog & Larson (1976). Animals were killed at approximately 6 pm using anaesthetic ether and their thyroid and associated tracheal region removed. This was finely chopped using a scalpel and digested with 1 ml of 2N KOH containing 2% KC10$_3$ in 25 mm diam. pyrex test tubes. After the contents of each tube had been mixed, it was dried at 90-95°C for 24 hours. The tubes were then transferred to a muffle furnace and heated to and kept at 625°C for one hour. The furnace door was opened for about 15 seconds after 5, 20 and 40 minutes in order to renew the air in the oven.

Following addition of 5ml of deionized water the samples were vortex mixed, centrifuged at 1500G for 10 minutes and iodine content of an aliquot of the supernatent determined. Reagent blanks were treated similarly and used in the construction of a standard curve.

Recovery checks with $^{125}$I were performed on some of
the thyroid gland samples from animals which had not been injected with $^{125}$I. A small amount of $^{125}$I (approximately 0.01 $\mu$Ci) was added to the thyroid digestate and an equal amount to another tube containing 1ml 2N KOH/KClO$_3$ plus 4ml deionized water. A 1ml aliquot of the supernatent from the ashed gland sample and a 1ml aliquot from the unheated KOH/KClO$_3$ solution were counted for two minutes in a Packard Auto-Gamma scintillation spectrometer. After subtracting background radioactivity, corrections were made for $^{125}$I recovery. The recovery was 76.8%±3.9 SEM (n=6).

The iodine content of the digested samples was assayed by the method of Riesco et al., (1976). Iodine catalyses the reaction of ceric ammonium sulphate and arsenious acid, and the rate of reaction was monitored in a Gilford 300-T-1 spectrophotometer by following absorbance at 420nm. The rate of reaction with standard solutions of KI and reagent blanks was compared with the digests of the gland and the concentration of iodine in the gland digests calculated (in $\mu$g l.gland$^{-1}$).

**Thyroid secretion rate**

Thyroid secretion rate, the product of radioiodine release rate and iodine content was calculated in $\mu$g l.day$^{-1}$. Assuming that all iodine leaves the thyroid as a component of thyroxine, thyroxine secretion rate (in nmol T$_4$day$^{-1}$) was calculated by multiplying the thyroidal iodine secretion rate by 1.97.
3.8 Statistical analysis

A two-tailed student's t-test was used to test for significant differences between treatments except for captive and free-ranging thyroid activity, where a paired t-test was used.
Chapter 4 Results and Discussion of the Cold Acclimation Study

4.1 Body mass

There was no significant difference in body mass between animals used in the thyroid activity experiments (Table 4.1) and metabolism experiments (Table 4.3).

4.2 Food energy intake of *A. stuartii*

During the metabolism experiments, there was no significant difference in food energy intake between cold and warm acclimated *A. stuartii* at 5°C or at 25°C. However, for both cold and warm acclimated *A. stuartii*, food energy intake was significantly higher at 5°C than at 25°C (Table 4.3).

4.3 Thyroid activity

No significant difference in thyroid radioiodine release rate or iodine content was detected between males and females, so the values for both sexes have been pooled. Radioiodine release rate was significantly higher in *A. stuartii* than in *M. musculus*, irrespective of acclimation temperature (Table 4.2 & Figure 4.1). There was no significant difference in radioiodine release rate between cold and warm acclimated *M. musculus*. However, the radioiodine release rate of cold acclimated *A. stuartii* (51.3%·day⁻¹) was significantly higher than (33.4%·day⁻¹) in warm acclimated *A. stuartii* (Table 4.2).
Table 4.1. Effects of cold and warm acclimation on body mass of *A. stuartii* and *M. musculus*.

<table>
<thead>
<tr>
<th>Acclimation status</th>
<th><em>A. stuartii</em></th>
<th><em>M. musculus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (g)</td>
<td>cold</td>
<td>warm</td>
</tr>
<tr>
<td></td>
<td>30.1*</td>
<td>27.1</td>
</tr>
<tr>
<td></td>
<td>(±2.1; 6)*</td>
<td>(±2.3; 7)</td>
</tr>
</tbody>
</table>

* Mean values.

b Numbers in parentheses represent ±S.E.M.; sample size.
Table 4.2. Effects of cold and warm acclimation on thyroidal radioiodine release rate, iodine content and thyroid secretion rate of *A. stuartii* and *M. musculus*.

<table>
<thead>
<tr>
<th>Acclimation status</th>
<th><em>A. stuartii</em></th>
<th><em>M. musculus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cold</td>
<td>warm</td>
</tr>
<tr>
<td>Radioiodine release rate (% day⁻¹)</td>
<td>51.3ᵃ,ᵇ,ᶜ,ᵈ</td>
<td>33.4ᵈ</td>
</tr>
<tr>
<td>(±7.2 ; 6)ᵇ</td>
<td>(±2.9 ; 7)</td>
<td>(±0.8 ; 7)</td>
</tr>
<tr>
<td>Iodine content (µg l. gland⁻¹)</td>
<td>0.93ᶜ,ᵈ</td>
<td>0.46ᵈ</td>
</tr>
<tr>
<td>(±0.06 ; 6)ᶜ</td>
<td>(±0.07 ; 7)</td>
<td>(±0.64 ; 7)</td>
</tr>
<tr>
<td>Thyroid secretion rate (µg l. day⁻¹)</td>
<td>0.47ᶜ,ᵈ</td>
<td>0.15</td>
</tr>
<tr>
<td>(±0.07 ; 6)ᶜ</td>
<td>(±0.02 ; 7)</td>
<td>(±0.02 ; 7)</td>
</tr>
<tr>
<td>(n mol T₄ day⁻¹)</td>
<td>0.93</td>
<td>0.30</td>
</tr>
<tr>
<td>(±0.14 ; 6)ᶜ</td>
<td>(±0.04 ; 7)</td>
<td>(±0.03 ; 7)</td>
</tr>
</tbody>
</table>

ᵃ Mean values.
b Numbers in parentheses represent ±S.E.M.; sample size.
c Significantly different from same species warm acclimated (p < 0.05).
d Significantly different from *M. musculus* acclimated to the same temperature (p < 0.05).
Table 4.3. Effects of cold and warm acclimation on metabolism and food energy intake of *A. stuartii* at 5 °C and 25 °C.

<table>
<thead>
<tr>
<th>Acclimation status</th>
<th>Cold (°C)</th>
<th>Warm (°C)</th>
<th>Cold (°C)</th>
<th>Warm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured at</td>
<td>5</td>
<td>5</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>N° of animals</td>
<td>7</td>
<td>7</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>29.5 ± 1.7</td>
<td>29.8 ± 2.2</td>
<td>30.9 ± 2.0</td>
<td>29.2 ± 2.3</td>
</tr>
<tr>
<td>Daily Metabolic rate (ml O₂·g⁻¹·hr⁻¹)</td>
<td>6.3 ± 0.2</td>
<td>7.0 ± 0.4</td>
<td>4.5 ± 0.7</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>Daily Metabolic rate (kJ·day⁻¹)</td>
<td>89.2 ± 3.0</td>
<td>100.3 ± 7.7</td>
<td>65.7 ± 6.3</td>
<td>48.5 ± 5.6</td>
</tr>
<tr>
<td>Food energy intake (kJ·day⁻¹)</td>
<td>118.3 ± 4.4</td>
<td>111.9 ± 9.8</td>
<td>96.0 ± 10.4</td>
<td>79.0 ± 12.1</td>
</tr>
</tbody>
</table>

a All values are mean.
b ±S.E.M.
c Significantly different from similarly acclimated group at 25°C.
Figure 4.1 Effects of cold and warm acclimation on thyroid radioiodine release rate of *A. stuartii* and *M. musculus*. Bars represent mean values and lines represent +S.E.M. with *N* written above each bar.
Thyroid iodine content was significantly higher in *M. musculus* than in *A. stuartii* irrespective of acclimation temperature (Table 4.2 & Figure 4.2). There was no significant difference in thyroid iodine content between cold and warm acclimated *M. musculus*. However, the thyroid iodine content of cold acclimated *A. stuartii* (0.93µg l.gland\(^{-1}\)) was significantly higher (by 83%) than (0.45µg l.gland\(^{-1}\)) in the warm acclimated *A. stuartii* (Table 4.2 & Figure 4.2).

There was no significant difference in thyroid iodine secretion rate between cold and warm acclimated *M. musculus* (Table 4.2 & Figure 4.3). There was also no significant difference in iodine secretion rate between the warm acclimated *A. stuartii* and either group of *M. musculus*. However, thyroid iodine secretion rate in the cold acclimated *A. stuartii* (0.47µg l.day\(^{-1}\)) was significantly higher (by 213%) than (0.15µg l.day\(^{-1}\)) in the warm acclimated *A. stuartii* (Table 4.2 and Figure 4.3). Assuming that all iodine leaves the thyroid as a component of thyroxine, then the thyroxine secretion rate (\(T_4\)SR) of cold acclimated *A. stuartii* is 0.9 nmol \(T_4\)day\(^{-1}\). Thyroid secretion rate in the other three groups ranged from 0.3 to 0.4 nmol \(T_4\)day\(^{-1}\).

### 4.4 Metabolic rate

There was no significant difference in daily metabolic rate (DMR) between cold and warm acclimated *A. stuartii* at 5°C. There was also no significant difference between cold and warm acclimated *A. stuartii* when these parameters were
Figure 4.2 Effects of cold and warm acclimation on thyroid iodine content of A. stuartii and M. musculus. Bars represent mean values and lines represent +S.E.M. with N written above each bar.
Figure 4.3. Effects of cold and warm acclimation on thyroid iodine secretion rate of *A. stuartii* and *M. musculus*. Bars represent mean values and lines represent S.E.M. with N written above each bar.
measured at 25°C. However, DMR was significantly higher when measured at 5°C than at 25°C, irrespective of acclimation temperature. (Table 4.3 & Figure 4.4). Table 4.3 indicates that DMR accounted for a higher percentage of food energy intake at 5°C than at 25°C. At 5°C, DMR was 75-90% of food energy intake whilst at 25°C, DMR was 61-68% of daily food energy intake.

The daily variation in metabolic rate of *A. stuartii* considered to be representative of each group are shown in Figure 4.5.

Although the MR measured at 25°C of some of the warm acclimated *A. stuartii* was higher during periods of darkness than light, this pattern was not exhibited by most warm acclimated *A. stuartii*, nor by the cold acclimated *A. stuartii*.

The MR at 5°C of the warm acclimated *A. stuartii* was generally high throughout the 24 hour period, although one *A. stuartii* did exhibit a markedly lower MR during the light period. The MR at 5°C of the cold acclimated *A. stuartii* was generally high throughout the 24 hour measuring period.

In some of the *A. stuartii* in which there were periods of high and periods of low MR, the decrease in MR was recorded when the lights were switched on in the morning. However, in others, the decrease occurred at other times of the day. Thus, there is no consistent pattern of increased MR during periods of darkness and decreased MR during periods of light. As can be seen from Figure 4.5, at no time did oxygen consumption drop to below 1ml O₂.g⁻¹.h⁻¹ which is the reported standard metabolic rate for this species (Dawson & Hulbert, 1970). I take this to indicate that there were no significant periods of torpor.
Figure 4.4. Effects of cold and warm acclimation on daily metabolic rate of *A. stuartii* at 5°C and 25°C. Bars represent mean values and lines represent ±S.E.M. with N written above each bar.
Figure 4.5. Daily variations in metabolic rate at 5°C and 25°C of cold and warm acclimated *A. stuartii*. The 12-h photocycle is indicated by the light and dark bars above the time scale.
4.5 Discussion

The effects of long-term cold exposure on thyroid function has been studied in another marsupial. Setchell (1974) monitored the rate of disappearance of injected $^{125}$I-labelled thyroxine in *M. eugenii* and reported that long-term cold exposure increased thyroxine secretion rate. However, this is the first time that the effects of cold acclimation on thyroid secretion rate have been studied in a very small marsupial.

The thyroid radioiodine release rates for both cold and warm acclimated *M. musculus* (Table 4.2) are similar to those obtained by Else, (1979) for *M. musculus* (9.2% day$^{-1}$) and by Withers and Hulbert (1987) for *Rattus norvegicus* (10.1% day$^{-1}$). The higher value of 33.4% day$^{-1}$ obtained for warm acclimated *A. stuartii* is similar to those obtained in two bandicoots, 32.3% day$^{-1}$ in *Isodon macrourus* and 18.9% day$^{-1}$ in *Perameles nasuta* (Hulbert & Augee 1982).

Higher thyroidal radioiodine release rates result in a greater potential for recycling of radioiodine and thus a greater potential for underestimating radioiodine release rate (Hulbert et al., 1987). Radioiodine recycling in the rabbit was found to be approximately 10%, with $^{131}$I release rates ranging from 5-24% day$^{-1}$ (Brown-Grant et al., 1954 b). Although the extent of radioiodine recycling in *A. stuartii* and *M. musculus* is unknown, Figure 4.3 indicates that the differences between cold acclimated *A. stuartii* and both groups of *M. musculus* are so large that the interspecies differences in thyroid iodine release rate shown in Table 4.3 are not solely due to recycling differences.
The thyroidal iodine content values for *M. musculus* (6.9-8.2 μg/l.100g⁻¹) obtained in the present study (Table 4.2) are similar to those obtained in rats, 6.4 μg.l.100g⁻¹ (Salter & McKay, 1944); 3.8 μg.l.100g⁻¹ (Wilson & van Zyl, 1967); 3.1 μg.l.100g⁻¹ (Galton & Nisula, 1969) & 3.5 μg.l.100g⁻¹ (Withers & Hulbert, 1987). The lack of a significant difference in thyroid iodine content between cold and warm acclimated animals, found in *M. musculus* (Table 4.2) has been reported in rats (Heroux & Brauer, 1965; Galton & Nisula, 1969). This is, however, the first time that thyroid iodine content has been determined in a marsupial. It is interesting that the thyroid iodine content of the marsupial is smaller than that of the similar-sized eutherian, irrespective of whether they are cold or warm acclimated. It is also evident, that although cold acclimation produced no significant change in thyroid iodine content of the eutherian, a significantly higher value was found in the cold acclimated marsupial.

From Table 4.2, the thyroxine secretion rate (T₄SR) was 0.99 nmole T₄.100g⁻¹.day⁻¹ in warm acclimated *M. musculus* and 1.28 nmole T₄.100g⁻¹.day⁻¹ in cold acclimated *M. musculus*. These values are not too dissimilar to 1.78 nmole T₄.100g⁻¹.day⁻¹ reported for *M. musculus* (Bauman, *et al.*, 1968) and values reported for *Rattus norvegicus*, 1.13-2.12 nmole T₄.100g⁻¹.day⁻¹ (Bauman *et al.*, 1968) and 0.75 nmole T₄.100g⁻¹.day⁻¹ (Withers & Hulbert, 1987) and for *Rattus rattus* 1.40 nmole T₄.100g⁻¹.day⁻¹ (Setchell, 1974). Thus, the T₄SR values in Table 4.2 are slightly lower than those in the
The lack of a significant difference in thyroid iodine secretion rate between cold and warm acclimated *M. musculus* (Table 4.2) indicates that they are not directly dependent on thyroid hormones per se for the increased thermogenesis reported by Reynolds & Hulbert (1982) in cold acclimated *M. musculus*. However, the maximum thermogenic response to noradrenaline demonstrated in cold acclimated *M. musculus*, an increase of approximately 5 ml O$_2$.g$^{-1}$.hr$^{-1}$ greater than the maximum in warm acclimated *M. musculus* (Reynolds & Hulbert 1982), indicates that classical brown fat NST could contribute significantly to their heat production. Furthermore, increased secretion of thyroid hormones is not required for NST. The presence of thyroid hormones is required merely to potentiate the action of noradrenaline in NST (Hemon et al., 1976).

Galton & Nisula, (1969), have suggested that increased food consumption, rather than cold exposure per se causes increased thyroid activity. The thyroid iodine secretion rate of the cold acclimated *A. stuartii* was 213% higher than that of the warm acclimated *A. stuartii* (Table 4.2). However, the daily food energy intake of the cold acclimated *A. stuartii* at 5°C was only 50% higher than that of the warm acclimated *A. stuartii* at 25°C (Table 4.2). It is therefore unlikely that increased food consumption alone, was the cause of increased thyroid secretion in the cold acclimated *A. stuartii*.

The present study indicates that the rate of thyroid secretion of *A. stuartii* increased after a period of cold acclimation but that no change was evident in *M. musculus*. I suggest, that *A. stuartii* and perhaps *M. musculus* increase
their thyroid secretion rate when first exposed to 5°C. Furthermore, that when BAT and thus the capacity for NST has increased in *M. musculus* its thyroid secretion rate returns to normal levels. However, that of *A. stuartii* remains high as it lacks the capacity for effective NST.

It is evident from Figure 4.5 that torpor is not an integral part of the energetic strategy of *A. stuartii* when food is readily available, irrespective of whether they are cold or warm acclimated. Furthermore, the higher daily metabolic rate of *A. stuartii* at 5°C, irrespective of whether they are cold or warm acclimated (Table 4.3) indicates a strategy of increased heat production in the cold, rather than one consisting only of reduced heat loss. It is also evident from Table 4.3 that cold acclimation does not increase the daily metabolic rate of *A. stuartii* at 5°C.

The lack of a significant difference in DMR at 25°C between cold and warm acclimated *A. stuartii* conflicts with results from other studies. Heat production at 30°C in restrained *A. stuartii* (Reynolds & Hulbert, 1982) and at 25°C and 30°C in unrestrained *Dasyuroides byrnei* (Smith & Dawson, 1984) was significantly higher in cold acclimated than in warm acclimated animals. Although thermal conductance of cold acclimated restrained *A. stuartii* was not significantly different from that of warm acclimated *A. stuartii* (Reynolds & Hulbert, 1982), thermal conductance of cold acclimated *D. byrnei* was significantly higher at the same temperature as was heat production (Smith & Dawson, 1984). However, DMR at 25°C in the present study was 35% higher in the cold acclimated than in the warm acclimated *A. stuartii*. The lack of a
significant difference between these two groups (p=0.173) may have resulted from the small sample size (n=2) in the cold acclimated *A. stuartii*. Tables 4.1 and 4.3 indicate that acclimation to cold does not result in altered body mass in *A. stuartii* and *M. musculus*. This finding, agrees with that of Reynolds & Hulbert (1982) and indicates that the daily metabolic rate of *A. stuartii* was not influenced by increases or decreases in body mass.

MR at 5°C of warm acclimated *A. stuartii* was 4.5ml O$_2$.g$^{-1}$.h$^{-1}$ when restrained (Reynolds & Hulbert, 1982) and 7.0 ml O$_2$.g$^{-1}$.h$^{-1}$ when unrestrained (Table 4.3). This indicates that restraint prevented warm acclimated *A. stuartii* from using activity to increase thermogenesis at 5°C and maintain a constant body temperature. Furthermore, it indicates that 36% of the energetic cost of heat production in unrestrained *A. stuartii* at 5°C (Table 4.3) results from activity. As none of the animals in either study were fasted, specific dynamic action would not be expected to be a component of this difference.

*A. stuartii* may adopt a near spherical posture to reduce heat loss (Wallis, 1982). Restrained *A. stuartii* at 5°C would not have been able to alter body posture to conserve body heat. This, along with possible disturbance to the arrangement of fur may be responsible for the higher metabolic cost 8.2ml O$_2$.g$^{-1}$.h$^{-1}$ of cold acclimated *A. stuartii* at 5°C when restrained (Reynolds & Hulbert, 1982), than 6.3ml O$_2$.g$^{-1}$.h$^{-1}$ when unrestrained (Table 4.3).

MR at 5°C of *A. stuartii* was 82% higher in cold acclimated than in warm acclimated animals (Reynolds &
Hulbert, 1982). As these *A. stuartii* exhibited no thermogenic response to noradrenaline and were also restrained (Reynolds & Hulbert, 1982), this difference was not due to activity or classical noradrenaline mediated NST. It is possible that shivering contributed to increased thermogenesis in the cold acclimated *A. stuartii*. However, heat production of restrained *A. stuartii* was also significantly higher at 30°C in cold acclimated than in warm acclimated animals (Reynolds & Hulbert, 1982). This indicates that increased thermogenesis is unlikely to have been caused by shivering alone. Thus, although cold acclimated *A. stuartii* may use shivering thermogenesis, they also use a non shivering thermogenic mechanism that is not mediated by noradrenaline. The increased thyroid secretion rate in cold acclimated *A. stuartii* (Table 4.2) suggests that thyroid hormones were responsible for at least some of the increased heat production reported by Reynolds & Hulbert, (1982) in restrained cold acclimated *A. stuartii* at both 5°C and 30°C.

Thyroid hormones stimulate metabolic rate in marsupials and eutherians (Setchell, 1974; Hulbert & Augee, 1982) and increases in thyroid radioiodine release rate have been reported within several hours of cold exposure in rats, rabbits and guinea pigs (Brown-Grant *et al.*, 1954; Brown-Grant 1956 & Yamada *et al.*, 1965). However, although the thyroid pituitary axis may be stimulated within hours of cold exposure, thyroid hormones do not exert a calorigenic effect until after several days (Ingbar & Woeber, 1974). Thus, the lack of a significant difference in MR between Cold acclimated and warm acclimated *A. stuartii* at 5°C (Table 4.3) was not due to an acute increase in thyroid activity in the warm acclimated *A. stuartii*. 
The higher food energy intake of *A. stuartii* at 5°C (Table 4.3), irrespective of whether they are cold or warm acclimated is consistent with a strategy of increased heat production in the cold. The increased food consumption of mammals kept in cold environments in similar experiments is well established (Bauman et al., 1968; Himms-Hagen, 1972). That cold acclimation did not increase the food energy requirements of *A. stuartii* at 5°C or at 25°C to levels greater than those of warm acclimated *A. stuartii* is also consistent with the daily metabolic rate results for these animals (Table 4.3).

It is evident that acclimation to cold can occur in marsupials as well as eutherians (Reynolds & Hulbert, 1982; Smith & Dawson, 1984), although acclimation to cold in *A. stuartii* is more concerned with increased heat production, rather than reduction of heat loss via torpor. However, *A. stuartii*, unlike many eutherians increases heat production, not by noradrenaline-mediated non-shivering thermogenesis, but probably by thyroid hormone calorigenesis. Warm acclimated *A. stuartii* may use activity to increase heat production in the cold.
5.1 Body mass

The body mass at capture of the *A. stuartii* used in the thyroid experiments increased significantly from 19.6g during February to 37.8g during August (Table 5.1). The body mass of *A. stuartii* increased significantly while in captivity during April and June but decreased significantly between release and recapture between February and June (Table 5.1).

The body mass of *A. stuartii* used in the metabolism experiments is shown in Tables 5.4, 5.5 & 5.6. The body mass of males (35.2-37.1g) was significantly higher than that of females (25.7-28.6) during July and this difference became even greater in August (45.2-46.6g in males and 25.7-27.8g in females). However, the body mass of females did not vary greatly throughout the second half of the year. There was a slight increase in body mass of females up to December values of 29.9-31.6g. The values for October and early November include the weight of the litters, and are thus higher than the weight of pre-reproductive females in August. There was a slight increase in combined mass of females and litters during October (Tables 5.4, 5.5 & 5.6).

5.2 Food energy intake

In the *A. stuartii* used in the thyroid experiments, the food energy intake in captivity increased significantly from 62.7 kJ.day\(^{-1}\) in juveniles during February to 118.1 kJ.day\(^{-1}\) in adults
Table 5.1. Seasonal variations in food consumption and body mass of fresh-caught *A. stuartii*.

<table>
<thead>
<tr>
<th>Time of year</th>
<th>Statusa</th>
<th>Body mass (g)</th>
<th>Food energy intake (kJ.day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Captured</td>
<td>Released</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb.</td>
<td>J</td>
<td>19.6b±0.7(6)</td>
<td>21.5±1.1(6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M + F</td>
<td>17.9-23.5d</td>
<td>18.5-24.8h</td>
</tr>
<tr>
<td>Apr.</td>
<td>M + F</td>
<td>24.7d±1.4(8)</td>
<td>29.3de±1.4(8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M + F</td>
<td>15.1-20.0d</td>
<td>13.0-20.1</td>
</tr>
<tr>
<td>Jun.</td>
<td>M + F</td>
<td>25.1d±1.7(8)</td>
<td>30.5de±1.8(8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M + F</td>
<td>13.6-20.4</td>
<td>7.9-15.6</td>
</tr>
<tr>
<td>Aug.</td>
<td>M + F</td>
<td>37.8d±3.8(6)</td>
<td>40.5d±3.8(6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M + F</td>
<td>13.4-18.9</td>
<td>11.2-20.2</td>
</tr>
<tr>
<td>Nov.</td>
<td>F + Y</td>
<td>34.7d±1.3(4)</td>
<td>32.9d±0.5(4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>16.0-21.2</td>
<td></td>
</tr>
<tr>
<td>Nov.</td>
<td>F</td>
<td>27.5d±1.2(4)</td>
<td>25.6d±1.1(4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>17.1-21.4</td>
<td></td>
</tr>
<tr>
<td>Feb.</td>
<td>F</td>
<td>27.7d±1.7(2)</td>
<td>31.0d±0.9(2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>17.9-23.5</td>
<td>18.5-24.8</td>
</tr>
</tbody>
</table>

a J= Juveniles; M= Males; F= Females; Y= Young.
b Mean values.
c Numbers in parentheses represent ±S.E.M.; sample size.
d Significantly different from February juveniles.
e Significantly different from capture.
f Significantly different from release.
g Mean min. and max. air temperature of captivity area.
h Mean min. and max. air temperature while free-ranging.
during August (Table 5.1). In mature females during February, it had decreased to 76.2 kJ.day\(^{-1}\) (Table 5.1). In the \textit{A. stuartii} used in the metabolism experiments, food energy intake of males was significantly higher in August (121.2-159.7 kJ.day\(^{-1}\)) than in July (106.1-130.9 kJ.day\(^{-1}\)). However, there was no significant difference for females between July (68.3-94.3 kJ.day\(^{-1}\)) and August (78.6-79.6 kJ.day\(^{-1}\)) (Tables 5.4, 5.5 & 5.6). Food energy intake values at 5\(^\circ\)C were generally higher than those obtained at ambient temperature or at 25\(^\circ\)C.

5.3 Thyroid activity in captivity of freshly-caught \textit{A. stuartii}

The radioiodine release rate of captive \textit{A. stuarti}, increased significantly from 17.9\%.day\(^{-1}\) in juveniles during February to 47.1\%.day\(^{-1}\) in mature animals during August (Table 5.2). The radioiodine release rates of females with pouch young during early November (33.5\%.day\(^{-1}\)) was significantly lower than the August value. The radioiodine release rate of females without pouch young captured late in November (20.3\%.day\(^{-1}\)) was significantly lower than that of females with young captured early in November (Table 5.2). The radioiodine release rate of mature females captured during February (14.2\%.day\(^{-1}\)) was significantly lower than in females late in November but not significantly different from that of juveniles during February (Table 5.2).

Thyroid iodine contents ranging from 0.57-1.51\(\mu\)g.l gland\(^{-1}\) are shown in Table 5.2 & Figure 5.2. There is more variation in thyroid iodine content of \textit{A. stuartii} in the seasonal study than in the cold acclimation study. No significant
Table 5.2. Seasonal variations in thyroidal radioiodine release rate of fresh-caught *A. stuartii* in captivity and free-ranging.

<table>
<thead>
<tr>
<th>Time of year</th>
<th>Statusa</th>
<th>Radioiodine release rate (% day⁻¹)</th>
<th>Captive</th>
<th>Captive values of free-ranging <em>A. stuartii</em></th>
<th>Free-ranging</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb.</td>
<td>J</td>
<td>17.9b</td>
<td>19.1</td>
<td>18.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(±6.9 ; 6)c</td>
<td>(±4.3 ; 4)</td>
<td>(±1.5 ; 4)</td>
<td></td>
</tr>
<tr>
<td>Apr.</td>
<td>M+F</td>
<td>28.5dea</td>
<td>29.4</td>
<td>24.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(±2.5 ; 8)</td>
<td>(±2.7 ; 7)</td>
<td>(±2.8 ; 7)</td>
<td></td>
</tr>
<tr>
<td>Jun.</td>
<td>M+F</td>
<td>43.8def</td>
<td>40.0def</td>
<td>17.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(±3.7 ; 8)</td>
<td>(±4.3 ; 4)</td>
<td>(±1.9 ; 4)</td>
<td></td>
</tr>
<tr>
<td>Aug.</td>
<td>M+F</td>
<td>47.1d</td>
<td>69.0</td>
<td>22.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(±5.6 ; 6)</td>
<td>(± ; 1)</td>
<td>(± ; 1)</td>
<td></td>
</tr>
<tr>
<td>Nov.</td>
<td>F + Y</td>
<td>33.5de</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(±2.0 ; 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov.</td>
<td>F</td>
<td>20.3e</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(±2.9 ; 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb.</td>
<td>F</td>
<td>14.2e</td>
<td>14.2</td>
<td>12.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(±1.5 ; 2)</td>
<td>(±1.5 ; 2)</td>
<td>(±3.5 ; 2)</td>
<td></td>
</tr>
</tbody>
</table>

a J= Juvenile; M= Male; F= Female; Y= Young.
b Mean values.
c Numbers in parentheses represent ±S.E.M.; sample size.
d Significantly different from February Juveniles (p<0.05).
e Significantly different from previous month's value (p<0.05).
f Significantly different from free-ranging *A. stuartii* (p<0.05).
Figure 5.1. Seasonal variations in thyroid radiiodine release rate of captive A. stuartii and the same animals while free-ranging. Bars represent mean values and lines represent +S.E.M. with N written above the bars.
Figure 5.2 Seasonal variations in thyroid iodine content of A. stuartii. Bars represent mean values and lines represent +S.E.M. with N written above each bar.
difference in thyroid iodine content was detected between *A. stuartii* killed within two days of capture and those killed about a week after capture, so these values were pooled.

The thyroid secretion rate of captive and free-ranging *A. stuartii* are given in Table 5.3. The thyroid secretion rate of captive *A. stuartii* increased from 0.10 µg l.day$^{-1}$ during February to 0.38 µg l.day$^{-1}$ during April and 0.35 µg l.day$^{-1}$ during June. A single value of 0.61 µg l.day$^{-1}$ is given for August. It decreased to 0.21 µg l.day$^{-1}$ during February (Table 5.3).

5.4 Thyroid activity in the field

A comparison of radioiodine release rates while free-ranging, with values obtained while in captivity for the same animals is shown in Table 5.2 & Figure 5.1. Thyroid radioiodine release rates of free-ranging *A. stuartii* were similar to those of captive animals during February and April. Radioiodine release rate of free-ranging *A. stuartii* increased slightly from 18.5%.day$^{-1}$ during February to 24.2%.day$^{-1}$ during April and decreased slightly to 17.4%.day$^{-1}$ during June. A single value of 22.7%.day$^{-1}$ was obtained for a male *A. stuartii* in August (Table 5.2). The June radioiodine release rate in captivity (40.0%.day$^{-1}$) is significantly higher than all free-ranging values and their corresponding values in captivity, except for August.

The calculated thyroid iodine secretion rates of free-ranging *A. stuartii* were similar to those of captive animals during February and April and lower during June and August (Table 5.3).
Table 5.3. Seasonal variations in calculated thyroid secretion rate of fresh-caught *A. stuartii* in captivity and free-ranging *A. stuartii*.

<table>
<thead>
<tr>
<th>Time of year</th>
<th>Status&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Radioiodine release rate (%·day&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Thyroid iodine content (µg·gland&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Calculated thyroid secretion rate (µg·day&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Captive</td>
<td>Free-ranging</td>
<td>Captive</td>
<td>Free-ranging</td>
</tr>
<tr>
<td>Feb</td>
<td>J</td>
<td>17.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.5</td>
<td>0.57&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.08;7)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Apr.</td>
<td>M</td>
<td>28.5</td>
<td>24.2</td>
<td>1.32&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.31;6)</td>
</tr>
<tr>
<td>Jun.</td>
<td>M+F</td>
<td>43.8</td>
<td>17.9</td>
<td>0.80&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.21;7)</td>
</tr>
<tr>
<td>Aug.</td>
<td>M+F</td>
<td>47.1</td>
<td>22.7</td>
<td>1.29&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.20;9)</td>
</tr>
<tr>
<td>Nov.</td>
<td>F + Y</td>
<td>33.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Nov.</td>
<td>F</td>
<td>20.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Feb</td>
<td>F</td>
<td>14.2</td>
<td>12.8</td>
<td>1.51&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.69;3)</td>
</tr>
</tbody>
</table>

<sup>a</sup> J = Juvenile; M = Male; F = Female; Y = Young.

<sup>b</sup> Mean values taken from Table 5.2.

<sup>c</sup> Numbers in parentheses represent ±S.E.M.; sample size.

<sup>d</sup> Significantly different from February Juveniles (p<0.05).

<sup>e</sup> Significantly different from previous month's value (p<0.05).
5.5. Metabolic rate

Daily metabolic rate (DMR) at ambient temperature cycle, of male *A. stuartii* was higher during August (79.9 kJ.day\(^{-1}\)) than July (63.0 kJ.day\(^{-1}\)) and higher than that of females during both of these months (Table 5.4 & Figure 5.3). DMR of females decreased significantly from July (52.9 kJ.day\(^{-1}\)) to August (42.2 kJ.day\(^{-1}\)) and then increased progressively to a significantly higher level (58.8 kJ.day\(^{-1}\)) in late November. DMR during December (44.1 kJ.day\(^{-1}\)) was significantly lower than that of November (Table 5.4 & Figure 5.3). Metabolic rate (MR) of males was (3.7 ml O\(_2\).g\(^{-1}\).h\(^{-1}\)) during July and August (Table 5.4 & Figure 5.4). August and October values of females were similar to those of the above males (Table 5.4 & Figure 5.4). However, female MR was significantly higher during July (4.3 ml O\(_2\).g\(^{-1}\).h\(^{-1}\)) and late November (4.2 ml O\(_2\).g\(^{-1}\).h\(^{-1}\)) and lower during December (2.9 ml O\(_2\).g\(^{-1}\).h\(^{-1}\)) (Table 5.4 & Figure 5.4).

The seasonal pattern in DMR of males and females measured at 25\(^{\circ}\)C (Table 5.5 & Figure 5.3) was similar to that at ambient temperature. However, DMR of females was slightly lower during July (43.5 kJ.day\(^{-1}\)) and higher during December (50.7 kJ.day\(^{-1}\)). No significant differences in MR at 25\(^{\circ}\)C were detected in males between July (3.4 ml O\(_2\)) and August (3.5 ml O\(_2\).g\(^{-1}\).h\(^{-1}\)) and females between July (3.1 ml O\(_2\).g\(^{-1}\).h\(^{-1}\)) and December (3.1 ml O\(_2\).g\(^{-1}\).h\(^{-1}\)), although MR of females in late November (4.4 ml O\(_2\).g\(^{-1}\).h\(^{-1}\)) was significantly higher than all other MR values at 25\(^{\circ}\)C (Table 5.5 & Figure 5.4).
Table 5.4. Metabolism and food energy intake at ambient temperature cycle of *A. stuartii* freshly-caught at various times of the year.

<table>
<thead>
<tr>
<th>Time of Year</th>
<th>Status*</th>
<th>N° of Animals</th>
<th>Body Mass (g)</th>
<th>Metabolic Rate (ml O₂.g⁻¹.hr⁻¹)</th>
<th>Food Energy Intake (kJ.day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.0-19.6</td>
<td>M</td>
<td>7</td>
<td>35.2bc±1.6</td>
<td>3.7c±0.1</td>
<td>63.0c±4.1</td>
</tr>
<tr>
<td>July</td>
<td>F</td>
<td>5</td>
<td>25.7±1.4</td>
<td>4.3±0.2</td>
<td>52.9±4.2</td>
</tr>
<tr>
<td>15.4-20.5</td>
<td></td>
<td></td>
<td>35.2bc±1.6</td>
<td>3.7c±0.1</td>
<td>63.0c±4.1</td>
</tr>
<tr>
<td>11.5-16.7</td>
<td>M</td>
<td>9</td>
<td>45.2cde±2.7</td>
<td>3.7c±0.1</td>
<td>79.9cd±4.0</td>
</tr>
<tr>
<td>August</td>
<td>F</td>
<td>3</td>
<td>25.7±1.8</td>
<td>3.4d±0.1</td>
<td>42.2±3.4</td>
</tr>
<tr>
<td>13.8-18.1</td>
<td></td>
<td></td>
<td>45.2cde±2.7</td>
<td>3.7c±0.1</td>
<td>79.9cd±4.0</td>
</tr>
<tr>
<td>Early</td>
<td>F+Y</td>
<td>4</td>
<td>29.8d±0.7</td>
<td>3.3d±0.2</td>
<td>46.9±2.3</td>
</tr>
<tr>
<td>October</td>
<td>24.8-28.8</td>
<td></td>
<td>29.8d±0.7</td>
<td>3.3d±0.2</td>
<td>46.9±2.3</td>
</tr>
<tr>
<td>Late</td>
<td>F+Y</td>
<td>3</td>
<td>33.3d±1.9</td>
<td>3.4d±0.1</td>
<td>54.9±5.1</td>
</tr>
<tr>
<td>October</td>
<td>22.0-24.5</td>
<td></td>
<td>33.3d±1.9</td>
<td>3.4d±0.1</td>
<td>54.9±5.1</td>
</tr>
<tr>
<td>November</td>
<td>F</td>
<td>4</td>
<td>29.1de±0.7</td>
<td>4.2d±0.2</td>
<td>58.8±1.9</td>
</tr>
<tr>
<td>22.8-26.0</td>
<td></td>
<td></td>
<td>29.1de±0.7</td>
<td>4.2d±0.2</td>
<td>58.8±1.9</td>
</tr>
<tr>
<td>December</td>
<td>F</td>
<td>4</td>
<td>31.6de±0.5</td>
<td>2.9d±0.2</td>
<td>44.1e±2.4</td>
</tr>
<tr>
<td>25.6-29.5</td>
<td></td>
<td></td>
<td>31.6de±0.5</td>
<td>2.9d±0.2</td>
<td>44.1e±2.4</td>
</tr>
</tbody>
</table>

Note:  
* M=Male, F=Female, F+Y=Female + Young  
  b All values are mean ± SEM.  
  c Significantly different from females in same month (p<0.05).  
  d Significantly different from same sex in July (p<0.05).  
  e Significantly different from same sex in previous month (p<0.05).  
  f Mean min. and max. ambient temperature during measurements.
Figure 5.3. Daily metabolic rate at ambient temperature cycle, 25°C and 5°C of A. stuartii fresh-caught at various times of the year. Bars represent mean values and lines represent S.E.M. with N written above each bar.
Figure 5.4. Mean metabolic rate over 24 hours at ambient temperature cycle, 25°C and 5°C of A. stuartii fresh-caught at various times of the year. Bars represent mean values and lines represent +S.E.M. with N written above each bar.
Table 5.5. Metabolism and food energy intake at 25°C of *A. stuartii* freshly-caught at various times of the year.

<table>
<thead>
<tr>
<th>Time of Year</th>
<th>Status&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of Animals</th>
<th>Body Mass (g)</th>
<th>Metabolic Rate (mLO₂.g⁻¹.hr⁻¹)</th>
<th>Food Energy Intake (kJ.day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July</td>
<td>M</td>
<td>7</td>
<td>36.4&lt;sup&gt;bc&lt;/sup&gt; ±1.8</td>
<td>3.4 ±0.2</td>
<td>59.7&lt;sup&gt;c&lt;/sup&gt; ±4.3</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
<td>26.8 ±1.7</td>
<td>3.1 ±0.3</td>
<td>43.5 ±4.8</td>
</tr>
<tr>
<td>August</td>
<td>M</td>
<td>6</td>
<td>46.6&lt;sup&gt;c&lt;/sup&gt; ±3.6</td>
<td>3.5 ±0.2</td>
<td>78.7&lt;sup&gt;cde&lt;/sup&gt; ±7.7</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2</td>
<td>27.8 ±0.8</td>
<td>3.1 ±0.2</td>
<td>41.1 ±4.3</td>
</tr>
<tr>
<td>Early October</td>
<td>F+Y</td>
<td>4</td>
<td>29.5 ±0.7</td>
<td>3.3 ±0.2</td>
<td>46.6 ±2.5</td>
</tr>
<tr>
<td>Late October</td>
<td>F+Y</td>
<td>3</td>
<td>33.0 ±1.8</td>
<td>3.5 ±0.1</td>
<td>55.5 ±5.1</td>
</tr>
<tr>
<td>November</td>
<td>F</td>
<td>4</td>
<td>28.9 ±1.0</td>
<td>4.4&lt;sup&gt;de&lt;/sup&gt; ±0.1</td>
<td>61.7&lt;sup&gt;d&lt;/sup&gt; ±0.9</td>
</tr>
<tr>
<td>December</td>
<td>F</td>
<td>4</td>
<td>30.3 ±0.8</td>
<td>3.5&lt;sup&gt;e&lt;/sup&gt; ±0.2</td>
<td>50.7&lt;sup&gt;e&lt;/sup&gt; ±2.0</td>
</tr>
</tbody>
</table>

Note: 
- <sup>a</sup> M=Male, F=Female, F+Y=Female + Young
- <sup>b</sup> All values are mean ± SEM.
- <sup>c</sup> Significantly different from females in same month (p<0.05).
- <sup>d</sup> Significantly different from same sex in July (p<0.05).
- <sup>e</sup> Significantly different from same sex in previous month (p<0.05).
The seasonal pattern of DMR at 5°C was similar to that at ambient temperature and at 25°C (Table 5.6 & Figure 5.3), although the values were significantly higher (76.4-115.0 kJ.day⁻¹). There was, however, no significant difference in DMR between late October (98.9 kJ.day⁻¹) and late November (95.6 kJ.day⁻¹) (Table 5.6 & Figure 5.3). There was no significant difference in MR of males or females between July and August (Table 5.6). Female MR increased significantly from 4.8 ml O₂.g⁻¹.hr⁻¹ in August to 7.2 ml O₂.g⁻¹.hr⁻¹ in late November (Table 5.6 & Figure 5.4). Tables 5.4 - 5.6 indicate, that at all times during the year, DMR accounted for a higher percentage of food energy intake at 5°C than at ambient temperature or at 25°C, (69-98% compared to 52-77%).

Daily variations in metabolic rate of *A. stuartii*, considered to be representative of each group are shown in Figures 5.5- 5.10. No consistent differences in light:dark metabolic pattern at ambient temperature, 25°C or 5°C were observed in male or female *A. stuartii* during July or August (Figures 5.5 - 5.7). Similarly, no consistent differences were observed in females with or without pouch young from July to December (Figure 5.8-5.10). However, the MR of lactating females during November was slightly lower during the light period (Figures 5.8-5.10). MR at 5°C fluctuated less than MR at ambient temperature or at 25°C. An exception to this was an individual male in late July (Figure 5.11) whose MR decreased in a step-wise pattern from approximately 7ml O₂.g⁻¹.h⁻¹ to 1.6ml O₂.g⁻¹.h⁻¹. It increased to 7.4ml O₂.g⁻¹.h⁻¹, decreased again to 1.5ml O₂.g⁻¹.h⁻¹ and then increased again to approximately 6ml
Table 5.6. Metabolism and food energy intake at 5°C of *A. stuartii* freshly-caught at various times of the year.

<table>
<thead>
<tr>
<th>Time of Year</th>
<th>Status</th>
<th>N° of Animals</th>
<th>Body Mass (g)</th>
<th>Metabolic Rate (ml O₂ g⁻¹ hr⁻¹, kJ day⁻¹)</th>
<th>Food Energy Intake (kJ day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July</td>
<td>M</td>
<td>5</td>
<td>37.1 ±0.4</td>
<td>90.4 ±5.3</td>
<td>130.9 ±3.1</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>3</td>
<td>28.6 ±3.1</td>
<td>76.4 ±5.1</td>
<td>94.3 ±7.1</td>
</tr>
<tr>
<td>August</td>
<td>M</td>
<td>9</td>
<td>45.5 ±2.3</td>
<td>115.0 ±6.4</td>
<td>159.7 ±9.0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>3</td>
<td>26.0 ±1.3</td>
<td>59.8 ±4.9</td>
<td>78.7 ±22.3</td>
</tr>
<tr>
<td>Early October</td>
<td>F+Y</td>
<td>3</td>
<td>29.6 ±1.0</td>
<td>83.9 ±3.0</td>
<td>85.6 ±5.7</td>
</tr>
<tr>
<td>Late October</td>
<td>F+Y</td>
<td>4</td>
<td>32.6 ±1.4</td>
<td>98.9 ±4.5</td>
<td>105.8 ±8.7</td>
</tr>
<tr>
<td>November</td>
<td>F</td>
<td>3</td>
<td>27.7 ±1.5</td>
<td>95.6 ±3.5</td>
<td>114.4 ±1.5</td>
</tr>
<tr>
<td>December</td>
<td>F</td>
<td>4</td>
<td>29.9 ±0.3</td>
<td>86.8 ±3.1</td>
<td>123.4 ±1.4</td>
</tr>
</tbody>
</table>

Note:  
- a M=Male, F=Female, F+Y=Female + Young  
- b All values are mean ± SEM.  
- c Significantly different from females in same month (p<0.05).  
- d Significantly different from same sex in July (p<0.05).  
- e Significantly different from same sex in previous month (p<0.05).
Figure 5.5. Daily variations in metabolic rate at ambient temperature cycle of representative male and female A. stuartii individuals fresh-caught during July and August. The photoperiod is indicated by the light and dark bars above the time scale.
Figure 5.6. Daily variations in metabolic rate at 25°C of representative male and female *A. stuartii* individuals fresh-caught during July and August. The photoperiod is indicated by the light and dark bars above the time scale.
Figure 5.7. Daily variations in metabolic rate at 5°C of representative male and female *A. stuartii* individuals fresh-caught during July and August. The photoperiod is indicated by the light and dark bars above the time scale.
Figure 5.8. Daily variations in metabolic rate at ambient temperature cycle of representative female and female plus pouch young A. stuartii fresh-caught during October, November and December. The photoperiod is indicated by the light and dark bars above the time scale.
Figure 5.9. Daily variations in metabolic rate at 25°C of representative female and female plus pouch young A. stuartii fresh-caught during October, November and December. The photoperiod is indicated by the light and dark bars above the time scale.
Figure 5.10. Daily variations in metabolic rate at 5°C of representative female and female plus pouch young A. stuartii fresh-caught during October, November and December. The photoperiod is indicated by the light and dark bars above the time scale.
Figure 5.11. Daily variation in metabolic rate at 5°C of a male A. stuartii fresh-caught during July. The photoperiod is indicated by the light and dark bars above the time scale.
0₂·g⁻¹·h⁻¹, where it remained until the end of the experiment, 10 hours later. These periods of reduced oxygen consumption may indicate bouts of torpor. However, this was an exception. In general no animal had a MR below 2 ml O₂·g⁻¹·h⁻¹. The SMR for this species is 1 ml O₂·g⁻¹·h⁻¹ (Dawson & Hulbert, 1970). The fact that no troughs fell below this level I take to indicate that there was no torpor for any significant period.

5.6. Discussion: Captive vrs. free-ranging

The increased body mass associated with maturation in *A. stuartii* (Table 5.1) is well documented (Wood 1970; Woollard, 1971; Barnett, 1973). The increase in body mass during captivity may be the result of an abundant food supply and decreased energy expenditure involved in obtaining food in captivity. Conversely, the low body mass of free-ranging animals compared to that of those in captivity may be associated with a reduced food supply and increased energy expenditure involved in obtaining food in the natural environment, especially during the colder months of the year. An increased demand for food is also likely as the size of the free-ranging animals increases during the year. Lee (personal communication) found that many recaptured *A. stuartii* and *A. swainsonii* showed small weight losses upon recapture and suggested that these animals may have spent more time than others in the traps without food.

Increased body mass (Table 5.1) and the lower environmental temperatures of autumn and winter are factors expected to result in increased food consumption of captive
A. stuartii (Table 5.1). It has been suggested that torpor in A. stuartii is an emergency response to low environmental temperature and food deprivation, rather than a daily event (Geiser, 1985). Thus, the incidence of torpor in A. stuartii is expected to be less in captivity, when food is supplied ad libitum than in free-ranging animals.

Observations on the timing of the disappearance of males from the population, size of pouch young of females caught early in October, combined with data on size at birth and length of gestation (Selwood, 1980) indicate that mating of A. stuartii in the Wollongong area occurs during early September. The dramatic increase in body mass observed in males prior to mating and the gradual and smaller increase in females after mating (Tables 5.1 & 5.4) confirm the findings of Woolley (1966). However, (Woolley, 1966) also points out that the body mass of female plus young declines for a few days after the birth of the young.

Figures 5.5-5.10 suggest that frequent torpor is not a characteristic of the life history of A. stuartii, as it is in arid-dwelling small marsupials such as Antechinomys laniger and Sminthopsis macroura (Geiser, 1985), and that there is no rigid diurnal MR pattern in A. stuartii. However, when a diurnal pattern did occur, there was a slight tendency for MR to be reduced during the day rather than at night. Wallis (1976) has also reported that A. stuartii showed bursts of activity during daylight hours as well as at night. The results of this study appear to conflict with those of Hall (1980 b), whose study, based largely on trapping, found that free-ranging A. stuartii were nocturnal. However, increased arthropod activity at night
and the scansorial behaviour of *A. stuartii* (Statham, 1982) suggested that the nocturnal activity exhibited in free-ranging *A. stuartii* may be a prey-dependent phenomenon.

The increase in DMR of males from July to August is modulated by a concurrent increase in body mass. This is evident in the lack of a significant difference in MR between July and August when measured at ambient temperature, 25°C or 5°C (Tables 5.4-5.6). The DMR of females is also modulated by body mass, as the seasonal pattern of MR is similar at 25°C and 5°C. MR at ambient temperature is significantly higher in July and lower in December than in August. Ambient temperatures during December measurements (25.6-29.5°C) were higher than (13.8-18.1°C) during August (Table 5.4). This would tend to reduce MR of females in December, compared to those of August. Although the average maximum and minimum temperatures were higher during measurements in July than in August (Table 5.4), the amount of time during which low temperatures were experienced was greater in July than in August. Unusually long periods of cold weather were experienced in Wollongong during July 1986. The first recorded snowfall in the wollongong area occurred during some of the July measurements. The smaller surface area to volume of males would reduce heat loss and perhaps any positive metabolic response to lower ambient temperatures compared to that of females. This may have contributed to the lack of a significant difference in male MR between July and August under similar conditions.

Although, October values of MR were derived from females plus litters, it is interesting that at ambient temperature and 25°C, these values are generally similar to
those from individuals at other times of the year. However, MR at 5°C of females with young is significantly higher than that of pre-reproductive females during July and August (Table 5.6). The lack of insulation and higher surface area to body volume ratio of the neonates would increase thermogenic requirements if high body temperatures were to be maintained, especially at ambient temperatures as low as 5°C. However, it is doubtful whether the pouch young would be able to thermoregulate effectively under these conditions. Their presence may have increased the thermoregulatory cost of the adult females.

MR measured at ambient temperature, 25°C and 5°C is significantly higher during November than during August, although there is no significant difference in body mass (Tables 5.4-5.6). It is possible that capture at this time of the year results in greater stress and in turn, greater energy expenditure than at other times of the year. An alternative explanation is that during November, a lactating female is feeding a litter with a total body mass which is relatively large compared to its own body mass. It is therefore possible that the greater MR of females during November is related to the increased demands of lactation. Lactation in small eutherians requires a substantial increase in energy (Fleming et al., 1981) and data available for some marsupial species indicates that the cost of lactation is also significantly higher than in non-reproductive females. Woolley (1982) has reported that small dasyurid species require more food when lactating, especially towards the end of the suckling period. Females with pouch young captured during this study generally possessed full litters. Little data is available concerning the mortality rate of *A. stuartii* young when they
are deposited in a nest. Wood (1970) suggested that the variations in mortality of pouch young she observed in a long term study were the result of variations in weather conditions. However, Dickman (1982) observed juvenile *A. stuartii* foraging in groups of up to 6 individuals during mid-December and communal nesting of up to 6 individuals (including the mother) until mid-March. These findings suggest that the energetic cost during late lactation in *A. stuartii* is probably high. The cumulative resting metabolic rate during lactation, of post-absorptive *D. virginiana* at thermoneutrality was 92% higher than that of non-reproductive, summer-acclimatized females (Fleming et al., 1981) The field metabolic rate of *A. swainsonii* during lactation, measured using the techniques of Nagy et al., (1978) was significantly higher than that of pre-mating females (Lee, personal communication). Determination of metabolic rate of *A. stuartii* during November and December, should indicate whether the increased metabolic rate observed in lactating *A. stuartii* in captivity in this study is a stress-induced artifact or whether it also occurs in the field.

A seasonal pattern in thyroid radioiodine release rate is evident in *A. stuartii* held in captivity (Figure 5.2), with maximum values recorded during June and August similar to those of the cold acclimated *A. stuartii*. Minimum thyroid radiodine release rate values were recorded during February in both juveniles and post-reproductive females. Some of the mean values recorded during the year were similar to or slightly less than those recorded for warm acclimated *A. stuartii* (see chapter 4). However, they were all higher than those recorded
for warm or cold acclimated *M. musculus*.

No seasonal pattern is evident in thyroidal radioiodine release rate of free-ranging *A. stuartii*. Thus, in *A. stuartii*, a species capable of torpor throughout the year, except perhaps during the post-mating period (Geiser, 1985), there is no seasonal “shut-down” of the thyroid gland as found in eutherian hibernators by Hulbert & Hudson, (1976) & Hudson & Deavers (1976).

There is considerable variation in thyroid iodine content during the year (Table 5.2 & Figure 5.2). The seasonal thyroid iodine content values overlap those of the warm and cold acclimated *A. stuartii*. Unfortunately, little data is available concerning the size and variability of the iodine pool of the thyroid. Brown-Grant et al., (1954 b) reported a decline in thyroid radioiodine release rate in rabbits over a 2-3 month period after arrival in their laboratory, although it is unclear whether these were wild-caught animals. They attributed this decline to an increase in thyroid iodine pool associated with increased dietary iodine intake. Variations in the abundance of particular arthropod species may vary the iodine content of the diet of *A. stuartii* and in turn, their thyroidal iodine content. Barnett (1973) reported that *A. stuartii* rarely consumed food on the first night after capture, but ate voraciously on succeeding nights. Fresh-caught *A. stuarti* in this study varied considerably in the amount of food consumed in the first 24 hours of capture. However, it is not known how much of this variability is a feature of an individual feeding pattern, and how much is due to the stress of capture and handling. In a study of the energy requirements of *A. swainsonii*, Cowan et al.,
(1974) found that individuals exhibited different feeding regimes, which were modified from day to day. As the time since last meal could influence thyroid iodine content, similar variations in the feeding regimes of *A. stuartii* could have contributed to the variability observed. The time in the trap, exposure of the trap to wind and moisture and other factors concerned with the demand for thyroid hormones such as ambient temperature regime would also be expected to contribute to variability of thyroid iodine pool. The small sample size and considerable variation in the time of cessation of lactation (Wood, 1970) may have contributed to the variation in thyroid iodine content shown in mature females in February (Table 5.3.)

As the thyoidal iodine pool of *A. stuartii* is relatively small compared to that of *M. musculus* (Tables 4.2 & 5.3), small changes in one or several of the above factors may have a significant influence on the thyoidal iodine content of *A. stuartii*.

Factors which could lead to increased thyroid activity of captive *A. stuartii* during the year are:

(i) increased body mass resulting in increased tissue demand for thyroid hormones;
(ii) decreased environmental temperature;
(iii) cold acclimatization of *A. stuartii* whilst free-ranging and in captivity;
(iv) variations in photoperiod;
(v) a combination of several of the above.

It is difficult to establish the differences in environmental conditions between captive and free-ranging
animals. Both groups were subject to similar photoperiod regimes, but it is likely that food availability would be decreased and energetic demands greater in free-ranging than in captive animals. As nest sharing in *A. stuartii* may persist until the male die off (Scotts, 1983), huddling may reduce the energetic demands during autumn and winter. Huddling, in small groups of sugar gliders has been reported to reduce their lower critical temperature ($T_{lc}$) from $27^\circ C$ to $16^\circ C$ and facilitate a reduction in weight specific metabolism (Fleming 1980). Morton (1978) suggested that nest sharing was the main adaptation to seasonal depression of temperature and food availability and that torpor was used only when foraging conditions were particularly poor.

Torpor has been shown to result in reduced thyroid gland activity (Rhodes, 1980). The depth, duration and incidence of torpor in *A. stuartii* increases during autumn and winter (Geiser, 1985). It is possible that the differences in thyroid radiiodine release rates and thyroid secretion rates between captive and free-ranging *A. stuartii* which are evident during April measurements result from torpor in the free-ranging animals. As the tendency for *A. stuartii* to enter torpor is greater when body mass is small (Geiser, 1985), torpor in some free-ranging *A. stuartii* may also have contributed to the greater variability in thyroid iodine content during the seasonal study. The decrease in environmental temperatures experienced during the period from February to August may have modulated thyroid secretion rate in the captive *A. stuartii*. Reduced food intake whilst free-ranging may have reduced the demand for thyroid hormones in free-ranging *A. stuartii*. The pattern of
increased thyroid activity of *A. stuartii* in captivity during the colder months of the year is similar to that found in *Didelphis virginianus* (Bauman & Turner 1966) and *M. eugenii* (Ralph 1972; Kaethner & Good 1975). However, the lack of a seasonal variation of thyroid activity in the free-ranging *A. stuartii* indicates the differences in laboratory and field experiments.

As this is the first time that thyroidal iodine secretion rate has been determined for free-ranging animals, there can be no comparison with values from other species. The only other study of thyroid function in free-ranging animals was concerned with the measurement of radioiodine release rate in rodents at different altitudes (Tryon *et al.*, 1968). They found no significant difference in radioiodine release rate between males or females, although, they did find that radioiodine release rate was significantly greater at 2,200m than at 2,600 or 2,900m. Their pooled thyroid radioiodine release results for two free-ranging eutherian species, *Thomomys talpoides* and *CiteLLus armatus* range from 11.4 to 16.9%.day⁻¹ which are similar to the values mentioned earlier for *M. musculus* and *R. norvegicus*. 
Chapter 6 General Discussion

Cold acclimation of *A. stuartii* has been previously studied by Reynolds & Hulbert (1982). These authors demonstrated that the metabolic rate at 5°C of restrained *A. stuartii* and *M. musculus* was significantly higher in cold acclimated than warm acclimated animals. However, although an increase in noradrenaline-mediated non-shivering thermogenesis was demonstrated in cold acclimated *M. musculus*, there was no thermogenic response to noradrenaline in the *A. stuartii*. The lack of physiologically significant deposits of BAT in marsupials in general (Rowlatt *et al.*, 1971) and the lack of thermogenic response to noradrenaline in *A. stuartii* (Reynolds & Hulbert, 1982) indicates that noradrenaline-mediated non-shivering thermogenesis is unlikely to contribute significantly to acclimation to cold in this species. However, the apparent absence of brown adipose tissue and noradrenaline-mediated non-shivering thermogenesis in marsupials is at present controversial.

As mentioned previously (see chapter one), a relatively small thermogenic response was reported in mature *A. stuartii* (Wallis, 1977) and *P. tridactylus* (Nicol, 1978) after injection of relatively large amounts of noradrenaline. However, Reynolds & Hulbert (1982) demonstrated the absence of a thermogenic response to noradrenaline in mature *A. stuartii*, even after cold acclimation. Gotts (1975), reported
non-shivering thermogenesis in *A. swainsonii* in males captured during winter and in cold acclimated juveniles. Loudon *et al.*, (1985) reported a 48% rise in metabolic rate of young *M. rufogriseus rufogriseus* after an injection of noradrenaline. Loudon *et al.*, (1985) also reported the presence of brown adipose tissue in *M. rufogriseus rufogriseus*. However, the brown adipose tissue and the thermogenic response to noradrenaline in *M. rufogriseus rufogriseus* were not detected until the young were developed enough to leave the pouch (Loudon *et al.*, 1985). The age of pouch exit (90 days in *M. rufogriseus rufogriseus*) has been suggested as corresponding to the developmental stage of eutherian birth (Rothwell & Stock, 1985) and thus the time when marsupial young require an effective thermoregulatory capacity. It may be that classical noradrenaline-mediated non-shivering thermogenesis is present in some marsupials at the time of pouch exit. As body size would be relatively small at the time of pouch exit, when they are no longer dependent on the thermogenic capacity of the mother, the potential for heat loss and thus the need for heat production would be great. However, the absence of significant deposits of brown adipose tissue in many marsupials (Rowlatt *et al.*, 1971) and the small thermogenic response or lack of response to noradrenaline in the studies mentioned above indicate that classical noradrenaline-mediated non-shivering thermogenesis in marsupials may be confined to a restricted period during development, perhaps only in some species. As pointed out earlier (see chapter one), the absence of sham-injected controls in the experiments involving injection of noradrenaline and the absence of data points on the graphs of
Gotts (1975) reduces the impact of these studies. However, the magnitude of the difference in thyroid secretion rate between cold and warm acclimated *A. stuartii* (Table 4.2) suggests that the calorigenic action of thyroid hormones may have comprised a significant proportion of the thermogenesis detected by Reynolds and Hulbert (1982). The similarity in thyroid iodine secretion rate between warm acclimated *A. stuartii* and cold acclimated *M. musculus*, the latter capable of considerable NST, and the higher thermogenesis at 30°C in the cold acclimated than in the warm acclimated *A. stuartii* (Reynolds & Hulbert, 1982), is circumstantial evidence supporting this idea. The significance of shivering thermogenesis in this particular situation is at present, unresolved.

A marsupial-eutherian comparison of thyroid function using radioiodine release rate has been carried out previously by Hulbert and Augee (1982). These authors maintained the bandicoots *Isoodon macrourus* and *Perameles nasuta* and the similarly sized rabbit *Oryctolagus cuniculus* under conditions of 12 hour light : 12 hour dark photoperiod and ad libitum food and water for 4 weeks at 22°C. They concluded that the thyroid was equally active in the bandicoot and rabbit under these conditions because it was responsible for the same proportion of oxygen consumption in each species. They showed that the bandicoots had higher radioiodine release rates than the rabbit and suggested that they had lower thyroidal iodine contents. Setchell (1974), after monitoring the disappearance of 125I-labelled thyroxine from plasma, had also reported no significant difference in thyroid secretion rate between a
variety of eutherian and a variety of marsupial species. The finding in the present study that thyroid secretion rates *A. stuartii* were not significantly different from those of *M. musculus* after warm acclimation, supports the suggestion by Setchell (1974) and Hulbert & Augee (1982), that in general, the thyroid activity of marsupials is not significantly different from that of eutherians. The present results are thus in agreement with the finding by Setchell, (1974) and Hulbert & Augee (1982) that the lower BMR of marsupials compared to eutherians is not the result of a relatively inactive thyroid.

However, it is apparent from Table 4.2 that the similarity in thyroid secretion rate between warm acclimated *A. stuartii* and both groups of *M. musculus* was achieved with a higher thyroid radioiodine release rate in the marsupial than in the eutherian. This higher thyroid radioiodine release rate appears to be a consequence of a smaller thyroid iodine content in the *A. stuartii*. The thyroid iodine content of *A. stuartii* in both the cold acclimation and the seasonal study was significantly smaller than that of *M. musculus* in the cold acclimation study (Tables 4.2 & 5.3). Table 6.1 compares data available for the thyroid radioiodine release rate of some eutherian, marsupial and monotreme species. It is interesting that marsupial radioiodine release rates are generally higher than those of eutherian or monotreme species. Notable exceptions are the rabbit (*O. cuniculus*) which has been suggested as having a relatively low thyroid iodine content (Hulbert & Augee, 1982) and the rabbit-eared bandicoot (*M. lagotis*), whose relatively low metabolic rate (Hulbert & Dawson, 1974) would be expected to enhance its survival in its
Table 6.1. A comparison of thyroid radioiodine release rates in eutherians, marsupials and monotremes.

<table>
<thead>
<tr>
<th>Species</th>
<th>Thyroid radiodine release rate (% day⁻¹)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eutherians</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mus musculus</em></td>
<td>9.8±0.8 (5)</td>
<td>Hulbert &amp; Else 1981</td>
</tr>
<tr>
<td><em>Mus musculus</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.9±0.8 (7)</td>
<td>This study</td>
</tr>
<tr>
<td><em>Mus musculus</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.2±0.5 (8)</td>
<td></td>
</tr>
<tr>
<td><em>Rattus norvegicus</em></td>
<td>10.1±0.7 (6)</td>
<td>Withers &amp; Hulbert 1987</td>
</tr>
<tr>
<td><em>Dasyproctus cuniculus</em></td>
<td>17.6 (30)</td>
<td>Brown-Grant <em>et al.</em> 1954</td>
</tr>
<tr>
<td><em>Thomomys talpoides</em></td>
<td>11.4-16.9 (27)</td>
<td>Tryon <em>et al.</em> 1968</td>
</tr>
<tr>
<td><em>Citellus armatus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Peromyscus leucopus</em></td>
<td>17.9±2.4 (6)</td>
<td>Rhodes 1980</td>
</tr>
<tr>
<td><em>Peromyscus leucopus</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.0±0.7 (5)</td>
<td></td>
</tr>
<tr>
<td><strong>Marsupials</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Anthechinus stuartii</em>&lt;sup&gt;w&lt;/sup&gt;</td>
<td>51.3±7.2 (6)</td>
<td>This study</td>
</tr>
<tr>
<td><em>Anthechinus stuartii</em>&lt;sup&gt;f&lt;/sup&gt;</td>
<td>33.4±2.9 (7)</td>
<td></td>
</tr>
<tr>
<td><em>Isoodon macrourus</em></td>
<td>33.2±4.9 (5)</td>
<td>Hulbert &amp; Augee 1982</td>
</tr>
<tr>
<td><em>Marmota leagota</em></td>
<td>11.6 (1)</td>
<td></td>
</tr>
<tr>
<td><em>Perameles nemius</em></td>
<td>18.9±1.5 (4)</td>
<td></td>
</tr>
<tr>
<td><strong>Monotremes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tachyglossus aculeatus</em></td>
<td>9.9±3.8 (4)</td>
<td></td>
</tr>
<tr>
<td><strong>Hibernators</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Spermophilus tridecimlineatus</em></td>
<td>2.3</td>
<td>Hulbert &amp; Hudson 1976</td>
</tr>
<tr>
<td><em>Spermophilus tridecimlineatus</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SEM (sample size)
<sup>b</sup> Cold acclimated
<sup>c</sup> Warm acclimated
<sup>w</sup> Warm acclimated
<sup>t</sup> Torpid
<sup>h</sup> During hibernation period of year
desert environment. Similarly, Hudson and Wang (1979) have suggested that the relatively inactive thyroid glands and low endogenous heat production of desert ground squirrels would be adaptive for animals exposed to heat loads. Further data from other eutherian and marsupial species may indicate whether marsupials in general have a relatively smaller thyroidal iodine content than eutherians. As the thyroid secretion rate of marsupials appears to be similar to that of eutherians, except after cold acclimation, the evolutionary significance of such a difference in thyroid iodine content between marsupials and eutherians is unclear.

In some eutherians there is no thermogenic response to noradrenaline (Chaffee and Roberts, 1971) and cold acclimation has little effect on BAT mass, although these species may stop shivering in the cold and apparently develop some form of NST. Thus, hypertrophy of BAT is apparently not required in all mammals (Chaffee & Roberts, 1971), not even in all eutherians. The results of the present study suggest that a comparison of thyroid secretion rates in cold acclimated eutherians that utilize NST and those that do not may reveal whether the latter rely upon higher thyroid secretion rates for increased thermogenesis. Similarly, a study of thyroid activity, BAT and the thermogenic response to noradrenaline during development in a wide variety of marsupials may indicate whether the lack of BAT and the capacity for effective NST and the use of a sustained increase in thyroid activity in cold acclimation are general marsupial characteristics.

It is interesting that MR measured at 5°C was higher in the cold and warm acclimated A. stuartii (Table 4.3) than in
those captured and measured during the winter months of July and August (Table 5.6). This suggests that free-ranging A. stuartii may be able to reduce heat loss to a greater extent than those maintained in the laboratory, even cold acclimated animals. Three mechanisms which could be associated with such a strategy are a reduction in thermal conductance and increase in fur length and/or thickness, the use of torpor and more efficient nest building. No difference in the pattern of nest-building was observed between cold and warm acclimated A. stuartii. Neither was a pattern of torpor evident in these groups. Winter-captured A. stuartii have significantly lower conductance and thicker fur than summer-captured A. stuartii (Wallis, 1977), but the lack of a significant difference in both of these parameters was reported between warm and cold acclimated A. stuartii when restrained (Reynolds & Hulbert, 1982). These results suggest that the greater ability of the fresh-caught A. stuartii to reduce heat loss is a significant factor in reducing their metabolic cost at 5°C compared to laboratory-held A. stuartii. However, Smith and Dawson (1984) have also suggested that restraint may have influenced the conductance measurements of A. stuartii by Reynolds and Hulbert (1982). Metabolic comparisons would have to be carried out under identical conditions for this issue to be resolved.

Tables 4.3 & 5.4-5.6 indicate that food energy intake values are higher than corresponding DMR values. However, as protein is not completely oxidised in the body (Schmidt-Nielsen, 1975), the combustion value for high protein protein foods such as the diet used in the present study will be an overestimate of the energy available to the animal. Table 6.3 summarizes some
Table 6.3. Daily food energy intake of several *Antechinus* species in captivity.

<table>
<thead>
<tr>
<th>Species</th>
<th>Time of year</th>
<th>Body mass (g)</th>
<th>N* animals</th>
<th>Temp. (°C)</th>
<th>Sex</th>
<th>Daily food energy intake kJ.day⁻¹</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. stuartii</em></td>
<td>July</td>
<td>35.2ᵃ</td>
<td>7</td>
<td>15.0-19.6</td>
<td>M</td>
<td>107</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.7</td>
<td>5</td>
<td>15.5-20.5</td>
<td>F</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>22.6</td>
<td>10</td>
<td>8</td>
<td>M+F</td>
<td>89</td>
<td>Nagy et al., 1976</td>
</tr>
<tr>
<td><em>A. flavipes</em></td>
<td></td>
<td>36</td>
<td>5</td>
<td>18-20</td>
<td>M</td>
<td>146</td>
<td>Woollard, 1971</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>5</td>
<td></td>
<td>F</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td><em>A. swainsonii</em></td>
<td>Feb</td>
<td>73</td>
<td>5</td>
<td>25</td>
<td>M</td>
<td>183</td>
<td>Inns, 1976</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>2</td>
<td></td>
<td>F</td>
<td>86</td>
<td>Cowan, 1974</td>
</tr>
</tbody>
</table>

ᵃ Mean
of the available data concerning food energy intake of three *Antechinus* species. The weight-specific daily food energy intake values are given, because of the differences in body mass between species and sexes. *A. stuartii* values from males and females in this study, approximately 3.0 kJ.g$^{-1}$.day$^{-1}$. were lower than 3.9 kJ.g$^{-1}$.day$^{-1}$ (Nagy et al., 1978) and 4.1-4.3 kJ.g$^{-1}$.day$^{-1}$ (Woollard, 1971). Food consumption studies of other species of *Antechinus* have recorded lower daily food energy intake values. Inns (1976), found that daily food energy intake was 2.3kJ.g$^{-1}$.day$^{-1}$ in *A. flavipes*, whilst Cowan (1974) reports a value of 2.3 kJ.g$^{-1}$.day$^{-1}$ for *A. swainsonii*. Both of these studies were at temperatures higher than those in the present study. Thus, the values for *A. stuartii* in this study, whilst lower than those of other studies are higher than those for *A. swainsonii* and *A. flavipes*.

Nagy et al. (1978) reported that 83% of ingested energy was metabolized by *A. stuartii* kept without water for a week at 8°C, but supplied with enough *Tenebrio* larvae to maintain constant body mass. Values of daily metabolic rate expressed as a percentage of food energy intake at 5°C in both the cold acclimation and seasonal studies are similar to the value of 83% reported by Nagy et al., (1978). Daily metabolic rate accounted for a higher percentage of food energy intake at 5°C than at ambient temperatures or at 25°C, in both the cold acclimation and seasonal studies (Tables 4.3 & 5.4-5.6). Although it was not directly measured, these results suggest that an increase in digestive efficiency may have occurred at 5°C in this study. Digestive efficiency seems to have increased from 61-68% in *A. stuartii* kept at relatively neutral temperatures of 25°C to
approximately 75-90% in *A. stuartii* kept at 5°C. An increase in digestive efficiency in the cold has also been reported in birds (Alaskan red-polls) during winter (Chaffee & Roberts 1971).

Lee (personal communication) suggested that a dramatic relaxation in competition for food would result from the male die-off in *A. swainsonii*. It was also suggested that in *A. swainsonii*, even when males are no longer competing for resources, the December food supply must be more than twice that of July or August, if juveniles were to persist (Lee, personal communication). Table 6.2 summarizes some of the data available for *A. stuartii*. It is evident that the daily metabolic rate of free-ranging and captive males has increased to considerably higher levels than that of females prior to mating. Food energy intake of captive males is also higher than that of females prior to mating. This suggests that male *A. stuartii*, if they were still alive would consume about the same amount of food as a lactating female during late October or November.

From both the cold acclimation and seasonal studies, it is evident that torpor is not an integral component of the energetic strategy of *A. stuartii* when food is available. These findings are similar to those of other studies. Geiser (1985), found that these forest-dwelling marsupials, which live in a highly predictable environment exhibit a lower incidence of torpor than arid-dwelling species. Wallis (1976), suggested that torpor in *A. stuartii* was an emergency measure under conditions of reduced food availability rather than a daily event. However, when food is readily available, *A. stuartii*, including warm acclimated animals are capable of maintaining for at least 24 hours, an average metabolic rate at 5°C that is seven times
Table 6.2  Summary of Captive and Free-ranging Metabolic rates of *A. stuartii*

<table>
<thead>
<tr>
<th>Status</th>
<th>Month</th>
<th>Body Mass (g)</th>
<th>No. of Animals</th>
<th>Daily Metabolic Rate (kJ.day⁻¹)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std. Met. Rate</td>
<td></td>
<td>36.5</td>
<td>6</td>
<td>17.6</td>
<td>Dawson &amp; Hulbert 1970</td>
</tr>
<tr>
<td>Premating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M C July</td>
<td>35 ± 2⁸</td>
<td>7</td>
<td>63 ± 4</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>F C July</td>
<td>26 ± 1</td>
<td>5</td>
<td>53 ± 4</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>M C Aug.</td>
<td>45 ± 3</td>
<td>9</td>
<td>80 ± 4</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>F C Aug.</td>
<td>26 ± 2</td>
<td>3</td>
<td>42 ± 3</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>M FR July</td>
<td>29</td>
<td>6</td>
<td>73 ± 7</td>
<td>Nagy et al., 1978</td>
<td></td>
</tr>
<tr>
<td>F FR July</td>
<td>18</td>
<td>8</td>
<td>49 ± 6</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>Mating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M FR Aug.</td>
<td>29</td>
<td>9</td>
<td>84 ± 5</td>
<td>Lee (personal communication)</td>
<td></td>
</tr>
<tr>
<td>F FR Aug</td>
<td>21</td>
<td>8</td>
<td>67 ± 9</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>Post-mating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F + Y C Early Oct.</td>
<td>30 ± 1</td>
<td>4</td>
<td>47 ± 2</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>F + Y C Late Oct.</td>
<td>33 ± 2</td>
<td>3</td>
<td>55 ± 5</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>F C Nov.</td>
<td>29 ± 1</td>
<td>4</td>
<td>59 ± 2</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>F C Dec.</td>
<td>32 ± 1</td>
<td>4</td>
<td>44 ± 2</td>
<td>&quot;</td>
<td></td>
</tr>
</tbody>
</table>

⁸ Mean ± SEM
M Male
F Female
Y Pouch young
C Captive
FR Free-Ranging
their standard metabolic rate. Thus, these small marsupials are very effective thermoregulators.
Summary

The thyroid gland activity of cold acclimated *A. stuartii* is significantly higher than that of warm acclimated *A. stuartii*. The thyroid gland activity of cold and warm acclimated *M. musculus* is not significantly different from that of warm acclimated *A. stuartii*. It is suggested that the increased thyroid gland activity reported to follow initial exposure to cold, is reduced to "normal" levels as brown adipose tissue and thus the capacity for noradrenaline-mediated non shivering thermogenesis develops in *M. musculus*. It is further suggested that thyroid activity in cold acclimated *A. stuartii* remains at an elevated level because they lack the capacity for noradrenaline-mediated non shivering thermogenesis.

The thyroid gland activity of free-ranging *A. stuartii* did not exhibit a seasonal pattern. However, a seasonal pattern was evident in *A. stuartii*, fresh-caught and maintained in captivity for a week. Thyroid activity in these captive *A. stuartii* increases during autumn and winter and decreases in reproductive females during spring and summer.

It is suggested that thyroid gland activity in *A. stuartii* is modulated by low ambient temperatures experienced during autumn and winter. The lower thyroid activity observed in some free-ranging *A. stuartii* may be associated with reduced food consumption and increased incidence of torpor in the natural environment.

The metabolic energy expenditure of male *A. stuartii* was 63 kJ.day⁻¹ during July, but increased to 80 kJ.day⁻¹ in
August due to increased body mass. Food energy intake increased from 107 to 140 kJ.day\(^{-1}\) during this period. The metabolic energy expenditure of females decreased from 53 to 42 kJ.day\(^{-1}\) during the same period, although food energy intake and body mass remained constant. In reproductive females, energy expenditure increases while the litter is attached. However, the maximum energy expenditure recorded was 59 kJ.day\(^{-1}\) after the litter had detached. Food energy intake was also high at this time, 88 kJ.day\(^{-1}\). Metabolic rate also tended to be higher during late lactation (in November) than in pre-reproductive females (in August), whether measurements were carried out at the ambient temperature cycle, 25°C or 5°C. This is interpreted as an increase in the energetic cost of lactation as well as that of thermoregulation.

Cold acclimation resulted in no significant difference in 5°C metabolic rate in unrestrained animals compared to a reported difference in restrained animals.
REFERENCES


Consumption of the Hamster (Mesocricetus auratus) at 25.5°C and 4.5°C. Gen. Comp. Endocrinology 10, 92-98.


and Thyroid Function in the Cold-Adapted Rat. Endocrinology 85, 79-86.


Heldmaier, G. (1975). The Effect of Short Daily Cold


Endocrinology 64, 462-464.


Martin, C.J. (1903). Thermal Adjustment and Respiratory Exchange in Monotremes and Marsupials - a


Riesco, G., Taurog, A. & Larsen, P.R. (1976). Variations in
the Response of the Thyroid Gland of the Rat to Different Low-iodine Diets: Correlation with Iodine Content of Diet. Endocrinology 99, No. 1, 270-280.


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