CHAPTER 6

FIELD WATER TURNOVER RATES

6.1 INTRODUCTION

This chapter examines seasonal patterns of water turnover in the field for each species of wombat. Water 'flux' represents water influx to the body water pool and water efflux from the body water pool to the environment. Thus, water flux rates measure the rate at which water passes through an organism. Measuring water flux rate is a first step in determining field metabolic rate, since calculations of metabolic rate (next chapter) require the water flux rate to be known. Water flux rates are inextricably linked to diet and habitat and so *per se* provide important information on aspects of an animal's physiology and interaction with its environment.

Animals are composed mostly of water and are open systems, continuously exchanging water with their environment. Most animals must maintain a relatively constant total body water volume, and so water flux rates reflect the ability to cope with variable water availability in the environment, which may include water excess or unavailability. Differences between water influx and efflux rates can be used to determine if an animal is in water balance or is water stressed. With knowledge of the diet, water influx to the body water pool can be partitioned into its various sources, which include free water that is drunk, preformed water in food, and water produced by metabolic processes. Water influx can also be used to calculate feeding rates or the quantity of drinking water required (if any) for a given diet. Water flux rates in the field

in this study were measured by using water which has been labelled with isotopes of hydrogen (Lifson and McClintock 1966). These isotopes also provided an estimate of the volume of the total body water pool (TBW) of an animal, which can give an indication of body condition (Sheng and Huggins 1979) and body fat (Woolnough *et al.* 1997).

The two genera of wombats inhabit vastly different habitats in terms of seasonal water availability; *Lasiorhinus* occupy hot semi-arid areas with either a Mediterranean or a wet-dry tropical rainfall regime, whereas *Vombatus* occur in cool-temperate mesic areas. *Lasiorhinus* are apparently able to survive in the dry season during periods when surface water is absent. *Lasiorhinus* and *Vombatus* therefore, could be expected to be markedly different in terms of their water metabolism. Field water flux rates have been previously investigated in only one of the three wombat species; the southern hairy-nosed wombat, by Wells (1973). The only other study investigating water flux rates in wombats was done by Barboza (1989) on southern hairy-nosed wombats and common wombats fed artificial diets under laboratory conditions.

6.2 METHODS

6.2.1 Sampling Procedure

Water flux rates were measured in free-living wombats of all three species using labelled 'heavy' water in which isotopes of hydrogen (deuterium or tritium) are incorporated (Lifson and McClintock 1966). This technique involves enriching the body water pool with an isotope of hydrogen and then measuring the rate at which the isotope is lost to the environment. The concentration of the hydrogen isotope (nearly all of which remains associated with water molecules) in the body water pool declines exponentially to the natural abundance (background) level as a result of dilution through the exchange of water between the animal and the environment. Unlabelled water enters the body water pool as free water in food, by drinking, through the formation of metabolic water and through exchange across body surfaces (primarily the lungs). Simultaneously, water is lost from the body pool from excretion, defecation, sweating and other external secretions and exchange across body surfaces.

The rate of isotope loss from the body water pool is a measure of the rate of water movement (water flux) through the animal.

Water flux rates can be measured using either tritium (^3H) or deuterium (^2H) isotopes of hydrogen. Tritium is a radioactive isotope (a weak beta-emitter with a physical half-life of 12.3 yrs), whereas deuterium is a stable (non-radioactive) isotope. I used tritiated water to measure water flux rates in common wombats and deuterium for both of the hairy-nosed species. Tritiated water was used in common wombats because it was cheaper to analyse than deuterium. Deuterium was used for southern hairy-nosed wombats because of the stringent permit requirements for the use of radioactive materials in South Australia. I used deuterium in the northern hairy-nosed wombat to avoid any (real or perceived) threats to this endangered species from the use of tritium.

Water flux rates in common wombats and in southern hairy-nosed wombats were measured twice a year, at the seasonal extremes of water and food availability (summer and winter). For reasons presented in the results section, water flux rates in northern hairy-nosed wombats were measured during the season of lowest water availability (winter), but not during other times of the year. Field trips were conducted during August '95 and January '96 for common wombats, during August '96 and February '97 for southern hairy-nosed wombats, and during September '95 and June/July '96 for northern hairy-nosed wombats (see Chapter 3 for details of field trips).

The study areas and methods used to capture and handle wombats are described in detail in Chapter 3. I took blood samples from the brachial vein, or sometimes from a vein in the leg, of anaesthetised animals using a 1 mL plastic tuberculin syringe with a 21 gauge needle. Blood was then transferred to a 1.5 mL O-ring sealed microtube and refrigerated or frozen, along with a sample of the isotope injection solution. An initial blood sample was taken from each animal to assess background isotope levels. Animals were then injected intraperitoneally with either 4.0 mL of deuterium or 1.0 mL of tritium (185 MBq / mL, equivalent to between 4.7 and 8.8 Mbq / kg), using a 1 mL or 5 mL glass syringe. Injection volumes for each syringe were calibrated in the laboratory by weighing a volume of distilled water

equivalent to that injected (assuming 1 mL of water = 1 g). The precision of injected volumes was assessed in the laboratory from a series of ten trial injections, and was found to have a coefficient of variation of less than 0.5%. All injections were made by myself, except for two injections in northern hairy-nosed wombats which were done by another experienced person. Following the isotope injections, animals were put into a hessian sack and left in a quiet place to allow the isotope to equilibrate within the body water pool (see section 6.2.3 below). Light anaesthesia was maintained for the duration of the equilibration period to reduce capture stress. During winter, anaesthetised animals were kept warm by using additional hessian sacks as blankets and in some instances by placing them in a heated room, heated car, or near a campfire. Animals were fitted with a radio collar if they were not already wearing one (see Chapter 3). At the end of the equilibration period, another blood sample was taken and the anaesthetised animals were released by placing them inside the burrow entrance to recover. Wombats which had been injected with isotope were recaptured between 7 days and 5 weeks later, depending on wombat species and season (see section 6.2.4 below), and a final blood sample taken.

6.2.2 Sample Analysis

Standard Solutions

The concentrations of isotope in the tritium and deuterium injection solutions were determined by preparing diluted 'standards' and analysing these using the methods described below for water distilled from blood samples. I prepared the standards for the injection solutions by diluting a 20 μL or 50 μL sample of injection solution with distilled water to make up 100.0 mL of standard solution. The diluent (distilled water) was analysed to determine its background isotope concentration, which was subtracted from the isotope concentration of the prepared standard. The concentration of the injection solution was calculated from the concentration of the standard and the dilution ratio used to prepare it.

Deuterium

Analysis of isotope levels in blood samples first involves extracting all of the water from the whole blood samples. I did this using the micro-distillation technique of Wood *et al.* (1975). Briefly, this technique involved drawing (by capillary action) approximately 45 μL of blood into a 70 μL glass hematocrit tube, one end of which was then flame sealed. The tube was placed, closed end first, into a 9" glass pasteur pipette. The pasteur pipette was then flame sealed under vacuum (by first flame sealing the large end of the pipette, then applying a vacuum to the small end whilst flame sealing it). The large end of the pipette (containing the hematocrit tube and blood sample) was then placed horizontally on a warm hotplate with the thin end protruding into cool air. The pipette was left for several hours until all of the water had distilled from the heated blood sample and condensed into the cool thin end of the pipette.

The next step involved splitting the distilled water molecules into hydrogen and oxygen. Five μL of the distilled water was placed in a glass tube with 150 mg of Zinc Reagent (Hayes and Johnson, Dept. Geological Sciences and Dept. Chemistry, Indiana University). The water sample and Zinc Reagent were frozen in liquid nitrogen (to prevent fractionation of the water sample under vacuum) and any remaining gas was evacuated (under 10^{-2} mbar vacuum) from the tube. The sample and zinc were then heated at 500 °C for 30 minutes to ensure complete reduction of the water molecules via the reaction:

$$H_2O + Zn \rightarrow H_2 + ZnO$$

The H_2 was then analysed using an Isotope Ratio Mass Spectrometer ('Optima' model, V. G. Isotech, Cheshire England) to determine the ratio of deuterium (^2H) to hydrogen (^1H). The ratio of deuterium in the sample was then expressed relative to a standard ratio (Standard Mean Ocean Water, or SMOW).

Tritium

I extracted water from blood using the micro-distillation technique as described above and also by vacuum sublimation (Vaughan and Boling 1961). The

Vaughan and Boling (1961) method involves placing a blood sample (approx 0.5 mL) in one end of an inverted 'U' shaped glass tube, evacuating the tube and then immersing the opposite end in liquid nitrogen. Water molecules leaving the blood are frozen when they reach the end submersed in liquid nitrogen. This process extracted all water from the blood sample in around 2 to 4 hours. Fifty μL of the distilled water was then placed into a plastic scintillation vial with 3 mL PCS (Phase Combined System, Amersham Corp, Ontario) scintillation fluid and the radioactivity 'counted' in a Beckman LS2800 Scintillation Counter to 1% error. All samples were analysed at the same time (to obviate the need to correct for physical decay of the isotope), together with prepared standard solutions.

6.2.3 Isotope Equilibration Period

Following injection, a period is required to allow the isotopically labelled water to disperse from the injection site and thoroughly mix (or equilibrate) within the body water pool. The time required for isotopes to equilibrate varies according to the site of injection (intraperitoneal, intramuscular, subcutaneous or intravenous), body size and, for ectotherms, body temperature (Nagy and Costa 1980; Nagy 1983; B. Green pers comm). Intravenous injections require the shortest equilibration periods whereas subcutaneous injections are likely to require the longest periods because of the slow dispersal of fluids from these tissues. Nagy (1983) states that an equilibration period of one hour is usually sufficient for small (300 g or less) animals given intravenous, intraperitoneal or intramuscular injections, though more time is needed for reptiles if they are cold. Animals above one kg require two to four hours and some ruminants may require ten hours (Nagy and Costa 1980; Nagy 1983). Wells (1973) concluded that isotopes administered via intramuscular injection in wombats equilibrated in around three hours. Barboza (1989) concluded that up to 30 hours may be necessary for isotope equilibration in common wombats and southern hairy-nosed wombats.

Because of the discrepancy between the equilibration periods arrived at by Wells (1973) and Barboza (1989), I conducted preliminary trials in common wombats to determine an appropriate equilibration period. Two captive and one wild wombat (all females by chance) were injected intraperitoneally with 0.5 mL of tritiated water (185

Mbq / mL). Serial blood samples were then taken for up to 12 hours following injection for analysis of blood isotope levels. All wombats were lightly anaesthetised for the duration of the trial (see Chapter 3 for details of anaesthesia). The two captive wombats (one approximately 12 years old) were studied at the Pet Porpoise Pool, Coffs Harbour NSW. I caught the wild wombat on the cattle grazing property 'Seymour', Nowendoc NSW. This animal was kept on-site in a hessian bag during the 6.5 hour trial and then released, still lightly sedated, down its burrow before dawn.

6.3.4 Recapture Interval

The time interval between isotope injection and recapture influences the magnitude of errors involved in isotope turnover estimates (Nagy 1980; Nagy 1983). If isotope levels of the recaptured animals are close to the initial (injection) level (the animal has been recaptured before sufficient isotope turnover has occurred) or are close to the background level (the animal was recaptured too late, and insufficient isotope remains in the body water pool), analytical errors have a large effect on the final estimate. Error is lowest after the concentration of isotope in the body water pool has declined to less than half the initial level, but is still significantly above background levels. The rate of isotope loss from the system is exponential and can be measured in biological half-lives. Nagy (1983) states that reliable estimates are still obtained when animals are recaptured between one and two biological half-lives for an isotope such as ^{18}O (oxygen-18), but up to five half-lives for tritium because it can be measured more accurately. Errors involved in estimates are also dependent on the initial dose of isotope injected (K. Newgrain pers comm). Biological half-life of ^{18}O varies with body mass and differs between major taxa (Nagy 1983). The predicted biological half-life ($T_{1/2}$) of ^{18}O (as $H_2{}^{18}O$) in a 30 kg terrestrial marsupial (such as a wombat) was estimated to be 14.1 days using the equation of Nagy (1983):

$$T_{1/2} = 0.151\ g^{0.44}$$

Animals ingesting food of high water content (such as lush green grass) are likely to have higher rates of water turnover and correspondingly higher rates of loss of isotopes and therefore shorter biological half-lives than predicted by such

generalised equations. Conversely, the biological half-life of ^{18}O in animals inhabiting arid environments may be longer than that predicted by the above equation.

Suitable recapture intervals were estimated for each wombat species based on a combination of factors. These factors included the injection dose of oxygen isotope planned to be administered, the predicted biological half-life of the ^{18}O isotope for a 30 kg marsupial (using the above equation), the measured or presumed water content of available forage and the low water turnover rates obtained by Wells (1973) for southern hairy-nosed wombats. The ^{18}O isotope was used in calculations instead of the hydrogen isotopes because I planned to use ^{18}O in conjunction with the hydrogen isotopes (for measurements of field metabolic rate, next chapter) and, because it is lost as CO_2 as well as water, its rate of loss is higher than that of the other isotopes. The higher loss rate and smaller doses of ^{18}O planned to be used (due to expense) compared to the hydrogen isotopes meant that recapture intervals were constrained by the ^{18}O isotope. A recapture interval of 10 to 14 days was set for common wombats for both summer and winter. For the two hairy-nosed species, a recapture interval of 10 to 14 days was set for the wet season, and three to four weeks for the dry season.

I conducted a preliminary trial on common wombats to gauge the accuracy of the recapture estimates for this species. I set traps on ten burrows over five nights and captured two wombats (one of each sex) on the cattle grazing property 'Seymour' and injected them with 2.0 ml of tritiated water. Unfortunately, I was unable to recapture either wombat over the next three weeks. Both wombats were handled prior to anaesthesia (to move them from the trap into a hessian sack), and it is probable that they had become extremely trap-shy. Neither animal was wearing a radio-collar as I had assumed that these individuals would return to the same burrows (or nearby burrows) within a week or two of capture. On two occasions wombats escaped by digging their way out past the traps, though I could not verify if these were the study animals.

I moved the ten traps to the Riamukka site and captured two individuals (both females), though one was too small (11 kg) for the trial and was released. The other, a 35.4 kg lactating female, was removed from the trap, anaesthetised, given 0.5 mL tritium and 4.0 mL ^{18}O, and released down the burrow wearing a radio-collar. This

animal was recaptured 11.6 days later in another burrow. A blood sample was taken and the collar (a prototype) removed as it had begun cutting into the neck of the animal. The biological half-life of the ^{18}O isotope in this animal was calculated (using the equation in section 6.2.5) to be 8.5 days, giving an optimum recapture interval of between 8 and 17 days which was close to the initial estimates of around 10 to 14 days.

6.2.5 Total Body Water Validation and Body Composition

Previous studies (e.g. Sheng and Huggins 1979; Rothwell and Stock 1979; Munks 1990) have shown that Total Body Water volume (TBW) based on isotope dilution space may be associated with some error. No studies have evaluated errors associated with estimates of TBW using isotope dilution space in wombats. I conducted a pilot trial in conjunction with A. Woolnough to validate estimates of TBW based on *in vivo* isotope dilution space against TBW derived by carcass desiccation (see Woolnough *et al.* 1997 for details). The wombat carcasses were also chemically analysed for fat content. For this trial we sacrificed wild southern hairy-nosed wombats which had been listed for destruction under a S.A. Department of Environment and Natural Resources permit granted to landowners. This research was approved by the Experimentation Ethics Review Committee of James Cook University and conforms with the Australian Code of Practice for the care and use of animals for scientific purposes.

Fifteen southern hairy-nosed wombats (eight females and seven males) were captured in cage traps or by 'stunning' (Robertson and Gepp 1982) in the Murraylands in South Australia (see Chapter 3). Stunning involves firing a high velocity .22 bullet above the wombat's head (at night using a spotlight) causing disorientation and so facilitating capture in a hand-held net. Once captured, wombats were anaesthetised (Zoletil 100, 5 $mg.kg^{-1}$) via intramuscular injection and a one mL blood sample was taken to determine background isotope levels. Animals were then given intraperitoneal injections of 0.5 mL deuterium and 0.5 mL ^{18}O and held while the isotopes equilibrated within the body water pool. Animals were then euthanased whilst still anaesthetised by an injection of sodium pentabarbitone (Lethabarb™). The

carcasses were taken to the autopsy laboratory at Morundi Wildlife Zoo where we shaved them and removed the viscera (to be analysed seperately). Care was taken to retain all body tissues and fluids. Blood samples were analysed for isotope concentrations (using the above methods) by Dr Brian Green and Keith Newgrain, CSIRO, Canberra, and carcass desiccation and fat determination was undertaken by Paul Eason at the Victorian Institute of Animal Science.

6.2.6 Calculations

I estimated the total volume of water in the body (total body water pool; TBW) of each animal at the time of isotope injection by comparing blood isotope levels (after isotopes had equilibrated within the body water pool) to the standard dilutions of the injected isotope solutions. I assumed that any changes in mass of the animal between capture and recapture were accompanied by similar relative changes in pool size (ie. TBW remained a constant proportion of total body mass), and that changes were linear over the capture-recapture interval. Rates of water influx and efflux were calculated from the decline in isotope concentrations in serial (capture and recapture) blood samples in conjunction with pool sizes using the equations of Nagy (1983).

The biological half-life of isotopes ($T_{1/2}$, time for isotope concentration to exponentially decline to half of the initial level) in wombats of each species were calculated using the standard exponential decay equation:

$$T_{1/2} = 0.693 / K$$

where K = ln(Initial Concentration) - ln(Final Concentration)/time

Concentration is the concentration of isotope in the initial and final blood samples, ln is natural logarithims and time is days between the Initial and Final samples.

6.3 RESULTS

6.3.1 Isotope Equilibration Period

The results of the blood isotope equilibration trials are shown in Figure 6.1. Serial concentrations of tritium in the blood of each of three common wombats are indicated by different symbols. Values are expressed as percentages of the final blood concentration for each individual to enable direct comparison, and a trendline has been fitted through the points as a visual aid. Following injection, blood isotope concentrations were high but decreased and stabilised after four or five hours, with little difference between these levels and those 24 hours after injection.

I chose four hours as a suitable equilibration time for two reasons; firstly, isotope levels in the blood had equilibrated (or were within one or two percent of the equilibration value) after this period, and secondly, four hours was about the maximum time I wished to have animals sedated/chemically restrained with anaesthetic drugs. Animals which have been anaesthetised for four hours require 2 or more hours to fully recover, during which time they are susceptible to injury from lack of coordination and disorientation. Longer periods of anaesthesia require longer recovery times. In addition, longer anaesthesia times mean that animals caught near dawn in summer are returned to their burrows during hotter periods of the day, increasing the chances of heat stress and dehydration (particularly for the two species which live in arid habitats). I assumed that equilibration times in common wombats would be similar to those of the two other wombat species.

6.3.2 Total Body Water Validation and Body Composition

Deuterium was used to validate TBW and body composition for reasons presented in 6.2.1. Deuterium was found to overestimate TBW in southern hairy-nosed wombats by 5.0 ± 2.9% and ^{18}O was found to underestimate TBW by 2.2 ± 2.7%. These accuracy's are within the range of values recorded for studies on other species (section 6.4.1). I have assumed that TBW estimates using isotope dilutions would be of similar accuracy for the two other wombat species.

Body fat averaged 8.1 ± 5.5% of body mass and varied widely between individuals, ranging between 2.6% and 19.3%. No significant differences were found in the percentage of body fat between sexes. Refer to Woolnough *et al.* (1997) for further details.

6.3.3 Biological Half-life of Hydrogen Isotopes

Biological half-life ($t_{1/2}$) of the hydrogen isotope did not differ significantly between southern hairy-nosed wombats (25.8 ± 5.8 days, n = 9) (mean ± SE) and northern hairy-nosed wombats (34.1 ± 7.1 days, n = 5), but was significantly longer in these species than in common wombats (10.0 ± 0.9 days, n = 14) (Kruskal-Wallis $H_{2,25}$ = 15.27, P = 0.0005, Kruskal-Wallis comparison of median rank) for data pooled from both seasons.

The $t_{1/2}$ of the isotope in common wombats did not differ significantly between sex or season (ANOVA sex: $F_{1,15}$ = 2.88, P = 0.11; season: $F_{1,15}$ = 0.19, P = 0.67). The $t_{1/2}$ in southern hairy-nosed wombats was significantly longer in the dry season (42.8 ± 5.4 days, n = 4) than in the wet season (12.2 ± 1.1 days, n = 5), but did not differ significantly between sexes (ANOVA season: $F_{1,5}$ = 40.73, P = 0.0014; sex: $F_{1,5}$ = 0.72, P = 0.43). Small sample sizes precluded tests for the effect of gender on half-life of the isotope in the northern hairy-nosed wombat, and no samples were obtained from this species during the wet season. Half-life of the isotope was not significantly different between common wombats and southern hairy-nosed wombats during the wet season (t_8 = -0.95, P = 0.37). During the dry season, half-life did not differ significantly between the hairy-nosed species, but both were significantly longer than in common wombats (Kruskal-Wallis $H_{2,15}$ = 13.04, P = 0.0015, Kruskal-Wallis comparison of median rank). Biological half-life of the oxygen isotope is given in section 8.3.1.

6.3.4 Body Mass and Mass Change

Body mass and body mass change for each wombat species during wet and dry seasons are shown in Tables 6.1, 6.2 and 6.3. Mean body mass of southern hairy-nosed wombats (24.14 ± 0.58 kg) and northern hairy-nosed wombats (28.01 ± 1.45 kg) were not significantly different, but both were significantly less than that of common wombats

(33.46 ± 0.89 kg) (ANOVA $F_{2,16} = 22.35$, $P = 0.0000$, Tukey HSD). These body weights were for dry season, the only season for which data is available for all three species. Body mass did not vary significantly between wet and dry seasons for southern hairy-nosed wombats (paired $t_3 = 1.30$, $P = 0.29$), but did for common wombats (paired $t_7 = -0.295$, $P = 0.02$) with each individual being, on average, slightly heavier (3 %) during the wet summer (34.33 ± 0.75 kg) than the dry winter (33.24 ± 0.98 kg).

Mass change during isotope turnover periods was significant for southern hairy-nosed wombats in both the dry (paired $t_3 = 20.20$, $P = 0.0003$) and wet seasons ($t_4 = 6.06$, $P = 0.0038$), but was not significant in either season for common wombats (dry season: paired $t_8 = 1.01$, $P = 0.34$; wet season: paired $t_4 = 0.38$, $P = 0.73$) or in the dry season for northern hairy-nosed wombats (paired $t_4 = 2.06$, $P = 0.11$). In the dry season, rate of mass loss during isotope turnover periods for southern hairy-nosed wombats (0.40 ± 0.06 % mass day^{-1}) was significantly greater than for common wombats (0.03 ± 0.12 % mass day^{-1}) but not significantly different to that in northern hairy-nosed wombats (0.29 ± 0.18 % mass day^{-1}) (Kruskal-Wallis $H_{2,15} = 6.87$, $P = 0.032$, Kruskal-Wallis comparison of median rank). Rate of mass loss during isotope turnover periods in the wet season were significantly different ($t_{10.4} = 8.58$, $P = 0.0000$) between southern hairy-nosed wombats (0.35 ± 0.06 % mass day^{-1}) and common wombats (0.02 ± 0.12 % mass day^{-1}).

6.3.5 Total Body Water

Total body water (TBW) volume estimated by hydrogen isotopes (deuterium or tritium) was, on average, 2.01% greater than that estimated by ^{18}O (mean ratio of H / ^{18}O TBW volume = 1.020). This difference was significant during the wet season (paired $t_{15} = 3.88$, $P = 0.0015$) but not for the dry season (paired $t_{18} = 1.34$, $P = 0.1972$). Unless otherwise stated, further analyses involving TBW are based on ^{18}O estimates. This is because ^{18}O was used in all TBW measurements and estimates based on ^{18}O are generally closer to true TBW volumes (estimated by carcass desiccation) than H isotopes (Nagy and Costa 1980; this study).

TBW volumes for each wombat species are shown in Tables 6.1, 6.2 and 6.3. On average, TBW formed 71.71 ± 0.01 % (n = 35) of body mass (all species combined). Mass specific TBW (TBW as % mass) did not differ significantly between species or

season for common wombats and southern hairy-nosed wombats (ANOVA species: $F_{1,26}$ = 0.01, P = 0.91; season: $F_{1,26}$ = 0.15, P = 0.71), nor did it differ significantly between all three species during the dry season; the season for which data is available for northern hairy-nosed wombats (ANOVA $F_{2,16}$ = 0.29, P = 0.75) (Figure 6.2). Seasonal changes in mass-specific TBW of individuals are shown in Figures 6.3 and 6.4. Within species, mass-specific TBW of individuals did not differ significantly between wet and dry seasons for either common wombats (paired t_7 = -0.57, P = 0.59) or southern hairy-nosed wombats (paired t_3 = 0.63, P = 0.58).

6.3.6 Water Flux Rates

Water influx and efflux rates for each wombat species are given in Tables 6.1, 6.2 and 6.3; mean seasonal water influx rates are shown in Figure 6.5. Water flux rates in one individual common wombat (female #8) during summer have been excluded from analyses due to the spurious values arising from insufficient isotope turnover; this individual was (unintentionally) recaptured too soon, and could not be recaptured after an appropriate release period. Water influx rates during the dry season were not significantly different between southern hairy-nosed wombats (12.11 ± 1.36 $mL.kg^{-1}.d^{-1}$) and northern hairy-nosed wombats (17.70 ± 3.78 $mL.kg^{-1}.d^{-1}$), but both were significantly less than that of common wombats (53.13 ± 4.49 $mL.kg^{-1}.d^{-1}$) (ANOVA $F_{2,14}$ = 29.68, P = 0.0000, Tukey HSD). During the wet season, water influx rates did not differ significantly between common wombats (57.37 ± 9.75 $mL.kg^{-1}.d^{-1}$) and southern hairy-nosed wombats (43.24 ± 4.67 $mL.kg^{-1}.d^{-1}$) (ANOVA $F_{1,8}$ = 1.71, P = 0.23). For common wombats, water influx rates varied significantly between sexes (males: 59.96 ± 5.21 $mL.kg^{-1}.d^{-1}$; females: 46.45 ± 7.14 $mL.kg^{-1}.d^{-1}$) but not between seasons (ANOVA sex: $F_{1,9}$ = 6.59, P = 0.030; season: $F_{1,9}$ = 0.05, P = 0.82). For southern hairy-nosed wombats, water influx rates varied significantly between seasons but not between sexes (ANOVA season: $F_{1,5}$ = 73.01, P = 0.0004; sex: $F_{1,5}$ = 5.51, P = 0.066).

Table 6.1 Mass change, total body water and water flux in common wombats during wet and dry seasons. Missing data indicate animals that were not captured or recaptured within a season.

Sex	Tag #	Mass (g)	Mass % change	TBW (mL)	TBW % Mass	H_2O Influx $mL.kg.d^{-1}$	H_2O Efflux $mL.kg.d^{-1}$
			DRY SEASON				
F	3	33400	0.05	26543	79.47	71.62	71.20
F	5	31600	-0.11	23600	74.68	51.15	51.95
F	8	34000	-0.08	22421	65.94	-	-
M	10	34200	-0.06	24927	72.89	62.61	63.05
M	13	33800	0.08	22595	66.85	54.04	53.48
M	15	35200	-0.11	26326	74.79	63.77	64.56
F	16	38200	-0.07	26772	70.08	31.51	31.97
M	18	28400	0.17	21128	74.39	46.02	44.73
M	21	32300	-0.20	22646	70.11	44.33	45.75
M	24	-	-	-	-	-	-
F	25	-	-	-	-	-	-
			WET SEASON				
F	3	34000	0.50	29455	86.63	35.10	30.81
F	5	33400	-	22669	67.87	-	-
F	8	34200	-	23412	68.46	-	-
M	10	34600	-0.09	23484	67.87	49.35	49.99
M	13	33800	-	23065	68.24	-	-
M	15	-	-	-	-	-	-
F	16	39000	-0.21	24683	63.29	42.85	44.15
M	18	31400	-	22040	70.19	-	-
M	21	34200	-0.08	24580	71.87	87.66	88.20
M	24	25800	-0.04	19576	75.88	71.89	72.16
F	25	32800	-	24591	74.97	-	-

Table 6.2 Mass change, total body water and water flux in southern hairy-nosed wombats during wet and dry seasons. Missing data indicate animals that were not captured or recaptured within a season.

Sex	Tag #	Mass (g)	Mass % change	TBW (mL)	TBW % Mass	H_2O Influx $mL.kg.d^{-1}$	H_2O Efflux $mL.kg.d^{-1}$
			DRY SEASON				
M	1	23600	-0.38	18000	76.27	12.98	15.86
F	2	23100	-0.37	15910	68.87	8.32	10.87
M	3	23200	-0.40	16383	70.62	12.34	15.15
M	4	26200	-	18749	71.56	-	-
F	5	24600	-0.45	16795	68.27	14.79	17.83
F	7	-	-	-	-	-	-
			WET SEASON				
M	1	22700	-0.15	16538	72.85	43.64	44.74
F	2	23500	-0.42	15976	67.98	34.59	37.45
M	3	23000	-0.31	16767	72.90	59.58	61.81
M	4	27000	-0.36	20520	76.00	44.72	47.46
F	5	23600	-0.51	16778	71.09	33.69	37.28
F	7	21600	-	16542	76.56	-	-

Table 6.3 Mass change, total body water and water flux in northern hairy-nosed wombats during the dry season.

Sex	Tag #	Mass (g)	Mass % change	TBW (mL)	TBW % Mass	H_2O Influx $mL.kg.d^{-1}$	H_2O Efflux $mL.kg.d^{-1}$
F	61	22600	-0.971	14540	64.34	8.94	15.19
M	32	29550	-0.392	20547	69.53	9.25	11.98
F	82	30500	-0.096	23011	75.45	23.41	24.13
F	152	27400	-0.01	19433	70.92	19.07	19.14
M	25	30000	0.01	21684	72.28	27.82	27.75

No significant relationships were found between water influx rate and either body mass or changes in body mass over the isotope turnover periods within each of the species, though weak (but significant) correlations were found when data was pooled from all species (linear regressions: water influx rate vs body mass, $r^2 = 0.20$, $F_{1,25} = 6.44$, $P = 0.0177$; water influx vs mass change, $r^2 = 0.22$, $F_{1,25} = 7.05$, $P = 0.014$). These correlations, however, are confounded by habitat differences.

No significant differences were found between water influx and efflux rates during the isotope turnover periods in either season for common wombats, nor for the dry season for northern hairy-nosed wombats, suggesting that these animals were in water balance. Water efflux rates were significantly greater than influx rates for southern hairy-nosed wombats in both the dry (paired $t_3 = -27.63$, $P = 0.0001$) and wet (paired $t_4 = -6.07$, $P = 0.0037$) seasons, indicating that these animals were in negative water balance. The difference between water influx and efflux rates (net loss of water) for southern hairy-nosed wombats was similar for both seasons (dry season, 2.8 $mL.kg^{-1}.day^{-1}$; wet season, 2.5 $mL.kg^{-1}.day^{-1}$), but dry season loss represents 23% of the water influx rate during that period whereas the wet season loss represents only 6% of the wet season water influx rate. For these calculations I have assumed that any change in mass was reflected by a similar change in TBW. It is possible that the absolute TBW pool remained constant and mass changed, though non-significant interseasonal mass changes (section 6.3.4) suggest that this was not the case.

Figure 6.6 shows the residual variation in water influx rates for the available data on other marsupial herbivores. The zero line represents the 'mean' or 'expected' water influx rate for any given body size. Superimposed on this regression are the residuals (or deviations from the 'expected' rate) for seasonal water influx rates of the wombats. The differences in water influx rates between wombats and other marsupial herbivores are discussed in section 6.4.3

6.3.7 Partitioning of Water Influx

Water influx to the body water pool can be from drinking, from ingesting preformed water (moisture) in food, from metabolic processes that produce water (such

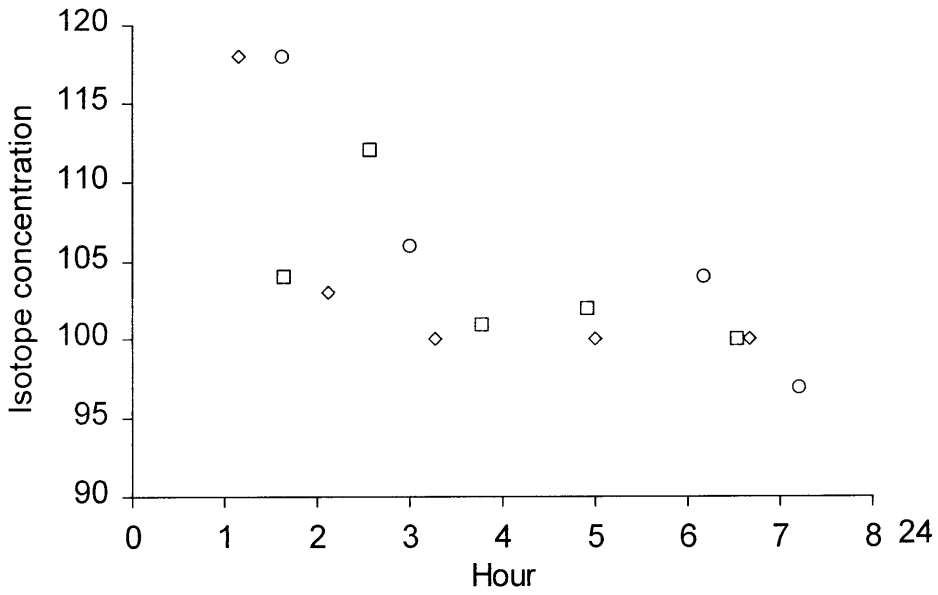

Figure 6.1 Relative blood tritium concentrations over time for 3 common wombats. Values are tritium counts expressed as a percentage of the final blood concentration for each animal.

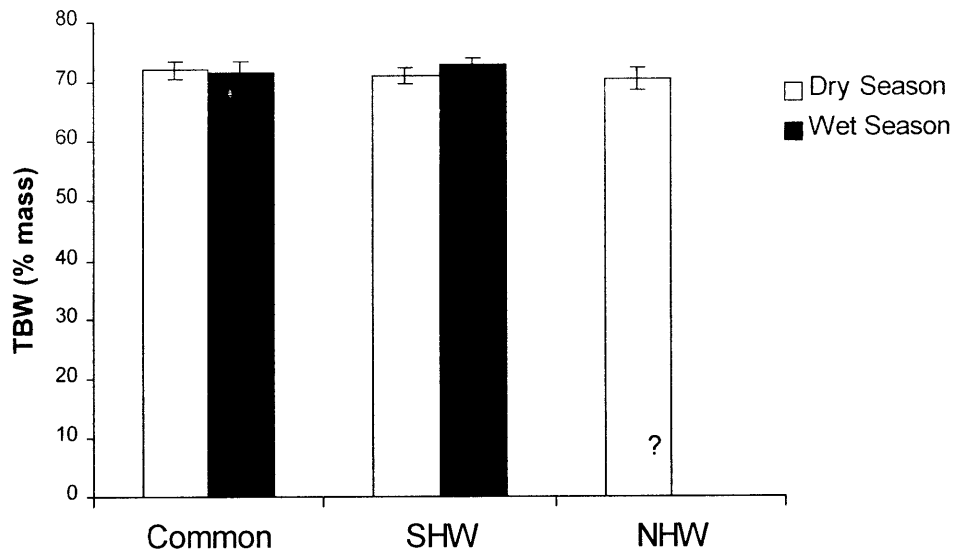

Figure 6.2 Mean seasonal total body water (TBW) volumes (as % total mass) for the three wombat species. Common = common wombat, SHW = southern hairy-nosed wombat and NHW = northern hairy-nosed wombat. Error bars are standard errors.

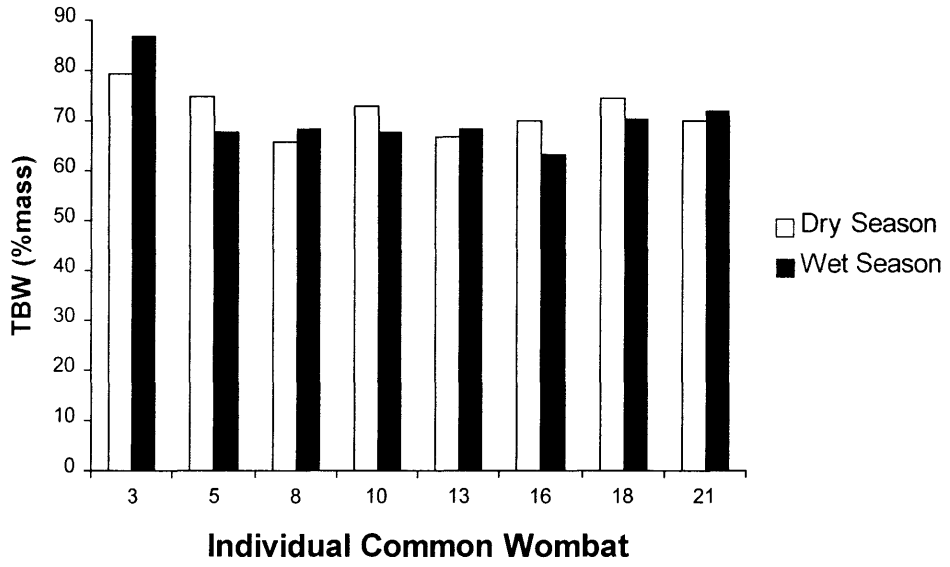

Figure 6.3 Seasonal total body water (TBW) volumes (as % total mass) for individual common wombats.

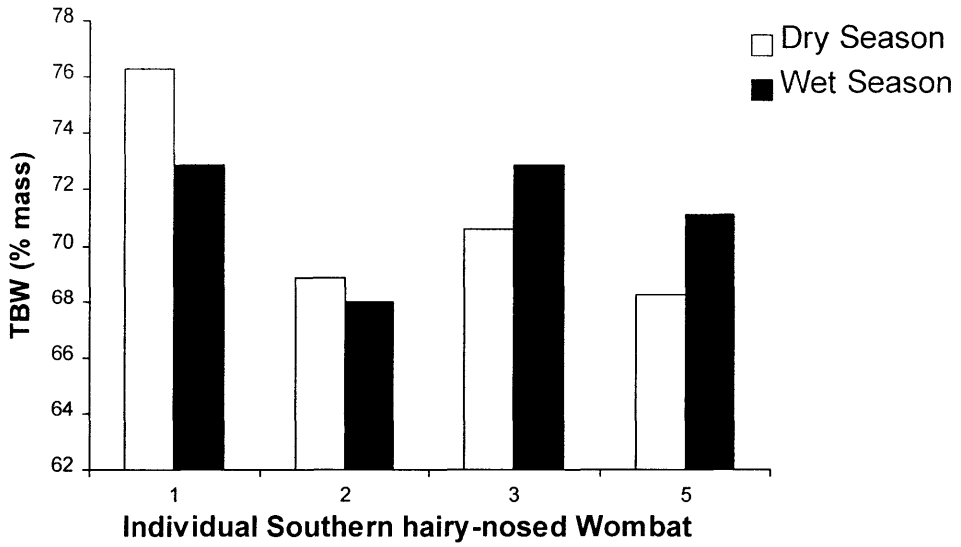

Figure 6.4 Seasonal total body water (TBW) volumes (as % total mass) for individual southern hairy-nosed wombats.

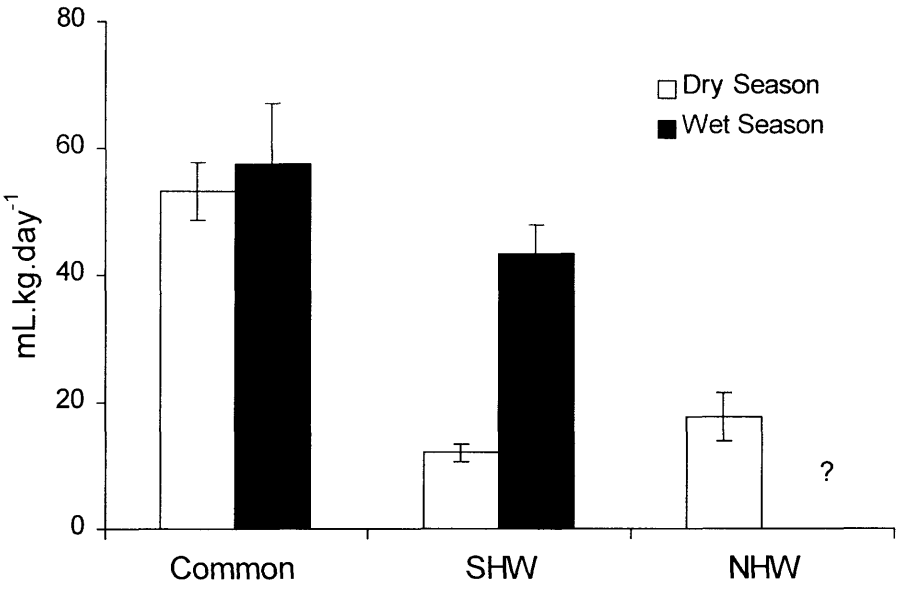

Figure 6.5 Mean seasonal water influx rates for the three wombat species. Common = common wombat, SHW = southern hairy-nosed wombat and NHW = northern hairy-nosed wombat. Error bars are standard errors.

as the oxidation of carbohydrates, proteins and fats in food and catabolism of body fat) and from exchange across body surfaces (primarily the lungs). Partitioning of water influx can provide important information such as whether the animal needs to drink free water in the field, or whether it can satisfy all of its water requirements from food sources. Water influx can be quantitatively partitioned if information is available on food moisture content, Field Metabolic Rate, and the amount of water derived from metabolic processes. From plant foods, metabolically produced water amounts to about 0.0295 mL per kJ of energy metabolised from this food source (Nagy 1983).

Common wombat

Water influx rates for common wombats during the dry winter averaged 1770 ± 160 $mL.day^{-1}$ on a whole animal basis. Grass species in the diet at this time contained 71% moisture (Chapter 4); each gram of grass eaten therefore yields 0.71 mL of preformed water. Common wombats required 2446 $g.day^{-1}$ of fresh grass to balance their energy expenditure of 5139 $kJ.day^{-1}$ during this period (Chapter 7). Water influx from this amount of grass via preformed water should therefore be about 2446 x 0.71 = 1737 $mL.day^{-1}$, and from metabolic water should be about 5139 x 0.0295 = 152 $mL.day^{-1}$, (as ca. 0.0295 $mL.day^{-1}$ of metabolic water is derived per kJ of energy matabolised from plants - see above), giving a total of 1889 $mL.day^{-1}$ which is within the water influx rate range of 1770 ± 160 $mL.day^{-1}$ given above).

Water influx rates for common wombats during the wet summer averaged 1885 ± 300 $mL.day^{-1}$. Wombats at this time were feeding on grass species that contained 60% moisture (Chapter 4). Common wombats required 3578 $g.day^{-1}$ of fresh grass to balance their energy expenditure of 10731 $kJ.day^{-1}$ during this period (Chapter 7). Water influx from this amount of grass (via preformed and metabolic water) therefore, should be about 3578 x 0.60 + 10731 x 0.0295 = 2463 $mL.day^{-1}$, which is 278 $mL.day^{-1}$ above the upper range calculated for daily water influx rate of 2185 $mL.day^{-1}$ (ie 1885 + 300 $mL.day^{-1}$).

Southern hairy-nosed wombats

No rain fell during the dry season isotope turnover period and free standing water was not available to southern hairy-nosed wombats. I therefore assumed that these wombats did not drink during the dry summer and obtained all of their water from preformed water in plants and from metabolic water. I also assumed that water derived from pulmonary exchange was negligible in this arid habitat. I measured the preformed water content of grasses at the site during the dry season by cutting, drying and weighing grass samples.

Water influx rates for southern hairy-nosed wombats during this period averaged 287 ± 36 $mL.day^{-1}$. Grasses at this time contained 32% moisture (Chapter 4); each gram of grass eaten therefore yields 0.32 mL of preformed water. Southern hairy-nosed wombats required 513 $g.day^{-1}$ of fresh grass to balance their energy expenditure of 3142 $kJ.day^{-1}$ during this period (Chapter 7). Water influx from this amount of grass (via preformed and metabolic water) therefore, should be about 513 x 0.32 + 3142 x 0.0295 = 257 $mL.day^{-1}$, which is within the daily water influx range of 287 ± 36 $mL.day^{-1}$.

Water influx rates for southern hairy-nosed wombats during the wet winter averaged 1035 ± 112 $mL.day^{-1}$. Grasses during this period contained 57% moisture (Chapter 4). Southern hairy-nosed wombats required 1728 $g.day^{-1}$ of fresh grass to balance their energy expenditure of 6689 $kJ.day^{-1}$ during this period (Chapter 7). Water influx from this amount of grass (via preformed and metabolic water) therefore, should be about 1728 x 0.57 + 6689 x 0.0295 = 1182 $mL.day^{-1}$, which is close to the upper range of the daily water influx rate of 1035 ± 112 $mL.day^{-1}$.

Northern hairy-nosed wombat

Rain did not fall during the dry season isotope turnover period and free standing water was not available to northern hairy-nosed wombats. I therefore assumed that all of their water was obtained from preformed water in plants and from metabolic water. Water derived from pulmonous exchange was likely to be negligable in this arid habitat.

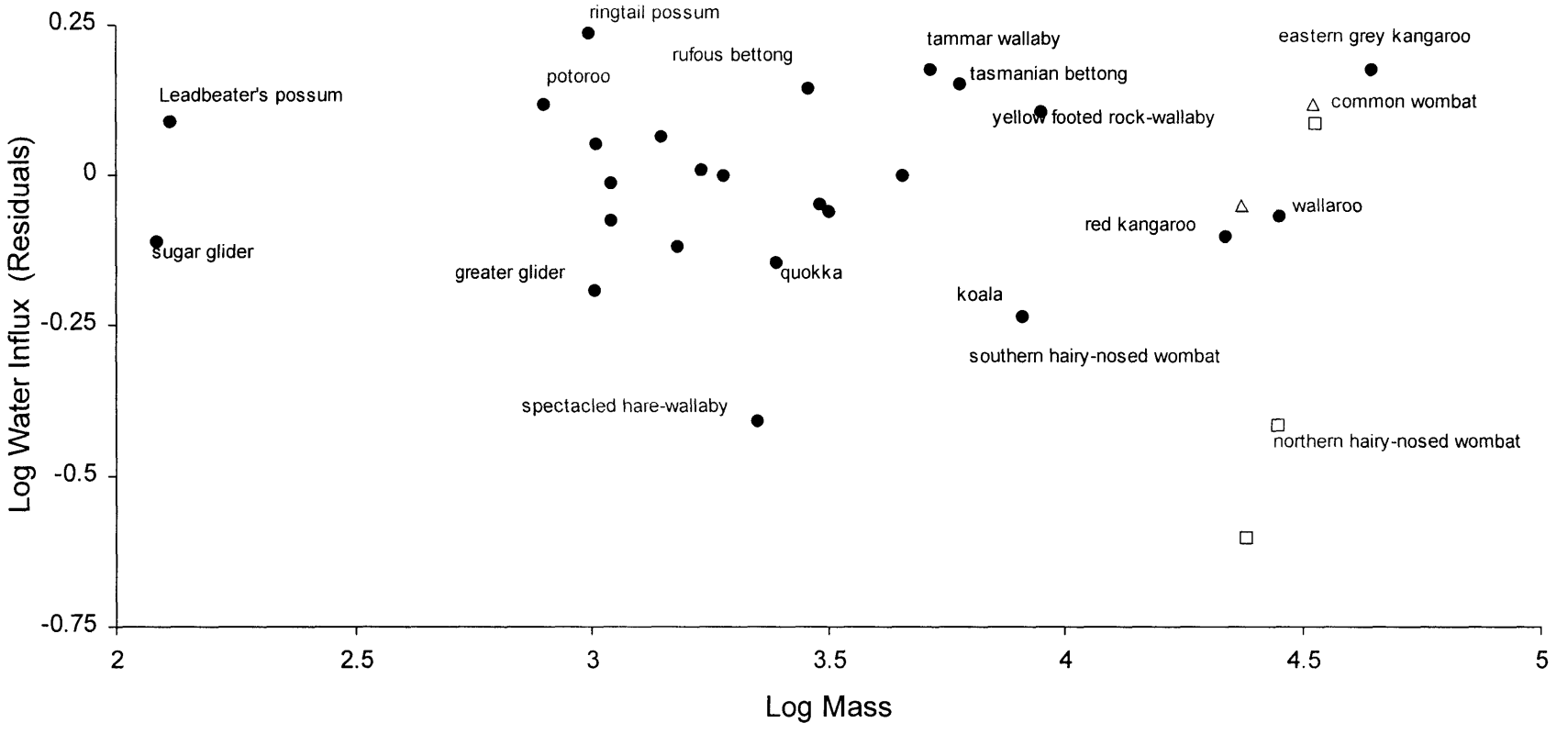

Figure 6.6 Water influx rates of herbivorous marsupials. Values are residuals from a regression of water influx rate ($mL.day^{-1}$) on body mass for 24 marsupial herbivores (names of some species are shown). The line represents the mean or 'expected' value for any body mass; only doubly-labelled water studies have been included. Seasonal values for wombats (open symbols) have been superimposed over the regression. For wombats: triangles = wet season, squares = dry season.

Water influx rates for northern hairy-nosed wombats during the dry winter averaged 509 ± 122 $mL.day^{-1}$. The water content of grass was not measured during this period. However, because northern hairy-nosed wombats apparently obtained all of their water from grasses, it is possible to calculate this water content based on the water influx rate and Field Metabolic Rate. Mass change for northern hairy-nosed wombats during isotope turnover periods was not significant, and so I assumed that these animals were in water and energy balance (ie. not catabolising fat reserves). Northern hairy-nosed wombats required 422 $g.day^{-1}$ of dry matter to balance their energy expenditure of 3802 $kJ.day^{-1}$ (Chapter 7). Metabolic water derived from this amount of plant food should be about 3802 x 0.0295 = 112 $mL.day^{-1}$, leaving 397 $mL.day^{-1}$ to be derived from preformed water in grass. The estimated water content (%) of grass that northern hairy-nosed wombats were eating during the dry season is therefore 397 / (397 + 422) = 0.48, or 48%, which means wombats would need to ingest about 819 $g.day^{-1}$ of this fresh grass to maintain water and energy balance.

6.4 DISCUSSION

6.4.1 Potential Errors

Total Body Water Volume

Both isotopes (deuterium and ^{18}O) used in the TBW validation trial in southern hairy-nosed wombats resulted in small though significant errors in estimates (Woolnough *et al.* 1997). Deuterium dilution space overestimated TBW by 5%, which is at the upper end of the range of errors found in other species (Sheng and Huggins 1979). This error may be due to errors in injection volumes, accuracy of reference standards and errors associated with analysis using mass-spectometry (K. Newgrain pers comm). Hydrogen isotope dilution space has been shown to generally result in TBW values greater than those measured directly from carcass desiccation (Rothwell and Stock 1979; Bakker and Main 1980; Nagy and Costa 1980; Green and Eberhard 1983; Munks 1990; Woolnough 1997), and has been attributed to exchange of these isotopes with non-aqueous H pools

such as those in protein and fat (Nagy and Costa 1980) and analytical errors (Arnould 1995; Woolnough 1997). Estimates of TBW using ^{18}O dilution space in the validation trial were closer to carcass desiccation values than those using deuterium. Other studies have also shown that estimates of TBW based on ^{18}O are generall closer to TBW measured by carcass desiccation (Nagy 1980; Nagy and Costa 1980).

Water Flux Rates

The isotopically-labelled water method for measuring water flux rates in animals involves a number of assumptions, which if violated could cause errors in water flux estimates (Lifson and McClintock 1965). These assumptions are:

1. Constant body water volume during the measurement period
2. Constant water flux rates
3. Isotope labels body water only
4. Isotope lost only as water
5. No isotopic fractionation occurs in the body
6. No water input via the skin or lungs

These assumptions and the associated potential errors have been evaluated by Nagy and Costa (1980). In most situations these potential errors are either small in magnitude or can be avoided by using appropriate experimental design and appropriate equations in the analysis (Kunz and Nagy 1989). The numerous laboratory studies undertaken to validate the tritiated water method indicate that tritiated water measurements of water flux in mammals are generally accurate to within -7 to +4% (data in Nagy and Costa 1980, Nagy 1980, Nagy 1989). It is more difficult to evaluate the validity of these assumptions in the field. In particular, body water volumes and water flux rates both probably fluctuate instead of being constant, though sensitivity analyses indicate that the magnitude of these errors are likely to be small unless fluctuations are very large (ie. >40%) (Nagy and Costa 1980). Analytical errors can be minimised by using larger doses of isotope (K. Newgrain, personal communication) and appropriate recapture intervals (Nagy and Costa 1980; Nagy 1980) (see also section 6.3.4).

Capture and Handling

One aim of this study was to investigate field water flux rates of free-living wombats under natural conditions. Human interference has the potential to affect natural (or 'normal') wombat behaviour and hence alter field water flux rates. In any study that assumes natural behaviour of study individuals, unquantified deviations in measurements resulting from human interference are a source of potential error.

In this study, trapping, handling and collaring possibly disrupted 'normal' behaviour patterns of study individuals. Wombats during these periods apparently had higher levels of activity (Chapter five). Without controlled experiment it is difficult to determine whether such activity is normal or not, and the effect on WTR. If wombat behaviour was indeed disrupted and resulted in higher than normal WTR (from increased activity as suggested in Chapter five), the significance for this study will be for more conservative results. In other words, for these inherently low WTR species, disrupted behaviour may result in normal WTR being even lower than that measured.

6.4.2 Total Body Water and Condition

Total body water volume estimated by hydrogen isotopes (deuterium or tritium) was, on average, slightly greater (2.01%) than that estimated by ^{18}O, and this difference was significant for the wet season but not for the dry season. The larger TBW estimates based on hydrogen isotopes compared to oxygen isotopes has been found in other studies, and the probable reasons are mentioned in the preceeding section. The results of the validation trials and of other studies (Nagy 1980; Nagy and Costa 1980) suggest that TBW estimates based on ^{18}O dilution space from this study are within 2 or 3% of true values.

An animal's physical wellbeing is often described as its 'condition', with the assumption that body condition is a good indicator of nutritional status, and that animals of higher body condition have increased fecundity and lower mortality (Brochu *et al.* 1988; Krebs and Singleton 1993). Various physiological and morphometric methods have been used to derive relative condition indices (eg Barnett *et al.* 1979; Bakker and

Main 1980; Krebs and Singleton 1993; Arnould 1995) with no single method accepted as standard. Condition is often measured in terms of body fat, since this is assumed to be directly related to an animal's overall energy balance and an indicator of resources able to be directed to reproduction or surviving adverse environmental conditions. The ratio of TBW to mass of an animal has been used as an estimator of body fat, based on the observation that lean body mass contains a reasonably constant proportion of water and TBW is inversely proportional to the percentage of body fat (Pace and Rathburn 1945; Green and Eberhard 1983; Woolnough *et al.* 1997). This is because fat contains little water, unlike the other body tissues, and thus animals which have more fat for their weight should have less water-containing tissue and hence proportionately less TBW.

Despite marked differences between the habitats of the hairy-nosed species (semi-arid) and the common wombat (mesic), there were no significant differences in mass-specific TBW (TBW as a % of mass) between the genera, nor were there any significant differences between the hairy-nosed species. This suggests that, on average, the percentage of body fat was similar for the three wombat species. Moreover, no significant differences were detected in mass-specific TBW for the same individuals between the dry and wet seasons for either common wombats or southern hairy-nosed wombats, suggesting that these individuals were able to maintain a similar level of body condition irrespective of season. For southern hairy-nosed wombats, this conclusion is supported by the lack of significant changes in body mass for individuals between seasons. Individual common wombats were slightly heavier (3%) during the wet winter than the dry summer, though the TBW data suggests that this was due to tissues other than fat. It is possible that TBW is not sufficiently accurate to detect small (< 3%) differences in body fat.

Mass-specific TBW was highly variable between individuals, even within the same species and season. For example, mass-specific TBW for common wombats during the dry season ranged from 66.85% to 79.47%, and wet season values ranged from 63.29% to 86.63%. This suggests that condition (in terms of body fat) may also have been highly variable between individuals.

The only other information on TBW and water flux rates of wombats in the field comes from a previous study at Brookfield CP on southern hairy-nosed wombats by Wells (1973). Wells found that mass-specific TBW in individual southern hairy-nosed wombats did not vary significantly between seasons, which is corroborated by the present study.

6.4.3 Water Metabolism

Water flux rates varied markedly between the two genera; water flux rates for the arid-adapted hairy-nosed species were lower than for the mesic-adapted common wombat. This difference was most pronounced during the dry season, when water influx rates for southern hairy-nosed wombats and northern hairy-nosed wombats were only 23% and 33%, respectively, of the influx rate for common wombats. Common wombats showed little difference in water influx rates between wet and dry seasons, whereas southern hairy-nosed wombats showed marked variation, with the dry season rate being only 28% of the wet season rate. Common wombats and northern hairy-nosed wombats were able to maintain water balance during isotope turnover periods, but southern hairy-nosed wombats were in negative water balance during isotope turnover periods in both seasons. Water influx rates for southern hairy-nosed wombats during dry and wet seasons in this study were similar to those obtained by Wells (1973).

These inter-genera and inter-seasonal differences in water flux rates appear to be related to seasonal water availability (free-standing and in food) of the different habitats. Common wombats in this study inhabited a mesic environment which did not have a true 'dry' season; the rainfall received during the 'dry' winter was only slightly less than the 'wet' summer. This site had a small permanent stream and the water content of food species was high throughout the year. The permanent availability of free-standing water for drinking and the relatively consistent moisture content of food resources is reflected in little inter-seasonal variation in water influx rates of common wombats. Common wombats appear to have been able to satisfy all their water requirements from food during the wet summer. During the drier winter, common wombats had access to drinking water to make up for any reduced dietary water intake (mostly due to lower

feeding rates). However, water intake from food (despite being lower than that of the wet season) was within the range of estimated total water intake during the dry season and so wombats may have not needed to drink.

In contrast, semi-arid habitats of the hairy-nosed species receive little rainfall annually and the variation between seasons is pronounced. Free-standing water is not present for much of the dry season at the sites studied, and thus wombats at this time of year depend largely on the moisture in food and water produced by metabolic processes to satisfy their water requirements. The results of this study suggest that these two species may not need to drink during the dry season provided sufficient food is available with a moisture content of at least 30% for southern hairy-nosed wombats and at least 44% for northern hairy-nosed wombats. These species might be able to survive on forage with lower water contents, though this was not investigated in the present study. The negative water balance found during isotope turnover periods for southern hairy-nosed wombats suggests that during the dry season this species may need to obtain some water through catabolism of fat reserves. Prolonged dry periods without free water may therefore adversely affect southern hairy-nosed wombats. Northern hairy-nosed wombats experience the driest time of the year during winter, whereas southern hairy-nosed wombats experience the driest time of the year during summer. It is probable that higher evapotranspiration rates during summer cause a reduced moisture content of grasses, which might explain the lower water turnover rates of southern hairy-nosed wombats. When drinking water is not available, wombats are apparently able to survive on forage of lower moisture content than can other grazing mammals. For example, Jarman (1973) estimated that impala *Aepyceros melampus* drank when the moisture content of grass fell below 66.7%. In the absence of drinking water, rabbits *Oryctolagus cuniculus* cease feeding when the moisture content of herbage falls below 55% (Cooke 1982), and Nagy (1994a) found that springbok *Antidorcas marsupialis* in the Kalahari desert require a forage moisture content of at least 67% to maintain water balance.

Water flux rates of southern hairy-nosed wombats during the dry season are extremely low; in fact, amongst the lowest recorded for herbivorous mammals. Water flux rates are strongly influenced by body mass, and so meaningful comparisons can only

be made between animals of similar body mass, or by using techniques which take into account the effect of body mass. Nagy and Peterson (1988) reviewed field water flux rates in mammals and obtained a general regression equation predicting water flux rate from body size (water influx $mL.day^{-1} = 0.874$ body mass $(g)^{0.71}$). Water influx rates of southern hairy-nosed wombats during the dry season are only 25% of the rate predicted for a 24 kg herbivorous mammal while wet season rates are 92% of the predicted value. This equation, however, is based on data from all seasons and habitats, and includes multiple representation of some species. Unfortunately, it is difficult to determine whether data came from wet or dry seasons in many of the original references, a problem no doubt faced by these authors when deriving this equation.

More recently, Green (1997) reviewed the available data on field water fluxes in marsupials, of which the herbivores included 15 macropodoids, 8 arboreal folivores and one Vombatid (*Lasiorhinus latifrons*, from the work of Wells 1973). Green was able to separate seasonal and taxa effects and derive equations predicting water influx for macropodoid and non-macropodoid herbivores during dry or wet seasons. Water influx rates for southern hairy-nosed wombats during the wet season are still only 38% of that predicted for a 24 kg grazing macropodiod during this season, and dry season water influx rates are only 25 % of that predicted for a dry season. To compare water influx rates of southern hairy-nosed wombats to the non-macropodoid herbivores, I recalculated the non-macropodoid equation leaving out the data for *Lasiorhinus* (to avoid multiple representation). Thus, including only arboreal folivores, Water Influx $mL.day^{-1} = 2.19$ body mass $(g)^{0.58}$. The average water influx rate of southern hairy-nosed wombats for the dry season was 38% of the predicted value for a 24 kg folivore, whereas wet season influx rates were about 36% greater than the predicted value. Although southern hairy-nosed wombats are grazers, their water turnover rates are much lower than those of the (grazing) macropodoids, and are closer to the generally low water influx rates predicted for marsupial folivores.

Water influx rates for northern hairy-nosed wombats during the dry season were also low. Their water influx rate was 41% of that predicted by the Nagy and Peterson

(1988) equation for a 28 kg herbivorous mammal, 39% of that predicted for a macropodoid during the dry season and 67% of that predicted for a marsupial folivore.

Water influx rates for common wombats were above the marsupial mean. Water influx rates were 25% (dry season) and 34% (wet season) greater than that predicted by the equation Nagy and Peterson (1988) for a 33 kg mammalian herbivore. The dry season water influx rate was 18% greater than that predicted for a 33 kg macropodoid in the dry season, but the wet season influx rate was only about half (52%) of that predicted for a macropodoid during the wet season.

When all available data are combined from field studies of water influx in marsupial herbivores (section 6.3.7), southern hairy-nosed wombats and northern hairy-nosed wombats are well below the marsupial mean, and common wombats are slightly above. The notion of a 'marsupial mean' can be useful for broad comparisons between species, though the currently available data set that it is based on has deficiencies. The 'marsupial mean' may be biased towards specific taxonomic groups, since not all taxa are represented and some taxa are more closely related than others. It may also suffer from seasonal and habitat bias, because species are not evenly represented amongst different habitats (eg. xeric and mexic) and seasons.

6.4.4 Summary

Water flux rates of *Lasiorhinus* during the dry season are amongst the lowest recorded for mammalian herbivores. During 'normal' dry seasons, these species can probably survive without drinking. Water flux rates for common wombats were comparable with other marsupial herbivores such as the macropodoids. Isotope equilibration times were found to be around 4 hours (similar to the results of Wells 1973), instead of up to 30 hours as suggested by other workers. Common wombats and northern-hairy wombats did not lose body mass during the isotope turnover periods, suggesting that these animals were in energy and water balance. Southern hairy-nosed wombats, however, lost body mass during isotope turnover periods in both seasons, suggesting they were in negative energy balance. Southern hairy-nosed wombats and common wombats were apparently able to maintain body condition between seasons