

**THE ISOTOPIC COMPOSITION OF KANGAROO  
TEETH AS A TRACER OF MIGRATION AND A FORENSIC  
TOOL**

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## **Abstract**

Isotopic analysis of dental and bone tissues can be used to reveal movement and diet in animals. However the suitability of these methods for tracing migration in an Australian animal has not yet been demonstrated. In this pilot study, Sr, C and O isotope profiles were created from 31 individuals selected from four species of kangaroos in South Australia (*Macropus rufus*, *M. fuliginosus*, *M. giganteus* and *M. robustus*). Primarily, enamel from fourth molars and bone apatite were sampled to document isotopic changes in individuals between the formation of the tissues in the tooth and bone. Variations in the strontium ( $^{87}\text{Sr}/^{86}\text{Sr}$ ), carbon ( $\delta^{13}\text{C}$ ) and oxygen ( $\delta^{18}\text{O}$ ) isotopes were used to assess movement of kangaroos across local geological and environmental conditions. Five individuals displayed a marked difference between enamel and bone apatite isotopic composition, indicating movement between distinct geological and environmental regions. The implications of this study to migration reconstruction in the past are discussed.

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## **1. INTRODUCTION**

Relatively few isotopic studies have been conducted on Australian fauna to date. These studies involved C and O analysis on macropods (Ayliffe & Chivas 1990, Murphy *et*

*al.* 2007; Murphy & Bowman 2007; Pate 1995, 1997, 1998; Prideaux *et al.* 2007), and wombats (Fraser *et al.* 2008).

While the use of Sr isotope analysis has become a common practice in the last decade in the study of migration and mobility of both prehistoric humans (e.g. Bentley *et al.* 2003, Ezzo *et al.* 1997; Grupe *et al.* 1997; Price *et al.* 2000, Schweissing & Grupe 2002, 2003; to name but a few) and other fauna (e.g. Britton *et al.* 2009; Balasse *et al.* 1999, 2001, 2002; Hoppe & Koch 2007; Pellegrini *et al.* 2008; Shaw *et al.* 2009), to the knowledge of the author, this is only the second such study to focus on the migration of a modern herbivore using Sr (Britton *et al.* 2009), and the first time Sr analysis has been used on any Australian fauna.

Kangaroos are the largest and most widespread of Australia's herbivores. The four common species of *Macropus* investigated in this study, the red kangaroo (*Macropus rufus*), the common wallaroo (*M. robustus*), the western grey (*M. fuliginosus*), and the eastern grey (*M. giganteus*), have a range that covers most of the continent and are very populous. This makes them ideal in the use of future continent-wide studies.

The presence of macropods in Australia has a large temporal depth, which is important in linking with existing faunal-orientated palaeo-environmental studies which have mostly focussed on the megafaunal extinctions (~46 ka). The frequent presence of *Macropus spp.* in the fossil record also has the potential to shed light on other major Quaternary events, such as the last glacial maximum, Younger Dryas and the Holocene Warm Period. And perhaps more importantly in terms of Sr analysis, the study of modern kangaroos will potentially produce the local biologically available strontium signature for rock units across Australia, which can be used to assess provenance, mobility and migration of prehistoric human populations.

This study expands on previous work conducted by Tom Brookman for his Honours Thesis (2008) and utilises the same suite of *Macropus* skulls which Specimens were collected in the Gairdner Torrens and Flinders Ranges areas and in a transect stretching from Woomera, South Australia to Portland Victoria, over a distance of approximately 900 km (Figure 1). Brookman used carbon and oxygen analysis on 383 samples taken from 52 teeth, taken from 30 animals, to evaluate seasonal changes in *Macropus* diet and environment in South Australia, to assess their potential for reconstructing palaeoseasonality.

In this study,  $^{87}\text{Sr}/^{86}\text{Sr}$  analysis was conducted on tooth enamel and bone apatite extracted from 31 animals, in order to determine if migration could be observed in *Macropus* over the broad geological disparities of South Australia. C and O analysis was also conducted to assess whether changes in  $^{87}\text{Sr}/^{86}\text{Sr}$  were supported by changes in  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ . C and O isotopes are well established to be correlated with diet and climate respectively (Balasse *et al.* 2001, Bryant & Froelich 1995, Kohn *et al.* 1996, 1998, Kohn & McIver Law 2006, Murphey & Bowman 2007, Murphey *et al.* 2007 and Passey *et al.* 2005, to name but a few).  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  were also compared with correlating data taking from the same animals from Brookman (2008) to assess the validity of the methods used in this study.

## 2. GEOLOGICAL SETTING

Kangaroo samples were assigned to three broad regions: southeast SA and southwest Victoria, Flinders Ranges and Gairdner/Torrens and Stuart Highway. These represent regions of vastly differing geology and climate. The individuals in the south-east of the transect were mostly collected as a control, Pate and Noble's (2000) isotope

analyses of bone collagen in kangaroos indicated that this region is dominated almost entirely by C<sub>3</sub> vegetation. This is supported by Brookman's data in Table 2, with low  $\delta^{13}\text{C}$  values for the kangaroos from this region. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of this region should also be relatively uniform, as the underlying marine carbonates have a  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio highly reflective of seawater.

The Flinders Ranges are composed of folded and faulted sediments deposited in a large sedimentary basin during the Neoproterozoic. The Ranges were folded and faulted during the Delamarian Orogeny. Contractual orogenesis commenced at  $514 \pm 3$  Ma and persisted for ~24 million years until  $490 \pm 3$  Ma, terminated by rapid uplift, cooling, and extension in association with post-tectonic magmatism (Foden *et al.* 2006). Dominant lithologies are, in order of decreasing abundance, siltstone, sandstone, dolomite, limestone, diamictite, magnesite and conglomerate. (PIRSA 2009) Granitoid intrusions occurred in the eastern Nackara and Fleurieu Arcs, and in basement inliers of the northern Flinders Ranges. Metamorphic grade varies from sub-greenschist to mid-amphibolite facies. On a broad scale the Flinders Ranges can be considered felsic silicastics, and as such, relatively high radiogenic  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios should be seen. In contrast, the southeast of the study area is dominated by Pleistocene carbonates. The large number of lithologies suggests a lot of variation will be seen  $^{87}\text{Sr}/^{86}\text{Sr}$  in specimens from across the region.

### **3. BIOAPATITE AND STABLE ISOTOPES**

#### **3.1. Biopatite**

The component of tooth enamel and bone used for analysis, calcium phosphate hydroxyapatite [ $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ], commonly referred to as bioapatite, functions in biominerals to resist compression and provide rigidity by forming an inorganic mineral

matrix. Bioapatite contains other anions such as carbonate, which commonly replaces the phosphate group; and cations, such as Sr and Pb, which exchange for Ca. Apatite constitutes 97% of tooth enamel by weight, and ~70% of bone (Hedges *et al.* 2005).

Enamel has <1% organic content and a very low porosity. The apatite in tooth enamel forms large and dense crystals (~1000 x 130 x 30 nm) with few defects or substitutions (Wang & Cerling 1994). Mineralisation is completed prior to tooth eruption, and enamel does not experience remodelling after formation. (Koch 1998). The accretion of enamel in permanent teeth is formed over a short period early in life, and incorporates trace elements from the diet of the individual during this finite process. Tooth enamel preserves incremental laminae that form at a variety of timescales. Tooth enamel accretes in 6 to 12 day growth layers, whose edges are marked by tiny ridges at tooth surfaces. In many large herbivores, a molar's enamel accretion can exceed one year (Ambrose 2006). This suggests a single tooth can often contain a complete record over a year of growth, and sequential teeth perhaps longer – allowing for overlap of growth times. (Balasse *et al.* 2002).

Bone contains ~70% bioapatite that forms in much smaller crystals than those in enamel (~100 x 20 x 4 nm). These crystals form in an organic structural matrix of the protein collagen, which makes up a further 24-26% of the composition of bone. Porosity increases from ~1% in enamel to ~40% in bone. Unlike enamel, bone is continually remodelled throughout life: it undergoes continual replacement of its inorganic phase. The ratio of re-absorption of old bone by osteoblasts and deposition of new bone, carried out by osteoclasts, determines the turnover rate of the bone (Hedges *et al.* 2005). Turnover rates of approximately 3% per year in cortical and 26% per year in trabecular bone have been estimated (Parfitt 1983). Stable isotope analysis of bone thus reflects the average of the later years of the life of the individual, in contrast to a snapshot during the

adolescence of the animal provided by enamel. Collagen is subject to degradation; consequently bone is more porous than enamel and is more susceptible to contamination and diagenesis.

### 3.2. Strontium Isotopes

Common substitution of strontium for calcium in the course of crystal genesis in bioapatite produces strontium concentrations on the order of 40-400 ppm (Schweissing & Grupe 2003b). The lack of remodelling in enamel means the Sr signature is preserved indefinitely. Furthermore, Budd *et al.* (2000) demonstrated that strontium in enamel is not measurably altered during diagenesis, further establishing enamel as an excellent medium for isotopic studies on both modern and ancient remains.

In nature, strontium occurs in the form of four stable isotopes. The most abundant is  $^{88}\text{Sr}$  (82.53%), followed by  $^{87}\text{Sr}$  and  $^{86}\text{Sr}$  (7.04% and 9.87%, respectively), while  $^{84}\text{Sr}$  is least abundant (0.56%).  $^{87}\text{Sr}$  forms in rocks as a decay product of radioactive  $^{87}\text{Rb}$ , which has a half-life of approximately  $4.7 \times 10^{10}$  years (Price *et al.* 2002). Relative variations in  $^{87}\text{Sr}$  abundance are calculated by a normalisation against the non-radiogenic isotope,  $^{86}\text{Sr}$ . The  $\delta$  notation is sometimes used to report strontium isotope data relative to modern seawater ( $^{87}\text{Sr}/^{86}\text{Sr}_{\text{sea water}} = 0.7092$ ), though more often, and in this study, the actual  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio is used.

Unlike light stable isotopes (e.g. carbon and oxygen) which fractionate in biological systems due to large mass differences, heavy stable isotopes such as strontium have a small relative mass difference and show negligible isotopic fractionation during transport in biochemical processes (Stille & Shields 1997). Consequently, the isotopic signal recorded in biominerals of bones and teeth are assumed to match the source of the strontium.

A distinction must be made between geological substrate strontium and biologically available strontium. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of herbivore enamel is directly related to the

$^{87}\text{Sr}/^{86}\text{Sr}$  ratio of ingested plants, water and ingested particulates over an herbivores local range (Valentine *et al.* 2008). Plants take up strontium from the soil, which is in turn derived from the bedrock. Bedrock  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios are controlled by age and chemical composition. Ratios of  $^{87}\text{Sr}/^{86}\text{Sr}$  generally vary between 0.700 and 0.750. Generally older geological units (>100 ma) and/or those with very high original Rb/Sr ratios (i.e. felsic continental crust) have very high  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios, whereas geologically younger and mafic rocks have  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of less than 0.706 (Price *et al.* 2000).

Despite significant heterogeneity in rocks, soils and plants in a given local area, animal skeletons display a remarkable homogeneity. At Grasshopper, Arizona, Price *et al.* (1994) showed that while the geology of the local site had a large variety in  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios, the variability in human and animal bones was less by several orders of magnitude, displaying a very low standard deviation. Sillen *et al.* (1998) demonstrated that strontium in whole soils in an area are highly variable, while plants are less variable across the region, but distinct from soils and rocks. This process, known as "biopurification", is the propensity of an organism to preferentially assimilate calcium in preference to strontium (Burton *et al.* 1995). Biopurification results in a reduction in the organism's Sr/Ca ratio relative to dietary ratios, which is reflected in their bone and teeth. Thus herbivores have lower ratios than those of the plants that they consume, and similarly carnivores have lower ratios than their diet. The result is an overall reduction in Sr/Ca with ascending trophic position, and acts to intergrate the Sr ratios of all organisms consumed (Price *et al.* 2002).

Several other factors are thought to contribute to differences between plant, soil, and bedrock strontium isotope composition. The presence of a significant dust component in the soil provides plants with a non-local source of Sr. Another possibility is differential weathering; differences in weathering rates of various components of the bedrock may

cause highly radiogenic muscovites and k-feldspars present to minimally contribute when compared to less radiogenic, faster weathering components (Sillen *et al.* 1998). These processes necessitate the measuring of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of local fauna to determine the composition of biologically available strontium, rather than the geological substrate strontium; preferably of an animal with a large enough geographical and diet range to average out the vegetation across the geological unit.

As previously mentioned, the decay of  $^{87}\text{Rb}$  is extremely slow, so there has been essentially no change in the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of natural materials in the last several hundred thousand years. This suggests that modern animals can provide the local biologically available strontium, acting as proxy for prehistoric species. Price *et al.* (2000) did a study of modern and prehistoric rabbit remains in Teotihuacan, Mexico, and found no difference in the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios.

### 3.3. Carbon and Oxygen isotopes

Bioapatite contains a small amount of  $\text{CO}_3^{2-}$  which substitutes for the  $\text{PO}_4^{3-}$  group. Serum  $\text{CO}_2$  and  $\text{HCO}_3^{1-}$  are the sources of these molecules and their isotopic composition reflects those of dietary carbon and ingested and metabolised water.

The oxygen isotope composition of enamel and bones reflects the  $\delta^{18}\text{O}$  of ingested water because it precipitates in equilibrium with body water. Because mammals maintain a body temperature of approximately  $37^\circ\text{C}$ , the isotopic composition of biogenic carbonates varies with ingested water. The other contribution is  $\text{H}_2\text{O}$  derived from metabolism, these small variations have little impact on the body water of large mammals (Koch *et al.* 1998), and is effectively constant on the timescale of interest here. There is strong evidence that the oxygen signal from meteoric water, contained in enamel, is a broad proxy for regional precipitation. Drinking water  $\delta^{18}\text{O}$  levels are well correlated with the isotopic composition

of local precipitation, such that tooth enamel records can be used as a climate proxy in species that derive most of their water directly from drinking. However, animals with low drinking water requirements (such as kangaroos) have a  $\delta^{18}\text{O}$  value that is closely related to relative humidity levels due to the effect of humidity on plant water-content (Ayliffe & Chivas 1990).

The fractionation that oxygen undergoes before it enters the body is complex, and includes many environmental factors such as latitude and longitude, temperature, evaporation, distance from water source and amount of rainfall. Many of these factors vary seasonally and produce a sinusoidal pattern in intra-tooth  $\delta^{18}\text{O}$  values. Thus in theory, enriched  $\delta^{18}\text{O}$  values should in kangaroo teeth represent summer precipitation, or hot and arid climates, and the less enriched values should represent winter precipitation, or wet and cool climates (Kohn et al. 1998). Differences in  $\delta^{18}\text{O}$  values would therefore be expected between animals living in the hot, dry northern part of South Australia compared to those in the wetter and cooler southern regions.

The carbon isotopic composition of teeth and bone is a reflection of diet. The  $\delta^{13}\text{C}$  values for carbon in the body of an herbivore, tends to reflect the average  $\delta^{13}\text{C}$  of the plant material consumed, with bones and teeth enriched with  $^{13}\text{C}$  by a constant value relative to the diet. If this fractionation is known, it is possible to distinguish between the relative amounts of  $\text{C}_3$  and  $\text{C}_4$  plants in the diet of an herbivore because these plant types have distinct  $\delta^{13}\text{C}$  values. The  $\text{C}_3$  photosynthetic pathway uses the Calvin Cycle to fix  $\text{CO}_2$  within its cells (Hedges et al. 2005).  $\text{C}_3$  plants account for 90% of all plant species and include trees, shrubs and winter rainfall grasses.  $^{13}\text{C}$  depletion in  $\text{C}_3$  plants causes a typical range in  $\delta^{13}\text{C}$  values of -20 to -30‰, with an average fractionation of  $\sim$  -27‰.

A second photosynthetic pathway known as the Hatch-Slack cycle is used by  $\text{C}_4$  plants, which include hot- and arid-region grasses, and crops such as corn and sugarcane.

C<sub>4</sub> plants are specially adapted to both low pCO<sub>2</sub> and arid conditions, and are much more efficient at fixing carbon than C<sub>3</sub> plants. Therefore, they fraction <sup>13</sup>C less and typically δ<sup>13</sup>C values are between -9 to -16‰, with an average composition of ~ -13‰.

Bioapatite carbonate is enriched in <sup>13</sup>C relative to diet. This fractionation has been measured or estimated in several previous controlled feeding experiments and field studies, with results ranging between about 6 and 15‰ (Passey *et al.* 2004). It is theorized that inter-species differences in bioapatite diet carbon isotope fractionation might arise primarily from differences in digestive physiology between species (Ambrose 1993). The factor most often implicated for causing interspecies differences in the fractionation between diet and metabolic CO<sub>2</sub> is inter-species differences in the amount of methane production by microorganisms in the digestive tract (Passey *et al.* 2004).

The enrichment has been calculated by Murphy *et al.* (2007) for kangaroos as 11.7 ± 0.6‰. Applying the values for C<sub>3</sub> and C<sub>4</sub> plants discussed above, a δ<sup>13</sup>C value of ~ -15‰ in enamel samples reflects a temperate grazer or browser. Such as in the south of SA, while a high δ<sup>13</sup>C value closer to -1‰ suggests a diet of semi arid grazing, seen in the northern end of the study area (Murphy 2007).

Carbon isotopes can also provide other information related to environment, because of the tendency of C<sub>3</sub> and C<sub>4</sub> plants to thrive in different climates, and other factors that affect the amounts of <sup>13</sup>C in the body. The presence of a canopy, light intensity, humidity, genetics and water stress are all known to have measurable effects on <sup>13</sup>C enrichment (Brookman 2008). Murphy and Bowman (2007) showed that seasonal water availability (SWA) was the dominant factor influencing C<sub>4</sub> relative abundance, with SWA explaining 76% and 69% of deviance in C<sub>4</sub> vs. C<sub>3</sub> values.

Unfortunately SWA was not available for this study, and instead an approximation of the relationship between the C<sub>4</sub> component of diet and δ<sup>13</sup>C is used according to

Hattersley's (1983) survey (figure 2). The shortfall of this survey is that Hattersley used C<sub>4</sub> abundance rather than biomass. For instance, the semi-arid Gairdner-Torrens area, from which the Stuart Highway samples were drawn contains a large percentage of C<sub>4</sub> species, however has a low actual biomass, which explains the lower than expected  $\delta^{13}\text{C}$  values Brookman observed in this area. Approximate C<sub>4</sub> relative abundances for each region are listed in table 1.

## **4. METHODS**

### **4.1 Kangaroo specimen collection and preparation**

This study was restricted to the four most common members of the kangaroo genus *Macropus*, the red kangaroo (*M. rufus*), the common wallaroo (*M. robustus*), the western grey (*M. fuliginosus*), and the eastern grey (*M. giganteus*). Skulls from 110 road-killed and culled individuals were collected throughout South Australia and southeastern Victoria (figure 1) in July and August 2007 (Brookman, 2008). An approximate time of death was assigned based on the level of decomposition, with age at death assigned using the molar progression index outlined in Kirkpatrick (1985) and Jackson (2003).

Molar progression is the forward movement of the series of two deciduous pre-molars, and four adult molars in the skull, during the life of an animal (McArthur & Sanson 1987). The diet of the larger macropods is very abrasive and wears the molars down over time (Staker 2006). The animal chews with the anterior molars only, and as these are worn they progress forward until they are shed completely and replaced by a posterior molar. The front rim of the eye orbit is used as a reference point for aging by molar progression; an aged kangaroo will eventually have all four molars moved completely past this point.

For C and O analysis, it is important to consider that teeth formed before weaning reflect the diet of an individual's mother. C and O undergo considerable fractionation during the biochemical processes in the body of an animal, so selecting teeth that formed after weaning minimises fractionation. Thus while it is common to perform Sr analysis on the first permanent molar, as this represents the earliest time in an animal's life that can be analysed, it was necessary to use dominantly older molars so that C and O analysis could be conducted on the same enamel samples.

In the selected kangaroo species, this means that the 3<sup>rd</sup> molars are often viable, but the 4<sup>th</sup> molars present the best option. The 3<sup>rd</sup> molar begins forming at ~393 days and the 4<sup>th</sup> molar at ~827 days after birth. Weaning occurs at ~540 days for *M. fuliginosus* and *M. giganteus*, and 409±74 days and ~360 days for *M. robustus* and *M. rufus* respectively (Dawson, 1995).

Given the time restraints of this study, it was necessary to narrow down the 110 specimens to approximately 40 that would produce the most useful data for analysis. This was later cut down to just 30. Individuals were primarily selected based on whether a fourth molar was present. Additional younger animals were selected to achieve a larger geographical spread, requiring a number of second and third molars to be analysed. While sampling aimed to reflect a varied population there were a number of instances of multiple sampling from a single animal. This allowed the analysis of isotopic relationships between left and right molars, and between consecutive teeth.

While some care was taken to select from each of the four species of Macropus, ultimately the inter-regional variation is considerably greater than any inter-species variations (Brookman 2008).

Enamel was extracted from 30 individual skulls (table 1). The preferred tooth for sampling was the largest/youngest molar in each skull, with most of the enamel from both

lophs of the molar removed. The enamel was removed and cleaned using a tungsten carbide drill bit, with care taken to remove all surface stains and dentine.

Apatite was extracted from 31 individual skulls (table 1). The preferred sampling location was the jugal bone of the zygomatic arc, the bone just below the eye socket. Several specimens contained only fragments of teeth, consequently for these specimens, a sample was taken from the maxilla. Each bone was cleaned and sampled using a tungsten carbide drill bit.

## 4.2 Stable Isotope Analysis

Samples of approximately 5 mg were prepared for isotope analysis, following the method outlined in Balasse *et al.* (2002) with modifications, at the University of Adelaide. Powdered samples were soaked in 10% H<sub>2</sub>O<sub>2</sub> at room temperature under constant air flow for 24 h in order to remove organic matter (collagen). Two cycles of H<sub>2</sub>O<sub>2</sub> treatment were required for bone samples to ensure all collagen was removed. Samples were then soaked in 1.5 ml of 0.1M acetic acid for four to six hours to remove secondary calcium carbonates, then rinsed five times with deionised water and dried in an oven for 24 h at 80°C.

The carbonate component of bioapatite was measured for oxygen and carbon isotopes via a dual inlet gas source isotope ratio mass spectrometry (IRMS) in the stable isotope laboratory at the University of Adelaide.  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  isotope data were acquired simultaneously on a Fisons Optima dual inlet mass spectrometer attached to an Isocarb preparation device at the University of Adelaide. Sample powder was reacted in a common, purified H<sub>3</sub>PO<sub>4</sub> bath at 90°C for 450 seconds. Evolved CO<sub>2</sub> was doubly-distilled cryogenically and measured against an in-house reference gas.  $\delta^{18}\text{O}$  was corrected for

$$\delta^{13}\text{C} = \left( \frac{\frac{\delta^{13}\text{C}}{\delta^{12}\text{C}}_{\text{sample}}}{\frac{\delta^{13}\text{C}}{\delta^{12}\text{C}}_{\text{reference}}} - 1 \right) \times 1000 (\text{‰})$$

equilibrium with H<sub>2</sub>O during the reaction using the Craig (1957) equation and both  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  samples were calibrated to Vienna peedee belemite (VPDB) reference standard using an in-house calcite standard and checked against the international reference standard, NBS-19. External error ( $1\sigma$ ) based on the standards was better than  $\pm 0.05\text{‰}$  for  $\delta^{13}\text{C}$  and  $\pm 0.1$  for  $\delta^{18}\text{O}$ . Standards were run in triplicate before each session and once after six consecutive samples. All measurements were reported in per mil (‰) units using the standard delta notation, e.g.

Sample splits were prepared for Sr isotope analyses at the University of Adelaide closely following methods described in detail in Halverson et al. (2007). Powdered samples of a minimum of 1.5 mg were weighed out and placed in 7 mL Teflon beakers. Samples were leached in 1 ml of 0.2M ammonium acetate for 30 minutes, and then rinsed twice with 1.5 ml of MQ H<sub>2</sub>O. Samples were then dissolved in 1.5 mL of 0.5M acetic acid and dried down on a hotplate at 80°C. Dried samples were then re-dissolved in 500  $\mu\text{l}$  3.5M HNO<sub>3</sub>, before loading into pre-conditioned microcolumns containing Eichrom Sr-spec. Loaded samples were then rinsed four times with 300  $\mu\text{L}$  3.5M HNO<sub>3</sub> to remove Ca. Sr was then eluted from the resin using two rinses of 500  $\mu\text{L}$  MQ H<sub>2</sub>O. Each sample was then centrifuged and filtered to remove any possible remaining resin or dust.

Samples were measured in 2% HNO<sub>3</sub> on a ThermoFinnigan Neptune multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS) in the University of Adelaide Laboratory for Trace Element and Isotope Analysis. Each sample was measured for ~12 minutes.  $^{82}\text{Kr}$  and  $^{83}\text{Kr}$  and  $^{85}\text{Rb}$  were measured to correct for interferences on  $^{84}\text{Sr}$  and  $^{87}\text{Sr}$ , respectively, and assumed exponential internal mass bias on the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio was corrected using  $^{86}\text{Sr}/^{88}\text{Sr} = 0.1194$ . The international strontium isotope standard NIST

SRM-987 (National Institute of Standards and Technology, Gaithersburg, USA) with an accepted  $^{87}\text{Sr}/^{86}\text{Sr}=0.71025$  was measured at the start and end of each run and between every four samples to establish analytical precision and ensure consistent output between runs. The average  $^{87}\text{Sr}/^{86}\text{Sr}$  of NIST SRM-987 over the course of analysis ( $n=31$ ) was 0.710243 with a  $2\sigma$  internal precision of 0.000015.

## 5. RESULTS AND DISCUSSION

### 5.1 Results

The results of the isotopic measurements are summarised in Table 1. The total isotopic range of all samples and all animals in this study is relatively large (0.7090–0.7163 for  $^{87}\text{Sr}/^{86}\text{Sr}$ , -20.54‰ and -5.85‰ for  $\delta^{13}\text{C}$  and -5.25‰ and 5.67‰ for  $\delta^{18}\text{O}$ ).  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios are plotted against latitude, and as a comparison of bone apatite (BA) to enamel apatite values in Figures 3 and 4 respectively.  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values are plotted against latitude, each other, and alongside Tom Brookman's  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  data in figures 5, 6 and 7 respectively.

While each specimen in this study was prepared for analysis of both enamel and bone apatite, for a number of reasons the final data contained gaps. The majority of missing Sr data is a result of there being insufficient weight of sample left after pre-treatment to remove organics, collagen and secondary calcium carbonates. A minimum of 1.5 mg of apatite was required for measurement on the MC-ICP-MS. Limited enamel on many of the teeth meant the sample could not be repeated with a larger initial sample weight. Furthermore, not all samples prepared ran successfully through the MC-ICP-MS and IRMS, due to low Sr or C content or technical difficulties with the instruments.

## 5.2. Strontium

### 5.2.1. SOUTHEAST SA AND VIC

Eight individuals were sampled from the southeast of South Australia, and southwest Victoria. The Sr ratios for these individuals ranged from 0.70900 to 0.71016, with a mean of 0.70962 and a standard deviation of  $2.75 \times 10^{-4}$ . The marine source of the Pleistocene carbonates that underlay this region, and the proximity to the ocean explains the low  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios exhibited by the eight kangaroos analysed. The Sr in the carbonates is nearly indistinguishable from seawater ( $^{87}\text{Sr}/^{86}\text{Sr} = 0.7092$ ).

R048 BA had the lowest  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio at just 0.70900. R048 was found slightly to the east of the other specimens collected from this area, and as can be seen on figure 1, is located just onto mafic Cenozoic rocks. This low Sr value is consistent with a study done by Price *et al.* (2000), which found mafic rocks to have  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of less than 0.706. In this case, sea spray has actually increased the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio, rather than mute it as seen in other regions.

The other seven individuals had  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios higher than seawater. It is likely that reworking and mixing of the marine carbonate with wind-blown materials has occurred, to increase the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio. A study by Dart *et al.* (2007) investigated the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of many carbonates across South Australia. They found that the dominant source of Sr in soil carbonates through inland Australia is marine aerosols driven onshore by winds and sea-spray. They found that there is a gradual increase in  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of regolith carbonates from the coast to 200 km inland, until the prevailing winds become the driving force. The soils that plants – and by association the fauna – receive their Sr from is thus heavily influenced by wind-blown material, and is not derived entirely from the bedrock – in this case marine carbonates.

Of the eight individuals, six were successfully analysed for both enamel and bone apatite  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio. R028, R029, R036, R038 and R050 had very similar  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios between bone and apatite samples, with differences ranging from 0.0001 to 0.00014. Multiple teeth were sampled from R042, a matched pair of fourth molars (LM4 and RM4), and consecutive molar RM3. A difference of 0.00012 occurred between LM4 and RM4, with the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of RM3 lying between the two. While left and right molars do not erupt simultaneously, the apatite production would have overlapped significantly over the year the molars took to form. A slight change in habitat and diet in the species may be to blame, and suggests the similarly sized differences seen in enamel and BA ratios of other individuals can be explained by small changes in the same location rather than movement of the individual to another location.

#### 5.2.2. FLINDERS RANGES

15 individuals from the Flinders Ranges were analysed. Values ranged from 0.71203 to 0.71415, with a mean value of 0.71309 and a standard deviation of  $6.02 \times 10^{-4}$ . 11 of these kangaroos were clustered closely together, in a range of 600 ha (seen on the inset of figure 1), with a mean Sr ratio of 0.71302 and a standard deviation of  $6.16 \times 10^{-4}$ . With the exception of R024, this range contains both the lowest and highest Sr ratios exhibited in the Flinders Ranges or Stuart Highway.

Paired left and right molars were sampled from R065 and R069. R069 RM4 and LM4 had similar values of 0.71378 and 0.71361 respectively, with a range of just 0.00017, a similar value to that seen in R042. On the other hand, R065 RM4 and LM4 had Sr ratios of 0.71465 and 0.71347 respectively, with a range four times larger than R069, of 0.00068. The bone apatite ratio fell approximately halfway in between these two values at 0.71392. A change in diet possibly brought on by a change in climate (eg. drop in rainfall) could

explain this. The kangaroo essentially does not eat the same vegetation in the same place all the time, with plants of different species and region exhibiting different Sr concentrations and ratios. The bone apatite value being averaged between the two is supportive of this.

R012 and R071 had significant differences between enamel and bone apatite Sr ratios of 0.00053 and 0.00108 respectively.  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  data for R012 shows a change from -5.85‰ and 1.00‰ in tooth RM4 for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  respectively, to -12.91‰ and 4.23‰ in BA. No  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  were recorded for R071 RM4 so a similar check for R071 was not possible. Brookman (2008) did analyse both R071 BA and RM4, with his numbers for the bone apatite a very close to match to those achieved in this study (-9.40‰ and 1.86‰ for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  compared to his results of -9.78‰ and 1.62‰). In general the results achieved by Brookman were close match when compared to the same samples in this study, suggesting that his values for R071 RM4 of -6.17‰ for  $\delta^{13}\text{C}$  and 1.64‰ for  $\delta^{18}\text{O}$  are applicable to this study. The change in Sr ratios is supported by these changes in this change in  $\delta^{13}\text{C}$ , suggesting that R071 did indeed undergo movement of some distance. The data for the three isotopes suggests they were both recent migrants into the area.

The remaining nine individuals had only small variation between enamel and bone apatite Sr ratios, ranging from as little as  $1.08 \times 10^{-5}$  to  $2.47 \times 10^{-4}$ .

### 5.2.3. TORRENS-GAIRDNER AND STUART HIGHWAY

Eight individuals were collected along the Stuart highway and make up the northern half of the transect from Woomera, South Australia to Portland Victoria. Strontium isotope ratios ranged from 0.71238 to 0.71445, with an average of 0.71307 and a standard deviation of  $6.58 \times 10^{-4}$ .

Multiple teeth were sampled from R024 and R027. R024 RM1, RM2 and RM3 and had similar Sr ratios, with a difference of only 0.00040 between the three molars, all three ratios however were significantly smaller than the bone apatite value, with a large difference of 0.00193 between R024RM1 and R024 BA. The bone apatite value for R024 closely matches the bone apatite value of R023 found in the same location. This is strong evidence that R024, a western grey kangaroo had migrated to the area between the beginning of the formation of RM3 at ~393 days old and the remodelling of the bone apatite in the last several years of life while the kangaroo. R024 was identified by molar progression to be 6.33 years old at death, so the bone apatite represents a value averaged over the ages of 3-6 years old. In western grey populations, it is young adult male which are the most likely to move over any distance. A movement of 85 km was recorded by a young adult male in NSW (Clancy & Croft 1990).

Two teeth were sampled from R027, with  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of 0.71259, 0.71372 and 0.71262 seen for BA, LM4 and RM3 respectively. This sequence suggests that the R027 had moved to a new region between formation of the third and four molars, then returned to its previous region during the last several years of its life. The sort of migration is almost certainly caused some form of climate change that affected the available forage enough to force the kangaroo to shift to a more desirable region. A study at Fowlers Gap Station (Croft 1991) found that some kangaroos would temporarily move home ranges during a dry year, while others would travel nightly from their home ranges to better feeding areas. This is strongly supported by the  $\delta^{13}\text{C}$  data, with values for RM1, RM2, RM3 and BA ranging from -18.45‰ to -2‰, and at a low of just -16.4‰ for LM4. The  $\delta^{13}\text{C}$  values strongly suggest that R027 shifted its home range during the formation of its fourth at approximately six to seven years of age, then returned to its original range shortly after until its death at 9.49 years old.

Of the seven individuals with bone and enamel apatite to compare, four display only minor differences and are likely local to the area they died in. R024 and R027 – as discussed above – and R013 exhibit a different  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio between bone and tooth enamel. R013 had difference of 0.00145 between BA and LM4. R013 was a red kangaroo of 5.08 years of age. Red kangaroos have been known to travel much larger distances than the other three *Macropus* species investigated. Mature red kangaroos move home-ranges at times, some local movements of 10-15 km, and others cover much larger distances. One radio collared red kangaroo was located some 250 km from its initial study site after being shot by a hunter (Croft 1991). The  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  of R013 BA and R013 LM4 support this, with a change of -12.7‰ to -9.8‰ of the  $\delta^{13}\text{C}$  values between BA and LM4, and 4.5‰ down to 2.5‰ seen in the  $\delta^{18}\text{O}$  values. The BA value represents an average over the ages of approximate 2-5, while LM4 was still erupting at time of death. The move to this region was thus quite recent or the bone apatite values would have approached those of LM4 as the bone was remodelled.

### 5.3. Carbon And Oxygen

#### 5.3.1. LATITUDE

Bone and Tooth enamel values were compared with the latitude at which the animals were found (figure 5). Based on a linear regression model, neither  $\delta^{13}\text{C}$  nor  $\delta^{18}\text{O}$  show a strong relationship with latitude. The variations in  $\delta^{18}\text{O}$  values for bone and enamel ( $R^2=0.41$  and  $0.36$  respectively) and  $\delta^{13}\text{C}$  of bone and enamel ( $R^2=0.49$  and  $0.36$  respectively) are thus only weakly explained by latitude. Given the known strong correlation with  $\text{C}_4$  species abundance in South Australia, it's possible that a more even spacing of samples could generate a better result. The majority of samples were taken

from the north, with few intermediate latitudes represented due to the lower abundance of kangaroos in the mid-transect relative to the north and south extremes.

A much larger range in  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values is seen in enamel than in bone apatite, which is to be expected given the averaging that occurs during bone remodelling. If the bone apatite values alone are considered, then  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values tend to decrease as latitude increases, towards the south.

Despite the weak linear relation between  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  and latitude, co-variation seen between  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  is great, with a similar shape in values and ranges seen when plotted against latitude (figure 5).

### 5.3.2 RELATIONSHIP BETWEEN $\delta^{13}\text{C}$ AND $\delta^{18}\text{O}$

The hot dry climates to the north of South Australia experience little rain and tend to be enriched in both  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ . Hattersley (1985) suggests that C4 plant abundance in the Torrens-Gairdner/Stuart highway region and Flinders Ranges are 53 and 81% respectively (table 1). This does not take into account the much higher biomass seen in the Flinders Ranges, which increases the amount C4 plants enriched in  $\delta^{13}\text{C}$  in the diets of the kangaroos. The cooler and wetter climate in the southern part of South Australia produces almost exclusively C<sub>3</sub> plants, with the increased rainfall also producing less enriched  $\delta^{18}\text{O}$  values.

This suggests that there should be a strong relationship between  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ . Both should be seen to be enriched in the north, and less enriched - or depleted - to the south. A comparison of  $\delta^{13}\text{C}$  to  $\delta^{18}\text{O}$  for bone apatite and enamel (figure 6) produced only a very weak correlation ( $R^2=0.147$  and  $0.203$  respectively). The natural variances between individuals seems too be great to concisely produce a linear relationship between of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ .

### 5.3.3. COMPARISON WITH BROOKMAN (2008)

It was important to see if the isotopic data produced in this study could be related to the values that Tom Brookman calculated. 22 bone or enamel apatite samples were analysed in both studies. Brookman performed microsampling of enamel on each tooth sample, so his values were averaged over the tooth for comparison with this study. Sample preparation was slightly different also, and isotopes were measured on a Finnegan MAT 252 isotope ratio mass spectrometer at the Illinois State Geological Survey. Values for the two studies were plotted (image 7) and show a strong correlation between the majority of samples.

### 5.3.4. DOMINANCE OF C<sub>3</sub> AND C<sub>4</sub> PLANT SPECIES BY REGION

As explained in section 3.3, an enrichment factor of  $\delta^{13}\text{C}$  of  $11.7 \pm 0.6\text{‰}$  is seen in kangaroos. A diet of pure C<sub>3</sub> species will generate  $\delta^{13}\text{C}$  values of approximate -15 to -20‰, and a diet of purely C<sub>4</sub> species will generate  $\delta^{13}\text{C}$  values of around -3 to -5‰ (Brookman 2008).  $\delta^{13}\text{C}$  values of bone and enamel apatite was plotted for each region of the study against  $\delta^{18}\text{O}$  (figure 8). The individuals collected from the southern part of South Australia all had  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values suggestive of a purely C<sub>3</sub> diet. Individuals from the Stuart highway and Flinders Ranges seem to have an intermediate diet of C<sub>3</sub> and C<sub>4</sub> plant species and vary greatly in  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ .

## 6. CONCLUSION

Despite the limitations of scale to South Australia, the results have promising implications for an Australia wide study. The evidence of mobility seen in R012, R013,

R024, R027 and R071 confirms that Sr can successfully be used to assess migration in kangaroos, and shows the potential to use modern kangaroos as a proxy for prehistoric fauna. Furthermore, comparison between the migratory individuals and the oxygen and carbon isotope values hint at the possibility of using oxygen and carbon isotope data to assess migration.

This study is the first demonstration that Sr isotopes can be effectively used in a modern population of an Australian fauna to show movement between different geological units. Differences seen between matched molars also demonstrate the level of inter-individual isotopic variation that can be expected. Differences between consecutive molars imply that analysis of multiple teeth of an individual will make more instances of migration become apparent. Further study into intra-tooth isotope analysis may allow the identification of summer and winter ranges, and also an expansion to an Australia wide study may allow the application of these techniques to archaeological specimens.

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## 8. FIGURE CAPTIONS

**Figure 1:** Kangaroo locations mapped on the surface geology of South Australia. The key geological units are: Yellow: Pleistocene carbonates, orange: Cambrian felsic siliclastics of the Adelaide hills and Flinders Ranges, green: Cenozoic mafic to ultramafics. A number of specimens were too tightly grouped to show at this scale, and can be seen on the inset. Modified from map produced online using *Map Connect*, Geoscience Australia.

**Figure 2:** C<sub>4</sub> species abundance in Australia from Hattersley (1983, 116) with the sample transect in blue.

**Figure 3:**  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of each sample plotted against latitude. The Southern Limit of the Adelaide Hill represents the latitude where bedrock geology changes from the felsic siliclastics of the Adelaide Hills, to the marine carbonates of the South.

**Figure 4:** Tooth enamel  $^{87}\text{Sr}/^{86}\text{Sr}$  plotted against bone apatite  $^{87}\text{Sr}/^{86}\text{Sr}$ . Line of  $x=y$  has been plotted to mark where an individual that has not undergone any migration should lie. Individuals of interest, i.e. those that have undergone migration, have points that lie away from  $x=y$ . Teeth from the same individuals are connected by dotted lines.

**Figure 5:**  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values for bone apatite (BA) and enamel plotted against latitude to establish if there was a positive correlation between them.

**Figure 6:**  $\delta^{13}\text{C}$  plotted against  $\delta^{18}\text{O}$  for enamel and bone apatite.

**Figure 7:** Comparison of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values for individuals investigated in both this study and by Brookman (2008).

**Figure 8:** Regional and environmental isotopic groupings of bone and enamel apatite. R027, R028 and R028 were assigned to the regional grouping of Adelaide and Surrounds, due to their geographical remoteness to the other Stuart Highway individuals.

## 9. TABLES

**9.1 Table 1** – C4 relative abundance, by species diversity, in areas from which Kangaroos were sampled (from North to South).

Subdivision	Region	% C <sub>4</sub>
GT	Gairdner-Torrens/Stuart Highway	82
FR	Flinders Ranges	51
SL	Southern Mount Lofty Ranges	31
MY	Murray	47
SE	South-East	23
DK	Grids D-K (SW Victoria)	18

**Source:** Modified from Hattersley (1983, 114-115).

**9.2 Table 2:** Summary of isotopic results ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$ ).

Identification		Location			Stable Isotope Data			Brookman Data <sup>3</sup>	
Sample <sup>1</sup>	Species <sup>2</sup>	°S	°E	Age (yrs) <sup>3</sup>	$^{87}\text{Sr}/^{86}\text{Sr}$	$\delta^{13}\text{C}_{\text{PDB}}$ (‰)	$\delta^{18}\text{O}_{\text{PDB}}$ (‰)	$\delta^{13}\text{C}_{\text{PDB}}$ (‰)	$\delta^{18}\text{O}_{\text{PDB}}$ (‰) <sup>4</sup>
<i>Region: Southeast SA and southwest VIC</i>									
R028 BA	Grey Kangaroo*	35.53917	139.3600	2.63	0.70981	-17.65	-0.56		
R028 LM2					0.70990	-13.99	-1.83		
R029 BA	Grey Kangaroo*	35.90201	139.4478	8.88	0.70934	-16.62	-1.84		
R029 RM4					0.70942	-10.57	-2.57	-14.84	-0.27
R036 BA	Grey Kangaroo*	38.07289	141.1571	7.25	0.70961	-15.90	-0.20	-18.31	-1.41
R036 LM2					0.70948	-19.42	-1.05		
R036 LM3					0.70957	-18.31	-2.32	-16.47	-3.25
R038 BA	Grey Kangaroo*	38.07546	141.1653	5.17	0.70949	-14.47	-5.25		
R042 BA	Grey Kangaroo*	38.07848	141.1772	7.75	0.70971	-13.07	-2.09	-14.74	-0.57
R042 LM4					0.70957	-14.75	-3.42	-17.00	-2.31
R042 RM1						-15.25	-2.57		
R042 RM2						-14.51	-2.85		
R042 RM3					0.70962	-14.75	-3.58		
R042 RM4					0.70969			-17.03	-1.93
R045 BA	Grey Kangaroo*	38.08595	141.1919	7.75	0.70956	-16.87	-1.98	-17.07	-1.81
R045 LM4						-11.27	-1.48	-16.95	-0.46
R048 BA	Grey Kangaroo*	37.97370	141.3806	6.33	0.70900			-17.92	-2.80
R048 LM4						-16.76	-4.95	-16.33	-1.22
R050 BA	Grey Kangaroo*	37.59233	141.1822	5.92	0.71002	-18.84	-1.12		
R050 LM4					0.71016	-8.21	-0.54		
<i>Region: Flinders Ranges</i>									
R003 BA	M. rufus (male)	31.41139	138.7110	17.57	0.71329	-11.47	2.34	-14.64	0.89
R003 LM4					0.71335	-10.08	-0.21	-11.09	1.75
R007 BA	?	31.41356	138.7296	16.18	0.71388	-10.99	1.03		
R007 RM4					0.71363				
R009 BA	?	31.64757	138.8082	12.62	0.71322	-9.22	4.43	-10.02	2.19
R009 RM4					0.71321	-8.10	1.98	-5.50	3.50

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R010 BA	?	31.21420	138.6589	5.52	0.71633	-6.96	3.90		
R012 BA	?	30.56554	138.8456	12.62	0.71302	-12.91	4.23		
R012 RM4					0.71249	-5.85	1.00		
R065 BA	M. robustus	31.40873	138.7347	12.44	0.71392	-12.74	3.13		
R065 LM4					0.71347	-11.18	-1.26		
R065 RM4					0.71415				
R069 BA	?	31.41695	138.7271	13.71	0.71392	-12.51	2.38		
R069 LM4					0.71361	-8.05	-2.78		
R069 RM4					0.71378	-8.34	-1.49		
R070 BA	M. rufus (male)	31.46183	138.7162	8.35	0.71392	-15.03	-1.03	-13.08	0.33
R070 RM4					0.71203	-12.58	-0.80		
R071 BA	?	31.45768	138.7200	10.70	0.71229	-9.40	1.86	-9.78	1.62
R071 LM4					0.71337			-6.17	1.64
R072 BA	?	31.44060	138.7300	11.62	0.71300	-10.82	-0.29		
R072 RM4					0.71302	-6.96	-1.27		
R084 BA	M. robustus	31.42793	138.7205	8.33	0.71306	-13.01	-0.18	-16.85	1.48
R084 RM3					0.71286	-11.30	-1.50		
R089 BA	?	31.43423	138.6983	11.62	0.71296	-11.96	0.96		
R089 RM3					0.71235	-9.92	0.51		
R094 BA	M. robustus	31.43405	138.7022	10.60	0.71210	-10.59	-0.47		
R094 LM4					0.71206	-10.08	-0.96		
R095 BA	M. robustus	31.43401	138.7022	13.48	0.71347	-7.94	-0.81		
R095 LM4						-5.90	-1.54		
R105 BA	?	31.42172	138.6936	10.70	0.71298	-7.83	-1.87		
R105 LM4					0.71281				
<i>Region: Gairdner/Torrens and Stuart Highway</i>									
R013 BA	M. rufus (male)	32.05733	137.4458	5.09	0.71383	-12.70	4.52	-12.78	4.70
R013 LM4					0.71238	-9.76	2.55		
R015 BA	M. fuliginosus	31.45726	137.036	7.25	0.71249	-10.15	1.66	-10.56	2.94
R015 RM4					0.71243			-10.61	2.60
R018 BA	M. rufus (male)	30.81252	136.9039	5.52		-6.96	3.90		

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R018 LM4					0.71233	-9.73	5.67		
R019 BA	<i>M. robustus</i>	31.17365	136.8413	2.93	0.71367	-13.51	3.35		
R019 LM2					0.71321	-11.52	2.51		
R020 BA	?	31.74889	137.2603	12.62	0.71310	-12.00	3.41		
R020 RM4					0.71357	-9.05	2.84		
R023 BA	<i>M. fuliginosus</i>	32.74887	138.1650	7.75	0.71408	-15.36	0.63	-11.03	0.63
R024 BA	<i>M. fuliginosus</i>	32.75972	138.1709	6.33	0.71445	-17.01	0.29	-16.61	1.90
R024 RM1					0.71252	-15.15	-0.19		
R024 RM2					0.71277	-14.43	-0.73		
R024 RM3					0.71292	-13.49	0.31	-13.49	2.78
R027 BA	<i>M. fuliginosus</i>	34.76587	138.9438	9.50	0.71259	-20.54	-0.87	-10.65	4.83
R027 LM4					0.71262	-16.38	-1.71		
R027 RM1						-20.00	-1.60		
R027 RM2						-19.48	-3.14		
R027 RM3					0.71372	-18.45	-2.06	-17.52	0.31

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1. Naming of enamel sampled followed the format L/R(left/right)M#,(molar 1/2/3/4). BA = bone apatite

2. Due to the morphological similarities and extensive overlap in range of *M. fuliginosus* and *M. giganteus* in the south-eastern section of this transect, a general classification of grey kangaroos was used. Previous studies have shown the two species to be broadly similar (eg. Wilson 1975).

3. A number of the individuals selected were first analysed for 13C and 18O by Tom Brookman (2008). Brookman microsampled each tooth, so enamel apatite values were averaged over each tooth to enable comparison.

4. Values from Brookman 2008.  $\delta^{18}OPDB$  values converted from  $\delta^{18}OVSMOW$  values.

## 11. Figures

Figure 1

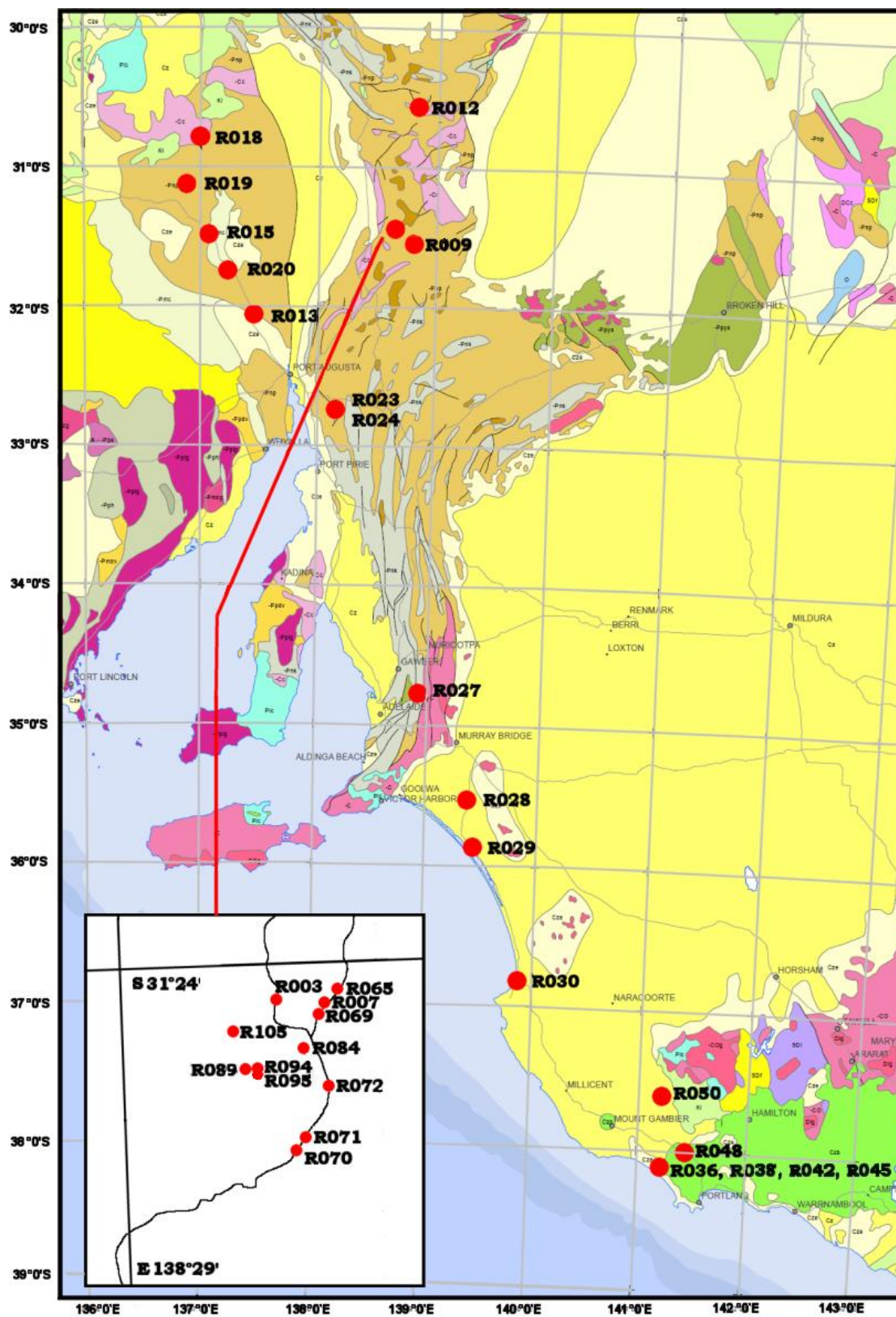


Figure 2

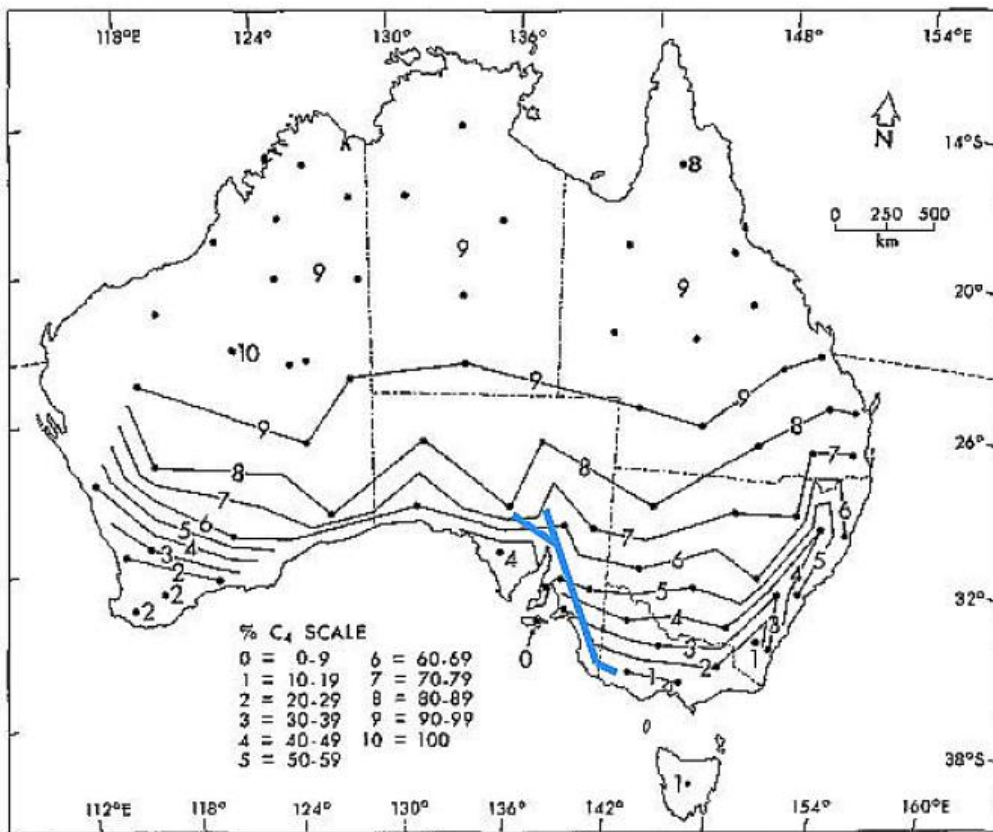


Figure 3

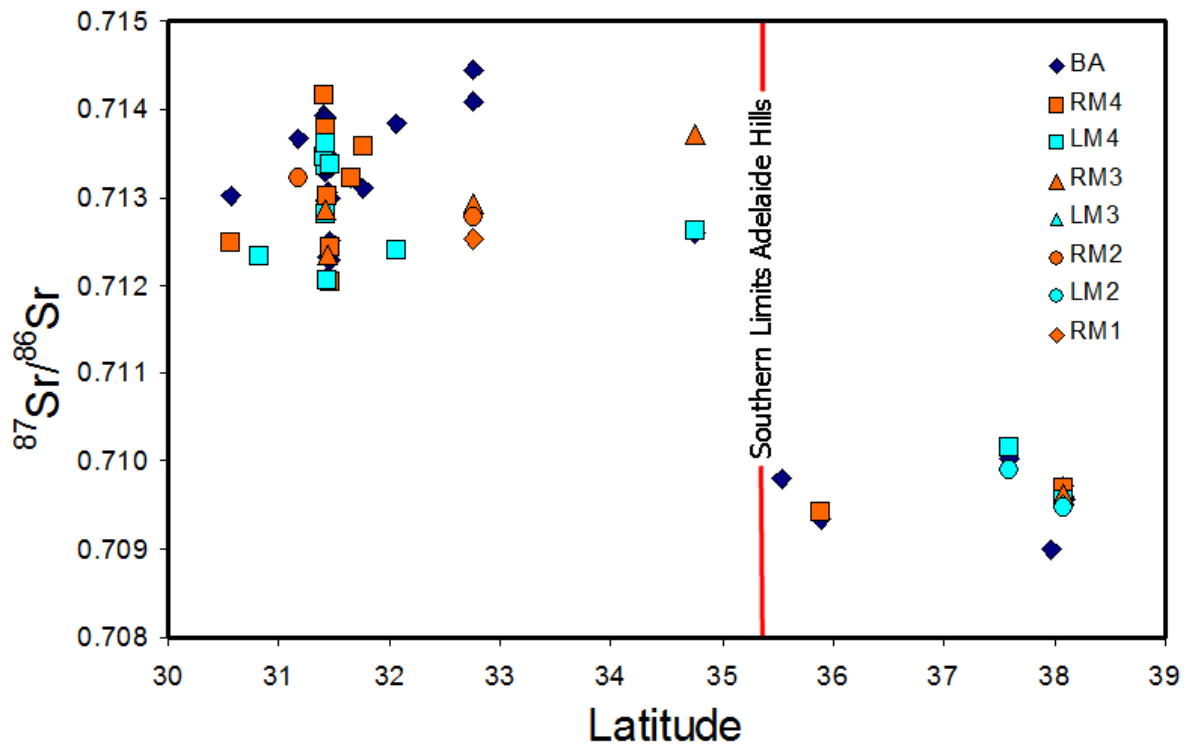


Figure 4

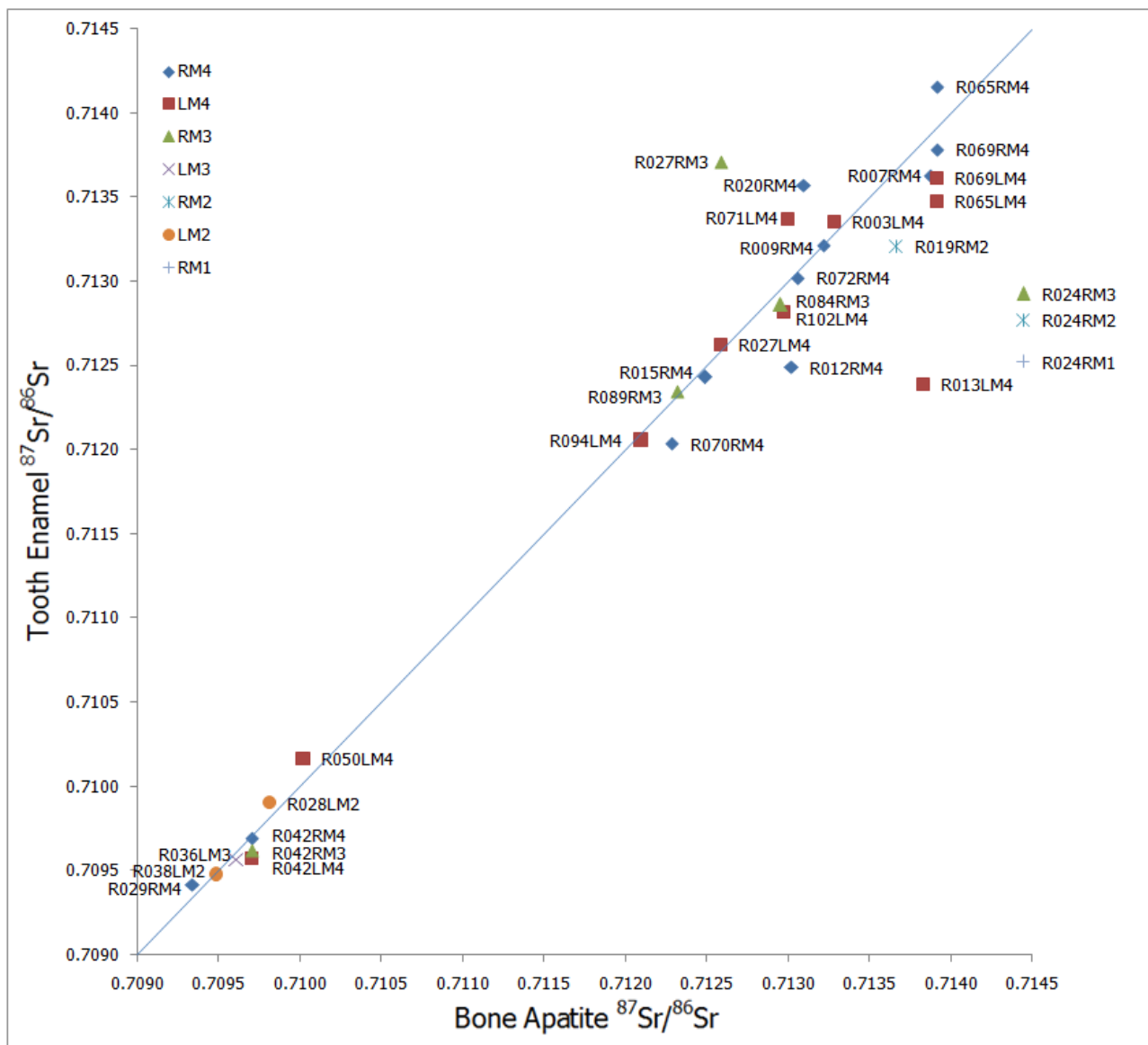


Figure 5:

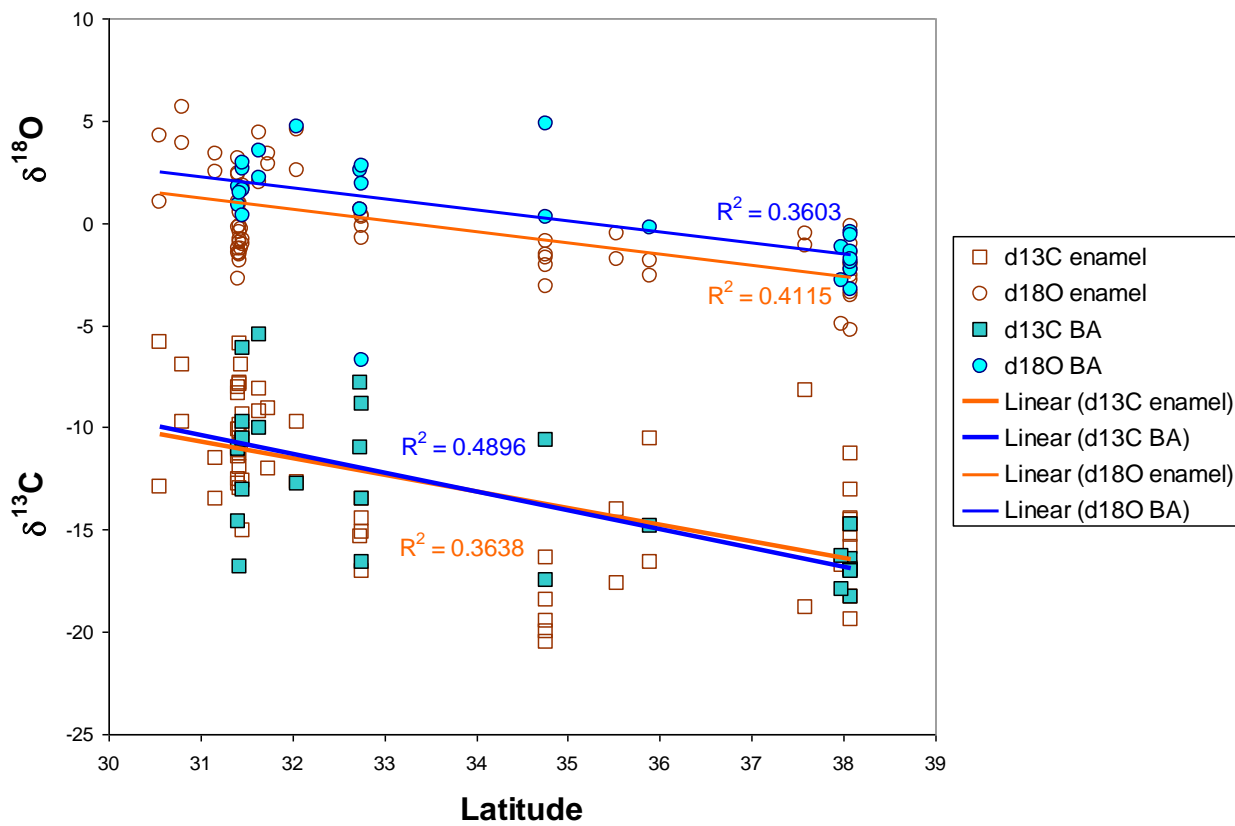


Figure 6:

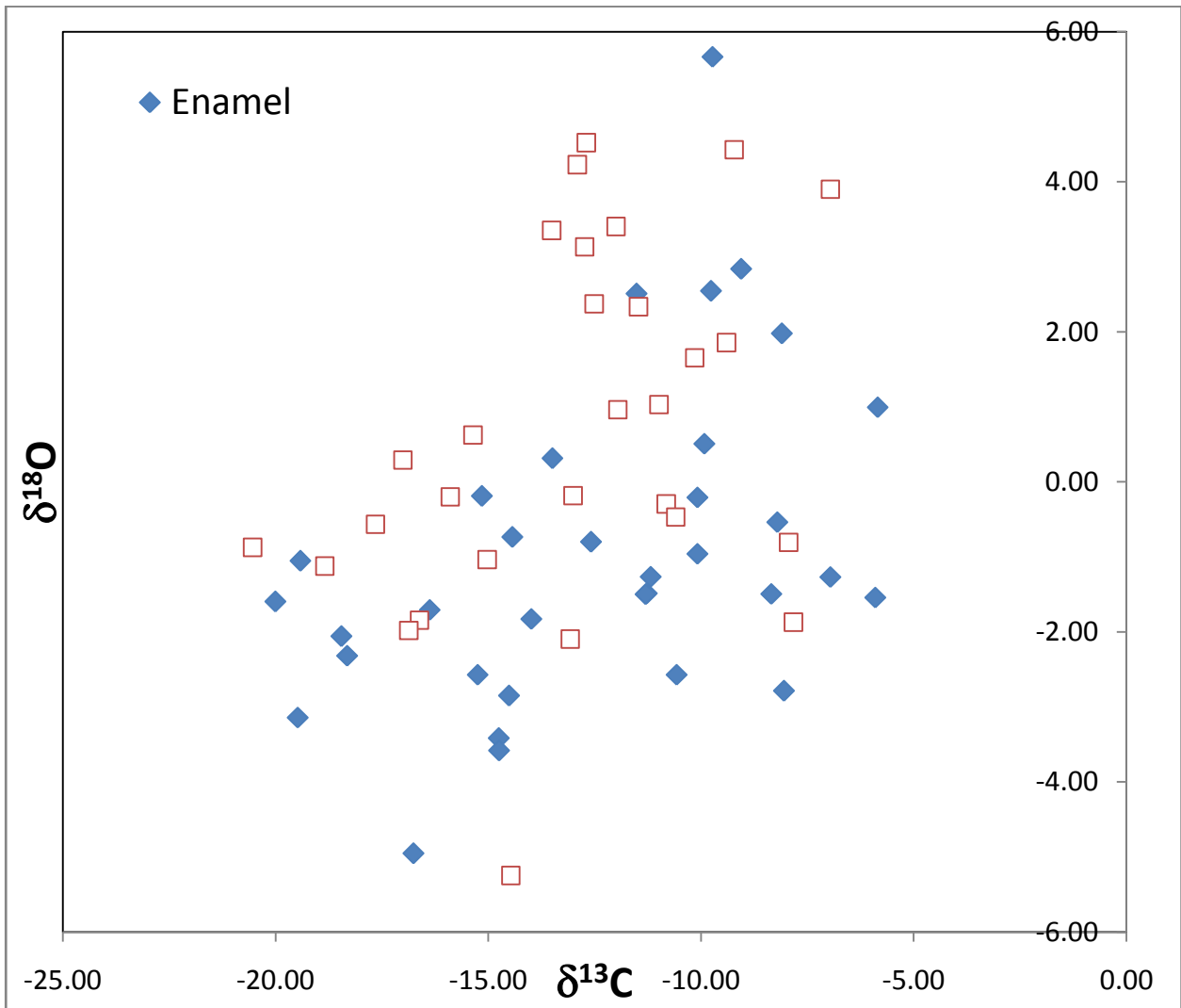


Figure 7

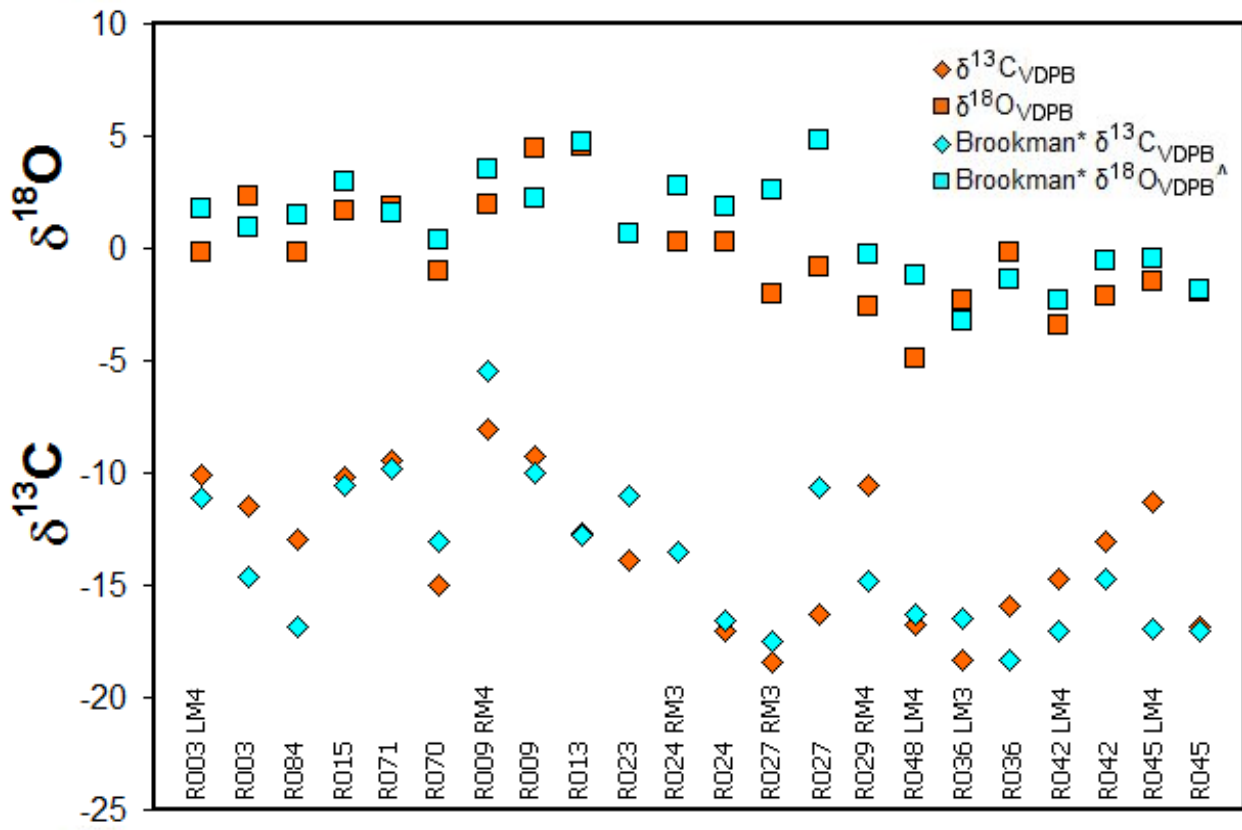
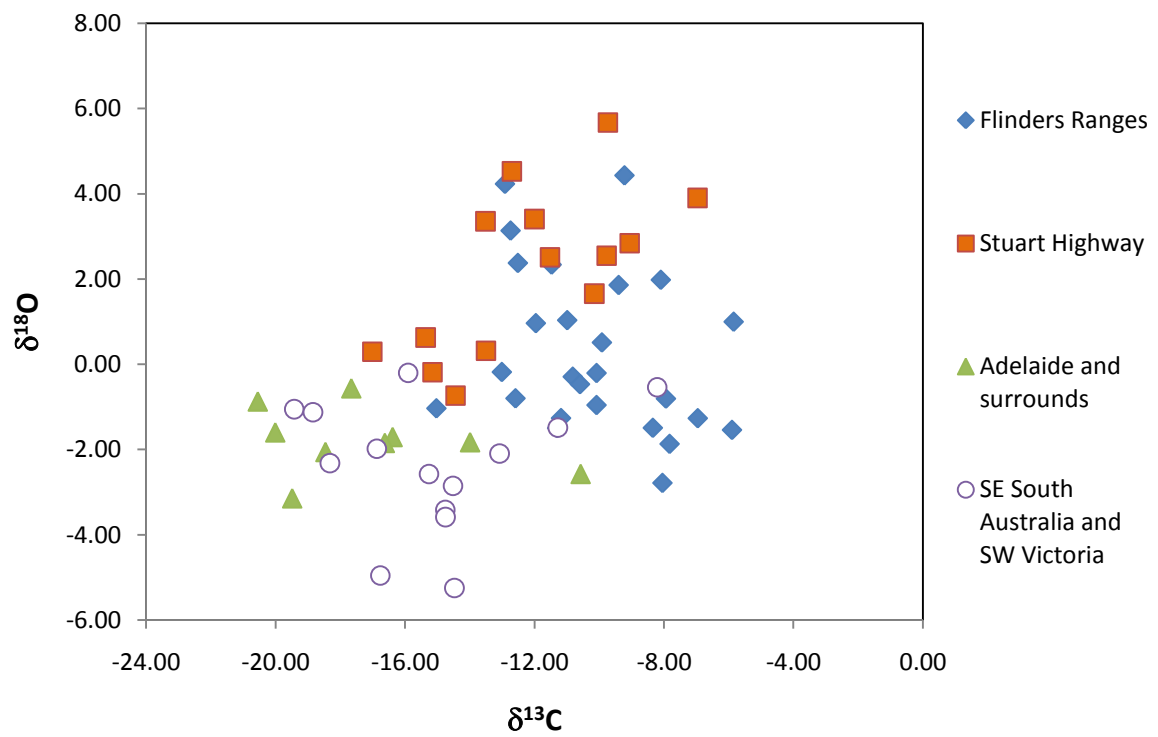


Figure 8



## 10. Appendices

### 10.1: Summary of initial data taken from Brookman (2008) and generated during inspection of each kangaroo specimen.

Sample	Species	Location			MI	Log Age (days)	Age (days)	Age (yrs)	T.O.D.	Molars (1 = fully erupted, p = partially erupted)								Mandibles	Notes
		oS	oE	Elevation (m)						LM1	LM2	LM3	LM4	RM1	RM2	RM3	RM4		
R003	M. rufus (male)	31.41139	138.71104	664	4	3.8074	6418.004	17.57154	Early 2007		1	1	1	1	1	1	1	2	
R007	?	31.41356	138.72955	681	4	3.7715	5908.81	16.17744	?		1	1			1	1	1	0	Teeth worn
R009	?	31.64757	138.80823	367	4	3.6638	4611.052	12.62437	Early 2007	1	1	1	1	1	1	1	1	0	
R010	?	31.21416	138.65891	539	3	3.3048	2017.437	5.523442	?/6/07	1	1	1 p						2	half maxilla missing
R012	?	30.56554	138.84555		4	3.6638	4611.052	12.62437	Early 2007	1	1	1	1		1	1	1	2	fragments
R013	M. rufus (male)	32.05733	137.44579	94	3	3.2689	1857.377	5.08522	?/6/07	1	1	1		1	1	1 p		1	only partial mandible
R015	M. fuliginosus	31.45726	137.03598	100	3	3.4228	2647.403	7.248193	?/6/07	1	1	1	1	1	1	1	1	1	Partial mandible
R018	M. rufus (male)	30.81252	136.90388	114	3	3.3048	2017.437	5.523442	Early 2007	1	1	1	1	1	1	1		1	RM4 removed
R019	M. robustus	31.17365	136.84126	122	2	3.0297	1070.73	2.931499	?/6/07	1	1	1		1	1	1		0	
R020	?	31.74889	137.2603	90	4	3.6638	4611.052	12.62437	May/June 07	1	1	1	1	1	1	1	1	2	
R023	M. fuliginosus	32.74887	138.16495	309	3	3.4522	2832.435	7.754785	?/6/07	1	1	1		1	1	1		0	
R024	M. fuliginosus	32.75972	138.17093	313	3	3.3641	2312.81	6.332129	Early 2007	1	1	1	1	1	1	1	1	1	
R027	M. fuliginosus	34.76587	138.9438	438	4	3.5402	3468.806	9.497073	?/6/07	1	1	1	1	1	1	1	1	1	
R028	Grey Kangaroo*	35.53917	139.35999	7	2	2.9827	960.9925	2.631054	23/6/07	1	1			1	1			2	



## 10.2: MC-ICP-MS data

Sample	Machine Corrected	Raw (Blank & Interference Corr)		Corrected	2 S.E.
	87Sr/86Sr	87/86	88/86	87/86	
R003-LM3	0.713496	0.728468	8.73146104	0.713350321	1.52566E-05
R005	0.713432	0.72776	8.71623092	0.713285836	1.43408E-05
R007	0.714022	0.728503	8.71932136	0.713875523	1.12919E-05
R007-RM4	0.713774	0.729401	8.74678519	0.713628353	1.11599E-05
R009	0.713368	0.728657	8.73895858	0.713222143	1.33911E-05
R009-RM4	0.713357	0.727854	8.7201635	0.713211307	1.12177E-05
R010	0.71648	0.732333	8.75068997	0.716333667	6.76918E-06
R012	0.71317	0.728979	8.75144734	0.713023894	3.59955E-06
R012-RM4	0.712634	0.727414	8.72726299	0.712488463	1.11539E-05
R013	0.71398	0.729654	8.74781514	0.713833769	9.60488E-06
R013-LM4	0.712531	0.727555	8.73302156	0.712384678	6.23065E-06
R013-RM4	0.712575	0.728306	8.74989348	0.712429525	5.34E-06
R015	0.712639	0.728203	8.74587759	0.712493072	9.27498E-06
R018-LM4	0.712474	0.728184	8.74945608	0.712327798	1.31336E-05
R019	0.713811	0.729485	8.7478425	0.713665067	6.62253E-06

R019-LM2	0.713352	0.728894	8.74497896	0.71320646	9.71432E-06
R020	0.713245	0.72878	8.74488631	0.713099155	1.09295E-05
R020-RM4	0.713716	0.729278	8.74527587	0.713570094	1.207E-05
R023	0.714221	0.728773	8.72092124	0.714075364	8.34916E-06
R024	0.714594	0.729594	8.73154509	0.714447878	6.64814E-06
R024-RM1	0.712667	0.727611	8.73110201	0.71252072	1.17735E-05
R024-RM2	0.712915	0.727538	8.72313163	0.71276901	1.09144E-05
R024-RM3	0.713071	0.727996	8.73041125	0.71292492	9.56255E-06
R027	0.712735	0.72799	8.73848247	0.712589416	7.13127E-06
R027-LM4	0.712766	0.728571	8.7515867	0.712619705	6.94672E-06
R027-RM2	0.714306	0.728887	8.72182517	0.714159566	3.18153E-05
R027-RM3	0.713861	0.728899	8.73252753	0.713715351	1.20386E-05
R027-RM4	0.713802	0.728711	8.72969666	0.713655667	4.20751E-05
R028	0.709958	0.724957	8.73E+00	0.709813017	8.17459E-06
R028-LM2	0.710047	0.725783	8.75137551	0.709901336	9.30105E-06
R029	0.709486	0.724655	8.73814501	0.709341049	7.69795E-06
R029-RM4	0.709563	0.725365	8.75318658	0.709418089	7.95096E-06
R036	0.709755	0.724142	8.7191963	0.709610096	4.67648E-06
R036-LM2	0.709623	0.723972	8.71839071	0.70947771	9.20316E-06

R036-LM3	0.70971	0.725161	8.7446695	0.709564578	8.68128E-06
R038	0.709635	0.724022	8.71928155	0.709489758	9.67873E-06
R042-LM4	0.709715	0.724738	8.73446963	0.709569647	8.92932E-06
R042-RM3	0.709763	0.72463	8.73070524	0.709617342	8.06137E-06
R045	0.70971	0.724907	8.73857066	0.709564441	6.72632E-06
R048	0.709145	0.723757	8.72490172	0.708999831	1.65005E-05
R050	0.710167	0.72539	8.73907534	0.710022147	7.28542E-06
R065	0.714066	0.72952	8.74253205	0.71391956	7.55021E-06
SRM987	0.710418	0.725817	8.74305391	0.710273022	6.829E-06
SRM987	0.710399	0.726194	8.75259474	0.710253712	6.39428E-06
SRM987	0.710409	0.726286	8.75452494	0.710263381	5.80627E-06
SRM987	0.710408	0.726321	8.7555083	0.710262166	5.47212E-06
SRM987	0.710413	0.726659	8.76340445	0.710267573	6.19039E-06
SRM987	0.710397	0.726545	8.76104605	0.710251903	7.57469E-06
SRM987	0.71041	0.725752	8.74196942	0.710264706	6.25753E-06
SRM987	0.710411	0.725808	8.74304848	0.71026732	5.98262E-06
SRM987	0.710405	0.725327	8.73167795	0.71025971	4.98231E-06
SRM987	0.710453	0.725352	8.73006573	0.710270207	7.62579E-06
SRM987	0.710399	0.725611	8.73861677	0.710253934	5.97931E-06

SRM-987	0.710421	0.72505	8.72472056	0.710275484	5.9349E-06
SRM-987	0.710404	0.725375	8.73276403	0.710258111	6.92482E-06
SRM-987	0.710405	0.725987	8.74747407	0.710259882	8.37873E-06
SRM-987	0.710399	0.726055	8.74927623	0.710253671	7.54295E-06
SRM-987	0.710395	0.726045	8.74916759	0.710249908	6.9344E-06
SRM-987	0.710408	0.726028	8.74844894	0.710262249	7.18807E-06