Chapter 6:
Development of Thermoregulation in *Petaurus breviceps*

6.1 Introduction:

Thermoregulation is a complex process involving the integration of a number of physiological systems, including the muscular, nervous and endocrine. Further, insulation and body size also play an important role in retaining any heat produced. In many small mammals, these systems are not fully developed at birth and, consequently, the young are ectothermic until the thermoregulatory processes begin to function. This is especially true for marsupial species, as the young are born in an extremely altricial state and have an extended period of development in the mother's pouch. Consequently, the time from conception to weaning in these species and rate of development is much slower than that of placental species (Lee & Cockburn 1985).

The development of thermoregulation can transpire within a few days, weeks or even months after birth, but is usually established by the time of weaning (Reynolds 1952; Morrison & Petajan 1962; Shield 1966; Hudson 1974; Maxwell & Morton 1975; Dolman 1980; Geiser *et al.* 1986; Geiser & Kenagy 1990). This process generally occurs in conjunction with an increase in body size and pelage (Morrison & Petajan 1962), and the activation of the thyroid gland (Setchell 1974; Hulbert 1988).

The young of *P. breviceps* are born between June and January (Fleay 1947; Smith 1979; Suckling 1984) after 16 days gestation and weigh approximately 0.19 g (Smith 1971). Like other marsupials, they are hairless with only the forelimbs and olfactory organs showing reasonable development. By ages 60-68 days, the young are unable to be fully contained within the pouch. However, at this stage they are still predominantly unfurred (Smith

1979). Further, some researchers have observed finding slightly furred, relatively

undeveloped young alone in nest boxes during the day (F. Geiser, pers. comm.).

Consequently, the possibility exists that the young of this species are exposed to a

significant thermal stress before the full development of endothermy. However, as yet, no

study has examined when these animals are able to maintain a stable T_b. Therefore, the

primary aim of this project was to investigate the development of thermoregulation in the

sugar glider.

6.2 Materials and Methods:

To determine the development of thermoregulation in *P. breviceps*, RMR, T_b, conductance

and body mass were measured in 10 juvenile sugar gliders which had been born in captivity.

Pouches of all mature females were checked weekly for the presence of young. However,

due to the small size of the neonates (10.8 mm crown-rump, 194 mg; Smith 1971; 1979),

combined with the increased aggression of females prior to and after birth, young were

sometimes not detected for a few weeks. Therefore, ages had to be determined using head

length and the regression equation provided by Smith (1979), as this parameter has been

found to be the most reliable correlate for estimating age in sugar gliders to an age of

100 days.

Gliders are permanently attached to the teat from birth until 40-65 days of age (Smith

1979). Therefore, to ensure that the young gliders could reattach to the teat, gliders were

not removed from their mothers until some fur, or at least pigment, was present on the

shoulders (age 51-65 days) and they were observed partly out of the pouch (age

60-68 days; Smith 1979). Measurements of body mass, metabolic rate and T_b were then

taken at approximately weekly intervals until the gliders could at least maintain a stable T_b

over a T_a range of 30-15 °C, after which measurements were taken at irregular intervals

until the gliders had obtained an adult mass. Unfortunately, as 4 of the gliders were not

born until near the completion of the study, full data sets are only available for 6 individuals.

Very young gliders were transferred from the aviary to the laboratory with their mothers and removed from the teat directly prior to being placed in the respirometry chambers at T_a 30 °C. However, once the young was found completely out of the pouch it was transferred alone to the laboratory on the morning of the measurement. A T_a of 30 °C was chosen as it is within the TNZ of adult gliders (see Chapter 3) and would probably cause little thermal stress to the young, although pouch temperatures are probably slightly higher. The gliders then remained at this T_a for approximately one hour before the T_a was reduced in 5 °C increments of approximately 1 h each. This procedure was subsequently repeated until either T_a 15 °C was reached, or metabolic rate or T_b decreased significantly, indicating that the animals were becoming hypothermic. Initially, respirometry chambers of 0.5 L, with flow rates around 10-13 L h^{-1}, were used, and 99 % equilibrium of the system was achieved after 10-13 min. However, as the gliders increased in size, they were subsequently placed in larger chambers (1-3 L) with concomitant increases in flow rate.

Two systems were used to determine the RMR of the juvenile gliders. One system (Ametek Applied Electrochemistry S-3A/I single channel oxygen analyser) was the same as that outlined in Chapter 2, while the other was a two channel Ametek Applied Electrochemistry S-3A/II oxygen analyser. This system permitted continuous measurements of two animals simultaneously, with readings taken every 3 minutes. A reference reading was taken every 30 minutes. Otherwise, the setup of the two systems was almost identical. Since very young gliders had a tendency to become hypothermic quickly, this second system was primarily used for the early measurements as it provided more frequent readings (one reading every 3 minutes compared to every 6, 9 or 12 minutes, depending on the number of animals used, in the original system) which allowed a safer monitoring of an animal's condition.

With the exception of one animal which was implanted with a small temperature-sensitive transmitter (mass 2.7 g) when it was 100 days old (mass 57 g), T_b were determined by inserting a 38 s.w.g. copper/constantan thermocouple probe rectally for 15-20 mm and reading from an Omega (Stamford) HH-71T electronic thermometer. The thermocouple had been calibrated over a temperature range 10-40 °C in a waterbath (Lauda RMT-6), to the nearest 0.1 °C, against a precision mercury thermometer (R6578, Dobros, Australia). Initially, T_b were measured immediately after removal of the animal from the chamber, once stable metabolic measurements had been obtained at each T_a (after approximately 1 h). Animals were returned to the chamber within 1 minute. However, once a glider's eyes opened and it became more aware of its surroundings, the animal took longer to settle after each disturbance. Consequently, the possibility existed that metabolic rates would be higher than normal due to the animal's agitation. Therefore, T_b were only determined at the beginning and end of each measurement once T_b were generally stable over the 30-15 °C T_a range. Conductance was calculated, using metabolic rate and T_b ($C=MR/(T_b-T_a)$, as mentioned in Chapter 2.

Energetic costs of lactation were analysed by comparing metabolic rates of lactating individuals, with either one or two young, to those of the same individuals when they were in a non-reproductive state. With one exception (Pb2 at T_a 14 °C) when the young was close to weaning, measurements were all made when the mothers were in the early-mid stages of lactation (up to 10 weeks after birth; lactation completed after approximately 17 weeks; Fleay 1947) and pouch young were present during these measurements. To be able to make valid comparisons between lactating and non-lactating females, adults needed to be in the metabolic chambers overnight without food or water, as these were the conditions for non-reproductive individuals. This combined with the need to avoid any deleterious effects to the young and the problem associated with separating the oxygen consumption of the young from that of the mother, meant that only the one measurement was made on an individual during the later stages of lactation. All comparisons were made over a similar T_a range and during the same months. However, it should be noted that as

only 2 lactating females were used for these measurements, with a total of 4 offspring from 3 pregnancies, these results must be viewed with caution.

6.3 Results:

6.3.1 Body mass:

Body mass of the juvenile gliders increased with time and, given a birth mass of 0.2 g (Smith 1971), displayed a typical sigmoidal growth curve (Fig. 6.1). At birth, gliders were small and hairless with relatively large heads and well developed forelimbs. Apart from increasing in size, they remained in this state for at least the first 15-20 days (Plate 6.1). Initial growth was slow compared to the later stages of development, as by the age of 60 days gliders had only increased their mass by approximately 13 g, again assuming the same mass at birth as that of Smith (1971). At this stage the eyes were still closed and the gliders only had a covering of very short, fine fur on the dorsal surface, with bare underside, patagium, legs and tail. Co-ordination did not appear to be well developed as the animals had trouble righting themselves, although they could move around quite freely. While they were more often partially exposed while attached to their mother, they could still be fully enclosed in the pouch at this age.

From ages 60 to 110 days, juvenile gliders substantially increased their mass, growing at a rate of approximately 1 g day^{-1}, after which the rate of increase began to taper off (Fig. 6.1). From ages 70 days onward, young gliders could no longer be fully enclosed within the pouch, but would firmly hold on to their mother's fur while suckling (Plate 6.2). The physical characteristics of the gliders rapidly developed over this time: eyes opened, fur grew to cover the entire body, and co-ordination improved, resulting in animals that resembled young adults (Plate 6.3)
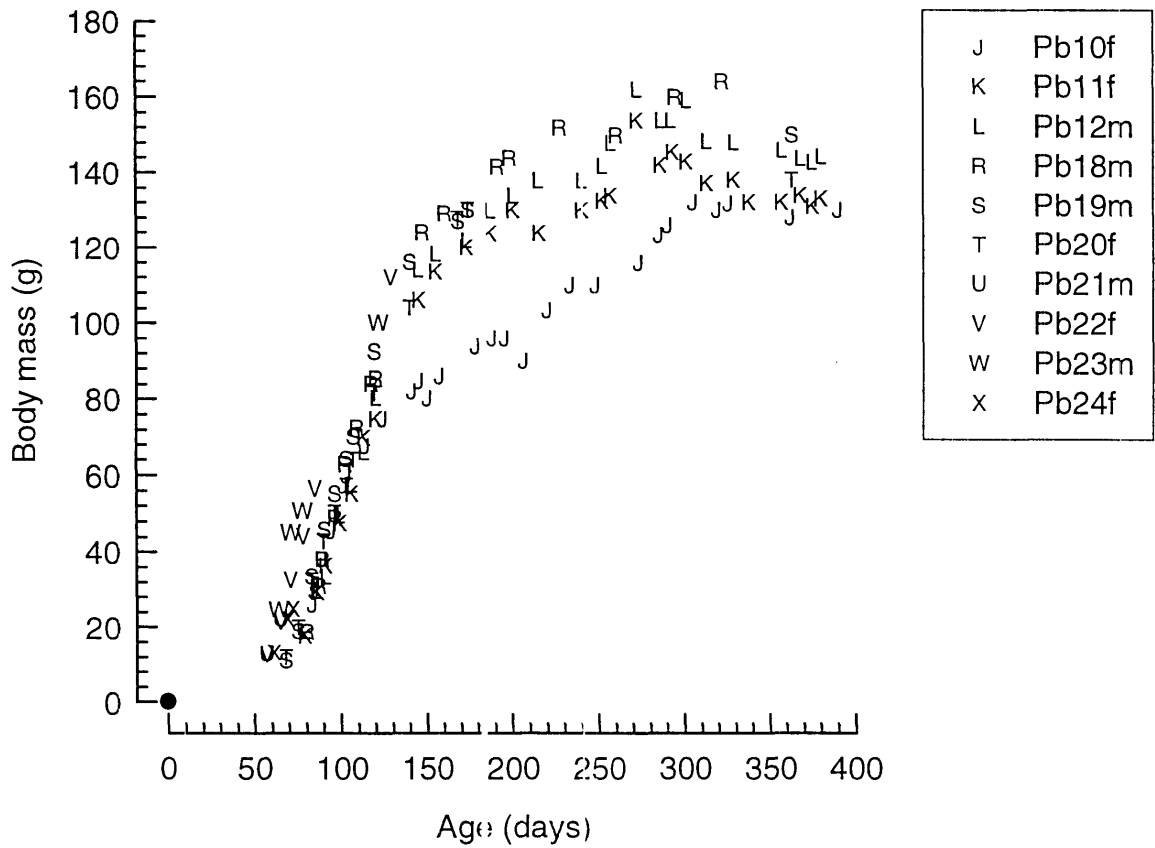
Fig. 6.1. Growth of *P. breviceps* born in captivity, measured as body mass (g). 'm' and 'f' in legend denote male or female gliders, respectively. The closed circle represents the birth mass of gliders (0.2 g) as determined by Smith (1971).

Plate 6.1. Two juvenile *P.breviceps*, still attached to teats and fully contained within the mother's pouch, aged approximately 40 days.

Plate 6.2. Juvenile *P. breviceps*, suckling on mother and aged approximately 90 days.

Plate 6.3. Juvenile *P. breviceps*, independent of its mother, aged approximately 125 days.
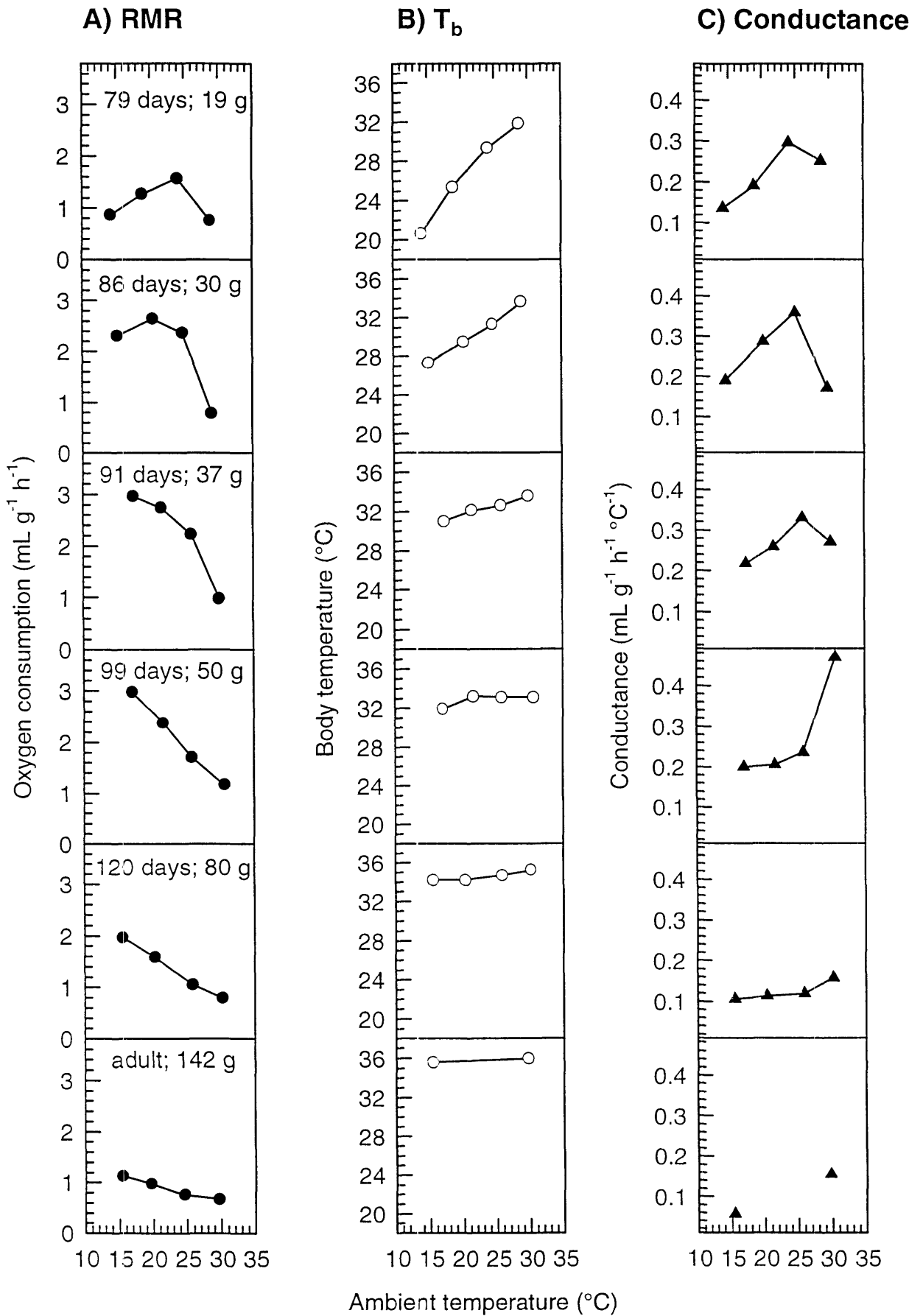
The rate of increase in mass of one glider (Pb10) was notably lower than that of the other gliders from age 120 days onward (Fig. 6.1). This was the only juvenile glider to have a transmitter implanted. However, the operation was performed approximately 7 weeks prior to the point where rate of growth started to decline and growth rate was similar to all other gliders up until this time. Further, the transmitter was removed when this glider was 195 days old and mass increase did not alter from that when the transmitter was still present. Another factor that might have a bearing on this decline in mass increase is that the animal's mother died at approximately the same time and it is possible that the lack of certain nutrients found in her milk slowed the rate of growth in this glider. However, the glider did eventually reach adult weight, and gave birth to a single young in her second breeding season.

Of the 7 litters born, 4 were of single young (Pb10, Pb18, Pb23 & Pb24), and there was an equal proportion of 5 males and 5 females. Each of the 3 litters of 2 young consisted of a male and a female. Apart from the one female glider mentioned above, males and females displayed no difference in their rates of increase in mass, at least until age 150 days (F=1.20 DF 1, 63 p=0.278, ANCOVA). Nor was there any difference in the mean mass of males and females at any given age below 150 days (F=1.72 DF 1, 64 p=0.195, ANCOVA). Therefore, the observed differentiation in the masses of mature males and females (see Chapter 3; Fig. 3.2) is probably due to the earlier cessation of growth by females once they approach adult size. No difference was observed in the rate of increase in mass between those gliders that were born in litters of one or two young.

6.3.2 Metabolism:

All sugar gliders followed the same basic pattern of development in their metabolic capability. As shown for Pb12, the gliders were unable to increase RMR as T_a decreased from 30 °C to 15 °C until the age of approximately 90 days (Fig. 6.2a). In the early measurements of gliders under 85 days old, RMR increased up to 50 % with the first 5 °C

Fig. 6.2. (On following page) Development of **A**) resting metabolic rate (RMR), **B**) body temperature (T_b), and **C**) conductance in a young glider (Pb12), born 16 August 1995. The glider was initially placed in the chamber in the morning at ambient temperature (T_a) 30 °C for approximately an hour, after which T_a was reduced in 5 °C increments, remaining at each step for an hour. To avoid elevating RMR, T_b, and calculated conductance of the adult glider was only obtained before and after the measurement. As conductance of the adult glider is a curvilinear response, no line was drawn between the two points.

A) RMR

B) T_b

C) Conductance

79 days; 19 g

86 days; 30 g

91 days; 37 g

99 days; 50 g

120 days; 80 g

adult; 142 g

Oxygen consumption (mL g^{-1} h^{-1})

Body temperature (°C)

Conductance (mL g^{-1} h^{-1} °C^{-1})

Ambient temperature (°C)

decrease in T_a (30-25°C), but subsequently declined at T_a below 25 °C (Figs. 6.2a, 6.3). The RMR of one glider aged 68 days declined after the initial decrease in T_a from 30 °C to 25 °C. However, its twin and younger gliders aged 57-64 days, which were of an equal and lower mass, respectively, did not show this response, but rather increased RMR with the initial drop in T_a. At this age (<65 days old) the young gliders appeared to shake their whole body rather than shiver, and generally just lay in the chamber without making any attempt to curl into a ball and thereby reduce surface area. However, with each successive measurement the ability to shiver improved, the gliders began to assume a 'ball' posture as T_a decreased, and metabolic heat production improved, as demonstrated by the increasingly lower T_a at which maximum RMR occurred.

At ages 85-95 days, when the gliders weighed around 45-50 g and short fur covered the entire body, RMR of the animals were at their maximum level and had increased to over twice those observed in gliders below 80 days of age (Figs. 6.2a, 6.3). Thereafter, as the glider grew and the amount of fur thickened, the slopes of the regressions between RMR and T_a became shallower until, at adult mass, regression equations were similar to those found in mature gliders at the same time of year.

This abovementioned pattern of an increase in RMR to very high values at 90-100 days followed by a steady decrease with age, and consequently size, was observed in all individual gliders at all T_a below 30 °C (Fig. 6.3). The responses at each T_a differed due to the values of the peak RMR, which were higher at T_a 20 °C and 15 °C than at T_a 25 °C. Further, due to the gradual acquisition with age of thermoregulatory ability at progressively lower T_a, these peaks in RMR occurred in slightly older gliders at T_a 15 °C compared to T_a 25 °C. At T_a 30 °C, which is within the TNZ for adult gliders, there was no observable peak in RMR, although values were slightly higher at ages 90-99 days (Fig. 6.3).
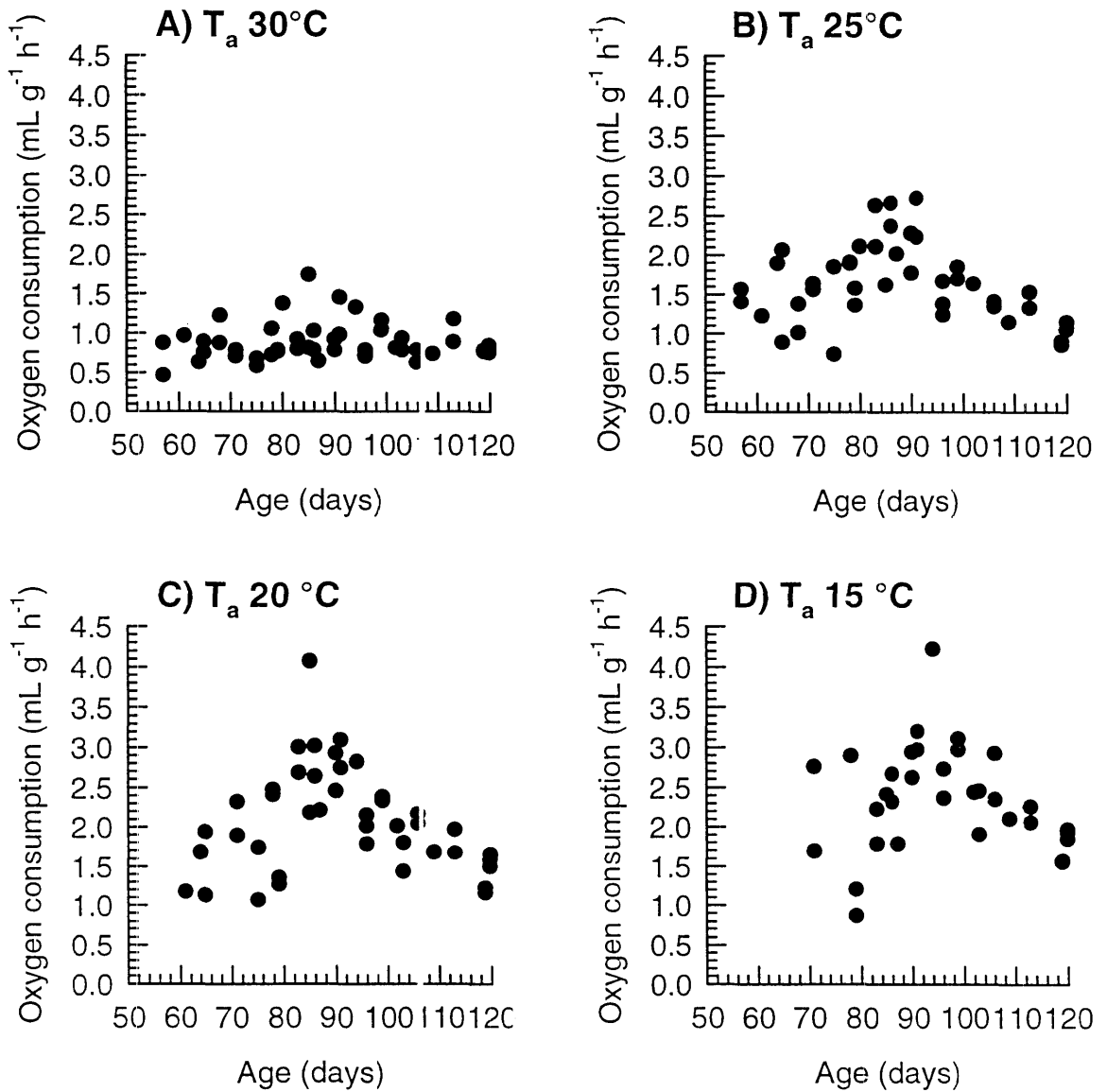
Fig. 6.3. Changes with age in resting metabolic rates (RMR) of juvenile *P. breviceps* at ambient temperatures (T$_a$) **A)** 30 °C, **B)** 25 °C, **C)** 20 °C, and **D)** 15 °C.

6.3.3 Body temperature:

As was observed with RMR, the ability to sustain a normothermic T_b gradually improved with age (Fig. 6.2b). At the time when most of the young glider's body could still be enclosed within the pouch, mean T_b on removal from the mother was 33.8±1.0 °C (ages <85 days; cf. adult normothermic, resting T_b 35.3±0.7 °C). However, as the gliders grew and more of the body became exposed (ages 85-90 days), this initial T_b decreased to 32.6± 1.1 °C, but subsequently increased to adult levels by age 110-120 days.

When gliders below the age of 95-100 days were placed alone in the chambers, T_b steadily decreased as T_a was lowered from 30-15 °C, though T_b was always at least 2 °C above T_a (Fig. 6.2b). The decline of T_b with T_a was particularly evident in gliders below the age of 85 days, despite the fact that metabolic heat production showed an initial increase. However, co-ordination at these ages, although only reasonably developed to begin with, did not appear to be affected, even at T_b as low as 20 °C. The rate of decrease in T_b slowed from 56-100 days, thus increasing the differential between T_b and T_a (Fig. 6.4), and at approximately 90-100 days of age T_b fell by only 1 °C over the 4 hour measuring period. Mean T_b at this stage was 33.1±0.6 °C, approximately 2 °C lower than that of adult gliders. Gliders that were older than 95-100 days showed a relatively constant T_b over the measuring period at all T_a. However, the differential between T_b and T_a continued to increase with further development because T_b increased from 33 °C to the adult T_b of over 35 °C (Fig. 6.4).

6.3.4 Conductance:

Conductance in gliders up to the age of 90 days increased when T_a was lowered from 30 to 25 °C, concomitant with the higher metabolic rates, increasing T_b and improved shivering ability observed up to this age (Fig. 6.2c). Due to their very small size and lack of significant fur covering, the rate of heat loss below the TNZ was at its maximum
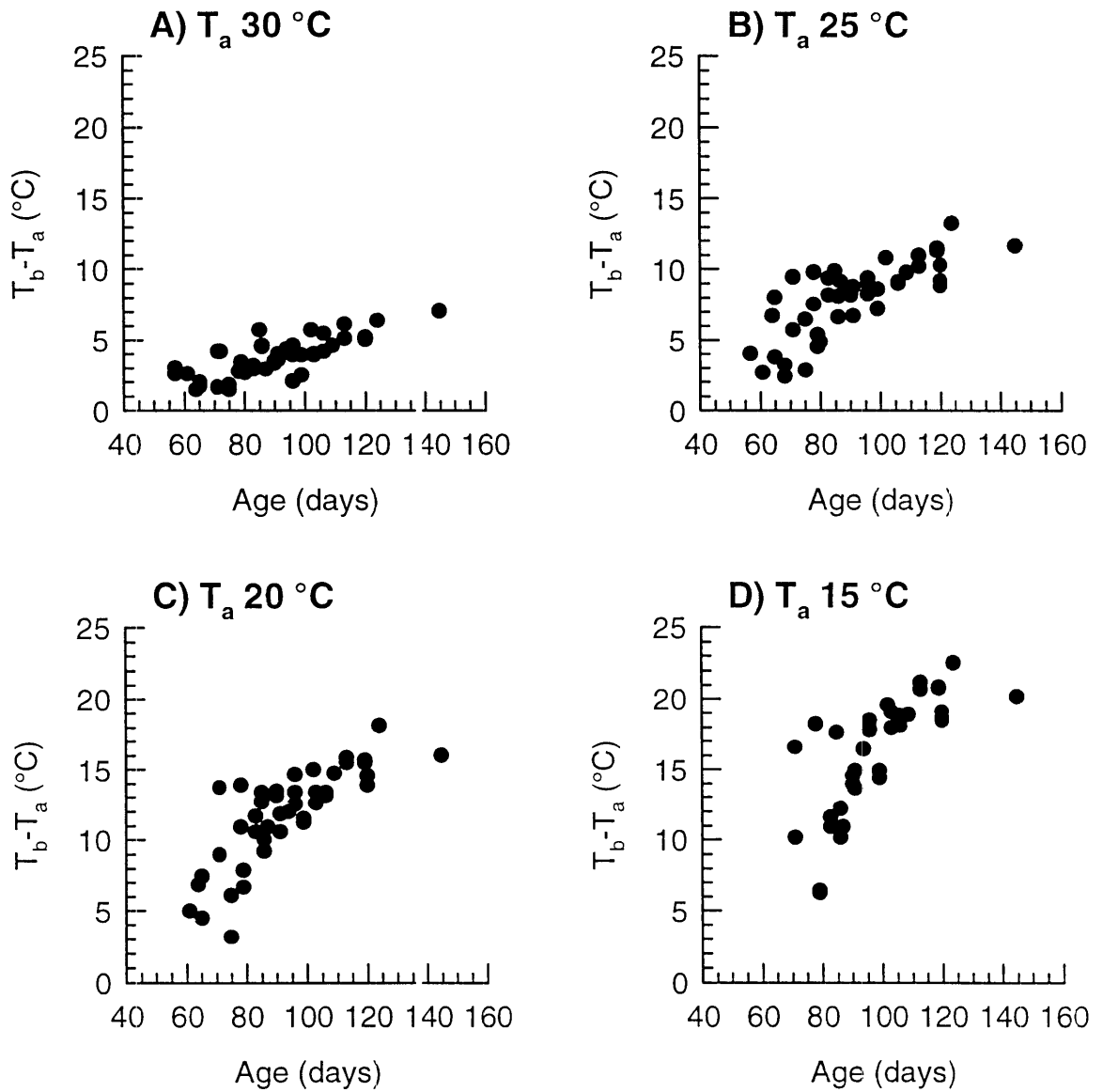
Fig. 6.4. Changes with age in the difference between body temperature (T_b) and ambient temperature (T_a) (ΔT) of juvenile *P. breviceps* at T_a **A)** 30 °C, **B)** 25 °C, **C)** 20 °C, and **D)** 15 °C.

(0.40-0.50 mL g^{-1} h^{-1} °C^{-1}) during this period (Fig. 6.5). Once T_a was lowered below 25 °C, conductance steadily declined. It was at these ages when T_b decreased markedly, and RMR also either declined or the rate of increase slowed as T_a dropped.

In gliders at approximately 90 days of age, when short fur covered the entire body, conductance showed a similar response to T_a as adult gliders over the T_a range 30-15 °C, but their overall rate of heat loss was much higher because of their smaller size and poor insulation (Figs. 6.2c, 6.5). It was at this age when RMR had reached its maximum level and increased linearly with decreasing T_a, and T_b were nearly stable over the T_a range tested. As the gliders increased in size and their fur covering grew longer and thicker, conductance values gradually declined to those levels found in adult animals (Fig. 6.5).

6.3.5 Lactation:

Metabolic rates of two females (Pb2 & Pb7), together with either one or two young ranging in ages from 5 days to 10 weeks, were measured at T_a 13.6-25.0 °C (Fig. 6.6). In addition, one of these mothers (Pb2) was also measured without its pouch young, at T_a 14.3 °C, during the period of weaning.

Over the T_a range measured, the RMR of the two lactating females were indistinguishable in both slope (F=3.95 DF 1, 26 p=0.057, ANCOVA) and elevation (F=2.77 DF 1, 27 p=0.108, ANCOVA) from those when they were in a non-reproductive state (Fig. 6.6). This was independent of the stage of lactation at the time of measurement and whether there were one or two young present.

In addition, ADMR of these animals was measured over the T_a range 13-16 °C. As no correlations were observed between ADMR and T_a, the means were compared and, as above, no difference was observed between the ADMR of lactating (1.70±0.49 mL g^{-1} h^{-1}) and non-lactating (1.94±0.28 mL g^{-1} h^{-1}) animals (t=0.91 DF=6 p=0.40, t-test).
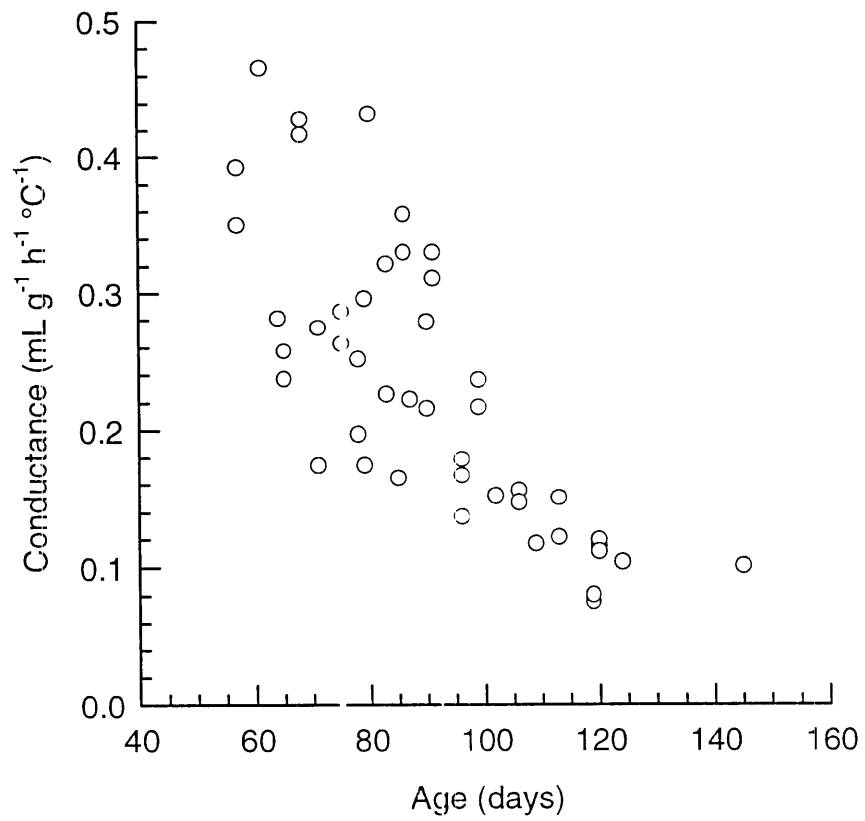
Fig. 6.5. Changes with age in the conductance of juvenile *P. breviceps* at ambient temperature (T_a) 25 °C.
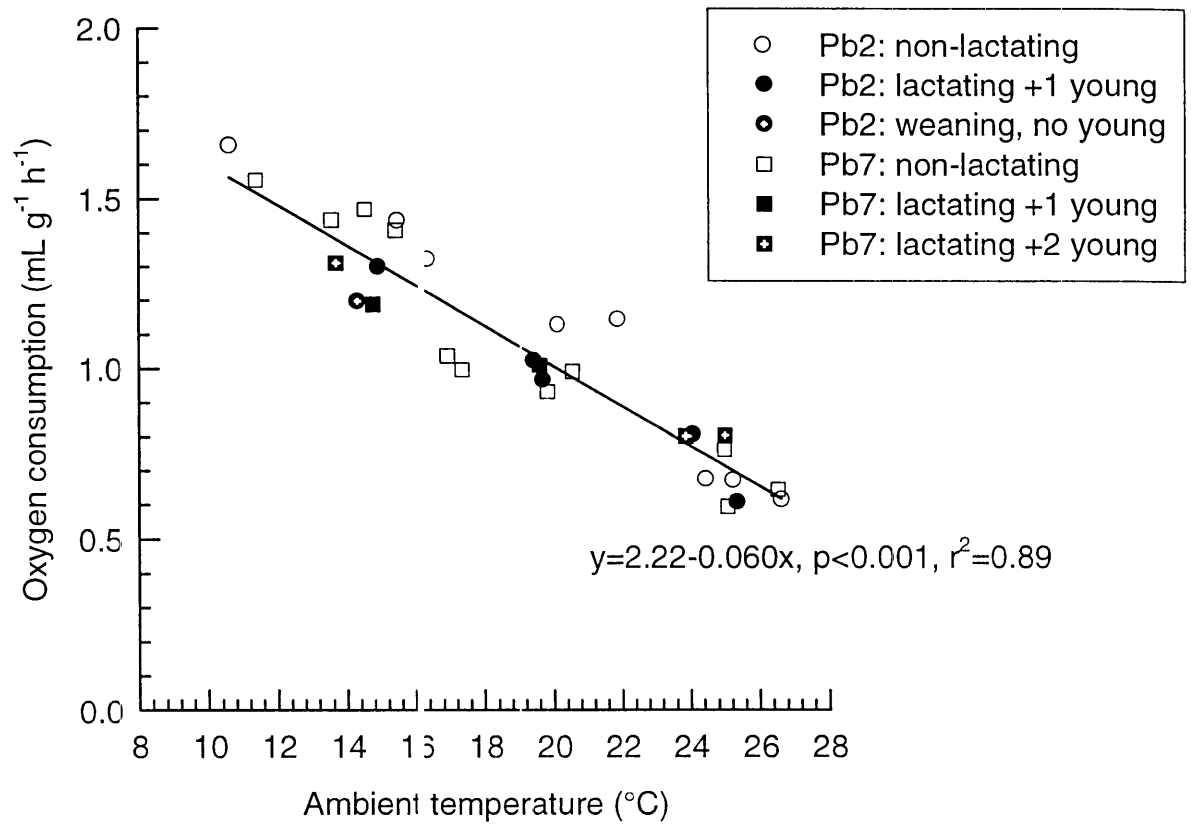
Fig. 6.6. Resting metabolic rates (RMR) cf two female *P. breviceps*, in a non-reproductive state and during lactation, as a function of ambient temperature (T_a).

6.4 Discussion:

Petaurus breviceps, like all other marsupials, gives birth to altricial young. Development is slow and it takes about 250 days from birth to grow to full size. During the early stages of pouch life they are obviously poikilothermic, and even by the age of 56 days possess only a very limited thermoregulatory ability which is restricted to T_a above 25 °C. However, by the age of 95-100 days, and when the gliders weighed only 36-42 % of adult mass, improvements in both heat production and retention resulted in the gliders being able to maintain a constant T_b over a T_a range of at least 30-15 °C, although at a slightly lower T_b than that of adults. Further growth was characterised by a steady decrease in mass-specific metabolic rate and a slight increase in T_b, presumably due to the decrease in conductance, until at an adult mass, adult levels were observed.

The growth rates and development observed in the current study appear to be similar to those of other sugar gliders born and reared in captivity (Smith 1979), suggesting that the experiments conducted here did not affect the development of the young. This is in contrast to Hudson (1974), who observed reduced growth rates in neonatal rodents, *Baiomys taylori*, periodically removed from their mothers and exposed for 2 hours to T_a 30 °C. It is likely that removal of young from their mothers has a more pronounced effect in rodents, as these species grow substantially more rapidly than marsupials which have a very slow rate of development (Tyndale-Biscoe 1979). It was noticeable in sugar gliders, however, that although litter mates developed at the same rate, eg. eye opening occurred within a day of each other, these same characteristics occurred at slightly different ages in individuals of different litters. However, the variation in development was generally within the ranges observed by Smith (1979). Therefore, some of the variability witnessed in the development of the thermoregulatory processes in this species may be due to natural variation.

It has been reported from observations of gliders in captivity that young at age 70 days are frequently left in the nest while the mother leaves to forage during the night (Smith 1971).

However, at this age the young gliders have only a rudimentary thermoregulatory ability

which is restricted to high T_a, whereas outside T_a may drop to as low as -6.0 °C during this

period (Bureau of Meteorology 1997). Consequently, it is possible that, in order to keep

the young gliders warm during this period, the foraging time of the mother is restricted to

short bouts, as has been observed with the dasyurid, *Phascogale tapoatafa*, which returns

to the nest more frequently and for longer periods during the early nestling stage, when the

young are without fur, compared to the later nestling stage (Soderquist 1993). Short

foraging forays might also explain the observations by Quin (1993) of the activity patterns

of female sugar gliders during the reproductive season. It was noticeable that some females

during the reproductive season commenced activity later, retired earlier and displayed

shorter bouts of activity throughout the night compared to gliders at other times of the year

(Quin 1993). However, it was not stated whether any of these females had young.

Thermal stress on the young gliders while the adult is away is partially reduced due to the

insulation of the nest slowing the decline in nest temperature. It is also possible that other

family members may remain behind with the young gliders to prevent them becoming

hypothermic. In fact, this was observed on one occasion during the present study when the

mother was found in one nest box with most of the other adult gliders, presumably after

being interrupted during a feeding bout, and her two young were found in another nest box

with their grandmother. Further, if young gliders called out when separated from their

mother, older siblings would come out to investigate and attempt to take them back to the

nest. Consequently, these calls may act as a cue for these older gliders to stay with the

young while the mother is away for a period of time.

The development of endothermy in *P. breviceps*, like that in a number of other species,

both placental (Hudson 1974; Maxwell & Morton 1975; Dolman 1980; Geiser & Kenagy

1990) and marsupial (Reynolds 1952; Morrison & Petajan 1962; Shield 1966; Geiser *et al.*

1986), is a gradual process. Initially, constant high T_b can be maintained only at high T_a,

close to the TNZ, because mass-specific metabolic rates are low for their body size

(Fig. 6.7). Subsequently, as animals age and increase in size, mechanisms for heat production continue to develop, conductance decreases, and thermal tolerance improves. Once endothermy is attained at 95-100 days (approximately 50 g), metabolism at T_a 30 °C, within the TNZ for adults though not technically BMR for the juvenile gliders, decreases as the gliders grow, in a manner similar to that of the size relationship found between BMR and mass for marsupials in general (Fig. 6.7).

For marsupials, the need to thermoregulate does not become necessary until the individual can no longer be contained within the pouch, as temperatures within these confines are generally within 0.5-1.0 °C of those of the mother (Morrison & Petajan 1962; Shield 1966; Gemmell & Johnston 1985; Gemmell et al. 1987). Consequently, the T_b of a poikilothermic neonate will also be close to that of the adult, without the need to burn fuel for maintenance of T_b. In addition, given the naked state and small size of these animals, the rate of heat loss would be so high as to make the establishment of endothermy at this stage of development highly inefficient. Therefore, remaining poikilothermic during this stage means that nutrients can be channelled into growth and development rather than into heat production.

Between the ages of 60-95 days a number of factors contributed to the development of endothermy in P. breviceps, the most important being the increase in body size due to the growth of the animals. This resulted in a decrease in the surface area to volume ratio, thereby reducing the amount of metabolic heat lost from the body. Heat loss was further reduced due to the development of fur that grew to encompass the whole animal during this period and become an effective layer of insulation. It is interesting to note that the majority of fur growth did not occur until a large portion of the young glider's body protruded from the pouch, and it may be that this is a mechanism to prevent overheating while contained in the pouch.
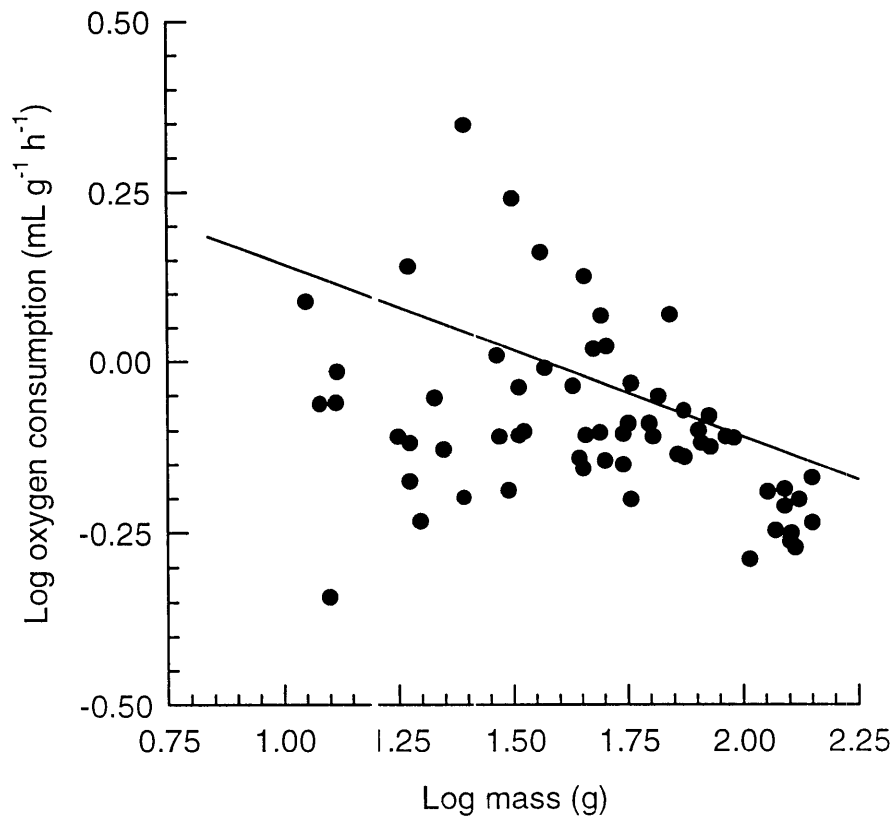
Fig. 6.7. Log resting metabolic rates (RMR) of juvenile gliders at ambient temperature (T$_a$) 30 °C as a function of log body mass. Solid line indicates the basal metabolic rates (BMR) of marsupials in general as calculated by Hayssen & Lacy (1985):

log BMR (mL g^{-1} h^{-1}) = 0.397-0.253 log body mass (g).

In addition to reducing the surface area of an animal, the larger body size also acts as a
factor in augmenting the absolute amount of heat produced, as an increase in muscle mass
will increase the animal's shivering capacity. It was noticeable that as physical co-ordination
improved, the degree of shivering at lower T_a also increased, whereas at earlier ages the
animal just shook or moved its body for a short period of time prior to lying still and letting
T_b decrease. However, it should be mentioned that the use of shivering is partially offset by
the increase in convective heat loss that results, thus reducing its overall effectiveness to
some degree.

An important change that has been observed to occur during the development of
endothermy is the activation of the thyroid gland (Setchell 1974; Hulbert 1988).
Considering that thyroid hormones are important in regulating metabolism, acting on all
body cells (Eckert & Randall 1983), the observed increase in heat production from ages
60-100 days in *P. breviceps*, was probably due to the activation of this gland during this
period.

In addition to oxidative metabolism and muscular action, a further source of heat
production in neonatal placental species is nonshivering thermogenesis via the utilisation of
brown adipose tissue (BAT) (Hull 1973). However, as yet BAT has only been found in the
young of one marsupial species, *Macropus rufogriseus* (Loudon *et al.* 1985). Even in this
case the amount of BAT was small. Further, the animals had been maintained in England at
T_a much lower than that to which they are usually exposed. Consequently, it is thought that
this may have influenced the presence and appearance of the adipose tissue (Gemmell &
Cepon 1993; Nicol *et al.* 1997) and further studies are warranted before the presence of
BAT can be unequivocally confirmed in neonatal marsupials. However, it is possible that
recently discovered uncoupling proteins that are associated with muscle rather than BAT
(Boss *et al.* 1997; Larkin *et al.* 1997) are important for nonshivering thermogenesis in
marsupials. In fact, research into the effects of vasoconstrictors in perfused muscle of
various species, including the marsupial, *Bettongia gaimardi*, would appear to indicate that

this tissue contributes to nonshivering thermogenesis in a wide range of vertebrates (Duchamp *et al.* 1993; Eldershaw *et al.* 1996; Steen *et al.* 1996).

Behavioural changes were also evident in the young gliders. They began to adopt the curled 'ball' posture observed in adults when they were about 70 days old. This posture reduces surface area, in particular the ventral surface and patagium which for a large period of time have much shorter fur than that of the dorsal surface, thereby reducing insulation when exposed. In fact, simply by adopting this posture a young opossum, *Didelphis marsupialis virginiana*, was able to maintain its T_b approximately 5 °C higher than that of an animal which remained outstretched (Morrison & Petajan 1962).

At the onset of endothermy, the T_b of the juvenile gliders was lower than that of the adult, as found in several other species (Reynolds 1952; Rosen 1975; Dolman 1980). Considering that endothermy is initially established at a markedly lower mass and when pelage is not as thick and long as that of adults, rate of conductance is much greater in these small animals. Therefore, by maintaining T_b a few degrees lower than mature individuals, juveniles can reduce the rate of heat loss by decreasing the differential between T_a and T_b. Furthermore, less nutrients are required for temperature regulation and more are able to be directed towards growth. This is particularly important for *P. breviceps* which, unlike *Baiomys taylori* whose growth rate slowed on attainment of endothermy (Hudson 1974), continues to grow at a high rate for at least another 40 days. During this period, conductance decreases and the set-point for T_b gradually increases to that of adults.

During the first ten weeks of lactation there was no apparent additional energetic cost to the mother in nurturing the developing young, although it should be pointed out that only two animals were measured during lactation in the present study. However, given the consistency of the measurements and their similarity to those for the same individuals in different years, they are deemed to be a reliable estimate of the metabolism of these gliders

during early-mid lactation. This observation is supported by the findings of Nagy &
Suckling (1985) who also observed no difference between the field metabolic rates of
non-lactating and lactating females with one and two young all estimated to be only a few
weeks old. These results are also in agreement with those of several other mammals studied
during pregnancy and lactation, in which it was found that it is not until during the latter
half of lactation that there is a significant energetic cost to the mother (Kenagy *et al.* 1989;
Munks & Green 1995; Green *et al.* 1997). However, it is possible that the metabolic rate of
a female during early-mid lactation is slightly increased, but is compensated for by the low
ectothermic metabolic rate of the young, thus resulting in an overall metabolic rate close to
that when the female is not lactating.

The pattern of energetic cost to the mother during lactation can be explained by the rates of
energy consumption of the developing young. During the early stages of lactation the rates
of milk consumption of the young are small due to the low growth rates, lack of
thermoregulation, and hence low metabolism. Therefore, the energetic costs of maintaining
the young to the mother during this period are negligible. However, once the young
animals begin to thermoregulate and leave the pouch, energy demands and, consequently,
food requirements also increase, which subsequently results in the latter half of lactation
being an energetically demanding time for the mother. Finally, during the period of weaning
the young subsists increasingly more on food other than that provided by the mother.
Therefore, less energy is required by the mother to produce milk and costs during this time
become closer to that observed during non-lactation.

Given the lack of obvious additional energetic needs during pregnancy and early lactation
of sugar gliders, it was surprising that there appeared to be an increase in the food
consumption of the mothers during this time. However, it seemed that these individuals
may have been employing a strategy to augment their fat content in preparation for the
increase in energy demands that occur during late lactation (Racey & Speakman 1987;

Munks & Green 1995), as body mass of both individuals during each lactation period initially increased, reaching a peak at approximately 10 weeks, and declined thereafter.

Petaurus breviceps commences its reproductive season during winter (June-July) (Suckling 1984; present study), often a period of high stress due to low T_a and restricted food availability. However, as there appears to be no significant additional energetic cost until after at least the tenth week of lactation, reproduction imposes no substantial energetic burden on these animals during this period. Assuming the cost of reproduction does become significant during the latter half of lactation (Kenagy *et al.* 1989; Munks & Green 1995; Green *et al.* 1997), this does not occur until spring, when the environmental conditions are such that thermoregulatory demands are reduced and food sources, in particular insects to cope with the increasing protein demands of the young, are more abundant. Further, these favourable environmental conditions prevail during the time when young are weaned and endothermy is attained, thus enhancing the survival chances of juvenile *P. breviceps*.

Chapter 7:
Effect of increased conductance due to exposure to a helox atmosphere

7.1 Introduction:

One of the most important components in the control of T_b is the ability of an animal to change its conductance. By reducing the rate of heat loss at cold T_a an animal is able to decrease the amount of energy required to maintain a normothermic T_b, whereas during high T_a an increase in conductance aids in the prevention of hyperthermia. Conductance may be altered in the short-term by changes to posture, evaporation, piloerection, peripheral circulation, and vasomotor control of the skin and underlying tissue (Scholander et al. 1950c). Further, nests and huddling may also be utilised to reduce surface area and, therefore, heat loss (Glaser & Lustick 1975). Long-term changes may be achieved through modifications to body size, pelage characteristics and fat deposition (Sealander 1972; Heldmaier & Steinlechner 1981; present study).

Conductance may also be altered artificially by exposing an animal to media that differs in conductivity from air. The conductivity of helium is approximately 6.5 times that of nitrogen (Hodgman et al. 1955). Consequently, a commonly used gas mixture for increasing conductance is helox, which comprises approximately 21 % oxygen and 79 % helium. Utilising this property of high conductivity, researchers have used this gas to elicit maximum metabolic rates of animals (Rosenmann & Morrison 1974; Rosenmann et al. 1975; Smith & Dawson 1985; Dawson et al. 1986; Dawson & Olson 1988; Hallam & Dawson 1993; Chappell et al. 1995; present study). In addition, helox has also been used to investigate the role of non-shivering thermogenesis in marsupials (May 1996), and various aspects of hibernation and torpor (Osborne & Milsom 1993; Geiser et al. 1996).

However, while helium is believed to be an inert carrier gas, there have been a number of conflicting reports of helox affecting some metabolic processes (see Brice & Welch 1983). Since BMR represents the total energy requirements for the maintenance of organ functions (Hainsworth 1981), one method of detecting if helox has any physiological effect, other than increasing conductance, would be to ascertain whether BMR is affected by a helox atmosphere. To the best of my knowledge this has not been determined previously. Consequently, *P. breviceps* were subjected to helox gas over a wide range of temperatures, including the TNZ, first to determine whether helox has any influences other than on the conductance of an animal, and secondly to look at the effect of that increased conductance on physiological variables.

7.2 Materials and Methods:

Measurements of metabolic rates, T_b and conductance in air and helox atmospheres were all performed according to the procedures outlined in Chapter 2. All animals used for these procedures had obtained an average mass of ≥ 140 g (males) and ≥ 115 g (females) and were, therefore, considered to be adults (Suckling 1995). No female was included that was known to be either pregnant or lactating.

For statistical analysis, data from air and helox measurements were first checked for seasonal differences, and were only divided into seasons if there was a significant result. Otherwise, data were pooled for the entire year. All mean values were analysed for differences between air and helox atmospheres using paired t-tests (Zar 1984).

7.3 Results:

7.3.1 Metabolism:

Metabolic rates of animals exposec to a helox atmosphere displayed a response to T_a that was qualitatively very similar to tha t of the same individuals when in air, with a distinct TNZ, in which BMR was observed, bordered on both sides by increasing RMR (Fig. 7.1).
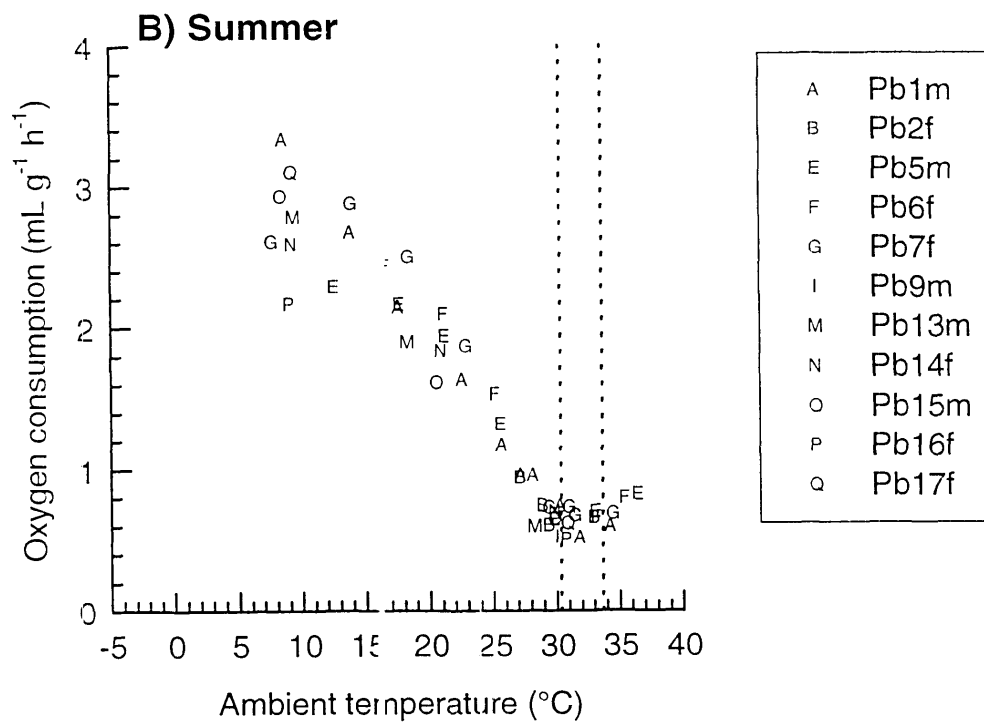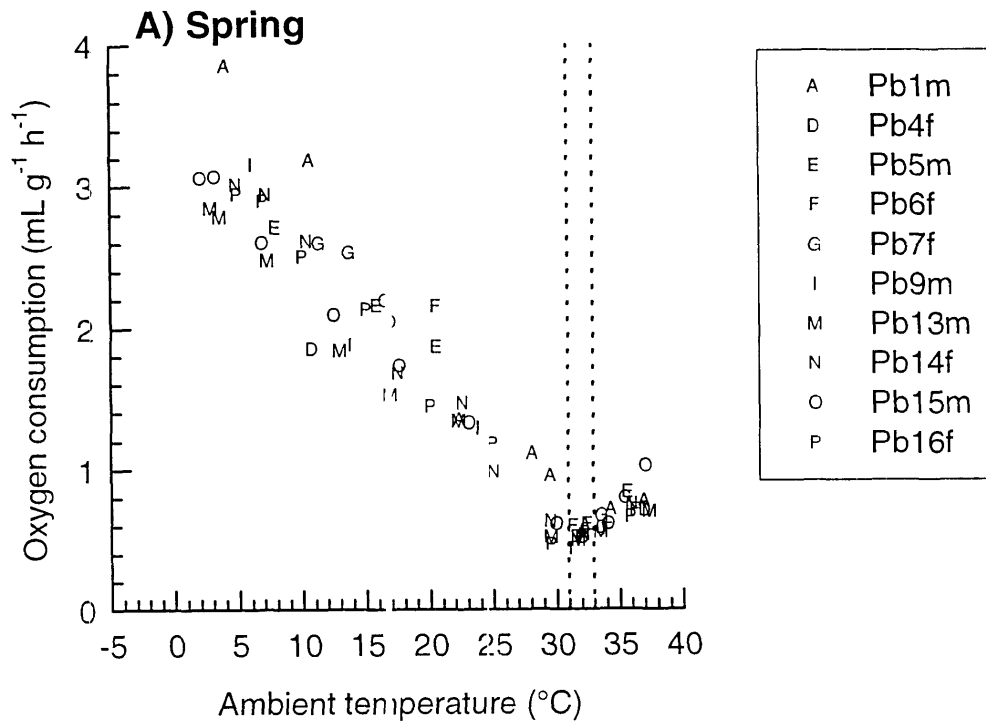
7.3.1.1 Basal metabolic rate:

Unlike the BMR measured in air, the mean BMR measured in helox did not show significant seasonal differences (Fig. 7.2; F=1.94 DF 3, 31 p=0.143, ANOVA), and the overall mean was 0.570±0.049 mL g^{-1} h^{-1} (N=12). As was found in air, the highest BMR in helox was observed in animals d iring summer (0.614±0.070 mL g^{-1} h^{-1}). However, in contrast to air, animals during the spring helox measurements had the lowest values (0.547±0.072 mL g^{-1} h^{-1}), while w nter and autumn values were intermediate and similar (0.561±0.034 mL g^{-1} h^{-1} and 0.561±0.070 mL g^{-1} h^{-1}, respectively).
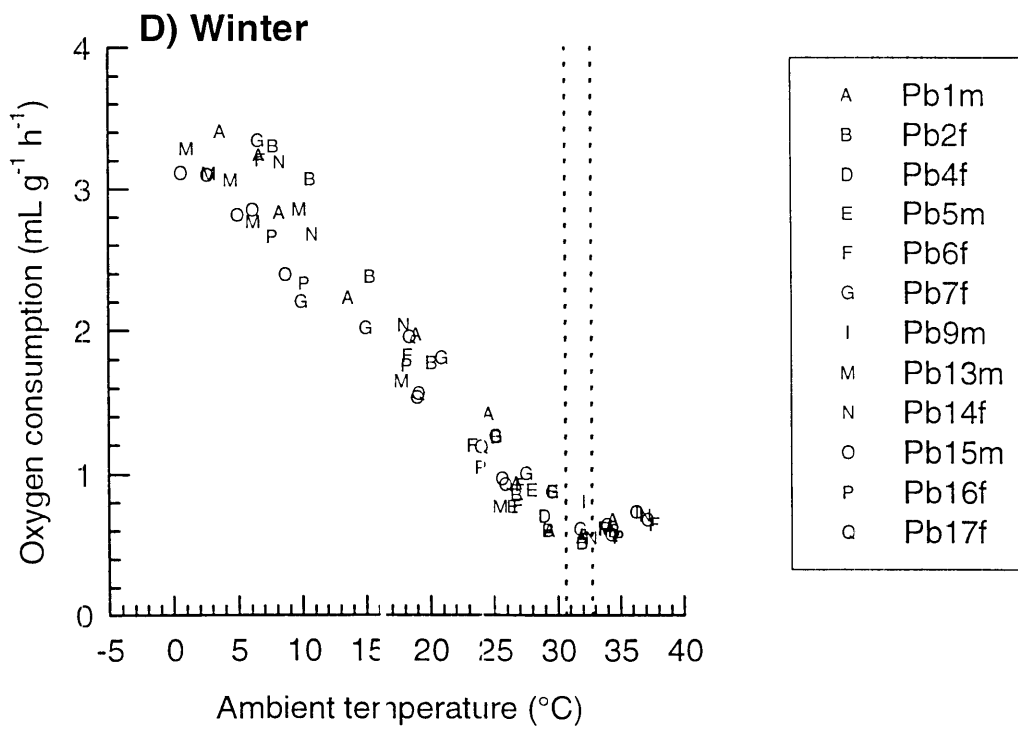
The mean BMR of animals in both air and helox atmospheres were indistinguishable for all seasons (Fig. 7.2; spring: t=1.24 D $^{=}$ 9 p=0.25; summer: t=0.46 DF 9 p=0.66; autumn: t=0.48 DF 8 p=0.65, paired t-test) except winter, when the BMR of all animals were consistently higher under helox gas compared to air (t=8.16 DF 5 p=0.0005, paired t-test).

7.3.1.2 Thermoneutral zone:

The T_{lc} in a helox atmosphere, as was found in air, were indistinguishable among seasons, with an overall mean of 30.8±1.1 °C (F=1.25 DF 3, 30 p=0.309, ANOVA). However, the T_{uc} also displayed no significant seasonal differences (F=2.39 DF 3, 19 p=0.101, ANOVA), which differs from obse vations in air, resulting in a mean T_{uc} of 33.1±0.3 °C.

Fig. 7.1. (On following pages) Res:ing metabolic rates (RMR) over entire range of ambient temperatures (T_a) measured for ind.vidual *P. breviceps* in helox atmosphere during A) spring, B) summer, C) autumn, and D) winter. Dashed lines indicate the thermoneutral zones (TNZ); 'm' and 'f' within lege id denote male and female gliders, respectively.

A) Spring

A	Pb1m
D	Pb4f
E	Pb5m
F	Pb6f
G	Pb7f
I	Pb9m
M	Pb13m
N	Pb14f
O	Pb15m
P	Pb16f

B) Summer

A	Pb1m
B	Pb2f
E	Pb5m
F	Pb6f
G	Pb7f
I	Pb9m
M	Pb13m
N	Pb14f
O	Pb15m
P	Pb16f
Q	Pb17f

C) Autumn

A	Pb1m
B	Pb2f
E	Pb5m
F	Pb6f
G	Pb7f
I	Pb9m
M	Pb13m
N	Pb14f
O	Pb15m
P	Pb16f
Q	Pb17f

D) Winter

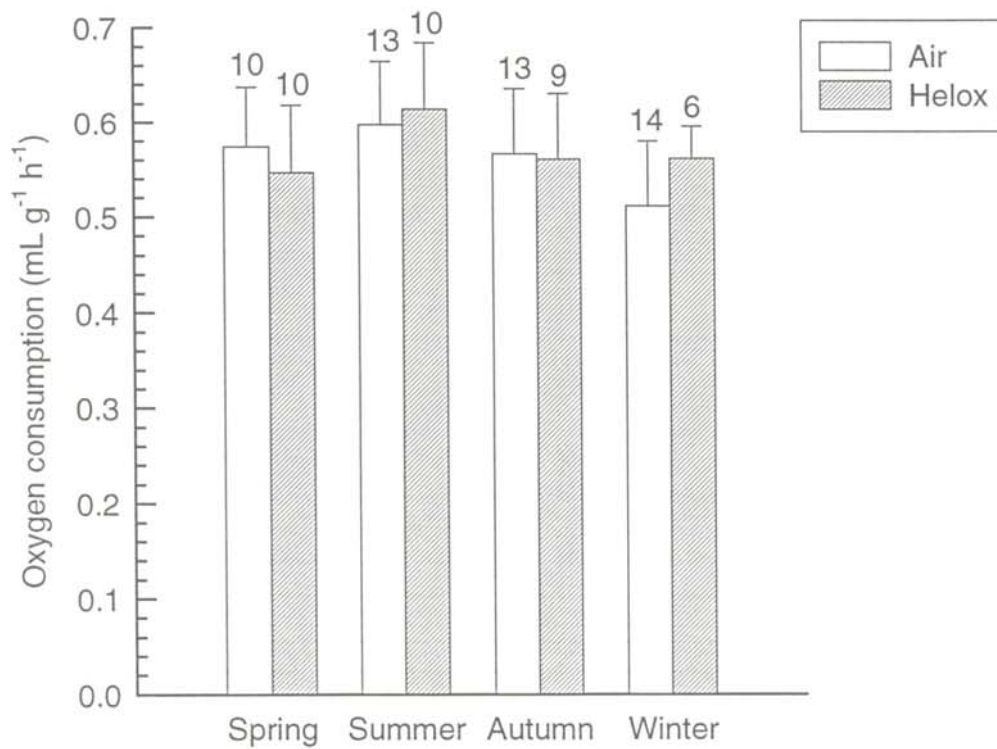| A	Pb1m
B	Pb2f
D	Pb4f
E	Pb5m
F	Pb6f
G	Pb7f
I	Pb9m
M	Pb13m
N	Pb14f
O	Pb15m
P	Pb16f
Q	Pb17f
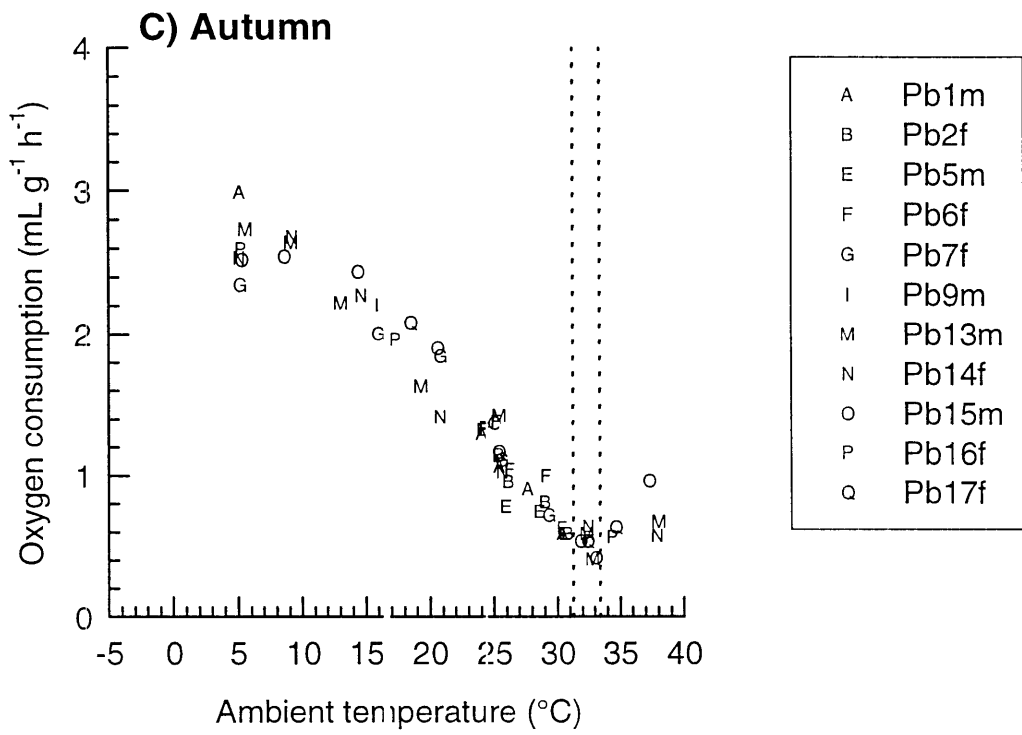
Fig. 7.2. Mean (±SD) basal metabolic rates (BMR) of *P. breviceps* in air and helox atmospheres during each season. Numbers indicate the number of individuals. Mean BMR of gliders in winter under a helox atmosphere was significantly higher than that of animals in air (t=8.16 DF 5 p=0.0005, paired t-test). During all other seasons BMR of animals were indistinguishable between the two atmospheres (p>0.05, paired t-tests).

TNZ ranges also did not differ among seasons (F=2.01 DF 3, 19 p=0.147, ANOVA), and the mean range was 2.1±0.6 °C.

The TNZ in helox occurred at a significantly higher T_a in comparison with the TNZ in air, with the mean T_{lc} shifted upward by 4.0 °C (t=11.00 DF 10 p<0.0001, paired t-test), and the T_{uc} by between 2.2 °C and 4.4 °C, depending on season (Table 7.1). The TNZ widths, however, did not differ significantly between air and helox (t=1.54 DF 9 p=0.16, paired t-test).

7.3.1.3 Resting metabolic rate:

7.3.1.3.1 Below the TNZ:

As in air, RMR below the T_{lc} of animals under a helox atmosphere showed an inverse linear response to decreasing T_a (Figs. 7.1, 7.3, Table 7.2). However, in contrast to animals in air, the slopes of the regression equations differed among seasons (Fig. 7.3, Table 7.2; F=5.95 DF 3, 164 p=0.001, ANCOVA). Slopes in summer were significantly steeper than those in spring, autumn or winter (p<0.01, Tukey). The regression equations did not differ between spring, autumn or winter (p>0.05, Tukey). Given that under the helox atmosphere animals react as if the T_a was much lower than that they are actually exposed to and that in air summer RMR were significantly elevated compared to the other seasons, this seasonal difference in slopes between summer and the other seasons is essentially the same as the seasonal difference in elevations found in air. Extrapolating these regression equations to the abscissa gave intercepts of the abscissa at 37.69, 35.88, 37.85 and 36.53 °C for spring, summer, autumn and winter, respectively.

As was expected, the slopes of the regression equations for RMR below T_{lc} of animals under helox atmosphere were significantly steeper than those under air (spring: F=78.84

Table 7.1 Mean (±SD) upper critical (T_{uc}) temperatures (°C), measured in air and helox atmospheres during each season, for *P. breviceps* (N = number of individuals; t and p values are for paired t-tests; means are for all individuals).

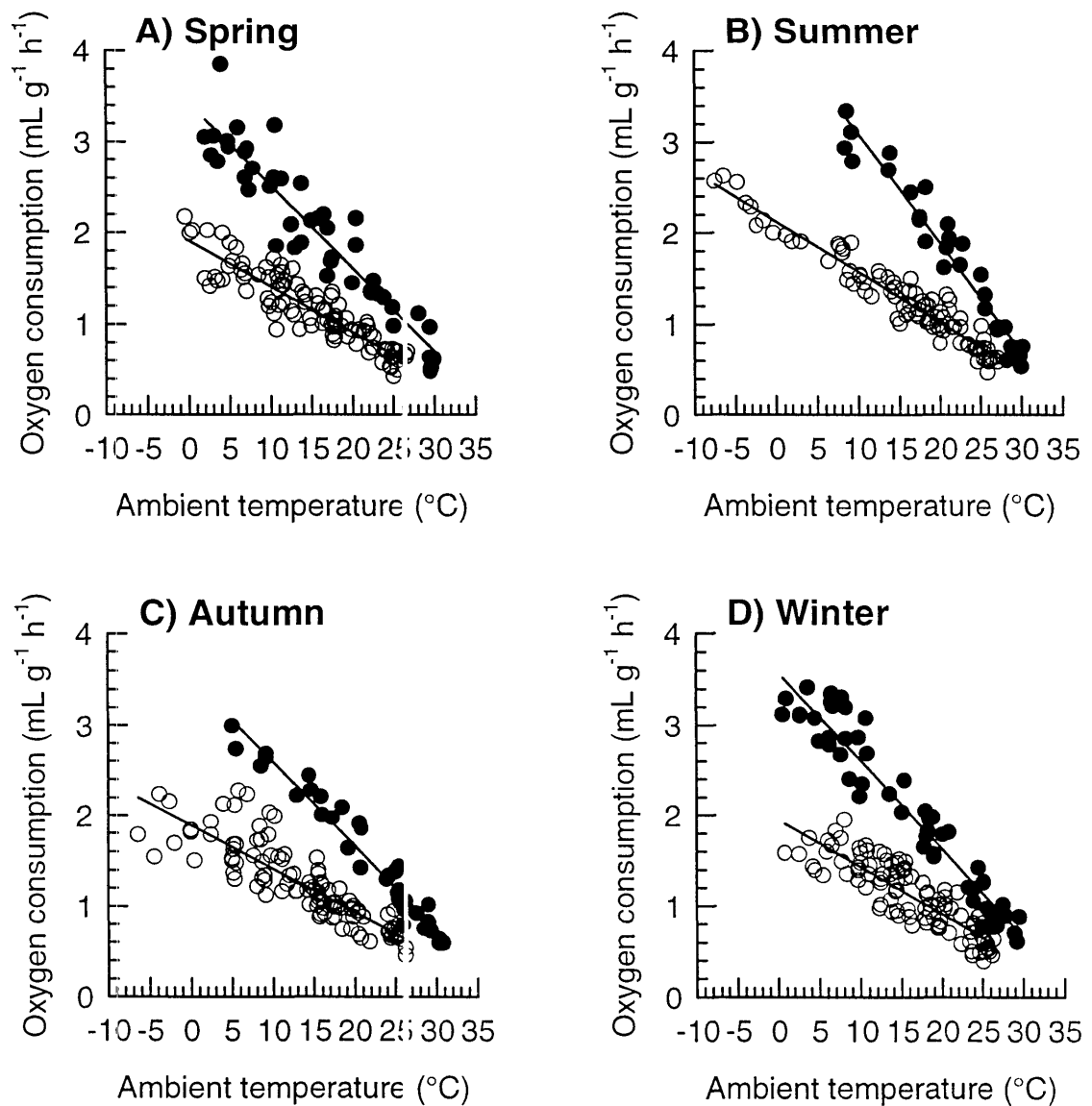| Season	Atmosphere	Mean (°C)	SD	N	t	DF	p
Spring	Air	30.80	1.20	6	4.50	4	0.011
	Helox	32.95	0.71	8			
Summer	Air	29.76	0.93	8	6.50	4	0.003
	Helox	33.63	0.76	5			
Autumn	Air	29.33	1.42	7	5.42	4	0.006
	Helox	33.36	0.20	5			
Winter	Air	28.31	1.62	8	4.51	4	0.011
	Helox	32.74	0.42	5			

Fig. 7.3. Resting metabolic rates (RMR) below the lower critical temperature (T_{lc}) of
P. breviceps in air (open circles) and helox (closed circles) atmospheres during A) spring,
B) summer, C) autumn, and D) winter. Regression equations are listed on the following
page in Table 7.2. During each season, the regression equations for animals under helox
atmosphere were steeper than those in air (p<0.001, ANCOVA). Further, the regression
line for animals in summer under helox was also steeper than those of animals in helox
during the other seasons (F=5.95 DF 3, 164 p=0.001, ANCOVA).

Table 7.2 Regression equations for RMR ($mL \ g^{-1} \ h^{-1}$) below T_{lc} versus T_a (°C) for *P. breviceps* during exposure to air and helox atmospheres (N = number of individuals; n = number of observations).

Season	Atmosphere	Regression equation	N	n	p	r^2
Spring	Air	y = 1.91-0.050x	13	101	< 0.001	0.83
	Helox	y = 3.43-0.091x	10	45	< 0.001	0.89
Summer	Air	y = 2.12-0.055x	13	92	< 0.001	0.93
	Helox	y = 4.27-0.119x	11	32	< 0.001	0.95
Autumn	Air	y = 1.89-0.048x	16	105	< 0.001	0.75
	Helox	y = 3.52-0.093x	11	39	< 0.001	0.95
Winter	Air	y = 1.94-0.052x	15	107	< 0.001	0.76
	Helox	y = 3.58-0.098x	11	56	< 0.001	0.94

DF 1, 142 p<0.001; summer: F=235.94 DF 1, 120 p<0.001; autumn: F=74.20 DF 1, 140 p<0.001; winter: F=114.41 DF 1, 159 p<0.001, ANCOVA).

7.3.1.3.2 Above the TNZ:

Unfortunately, because the TNZ in helox was shifted to a higher T_a than in air and there was a great possibility that the animals would die from heat stress if subjected to much higher T_a, RMR data above the T_{uc} are relatively scarce, especially in summer and autumn, and restricted to a rather narrow temperature range (Figs. 7.1, 7.4). Nevertheless, RMR above the T_{uc} appeared to be a linear function of T_a during winter and spring. T_a and RMR were not correlated during either summer or autumn, but this was most likely due to the low number of data points (4 in summer and 5 in autumn) and the narrow range. However, regression analysis revealed no significant differences between any season in either slope (F=0.76 DF 3, 29 p=0.525, ANCOVA) or elevation (F=2.19 DF 3, 32 p=0.109, ANCOVA). Consequently, a common regression line was calculated, although a high level of scatter was present around the line (y = -0.58 + 0.036x, p=0.002, r^2=0.25).

Due to the observed seasonal differences in the elevation of regression lines for RMR above the T_{uc} for animals in air, comparisons between air and helox equations had to be calculated for each season (Fig. 7.4, Table 7.3). With the exception of summer measurements, slopes and elevations did not differ. In summer, the elevation of the helox regression equation was significantly lower than that of air (F=4.69 DF 1, 22 p=0.041, ANCOVA). However, this was probably due to the difference in the ranges of T_a at which the animals were measured, and when the equations were compared over the same T_a range (34-37 °C), this significance disappeared.
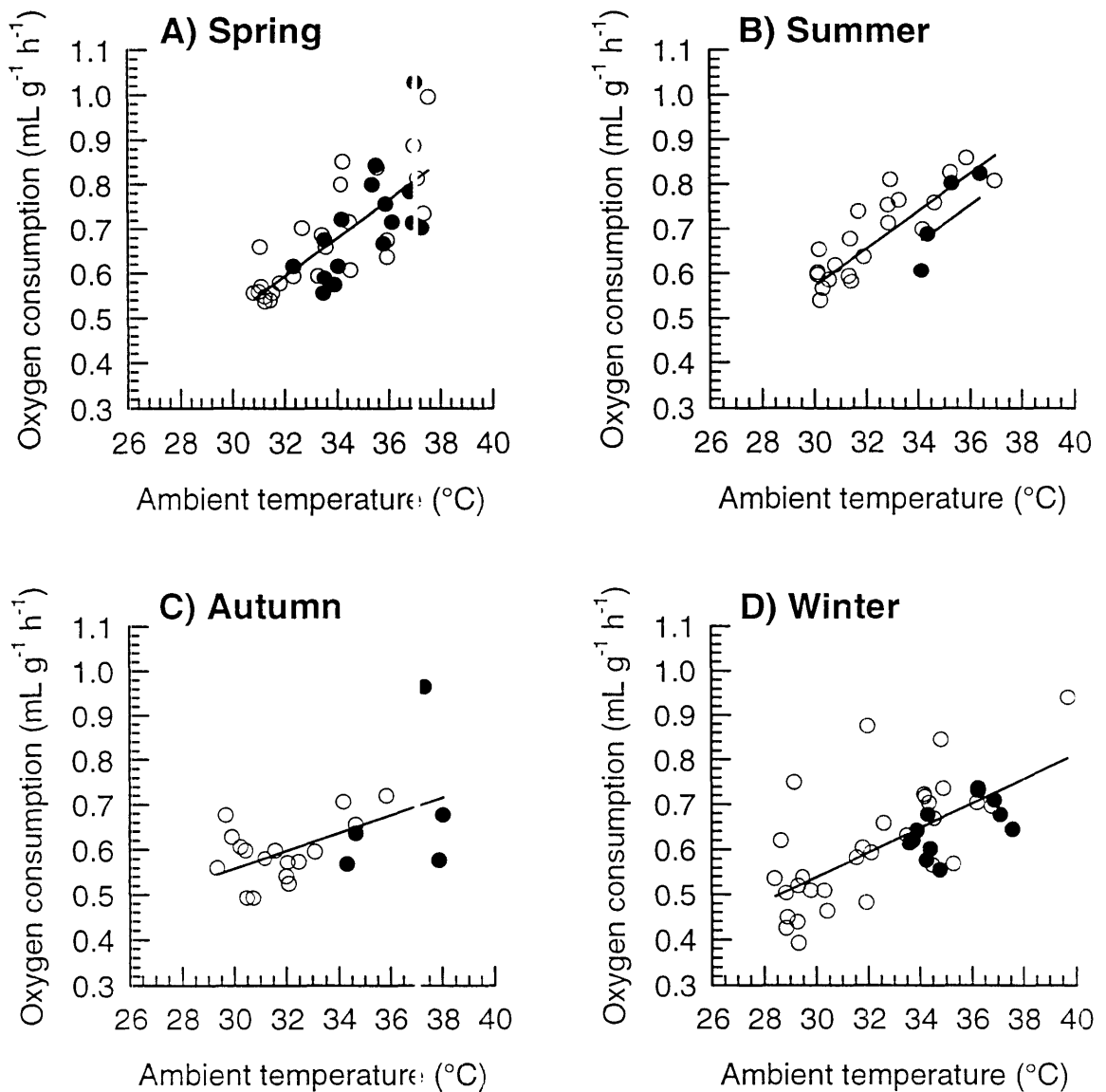
Fig. 7.4. Resting metabolic rates (RMR) above the upper critical temperature (T_{uc}) of
P. breviceps in air (open circles) and helox (closed circles) atmospheres during A) spring,
B) summer, C) autumn, and D) winter. Regression equations are listed on the following
page in Table 7.3. The regression line for animals under helox atmosphere was significantly
higher than that of animals in air during summer only (F=4.69 DF 1, 22 p=0.041,
ANCOVA).

Table 7.3 Regression equations for RMR ($mL\ g^{-1}\ h^{-1}$) above T_{uc} versus T_a (°C) for *P. breviceps* during exposure to air and helox atmospheres (N = number of individuals; n = number of observations).

Season	Atmosphere	Regression equation	N	n	p	r^2
Spring	Air/Helox	y = -0.78+0.043x	10	40	< 0.001	0.55
Summer	Air	y = -0.69+0.042x	10	21	< 0.001	0.74
	Helox	y = -0.76+0.042x	4	4	0.088	0.83
Autumn	Air/Helox	y = -0.031+0.020x	11	22	0.011	0.28
Winter	Air/Helox	y = -0.28+0.027x	11	43	< 0.001	0.45

7.3.2 Body temperature:

7.3.2.1 Below the turnpoint:

The mean T_b of normothermic resting animals in helox below the turnpoint, the T_a above which T_b started to rise in response to increasing T_a, showed no seasonal difference (Fig. 7.5; F=0.26 DF 3, 74 p=0.26, ANCOVA), and the overall mean T_b was 35.5±0.7 °C. Although only 0.4 °C higher, this value differed significantly from that of the mean T_b of the same animals in air (mean air T_a: 35.1±0.3 °C; t=5.08 DF 7 p=0.0015, paired t-test) because all individuals displayed a slight increase in T_b when the atmosphere was changed to helox.

7.3.2.2 Turnpoint:

The turnpoint for T_b under helox, like that in air, showed seasonal differences (Fig. 7.5, Table 7.4; F=22.74 DF 2, 10 p<0.001, ANOVA). The turnpoint in winter occurred at a significantly lower T_a than those in both spring and summer (p<0.01, Tukey). Unfortunately, due to problems with transmitters there were insufficient data to determine turnpoints for individuals during autumn and data had to be pooled, thus precluding it from the seasonal analysis. This T_b turnpoint was, however, very similar to that observed in winter. The turnpoints for each season under helox all occurred at T_a that were 2.3-2.8 °C higher than those in air (Table 7.4) and were all between 0.11-2.2 °C below the T_{lc}.

7.3.2.3 Above the turnpoint:

Above the turnpoint, T_b increased linearly and, while the slopes of the regression lines were similar (F=2.09 DF 3, 22 p=0.131, ANCOVA), the elevations differed among seasons, with summer values slightly lower than those observed during the other three seasons (F=7.55 DF 3, 25 p=0.001, ANCOVA)(Fig 7.5). The regression lines above the turnpoint were significantly different between animals in air and helox during both spring and summer
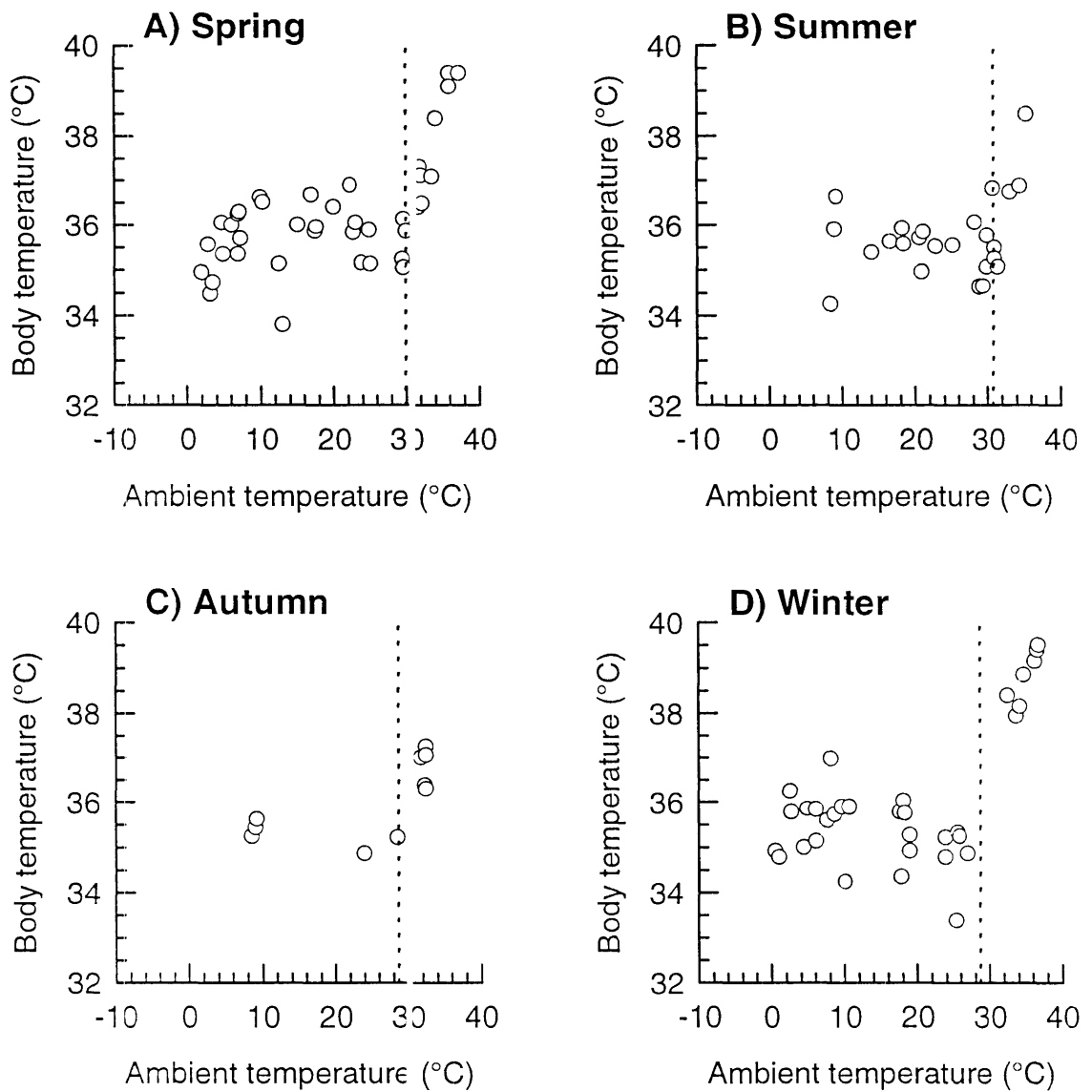
Fig. 7.5. Resting body temperatu·es (T_b) of *P. breviceps* in a helox atmosphere during A) spring, B) summer, C) autumr, and D) winter. Dashed line indicates the turnpoint, the ambient temperature (T_a) at whic·ı T_b started to rise in response to increasing T_a. Regression equations for data abcve the turnpoint are listed on the following page in Table 7.5.

Table 7.4 Resting body temperatu e (T_b) turnpoints for *P. breviceps* during each season in air and helox atmospheres (N = number of individuals; n = number of observations; p-values based on paired t-test; as data were pooled for animals in autumn, no SD or statistical tests could be determinec).

Season	Atmosphere	Turnpoint (°C)	SD	N	n	t	p
Spring	Air	27.13	0.95	5	70	5.06	0.015
	Helox	29.90	0.28	4	40		
Summer	Air	28.44	0.43	4	57	5.24	0.014
	Helox	30.70	0.57	4	24		
Autumn	Air	26.11	0.81	6	40	-	-
	Helox	28.70	-	6	10		
Winter	Air	26.33	0.84	5	65	6.49	0.007
	Helox	28.68	0.46	5	33		

Table 7.5 Regression equations fo T_b above turnpoint of *P. breviceps* in air and helox atmospheres (N = number of individuals; n = number of observations).

Season	Atmosphere	Regression equation	N	n	p	r^2
Spring	Air	$y = 23.7 + 0.42x$	5	18	< 0.001	0.91
	Helox	$y = 18.7 + 0.57x$	4	11	< 0.001	0.92
Summer	Air	$y = 22.7 + 0.45x$	4	17	< 0.001	0.70
	Helox	$y = 21.6 + 0.45x$	4	6	< 0.001	0.90
Autumn	Air/Helox	$y = 25.9 + 0.33x$	6	15	< 0.001	0.77
Winter	Air/Helox	$y = 22.8 + 0.45x$	5	22	< 0.001	0.87

(spring: slopes significant, $F=5.60$ DF 1, 25 $p=0.026$; summer: intercepts significant, $F=10.91$ DF 1, 20 $p=0.004$, ANCCVA). However, for animals in autumn and winter, the air and helox regression lines were indistinguishable (Table 7.5).

7.3.3 Conductance:

Similar to that in air, the conductance of animals in helox showed a curvilinear response to increasing T_a, though this relationship was not as strong in summer because much more scatter occurred between T_a 10-30 °C (Fig. 7.6). As observed in air, the point where conductance started to markedly increase appeared to correlate with the T_{lc} under helox atmosphere (i.e. T_a 30-31 °C), although in winter this turnpoint appeared to occur at a higher T_a (34 °C). However, this was most probably due to the small number of data in this region. Again, due to problems with transmitters, very few data were obtained during autumn.

Due to the abovementioned curvilinear relationship, conductance data for the entire T_a range measured (0.6-37.3 °C) were transformed to obtain linear regressions prior to statistical analysis. No seasonal differences in either elevation or slope were detected over this T_a range when all four seasons were included. However, when the limited data from autumn were removed from the analysis, the elevation for conductance in summer was significantly higher than that during spring (F=3.44 DF 2, 93 $p=0.036$, ANCOVA; $p<0.05$, Tukey), while conductance during winter was not different from either spring or summer ($p>0.05$, Tukey).

A comparison between conductance in air and helox during each season over the entire T_a range, again after the data were transformed to obtain linear regressions, revealed that the slopes of the regression equations for conductance in spring, summer and winter were steeper for animals under an air atmosphere compared to those under a helox atmosphere (spring: F=31.14 DF 1, 106 $p<0.001$; summer: F=4.87 DF 1, 75 $p=0.03$; winter F=12.90
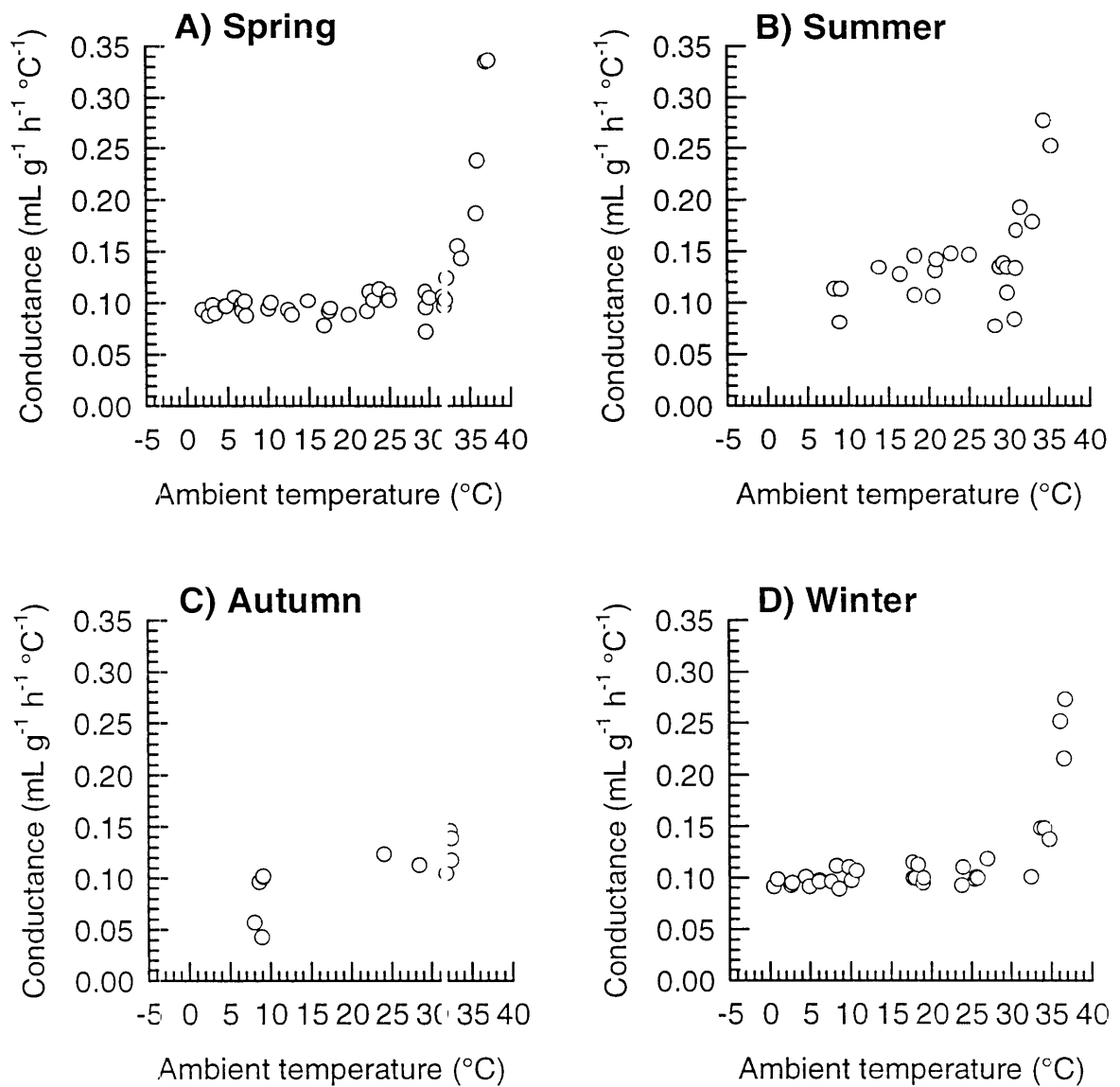
Fig. 7.6. Resting conductance for *P. breviceps* in a helox atmosphere during **A)** spring, **B)** summer, **C)** autumn, and **D)** w nter over the entire range of ambient temperature (T_a) measured.

DF 1, 92 p=0.001, ANCOVA). During autumn, probably due to the shorter range of T_a, the slopes were indistinguishable but the elevation was significantly higher in animals under helox atmosphere (F=22.26 DF 1, 47 p<0.001, ANCOVA).

7.3.3.1 Below the TNZ:

Below the helox T_{lc} a linear relationship between T_a and conductance occurred during winter only. Regression analysis showed that, like in air, there was a seasonal difference in the elevations (F=13.53 DF 3, 73 p<0.001, ANCOVA), with conductance in summer higher than that in spring (p<0.05, Tukey). Elevations for all other seasons were similar (p>0.05, Tukey). As conductance below T_a 15 °C in helox displayed no linear relationship with T_a, data from each season were averaged. These means differed among seasons (Fig. 7.7, Table 7.6; F=3.12 DF 3, 33 p=0.039, ANOVA). As was expected, mean minimum conductances of animals subjected to helox were significantly higher (1.7 to 1.8-fold) than those of animals in air (Fig. 7.7, Table 7.6).

7.3.3.2 Within the TNZ:

Within the TNZ, T_a and conductance were not correlated in any season or atmosphere. Seasonal differences were only detected for mean conductance values within the TNZ of animals in air (Fig. 7.8; F=4.26 DF 3,12 p=0.029, ANOVA), when mean conductance in winter was lower than that in summer (p<0.05, Tukey). No other seasonal difference was detected in either air or helox.

A comparison of the mean conductance values of both air and helox for each season revealed that conductance in helox was higher than in air during all seasons except for summer (Fig. 7.8, Table 7.7). However, summer helox conductance values were still approximately 30 % higher than those of the same animals in air, and were probably not significantly different due to the higher degree of variance observed in animals subjected to
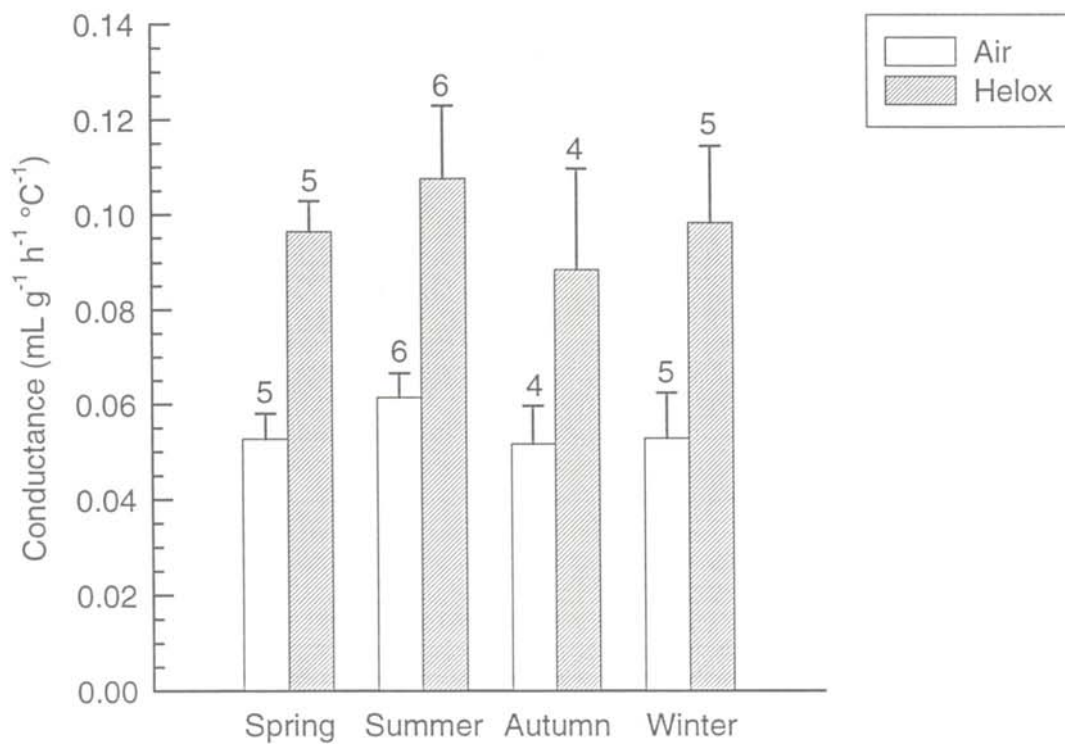
Fig. 7.7. Mean (±SD) minimum conductance of *P. breviceps* in air and helox atmospheres during each season. Numbers indicate the number of individuals. Conductance of animals in a helox atmosphere was significantly higher than those in air (see Table 7.6 below).

Table 7.6 Mean minimum conductance of *P. breviceps* during each season in air and helox atmospheres (N = number of individuals; n = number of observations; p-values based on paired t-test).

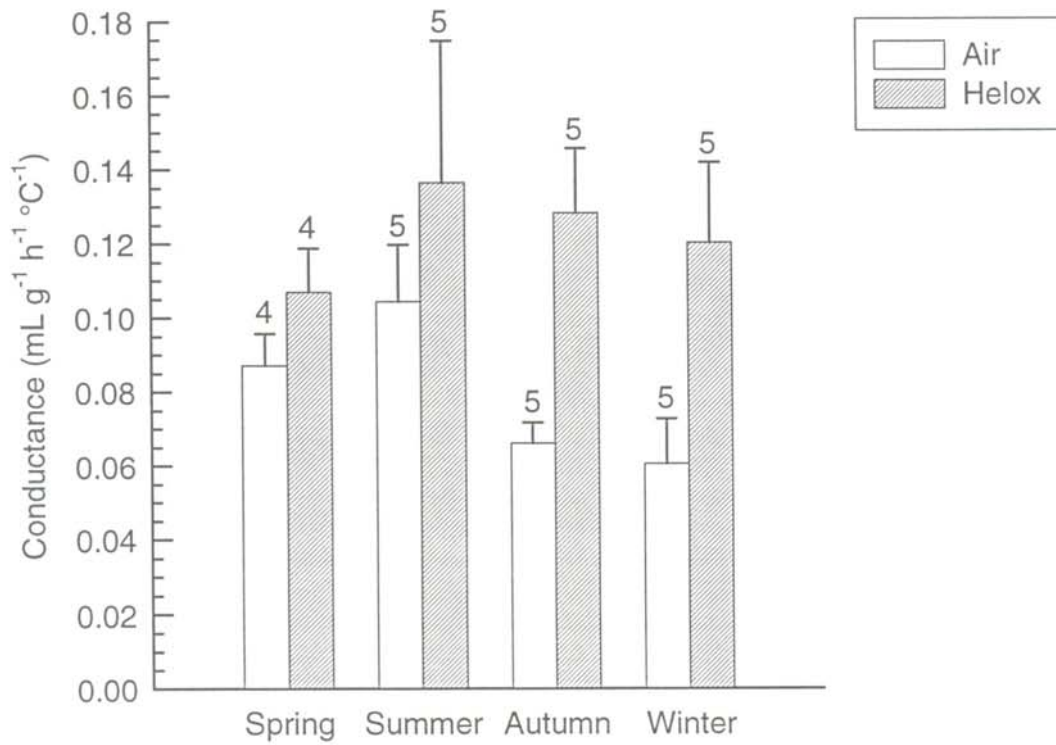| Season	Atmosphere	Conductance (mL g-1 h-1 °C-1)	SD	N	n	t	p
Spring	Air	0.053	0.005	5	15	43.09	< 0.001
	Helox	0.096	0.006	5	12		
Summer	Air	0.062	0.005	6	9	8.83	< 0.001
	Helox	0.108	0.015	6	6		
Autumn	Air	0.052	0.008	4	13	4.03	0.028
	Helox	0.089	0.021	4	5		
Winter	Air	0.053	0.010	5	11	6.39	0.003
	Helox	0.098	0.016	5	12		

Fig. 7.8. Mean (±SD) conductance within the TNZ of *P. breviceps* in air and helox atmospheres during each season. Numbers indicate the number of individuals. Conductance of animals in a helox atmosphere was significantly higher than those in air during all seasons except summer (see Table 7.7 below).

Table 7.7 Mean conductance within the TNZ of *P. breviceps* during each season in air and helox atmospheres (N = number of individuals; n = number of observations; p-values based on paired t-test).

Season	Atmosphere	Conductance (mL g^{-1} h^{-1} °C^{-1})	SD	N	n	t	p
Spring	Air	0.087	0.009	4	4	3.54	0.038
	Helox	0.107	0.012	4	4		
Summer	Air	0.105	0.015	5	5	2.39	0.075
	Helox	0.137	0.038	5	5		
Autumn	Air	0.066	0.006	5	5	9.99	< 0.001
	Helox	0.128	0.017	5	5		
Winter	Air	0.061	0.012	5	5	5.00	0.008
	Helox	0.120	0.022	5	5		

helox atmosphere. These results are especially interesting when it is considered that despite

the higher conductances, BMR between animals in air and helox were indistinguishable in

all seasons, except winter.

7.3.3.3 Above the TNZ:

Above the TNZ, the slopes for conductance of animals during spring, summer and winter

were indistinguishable ($F=0.18$ DF 2, 18 $p=0.84$, ANCOVA). However, as in air, the

elevations differed among seasons ($F=9.06$ DF 2, 20 $p=0.002$, ANCOVA), as the

conductance in summer was higher than that during spring and winter ($p<0.05$, Tukey).

Unfortunately, due to the limited data, autumn could not be included in this seasonal

analysis.

Despite the higher conductive properties of helium, no difference was found in the slopes

or elevations of the regression equations between air and helox during each season above

the TNZ. Therefore, the observed difference in slopes over the entire T_a range, or elevation

in the case of conductance in autumn, was entirely due to the differences found in

conductance below the TNZ.

7.4 Discussion:

Petaurus breviceps responded to helox gas in a manner akin to that of animals in air, with

metabolic rates, T_b and conductance all showing a similar qualitative pattern in the two

atmospheres. Of particular importance was also the overall quantified lack of difference in

BMR of animals in air and helox atmospheres, as this would appear to confirm that helox

gas produces no physiological effects other than altering an animal's conductance.

The lack of difference in BMR of *F. breviceps* in air and helox atmospheres may also help to resolve the on-going debate about whether the value of the mass exponent for scaling BMR should be proportional to size by a factor of 0.75 (Kleiber 1961; Schmidt-Nielsen 1984), or closer to a surface area relationship of 0.67 (Heusner 1991). For sugar gliders, over the small mass range of 52 g, he mass exponent was calculated to be 0.62 (see Chapter 3), suggestive that metabolism is proportional to the surface area of the animal. However, conductance is inversely related to surface area (Scholander *et al.* 1950c; Bradley & Deavers 1980). Therefore, as the BMR of the gliders was similar under air and helox atmospheres, although conductances were markedly higher in the latter, it would appear that surface area is not the reason for the observed relationship between BMR and body mass, and some other factor must be responsible.

While BMR was similar in helox and air for most seasons, animals during winter had higher BMR under a helox atmosphere. However, this may have been due to methodology. As helox gas is expensive, animals were not subjected to a continuous helox atmosphere throughout the entire period within the respirometry chamber. Rather the animals were first subjected to air, until T_a stabilised and RMR were determined, before the gas was changed over to helox. Once the measurement of RMR in helox had been obtained, air was again pumped through the chambers while T_a was raised or lowered and the process repeated. Although the animals were allowed to equilibrate to these changes in atmosphere, given that winter animals had the highest T_a differential between the TNZ in air and helox (T_{uc} air-T_{lc} helox: 2.3 °C), there may still have been some residual after-effects of the comparatively high T_a in air compared to helox. Consequently, this may have resulted in a slightly higher BMR under helox gas during this season. Considering the response of the animals during the other three seasons, it, therefore, seems reasonable to assume that helox has no extraneous physiological effects.

The transposition of the TNZ in helox atmosphere, and concomitant points of increase for T_b and conductance, to a higher T_a was to be expected since the response to T_a of

conductance was not changed. Rather conductance, at least below the TNZ, was merely increase by a factor of 1.7-1.8. Due to this higher rate of heat loss, metabolic rate must increase at a higher T_a in order to maintain a stable T_b. Further, this higher rate of heat loss also means that the start of active cooling is also delayed until a warmer T_a. Consequently, the T_{lc} and T_{uc} are both transposed to a higher T_a, thereby shifting the TNZ upwards.

At T_a below thermoneutrality an increase in conductance at a given temperature results in a substantial increase in metabolic rate (Herreid & Kessel 1967; Dawson & Olson 1988; Hallam & Dawson 1993). Moreover, as the slope of the regression line of RMR versus T_a below the T_{lc} is similar to conductance, the differential between metabolism in air and helox increases as T_a lowers (Rosenmann & Morrison 1974). These reactions are due to the facilitation of heat transfer from the body in the more conductive medium (helox), resulting in the animals of this size responding to defend their normothermic T_b metabolically as if they were at a T_a equivalent to 20-30 °C lower than the actual measured T_a. Consequently, it is this property of the gas that elicits maximum metabolic rates at relatively high T_a with a substantially reduced risk of freezing injury to the animals (Rosenmann & Morrison 1974; Rosenmann et al. 1975; Dawson et al. 1986; Dawson & Olson 1988; Hallam & Dawson 1993; Chappell et al. 1995; present study).

Since RMR was increased under helox gas in order to defend normothermic T_b, it was perhaps unexpected to find that mean T_b were also slightly higher in helox compared to those in air. There are two possible explanations for this occurrence. First, the animals may have metabolically overcompensated for the substantial increase in conductance. Therefore, more heat was being produced than lost and T_b increased. Secondly, T_b may actually have a cubic curvilinear response to declining T_a (Lovegrove et al. 1991b). From high T_a, T_b decreases to the TNZ, then slightly increases as T_a lowers below the T_{lc}, before declining towards hypothermia. However, this response often only becomes evident when animals are measured over a range of T_a encompassing their lower thermal limit (Fig. 7.9).
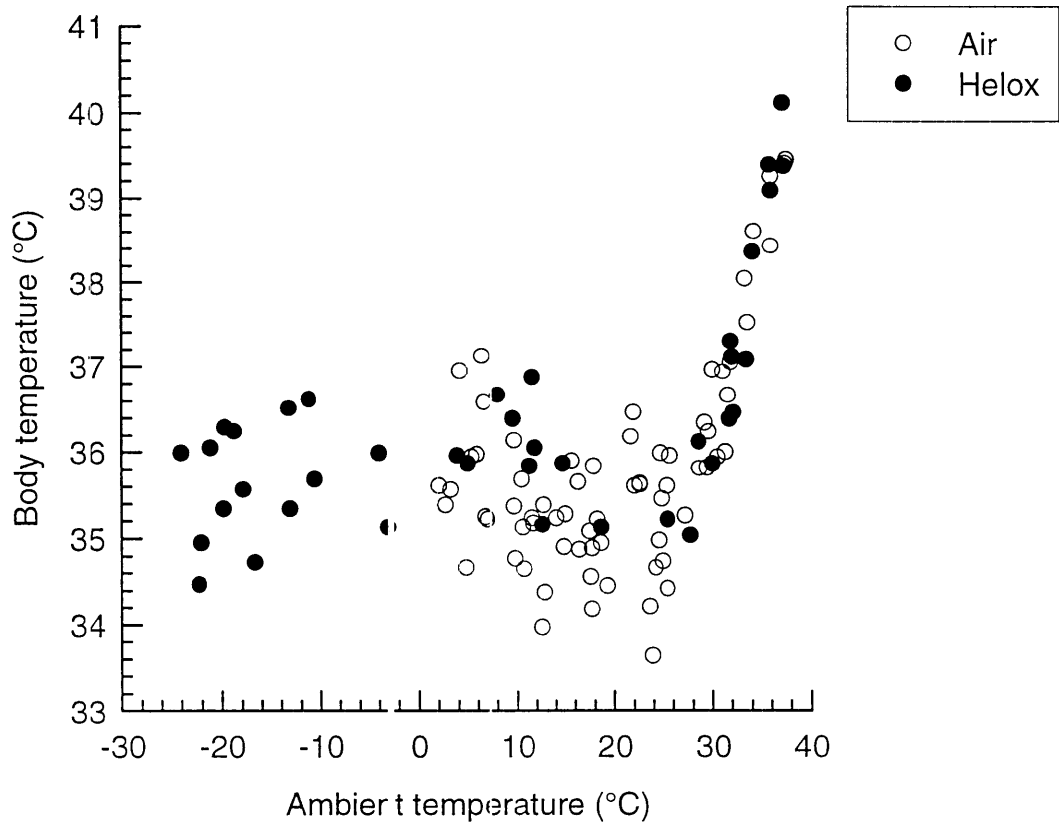
Fig. 7.9. Body temperatures (T_b) of *P. breviceps* in air and helox atmospheres over the range of effective ambient temperature (T_a) the animals could withstand during spring. T_b of the gliders decreased from high T_a to minimum values at T_a close to the lower critical temperature (T_{lc}). As T_a decreased below the T_{lc}, T_b increased slightly before declining just prior to the animals entering hypothermia.

Consequently, the higher mean T_b n helox may have been due to the inclusion of a higher proportion of T_b from the upper portion of the curve below T_{lc} than those in air.

Helox gas appeared to have little influence on either the conductance or metabolism of sugar gliders at T_a above the T_{uc}. This is probably due to the greater contribution of evaporative cooling used by this species at high T_a. Sugar gliders, when exposed to high T_a, initially spread saliva over their forelimbs. As T_b continues to increase, this salivation extends to cover the hindlimbs, abdomen, scrotal region and underside of the tail, and may be used in combination with vasodilation and open-mouthed panting (Robinson & Morrison 1957; Fleming 1980; present study). Consequently, since evaporative cooling is not affected by the helox atmosphere (Rosenmann & Morrison 1974), the predominance of evaporative cooling over conductance by sugar gliders as a method to remove heat at T_a above the TNZ means that there is little effect on the metabolism of this species under helox gas at these high T_a.

The primary influence of helox gas is on surface insulation, with a thicker layer of fur or feathers emphasising the conductivity effect of the gas (Rosenmann & Morrison 1974). Therefore, the ratio between conductance in air and helox can be used to assess the effectiveness of an animal's body covering. In *P. breviceps*, this ratio was higher in winter and spring (1.85 and 1.81, respectively) than during summer (1.74) or autumn (1.71), thus indicating that the fur of this species is a more effective insulator during the former seasons. It would be expected that fur in autumn should also have a higher insulation value than that during summer, especially since minimum T_a are on par with those during spring in the New England Tableland region (Bureau of Meteorology, 1997). However, it should be noted that these calculations only take into account fur and not tissue insulation (Rosenmann & Morrison 1974), which in gliders is probably highest during the autumn months (see Chapter 3). Consequently, it is probably the tissue insulation that gliders rely on to reduce heat loss in autumn rather than thicker fur.

Common uses for helox gas include the prevention of nitrogen narcosis in divers (Baddeley & Flemming 1967), induction of hypothermia (Werner 1992; Osborne & Milsom 1993), elicitation of maximum metabolic rates without the risk of tissue injury (Rosenmann & Morrison 1974; present study), and investigations into the various aspects of torpor (Osborne & Milsom 1993; Geiser *et al.* 1996). Consequently, it is an important requirement that this gas does not have any physiological side-effects. From the data presented here, it would appear that the only physiological parameter that was altered during helox exposure was that of conductance, primarily via increased heat transfer through the surface insulation layer.

Chapter 8:
Final Discussion

Petaurus breviceps, from the New England Tablelands region of NSW, alter many aspects of their thermal physiology, morphology and behaviour on a seasonal and daily basis in order to cope with the high energetic costs associated with endothermy. By confining their activity period to the scotophase, this species is able to avoid the heat of the day and, therefore, most adaptations are related to conserving heat. These adaptive strategies involve seasonal changes to body mass and composition, metabolism, T_b, conductance, and activity patterns, as well as the differential use of nests and communal huddling. Further, despite commencing in winter, reproduction is timed so that the period of highest energetic demand, to both the mother and the young, occurs at a time when environmental conditions are most favourable in terms of T_a and food availability. This, therefore, helps to reduce the energetic burdens associated with this process.

Given that some of these strategies are utilised opportunistically in response to acute stresses caused by an unpredictable environment, while others regularly occur as a response to predictable cycles, in particular seasonal and daily changes in T_a and climate, the stimuli which initiate the use of such strategies presumably vary. Among the possible stimulative factors, endogenous rhythms (circadian and circannual), photoperiod, T_a and nutrient supply are probably the primary cues.

When maintained under constant environmental conditions, some species continue to display their regular thermoregulatory and reproductive cycles, indicating that the occurrence of these events is controlled by internal factors (Pengelley & Fisher 1963; Aschoff & Pohl 1970; Enright 1970; Refinetti 1996). However, often these free-running periods are not quite synchronous with the cycle and, therefore, may require entrainment from some external stimulus (Heller & Poulson 1970; Mrosovsky 1978; Francis & Coleman

1990). Further, the cycle may not express itself under certain environmental conditions. The endogenous rhythm, therefore is one that allows an animal to prepare for future events while also permitting it a degree of flexibility should the environment vary (Heller & Poulson 1970; Kenagy 1986). Consequently, an animal may still utilise external environmental factors, despite the internal regulation.

Perhaps the most common environmental stimulus used as a cue for seasonal biological events is photoperiod. Although other external factors show seasonal changes, the extent of these may vary from year to year. Photoperiod, however, maintains a constant annual cycle that can, therefore, be used as a precise and reliable cue. Consequently, photoperiod has been found to stimulate changes in a number of variables, including diurnal T_b rhythms (Haim & Levi 1990; Haim et al. 1997), metabolism (Andrews & Belknap 1985; Corp et al. 1997), NST and the amount of BAT (Blank et al. 1988; Haim & Levi 1990; Ellison et al. 1992; Haim 1996), torpor occurrence, depth and duration (Morrison 1964; Heldmaier & Steinlechner 1981; Steinlechner et al. 1986; Kirsch et al. 1991), food consumption (Dark et al. 1983; Haim & Levi 1990; Hope et al. 1997b), body mass (Dark et al. 1983; Steinlechner et al. 1983; Reynolds & Lavigne 1988; Haim et al. 1995), pelage characteristics (Goldman et al. 1985), thermal conductance (Reynolds & Lavigne 1988; Haim et al. 1995; Corp et al. 1997), migration (Rowan 1938), activity (Lyne 1981; Goldingay 1984; Holloway & Geiser 1996a), and reproduction (Rowan 1928; Godfrey 1969; Sadleir 1972; Goldman et al 1986; Gemmell 1990, Holloway & Geiser 1996b).

Photoperiod provides a number of signals that an animal may use as cues to modify its physiology and behaviour. While some species may respond to a critical day length (Holloway & Geiser 1996b), others will react to the light intensity (Vriend & Lauber 1973) or the rate of change of photoperiod (McAllan & Dickman 1986). The pineal gland subsequently reacts to these signals by changing the levels of the hormone melatonin and the observed changes are, therefore, a response to endocrine regulation (Steinlechner et al. 1983; Goldman et al. 1986; Steinlechner 1990).

Given the increased energy demands with colder T_a, it is not surprising that temperature also acts as a stimulus to initiate certain changes in an animal's thermal physiology and behaviour. These can include alterations to activity levels (Francis & Colman 1990; Körtner & Geiser 1995b), huddling and nesting behaviour (Lynch 1973; Batchelder et al. 1983, Heath & Lynch 1983), and the use of torpor (Heath & Lynch 1983; Ruf et al. 1993). Further, T_a may also have an effec on certain physiological aspects of torpor, such as depth and duration (Twente & Twente 1965; French 1985; Barnes & Ritter 1993; Geiser & Broome 1993; Holloway & Geiser 1995). However, while certain regular cycles such as annual moult (Heath & Lynch 198; Ruf et al. 1993) and body mass (Stokkan et al. 1995) may also be partly controlled by T_a, thermoregulatory changes in response to T_a are more likely to initiate an immediate reaction.

As with T_a, thermoregulatory responses to food availability are generally instantaneous. Reduction in activity (Holloway & Geiser 1996a), decrease in metabolic rates (Song & Geiser 1997) or an increase in torpor usage or duration (Pengelley & Fisher 1963; Otsu & Kimura 1993; Ruf et al. 1993; Song & Geiser 1997) are usually the result of a shortage in food supply. Further, low T_a and reduced food availability are often inextricably linked and combine to accentuate these responses (Hudson 1973; Morton 1978a).

This interrelation between environmental factors in the field may also include photoperiod, i.e. a short photoperiod coincides with low T_a and reduced food availability, thus making it difficult to discern which parameter is acting as the stimulative factor. However, given the seasonal onset of anticipatory changes in P. breviceps, as seen with the reduction in activity and increase in body mass prior to the onset of highly energetically stressful conditions, it is likely that either photoperiod or endogenous rhythms, or a combination of the two, are responsible for initiating the observed seasonal changes in the thermoregulatory physiology, morphology and behaviour of this species. Further, as sugar gliders held under constant light maintained their diurnal rhythms of activity, metabolism and T_b, with a free-running period length of 24.2 h (Kleinknecht et al. 1985), it would appear that diurnal changes are

also under the control of an endogenous cycle which is presumably entrained by photoperiod. Short-term reactions, however, such as use of torpor, nesting and huddling, and some changes in activity levels are probably a response to low T_a and food availability.

Within the New England Tablelands region, sugar gliders experience a relatively pronounced and predictable seasonal change in climate (see Fig. 3.1). However, even in summer these animals may occasionally experience T_a below 3 °C (Bureau of Meteorology 1997). Therefore, the reliance on set cues allows this species to prepare for the cold and energetically stressful winter season, while the short-term responses allow flexibility in case of unpredictable conditions and during times when the initially prepared responses are insufficient to cope with an increased energy demand.

The ability to adapt and adjust to changes in the environment has enabled *P. breviceps* to successfully inhabit a wide region of forests and woodland throughout eastern and northern Australia, New Guinea, West Irian and surrounding islands (Smith 1973; Flannery 1990; Suckling 1995). As long as old trees, primarily *Eucalyptus*, with fissures suitable for nesting sites are present, this species is even able to flourish in small remnant strips and patches of forest within cleared agricultural areas (Suckling 1984). Perhaps the most important factor limiting its distribution, therefore, is aridity combined with heat. Approximately 50 % of the water required to maintain water balance in sugar gliders from temperate regions is obtained by drinking free-standing water (Nagy & Suckling 1985). Furthermore, this species has water influx rates about 40-57 % higher (Nagy & Suckling 1985; Quin 1993) than those predicted for a similar-sized mammal (Nicol 1978). Consequently, given the reliance on evaporative cooling to maintain a stable T_b at high T_a, this species would presumably require an even higher water intake in hotter regions which may, therefore, restrict it to only those areas with relatively high or reliable levels of rainfall, or permanent water sources.

The size of *P. breviceps* generally decreases as latitude decreases (Smith 1973), in agreement with Bergmann's rule. This may possibly be an adaptation to the warm T_a experienced in these regions. A smaller size means a relatively larger surface area and, therefore, a higher conductance. Consequently, as was observed in individuals during summer in the present study (see Chapter 3), the T_{uc} of these animals is probably higher compared to those animals at high atitudes and evaporative cooling is thus not required until a warmer T_a is reached. Given the lack of marked seasonal changes in T_a and the fact that sugar gliders at the low latitudes do not have a restricted breeding season (Johnson 1964; Flannery 1990), it is also probable that animals within these regions do not display the high degree of seasonal changes in thermal physiology and behaviour observed in the present study. It is also possible that the seasonal changes are opposite to those observed in cold-climate species and are driven by food availability, as observed in the subtropical blossom-bat, *Syconycteris australi.* (Coburn & Geiser 1998). However, this can only be resolved through further investigation.

In conclusion, *P. breviceps* within he New England Tablelands district, respond to the marked seasonal changes in climate of the region and possible reduction in food availability in a variety of ways. Initially this species prepares for winter during autumn by decreasing activity and increasing food intake. This results in an increase in body mass due to an increase in fat content, a decrease in conductance and, consequently, a reduction in metabolic rates. Further, maximum heat output is increased allowing the animals a greater tolerance to the cold. Superimposed on these adaptations, sugar gliders can utilise torpor, nests, huddling and further reductions in activity. Consequently, the use of these strategies has enabled *P. breviceps* to successfully occupy this energetically stressful region. Further, the ability of *P. breviceps* to adapt to various conditions has allowed it to inhabit a wide variety of habitats in climatic conditions ranging from cold-temperate to tropical, making it one of the most successful and widely distributed Australasian marsupials.