The assumed relationship between Macropus $\delta^{18}O$ values and relative humidity is largely based on the general observation that Macropus do not necessarily need to drink and may gain their water from the grass leaves they eat. Leaf-water is isotopically enriched due to evaporative processes that are inversely related to relative humidity, thus the relationship is highly plausible and has been observed for mammals on other continents (Cormie et al. 1994; Huertas et al. 1995). However, the dataset obtained in this study suggests the relationship is more complex, especially across the northern regions of Australia. For Macropus enamel carbonate $\delta^{18}O$ values, the strongest negative correlations were between precipitation and evaporation (and therefore also moisture balance), which overall, is indicative of environmental ‘moisture availability’.

An attempt to find strictly linear relationships between meteorological variables and Macropus $\delta^{18}O$ values is hampered by the fact that there may not be clear or direct linkages between them. The sensitivity of Macropus metabolic water to changes in climate and diet is not known. Differences in Macropus drinking habits, behaviour and other physiological responses compound and make quantifying these relationships in this manner extremely difficult. Furthermore, attempting to find relationships between rainfall $\delta^{18}O$ values is limited by the low level of variability in the geographical distribution of rainfall $\delta^{18}O$ values in Australia (Hughes and Allison 1984). Furthermore, there is a lack of rainfall $\delta^{18}O$ data, especially on small regional geographic and topographic scales, available for comparisons. In light of these limitations, this study has observed the trends that prevail between Macropus $\delta^{18}O$ data and different habitats; overall, they are qualitative rather than quantitative.

Attempts have been made to solve the ‘isotopic equation’ for species-specific responses to meteoric water $\delta^{18}O$ values, which can be used to quantify palaeoprecipitation levels from fossil $\delta^{18}O$ values (e.g. Longinelli and Nuti 1973; Luz et al. 1990; Kohn 1996). More specific equations have been derived for mammals that do not rely as heavily on surface meteoric water sources, for example drought tolerant animals such as the kangaroos (Ayliffe and Chivas 1990 (see Equ. 3, pg 2607); Kohn 1996). A refinement of this relationship for the macropods was not attempted in this study. It is well known that the both moisture sources and water utilisation of the Macropus is highly habitat dependent (Blaney et al. 2000; Dawson et al. 2000a; 2000b; McCarron et al. 2001), as such, the animal itself can not always be considered a constant. For example in southern Australia, the kangaroos have greater access to free water (and are often seen drinking) so they may gain a greater proportion of oxygen from surface water sources. As well, during the summer the temperatures are lower than in the north so they may sweat or pant less. In contrast, the kangaroos in the arid regions of central and northern Australia rarely have free water to drink, and they demonstrate different behavioural and physiological adaptations to survive under extreme heat and water stress. To formulate an accurate equation, many input and output
variables would need to be considered (see Section 2.5.3.1). If the magnitudes and proportions of each input and output variable that need to be quantified can vary markedly depending on what environment the kangaroo lives in, this means that a single equation that defines all *Macropus* may not be appropriate. To begin to overcome these constraints, separate equations for each *Macropus* species within major bioregions need to be established, before quantitative interpretations of climate variables could be gained from *Macropus* δ18O values.

### 5.5.4 A multi-isotope approach to define *Macropus* habitats and climate

The *Macropus* carbon, nitrogen and oxygen stable isotope values were plotted against latitude to observe the nature and location of the isotopic changes from the tropical and sub-tropical north to the more temperate south (between 136.5-148° E). In addition, the paired isotope values were plotted relative to latitude to demonstrate how the isotopes co-vary across these zones. These relationships are illustrated in Figure 5-32 A to E.

The change in isotope values over latitude reiterates the results and trends discussed previously: in particular, it shows the greater variability in δ15N and δ18O values in the northern areas above ~25 degrees latitude. Across this traverse, the nitrogen and oxygen isotopes co-varied positively ($r^2=0.6$, $P<0.001$) but the carbon and oxygen and the carbon and nitrogen isotopes did not ($r^2=0.3$ and $r^2=0.1$ respectively, both $P<0.5$). Figure 5-32 D-F show that the boundary between the northern more warmer ‘tropical’ north and the cooler more temperate south are best defined by the carbon isotope values. This reflects the well established and empirically observed relationships between the climatic conditions that define the distribution of C3 and C4 plants across the continent, and globally. Despite their greater variability, the nitrogen and oxygen isotopes give additional information regarding the habitat’s dryness and overall aridity.
Figure 5-32. A to C show plots of *Macropus* $\delta^{13}$C, $\delta^{15}$N and $\delta^{18}$O values against latitude (specimens were located between 136-148°E). D to F show 3-dimensional comparisons of paired isotopes against latitude, the data from above and below 25° South were coloured red and green, respectively, to emphasise the the ranges and trends in isotopic values in these different areas.
5.6 Summary and conclusions

This research on the stable isotope ratios in *Macropus* has examined isotopic variability on two levels; intra-population and intra-tooth variability. Intra-population variability in all three isotopes averaged around 3 to 4%, which shows that considerable variability exists in modern populations living at the same site. The examination of intra-population isotopic variability considered factors such as age, habitat and species and the findings are summarised as follows:

- Bone collagen $\delta^{13}C$ and $\delta^{15}N$ and enamel carbonate $\delta^{18}O$ values did not correlate with age, especially when individuals aged over 3 years were considered. An age-dependent relationship within the *Macropus* bone collagen isotope data was not apparent most likely because the bone has remodelled since youth. However, evidence for age-related trends remained permanently recorded in the teeth tissues.

- Variation was greater at the sites and in regions that have mixed C$_3$/C$_4$ plant availability.

- Bone collagen $\delta^{13}C$ results elucidated differences in the dietary niches of the three co-existing *Macropus* species. However, evidence of niche partitioning was only discernible in the northern C$_4$ grass dominated. In contrast, there were no recognisable differences in the $\delta^{15}N$ and $\delta^{18}O$ values between species. Thus, carbon stable isotope analysis may be a tool to help identify the dietary overlap, competition and feeding niches of the fossil *Macropus* species at local and regional scales.

The findings from the examination of inter-tooth variability are summarised as follows:

- Enamel carbonate $\delta^{13}C$ inter-tooth study showed that juvenile teeth were approximately 3.3% ± 1.7% more depleted than the adult-formed teeth. This trend was common across all habitats and species, which suggests that there is a physiological age-related basis for this observation.

- Dentine collagen $\delta^{15}N$ inter-tooth study observed that juvenile teeth were approximately 1 ± 0.8% more enriched than the adult teeth. A larger sample size is required to validate if this apparent enrichment is due to tropic-level via milk consumption.

The levels of intra-population and inter-tooth variability observed in this study were large enough to affect the accuracy of palaeodietary and palaeoecological interpretations of fossil *Macropus* significantly. In light of this, two sampling procedures for the *Macropus* are suggested: firstly, sample sizes of at least ten or more specimens need to be analysed to begin to obtain a meaningful estimation of the population's average isotope values. Secondly, to maintain consistency for both temporal and spatial interpretations of enamel stable isotope values, sampling should analyse and compare the same tooth type. The most suitable tooth types to analyse are the adult third and fourth molars because they represent the adult diet isotopic values.
The relationships between carbon, nitrogen and oxygen isotopes and the climate variables and habitats are summarised as follows:

- The carbon isotope values of modern *Macropus* followed the general geographic patterns of C$_3$ and C$_4$ grass distribution in Australia. Thus, fossil *Macropus* $\delta^{13}$C values will be a valuable proxy for estimating the relative levels of C$_3$ and C$_4$ plants in past environments.

- The nitrogen isotope values of modern *Macropus* became more enriched as the environments became drier and less humid. Within this study, the 500mm annual precipitation boundary, which marks the transition between the semi-arid and wetter temperate zones, was the point where $\delta^{15}$N values diverged most sharply; specifically, below 500mm the $\delta^{15}$N values were more enriched than 6‰, and above 500mm the $\delta^{15}$N values were more depleted than 6‰. Although, the relationship between *Macropus* $\delta^{15}$N values and annual precipitation was not strictly linear, *Macropus* $\delta^{15}$N values can be used to infer relative levels of moisture availability in the environment. Specifically, $\delta^{15}$N values can provide a quantitative indicator of annual precipitation that indicates occupation within either of these contrasting precipitation and ecological zones.

- The oxygen isotope values of modern *Macropus* became more enriched as the environments became drier and less humid. Although $\delta^{18}$O values did not correlate strongly with local precipitation or relative humidity, they can still provide qualitative information about palaeoenvironmental conditions.

- Overall, the isotope-climate relationships are stronger in the southern C$_3$ plant dominated regions than in the northern mixed C$_3$/C$_4$ plant and C$_4$ grass dominated regions. Hence, there are differences in the response of *Macropus* stable isotope values to environmental factors between these two climatic zones that need to be investigated further. Carbon isotope ratios are the most appropriate isotope to differentiate between these two climatic zones in palaeoecological studies.

In conclusion, this study has examined the levels of variability in *Macropus* stable isotope values and what factors within and between populations has influenced these. In the future, well designed sampling protocols will lessen the additional biases of low sample size and incompatible comparisons between teeth or species. This study has shown that *Macropus* stable isotope values are influenced by climate; and a multi-isotope analysis approach will provide a better overall picture of the habitat types and environmental conditions than a single isotope type will. In conclusion, the stable isotope analysis of fossil *Macropus* has the potential to provide valuable palaeoenvironmental information on both qualitative and quantitative levels.
CHAPTER 6

6  A STABLE ISOPOE STUDY OF MODERN WOMBATS

This section presents the isotopic study of the bone collagen, enamel carbonate and faeces of modern wombats (genus *Vombatus* and *Lasiorhinus*) collected from five locations in the northern and southern parts of eastern-Australia. Wombats are small grazing herbivores that inhabit a wide range of environments within contrasting climatic zones, such as: semi-arid, tropical, temperate bushlands, grasslands, and alpine herb-fields. Wombats are common in the Quaternary fossil record and are a key species for palaeoecological research. Consistent with the preceding studies of the koalas and kangaroos, the relationships between modern wombat stable isotope ratios and their diets and environments were examined.

A major focus of this research on wombats is the analysis of carbon and oxygen isotopes in tooth enamel. In regards to their dentition, the wombats are unique among the living marsupials because their teeth grow continuously throughout life. Therefore, the $\delta^{13}C$ and $\delta^{18}O$ values along a tooth represent a time sequence of isotopic variations relating to dietary changes during its growth. This part of the project examines the utility of the isotopic signatures in wombat teeth for providing evidence of seasonality in diet and climatic conditions on a fine intra/inter-annual time scales. Stable isotope proxy records of seasonality in the terrestrial environment provide a more detailed picture of the nature of short term climate changes that have shaped the biogeography of a given area. As such, these high resolution records are highly sought because they are valuable palaeoecological tools.
The objectives of this study on wombats were to:

1. Identify the relationships between $\delta^{13}C$ values of the wombats’ diet and bone collagen and enamel carbonate tissues. This part of the study included analysis of faeces to determine diet $\delta^{13}C$ values and calculated the isotopic enrichment between diet and these two tissues, and also the spacing between tissues.

2. Investigate the levels of intra-population variability in bone collagen $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ values.

3. Micro-sample incisor enamel in order to determine the magnitude of variability in $\delta^{13}C$ and $\delta^{18}O$ values along the tooth and examine these variations in light of seasonal diet and monthly climate changes. This part of the study also included an attempt at refining the growth rate of wombat incisors so that stable isotope values could be matched with Calendar time.

4. Examine how the $\delta^{13}C$ and $\delta^{15}N$ values in bone collagen correlate to mean annual climate data and whether relationships reflect environmental signals such as moisture availability and habitat types.

6.1 Background to wombat ecology

Wombats are large (~20-40kg) burrowing, herbivorous marsupials. There are three extant allopatric species from contrasting habitats: *Vombatus ursinus* (common wombat) lives in the more mesic regions of the coast and ranges of south-eastern Australia; *Lasiorhinchus latifrons* (southern hairy-nosed wombat) has a patchy distribution in the semi-arid regions of southern Australia, and *Lasiorhinus krefftii* (northern hairy-nosed wombat) lives in the semi-arid tropical regions of inland north central Queensland. The northern hairy-nosed wombat is restricted to a single population of approximately 113 individuals in the Epping Forest National Park (NP) and is currently classified at critically endangered (Dr A Horsup, pers com., 2003). These present day wombat distributions are shown in Figure 6-1 (below).

Wombats are territorial and live within large community burrow structures, with home ranges that extend approximately 5 to 6 hectares (Wells 1978; Horsup 1999). In some regions common wombats may travel up to 3km to graze and have ranges up to 25 hectares (Strahan 2000). They are largely nocturnal, especially in the semi-arid regions and during the hot summer months.

6.1.1 Diet selection and forage availability

Wombats feed mainly on native perennial grasses and smaller quantities of sedges and forbs. An analysis of northern hairy-nosed wombat faeces identified that dicotyledons contributed to less
than 10% of the northern hairy-nosed wombat diet (Woolnough and Johnson 2000). A similar faeces study by Mallet and Cooke (1986) observed, that common wombats in the southeast of South Australia (not a location examined in this study) ate mostly grasses in summer (>75%), while in winter, the proportion of sedges increased (~63%). Although wombats have a preference for grasses, they have been observed digging roots, eating saltbush and taking bark off trees during times of extreme drought and lack of green pastures (R.T. Wells pers. com., 2004; Wells 1989).

The quality of the wombat’s grass diet may change seasonally. Rainfall and pasture growth is highly seasonal in the semi-arid regions, and the proportion of fibrous cellulososes and lignin to nutritious cell wall contents increases as the grass ages and dries off in the summer months (Hume 1999). A detailed pasture evaluation at Epping Forest NP found that the nutritive value of native grasses decline during the dry seasons, and in particular, total nitrogen content declines by ~36% (Woolnough and Foley 2002). Forage availability and quality in the mesic regions, which are inhabited by the common wombat, is more abundant than in the semi-arid regions. In the alpine regions, new grass growth declines during the snow covered winter months from June to late August (Mallett and Cooke 1986; Green and Obsorne 1994; Hume and Barboza 1998).

6.1.2 Digestion and metabolism

The wombats’ grass diet is very fibrous and high in abrasive silica inclusions, yet wombats have an efficient masticatory system that releases nutrients from leaf cells and reduces digesta particle size. Wombats are hindgut fermenters: their digestive physiology comprises a small stomach, vestigial caecum and small intestine, but they have a large and extensive proximal colon (Barboza and Hume 1992). Leaf cell contents are digested in the simple small stomach and small intestine. The carbohydrates in cellulose of cell walls are broken down into short chained fatty acids (SCFA) by microbial fermentation in the proximal colon. SCFA production provides a large proportion of the wombats’ energy requirements and is equivalent to 30 to 33% of their digestible plant intake (Barboza and Hume 1992; Barboza 1993).

Wombats consume a low nutrient diet, yet it is higher in crude protein, has few secondary metabolites (such as phenolics and tannins) and is more easily digested than the Eucalyptus leaves eaten by koalas (see section 4.1.2). For example, maximum crude protein levels of Stipa nitida grass (eaten by the southern hairy-nosed wombat) and Austrodonthonia sp. (wallaby grasses, eaten by the common wombat) are approximately 11% and 12-18%, respectively, compared to the 6 to 9% of some Eucalyptus species (Cork 1986; Archer and Robinson 1988; Hume 1999).
The wombats’ maintenance requirements for nitrogen are low: approximately 201 and 158 mg/kg\(^{-0.75}\)d\(^{-1}\) for \textit{Lasiorhinus} and \textit{Vombatus}, respectively (Barboza et al. 1993). Low nitrogen in the diet is compensated for by low rates of nitrogen loss in urine and faeces and high rates of endogenous urea recycling. For example, in wombats on low protein diets, up to 78% of their synthesised urea is recycled, and between 49 to 60% may be incorporated into microbial protein (Barboza et al. 1993; Hume 1999).

Wombats lead an energy conserving lifestyle. They are mainly nocturnal and shelter from the climatic extremes in their burrows. Northern hairy-nosed wombats sometimes spend less than 6 hours each day out of their burrows feeding (Horsup 1999). As might be expected, the basal and field metabolic rates (BMR and FMR, respectively) of wombats are low (Hume 1999). A study by Evans et al. (2003) measured the seasonal FMR of all wombat species to be lower than that recorded for any other Australian marsupial, and in particular, \textit{Lasiorhinus latifrons} has a FMR 40% lower than any other mammal. Their daily energy requirements (~140 kJ/kg\(^{-0.75}\)d\(^{-1}\)) are only 32% of that estimated for macropods (Barboza et al. 1993).

The study by Evans et al. (2003) also measured the water flux rates for all three species of wombats and found that preformed water in food is their main source of water in both the dry and wet seasons. However, wombats have been observed drinking from streams (Strahan 2000; personal observations, Tharwa ACT 2002). In semi-arid habitats, surface waters are extremely rare and ephemeral, so the hairy-nosed wombats are less likely to drink free water. In light of these observations, wombats are considered to be non-obligate drinkers, yet their specific drinking habits may be site-dependent.

### 6.1.3 Life history

The three wombat species do not have the same or a strictly defined breeding season. Common wombats, in southern South Australia and the alpine region in Victoria, have been observed breeding from autumn to early winter, with weaning beginning in spring (Mallett and Cooke 1986). It appears that reproduction in the hairy-nosed wombats is more likely to occur in response to new pasture growth, which has the capacity to provide adequate nutrition for the additional demands of lactation on females and grazing of new joeys (Crossman et al., 1994; R.T. Wells pers com. 2004). Most births of the southern hairy-nosed wombat occur from late September to December (Strahan 2000), and from November to April for the northern hairy-nosed wombat (Horsup 1999). Wombats usually give birth to a single joey after approximately 21 days gestation. Pouch life lasts approximately 6 to 9 months and lactation for approximately 9 months. The joeys remain with the mothers for approximately 11-12 months (Wells 1978a; Mallett and Cooke 1986; Wells 1989; Horsup 1999).
6.1.3.1 Tooth formation

All wombat teeth are rootless and grow continuously. This has been considered a successful morphological adaptation that enables wombats to exploit an abrasive silica rich grass diet. The wombat’s teeth begin to form before weaning, and as the juvenile begins to eat grass, the enamel cusps are quickly obliterated and the softer dentine core is exposed. Only the labiodental side of the incisor teeth are enamel covered, the softer dentine is exposed on the upper surface and wears to create a spatulate-like upper surface with a hard enamel lower edge. Wombats can not be aged easily via their teeth (as is done for koalas and kangaroos), however, an adult’s lower incisor teeth are approximately 6 to 8 cm long, and the molars are around 3 to 4 cm long; most of the tooth’s length is encapsulated within the alveolar. Figure 6-2 (below) shows adult wombat incisor teeth that were sampled in this study.

6.1.3.2 Wombat teeth growth rate

Wombat teeth growth rates have not been reported in literature; however, a growth rate of 0.1 mm/d has been estimated for adult wombat teeth by Dr G. Sanson at Monash University (G. Sanson, pers com. November, 2004). This unpublished research also found that tooth growth rates may vary; in particular, that growth rates increase with the abrasiveness of the diet. From this observation (as it has not been tested), it could be suggested that the teeth of wombats living in the semi-arid regions grow faster than the teeth of wombats living in the more mesic highly vegetated regions. This is because the dry arid grasses are very fibrous and less plant cover results in abrasive soil particles being ingested along with food, which causes faster tooth wear.

6.2 Potential factors influencing stable isotopes in wombats

Carbon isotopes in body tissues of grazing herbivores are related to the proportions of C₃ and C₄ grass in the diet (DeNiro and Epstein 1978). The abundance of C₃ and C₄ grasses is related to climate (Johnston 1996), and their distributions in the Australian continent have been documented by (Hattersley 1983). Hattersley’s map (Figure 3-2 in Section 3) provided a general indication of the C₃ and C₄ grass species composition in each wombat habitat (see Table 6-1).

This study analysed bone collagen and enamel carbonate so the variations in δ¹³C isotope signatures of these two tissues could be compared. Bone collagen δ¹³C values represent a longer-term average of an individuals’ dietary intake because it is constantly remodelled through-out life. In contrast, enamel forms once, therefore the serial intra-tooth δ¹³C values represent a temporal sequence of dietary intake that should indicate how diet δ¹³C values fluctuate over time. In habitats where the proportions of C₃ and C₄ grasses change seasonally, it
is expected that there will be a change in the $\delta^{13}C$ values along the teeth. Because $C_3$ plants grow in cooler and moister conditions than $C_4$ plants, it is predicted that in some regions the wombats' intra-tooth $\delta^{13}C$ values will be relatively more enriched during the summer months and more depleted during the winter months. This study could also examine how the seasonal change teeth $\delta^{13}C$ values compare to the more time averaged $\delta^{13}C$ values represented in bone collagen.

The $\delta^{15}N$ of bone collagen of herbivores records the $\delta^{15}N$ of the plant dietary proteins (DeNiro and Epstein 1981). Within the Australian continent, Gröcke et al. (1997) identified a strong negative correlation between Macropus bone collagen $\delta^{15}N$ values and precipitation. Although this relationship was not evident in the koala data presented previously, this relationship was still assessed for wombats.

The $\delta^{18}O$ of enamel carbonate reflects the $\delta^{18}O$ of mammalian body waters, and in those species that drink freely, it varies in accordance with the $\delta^{18}O$ of meteoric waters (Longinelli and Nuti 1973). Wombats are non-obligate drinkers so their $\delta^{18}O$ values should be heavily influenced by the $\delta^{18}O$ values of leaf water, which is enriched greatly in $^{18}O$ over meteoric water (Roden and Ehleringer 1999). The leaf water $\delta^{18}O$ values in grasses eaten by wombats could not be measured in this study; therefore, it is not known how leaf water $\delta^{18}O$ values change seasonally. The $\delta^{18}O$ values of local rainfall and surface waters are also unknown. Previous studies have observed that the $\delta^{18}O$ values of mammals in arid and drought affected areas correlate more strongly with humidity levels than with meteoric water values (Ayliffe and Chivas 1990; Kohn 1996). During some seasons, wombats may be exposed to free water, so it is possible that changes in moisture sources may affect wombat $\delta^{18}O_{\text{enamel}}$ values. At this stage, it is difficult to predict the effects different climatic conditions have on wombat $\delta^{18}O$ values. Typically, interpretations of sinusoidal patterns of intra-tooth $\delta^{18}O$ values assign the more enriched values as representing summer and the less enriched values as representing winter (Fricke and O'Neil 1996; Stuart-Williams and Schwarcz 1997). The wombat intra-tooth $\delta^{18}O$ values were assessed for this relationship with seasonality.
6.3 Sample locations and methodology

6.3.1 Study site locations and sampling

Adult wombat bone, incisor teeth and faeces samples were collected between August 2001 and February 2003 from the five locations shown in Figure 6-1. The wombat samples from Epping Forest National Park (NP) site were obtained from an existing collection belonging to the Queensland National Parks and Wildlife Service, these were collected between November 1996 and December 2001.

![Map of Australia showing wombat populations](image)

**Figure 6-1.** Present-day distribution of wombats in Australia and the location of specimens analysed in this study.

The $\delta^{13}$C and $\delta^{15}$N compositions of the wombats' diet were determined by analysing wombat faeces. A collection of 14 northern hairy-nosed wombat faeces samples from Epping Forest NP were obtained from Dr A Horsup at Queensland Parks and Wildlife, Rockhampton. These faeces had been collected on a seasonal basis between January 1999 and December 2001. Five samples were collected from the Tharwa area between June 2002 and February 2003. Six samples were collected from the Saw Pit Creek and Charlotte’s Pass areas in Mt Kosciusko NP between May 2002 and February 2003.

6.3.1.1 Wombat incisor teeth microsampling

A total of eight incisors from different wombats were micro-sampled, which provided 384 subsamples on which $\delta^{13}$C and $\delta^{18}$O values were analysed. At least one incisor was sampled from
each population. Subject to availability, two incisors were sampled from the Brookfield CP population and three from the Braidwood population. The wombat’s year and month of death were recorded, except for specimen W1016 from Epping Forest NP.

The lower incisors were sampled because it is the straightest and longest tooth in the jaw - two incisors are shown in Figure 6-2. The first 1-1.5 cm at the base if the tooth comprises less crystalline enamel and is higher in organic material; this portion was discarded if it was pliable and discoloured. The length of each incisor was measured before cutting. The incisor was first cut in half lengthwise and the dentine was removed from the lower enamel bearing side using a diamond cutting burr. The incisor was then cut, perpendicular to the growth axis, into approximately 1-1.5mm wide sub-samples using a 200μm thick diamond blade. The width of each sub-sample and the remaining length of tooth were recorded after each cut. This ensured that the position of each sub-sample along the tooth was documented.

![Wombat incisor sample](image)

**Figure 6-2.** Wombat lower incisors and cutting lines.

6.3.1.2 Incisor tooth growth rate and time resolution

The calendar time represented by each tooth was calculated based on the growth rate of 0.1mm/d (G. Sanson, pers. com., 2004) and the animal’s date of death. For example, a 7.5cm lower incisor could represent approximately 2 years growth. Due to the uncertainty in the growth rate, this was considered a preliminary estimation. Stuart-Williams and Schwarcz (1997) constrained the growth rate of beaver incisors, which also grow continuously, by matching the isotopic data to seasonal fluctuations. This approach was also investigated as a possible method to determine the growth rates of wombat incisors in this study. This method was limited to those specimens that had a marked isotopic variability (i.e. >2‰ in δ¹⁸O) with a repeated pattern in isotope vales.
Even though the growth rate can be refined to calendar time, two additional uncertainties remain. First is the time resolution represented in each sub-sample and the second is the time lag between the isotopic signals of ingested foods/water being incorporated into the enamel tissues.

The amount of time represented in a given intra-tooth sub-sample depends on several factors. A 1-1.5 mm sub-sample, based on the growth rate of 0.1 mm/d, could represent approximately 10 to 15 days growth. However because enamel layers are deposited at an angle to the dentine-enamel junction (DEJ) and not perpendicular to the growth axis (Hillson 1986), the sub-samples taken here contain enamel from layers formed over longer time spans. Although enamel secretion is immediate, subsequent enamel maturation can occur heterogeneously over a period of a few weeks to months. As discussed previously (in section 2.2.3.1 and detailed in Wiedemann et al. 1999; Balasse, 2003), these factors will result in a certain degree of time averaging and a possible dampening of the amplitude of isotopic signals.

The equilibration time for isotopic changes in the diet or water intake to be reflected in wombat enamel tissues is unknown. This timing can be species specific, and may also be dependent on body size and metabolic rate. For example, Sharp and Cerling (1998) observed that it took between 10 to 14 days in a human volunteer, for an isotopic change in ingested water δ¹⁸O values to be detected in respired CO₂. Bryant and Froelich (1995) suggested the equilibration rate is faster in animals with high metabolic rates; wombats have a very low metabolic rate (see Section 6.1.2), which may mean that their isotopic turnover is slower than other mammals.

These two uncertainties can only be resolved with a detailed histological examination of wombat enamel, and experiments involving ingestion of fluorescent dyes (eg. oxy-tetracycline) and isotopic markers. Nonetheless, this study has accomplished a relatively high sampling resolution, more likely to be in the order of a couple of weeks rather than months.

### 6.3.2 Analytical procedures

Sample preparation and isotopic analysis of wombat faeces, enamel and bone collagen were undertaken using the methods described in Section 3.2. It is noted here that the δ¹⁵N analysis of wombat faeces was unsuccessful, because only three of the 25 samples yielded enough nitrogen gas for isotopic measurement. Consequently, δ¹⁵N values of wombat faeces are not included here.
6.3.3 Study site climate information

The five study sites have varying climatic conditions, which are summarized in Table 6-1. The proportions of C$_4$ grasses at each site have been estimated from the percentage C$_4$ grass distribution map of Hattersley (1983), which is shown above in Figure 3-2. The mean monthly precipitation, temperature, evaporation and 3pm relative humidity data for each site are shown in Figure 6-3 (below).

Table 6-1 The climate summary for the six wombat populations

<table>
<thead>
<tr>
<th>Wombat population location</th>
<th>Location (decimal degrees)</th>
<th>Climate description</th>
<th>Dominant rainfall season/ surface water availability</th>
<th>Rainfall (mm)</th>
<th>Temp. ($^\circ$C): max. [min.]</th>
<th>3pm % relative humidity</th>
<th>%C$_4$ grass$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Tharwa</td>
<td>S 35.503 E 148.987</td>
<td>Cool temperate: mild summers, cool winters</td>
<td>Uniform, small ephemeral creeks</td>
<td>830</td>
<td>18.6 [5.9]</td>
<td>50.3</td>
<td>20-30</td>
</tr>
<tr>
<td>5. Epping Forest National Park</td>
<td>S 23.3999 E 146.683</td>
<td>Topical semi arid: hot humid summers, dry warm mild winters</td>
<td>Summer, no permanent water courses or ponds</td>
<td>576 (± 228)</td>
<td>29.3 [14]</td>
<td>36.9</td>
<td>&gt;90</td>
</tr>
</tbody>
</table>

Climate data was obtained from ANUCLIM and Bureau of Meteorology Climate Data CD.
* Estimated % C$_4$ grass species based on data from Hattersley (1983)

Although wombats are non-obligate drinkers and consequently their δ$^{18}$O values are not expected to reflect the δ$^{18}$O values of meteoric rainfall directly, the rainfall δ$^{18}$O values measured at Brisbane, Melbourne and Adelaide have been included in Figure 6-4 to show the annual variation in mean monthly δ$^{18}$O values. These three sites are the locations closet and most relevant to the wombat populations used in this study. This figure shows the δ$^{18}$O values at all sites are more depleted in the late autumn and winter months of April to July and more enriched in the summer months of December and January. The overall annual range of values is approximately 2.5 to 3‰. The southern more temperate sites of Melbourne and Adelaide generally have lower δ$^{18}$O values than Brisbane in the north.
Figure 6-3. Mean monthly climate data for the five sample locations

Figure 6-4. The mean monthly \(\delta^{18}O\) precipitation values at Adelaide, Melbourne and Brisbane. The box plots show the annual mean (dotted line), median values, and 5th and 95th percentile ranges. Data is from the Global Network of Isotopes in Precipitation at http://isohis.iaea.org
6.3.4 Comparison of stable isotope results with climate data

To examine the relationships between climate variables and the stable isotopes in wombat bone collagen, the five populations mean $\delta^{15}$N and $\delta^{18}$O values were plotted against the following mean annual climate data of each location: a) precipitation, b) potential evaporation, c) maximum daily temperature, d) 3pm % relative humidity, and e) moisture balance (rainfall minus evaporation).

In contrast to the koala and Macropus studies, wombat bone collagen $\delta^{13}$C values were not correlated with these climate variables because wombat bone collagen $\delta^{13}$C variability is primarily influenced by the proportions of C$_3$ and C$_4$ grass in the diet, therefore, correlations between $\delta^{13}$C values and climate data per se will not reflect the first order plant-climate relationships accurately. Furthermore, this observation was previously recognized in the $\delta^{13}$C results in the Macropus study (Chapter 5).

An estimate of the incisors growth rate was needed to help establish the time period represented by each tooth, so that comparisons with actual monthly climate data could be considered. An attempt to constrain the growth rates more precisely were made using the wombat's date of death and the timing of its peak intra-tooth $\delta^{13}$C values with the previous seasons - samples from the Braidwood site were used. Undoubtedly, a circular argument is evoked when this derived growth rate is then used to extrapolate evidence for seasonal responses between isotopes and climate. For that reason, it must be noted that the derived growth rate used below served to provide a temporal setting in which to examine whether the variation in isotopic values met with expected seasonal trends and models of isotopic responses to climate that have been observed in other studies. The date of death was unknown for specimen W1016 from Epping Forest NP, therefore this specimen's isotope values were only compared to the ranges of annual mean monthly climate data.

6.3.4.1 Presentation of incisor intra-tooth data

In contrast to the koala and Macropus chapters, when the wombat incisor intra-tooth isotopic values are compared to monthly climate data, both the $\delta^{13}$C and $\delta^{18}$O ratios are presented together. This not only avoids a repetition of plots, but also enables the temporal co-variations in both isotopes to be observed.
6.4 Wombat stable isotope results

6.4.1 Carbon isotope results

6.4.1.1 Bone collagen and faeces

The faeces and bone collagen carbon isotope results for the five wombat populations are presented in Table 6-2. The raw data are included in Appendix C (Tables C-1 and C-2). In this dataset, the $\delta^{13}C$ values of faeces and bone collagen ranged from -13.3 to -30.1‰ and -8.96 to -25.4‰, respectively.

Table 6-2. Faeces and bone collagen carbon stable isotope results

<table>
<thead>
<tr>
<th>Wombat populations</th>
<th>Mean $\delta^{13}C$ faeces</th>
<th>Mean $\delta^{13}C$ bone collagen</th>
<th>Isotopic enrichment between faeces and bone collagen</th>
<th>% of range of $\delta^{13}C$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Brookfield Conservation Park (CP)</td>
<td>n/a</td>
<td>-22.3</td>
<td>n/a</td>
<td>1.3</td>
</tr>
<tr>
<td>Group mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of samples</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Mt. Kosciusko National Park (NP)</td>
<td>-29.1</td>
<td>-24.0</td>
<td>5.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Group mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.2</td>
<td>0.9</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Number of samples</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Tharwa</td>
<td>-26.9</td>
<td>-21.5</td>
<td>5.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Group mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.9</td>
<td>1.0</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Number of samples</td>
<td>5</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Braidwood</td>
<td>n/a</td>
<td>-20.7</td>
<td>n/a</td>
<td>4.6</td>
</tr>
<tr>
<td>Group mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of samples</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Epping Forest National Park (NP)</td>
<td>-14.3</td>
<td>-9.6</td>
<td>4.7</td>
<td>2.1</td>
</tr>
<tr>
<td>Group mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.7</td>
<td>0.6</td>
<td>0.9</td>
<td></td>
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<tr>
<td>Number of samples</td>
<td>14</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean value</td>
<td></td>
<td>5.0</td>
<td></td>
<td>2.4</td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td>0.4</td>
<td></td>
<td>0.3</td>
</tr>
</tbody>
</table>

The $\delta^{13}C$ values of faeces from Epping Forest NP, Mt Kosciusko NP and Tharwa are shown in Figure 6-5. The faeces $\delta^{13}C$ values clearly defined the northern hairy-nosed wombats as mostly $C_4$ grass consumers and the common wombats at the two New South Wales sites as mostly $C_3$ plant consumers. At the Epping Forest site, the range of faeces $\delta^{13}C$ values was 2.4‰ and there was no distinct seasonal pattern in isotope values over the 23 month sample period. The Mt Kosciusko and Tharwa sites had larger ranges in faeces $\delta^{13}C$ values than the Epping Forest site; 3.1 and 4.5‰, respectively. Although the samples from these two sites represent a shorter 10 month period, a distinct seasonal change is evident- faeces $\delta^{13}C$ values gradually became more enriched towards the summer months. This change was larger at the Tharwa site than the Mt Kosciusko site.
Figure 6-5. Carbon isotope values of wombat faeces from the three sampling sites.

The box-plots in Figure 6-6 (below) compare the spread of the bone collagen $\delta^{13}C$ values ($\delta^{13}C_{\text{collagen}}$) of each wombat population. Most noticeably, the northern hairy-nosed wombats at Epping Forest NP are isotopically distinct from the southern populations of common and southern hairy-nosed wombats at sites 1 to 4. An ANOVA test found that the $\delta^{13}C_{\text{collagen}}$ values of wombats from sites 1 to 4 are still significantly different ($P \leq 0.001$). The post-hoc Tukey HSD test found that the $\delta^{13}C_{\text{collagen}}$ values of wombats from Brookfield CP, Tharwa and Braidwood were not significantly different to each other ($P \geq 0.05$). The most depleted $\delta^{13}C$ values of the Mt Kosciusko NP wombats separate this group from the others.

Figure 6-6. Boxplots of bone collagen $\delta^{13}C$ results from each wombat population
6.4.1.2 Bone collagen δ\textsuperscript{13}C intra-population variability

The largest intra-population range in δ\textsuperscript{13}C\textsubscript{B-collagen} values was 4.6‰ in the Braidwood population and the smallest range was 1.3‰ in the Brookfield CP population. The mean intra-population range in wombat δ\textsuperscript{13}C\textsubscript{B-collagen} values was 2.4 ± 0.3‰.

6.4.1.3 Isotopic enrichment between diet δ13C and bone collagen δ\textsuperscript{13}C

The faeces δ\textsuperscript{13}C values have been used to determine wombat δ\textsuperscript{13}C diet values. The calculated values for isotopic enrichment between diet and δ\textsuperscript{13}C\textsubscript{B-collagen} of the three populations are included in Table 6-2. The data from the Epping Forest NP site provide the most accurate estimation of the diet to bone collagen enrichment, this figure was 4.7 ± 0.9‰. This is because firstly, the faeces were collected over a longer period (23 months compared to 10) so the mean of these samples (N=14) was a good estimate of the mean annual plant diet δ\textsuperscript{13}C values. Secondly, the Epping Forest bone collagen sample size was larger (N=17) than sites 2 and 3 (N=5 and 6, respectively).

6.4.1.4 Incisor intra-tooth δ\textsuperscript{13}C results

The δ\textsuperscript{13}C results of the intra-tooth microsampling analysis are summarised in Table 6-3. This table includes the mean, minimum, maximum and range of isotopic values of each incisor. The raw data for each incisor are included in Appendix C (Tables C-3 to C-10). Table 6-3 also includes the δ\textsuperscript{13}C\textsubscript{B-collagen} value of each wombat for comparison. Similar to the findings of the δ\textsuperscript{13}C\textsubscript{B-collagen} results, the mean of the intra-tooth δ\textsuperscript{13}C\textsubscript{enamel} values identified the wombats from Brookfield CP, Mt Kosciusko NP, Tharwa and Braidwood as predominantly C\textsubscript{3} plant consumers and the wombat at Epping Forest NP as C\textsubscript{4} grass consumers. The common wombat in the alpine region at Mt Kosciusko NP (W506) had an extremely depleted mean intra-tooth δ\textsuperscript{13}C\textsubscript{enamel} value (-16.59‰), which indicated it most likely consumed a 100% C\textsubscript{3} plant diet.

The sequence of intra-tooth δ\textsuperscript{13}C values of each wombat incisor are shown in Figure 6-7. This plot shows the range and amplitude of isotopic variation along each tooth, from base to crown. It is important to note that the isotopic values at any given point along the x-axis might not be contemporaneous, because most of the wombats lived at different times.

The range of intra-tooth δ\textsuperscript{13}C values was smaller in the wombats from Brookfield CP, Mt Kosciusko NP and Epping Forest NP (-1.6, 1.3 and 1.5‰, respectively) than in the wombats from Tharwa and Braidwood (3.2 and 6.5‰, respectively). The amplitude and pattern of intra-tooth δ\textsuperscript{13}C variation in specimens W450 and W52 from Braidwood, which died at the same time (September 2001), are strikingly similar. These two specimens also had the largest variations in δ\textsuperscript{13}C values of 7.49 and 7.98‰, respectively. This indicated that during the time represented by the tooth there was a substantial change in the proportion of C\textsubscript{3} and C\textsubscript{4} grasses eaten by these wombats.
### Table 6.3. Wombat intra-tooth microsample carbon isotope results

<table>
<thead>
<tr>
<th>Wombat population</th>
<th>Number of microsamples</th>
<th>Length of incisor (cm)</th>
<th>Mean δ¹³C of all microsamples</th>
<th>Standard deviation</th>
<th>Minimum δ¹³C value</th>
<th>Maximum δ¹³C value</th>
<th>% range δ¹³C values</th>
<th>δ¹³C collagen</th>
<th>Collagen to enamel spacing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Brookfield CP</td>
<td>W69</td>
<td>26</td>
<td>-13.66</td>
<td>0.44</td>
<td>-14.54</td>
<td>-12.64</td>
<td>1.90</td>
<td>-22.87</td>
<td>9.21</td>
</tr>
<tr>
<td></td>
<td>W633</td>
<td>60</td>
<td>-13.63</td>
<td>0.42</td>
<td>-14.34</td>
<td>-13.04</td>
<td>1.30</td>
<td>-21.63</td>
<td>8.00</td>
</tr>
<tr>
<td>2. Mt Kosciusko NP</td>
<td>W506</td>
<td>50</td>
<td>-16.59</td>
<td>0.30</td>
<td>-17.33</td>
<td>-16.00</td>
<td>1.33</td>
<td>-23.95</td>
<td>7.36</td>
</tr>
<tr>
<td>3. Tharwa</td>
<td>W881</td>
<td>52</td>
<td>-13.86</td>
<td>1.00</td>
<td>-15.23</td>
<td>-12.02</td>
<td>3.21</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>4. Braidwood</td>
<td>W52</td>
<td>38</td>
<td>-13.71</td>
<td>2.29</td>
<td>-17.20</td>
<td>-9.71</td>
<td>7.49</td>
<td>-21.73</td>
<td>8.02</td>
</tr>
<tr>
<td></td>
<td>W450</td>
<td>43</td>
<td>-12.37</td>
<td>2.26</td>
<td>-17.08</td>
<td>-9.10</td>
<td>7.98</td>
<td>-19.07</td>
<td>6.70</td>
</tr>
<tr>
<td></td>
<td>W873</td>
<td>39</td>
<td>-13.03</td>
<td>1.28</td>
<td>-14.56</td>
<td>-10.6</td>
<td>3.96</td>
<td>-20.76</td>
<td>7.73</td>
</tr>
<tr>
<td>5. Epping Forest NP</td>
<td>W1016</td>
<td>60</td>
<td>-1.62</td>
<td>0.36</td>
<td>-2.45</td>
<td>-0.96</td>
<td>1.49</td>
<td>-9.33</td>
<td>7.81</td>
</tr>
</tbody>
</table>

Mean % spacing: 7.83

Standard deviation: 0.76

![Figure 6-7. Wombat intra-tooth microsampling analysis δ¹³C results](image)

**Figure 6-7.** Wombat intra-tooth microsampling analysis δ¹³C results

#### 6.4.1.5 Spacing between bone collagen δ¹³C and incisor enamel δ¹³C

The spacing between the δ¹³C values of bone collagen and incisor enamel carbonate (Δ¹³C_BC-EC) were calculated using the mean of each wombat’s intra-tooth δ¹³C microsample values and the individual’s δ¹³C_B-collagen value (not the population mean δ¹³C_B-collagen value). The mean Δ¹³C_BC-EC value of the eight wombats was 7.8 ± 0.8%o.
6.4.2 Nitrogen isotope results

The bone collagen nitrogen isotope results are presented in Table 6-4. The raw data are included in Appendix C (Table C-1). In this dataset, the entire range in bone collagen $\delta^{15}N$ values was from 1.8 to 10.2 $\%e$. The box plots in Figure 6-8 (below) compare the spread of $\delta^{15}N$ values from each wombat population. The northern hairy-nosed wombats at Epping Forest have the highest population mean $\delta^{15}N$ value (8.3 ± 0.9$\%e$), and it is visually apparent in Figure 6-8 that this population is significantly different to the other wombat populations. An ANOVA test found that the $\delta^{15}N$ values of wombats from sites 1 to 4 are still significantly different (P≤0.002). The post-hoc Tukey HSD tests found that the $\delta^{15}N$ values of wombats from Mt Kosciusko NP, Tharwa and Braidwood were not significantly different to each other (P=0.164).

<table>
<thead>
<tr>
<th>Wombat populations</th>
<th>Mean $\delta^{15}N$ bone collagen</th>
<th>$%e$ range of $\delta^{15}N$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Brookfield CP</td>
<td>5.7</td>
<td>1.6</td>
</tr>
<tr>
<td>Group mean</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of samples</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>2. Mt Kosciusko NP</td>
<td>3.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Group mean</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of samples</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>3. Tharwa</td>
<td>4.7</td>
<td>4.6</td>
</tr>
<tr>
<td>Group mean</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of samples</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>4. Braidwood</td>
<td>3.9</td>
<td>4.2</td>
</tr>
<tr>
<td>Group mean</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of samples</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>5. Epping Forest NP</td>
<td>8.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Group mean</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of samples</td>
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<td>3.3</td>
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</tr>
<tr>
<td>Standard deviation</td>
<td>1.1</td>
<td></td>
</tr>
</tbody>
</table>

6.4.2.1 Bone collagen $\delta^{15}N$ intra-population variability

The largest intra-population range in $\delta^{15}N$ values was 4.6$\%e$ in the Tharwa population and the smallest range was 1.6$\%e$ in the Brookfield CP population. The mean intra-population range in wombat $\delta^{15}N$ values was 3.3 ± 1.1$\%e$.  

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6.4.2.2  Relationships between wombat bone collagen $\delta^{15}N$ values and climate variables

The relationships between wombat $\delta^{15}N$ values and mean annual climatic variables are shown in Figure 6.9 A-E. The relationships between $\delta^{15}N$ values and evaporation, maximum temperature, 3pm relative humidity and moisture balance were strong. All correlations were statistically significant ($P \leq 0.05$) and the linear regression $r^2$ values were greater than 0.77. The correlation between $\delta^{15}N$ and rainfall was significant ($P < 0.05$), but, the linear relationship between them was weak ($r^2 = 0.3$).

The correlations and linear regressions were computed for the wombat populations that eat a predominantly C$_3$ plant diet (i.e. Sites 1 to 4, excluding Site 5) to see if the effects of removing the C$_3$-grass-eating wombats at the Epping Forest NP site changed the relationships observed previously. These are listed in Table 6-5 (below). All relationships between the five climate variables and $\delta^{15}N$ of the C$_3$ plant consuming wombats remained strong and the linear relationship between rainfall and $\delta^{15}N$ increased to $r^2 = 0.75$.

These results strongly suggest that relative levels of moisture or aridity in the local environment can explain the inter-population variability in $\delta^{15}N$ values. The wombats from the two semi-arid sites, Brookfield CP and Epping Forest NP, had the most enriched $\delta^{15}N$ values ($5.7 \pm 0.7\%o$ and $8.3 \pm 0.9\%o$, respectively), whereas the wombats from the cool alpine region in Mt Kosciusko NP had the lowest mean value ($3.5 \pm 0.9\%o$). Although the $\delta^{15}N$ values at the driest site, Brookfield CP, were not as enriched as the values at the Epping Forest NP site, the relationship between elevated $\delta^{15}N$ values and climatic variables, which indicate greater habitat ‘dryness’ and aridity, was significant.

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Figure 6-9 A to E. Plots of wombat population mean $\delta^{15}$N values against climate
Table 6-5. Statistical computations between wombat δ¹⁵N values and climate variables for wombat sites C3 plant dominated sites (Sites 1-4, excluding Site 5: Epping Forest NP)

<table>
<thead>
<tr>
<th>Climate variable</th>
<th>Rainfall</th>
<th>Potential evaporation</th>
<th>Maximum temperature</th>
<th>3pm relative humidity</th>
<th>Moisture balance</th>
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<tr>
<td>Pearson's correlation coefficient</td>
<td>-0.480</td>
<td>0.496</td>
<td>0.500</td>
<td>-0.594</td>
<td>-0.515</td>
</tr>
<tr>
<td>P value</td>
<td>P=0.004***</td>
<td>P=0.002***</td>
<td>P=0.002***</td>
<td>P=0.001***</td>
<td>P=0.002***</td>
</tr>
<tr>
<td>Linear regression r²</td>
<td>r²=0.75</td>
<td>r²=0.8</td>
<td>r²=0.88</td>
<td>r²=0.96</td>
<td>r²=0.8</td>
</tr>
<tr>
<td>Linear regression (equation)</td>
<td>(r²=0.32)</td>
<td>(r²=0.93)</td>
<td>(r²=0.97)</td>
<td>(r²=0.77)</td>
<td>(r²=0.77)</td>
</tr>
<tr>
<td>y = 0.003x + 6.5</td>
<td>y = 0.003x + 0.14</td>
<td>y = 0.5x + 1.2</td>
<td>y = -0.1x + 9.6</td>
<td>y = 0.002x + 3.2</td>
<td></td>
</tr>
</tbody>
</table>

* P value is at 0.05 significance level, ** P value is at 0.01 significance level.

6.4.3 Oxygen isotope results

6.4.3.1 Incisor intra-tooth δ¹⁸O results

The δ¹⁸O results of the intra-tooth microsampling analysis are summarised in Table 6-6. This table includes the mean, minimum, maximum and range of isotopic values of each incisor. The raw data for each incisor are included in Appendix C (Tables C-3 to C-10).

Table 6-6. Wombat intra-tooth microsample oxygen isotope results

<table>
<thead>
<tr>
<th>Wombat population specimen ID</th>
<th>Number of micro-samples / incisor</th>
<th>Length of incisor (cm)</th>
<th>Mean δ¹⁸O of all micro-samples</th>
<th>Standard deviation</th>
<th>Minimum δ¹⁸O value</th>
<th>Maximum δ¹⁸O value</th>
<th>% range of δ¹⁸O values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Brookfield CP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W69</td>
<td>26</td>
<td>6.2</td>
<td><strong>1.32</strong></td>
<td>0.99</td>
<td>0.09</td>
<td>3.82</td>
<td>3.73</td>
</tr>
<tr>
<td>W633</td>
<td>60</td>
<td>7.2</td>
<td>1.18</td>
<td>0.64</td>
<td>0.01</td>
<td>2.50</td>
<td>2.49</td>
</tr>
<tr>
<td>2. Mt Kosciusko NP</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>W506</td>
<td>50</td>
<td>7.5</td>
<td><strong>-1.96</strong></td>
<td>0.68</td>
<td>-2.99</td>
<td>-0.80</td>
<td>2.19</td>
</tr>
<tr>
<td>3. Tharwa W881</td>
<td>52</td>
<td>7.9</td>
<td><strong>-2.32</strong></td>
<td>1.24</td>
<td>-4.46</td>
<td>-0.32</td>
<td>4.14</td>
</tr>
<tr>
<td>4. Braidwood W52</td>
<td>38</td>
<td>7.3</td>
<td><strong>-1.54</strong></td>
<td>0.64</td>
<td>-3.00</td>
<td>-0.45</td>
<td>2.55</td>
</tr>
<tr>
<td>W450</td>
<td>43</td>
<td>7.2</td>
<td><strong>-2.19</strong></td>
<td>0.94</td>
<td>-4.22</td>
<td>-0.78</td>
<td>3.44</td>
</tr>
<tr>
<td>W873</td>
<td>39</td>
<td>7.7</td>
<td><strong>-2.01</strong></td>
<td>1.64</td>
<td>-4.77</td>
<td>2.25</td>
<td>7.02</td>
</tr>
<tr>
<td>5. Epping Forest NP W1016</td>
<td>60</td>
<td>7.8</td>
<td><strong>2.03</strong></td>
<td>0.86</td>
<td>0.19</td>
<td>3.93</td>
<td>3.74</td>
</tr>
</tbody>
</table>

The mean of the intra-tooth δ¹⁸O enamel values of the semi-arid wombats from Brookfield CP and Epping Forest NP have more enriched δ¹⁸O values (~0.95 and 2.03‰, respectively) than the wombats from Mt Kosciusko NP, Tharwa and Braidwood (~1.96, -2.32 and -1.91‰, respectively). These more enriched δ¹⁸O values may be indicative of increased evaporative enrichment of surface waters and leaf-water δ¹⁸O values due to the drier conditions and higher temperatures at these two sites.

The sequence of intra-tooth δ¹⁸O values of each wombat incisor are shown in Figure 6-10. Similar to the description for Figure 6-7 (carbon), this graph demonstrates the range and
amplitude of isotopic variation along each tooth and the isotopic values at any given point along the x-axis are not necessarily contemporaneous, because the wombats lived at different times.

The intra-tooth ranges in δ¹⁸O values were generally less variable than the ranges in δ¹³C intra-tooth values. The largest intra-tooth δ¹⁸O range was 7.02‰ in W873 at Braidwood; however, the other two wombats from Braidwood, W450 and W52, had much smaller intra-tooth δ¹⁸O ranges of 3.44 and 2.55‰, respectively. Although from the same location, W873 lived in the year preceding W450 and W52 and it may have experienced a different set of climatic conditions. The smallest intra-tooth range in δ¹⁸O values was 2.19‰ in the wombat from Mt Kosciusko NP. The most apparent trend in the δ¹⁸O data so far is that the δ¹⁸O values in wombat teeth from the inland more arid sites at Braidwood and Epping Forest are positive values and overall, more positive than the other wombats from the coastal and mountainous regions.

![Wombat δ¹⁸O inset](image)

Figure 6-10. Wombat intra-tooth microsampling analysis δ¹⁸O results

6.4.3.2 Relationships between wombat δ¹⁸O values and climate variables

The mean of each incisor’s microsample values was used to plot wombat δ¹⁸O against the climate variables. These relationships are shown in Figure 6-11 A-E. Wombat δ¹⁸O values were negatively correlated with precipitation, relative humidity and moisture balance. The δ¹⁸O values were positively correlated with evaporation and temperature. All relationships were significant, P≤0.01. Although these correlations used a derived mean value, they show that strong climate to isotope relationships exist. The wombat δ¹⁸O values became more enriched as the environmental conditions became drier, warmer and less humid.
CHAPTER 6. STABLE ISOTOPES IN WOMBATS

Figure 6-11 A to E. Plots of wombat mean $\delta^{18}O$ values against climate. The $\delta^{18}O$ values are the mean of the incisor microsample values. The error bars represent the standard deviation.
6.4.4 Estimation of incisor tooth growth rate based on intra-tooth $\delta^{13}$C values

An attempt was made to refine the calendar time represented in the wombat’s incisor by examining the intra-tooth $\delta^{13}$C patterns. W450 and W52 from Braidwood have the most marked intra-tooth $\delta^{13}$C variations - their incisor $\delta^{13}$C values form a sequential smooth curve from base to tip with the most enriched $\delta^{13}$C values (~9.2‰) occurring at approximately 45mm from the tooth base (see Figure 6-7 above). If the most enriched $\delta^{13}$C values represent the time at which a greatest proportion of $C_4$ plants were being consumed, then it is possible to place this particular period (‘$C_4$ peak’) in calendar time because the months in which $C_4$ grasses are more prevalent at Braidwood can be determined by examining the monthly climate data and local plant records. The average percentage of $C_4$ grasses at Braidwood is approximately 30%. $C_4$ grasses are most abundant towards the middle and end of summer, from late December through to March, when mean daily temperatures are above 21°C and there is enough rainfall to promote new plant growth (Hattersley 1983; Garden et al. 2001; Mitchell et al. 2001).

6.4.4.1 Estimation of incisor growth rate using W450 and W873

If the 73mm long tooth of W450 represented approximately 2 years enamel growth, it would be expected that the ‘$C_4$ peak’ would be repeated. However, this clearly was not evident, which suggested that the incisor growth rate was faster than 0.1mm/d. The pattern of intra-tooth $\delta^{13}$C values of W873 (see Figure 6-7 above) has one single ‘$C_4$ peak’ between two troughs of lower $\delta^{13}$C values, which further illustrated that less than two years were represented in that 70mm length of tooth.

Given the date of death (20th September 2001) and using the growth rates of 0.1, 0.15 and 0.2mm/d, a series of monthly time-scales were positioned against the intra-tooth $\delta^{13}$C ‘curve’ of W450 to illustrate the possible monthly timings of the more enriched $\delta^{13}$C ‘$C_4$ peak’. These timescales are shown Figure 6-12.

As is shown in Figure 6-12, a growth rate of 0.1mm/day placed the ‘$C_4$ peak’ in mid-June 2000, which was highly unlikely because this was the winter season. Furthermore, the ‘June 2001’ $\delta^{13}$C values were not similar. A growth rate of 0.15mm/d placed the $C_4$ peak in late spring in late-November 2000, which was one to two months before the peak of summer. A growth rate of 0.2mm/d placed the $C_4$ peak at early February, which was the end of mid summer and was likely to be season when a large proportion of $C_4$ grasses were growing. From these observations, the incisor growth rate for wombats at Braidwood is between 1.5 and 0.2mm/d, and it can be further inferred from this that a 73mm long tooth may eventually be replaced over a 12-16 month period.
Figure 6-12. Estimation of the actual months and year(s) represented in the lower incisor of W450 from Braidwood. Based on the date of death and three different growth rates- 0.1, 0.15 and 0.2mm/d- the scale bars show the predicted timing of a seasonal C₄ peak in δ¹³C values using each growth rate.

6.4.5 Wombat intra-tooth δ¹³C and δ¹⁸O variation and environmental seasonality

The sequential microsampling of the wombat incisors has shown that there were isotopic variations in δ¹³C and δ¹⁸O values along the teeth, and the magnitude of these variations was different for each site. There was little scatter in the stable isotope data of each tooth, which suggested the micro-sampling strategy and analysis was successful at capturing the temporal changes in isotope values clearly. The main question that remained to be explored was: are these intra-tooth isotopic variations seasonal? In order to examine this, the intra-tooth isotope results of each wombat’s incisor were compared to the monthly climate data prevailing when the tooth was formed. Beginning with the date of death and using the average growth rate of 0.15mm/d, the calendar time represented in each tooth was estimated. The intra-tooth isotope values, as well as the relative amplitudes and patterning of isotopic variation, were examined in relation to the timing of peak seasonal summer and winter climate variations.

The estimated incisor growth rate of 0.15mm/day may have been inappropriate for some wombats. Incorrect growth rates, and the time lags between isotopic signals being ingested and incorporated into wombat body tissues, would cause isotope values to not correspond exactly or appear out of phase with the monthly climate data. Nonetheless, calendar time based on this growth rate provided a starting point to begin interpretations in this study.
6.4.5.1 Site: Brookfield Conservation Park (W69 and W633)

The two specimens W69 and W633 from the semi-arid Brookfield CP site died within less than two months of each other, late December 2001 and early February 2002, respectively. Figure 6-13 A, shows the sequence of intra-tooth \(\delta^{13}\text{C}\) and \(\delta^{18}\text{O}\) values for these two wombats, and Figure 6-13 B shows the corresponding climate data. The pattern and amplitude of both \(\delta^{13}\text{C}\) and \(\delta^{18}\text{O}\) intra-tooth values of the two Brookfield wombats were very similar. A deviation of approximately 1\(\%\text{e}\) in \(\delta^{13}\text{C}\) values occurred near the incisor crowns, and the \(\delta^{18}\text{O}\) values at the base of the teeth differed by approximately 1.4\(\%\text{e}\) – and in both instances W69 had the more enriched values.

6.4.5.1.1 Site 1 Brookfield CP intra-tooth \(\delta^{13}\text{C}\) variation and seasonality

The mean range of intra-tooth \(\delta^{13}\text{C}\) values for the Brookfield CP wombats was 1.6\(\%\text{e}\), and even within this small range, a low undulating cyclical pattern in the \(\delta^{13}\text{C}\) values was evident (Figure 6-13 A). Describing the patterns from crown to base, relative to the intra-tooth mean (-13.6\(\%\text{e}\)) the \(\delta^{13}\text{C}\) values were depleted in the spring and early summer (December) of 2000, but became enriched (+1\(\%\text{e}\)) at the end of summer and into autumn in 2001. Contrary to expectations, the \(\delta^{13}\text{C}\) values remained relatively enriched over the winter months (June to August 2001), and they did not deplete again until the following spring. This apparent inconsistency could be due to an incorrect growth rate being used to estimate the month of enamel formation. As well, enamel growth rates may have varied during the year. Increased growth rates over the summer would result in the enriched values being represented in a longer portion of the intra-tooth series. At the base of the teeth just prior to death, the \(\delta^{13}\text{C}\) values became enriched as the middle of summer approached.

Hattersley (1983) estimated this region could have around 40-50\% C\(_4\) grass species. The mean \(\delta^{13}\text{C}\) value of all intra-tooth microsamples for both W69 and W633 was -13.6\(\%\text{e}\), and all microsample values were more negative than -12.64\(\%\text{e}\). This indicates these wombats ate mostly C\(_3\) plants throughout the year, and there is little isotopic evidence to suggest that they consumed more than about 10\% C\(_4\) grasses at any given time. The population’s mean \(\delta^{13}\text{C}_{\text{B-collagen}}\) value of -22.23 \(\pm\) 0.5\(\%\text{e}\) concurs with this. Environmental interpretations based on bone or tooth \(\delta^{13}\text{C}\) values can not preclude the presence of more summer C\(_4\) grasses at Brookfield CP because these wombats may have selectively fed on C\(_3\) plants. Field observations at Brookfield CP recorded that L. ursinus eats mainly Stipa nitida (Wells 1978), a C\(_3\) perennial winter/spring growing grass (Watson and Dallwitz 1985), and if there is no new green pick during the dry summer months they feed on the dried-off winter grasses and sedges.
Climate data show that the semi-arid habitat Brookfield CP is winter rainfall dominated (Bureau of Meteorology 2001, at http://www.bom.gov.au/climate/environ) and the prime growing season occurs in the cool winter/spring, which favours mainly C₃ grasses. During the years when the wombats lived, the summer and autumn rainfalls for all months, except January 2001, were below the mean monthly averages (see Figure 6-13 B). Although summer average temperatures were conducive to C₄ plant growth (>21°C), and a seed-bank of C₄ grass species probably exists, the insufficient summer rainfall most likely discouraged their growth.

The lack of more enriched δ¹³C isotope values indicates that during the 2000 to 2002 period the plant biomass at Brookfield CP was dominated by C₃ species. The small amplitude in δ¹³C values (1.6‰) showed no significant seasonal change in consumption of C₃ and C₄ plants, which is probably because this habitat supports a single winter C₃ plant growth season and the extremely dry summers often depress the proliferation of new C₄ grasses.

6.4.5.1.2 Site 1 Brookfield CP intra-tooth δ¹⁸O variation and seasonality

The mean amplitude of intra-tooth δ¹⁸O values was 3.1‰, almost double that of the δ¹³C values. A well defined cyclical pattern in the δ¹⁸O values was also evident. Describing the values from crown to base, relative to the intra-tooth mean (1.3‰), the δ¹⁸O values gradually became more enriched as spring ended (November 2000), and reached a peaked value of approximately 1.7‰ during the summer (January to February 2000). The δ¹⁸O values then gradually depleted during autumn and were most depleted (~0.1‰) during the winter of 2001. The δ¹⁸O values became more enriched again during late spring and summer of 2002.

This sinusoidal pattern of intra-tooth δ¹⁸O values shows a marked seasonal relationship with the corresponding climate data. The δ¹⁸O values are more depleted when the climate was cooler, wetter and more humid, and more enriched when the climate was warmer, drier and less humid. Evaporation exceeds rainfall for most of the year at Brookfield CP and surface water is rarely available for the wombats to drink; thus, it is unlikely that intra-tooth δ¹⁸O values track seasonal local meteoric δ¹⁸O values. These wombats would have obtained most of their water from fluids in leaves, which is relatively enriched in δ¹⁸O due to evaporation, and negatively correlated with relative humidity (Barbour and Farquhar 2000). Although there are no δ¹⁸O leaf data available from Brookfield CP, the intra-tooth δ¹⁸O values follow a very similar, yet inverse, sinusoidal pattern as the monthly relative humidity values. The relative humidity at Brookfield CP varies by approximately 25% between summer and winter (Figure 6-13 B); therefore, if wombat intra-tooth δ¹⁸O values were responding to seasonal changes in relative humidity via the ingestion of leaf water). This 25% variation equated to an approximate 2‰ amplitude change in wombat incisor δ¹⁸O values.

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Figure 6-13. Site 1 Brookfield CP: intra-tooth δ¹³C and δ¹⁸O values and associated monthly climate data
6.4.5.2 Site 2: Mt Kosciusko National Park (W506)
The specimen, W506, from the alpine region of Mt Kosciusko NP died in early December 2001. Figure 6-14 A, shows the sequence of intra-tooth $\delta^{13}$C and $\delta^{18}$O values for W506, and Figure 6-14 B shows the corresponding climate data.

6.4.5.2.1 Site 2 Mt Kosciusko NP intra-tooth $\delta^{13}$C variation and seasonality
The sequence of intra-tooth $\delta^{13}$C isotopic values of W506 had a small range of 1.33‰, and the flattest isotopic profile of all the wombat incisors analysed. W506 had the most depleted $\delta^{13}$C values - the mean of all microsamples was -16.59‰ and all values were less than -16‰. The intra-tooth $\delta^{13}$C profile indicated W506 fed entirely on C$_3$ plants throughout the year and there is no evidence that it ate C$_4$ grasses. This finding is consistent with expectations, because the alpine environment has a dominant cool late spring and early summer growing season that strongly favours C$_3$ plants (Weare and Morgan 2001). In addition, the mean daily maximum temperatures at Mt Kosciusko NP only reach approximately 21°C during the summer months, and rarely above ~25°C (see Figure 6-14 B), therefore, C$_4$ grasses are uncommon in this habitat.

6.4.5.2.2 Site 2 Mt Kosciusko NP intra-tooth $\delta^{18}$O variation and seasonality
The amplitude of intra-tooth $\delta^{18}$O values of W506 was 2.19‰ and a fluctuating pattern was more pronounced than in the intra-tooth $\delta^{13}$C values. Describing the intra-tooth pattern from crown to base, relative to the mean value (-1.96‰) the $\delta^{18}$O values became gradually enriched over the spring and were the most enriched during the mid summer of 2000. The $\delta^{18}$O values decreased over autumn and were the most depleted during the winter months of June and July in 2001. In early spring 2001 the $\delta^{18}$O values made an unusually sharp 2‰ rise and rapidly depleted again before summer began in December 2001.

The pattern of intra-tooth $\delta^{18}$O values of W506 shows a relationship with the monthly climate data from Mt Kosciusko NP. The summer period was characterized by more enriched intra-tooth $\delta^{18}$O values and the winter period by more depleted values. The intra-tooth $\delta^{18}$O values also followed an inverse trend in relation to changes in the actual monthly relative humidity data for 2000/2001. However, the amplitude of $\delta^{18}$O variation relative to the annual relative humidity variation (~33%) was lower than that observed at Brookfield CP.

At this site, the wombats most likely ingest water from sources other than leaf water because they are exposed to free water in the form of snow-melt water, alpine bogs and more frequent rainfall events. These sources are likely to be isotopically depleted relative to leaf water $\delta^{18}$O values, which might then modulate the effects of enriched leaf water $\delta^{18}$O values on intra-tooth
\( \delta^{18}O \) values. There are no meteoric rainfall or plant leaf water \( \delta^{18}O \) data available for comparison, but the cooler temperatures and moist alpine climate would also result in less evaporative enrichment of leaf water \( \delta^{18}O \). Over the year, multiple sources of \( \delta^{18}O \) input may have influenced wombat intra-tooth \( \delta^{18}O \) values at Mt Kosciusko NP. It is unclear what caused the unusual 2\% variation at the base of the tooth; it might have been a localised climate event or a physiological response. Overall, a seasonal fluctuation in wombat intra-tooth \( \delta^{18}O \) values was evident at this site.
Figure 6-14 Site 2 Mt Kosciusko NP: intra-tooth $\delta^{13}$C and $\delta^{18}$O values and associated climate data
6.4.5.3 Site 3: Tharwa (W881)

The specimen W881 from the cool temperate region of Tharwa died in early March 2003. Figure 6-15 A, shows the sequence of intra-tooth $\delta^{13}C$ and $\delta^{18}O$ values for W881, and Figure 6-15 B shows the corresponding climate data. Seasonal faeces $\delta^{13}C$ values from Tharwa were included in Figure 6-5 (above).

6.4.5.3.1 Site 3 Tharwa intra-tooth $\delta^{13}C$ variation and seasonality

The sequence of intra-tooth $\delta^{13}C$ values of W881 had a distinct cyclical pattern with a maximum amplitude of 3.21‰. The mean $\delta^{13}C$ value of all microsamples was -13.86‰ and all values were less than -12.02‰, which indicated W881 fed predominantly on C3 plants but had consumed about 15% C4 grasses during some parts of the year. The intra-tooth $\delta^{13}C$ values were most enriched during the summer and autumn and most depleted during winter and early spring. The $\delta^{13}C$ values of the 2001/2002 summer were approximately 1.2‰ more depleted than the following 2002/2003 summer. The timing of this cyclical pattern in $\delta^{13}C$ values strongly indicates that during the summer periods, there was an increase in the proportion of C4 grasses at Tharwa. This seasonal pattern of $\delta^{13}C$ values was also observed in the $\delta^{13}C$ values of wombat faeces collected from June 2002 to March 2003 (see Figure 6-5, above). The summer faeces $\delta^{13}C$ values were ~4‰ enriched over the winter values.

In the Tharwa region, the annual rainfall (~870 mm) is uniformly distributed throughout year. During the summer/autumn season there is often enough moisture and temperatures are warm enough that some C4 grasses grow (Garden et al. 2001). This seasonal change in C3 and C4 grass composition has clearly been recorded in the wombat’s intra-tooth $\delta^{13}C$ values. The wombats at the Tharwa site lived near a small creek (often ephemeral) at the base of an open-forested hill slope. Rainfall recorded at the nearby Tidbinbilla climate station (source of weather data for Tharwa) during the spring/summer of 2002/2003 was well below average (see Figure 6-15 B) and the region appeared dry, but there was evidently soil moisture available in the wombat’s local habitat because sparse green forage was seen growing. This must have enabled C4 plants to grow this season despite the extreme lack of rainfall that summer.

6.4.5.3.2 Site 3 Tharwa intra-tooth $\delta^{18}O$ variation and seasonality

The serial intra-tooth $\delta^{18}O$ values of W881 had a distinct cyclical pattern similar to the profile of intra-tooth $\delta^{13}C$ values, and the maximum amplitude was 4.14‰. W881 had the most depleted $\delta^{18}O$ values of all the wombats analysed- the mean $\delta^{18}O$ value of all microsamples was -2.32‰ and all values were less than -0.32‰.
The pattern of intra-tooth $\delta^{18}O$ values of W881 shows a relationship with the monthly climate data from the Tharwa region. Similar to the observations from the other sites discussed so far, the intra-tooth $\delta^{18}O$ values were enriched during the warmer, drier and less humid summer months and depleted during the cooler, wetter and more humid winter months. This pattern appears clearly seasonal. The amplitude of isotopic change (4.14‰) was greater than that observed in the intra-tooth $\delta^{18}O$ of the Brookfield CP, Epping Forest NP and Mt Kosciusko NP wombats. Although considered non-obligate drinkers, the wombats at Tharwa are exposed to free water at the nearby creek and as rainfall (e.g. present on grass leaves), therefore it was possible that there was a seasonal change in the moisture sources that effected wombat $\delta^{18}O$ values.
Figure 6.15 Site 3 Tharwa intra-tooth δ\textsuperscript{13}C and δ\textsuperscript{18}O values and associated climate data
6.4.5.4 Site 4: Braidwood (W52, W450 and W873)

At the cool temperate Braidwood site, specimens W52 and W450 died at the same time in late September 2001, and W873 died in early October 2002. The intra-tooth values therefore provided a continuous isotopic record spanning nearly two and a half years. Figure 6-16 A, shows the sequence of intra-tooth $\delta^{13}$C and $\delta^{18}$O values for these three wombats, and Figure 6-16 B shows the corresponding climate data.

6.4.5.4.1 Site 4 Braidwood intra-tooth $\delta^{13}$C variation and seasonality

The sequence of intra-tooth $\delta^{13}$C values for the three Braidwood wombats shows a defined cyclical pattern. Similar to the observations at the Tharwa site, $\delta^{13}$C values are most enriched over the summer/autumn period and most depleted during the winter/spring period. The mean of all microsamples was -13.03‰, which indicated these wombats ate a predominantly C$_3$ diet, with about a 10% C$_4$ grass component. This is supported by the population’s mean $\delta^{13}$C$_{bi-collagen}$ value of -20.66‰. However, the intra-tooth $\delta^{13}$C values indicated that these wombats ate a larger proportion of C$_4$ grasses during the summer, perhaps up to about 35%.

Hattersley (1983) suggests that this area has around 30-40% C$_4$ grasses, and field observations record that C$_4$ species are present in the region (Garden et al. 2000; Garden et al. 2001). Similar to the Tharwa area, the annual rainfall at Braidwood is fairly uniform during the year. Coupled with warm temperatures, the growth of new summer C$_4$ grasses is often prolific. The cyclical pattern of intra-tooth $\delta^{13}$C values clearly showed that the wombats consumed different proportions of C$_3$ and C$_4$ plants during the year, which reflects a seasonal change in C$_3$ and C$_4$ grass composition in the Braidwood area.

The maximum amplitude of intra-tooth $\delta^{13}$C values was different for the two years. The range of $\delta^{13}$C values represented by W52 and W450 was 7.8‰, and for W873 it was 3.96‰. 2001 winter values were not as depleted as those in 2000, and the 2002 summer values not as enriched as those in 2000/2001. These differences show that during the winter of 2000 C$_3$ plants with extremely depleted $\delta^{13}$C values dominated the Braidwood biomass, and during the summer of 2001/2002 there was a smaller input of C$_4$ grasses compared to the previous summer. The winter of 2000 was not unusually wetter or colder than 2001, but the actual potential evaporation was much lower (see Figure 6-16 B). W450 and W52 lived on the south side of a hill, whereas W873 lived on a warmer more exposed north westerly facing slope; therefore, slight differences in habitat may have influenced plant $\delta^{13}$C values.
6.4.5.4.2 Site 4 Braidwood intra-tooth $\delta^{18}O$ variation and seasonality

The sequences of intra-tooth $\delta^{18}O$ values of wombats from the Braidwood site did not have the cyclical patterns of summer high values and winter low values observed in teeth from the other sites discussed so far. Although the mean value of all $\delta^{18}O$ microsamples for each wombat differed by only 0.65‰ (see Table 6-6), the ranges and patterns of $\delta^{18}O$ values along each tooth were very different. The range in $\delta^{18}O$ values for W450 and W52 were 3.44‰ and 2.55‰, respectively, and for W873 it was 7.02‰. Interestingly, the isotopic patterns of W450 and W52, which coexisted, were quite different. At base of W52 and the crown of W873 the $\delta^{18}O$ values differed by nearly 3‰. The $\delta^{18}O$ values of W450 and W873 were relatively depleted over the winter of 2001, however, the winter 2000 values of W450 were actually similar to the $\delta^{18}O$ values that W873 had in the late summer of 2001/2002. The intra-tooth $\delta^{18}O$ pattern of W873 was stepped and not cyclical, the values plateaued slightly over the summer/autumn of 2002 and then gradually became approximately 4.5‰ more enriched towards the spring of 2002.

These complex patterns of serial intra-tooth $\delta^{18}O$ values at the Braidwood site are difficult to interpret. The large variations indicated that coexisting wombats can record very different intra-tooth $\delta^{18}O$ values; these may be related to differences in the sources of moisture, diet selectivity, behaviours or physiologies. The actual climate data showed that Braidwood experienced below average rainfall for many months when these wombats lived (see Figure 6-16 B). The most persistent rainfall deficit and lower then average relative humidity values occurred during 2002 when W873 lived, which may explain the unexpected rise in wombat $\delta^{18}O$ values during that year. At the Braidwood site, multiple moisture sources or seasonal and annual changes in moisture sources may have influenced wombat $\delta^{18}O$ values. Detailed data on plant leaf and local rainfall $\delta^{18}O$ values are needed to begin to determine the more specific effects these different environmental factors have on wombat $\delta^{18}O$ values.
Figure 6.16. Site 4 Bridgedale: intra-annual 18C and 18O values and associated monthly climate data.
6.4.5.5 Site 5 Epping Forest NP (W1016)
The month of death of specimen W1016 from the semi-arid tropical grassland site of Epping Forest NP was unknown, so comparisons with actual monthly climate data were not possible. Figure 6-17A shows the sequence of intra-tooth $\delta^{13}C$ and $\delta^{18}O$ values for W1016, Figure 6-17 B shows the mean monthly climate data for a one year period.

6.4.5.5.1 Site 5 Epping Forest NP intra-tooth $\delta^{13}C$ variation and seasonality
The sequence of intra-tooth $\delta^{13}C$ values of W1016 had a small range of 1.49‰ and a flat isotopic profile similar to that observed in W506 from Mt Kosciusko NP. W1016 had the most enriched $\delta^{13}C$ values, the mean of all microsamples was -1.62‰ and all values were more positive than -2‰, which clearly indicated it consumed an exclusive C$_4$ grass diet. Correspondingly, the series of northern hairy-nosed wombat faeces $\delta^{13}C$ values (see Figure 6-5 above) varied by only 2.4‰. Both the seasonal faeces and serial intra-tooth $\delta^{13}C$ values indicate that the Epping Forest NP wombats ate a rather constant diet of approximately 90% C$_4$ grasses throughout the year. This finding was consistent with expectations, because the semi-arid hot tropical grassland habitat at Epping Forest NP is dominated by C$_4$ grasses (Woolnough and Foley 2002). Hattersley (1984) also considered this area has 90% or more C$_4$ grasses. Field observations and seasonal faecal analyses recorded that the northern hairy-nosed wombats consistently feeds on native grasses such as Aristida spp. and Enneapogon spp and the introduced buffle grass Cenches ciliaris, which are all C$_4$ species (Woolnough and Johnson 2000).

6.4.5.5.2 Site 5 Epping Forest NP intra-tooth $\delta^{18}O$ variation and seasonality
The range of intra-tooth $\delta^{18}O$ values of W1016 was 3.74‰ and the mean $\delta^{18}O$ of all microsamples was 2.03‰. An undulating pattern of $\delta^{18}O$ values was observed but it was not possible to compare these to actual months or years. From crown to base, the first 55mm of tooth had a gradual ~3‰ enrichment in $\delta^{18}O$ values; this length of tooth equals approximately 1 year of tooth growth based on a growth rate of 0.15mm/d. For the remaining part of the tooth, the values fluctuated for approximately 1‰ and then rapidly depleted again before death. In such a highly seasonal, tropical location, with distinct wet and dry seasons, an oscillation in $\delta^{18}O$ values was expected. Quite possibly, the incisor growth rate of W1016 was faster than 0.15mm/d and this series of intra-tooth $\delta^{18}O$ values may represent an annual cycle. Ingesting abrasive soil and dry coarse grasses in this semi-arid habitat may increase tooth wear, which necessitates increased tooth growth (G. Sanson, pers.com).

Epping Forest NP is summer rainfall dominated, and even during the wet season, rain that collects in small puddles quickly evaporates because of the high temperatures. There is little
available surface water, therefore the northern hairy-nosed wombats must obtain the majority of their moisture from plant leaf water and metabolically from food (Horsup 1999). It is unknown whether $\delta^{18}O$ values of the C$_4$ grasses in this tropical savannah site become more enriched during the summer months because high temperatures increase transpiration, or whether these effects are counterbalanced by the higher rainfall and humidity levels, which may cause a relative depletion in $\delta^{18}O$ values. The tropical C$_4$ grasses are better adapted to high temperatures (Helliker and Ehleringer 2002; Winslow et al. 2003) and the summer rainfall at this site is low (<300mm) so relative humidity might have quite an influential effect on leaf $\delta^{18}O$ values.

At Epping Forest NP, the annual relative humidity varies by approximately 18%. It is highest during the summer months, yet it remains high through early winter, and is lowest at the beginning of spring. This pattern contrasts with the cyclical patterns of relative humidity observed at the southern sites (see Figure 6-3 above). The pattern and amplitude of intra-tooth $\delta^{18}O$ values of W1016 is most similar to the variation in monthly relative humidity values. If the W1016 $\delta^{15}O$ values represent relative changes in humidity, then possibly it died at the start of summer. However, if the high summer temperatures cause increased leaf water $\delta^{18}O$ enrichment, and the influence of relative humidity is less, the rapid depletion of the more enriched $\delta^{18}O_{exhale}$ values at the base of the tooth could represent the end of a summer period. Clearly, the relationships between the oxygen isotope ratios in C$_4$ grasses and various climatic parameters in this tropical grassland region are required before better interpretations of wombat intra-tooth $\delta^{18}O$ values can be made.
Figure 6.17. Site 5 Epping Forest NP: intra-tooth $\delta^{13}$C and $\delta^{18}$O values and associated monthly climate data.
6.5 Discussion

Bone collagen δ¹³C values indicated the C₃ plants dominate the diets of wombats in the southern and south-eastern regions and C₄ plants dominate in the tropical northern regions. These findings agreed with wombat ecology studies and local plant species lists. The intra-tooth δ¹³C values indicated the C₃ and C₄ composition of the wombat’s diet at some sites varied seasonally.

In the southern regions, enrichment of δ¹³C values were interpreted as an increase in the consumption of C₄ plants during the warmer months. This enrichment might not be solely due to C₄ plants. Pate and Krull (in press) recently observed that δ¹³C values of a δ¹³C pasture grass species (Barely grass, *Hordeum leporinum*), in South Australia, became more enriched with decreasing rainfall - a 3.85‰ change in grass δ¹³C values occurred over a change in mean rainfall of 807 mm. This isotopic change was over a geographic range, so it does not necessarily represent the seasonal changes C₃ grass δ¹³C values at one site. It does highlight, however, that a portion of enrichment seen in the intra-tooth series may also be due to the consumption of C₃ plants that have become enriched due to water stress or the seasonal replacement of C₃ species with better water stress tolerances. In regard to this, the previous study of koalas (Chapter 4) found that even in this obligate C₃ browser the bone collagen δ¹³C values were more enriched in the drier environments, which strongly reflect how relationship between plant δ¹³C values and relative moisture levels in the environment.

Wombat bone collagen δ¹⁵N values were positively correlated with potential evaporation and maximum daily temperatures, and negatively with annual moisture balance and 3pm relative humidity (see Figure 6-9). These findings contrasted with the previous koala study but were in better agreement with the observations of Gröcke and Bocherens (1996), which found that *Macropus* δ¹⁵N collagen values increased with decreasing rainfall. Wombats have a grass diet more similar to the *Macropus* and they inhabit a wider range of climatic extremes than koalas, especially areas with low rainfall in northern and southern Australia. Several authors have demonstrated positive correlations between water stress and δ¹⁵N in plants (Heaton 1987; Schwarcz et al. 1999). The grasses on which wombats feed are shallower rooted and may reflect the local surface hydrological regime more closely, especially ‘dryness’. Therefore, plant δ¹⁵N enrichment due to aridity is passed on and recorded in the tissues of some grazers more distinctively than browsers or mixed C₃/C₄ feeders.

The patterns of intra-tooth δ¹⁸O values of wombat from Brookfield CP, Tharwa and Mt Kosciusko NP showed cyclical-like trends that are most likely related to seasonality. The δ¹⁸O values were more depleted in the cooler wetter more humid winter months and more enriched in the warmer drier and less humid months. These findings were similar to the seasonal patterns of serial intra-tooth δ¹⁸O values that have been predicted and observed in studies of similar high-
crowned teeth (Fricke and O’Neil 1996; Stuart-Williams and Schwarcz 1997; Fricke et al. 1998).

This sinusoidal pattern, however, was not clear at all sites, which raised the questions: what determines wombat δ¹⁸O values and how does this vary between sites? Wombats are considered non-obligate drinkers and they obtain the majority of their water from foods (Evans et al. 2003). Their low metabolism and burrowing lifestyle also helps them conserve water. At many sites in this study, there is little if any free water available. Therefore, wombat δ¹⁸O values were not expected to track seasonal changes in meteoric δ¹⁸O values. Instead, they more likely to record seasonal changes in leaf water values, which are enriched over source waters due to transpiration and respond the changes in relative humidity (Barbour and Farquhar 2000; Barbour et al. 2004). The first order observation is that wombat δ¹⁸O values are more enriched in the drier and inland semi-arid site (in both southern and northern Australia), than in the cooler southern alpine less humid temperate climates. At some sites, it is possible that wombats do ingest some free water during the year, so there may be seasonal changes in moisture sources. For example, in the wetter periods free water is available on leaf surfaces and small ponds, and in the drier periods internal leaf water is the major source of moisture. Interestingly, at most sites the range of wombat intra-tooth δ¹⁸O values was larger than the 2-3.5‰ annual variation in meteoric rainfall δ¹⁸O values observed at Adelaide, Melbourne and Brisbane (see Figure 6-4 above). Overall, it is difficult to quantify and assess the effects of different geographic and climatic factors. Data for seasonal local meteoric or surface water δ¹⁸O values and leaf diet δ¹⁸O values are required from all sites to investigate and model the many different effects these factors have on the temporal change in the oxygen isotope composition of wombat tooth enamel.

6.5.1.1 Effects of different incisor growth rates and sampling technique

The growth rate of 0.15mm/d helped to place the isotopic values obtained from along the tooth in calendar time so they could be examined in relation to known seasonal climate variations. This provided a guide only. It assumed that incisor growth rates were constant for each location and throughout the year, and this may not be accurate. In this study, the growth rate was refined using the δ¹³C values of wombats from Braidwood (see section 6.4.4); so, although it was appropriate for the common wombats at Braidwood, Tharwa and Mt Kosciusko NP, it may have been less appropriate for the hairy-nosed wombats from the semi-arid areas at Brookfield CP and Epping Forest NP. It has been suggested that wombat incisor growth rates may vary depending on the habitat and abrasiveness of the diet (G. Sanson, pers com., see Section 6.1.3.2). Furthermore, mammalian enamel growth rates may also vary seasonally (Rinaldi and Cole 2004). Thus, the lack of an ‘expected’ seasonal correlation with monthly climate data
could be misinterpreted if only one growth rate is considered. Nevertheless, the data show seasonality in stable isotope ratios.

In addition, it is unknown how much the amplitudes of intra-tooth isotopic signals were attenuated due to sampling. The seasonal peaks may have been dampened and small yet significant seasonal isotopic fluctuations may have gone undetected. Despite these shortcomings, the data clearly support the hypothesis that wombat incisors record evidence of seasonality in carbon and oxygen isotope values.

6.5.1.2 Intra-tooth stable isotope reproducibility

The coexisting wombats, which were sampled at each of the Brookfield CP and Braidwood sites, had similar intra-tooth $\delta^{13}C$ values and patterns. These data showed that teeth of different individuals living in the same environment record similar carbon isotope signals; such ‘reproducibility’ adds confidence to the reliability of the $\delta^{13}C$ analysis of single teeth from other locations. In contrast, the differences in the three intra-tooth $\delta^{18}O$ series at Braidwood indicated that coexisting wombats can actually record very different intra-tooth $\delta^{18}O$ values. These may be related to differences in the sources of moisture and diet selectivity, as well as the behaviours and physiologies of individuals. These findings imply that more than one tooth from each site should be sampled to assess levels of intra-site intra-tooth $\delta^{18}O$ variability.

6.6 Summary and conclusions

The analyses of faeces, bone collagen and enamel $\delta^{13}C$ values enabled the calculation of the following isotopic enrichments and isotopic spacing between tissues, which are:

- Carbon diet to bone collagen enrichment: $4.7 \pm 0.9\%e$
- Isotopic spacing between bone collagen and enamel: $7.8 \pm 0.8\%e$
- Therefore, the carbon diet to incisor enrichment is approximately: $12.7 \pm 1.2\%e$

Wombat bone collagen and enamel $\delta^{13}C$ values both provided a good indication of the proportions of C$_3$ and C$_4$ grasses consumed. The bone collagen $\delta^{13}C$ values indicated the dominant plant group biomass in each habitat, which corroborated with modern ecological studies of wombat diet and plant availability. Thus, fossil wombat $\delta^{13}C$ values will be good palaeoenvironmental proxies that can be use to interpret the relative proportions C$_3$ and C$_4$ grass types in past environments.
Wombat bone collagen δ¹⁵N values showed highly significant relationships with climate variables that indicate aridity and low moisture levels, and as such are good palaeoclimatic indicators.

The sequential and contiguous sampling of wombat incisors used in this study produced a high level of isotopic resolution. A series of wombat incisor intra-tooth δ¹³C values provides a detailed record of how the diet δ¹³C values changed throughout a 1 to 1.25 year period in the individual’s life. Correlations with actual diet (δ¹³C in faeces) and monthly climate data have shown these intra-tooth changes are highly seasonal and record detailed environmental information regarding the seasonality of diet, plant growth and moisture levels in the environment.

Carbon intra-tooth profiles formed two main types:
1. incisor teeth series with low isotopic variability where characteristic of environments with a single plant growing season; dominated by either C₃ (eg. alpine or semi-arid Mediterranean regions) or C₄ (eg. tropical regions) plants.
2. incisor teeth series with high isotopic variability where characteristic of environments that have uniform and sufficient rainfall which promotes and sustains winter and a summer plant growing seasons (eg. cool and warm temperate regions).

Oxygen intra-tooth data produced these main findings:
1. Wombat δ¹⁸O values are more enriched in the drier and inland semi-arid sites than in the cooler southern alpine less humid temperate climates.
2. Oxygen intra-tooth profiles in the southern regions of Australia were strongly related to seasonal changes in both relative humidity and temperature.
3. Oxygen intra-tooth profiles of the northern tropical and southern semi-arid regions also indicated relationships with seasonal changes in relative humidities.

These intra-tooth isotope results from modern wombats provided an opportunity to examine how teeth enamel records evidence for environmental seasonality on a fine time scale. Further research on incisor growth rates and the primary inputs that control oxygen isotopes are needed to quantify the influences of specific climate variables on wombat stable isotope values. At this stage the data from this study has firmly established that the intra-tooth variation in δ¹³C and δ¹⁸O values at least provide qualitative estimates of seasonality in Australian environments. Collectively, the stable isotope analysis of fossil wombat bone collagen and enamel can be used to test hypotheses about past environmental variability on both longer-term and intra-annual seasonal time scales.
CHAPTER 7

A COMPARISON OF STABLE ISOTOPES IN COEXISTING SPECIES

7.1 Outline

This chapter presents a comparison of the stable isotope values from the three genera detailed in the previous chapters; the koala, *Macropus* kangaroos and wombats. This multi-species comparison has two main parts. The first part examines the similarities and differences in the stable isotope ratios and the range of intra-population isotopic variation of different species living at the same site. There were seven sites where co-existing genera where sampled (see Figure 7-1). At most sites, comparisons could only be made between the two ‘grazers’, the wombats and the kangaroos. All three genera could only be compared within one region; specimens from the Springsure, Emerald and Epping Forest NP sites in northern Queensland were grouped so the isotopic relationships between the ‘browsers’ (i.e. the koalas) and both ‘grazers’ could be examined.

The second part of this chapter compares the relationships between the isotope ratios of the three genera and their local climate data. The mean population bone collagen $\delta^{13}$C and $\delta^{15}$N, and enamel $\delta^{18}$O values of each of the genera, from all sites sampled across eastern Australia, were plotted together against local climate data. This enabled a comparison of how the stable isotope values of the different species reflect similar climatic conditions on the continental scale. Similar to the preceding chapters, the climate variables used were: precipitation, potential evaporation, maximum daily temperature, 3pm relative humidity and moisture balance.
7.2 Comparison of stable isotope ratios in co-existing genera

The data have been presented as bivariate plots of the two isotopes measured in each tissue. It is acknowledged that sample sizes from these seven sites are small. Figure 7-2 A-G show the bone collagen $\delta^{13}C$ and $\delta^{15}N$ values, and Figure 7-3 A-D show the enamel $\delta^{13}C$ and $\delta^{18}O$ values. In Figure 7-3, for wombats, the mean of each incisor’s intra-tooth microsample values has been plotted, and the error bars represent the full range of intra-tooth isotope values of that individual. In Figure 7-3, for kangaroos, the stable isotope values from the molar-4 were plotted.

![Map of Australia](image)

Figure 7-1. Location of the seven sites in Australia where the stable isotopes of co-existing genera were compared.