Chapter One: General Introduction and Aims of the Thesis

1.1 Homeothermy in Mammals

1.1.1 Homeothermy: Advantages, Achievement, and Adaptations

Many mammals are homeothermic. They maintain a relatively high and constant body temperature (T_b) throughout their adult life (Bartholomew 1982). This contrasts with the highly environment-dependent poikilotherms in which T_b and biological reaction rates vary with the fluctuations of environmental temperature (T_a; Bartholomew 1982). The high and stable T_b of mammals allows them to have a constant internal thermal milieu, and thus optimum physiological functions. Therefore, mammals can achieve a great level of activity, a high rate of growth, and thus a fast adaptation to changing situations over evolutionary time (Hulbert 1993). With these advantages, mammals have freed themselves from many environmental constraints and have exploited a large variety of habitats (Eisenberg 1981). Obviously, homeothermy in mammals presents a very important progress in evolution (Eisenberg 1981).

The achievement of mammalian homeothermy relies on the development of many different thermoregulatory mechanisms, in particular, endothermic heat production (Hulbert 1980a; Ruben 1995). In contrast to ectothermic animals, in which most body heat is derived from external heat sources (Schmidt-Nielsen 1990), mammals are capable of generating a large amount of heat via internal heat production (Schmidt-Nielsen 1990). Even the minimum metabolism of resting mammals for maintaining organ function is approximately four to five times that of similar-sized ectotherms at the same T_b (Dawson and Hulbert 1970; Hulbert 1980b). This remarkably high level of basal metabolic rate (BMR) is not only due to the larger total mitochondrial membrane surface area of mammalian tissues (Else and Hulbert 1985), but also related to some intrinsic properties of their cellular membranes and mitochondrial activity

(Hulbert and Else 1989, 1990; Clausen et al. 1991; Hulbert 1993). All of these are influenced by the highly active thyroid functions (Hulbert 1987, 1993).

The maintenance of T_b in the cold is largely dependent on the high metabolic capability of mammals to increase their endogenous heat (Feist and White 1989). This response is controlled by the central neural system in which two populations of preoptic and hypothalamic nuclei (POH) sense and collate the difference between POH and threshold temperature (T_{set}), and activate appropriate thermoregulatory mechanisms (Hammel et al. 1958; Hammel 1968; Bligh 1973). Below the lower critical T_a (T_{lc}) of the thermoneutral zone (TNZ), MR increases to compensate for the heat loss proportional to the thermal differential (ΔT) between T_b and T_a (Hammel 1968; Alexander 1979).

The increase of regulatory heat production is accomplished by means of non-shivering thermogenesis and shivering thermogenesis (Horwitz 1989; Heldmaier et al. 1990). Non-shivering thermogenesis is primarily important in small placentals (Heldmaier et al. 1982, 1986; Horwitz 1989). It is mainly associated with the uncoupling protein of mitochondria of brown adipose tissue (BAT), and is activated by the sympathetic nervous system (Hayward 1971; Janský 1973; Rothwell and Stock 1979; Nedergaard and Cannon 1984; Cannon and Nedergaard 1985; Heldmaier et al. 1986; Nicholls et al. 1986; Horwitz 1989). However, BAT and the uncoupling protein appear to be restricted to several orders of placentals (Smith and Horwitz 1969; Néchad 1986; Klaus et al. 1991; Hayward and Lisson 1992). In marsupials and other mammals that do not possess BAT, other mechanisms, as for example vascular control of resting muscle thermogenesis and heat production of liver and other inner organs, appear to be important (Colquhoun and Clark 1991; Hayward and Lisson 1992; Eldershaw et al. 1996). Primarily due to an increased capacity for non-shivering thermogenesis, the thermogenic capacity for T_b regulation can be improved in winter (Wunder 1984; Merritt 1986; Heldmaier et al. 1986, 1989, 1990; Klaus et al. 1988).

Shivering thermogenesis results from the heat that is released during tremor of skeletal muscles (Heldmaier et a.. 1986, 1990; Horwitz 1989). For small placentals, shivering thermogenesis is an additional source of heat during severe cold exposure (Heldmaier et al. 1986, 1990; Horwitz 1989). Nevertheless, in small marsupials it may be an important heat source to cope with even moderate cold (May 1993).

Since mammals rely on endothermic thermogenesis to elevate T_b above T_a, a highly effective insulation becomes necessary for them to keep the endogenous heat from dispersing. With the improvement of insulation, homeothermy in mammals can be maintained efficiently in the cold (Feist and White 1989). The increase of body insulation, or the decrease of total body conductance (C), has been accomplished in mammals by the development of fur, which traps an insulating layer of air between the body and the environment (Scholander et al. 1950b; Hammel 1955). It has been documented that mammals generally have a conductance that is several times lower than that of similar-sized ectotherms (Herreid and Kessel 1967; Hulbert 1980a; Aschoff 1981).

Since decreasing heat loss is of great importance for mammals to reduce metabolic costs of heterothermy in the cold, mammals have evolved many other adaptations to keep body heat (Schmidt-Nielsen 1964; Feist and White 1989). Behavioural adaptations may include changes in daily and seasonal activity patterns, selection of a protective microhabitat, nest building, nest-sharing and huddling, as well as seasonal migrations for those species capable of long distance movement (Kenagy 1973; Whitney 1976; Heldmaier et al. 1982; Vogt and Lynch 1982; Madison 1984; Schmidt-Nielsen 1990; Merritt 1995).

Long-term morphological acaptations of many larger mammals that reside in cold-dominated regions include increased body size and shortening of appendages to reduce the relative surface area, thus reducing thermal conductance and heat loss (Bradley and Deavers 1980). Bergmann (1847) and Allen (1877) attempted to explain

the determination of body size and body shape by their well-known climate rules. These rules claim that races of mammals in a cold climate tend to be larger and tend to have shorter appendages than races of the same species in a warmer climate. Nevertheless, the thermal advantage appears to be only one of many dominating factors for the determination of body size and body shape (Feist and White 1989). For instance, prey size appears to be a good predictor for the body size of carnivores, and the size of appendages is not always related to geographical features of a mammal's residence (McNab 1971; Stevenson 1986).

Besides the long-term changes, heat loss can also be reduced by improving whole body insulation on a seasonal basis. Mammals may accumulate a large amount of subcutaneous fat before winter (Davis 1967; Kenagy 1973). Abundant body fat acts as an insulator, but also reduces the relative surface area for heat transfer and can be used as fuel (Hainsworth 1981; Schmidt-Nielsen 1984; Florant et al. 1989).

Furthermore, mammals that experience harsh winters usually change the thickness, density and texture of their pelage seasonally to obtain a more insulative pelage during winter (Scholander 1950a; Irving 1972; Johnson 1984). Changes of fur colour also appear to be advantageous for resisting heat loss, at least in some medium-sized species (Feist and White 1989). For example, the white pelage colour of some arctic hares and foxes during winter may transfer more radiation to the skin under windy and sunny conditions in the cold weather (Walsberg et al. 1978).

In addition, many animals employ regional heterothermy, during which a constant core T_b is maintained, but a cooling of body extremities is tolerated (Irving and Krog 1955; Guard and Murrish 1975; Baust and Brown 1980; Henshaw 1986). Regional heterothermy reduces the thermal gradient between the body surface and the environment, and thus less heat from the body is lost. The regional heterothermy of mammals often involves an adjustment of the peripheral circulation, which changes the amount of heat loss from different parts of the body.

1.1.2 Homeothermy: Disadvantage and Challenges for Small Mammals

Even though heat loss can be successfully decreased by these adaptations to the cold, the endogenous heat production of mammals that is required to maintain homeothermy is still energetically expensive. Therefore many small-sized mammals survive much shorter times during starvation than similar-sized ectotherms (Bartholomew 1982; Hanski 1984). There is general agreement that this high energy expenditure is probably the major disadvantage of endothermic heterothermy (Hulbert 1993; Louw 1993). Obviously, to fulfil the need of high rate of metabolism, a sustained large amount of food is required. However, in the wild, the availability of free energy that animals can convert into physiological work and heat varies in both time and space. This is further complicated by seasonal and daily variations in T_a and in the availability of water (Louw 1993). Hence, homeothermy can be an energetic burden and may reduce the chance of survival of a mammal, particularly if several environmental stresses are encountered simultaneously (Withers 1992).

Homeothermy is most challenging for small mammals. Because small mammals have a large surface area to body mass ratio and thus a relatively high thermal conductance, they tend to have a high rate of heat loss (Scholander et al. 1950a, b; Bradley and Deavers 1980; McNab 1980, 1983; Schmidt-Nielsen 1984). In addition, a small body size restricts the capacity to carry thicker fur thus limits pelage insulation, as well as fat storage (Schmidt-Nielsen 1984). In the cold, therefore, small mammals must produce much more heat per gram of tissue than large mammals to compensate for this great heat loss (Kleiber 1961; McNab 1983). The high heat production requires high food intake which is often restricted by the inability for long distance movement and migration of small species (Bartholomew 1982). Therefore, small mammals are not always able to avoid unfavourable climatic conditions, and can be frequently exposed to periods of food shortage and lack of fuel for thermogenesis

(McNab 1983). Thus, small mammals require other thermophysiological adaptations to overcome these energetic limitations.

1.2 Hibernation and Daily Torpor in Mammals

1.2.1 Heterothermy: A Thermoregulatory Adaptation

The constant high energy drain of endothermy can be avoided if mammals give up their homeothermy temporarily, and become torpid. This strategy has been adopted by some mostly small-sized mammals and birds, known as heterothermic endotherms. Heterothermic endotherms do not maintain a steady high T_b all the time, although they may have a similar thermogenic capacity as their homeothermic relatives (Wang and Abbotts 1981; Chappell and Bachman 1995). Heterothermic mammals periodically abandon homeothermy and become dormant allowing their T_b to fall to levels that are often only a few degrees above their surroundings (Lyman 1982a; Barnes and Ritter 1993). This thermal response appears to be similar to that of poikilotherms in which T_b passively follows T_a (Bartholomew 1982; Buffenstein and Yahav 1991). A low MR, low T_b and a small ΔT between T_b and T_a is characteristic for torpid mammals (Lyman et al. 1982). Due to this decrease of metabolism during torpor, less fuel is consumed. As has been revealed by a field study, during a hibernation season that is nearly two thirds of the year, animals may spend only about 15% of annual energy expenditure (Kenagy et al. 1989). Because torpor can substantially decrease energy expenditure, it provides a temporary solution for small mammals to escape environmental constraints, in particular, reduced food availability (Wang 1978, 1979; Kenagy 1989; Kenagy et al. 1989).

Since superficially a heterothermic mammal reverses the evolutionary process from poikilothermy to homeothermy, it was originally believed that heterothermy is the consequence of inadequacy or poorly developed homeothermic thermoregulation

(Kayser 1961; Cade 1964). Torpor was considered to occur only in those groups of mammals which are phylogenetically most primitive (Kalabukhov 1956; Cade 1964).

However, later studies revealed that mammalian torpor is substantially different from poikilothermy (Tucker 1965). In contrast to poikilothermic animals, torpid animals are able to thermoregulate using metabolic heat production (Heller and Hammel 1972; Hudson 1973, 1978; Heller and Colliver 1974; Florant and Heller 1977; Florant et al. 1978; Heller et al. 1978). Proportional increase of heat production has been observed when mammals are torpid at a T_a that is below the critical temperature (T_{tc}) for thermoregulation (Wang and Hudson 1970, 1971; Heldmaier and Ruf 1992), and during torpor whenever the temperature of POH falls below a T_{set} (Heller and Colliver 1974; Florant and Heller 1977; Pivorun 1986). In addition, torpid mammals are capable of regaining their normothermic T_b spontaneously or whenever disturbed, by using only endogenous heat (Heller and Hammel 1972; Lyman 1982b). It is thus obvious that heterothermic mammals are not only able to regulate T_b during normothermia, but can also survive a lowering of the thermostat T_{set} and have a broader thermosensitivity (Florant et al. 1978; Wünnenberg et al. 1986). It has been proposed that the same populations of POH neurons of the central neural system are responsible for thermoregulation during torpor and during normothermia (Hammel 1967; Heller and Colliver 1974; Heller 1979), and the effective heat production of heterothermic mammals can be activated or deactivated at different T_b levels (South et al. 1978; Heller 1979). This advanced central neural control of torpor suggests that mammalian torpor is an advancement of thermoregulation (Heller and Colliver 1974). It appears that heterothermic mammals are better adapted to energetically restrictive situations and variable T_as than the strictly homeothermic mammals.

1.2.2 Evolution of Mammalian Torpor

It is now generally accepted that all living mammals, including the Monotremes (Prototheria), the Marsupials (Metatheria) and the Placentals (Eutheria), belong to the group Theria (Dawson 1989). Extant mammals most likely were derived from a common Cynodont ancestor, probably within the family Galesauridae in the late Triassic over 200 million years ago (McKenna 1969; Crompton and Jenkins 1973). Monotremes probably split from the branch leading to marsupials and placentals about 180 million years ago (Eisenberg 1981). Marsupials are likely to have branched from the placentals about 120 million years ago (Archer 1984). Probably in the late Cretaceous, Australian marsupials separated from South American marsupials (Archer 1984).

As an advanced thermoregulatory adaptation, torpor is not only associated with mammals which are taxonomically primitive. Torpor has been adopted successfully by members of all three mammalian subclasses. In monotremes, torpor has been observed in the short-beaked echidna, *Tachyglossus aculeatus* (Grigg et al. 1992; Nicol et al. 1992; Grigg and Beard 1996). In marsupials, torpor has been reported for 9 families (Dawson 1989; Geiser 1994; see below for details). In placentals, torpor has been reported for 5 orders. These are the Insectivora (Kayser 1964; Vogel 1976; Tähti 1978), Chiroptera (Microchiroptera and Megachiroptera; Hock 1951; Coburn and Geiser 1996), Primates (Schmid 1996; Ortmann et al. 1997), Carnivora (Hock 1960; Harlow 1981; Fowler and Racey 1988), and Rodentia (Morrison and Ryser 1962; Wang and Hudson 1970; 1971; Buffenstein 1984b; Barnes et al. 1986a, b; Ruf 1993; Körtner and Heldmaier 1995; Shenfor and Snyder 1996). Clearly, heterothermy is found in a diverse number of mammalian groups (Wang 1989).

These heterothermic mammals may be found in the arctic, boreal and temperate regions; arid and semi-arid places where shortage of food and water occur periodically (Wang 1989). They also can be from subtropical and tropical climates where daily and

seasonal challenges disrupt feeding patterns and create a temporary energy shortage (McNab 1978; Wang 1987, 1989; Coburn and Geiser 1996). Although torpor and evolution of endothermy are linked, it is not clear whether torpor is a monophyletic event or whether it has evolved polyphyletically in different groups of mammals.

Mrosovsky (1971, 1990) has suggested that mammalian heterothermy has most likely evolved from heterothermic ancestors of present-day homeotherms. It has been theorised that torpor in endotherms is a monophyletic evolution of plesiomorphic traits (Augee and Gooden 1992). Recent studies indicate some plasma protein genes are expressed only in torpid animals (Serre et al. 1992, 1995; Martin et al. 1993). Thus it has been proposed that mammalian torpor is probably the recurring expression of early traits, and has evolved from "a common pattern of circadian heterothermy that was facilitated by the advent of endothermic thermogenesis" (Malan 1996).

Alternatively, it has been proposed that torpor has evolved after precise control of T_b had been attained. It is seen as a thermoregulatory strategy of advanced mammals as a result of evolutionary convergence (Hudson 1967, 1973, 1978). Hudson (1973) has suggested that torpor represents a specific adaptation for each different ecological niche, and thus can have "repeatedly evolved depending upon the selective pressure" (Hudson 1973).

Therefore, it is possible that torpor has evolved independently many times in mammals, in some cases from primitive endothermy, and in others from physiologically advanced homeotherms (Bligh 1973).

1.2.3 Relationships between Hibernation and Daily Torpor

Heterothermic mammals can be divided into two general categories on the basis of their torpor patterns (Geiser and Ruf 1995). Daily heterotherms are the species that display shallow, daily torpor with minimum body temperature (T_{brnin}) defended during torpor generally between 10°C to 25°C and torpor bouts of less than 24 h

(Hudson 1973, 1978; Geiser and Ruf 1995). Hibernators are the species that display deep, prolonged torpor (hibernation), with T_{bmin} generally between 2°C and 10°C and torpor bouts of several days or weeks (Hudson 1973; Wang 1987; Geiser and Ruf 1995). There is general agreement that patterns of hibernation and daily torpor are qualitatively similar but quantitatively different (Hudson 1973, 1978; Wang 1989; Geiser and Ruf 1995). By analysing data of 103 mammalian and avian heterothermic species exhibiting either daily torpor or hibernation, Geiser and Ruf (1995) concluded that the differences of the means of many physiological variables, including the minimum metabolic rate during torpor (TMR) and the T_{bmin} between daily heterotherms and hibernators are significant. In particular, the maximum torpor bout duration and the minimum TMR differ clearly by more than 10-fold. The significant differences of these physiological variables thus warrant the distinction between shallow daily torpor and deep prolonged hibernation as two different physiological states of torpor in endotherms (Geiser and Ruf 1995).

Nevertheless, the separation of the physiological variables between the two torpor patterns is sometimes difficult. This is because some physiological variables for the two torpor patterns, as for example T_{bmin}, may overlap. Another complicating factor is that many hibernators may display short bouts of torpor. Some hibernators display torpor on a daily basis during summer or at the beginning and the end of a hibernating season, but prolonged bouts during the mid-hibernating season (Twente and Twente 1965; Wang 1979; Barnes and Ritter 1993). Some become torpid on a daily basis at relatively high T_as, and are torpid for long time at low T_as (French 1982a, 1985). To date there are no data to clarify whether the short bout torpor in hibernators and torpor in daily heterotherms are physiologically identical or different.

It is not known whether daily torpor of daily heterotherms and hibernation of hibernators follow an evolutionary or a physiological progression, or if they represent an evolutionary convergence that developed independently (Lyman et al. 1982; Geiser

and Ruf 1995). In the literature, hibernation and daily torpor have been frequently treated as a continuum representing different degrees of depth of mammalian torpor (Twente and Twente 1964; Hudson 1978). Apparently, hibernators can tolerate a greater extent of T_b reduction and are capable of both daily torpor and hibernation. Thus hibernators have been considered to be in a more advanced stage in the development of torpor. Daily heterotherms have been considered to be in a less advanced stage of torpor development, since they have not evolved the ability to tolerate the low T_b that is common in deep hibernation (Hudson 1973).

However, daily torpor and hibernation may not really represent an evolutionary progression (Lyman et al. 1982). Both torpor patterns may be employed by species that are phylogenetically advanced as well as species that are phylogenetically primitive. Since the origins of mammalian torpor are unclear, the ability to withstand a lower T_b during torpor does not necessarily suggest that hibernation is a more advanced stage in the development of torpor. Furthermore, the resistance of the heart and other organs to cold exposure does not appear to be a continuum, but hibernators and daily heterotherms appear to represent two groups (Lyman et al. 1982). While organs of hibernators may function at a T_a as low as 0°C, a temperature that is slightly lower than 25°C can be lethal for tissues of daily heterotherms (Lyman 1964; Hudson and Eller 1974; Geiser et al. 1989). These differences of cold tolerance of tissues may reflect the differences in the response of cell membranes to low temperature between hibernators and daily heterotherms (Raison and Lyons 1971, 1988; Geiser and McMurchie 1984; Aloia et al. 1986; Raison et al. 1988; Aloia and Raison 1989).

In addition, differences in morphological and physiological preparations for torpor are also obvious between hibernation and daily torpor. Hibernators usually exhibit torpor when sufficient amounts of body fat and/or food have been accumulated (Hoffman 1964; Kenagy 1980, 1989; Murie and Boag 1984; Grahn et al. 1994; Körtner and Heldmaier 1995). Hibernating bats seem to be the exception since

they do not normally accumulate a large amount of body fat. However, the amount of BAT in bats also undergoes annual cycles and BAT peaks usually appear before the hibernation season. The physiological preparations of hibernation appear to be associated with strictly circannual rhythms, which control various biological functions, including neuroendocrine activity and thermal behaviour of membrane enzymes (Strumwasser et al. 196'; Mrosovsky 1978, 1986; Hulbert and Hudson 1976; Pengelley et al. 1978; Augee et al. 1984; Zucker et al. 1993). Daily torpor of daily heterotherms, however, is more frequently related to an acute internal or environmental energy shortage (Morhardt and Hudson 1966; Buffenstein 1985; Geiser 1988b). Changes of neuroendocrine functions associated with daily torpor are not evident (Hudson 1981; Wang 1989). These distinct differences between hibernators and daily heterotherms do not seem to support the notion that hibernation developed from daily torpor. In contrast, they suggest that the two torpor patterns are a physiological response to the ecological needs of a species (Wang 1987).

This argument is supported by the fact that torpor patterns are obviously affected by environmental and morphological influences. Hibernation usually occurs in areas where the low winter T_as represent a regular and predictable challenge (Lyman 1982b; Wang 1987). In contrast, daily torpor often occurs in climates that are characterised by more significant daily fluctuations of environmental stresses, although it is not restricted to these environments (Lyman 1982b; Wang 1987). In addition, daily torpor is more common in small-sized species that can store less internal fuel than the relatively large hibernators (Geiser and Ruf 1995). Employment of daily torpor may enable these small mammals to overcome short-term food shortage and provide enough time to forage and feed. Nevertheless, the apparent adaptation to the environment does not exclude the possibility of a phylogenetic contribution to the determination of torpor pattern. Unfortunately, our present

knowledge is too limited to answer how closely daily torpor and hibernation are related phylogenetically and physiologically.

1.2.4 Thermoregulation and Torpor of Marsupials

Thermoregulation and torpor of marsupials are of great interest because they provide potential insight into evolution of both mammalian homeothermy and heterothermy (Dawson 1989; Geiser 1994). Because marsupials appear to have a lower resting T_b and BMR than placentals (Hulbert 1980a), they were originally considered as a group of functionally primitive mammals with an imperfect ability of maintaining homeothermy (Sutherland 1897; Martin 1902; Brown 1909; Johansen 1962).

However, this view of a primitive thermal physiology has been challenged (Dawson 1969; Dawson and Hulbert 1969; 1970; MacMillen and Nelson 1969; McNab 1988). Most of the marsupial species investigated possess an excellent ability of normothermic thermoregulation (Bartholomew 1956; Dawson and Hulbert 1969; Dawson and Olson 1988). While marsupials show a relatively low BMR compared to placentals (MacMillan and Nelson 1969; Dawson and Hulbert 1970; McNab 1988), they may even have a capacity for a higher metabolic scope than placental mammals (Baudinette et al. 1976; Baudinette 1982; Dawson and Dawson 1982; Reynolds and Hulbert 1982; Hinds and MacMillen 1984). In addition, the generally lower BMR and T_b of marsupials may largely reflect the influences of the arid environment and the zoogeographical zone in which marsupials evolved (Dawson 1989; Lovegrove 1996), and may be an adaptation to conserve water and/or to avoid hyperthermia (McNab and Morrison 1963; MacMillen and Christopher 1975; Schmidt-Nielsen 1990; Lovegrove et al. 1991). When compared with similar-sized placentals that have similar food preferences and are from similar habitats, the BMRs do not appear to differ among different mammalian groups (McNab 1978, 1986; Hayssen and Lacy 1985; Buffenstein and Jarvis 1985; Lovegrove 1986, 1996).

Although it has been known for many years that torpor occurs in some Australian marsupials (Hickman and Hickman 1960; Bartholomew and Hudson 1962; Morrison 1962, 1965; Morrison and McNab 1962, Godfrey 1966, 1968), the widely held belief that marsupial torpor was an uncommon phenomenon persisted until recently. This view was apparently strengthened by the assumption that the mild environmental conditions in Australia do not provide adequate selective pressure for the evolution of torpor. Nevertheless, marsupials that reside in southern and central Australia may encounter severe climatic conditions, including snow and drought. Accordingly, during the past two decades many marsupials have been found to exhibit torpor. Torpor has now been reported in 9 families of small marsupials (Geiser 1994), and it has become clear that torpor, especially daily torpor, is widely used by many marsupial species (Dawson and Wolfers 1978; McNab 1978; Wallis 1979, 1982; Fleming 1980; Dawson 1989; Geiser 1994). This common occurrence of torpor in marsupials may be correlated with the low BMR that is common for marsupials (McNab 1983; Lovegrove 1996), since a low BMR, and thus a high T_{lc}, may facilitate torpor at high T_as (Buffenstein 1985; Buffenstein and Jarvis 1985).

Australian marsupials that display daily torpor include small possums (Petauridae), the honey possum (Tarsipedidae), and carnivorous marsupials (Dasyuridae). Daily torpor in the Dasyuridae has been observed in at least 20 species, which comprises more than one-third of the species in the family (Geiser 1994). In addition, the numbat (Myrmecobiidae) and the marsupial mole (Notoryctidae) probably also display daily torpor (Wood-Jones 1923; Serventy and Raymond 1973; Geiser 1994). In addition, daily torpor has also been reported for several opossums (Didelphidae) from South America (Morrison and McNab 1962; Geiser 1994). Deep hibernation in Australian marsupials, however, has only been found in pygmy-possums (Burramyidae) and the feathertail glider (Acrobatidae; Fleming 1985a, b; Geiser and Broome 1991; Jones and Geiser 1992; Körtner and Geiser 1995, 1996).

Hibernation has also been observed in *Dromiciops australis* (Microbiotheriidae) of South America (Rosenmann and Ampuero 1981; Geiser 1994).

In the laboratory, daily torpor and hibernation in many marsupials can occur at any time of a year. In a few species torpor frequency and torpor depth do not change with season, although there are many others in which torpor may be restricted or enhanced in winter (Dawson and Wolfers 1978; Morton and Lee 1978; Geiser and Baudinette 1987; Frey 1991). Among the species that display spontaneous daily torpor, many become torpid at moderate T_as, while torpor in some species has been observed only at relatively low T_as (Geiser and Baudinette 1987). These specific characteristics of marsupial torpor appear to be mainly related to the climatic conditions of their environment, where changes in T_a and food and water availability are usually unpredictable rather than seasonal, depending largely on variable rainfall (Lee and Cockburn 1985; Nicol and Andersen 1996). Consequently, the seasonality of torpor of Australian marsupials is far less distinct than in most placentals from the northern hemisphere that experience seasonally harsh conditions (Geiser 1994).

Despite these specific differences, it is generally accepted that the general patterns of hibernation and daily torpor of marsupials do not differ from those known for placental and monotreme heterotherms (Geiser 1994). The differences in physiological variables such as the minimum TMR, torpor bout duration, and T_{bmin} between marsupials and placentals are not as pronounced as those between daily torpor and hibernation of all mammals (Geiser and Ruf 1995). Thus patterns of hibernation and daily torpor in marsupials are parallel to those in other mammalian groups. As in placental mammals (Lyman 1982c; Wang 1987), little is known in marsupials about whether daily torpor and hibernation are closely associated or whether they are quite separate types of thermoregulation.

1.3 Aims of the Thesis

1.3.1 Significance and General Aims

Although it is known that physiological variables such as the minimum TMR during hibernation and daily torpor differ quantitatively (Hudson 1973, 1978; Geiser and Ruf 1995), the reasons for these differences are not well understood (Morrison 1993). Several hypotheses have been proposed to explain how heterothermic endotherms alter their physiological functions to achieve low metabolic rates. These include depression of metabolism due to respiratory acidosis (Malan 1988; Milsom 1993), temperature effect on metabolic rate together with metabolic inhibition (Malan 1986; Geiser 1988a), and metabolic downregulation associated with a reduction of ΔT (Heldmaier and Ruf 1992) or thermal conductance (Snyder and Nestler 1990).

However, in the past, most studies on thermal and metabolic physiology of torpor have concentrated on a few hibernating species, mainly rodents and bats (Lyman et al. 1982). Not many systematic attempts have been made to investigate interrelations between physiological variables during torpor in species beyond a limited number of rodents hibernators (Morrison 1993). Therefore, the above hypotheses have not been examined adequately in various groups of mammalian heterotherms that exhibit hibernation and daily torpor. According to Morrison (1993), information to assess the current controversy about the reduction of metabolism during torpor are still insufficient. Largely due to lack of evidence from comparable experimental approaches during hibernation and daily torpor, it is most difficult to address whether different physiological processes are involved in the control of physiological variables in animals of different torpor categories (Wang 1987).

Moreover, due to the effect of T_a on thermoregulation, meaningful conclusions about thermophysiology in endotherms can only be made if measurements are conducted over a wide range of T_a (Bartholomew 1982). This applies particularly to mammalian torpor physiology and energetics, since T_a influences almost all

physiological variables during torpor. Nevertheless, investigations on physiological variables during torpor as function of T_a are often difficult to perform, because many experimental animals display torpor only over a narrow T_a range. In contrast, many small marsupials display torpor over wide ranges of T_a (Dawson 1989; Geiser 1994), and therefore represent an ideal group of animals for investigating physiological variables during torpor and their relationships with T_a. Furthermore, since both patterns of daily torpor and hibernation in marsupials appear to be similar to those in placentals (Geiser 1994; Geiser and Ruf 1995), small marsupials provide ideal subjects for the comparative study of physiology of mammalian hibernation and daily torpor.

The general aims of the present thesis are to investigate the differences between hibernation and daily torpor of two similar-sized small Australian marsupials *Cercartetus nanus*, a hibernator; and *Sminthopsis macroura*, a daily heterotherm. To achieve this, the MR, T_b, ΔT and C of the animals in association with their torpor patterns were determined over T_as ranging from the T_{lc} to below the T_{tc} during torpor entry, steady-state torpor, and arousal under the same experimental conditions. The interrelations between these variables were investigated during different phases of torpor and in different T_a ranges. In addition, behaviour of T_a selection and relationships between energy expenditure and duration of torpor and arousal were determined at different T_as. The possible differences of thermophysiology during both normothermia and torpor between marsupials and placentals were also addressed.

1.3.2 Specific Aims

The first part of this work (chapter 3) is to investigate the interrelations between MR, T_b, ΔT, and C during normothermia and during the steady-state hibernation and daily torpor of the two species. It emphasises on comparison of differences of MR reduction of the hibernator and the daily heterotherm. It also addresses whether

physiological variables during torpor and normothermia of the two marsupial species differ from those of placental heterotherms.

The second part of the work (chapter 4) aims to investigate the time course of changes of MR and T_b during transient states between normothermia and torpor. The interrelations between these variables during entry into and arousal from torpor in the two species are investigated. It also analyses the influences of other factors such as T_a and conductance on cooling and rewarming rates. In addition, it addresses the possible differences between the hibernator and the daily heterotherm regarding cooling and rewarming rates and the employment of the thermogenic capacity during rewarming process.

The third part of the work (chapter 5) is to provide detailed information on energy conservation by the use of hibernation and daily torpor and the influence of T_a upon energy savings. In addition, it analyses how torpor bout duration is related to energy expenditure during daily torpor and hibernation. It also investigates influences of arousal duration on energy expenditure during torpor.

The last part of the work (chapter 6) is to investigate the interrelations between torpor and temperature selection under different food regimes in the two species. It is the first study on temperature selection in marsupial heterotherms and on whether energetic benefits explain this behaviour of T_a selection during hibernation and daily torpor. It also provides information on relationships between torpor and activity when these marsupials have to cope with energy crises.

Chapter Two: General Materials and Methods

2.1 Experimental Animals

2.1.1 *Sminthopsis macroura*

The experimental animal for studying thermoregulation and energetics in relation to daily torpor was *Sminthopsis macroura* (plate 1). The stripe-faced dunnart or Darling Downs dunnart, *Sminthopsis macroura*, (synonyms: *S. froggati, S. larapinta*) is a polyprotodont marsupial of the family Dasyuridae. It is mouse-sized, weighing 15 - 40 g, with a head and body length of 70 - 100 mm. Its specific name refers to a carrot-shaped tail which is 80 - 110 mm in length and is used for fat storage, as is the case in other species of the genus. The animal has a fox-like head with large eyes. Its forelimbs and hindlimbs are of similar length. The common name of the animal is derived from a stripe line of dark hair running from between the eyes to between the ears. Similar to other members of the family, *S. macroura* has a dental formula of 4/3, 1/1, 3/3, 4/4 = 46 (Hyett and Shaw 1980). Its incisors are small and pointed, the canines are large and have a sharp edge, and the molars have three sharp cusps adapted to an insectivorous/carnivorous diet.

S. macroura is native to mainland Australia. It is distributed in central and northern Australia (Fig. 2.1), where it mainly occurs in low shrubland and grassland on clay, sandy or stony soil. Most of its habitats are arid and semiarid areas with highly variable rainfalls and pronounced daily temperature fluctuations (Morton 1983; Morton et al. 1983).

The behaviour of *S. macroura* in the wild is largely unknown. However, the animal is probably nocturnal and is active only during the night time, and feeds by preying on insects and other small invertebrates (Morton 1982; Morton et al. 1983).

During the day time, it probably shelters in cracks in the soil or under rocks or logs to avoid temperature extremes and of course from predators (Morton et al. 1983).

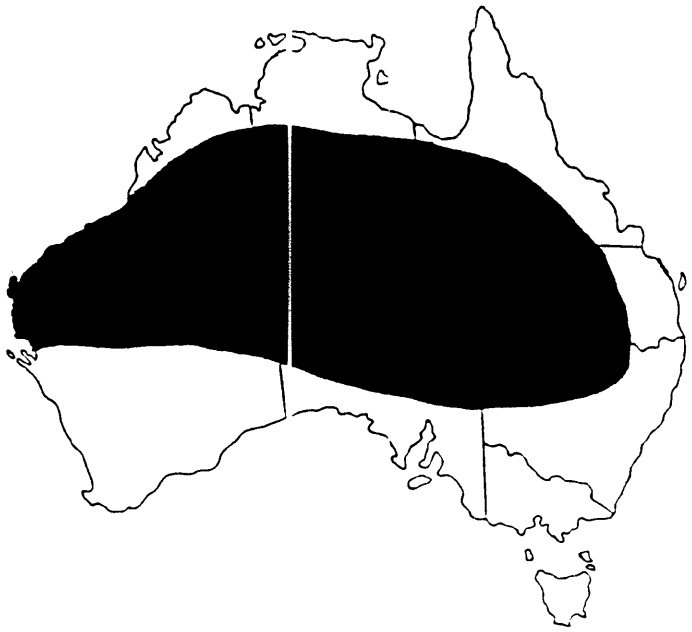

Fig. 2.1. Distribution range of the stripe-faced dunnart, *Sminthopsis macroura* (from Strahan R., ed. Complete Book of Australian Mammals 1983).

Breeding is seasonal from late winter to late summer. Captive studies have shown that females will attempt to raise two litters during this time (Lee et al. 1982; Woolley 1990). The usually litter size is 6 - 8 (Lee et al. 1982; Woolley 1990). In captivity, the gestation period is 12 days, and young were weaned at about 70 days (Woolley 1990). Neonates can be fully enclosed by the marsupium which is poorly developed and opens posteriorly (Woolley 1982, 1990).

Thermal Physiology and Torpor

The first experimental information on torpor and thermal physiology of *S. macroura* stems from Morrison's work in 1965. He measured deep rectal temperature

of several dasyurid species, including *S. macroura*, when fed and watered *ad libitum*. He found a substantial daily body temperature cycle in *S. macroura* with the lowest values appearing around midday and highest values at midnight (Morrison 1965).

This was followed by the study of Godfrey (1966, 1968) who measured continuously the animal's surface temperature using a thermistor that was mounted on a copper plate in the floor of the animal's nest box (Godfrey 1966, 1968). She reported that spontaneous torpor occurred regularly and the animal usually entered torpor around the time of sunrise and aroused at about midday.

Geiser and Baudinette (1985, 1987) subsequently carried out more detailed physiological studies to determine the fluctuation of MR during a torpor cycle. They demonstrated the ability of torpid *S. macroura* to regulate body temperature at a T_{bmin} of about 15°C, and observed torpor bouts of up to 17 h. They also reported that the timing of daily torpor of *S. macroura* is influenced by T_a and photophase. Although spontaneous torpor occurred throughout the year, it was more common and deeper in winter. In addition, food restriction enhanced the incidence of torpor (Geiser and Baudinette 1985, 1987).

Provenance and Maintenance in the Laboratory

The animals used in this study were adult males (1 - 2 years) and were obtained from a breeding colony at La Trobe University, Melbourne. Animals were maintained in the Department of Zoology at the University of New England individually in cages (30 × 22 × 14 cm). The cages contained sawdust, shredded paper and nest-boxes and were cleaned once every week. The animals were fed with canned dog food mixed with macerated cat food pellets supplemented with calcium and vitamins. Occasionally, *Tenebrio* larvae and boiled chicken eggs were provided. The animals were weighed weekly, as well as before and after each experiment. The photoperiod throughout the experiments was 12L : 12 D (lights on 0600h - 1800h). Details about

T_a, feeding routine and BM of the animals are provided in the "Materials and Methods" section of chapters 3 - 6.

2.1.2 *Cercartetus nanus*

The experimental animal used in the project for studying thermoregulation and energetics in association with hibernation was *Cercartetus nanus* (plate 2). The eastern pygmy-possum, or dormouse possum, *Cercartetus nanus*, (Synonyms: *Cercaertus nanus*) is also small. The animal has large eyes, big oval ears and pinkish nose. It has a long and prehensile tail and grasping feet suitable for climbing trees, and a brush-tipped tongue appropriate for gathering pollens. Its head and body length is 70 - 110 mm, tail length is 75 - 105 mm, and the tail can be used for fat storage. The animal weighs 15 - 45 g depending on the amount of fat storage. The dentition of the species is 3/1, 1/0, 3/3, 3/3 = 34 (Hyett and Shaw 1980).

Cercartetus nanus is distributed in Tasmania and along the southern-east coast of mainland Australia (Fig. 2.2). It is mainly found in rainforest, sclerophyll forests, or tree heath. It nests in small tree hoes, and each individual uses several nests (Turner and Ward 1983). The animal is generally nocturnal, becoming active a few hours after dusk. It feeds mainly on nectar and pollen of banksias, eucalypts and bottlebrushes, but fruits and insects are also taken when blossoms are less abundant (Turner 1984; Huang et al. 1987).

In the wild, breeding takes place from winter to late spring in Tasmania, and from spring to autumn, or to early winter, in mainland Australia (Turner and Ward 1983; Ward 1990; Bladon 1993). Females usually have 2 litters per year, but some have three (Ward 1990). Litter size is usually 4-5, but ranges from 2 to 6 (Ward 1990). The pouch is fully developed and opens anteriorly. Young stay in the pouch for about 6 weeks and become independent when they reach half of adult mass (Turner and Ward 1983). Males are reproductively active throughout the year (Ward 1990).

The animal increases BM to the end of summer, and the base of tail usually becomes noticeably thickened (Turner and Ward 1983).

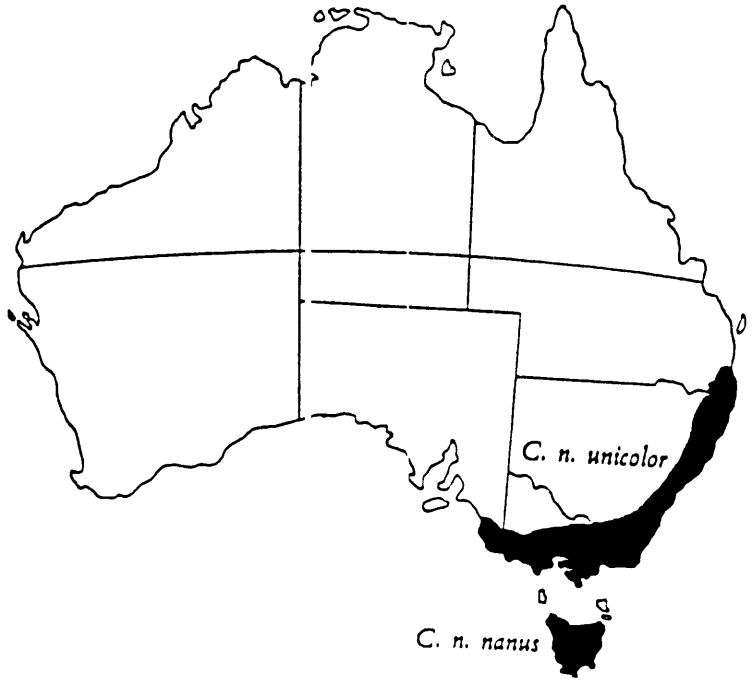

Fig. 2.2. Distribution range of the eastern pygmy-possum, *Cercartetus nanus* (from Strahan R. ed. Complete Book of Australian Mammals 1983).

Thermal Physiology and Torpor

As in other members of the family, the ability to become torpid during cold weather is well developed in *C. nanus*. When kept in outside cages, the animal regularly enters torpor in winter even under seemingly ideal conditions (Hickman and Hickman 1960). Wakefield (1970) reported that the animal is dormant more frequently during rain periods, and this was explained by the influence of high humidity upon its thermophysiology. However, other observations suggest that *C. nanus* may enter torpor during all seasons regardless of T_a and food conditions in the laboratory (Wallis 1979).

The first systematic physiological measurements on *C. nanus* were conducted by Bartholomew and Hudson in 1962. They investigated several variables including MR, T_b, water loss and heart rate in both normothermic and torpid individuals. They found that the BMR of this species was approximately only one-third of that expected for a similar-sized placental. However, the animals studied were obese, with an average body mass of 70 g and the high fat content might have caused an under-estimated BMR (Bartholomew and Hudson 1962). During torpor, T_b, MR and heart rate were all low indicating that the hibernation pattern of this species does not differ from that of placental hibernators (Bartholomew and Hudson 1962).

In a subsequent study, Geiser (1993a) determined the minimum TMR (0.018 mL g^{-1} h^{-1}), T_{bmin} (1.3°C) during torpor and the TBD (up to 27 days) of *C. nanus* collected in Victoria and Tasmania. In addition, the study suggests that in the laboratory *C. nanus* entered torpor from winter to summer when exposed to low T_as. Thus it seems that torpor in *C. nanus* is not strictly controlled by a circannual cycle. However, T_a has a strong effect on both torpor frequency and torpor bout duration (Geiser 1993a).

Provenance and Maintenance in the Laboratory

The animals used in this project were collected near Dorrigo, New South Wales, Australia (30°22'S, 152'45'E) in July 1993, July 1995 and May 1996. Since *C. nanus* are trap-shy, wooden nestboxes filled with bedding materials were hung on the trees in Banksia-dominated areas, and were eventually accepted by the resident population of *C. nanus*.

After collecting, animals were held individually in cages (30 × 22 × 14 cm) in a T_a and photoperiod controlled room in the Department of Zoology at the University of New England. The cages contained sawdust and shredded paper as bedding material. The animals were fed apples, walnuts, sunflower seeds and a mixed paste of baby cereal and honey, supplemented with calcium and vitamins. Water was provided *ad*

libitum. The cages were cleaned regularly and the animals were weighed once every week. The photoperiod was 12L : 12 D (lights on 0600h - 1800h) throughout the experiments. Details about T_a, feeding regimes and BM of the animal are provided in the "Materials and Methods" section of chapters 3 - 6.

2.2 Simultaneous Record of MR, T_b, and T_a

2.2.1 Metabolic Rate, MR

MR, measured as rate of oxygen consumption ($\dot{V}o_2$) was determined by open-flow respirometry. The equipment consisted of an oxygen analyser, a mass flowmeter (FMA-5606, Omega, Stamford), respirometry chambers (0.5 L in volume), a temperature-controlled cabinet (± 0.5°C), rotameters (7908, Aarlborg, New York), air pumps, and desiccator (silica gel) tubes.

The flowrate of outside air through the respirometry chambers was controlled by the needle valve of rotameters, and measured continuously by the mass flowmeter after moisture were removed by the desiccator. Sub-sample of dry air was subsequently pumped into the oxygen analyser for analysing oxygen contents. The oxygen content of air leaving the respirometry chamber was consecutively compared to that of the reference air.

Two different systems, system A (Fig. 2.3), and system B (Fig. 2.4) were applied in the experiments.

System A: This system was based on an Ametek Applied Electrochemistry S-3A/I (Pittsburgh) oxygen analyser, fitted with a high resolution output board (80335SE). It permitted the measurement of three animal channels and one reference channel in sequence. Scanning in 3-min intervals was achieved via solenoid valves controlled by a computer. Each channel was read every 12 min. Outputs from flowmeter and oxygen analyser were recorded via a 14-bit analog to digital interface card (Flytech) fitted to a personal computer.

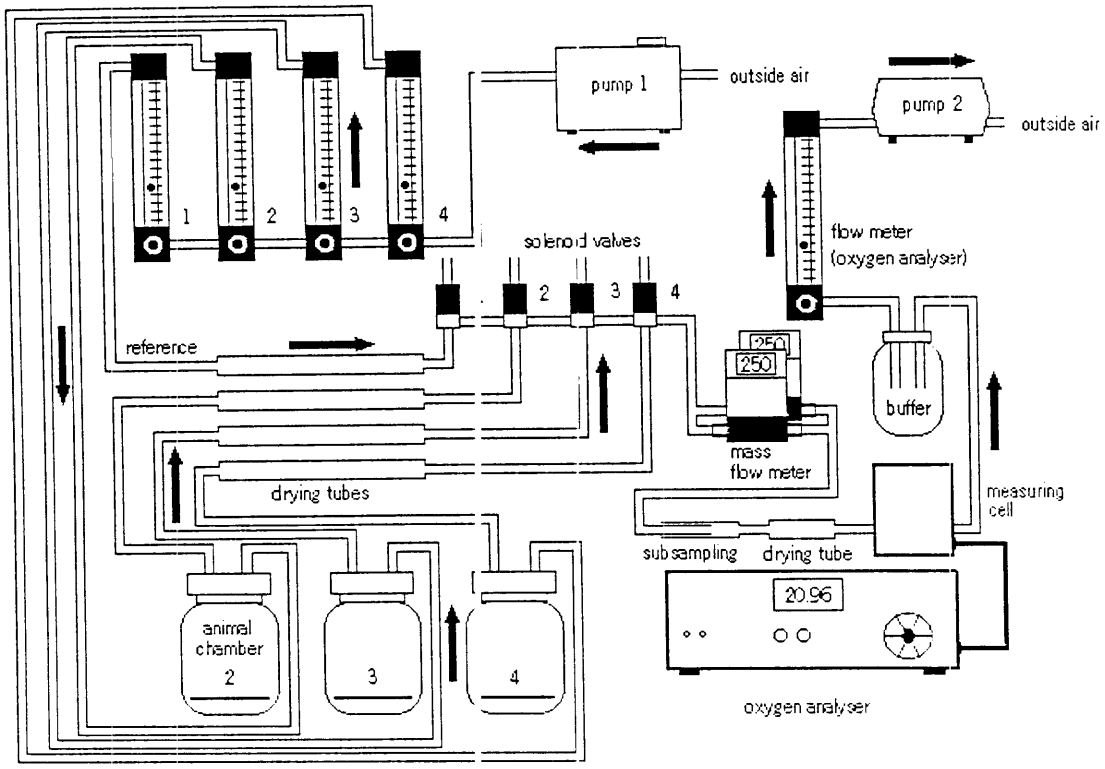

Fig. 2.3. Details of system A of the respirometry equipment.

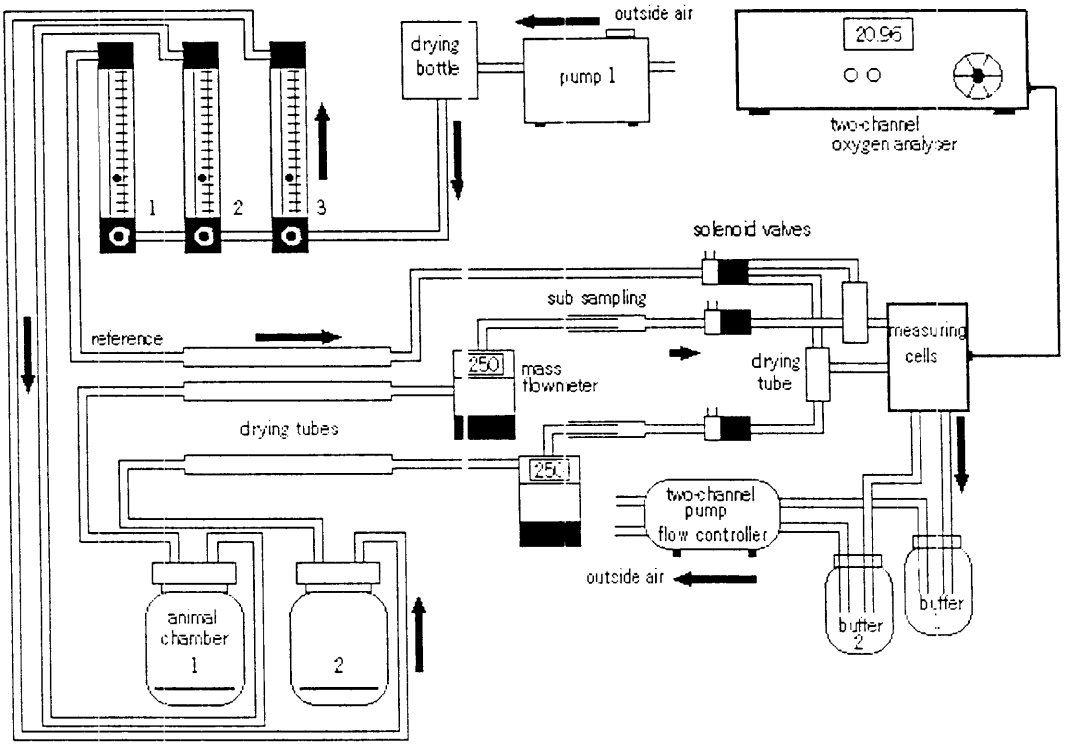

Fig. 2.4. Details of system B of the respirometry equipment.

System B: This system was based on an Ametek Applied Electrochemistry S-3A/II (Pittsburgh) oxygen analyser. It permitted continuous measurements of two animals simultaneously. Readings were taken every 3 min and every 30 min a reference reading was taken. Analog outputs from the two mass flowmeters and the oxygen analyser were interfaced to a computer via an A/D converter (DT100F logger, Data Electronics, Australia).

$\dot{V}O_2$ values were calculated according to equation 3a of Withers (1977) and presented in STPD form (0°C, 760 Torr, dry). The RQ was assumed to be 0.85, which would result in a maximum error of ± 3% in $\dot{V}O_2$ if the RQ was 0.7 or 1.0 (Withers 1977). The animals were weighed before and after the experiments and a linear decrease of BM throughout each experiment was assumed for calculation of mass-specific MR.

For determination of the maximum $\dot{V}O_2$ ($\dot{V}O_{2max}$), the animals were exposed to He-O_2 (79% Helium and 21% Oxygen), which is a more conductive gas than air without side effects on the animal's circulatory or respiratory system (Rosenmann and Morrison 1974). For these measurements, data processing was similar to that for air, but a correction factor for the mass flowmeter output was applied because of the different physical properties of this gas from air (Geiser et al. 1996).

2.2.2 Body Temperature, T_b

Small temperature-sensitive transmitters (Minimitter Model X-M, accuracy ± 0.1°C, 1.6 to 2.3g), fitted with fresh 1.5V silver-oxide batteries and sealed with wax were used to continuously monitor T_b of the animals.

The temperature-dependent click frequency of the transmitters was calibrated to the nearest 0.1°C against a certified calibrated precision mercury thermometer (R6578, Dobros, Australia) in a Lauda RMT-6 waterbath between 1 and 40°C. The transmitters were normally calibrated only before they were implanted into an animal's body, however, on several occasions they were recalibrated on removal from the animals' body at the conclusion of the experiment. The equation coefficients for the best linear fit, as well as the polynomial curve fit, between temperature and logarithmic intervals were recorded for obtaining T_b values.

Additionally, the time-lag of the transmitter signal was determined by immersing a transmitter equilibrated at about 10°C into a 35°C waterbath. A temperature differential of less than 0.1°C was reached within 1 min, showing that the response time of transmitters is very rapid.

Transmitters were surgically implanted into the intraperitoneal cavity under Halothane or Forthane (isoflurane) anaesthesia. An incision of about 1 cm was made in both the skin and muscle layers of the abdomen. The sterile transmitter was inserted and the incisions were sutured. Antibiotics, which were mixed with animal's food or water, were provided up to 48 h before and/or after implantation. After the surgery, the animals were allowed at least seven days for recovery in a quiet room at T_a of 22 - 25°C. BM, food consumption, and T_b were monitored closely during this period.

An antenna, consisting of a ferrite rod, was placed underneath each chamber for receiving transmitter signals. The antenna was multiplexed to a receiver (car radio). The transmitter signal was transformed to a square-wave signal after background noise was subtracted.

2.2.3 Ambient Temperature, T_a

The T_a in the respirometry chamber was measured to the nearest 0.1°C by a thermocouple inserted about 1 cm into the respirometry chamber. For system A of the respirometry equipment, thermocouple output was amplified by a digital thermometer (Omega DP116). For system B of the respirometry equipment, thermocouple output was interfaced to a computer via a A/D converter (DT100F logger) .

Analog outputs from transmitter receiver and digital thermometer of each animal were interfaced to a personal computer, and were recorded in the same time intervals as the MR measurements (chapter 2.2.1).

2.3 Simultaneous Record of T_b and Activity in a Temperature Gradient

2.3.1 Set-up of the Temperature Gradient

Temperature selection by the animals was investigated in a temperature gradient which measured 125 × 17 × 19 cm (Fig. 2.5). The metal floor of the gradient was covered with a thin layer (1 - 2 mm) of fine sand. A temperature gradient was generated by cold water pumped through copper coils in the metal floor of the gradient at one end and heating at the other end was achieved with a 150 W infrared ceramic lamp. A piece of transparent perspex was used to cover the gradient to permit light entry and to reduce heat transfer. Fresh air was pumped continuously into the gradient. Three pieces of insulating cardboard, shielded with an aluminium sheet to limit transmitter signals to only one compartment each, separated the gradient into four compartments of the same size. A small gate, positioned alternatively on the left or the right corner of each cardboard, connected the compartments (Fig. 2.5). A nest-box and a water bottle were placed inside each of the compartments.

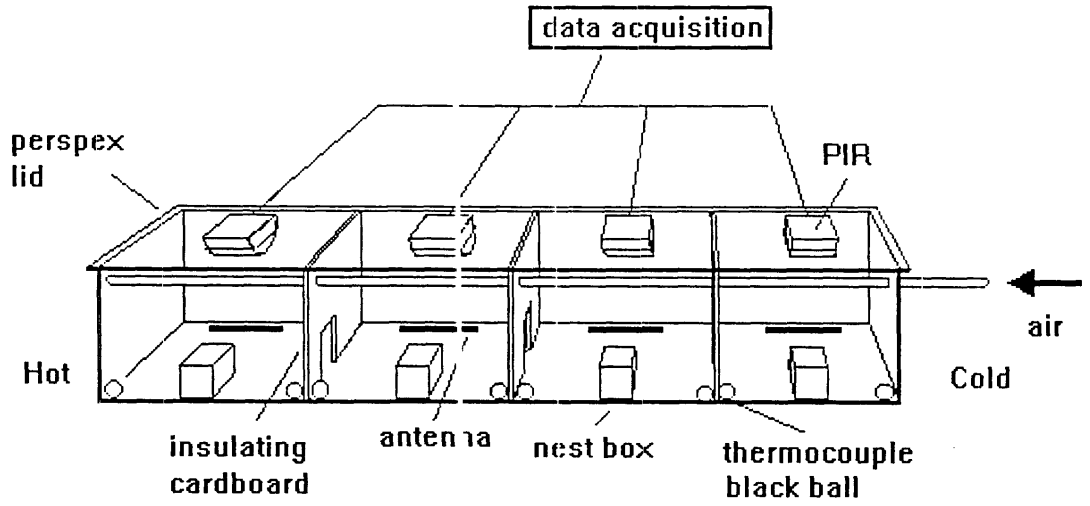

Fig. 2.5. Longitudinal view of the set-up of the temperature gradient. A temperature gradient was generated by cold water pumped through copper coils in metal floor of the gradient at one and heating on the other end was achieved with an infrared ceramic lamp.

To measure the operative black body temperature, calibrated thermocouples were placed in black ping-pong balls (Chappell and Bartholomew 1981; Seely et al. 1990). These thermocouples reflect the various aspects of an animal's heat transfer including radiation (Unwin 1980). Two thermocouples were fixed to the floor of each compartment. T_a of the warmest and the coldest end of each compartment was read to the nearest 0.1°C in 1-h intervals, and readings were stored on a data logger (Electronic Services Unit, University of New England).

Two thermal gradients were used for the experiments, one with a range of low T_as from about 5 to about 25°C, the other with a range of high T_as from about 20 to about 35°C. During each experiment, the T_a at each end of compartments of the gradient was recorded continuously. Average T_as are provided in Table 6.1.

2.3.2 T_b and Activity Measurements

For monitoring T_b in the temperature gradient, transmitter antennae consisting of a copper wire wound around a ferrite rod were placed within each compartment and multiplexed to a receiver (Fig. 2.5). The transmitter signal was transformed to a square-wave signal after background noise was subtracted. Compartments were scanned every 6 min.

Locomotor activity was monitored by passive infrared detectors (PIR, Jaycar Electronics LA-5017), which were placed on top of each compartment (Fig 2.5). A PIR is triggered by movements of an object with a temperature different from the surroundings (Ruf et al. 1991; Körtner and Geiser 1995). Activity of animals in each compartment was scanned and summed up over 6-min periods.

T_b and activity readings were stored on a personal computer, and location of the animal was determined by both T_b and activity signals from the compartments. Details about the measurements are provided in chapter 6.2.

2.4 Definition and Calculation

2.4.1 Washout of Respirometry System and Instantaneous $\dot{V}o_2$

During entry into and arousal from torpor, $\dot{V}o_2$ changes continuously and rapidly, thus no steady-state values can be obtained directly due to the mixing of gases in the respirometry chamber. However, the washout to a new equilibrium is an exponential function of time, and for a specific system with a given chamber size and flowrate, the rate of washout is constant (Bartholomew et al. 1981). Therefore, instantaneous $\dot{V}o_2$ were calculated for all measurements obtained in 3-min intervals regarding torpor entry and arousal.

To measure the washout characteristics for the respirometry system, twelve 1.4 Volt, 540 mAh zinc-air batteries in a respirometry chamber were connected in parallel. When the circuit was closed the batteries showed a constant rate of oxygen

consumption, and after disconnection the oxygen consumption dropped instantly to zero. However, the recorded values showed an exponential decline of $\dot{V}O_2$ due to gas mixing in the chamber. The derived curve characterises the washout of the system and correction factor can be calculated accordingly (Bartholomew et al. 1981). Correction factors for system A and system B of the respirometry equipment were recorded at the various flowrates used for measuring animals (Table 2.1).

Additionally, the lag-time of the respirometry equipment was determined by the time between disconnecting the batteries to the recorded start of $\dot{V}O_2$ decline (Table 2.1).

2.4.2 Resting Metabolic Rate (RMR), Basal Metabolic Rate (BMR), Thermoneutral Zone (TNZ) and Lower Critical Temperature (T_{lc})

All RMR and BMR measurements were carried out during the resting phase of the animals (i.e. the light phase). RMR values were determined from the mean of the lowest consecutive $\dot{V}O_2$ values over a minimum of 30 min in normothermic resting individuals whose T_bs were not lower than 33°C. RMRs were normally measured in the light phase after the animals had been in the chamber for about 2 hours.

Measurements of BMR, which is an estimation of the minimum energy requirement of an fasted, resting animal for maintenance of basic biological functions, but without cost of thermoregulation, were carried out between 0930h - 1700h in the light phase after the animals had been in the respirometry chambers for at least 2 hours. $\dot{V}O_2$ was measured at T_as between 25 and 36°C. The T_a was increased progressively in steps of about 1.5°C with each step lasting about 2 hours.

BMR for each individual was calculated using the lowest consecutive steady-state VO_2 values over a minimum of 30 m in each.

Table 2.1 Correction Factors for the Instantaeous $\dot{V}o_2$ with a 0.5-L Chamber

respirometry	**system A**	**system B**	**system B**
flowrate (mL min^{-1})	200	200	100
time-lag (min)	1.0	1.5	2.5
new equilibrium	a = 18.515	a = 6.180	a = 11.343
($y = a \times 10^{bx}$)	b = - 0.059	b = - 0.072	b = - 0.023
(x: time, y: $\dot{V}o_2$)			
Z (for 3 min)	0.5578	0.6289	0.2722

Note: The equation for calculating the instantaneous $\dot{V}o_2$ was:

$$A_i' = A_{i-1} + (A_i - A_{i-1}) / Z_i$$

A_i': calculated instantaneous $\dot{V}o_2$;

Ai: measured $\dot{V}o_2$ at time i;

A_{i-1}: measured $\dot{V}o_2$ at time i-1;

Z_i: value of Z for different time intervals of measurements.

The overall BMR of a species was determined as the mean of the BMR values of each normothermic individual.

T_{lc} is defined as the lowest T_a within the TNZ of a normothermic animal (Bartholomew 1982). The T_{lc} for each individual was determined by the intercept of two regressions fitted through the split data set of the RMR against T_a, whereby the lowest sum of the residual sums of squares for the two regressions was considered to be the best fit (Yeager and Ultsch 1989). The mean T_{lc} of both species was calculated from the T_{lc} values of all individuals.

The upper critical temperature (T_{uc}) was determined as the T_a above T_{lc} at which RMR started to increase with T_a.

TNZ was determined for each normothermic individual as the T_a range between T_{lc} and T_{uc} in which RMR values were constantly the smallest.

2.4.3 Torpor and Torpor Bout Duration (TBD)

Torpor was defined by a T_b below 32.0°C and/or reduction of MR below 75% of the RMR at the same T_a. For measurements within the TNZ, T_b equal to 32.0°C was used as threshold.

TBD was calculated from the time when $\dot{V}o_2$ fell below 75% RMR during torpor entry to the time when $\dot{V}o_2$ rose above 75% RMR during arousal. When only T_b measurements were available, torpor bout duration was the time period during which $T_b < 32.0$°C.

2.4.4 Metabolic Rate during Torpor (TMR) and The Lowest Body Temperature during Torpor (T_{bl})

TMR was determined from the mean of the lowest consecutive $\dot{V}o_2$ values over a minimum of at least 30 min in a steady-state torpor bout lasting for at least 2 hours.

T_{bl} was defined as the lowest T_b during a bout of torpor at a given T_a. It was determined over the same period at the time when TMR was determined during a torpor bout.

2.4.5 Critical Temperature during Torpor (T_{tc}), Minimum Metabolic Rate during Torpor (TMR_{min}), and Minimum Body Temperature during Torpor (T_{bmin})

The T_{tc} was defined as the T_a that separates thermoregulating and non-thermoregulating animals during torpor. At the T_{tc} the TMR of a torpid animal was at its minimum.

T_{tc} was determined as the intercept of two linear regressions fitted to TMR versus T_a (Yeager and Ultsch 1989) of all individuals.

TMR_{min} was defined as the smallest TMR of a torpid animal measured at the T_{tc}.

T_{bmin} was defined as the smallest T_b value of a torpid animal measured at the T_{tc}.

2.4.6 Cooling Rate, Overall Rewarming Rate, and Maximum Rewarming Rate

Cooling rate during torpor entry was defined as the rate of decrease in T_b from the time when T_b started to decrease continuously to the time when the decrease of T_b was less than 5% between two consecutive readings. Cooling time was defined as the time period used for calculating cooling rate.

Overall rewarming rate during arousal from torpor was defined as the rate of increase in T_b from the time when T_b started to increase continuously to the time when T_b reached 33.0°C. Overall rewarming time was the time period for calculating the overall rewarming rate.

Maximum rewarming rate was taken over a 12-min interval in which T_b showed the biggest increase during arousal from torpor.

2.4.7 Maximum Metabolic Rate ($\dot{V}o_{2max}$) and Maximum MRs at Arousal Peak and Activity Peak

Cold induced $\dot{V}o_{2max}$ was calculated from the mean of the highest consecutive MRs over 24 min during He-O_2 exposure at the T_a below which the onset of hypothermia occurred.

Maximum MR at arousal peak was calculated using the mean of 4 consecutive MR readings over 12 min during the time when the maximum rewarming rate was reached.

Maximum MR at activity peak was determined as the mean of the 4 highest consecutive nocturnal MR over 12 min with the assumption that periods of maximal activity correspond with the highest oxygen consumption.

2.4.8 Mass-specific Apparent Conductance (C), and Temperature Quotient (Q_{10})

The mass-specific apparent conductance (C), which is a measure of all kinds of heat loss including respiratory evaporation, was calculated from readings of MR, T_b and T_a using the equation:

$$C = MR / (T_b - T_a)$$ (Schmidt-Nielsen 1990).

The temperature quotient (Q_{10}), which is an expression of the effect of temperature on rates, was calculated for MR at different T_bs according to the equation:

$$Q_{10} = (MR_1 / MR_2)^{(10 / T_{b1} - T_{b2})}$$ (Schmidt-Nielsen 1990).

2.5 Statistics and Computer Software

2.5.1 Statistics

Unless otherwise noted, data in the experiments are presented as the mean ± 1 standard deviation (SD) for the number of experimental animals (N). Differences between two independent means were examined using a Student's t-test. Regressions were determined by the method of least squares. Paired comparisons of regressions were conducted using Student's t-test (Zar 1984). Data obtained from the same individual at the same T_a were averaged for statistical analyses. "n" indicates the total number of observations.

Selection of the appropriate regression models (linear or exponential) was made by comparing the coefficient of determination (r^2) for the linear model with that for the regression of the predicted y-value for the exponential model versus the measured y-value. Direct comparison of the r^2 values for the linear regression and the exponential regression could not be made because the total sum of squares of the logarithmic form of the exponential model does not equal the total sum of squares of the linear regression. Equality of the total sum of squares is essential if r^2 values are to be compared (Doran and Guise 1984).

Torpor frequencies under different experimental treatments were examined using Chi-square analysis and log-linear modelling (Crawley 1993). The impact of experimental treatments were analysed by ANOVA. Pairwise comparisons of means

for ANOVA were made with Tukey's test. *A posteriori* comparisons were conducted for 2-way and 3-way ANOVA.

Further details on statistics used are provided in the result chapters.

2.5.2 Computer Software

A range of programs written in "VISUAL BASIC" (Microsoft) for controlling solenoid valves, for data acquisition, data storage and data analysis were written by G. Körtner, B. Lovegrove and T. Ruf. Most statistical analyses were carried out using the statistical packages "MINITAB" (minitab) and "GLIM" (nag), as well as a "VISUAL BASIC" program written according to the details provided by Yeager and Ultsch (1989). Figures were created using software packages "SIGMA PLOT" (Jandel), "WORKS" (Microsoft), and "PAINT BRUSH" (Microsoft).