Oxygen and carbon stable isotope ratios of kangaroo tooth enamel

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OXYGEN AND CARBON STABLE ISOTOPE RATIOS OF KANGAROO TOOTH ENAMEL

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ABSTRACT

Stable isotopes are distributed in plants and organisms of an environment in different proportions and are often used to differentiate between environmental settings. Oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) isotope ratios are known as recorders of climate and vegetation changes. $\delta^{18}O$ ratios vary with precipitation, evaporation and transpiration processes while $\delta^{13}C$ ratios differ between plant groups. Therefore, by analysing $\delta^{18}O$ and $\delta^{13}C$ ratios in herbivore tooth enamel, inferences about animals' diet and ecology, as well as reconstructions of past climates can be made. $\delta^{18}O$ ratios of herbivores often reflect precipitation $\delta^{18}O$ values however, previous studies of kangaroos and other arid-adapted species show this is not always true. Kangaroos are mainly non-obligate drinkers and ingest the majority of water from food, primarily grasses. A strong relationship between $\delta^{18}O$ ratios of kangaroo tooth enamel and relative humidity is known, with leaf water suggested as a contributor. To test this theory, two isoscapes, one with precipitation $\delta^{18}O$ ratios and one modelling leaf water values, were constructed and compared to a meta-analysis of published kangaroo $\delta^{18}O$ values. Significant correlations were found between $\delta^{18}O$ ratios of tooth enamel and modelled leaf water (R^2=0.40) compared to precipitation values (R^2=0.020). Kangaroo $\delta^{18}O$ ratios also showed significant correlation with relative humidity (R^2=0.46) and aridity (R^2=0.37). No significant interspecies differences in slopes were found when compared to the group regression, which indicate that kangaroos all reflect similar proportions of modelled leaf water. Tooth enamel $\delta^{13}C$ ratios reflect relative distributions of C3 and C4 grasses, however, species differences also relate to diet preferences and ecological traits. These analyses show that $\delta^{18}O$ and $\delta^{13}C$ ratios of kangaroo tooth enamel reflect modelled leaf water values and vegetation, and can be used to make inferences about past climates' relative humidity to more accurately reconstruct past environments.

KEYWORDS

Kangaroos, isotope ratios, tooth enamel, precipitation, leaf water, isoscapes, oxygen, carbon, relative humidity.

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1. INTRODUCTION

Understanding past changes in climate and how biota has responded to them is crucial for predicting possible consequences of contemporary climatic changes. Through analysing proxies for climatic change embedded within organisms, such as stable isotopic signatures in animal tissue, these changes can be effectively tracked (Koch, 1998). Stable isotopes reflect the environments organisms inhabit and can be used to infer ecosystem structures, ecology of organisms and local climate conditions. To adequately interpret geological archives, it is essential to have an excellent understanding of what factors determine patterns of isotopic variation in modern biological systems.

Plants and animals take up different proportions of stable isotopes, and the isotopic compositions are used to differentiate between a range of environmental settings (Dawson & Siegwolf, 2007). Oxygen's stable isotope ratios are controlled by several climatic factors including precipitation, surface water evaporation and evapotranspiration from leaves (Gat, 1996). Carbon isotope ratios vary among plants depending on the photosynthetic pathway (C3, C4 and CAM) and can differentiate between types of vegetation (Michener & Lajtha, 2007).

Variations in carbon and oxygen isotope ratios in plants and water sources are also reflected in tissues of organisms that ingest them. Herbivores are useful recorders of variations in carbon and oxygen isotope ratios as water and food, once ingested, are incorporated into organic tissues such as tooth enamel (Sponheimer & Lee-Thorp, 2001; Lang, 2006). Oxygen isotope ratios in herbivore tooth enamel can be a mix of two main sources; leaf water and surface water (Bryant & Froelich, 1995). The relative proportions of these sources in any given organism vary due to climatic processes, which control precipitation and evaporation, and biology differences due to adaptations among species (e.g. Hoppe, 2006). Therefore, the values range among different organisms. As carbon isotope ratios vary among plant species, herbivore tooth enamel reflects the diet and habitat of that mammal (Hopley et al., 2006).

Kangaroos are an abundant and widespread Australian herbivore and have adapted to survive under a range of climate conditions including deserts, grasslands and woodlands. A kangaroo's diet largely depends on habitat (Brookman & Ambrose, 2012) and body mass, as smaller species tend to be browsers, consuming more shrubs and foliage, compared to larger species, which are mainly grazers, ingesting grasses (Arman & Ayliffe, 2015). The oxygen isotope ratios of kangaroo tooth enamel have been found to not correlate well with oxygen isotope ratios of local precipitation, possibly due to the lower water requirements of arid-adapted kangaroos and the potential influence of leaf water on their isotopic signatures (Murphy, 2006). Instead, oxygen isotope ratios in kangaroo tooth enamel have shown a strong correlation with relative humidity (Ayliffe & Chivas, 1990; Fraser, 2005), which suggests contribution from leaf water. However, it is still unclear to what extent the oxygen isotope ratios in kangaroos reflect precipitation or leaf water oxygen isotope ratios. Kangaroos form a crucial part of Australia's ecological history and are essential to understanding climate changes in Australia. By analysing stable isotopes in kangaroo tooth enamel both modern and past climate conditions can be reconstructed.

Isoscapes are used to visualise spatial variations in isotope ratios on maps. Using interpolation techniques to predict values between data points, isoscapes plotting precipitation oxygen isotope ratios have been generated for Australia (Bowen, 2010; Hollins et al., 2018). The precipitation isoscape generated by Hollins et al. (2018) uses a more detailed method than Bowen and Revenaugh (2003), including a higher number of sampling stations measuring oxygen isotope ratios across Australia and incorporating more complex climatic parameters. The website, IsoMAP, can be used to plot precipitation and leaf water isoscapes, using oxygen isotope ratios of precipitation, temperature and vapour pressure (IsoMAP, n.d.).

Analysing oxygen and carbon isotope ratios in modern kangaroo tooth enamel, and understanding how they reflect the environment, can underpin a potential proxy to reconstruct an ecological history of fossilised kangaroo remains in Australia. This project generates a precipitation and leaf water isoscape for Australia and compares these maps with a metanalysis of published kangaroo tooth enamel data to understand which source is most strongly reflected in the oxygen isotope ratios. The kangaroo oxygen isotope ratios are also compared with climate variables such as aridity, relative humidity, mean annual temperature (MAT) and mean annual precipitation (MAP) to understand whether there is a stronger influence from the environment or differences between kangaroo species. Using this approach, the following hypotheses are examined:

- Kangaroo tooth enamel more strongly reflect leaf water values than precipitation oxygen isotope ratios
- Species exhibit differences in oxygen isotope ratios, which reflects diet and water sources related to their preferred habitat
- Kangaroo carbon isotope ratios reflect the relative distribution of C3 and C4 grasses and variations in species’ diet

2. BACKGROUND

2.1. Review of isotopes

Elements are identified by the number of protons in the nucleus, known as the atomic number. However, the number of neutrons determines the atomic mass, which varies between an element's isotopes. Stable isotopes do not undergo radioactive decay, preserving their original isotopic signature overtime and are thus crucial in paleoenvironmental reconstructions. Environmental conditions can be inferred by understanding how the proportion of heavy to light stable isotopes are partitioned among substances. This partitioning is known as fractionation (Dawson & Siegwolf, 2007). In order to analyse the changes in isotope mixtures, ratios of rare to abundant isotopes are measured relative to globally accepted standards, and then amplified by 1000 as the difference is very small. This process is written in the following delta notation in per mille (‰) units for isotope X (eq. 1):

$$\delta X = ((Rsam/\ Rstd) - 1) * 1000 \quad \text{[eq. 1]}$$

where R is defined here as the ratio between the abundance of heavy to light isotopes for sample (Rsam) and standard (Rstd). Isotope standards are used as global references, to which other samples are compared. For carbon, the $\delta^{13}C$ value is usually measured relative to VPDB (from the Pee-Dee Belemnite formation), while for oxygen, the $\delta^{18}O$ value can be compared to either VPDB or SMOW (Standard Mean Ocean Water) (Werner & Brand, 2001). Isotope ratios are analysed using Isotope Ratio Mass

Spectrometry (IRMS), which separates molecules containing different isotopes based on the mass-to-charge ratio (Michener & Lajtha, 2007).

2.2. Environmental variations in isotopes

2.2.1. OXYGEN ISOTOPE RATIOS IN PRECIPITATION

Oxygen isotopes are fractionated during the water cycle, with lighter ^{16}O isotopes being preferentially evaporated and heavier ^{18}O isotopes preferentially condensed (Craig & Gordon, 1965). The general process involves water vapour being evaporated from the ocean surface and stored as vapour masses (clouds). These structures are moved by the wind, with more water vapour being taken up, until it is eventually precipitated over land. $\delta^{18}O$ ratios of precipitation therefore depend on several environmental factors including; latitude, altitude and the amount effect (Gat, 1996). As clouds continuously precipitate over a landmass, $\delta^{18}O$ ratios become fractionally lighter, as majority of the heavier isotopes have already condensed as precipitation, known as the continental effect (Sharp, 2007). $\delta^{18}O$ ratios generally decrease with increasing latitudes; therefore, areas closer to the equator have relatively high ratios (Liu et al. 2010). Altitude is another important factor, as there is a clear relationship between increasing elevation and decreasing $\delta^{18}O$ ratios. Similarly, temperature decreases with increasing elevation, which reduces the amount of vapour storage available in clouds, causing more rainout as vapour condenses (Sharp, 2007). The amount effect relates to the quantity of precipitation that occurs within a period of time (Kendall & McDonnell, 2003). For example, in areas such as the tropics where there are high precipitation amounts causing significant rainout periods, the $\delta^{18}O$ ratios are relatively light, as a higher proportion of vapour is condensed with limited opportunity to fractionate (Sharp, 2007).

2.2.2. OXYGEN ISOTOPE RATIOS IN LEAF WATER

$\delta^{18}O$ ratios in leaf water vary due to the climatic conditions of the environment plant species inhabit, particularly the combined effects of temperature variation and vapour pressure deficit (Cernusak et al., 2016). Both these climatic conditions control isotopic enrichment and affect transpiration (evaporation from leaves), which in turn affects the $\delta^{18}O$ ratio of leaf water (Horita et al., 2008). The Craig-Gordon model uses changes in relative humidity to model $\delta^{18}O$ ratios of leaf water at steady state, with equal amounts of water entering and leaving the plant system (Craig & Gordon, 1965). IsoMAP incorporates the Craig-Gordon model to calculate $\delta^{18}O$ values of leaf water for each grid cell (eq. 2),

$$\delta^{18}\mathrm{O}(\text{leaf water}) = \alpha[\alpha_k R_S(e_i - e_s/e_i) + \alpha_{kb} R_S(e_s - e_a/e_i) + R_A(e_a/e_i)]$$

(West et al., 2008) [eq. 2]

where α is the equilibrium fractionation, α_k is the kinetic fractionation, R_S is the $^{18}O/^{16}O$ ratio of source water, e_i is the internal leaf vapour pressure, e_s is the leaf surface vapour pressure, α_{kb} is the fractionation factor for diffusion through a boundary layer, e_a is the atmospheric vapour pressure, and R_A is the $^{18}O/^{16}O$ ratio of atmospheric water vapour. The Péclet effect is also an important factor, largely controlled by transpiration rates and temperature variations, and is used to prevent overestimation of $\delta^{18}O$ values in leaves

(West et al., 2008; Cernusak et al., 2016). In general, leaf water is more enriched in heavier oxygen isotopes relative to surface water, due to the preferential loss of lighter oxygen isotopes during evaporation (Roden & Ehleringer, 1999).

2.2.3. ISOSCAPES

The term isoscape was coined by West and Bowen (2010) and refers to the spatial mapping of isotopes over a landscape of some measurable size. $\delta^{18}O$ ratios are commonly used in isoscapes as they can be easily measured from precipitation and are known to show patterns associated with ecology, climate change, biogeochemistry and hydrology (West et al., 2010). These precipitation values can be tailored to suit the investigation being undertaken, from annual variations in short-term climate changes (e.g. seasonality), to weighted-average values measuring longer-term global climate changes (Bowen, 2010; Liu et al., 2013; Bowen et al., 2014). A precipitation isoscape for Australia has been developed by Hollins et al. (2018), which differs from the Bowen and Revenaugh (2003) model, by grouping data by climate regions to account for Australia's complex precipitation sources. Additional data from inland sampling stations was also included, resulting in more detailed predictions. Isoscapes can also be generated to reflect $\delta^{18}O$ ratios of modelled leaf water by using calculations from the Craig-Gordon model (West et al., 2008).

The website IsoMAP (Bowen et al., 2020), allows users to generate their own isoscapes based on precipitation or leaf water $\delta^{18}O$ ratios. Precipitation isoscapes can be created based on annual weighted-average $\delta^{18}O$ ratios sourced from Global Network of Isotopes on Precipitations (GNIP) sampling stations. Users can also incorporate various independent climate variables used to predict the interpolated $\delta^{18}O$ ratios according to the investigation undertaken (IAEA, n.d.). Leaf water isoscapes incorporate a precipitation isoscape as well as temperature and vapour pressure grids from the World Meteorological Organisation (New et al., 2002). Using interpolation methods, data points between sampling stations are predicted and mapped in a grid where variations in isotope ratios can be visualised (Bowen et al., 2020).

2.2.4. CARBON ISOTOPE RATIOS IN PLANTS

Plants preferentially take up higher proportions of lighter ^{12}C and are relatively depleted in heavier ^{13}C isotopes. However, the isotopic ratios depend on which photosynthetic pathway is used; C3, C4 or CAM (Michener & Lajtha, 2007). C3 plants use the ancestral method of carbon fixation, with the enzyme rubisco securing CO_2. However, under higher temperatures and lower atmospheric CO_2 conditions, rubisco cannot always differentiate between CO_2 and O_2, which leads to photorespiration and water loss in the plant (Edwards et al., 2010). C4 plants also use rubisco to fix CO_2, however, this is stored in a separate location called the bundle sheath cell. Here, the PEP carboxylase enzyme takes up CO_2 and concentrates it with rubisco where O_2 is not present, preventing photorespiration, although at a higher energy cost (Fry, 2006). CAM plants are similar to C4 plants as both have bundle sheath cells, however, they only open the stomata at night to further reduce water loss (Dawson & Siegwolf, 2007).

These differences between C3, C4 and CAM affect the isotope ratios and their optimal habitats (Edwards et al., 2010). C3 plants thrive in cool, closed environments, with $\delta^{13}C$ ratios between -20 to -34‰ (Unkovich et al., 2001). C4 plants prefer warm, open habitats, with higher $\delta^{13}C$ ratios than C3 plants, ranging from -9 to -17‰ (Unkovich et al., 2001). CAM plants are rarer, more complex and have $\delta^{13}C$ values that vary between both C3 and C4 ranges depending on species and environmental conditions (Michener & Lajtha, 2007). CAM plants do not form a major proportion of plants in this project's study site and as such, they will not be included in this project.

Australia has a general gradient of C4 plants in the north to C3 plants in the south (Murphy & Bowman, 2007). C4 plants are mainly grasses, shrubs and forbes, while C3 plants include all trees, most shrubs and cool season grasses. The abundance of C4 and C3 plants reflect variations in Australia's climate and seasonal water availability (Hattersley, 1963). Water stress is also an important influencing factor, as the $\delta^{13}C$ ratios in C3 plants become more enriched in heavier ^{13}C when there is less water available, which skews the data towards C4 characteristics (Tieszen, 1991).

2.2.5. STABLE ISOTOPES IN HERBIVORES

Stable isotope ratios of $\delta^{18}O$ and $\delta^{13}C$ in herbivores reflect body water and diet, and can be used to infer climate conditions of environments. $\delta^{18}O$ ratios in a herbivores' body water fluctuates due to water source differences. There are three main oxygen inputs that make up an herbivore's body water; atmospheric oxygen, metabolic oxygen from food, and free water, such as surface water and leaf water (Bryant & Froelich, 1995). Free water is thought to be the largest source of oxygen in herbivore body water (Kohn, 1996). An herbivore's body oxygen correlates with body mass as larger animals have higher energy requirements and faster water turnover rates (Langlois et al., 2003). Therefore, smaller animals are less dependent on free water for drinking than larger animals. A relationship between the $\delta^{18}O$ ratios of arid-adapted mammals and relative humidity has already been noted (Ayliffe & Chivas, 1990; Murphy & Bowman, 2007). As these mammals are less dependent on surface water, their $\delta^{18}O$ ratios are thought to be influenced more by leaf water values (Clementz et al., 2008; Lang et al., 2013). $\delta^{13}C$ ratios reflect the abundance of C3 and C4 plants in an herbivore's diet, which can be used to infer the environment type the animal inhabits (Dawson & Siegwolf, 2007).

The $\delta^{18}O$ and $\delta^{13}C$ ratios of herbivore tissues, especially tooth enamel, have been used to show relationships between various environmental controls such as average temperatures (Murphy & Bowman, 2007), vegetation (Brookman & Ambrose, 2013), relative humidity (Ayliffe & Chivas, 1990) and aridity (Levin et al., 2006). To combat harsher environmental conditions, some herbivores have also evolved different ecological adaptations, including efficient colon and kidney functions that conserve water and regulate body temperatures (Maloiy, 1973; Sponheimer & Lee-Thorp, 1999).

2.2.6. ECOLOGY OF KANGAROOS

Kangaroos are an abundant and widespread Australian marsupial (Figure 1). They can survive in a range of habitats and climate conditions due to the ecological adaptations that species have developed, including thermoregulation and foregut fermentation (Dawson, 2012). A kangaroo processes food in the foregut, which ferments the grass and allows it to pass through the digestive system with little energy expenditure. This adaptation helps kangaroos survive on dry grasses and shrubs that constitute much of arid central Australia. All kangaroos have good thermoregulation control, which is essential in dry climates as it reduces the amount of water lost through sweat and panting (Van Dyck & Strahan, 2008). The diet and habitat of kangaroos is generally determined by their size. Larger species such as *Macropus fuliginosus*, *Macropus giganteus, Macropus robustus*, and *Macropus rufus* are predominantly grazers that receive the majority of water from food, and are able to feed off of chenopods during drought periods (Ealey, 1967; Arman & Prideaux, 2001). Smaller species such as *Macropus agilis, Macropus bernardus, Macropus irma, Macropus rufogriseus, Wallabia bicolor* and *Onychogalea unguifera* are mainly browsers, consuming a higher percentage of shrubs and foliage in addition to grasses (Table 1) (Arman & Prideaux, 2001; Van Dyck & Strahan, 2008). Kangaroo teeth form progressively from the crown to the base of the tooth with the premolars, the first and second molars (M1 and M2) erupting during the weaning period (Brookman & Ambrose, 2012). Final molars, M3 and M4, erupt by 5-6 years of age and are used to infer climate conditions during the last several months of tooth formation (Sharman et al., 1964; Kido et al., 2018; Skippington et al., 2018).

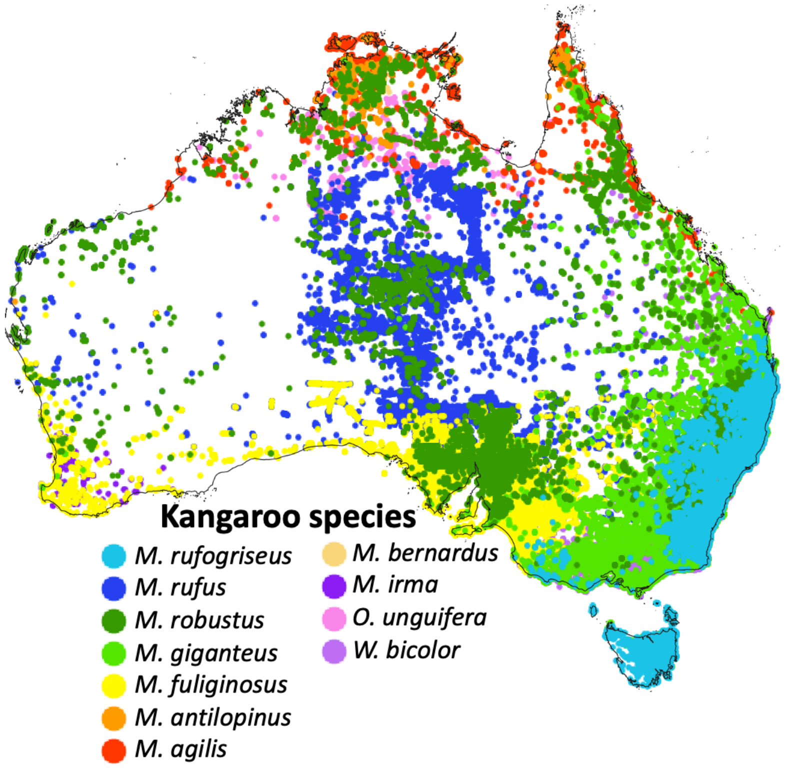

Figure 1: Records of kangaroo species occurrences downloaded by species from the Atlas of Living Australia via the spatial portal (2020a, 2020b, 2020c, 2020d, 2020e, 2020f, 2020g, 2020h, 2020i, 2020j, 2020k). Kangaroos are separated by colour and illustrate the observed habitual range of each species.

Table 1: Kangaroo ecology summary based on species profiles by Van Dyck and Strahan (2008), diet reconstructions by Arman and Prideaux (2015) including the classification of the feeding style (either grazer (G) or mixed feeder (MF)), and the C3 and C4 plant abundances in diet calculated using the 11.7‰ offset from Murphy (2006).

Species	Common name	Location	Habitat	Diet	Weight (kg)	C4 & C3 plants in diet (%)
Macropus agilis	Agile Wallaby	Northern NT & QLD	Open forests, grasslands, sand dunes and hills	100% grasses, G	9 - 27	50% C4 and C3 plants
Macropus antilopinus	Antilopine Wallaroo	Northern NT & QLD	Tropical woodlands, eucalypt woodlands, open forests	80% grasses and 20% browse, G	14 - 51	100% C4 plants
Macropus bernardus	Black Wallaroo	Northern QLD & NT	Sandstone plateau, spinifex grasses, sandstone heath, woodlands, rainforests	Predominantly grasses, G	13 - 21	50% C3 and C4 plants
Macropus fuliginosus	Western Grey Kangaroo	South & western Australia	woodlands, open forests, coastal heathlands, open grasslands	80% grasses, 20% browse, G	17 - 72	100% C3 plants
Macropus giganteus	Eastern Grey Kangaroo	Eastern coast	Forest fringes, woodlands, sclerophyll forests, shrubland and heathland	90% grasses, 10% browse, G	17 - 85	50% C3 and C4 plants

Species	Common name	Location	Habitat	Diet	Weight (kg)	C4 & C3 plants in diet (%)
Macropus robustus	Common Wallaroo	All of mainland Australia	Steep escarpments, rocky hills, caves	85% grasses, 15% browse, G	6.25 - 60	50% C3 and C4 plants
Macropus rufogriseus	Red-necked Wallaby	TAS to QLD	Eucalypt forests, heath, sedge and communities	20% grasses, 80% browse, MF	11 - 26.8	100% C3 plants
Macropus rufus	Red Kangaroo	NSW & QLD	Open plains, flood-outs of creeks and sheep rangelands	80% grasses, 20% browse, G	17 - 92	50% C3 and C4 plants
Onychogalea unguifera	Northern Nailtail Wallaby	Northern region	Woodlands, shrublands and grasslands	50% browse, 50% grasses, MF	4.5 - 9	100% C3 plants
Wallabia bicolor	Swamp Wallaby	East & southern Australia	Forests, woodlands and heath	Pasture, shrubs and bushes, MF	10.3 - 20.5	80% C3 plants

2.2.7. OXYGEN ISOTOPE RATIOS IN KANGAROOS

Kangaroos are mainly non-obligate drinkers and can receive most of the required water from their food (Jarman & Evans, 2010). The proportion of leaf water to drinking water is thought to vary with ecological and physical factors including body weight, age and habitat (Bryant & Froelich, 1995; Kohn, 1996; Langlois et al., 2003). A strong relationship between relative humidity and the $\delta^{18}O$ ratios of kangaroo bone collagen

(Ayliffe & Chivas, 1990) and tooth enamel (Murphy & Bowman, 2006) has previously been described, which suggests that leaf water is a contributing factor. The aridity of an environment, measured as the ratio of precipitation and potential evapotranspiration, also affects the enrichment of ^{18}O in kangaroo tooth enamel as $\delta^{18}O$ ratios increase with aridity (Levin et al., 2006; Skippington et al., 2018). M1 and M2 are known to be affected by weaning, where milk from mothers influence the $\delta^{18}O$ ratios in early teeth (Murphy et al., 2007).

2.2.8. CARBON ISOTOPE RATIOS IN KANGAROOS

$\delta^{13}C$ ratios among kangaroo species vary due to differences in diet and habitat. Therefore, kangaroos who inhabit southern regions of Australia with more C3 plants have lower $\delta^{13}C$ ratios than species in the north, where C4 plants are more abundant (Murphy et al., 2007). Habitat is also important, as kangaroo species living in arid regions of Australia, where there is less available surface water, will eat a higher proportion of grasses than species living in cooler environments (Fraser, 2005). If the amount of water in a kangaroo's regular diet drops below 70%, they are known to also consume chenopods (Jarman & Evans, 2010). Water stress on C3 plants in arid environments can influence the $\delta^{13}C$ ratios of kangaroo tooth enamel and reflect a higher bias towards C4 plants in a kangaroo's diet (Fraser et al., 2008). The weaning effect additionally impacts the $\delta^{13}C$ ratios similar to $\delta^{18}O$ ratios (Witt & Ayliffe, 2001).

3. METHODS

3.1. Kangaroo tooth enamel database construction

Data on kangaroo tooth enamel was compiled from two PhD theses, Murphy (2006) and Fraser (2005), and two papers, Prideaux et al. (2007) and Prideaux et al. (2009). All four data sources analysed $\delta^{18}O$ and $\delta^{13}C$ ratios from kangaroo tooth enamel by bulk sampling the enamel after it had been cleaned and crushed. Murphy (2006) and Fraser (2005) analysed the isotope ratios using a Kiel carbonate device attached to a Finnigan 251 IRMS, while Prideaux et al. (2007) and Prideaux et al. (2009) used the newer MAT-252 model with a dual inlet. All four papers measured $\delta^{18}O$ and $\delta^{13}C$ ratios of tooth enamel carbonate and sampled primarily M3 and M4 molars. Due to high similarities between these stable isotope measurement methods, the data was combined into a single dataset used for this analysis.

Data for a total of 777 kangaroo roadkill samples from 11 species was compiled in an Excel spreadsheet, each assigned the latitude and longitude coordinates of the sampling location, identified kangaroo species (or 'unknown' if unidentified) and the molar used for carbon and oxygen isotope analysis. If multiple teeth were measured from one kangaroo, the average of those values was used. Samples that measured the isotope ratios from the M1 and M2 were excluded from this study due to the weaning effect (Murphy et al., 2007; Witt & Ayliffe, 2015). Kangaroo $\delta^{18}O$ ratios were converted from the original PDB values to SMOW values when compared with precipitation and leaf water $\delta^{18}O$ ratios to maintain continuity between scales (eq. 3).

$$\delta^{18}\mathrm{O(SMOW)} = 1.03091 * \delta^{18}\mathrm{O(PDB)} + 30.91 \text{ (Sharp 2007) [eq. 3]}$$

3.2. Modelling isotope trends

3.2.1. PRECIPITATION ISOSCAPE IN ISOMAP

IsoMAP was used to generate a precipitation isoscape with a step-by-step process involving adjustable timeframes and several independent variable options (Figure 2) (Bowen et al., 2020). The time period, 1962-2004, was chosen as it contained the highest amount of data for the maximum number of sampling stations in Australia (nine stations) (Figure 4a). The independent variables, which determined the interpolated values between sampling stations, were chosen based on parameters used by Bowen and Revenaugh (2002) of latitude squared, absolute latitude and altitude (eq. 4).

$$\delta^{18}\mathrm{O}(\mathrm{precipitation}) = a * \mathrm{Latitude}^2 + b * \mathrm{absolute\ latitude} + c * \mathrm{altitude} \text{ [eq. 4]}$$

This method produced significant statistical results (*p*-value <0.05) meaning there was sufficient correlation for a geostatistical model to be produced, in addition to the standard statistical model available (Bowen et al., 2014). The geostatistical model is preferred as it incorporates the spatial locations of sampling stations into the regression analysis, influencing the predicted values and is thought to produce more accurate results (Bowen, 2010).

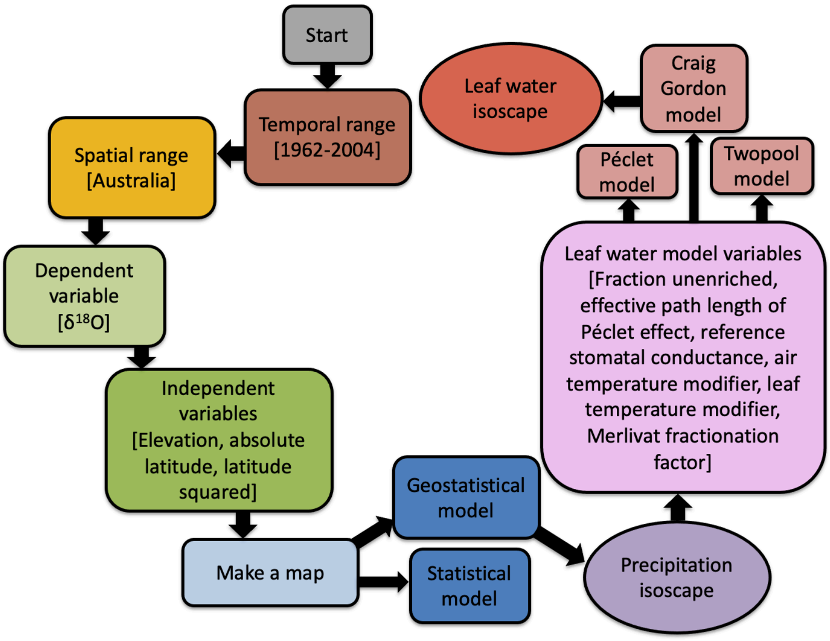

Figure 2: A flow chart showing the step-by-step process to generate a precipitation and leaf water isoscape for Australia using IsoMAP. Parameters selected are shown in parentheses. Rectangles indicate the steps taken and ovals represent the isoscape created.

3.2.1.1. Comparing IsoMAP and Hollins precipitation isoscapes

Hollins et al. (2018) calculated a regression equation for precipitation $\delta^{18}O$ values (eq. 5) similar to IsoMAP, based on the Bowen and Revenaugh (2002) paper, with the addition of distance from the coast.

$$\delta^{18}\mathrm{O} = a * (\mathrm{Latitude}^2) + b * (\mathrm{Absolute\ latitude}) + c * (\mathrm{Altitude}) + d * (\mathrm{Distance\ from\ the\ coast})\ (\mathrm{Hollins\ et\ al.,\ 2018})\ [\mathrm{eq.\ 5}]$$

The IsoMAP and Hollins et al. (2018) precipitation isoscapes were compared using the ‘sample raster value’ function in the 3.10 version of the GIS modelling program, QGIS. Data points from the Hollins et al. (2018) isoscape were directly compared with the spatially closest value in the underlying IsoMAP raster layer.

3.2.2. ISOMAP LEAF WATER ISOSCAPE

The leaf water model in IsoMAP calculated the leaf-water ^{18}O enrichment and added it to the precipitation isoscape using the parameters described in Figure 2. It also incorporated Craig-Gordon's steady state model (eq. 2) and climate grids of monthly air temperatures and vapour pressure (West et al., 2008).

3.2.3. HOLLINS ET AL. (2018) LEAF WATER ISOSCAPE

The leaf water enrichment factor modelled in IsoMAP was calculated for every grid cell by subtracting the precipitation $\delta^{18}O$ ratios from the leaf water $\delta^{18}O$ ratios (eq. 6). This enrichment factor was then added to the Hollins et al. (2018) precipitation isoscape to produce a modelled leaf water isoscape (eq. 7) where precip = precipitation.

$$\delta^{18}\text{OIsoMAP}_{\text{leaf water}} - \delta^{18}\text{OIsoMAP}_{\text{precip}} = \text{Leaf water enrichment factor} \quad \text{[eq. 6]}$$

$$\text{Leaf water enrichment factor} + \delta^{18}\text{OHollins}_{\text{precip}} = \delta^{18}\text{OHollins}_{\text{leaf water}} \quad \text{[eq. 7]}$$

3.3. Analysing isotopic trends

3.3.1. COMPARING MULTIPLE DATASETS IN QGIS

$\delta^{18}O$ and $\delta^{13}C$ ratios of kangaroo tooth enamel were compared with the precipitation and leaf water isoscapes and various climate data using the same 'sample raster values' function in QGIS described previously.

3.3.2. KANGAROO AND CLIMATE DATA

$\delta^{18}O$ and $\delta^{13}C$ ratios of kangaroo tooth enamel were plotted by species on maps in QGIS, which reflected both the variation in isotope ratios and relative habitat ranges. $\delta^{13}C$ and $\delta^{18}O$ ratios of kangaroo species were also compared with climate data including; MAT (supplementary Figure 15), MAP (supplementary Figure 16), relative humidity, and aridity (supplementary Figure 17) to see which climate variables most strongly influenced the kangaroo data (Table 2). The 11.7‰ offset between kangaroo tooth enamel $\delta^{13}C$ ratios and diet calculated by Murphy (2006), was also applied to the $\delta^{13}C$ ratios, then compared to average isotope ratios of C3 and C4 plants to estimate the proportions of grasses in kangaroo diets. The $\delta^{13}C$ ratios of individual species were compared with Australia's eight ecoregions, defined by the World Wildlife Foundation (WWF) as areas of similar ecosystems, climate and vegetation (Department of Agriculture, Water and the Environment, n.d.). The $\delta^{18}O$ values were compared with Australia's aridity index to relate species' diets with different habitats and climates. To determine the significance of these relationships, R^2 values and *p*-values were calculated based on linear regression graphs in the statistical program MATLAB.

Table 2: Climate data used to compare with kangaroo tooth enamel isotope data including, the data sources, data type and time-period they cover.

Climate data	Source	Time-period	Data type
Aridity	Grid downloadable from CSIRO website, (https://data.csiro.au/collections/collection/CIcsiro:32116v2/DItrue)	1976-2005	Aridity index
Relative humidity	Grid downloadable from the Bureau of Meteorology, (http://www.bom.gov.au/jsp/ncc/climate_averages/relative-humidity/index.jsp)	1978-2005	%
MAT	Grid downloadable from the Bureau of Meteorology, (http://www.bom.gov.au/jsp/ncc/climate_averages/rainfall/index.jsp)	1961-1990	°C
MAP	Grid downloadable from the Bureau of Meteorology, (http://www.bom.gov.au/jsp/ncc/climate_averages/temperature/index.jsp)	1981-2010	mm
Ecoregions	Grid downloadable from Welcome to Ecoregions 2017, (https://ecoregions2017.appspot.com/)	n.d.	Environmental setting

4. RESULTS

4.1. Modelling isotope trends

4.1.1. ISOMAP PRECIPITATION ISOSCAPE

A precipitation isoscape for Australia generated in IsoMAP was based on $\delta^{18}O$ values of precipitation from nine sampling stations (Figure 3a). The *p*-value calculated for the statistical model was <0.05 (Table 2) allowing a geostatistical model to be generated (Figure 3c). This isoscape was based on the regression equation (eq. 8) which determined the standard deviation, t-value and *p*-value for each parameter (Table 3).

$$\delta^{18}\mathrm{O} = -3.70939 - 0.00170963 * (\text{elevation}) - 0.00479098 * (\text{latitude}^2) + 0.137013 * (\text{absolute latitude}) \quad \text{[eq. 8]}$$

Table 3: Geostatistical data generated from the precipitation isoscape in IsoMAP (Dangerfield, 2020)

Variable	Estimate	Standard deviation	t-value	*p*-value
Intercept	-3.70939	0.76141	-4.87174	1.31829e-06
Elevation	-0.00170963	7.25784e-05	-23.5557	0
Latitude^2	-0.00479098	0.000620885	-7.71637	3.33067e-14
Latitude	0.137013	0.0449432	3.04859	0.0023701

Overall, the IsoMAP precipitation isoscape shows a relatively small change in $\delta^{18}O$ ratios across Australia, with approximately 85% of values between -3 and -4‰, an average of -3.81‰ and standard deviation of 0.6‰. The most significant variation occurs in the south-eastern mountainous regions of New South Wales, Victoria and Tasmania where there is a shift towards more negative isotope ratios of up to -6‰. The isoscape metadata and map is accessible for viewing and downloading on the IsoMAP website, published under the job key: 79875 (IAEA, n.d.; Dangerfield, 2020a).

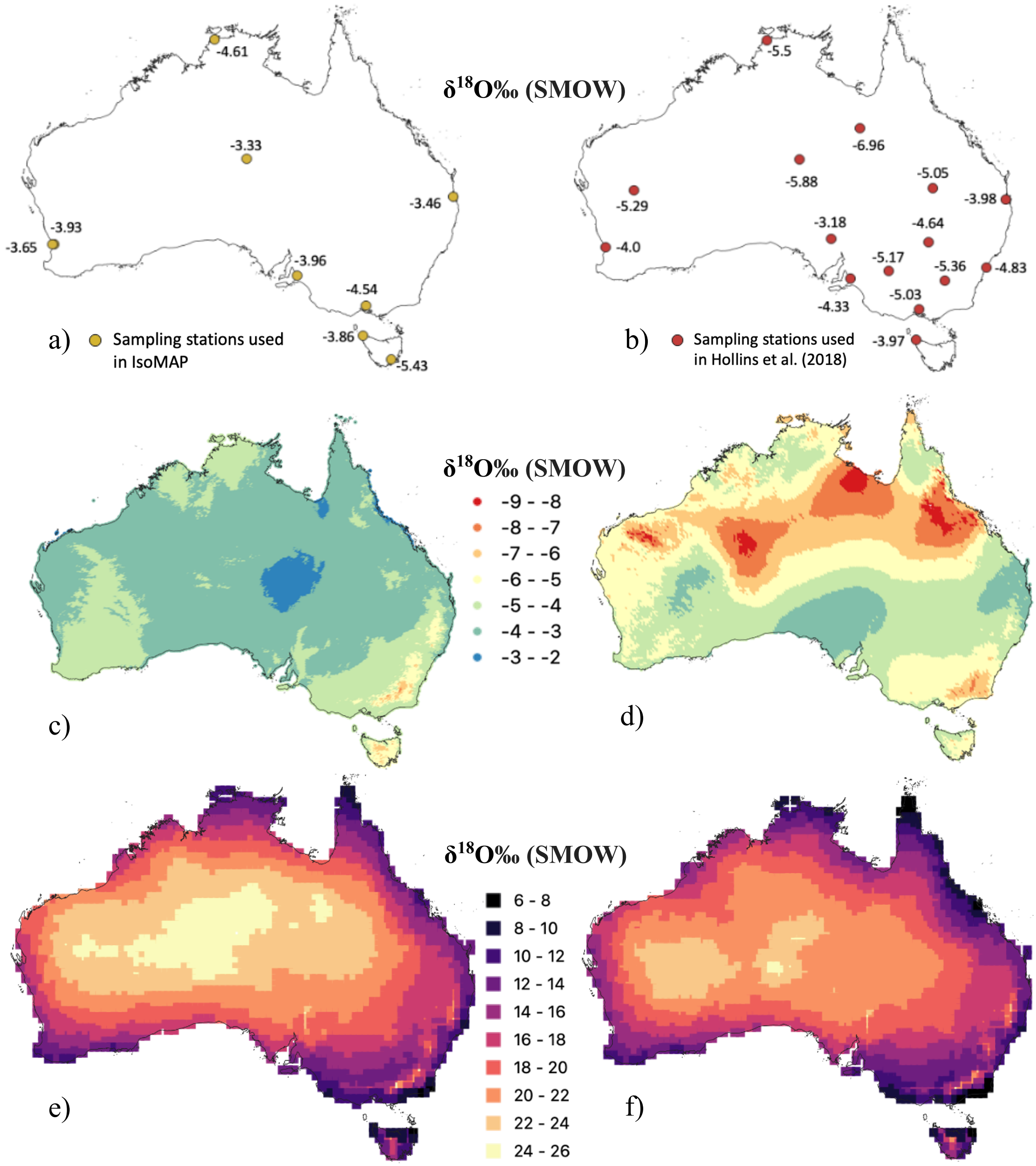

Figure 3: Sampling stations that measure the $\delta^{18}O$ ratios of precipitation in Australia used in (a) IsoMAP and (b) Hollins et al. (2018) precipitation isoscape construction. (c) The precipitation isoscape generated in IsoMAP (d) the precipitation isoscape from Hollins et al. (2018) (e) the leaf water isoscape generated in IsoMAP and (f) the leaf water isoscape using precipitation data from Hollins et al. (2018) model.

4.1.2. LEAF WATER ISOSCAPES

The IsoMAP leaf water isoscape reflects a steady increase in $\delta^{18}O$ values towards the centre of Australia, ranging from 4‰ in coastal regions to 28‰ in central Australia (Figure 3e). Higher values in the south-eastern mountains break this otherwise concentric pattern. The isoscape metadata and map is available on IsoMAP, published under the job key: 79884 (IAEA, n.d.; Dangerfield, 2020b). Hollins modelled leaf water isoscape reflects very similar patterns to IsoMAPs' despite differences between the precipitation isoscape datasets (Figure 3f).

4.1.3. COMPARISON OF ISOMAP AND HOLLINS PRECIPITATION ISOSCAPES

The Hollins et al. (2018) precipitation isoscape (Figure 3d), based on precipitation $\delta^{18}O$ values from sampling stations (Figure 3b), with an average of -5.31‰ and standard deviation of 0.94‰, which is significantly less than the IsoMAP isoscape. It shows three distinct circular areas in mid-northern Australia, which have more negative $\delta^{18}O$ ratios than other regions. The difference map comparing the IsoMAP and Hollins et al. (2018) precipitation values (Figure 4) shows that the highest similarities are in the mid-southern (including the mountainous region of New South Wales and Victoria) and upper northern regions. The greatest dissimilarities between these isoscapes are in the mid-northern sections of Australia, reaching differences of 5‰ between the datasets. Although IsoMAP and Hollins et al. (2018) precipitation isoscapes appear to have notable differences (Figure 4a) the leaf water values were very similar (Figure 3e and 3f), reflecting an almost constant offset between the two leaf water isoscapes (Figure 4b).

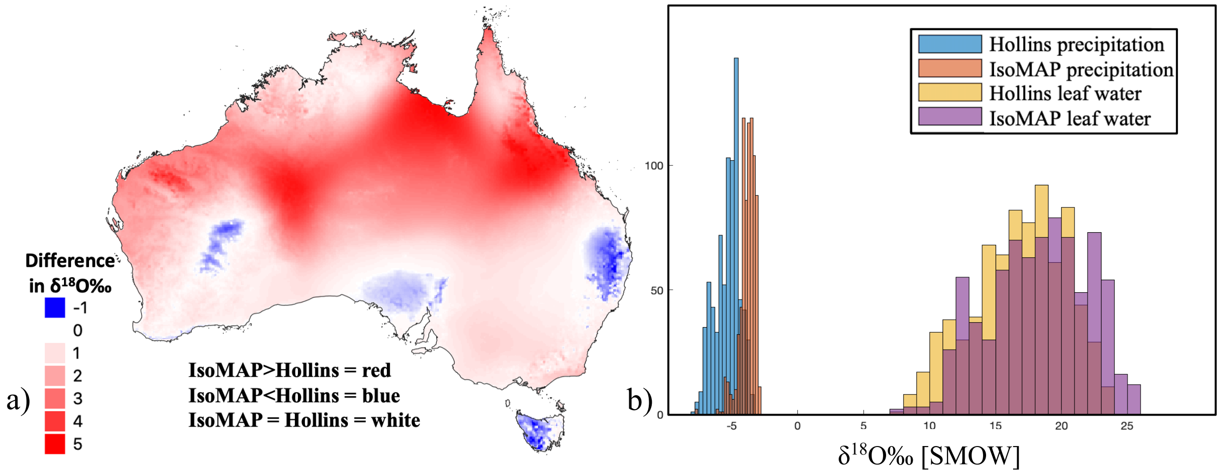

Figure 4: (a) Difference map comparing the $\delta^{18}O$ ratios of IsoMAP and Hollins precipitation isoscapes in QGIS and (b) histogram comparing the IsoMAP and Hollins precipitation and leaf water isoscape $\delta^{18}O$ values generated in MATLAB.

4.1.4. KANGAROO DATA

Kangaroos inhabit different areas of Australia (Figure 5) and the $\delta^{18}O$ and $\delta^{13}C$ ratios of tooth enamel shows differences between species, which correlate with aridity and latitudinal changes in vegetation (Figure 6 and supplemental Figure 14). Kangaroo species inhabiting central arid regions of Australia, including *M. rufus* and *M. robustus*, have higher $\delta^{18}O$ ratios compared to coastal species in more humid areas and the mountainous regions of south-east New South Wales, Victoria and Tasmania (Figure 7 and supplemental Figure 14b). The $\delta^{13}C$ values reflect a latitudinal change in C3 and C4 grass distributions (Figure 6b, 6d & 8, supplemental Figure 14a). Kangaroo species with more negative $\delta^{13}C$ ratios, reflect a C3 diet compared to species with less negative $\delta^{13}C$ ratios, indicating a C4 diet (Table 1, Figure 6b & 6d).

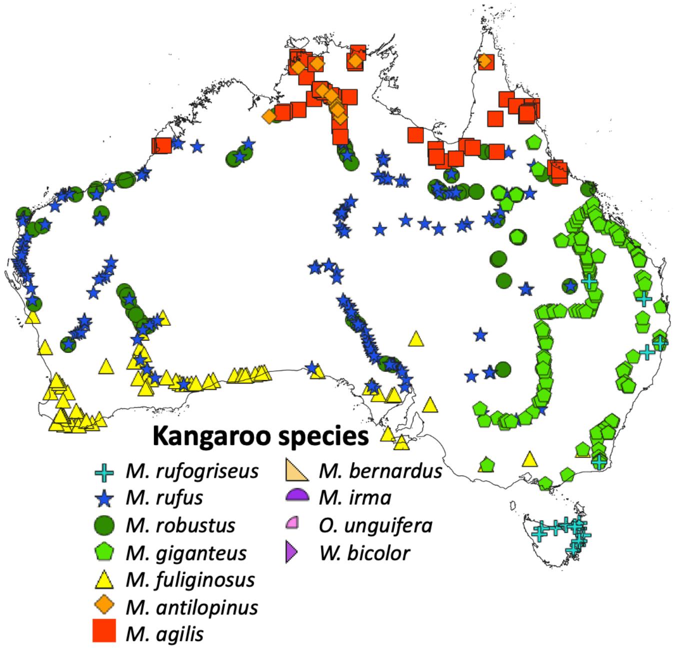

Figure 5: Sampling locations of kangaroo tooth enamel, coloured by species, and compiled from Murphy (2006), Fraser (2005), Prideaux et al. (2007) and Prideaux et al. (2009).

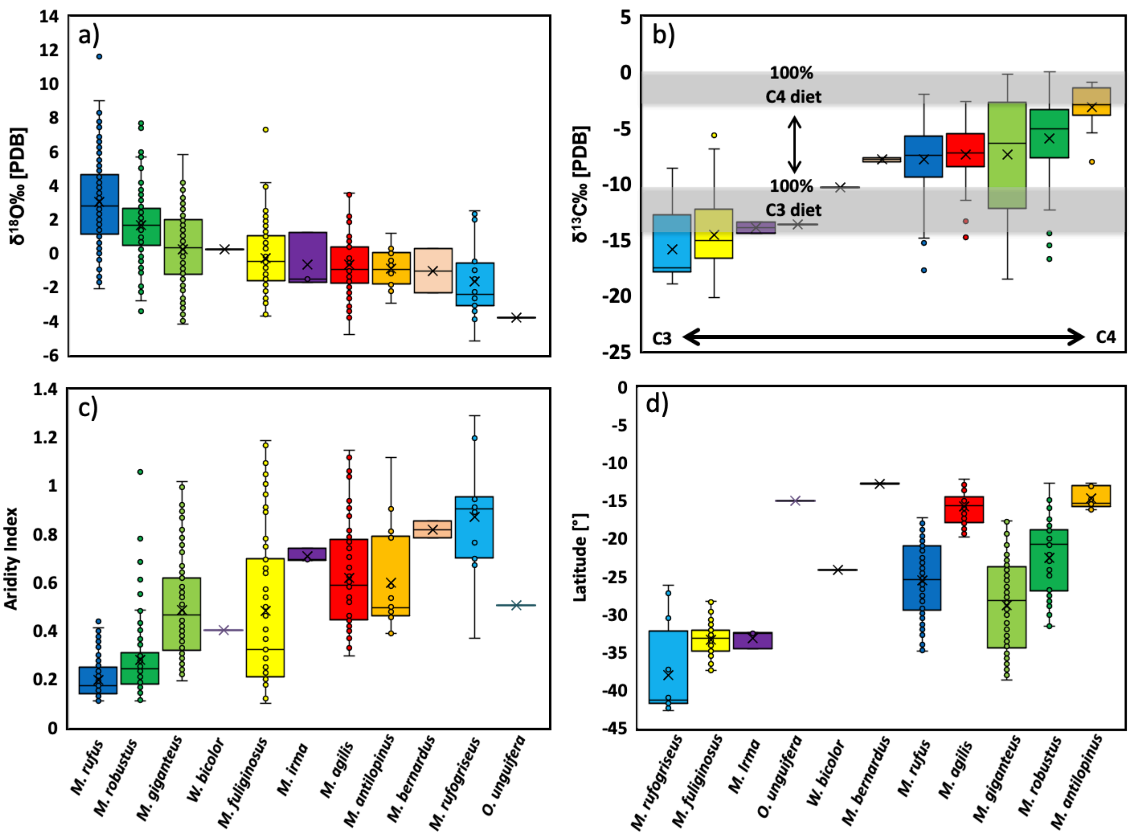

Figure 6: (a) $\delta^{18}O$ and (b) $\delta^{13}C$ ratios from kangaroo tooth enamel plotted by average isotope ratios for each species, (c) aridity index and (d) latitude ranges for each species. The grey zones in (b) are based on the average and standard deviation ranges of $\delta^{13}C$ ratios for C3 and C4 plants using the 11.7‰ offset between kangaroo tooth enamel and diet calculated by Murphy (2006).

4.1.5. KANGAROO DATA BY SPECIES

The $\delta^{18}O$ and $\delta^{13}C$ ratios were also plotted by individual species to investigate the relationships between diet and habitat (Figure 7 & 8). Species with a small number of sampling locations were included on a single map.

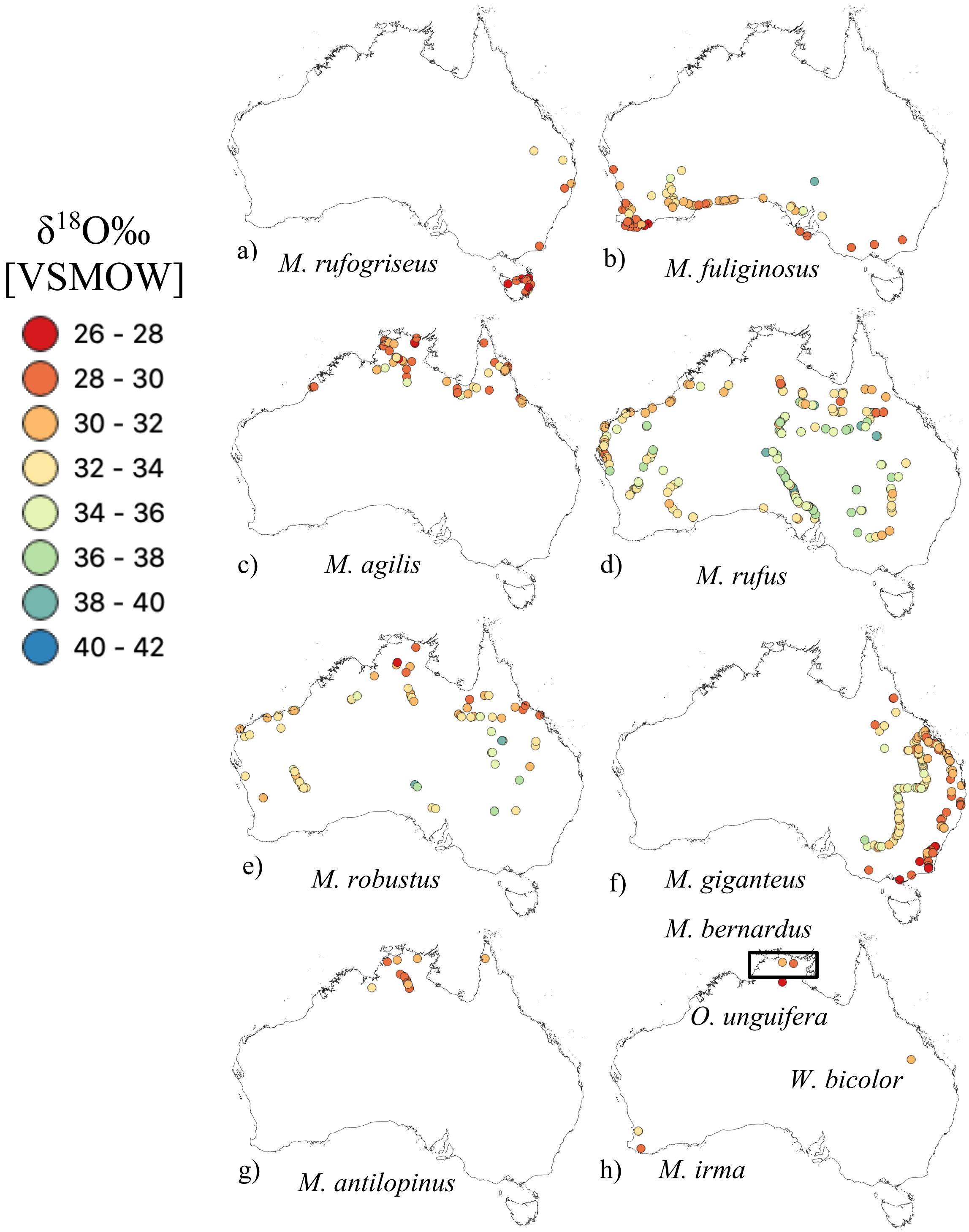

Figure 7: Kangaroo $\delta^{18}O$ ratios by individual species for (a) *M. rufogriseus* (b) *M. fuliginosus* (c) *M. agilis* (d) *M. rufus* (e) *M. robustus* (f) *M. giganteus* (g) *M. antilopinus* and (h) *W. bicolor, O. unguifera, M. irma* and *M. bernardus*.

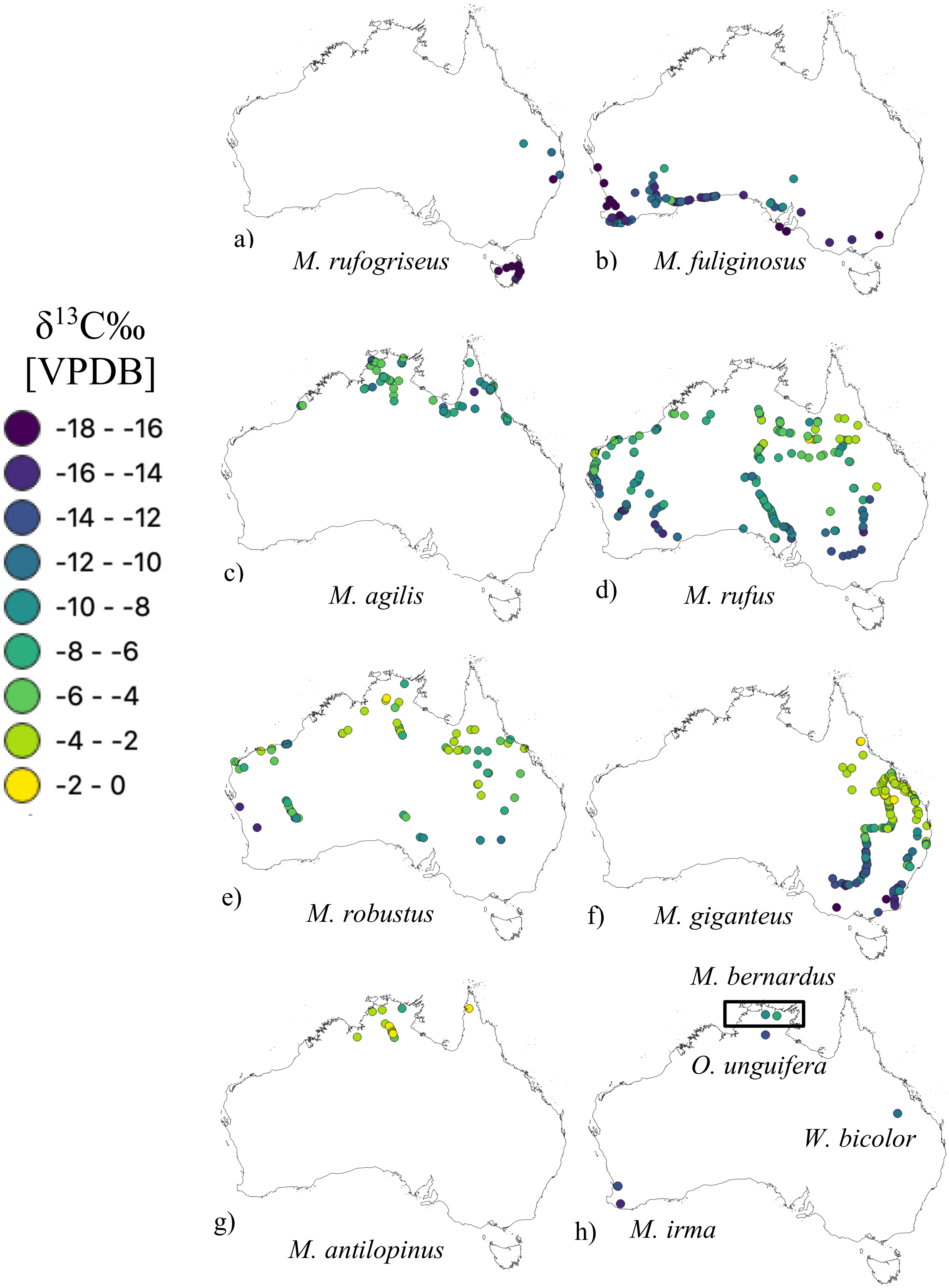

Figure 8: Kangaroo δ^{13}C ratios by individual species for (a) *M. rufogriseus* (b) *M. fuliginosus* (c) *M. agilis* (d) *M. rufus* (e) *M. robustus* (f) *M. giganteus* (g) *M. antilopinus* and (h) *W. bicolor, O. unguifera, M. irma* and *M. bernardus*.

M. agilis, *M. antilopinus*, *M. bernardus, W. bicolor* and *O. unguifera* appeared to inhabit the tropical regions of northern Australia (Figure 9). *M. rufus*, *M. robustus* and *M. giganteus* cover several regions from tropics and temperate grasslands, to forests and deserts. *M. rufogriseus* seemed restricted to temperate forests in cooler regions of Australia, while *M. fuliginosus* dwells in mediterranean forests, woodlands and scrubs along the southern coast. C4 vegetation is more abundant in tropical areas of Australia, while C3 plants dominate temperate regions (Figure 9).

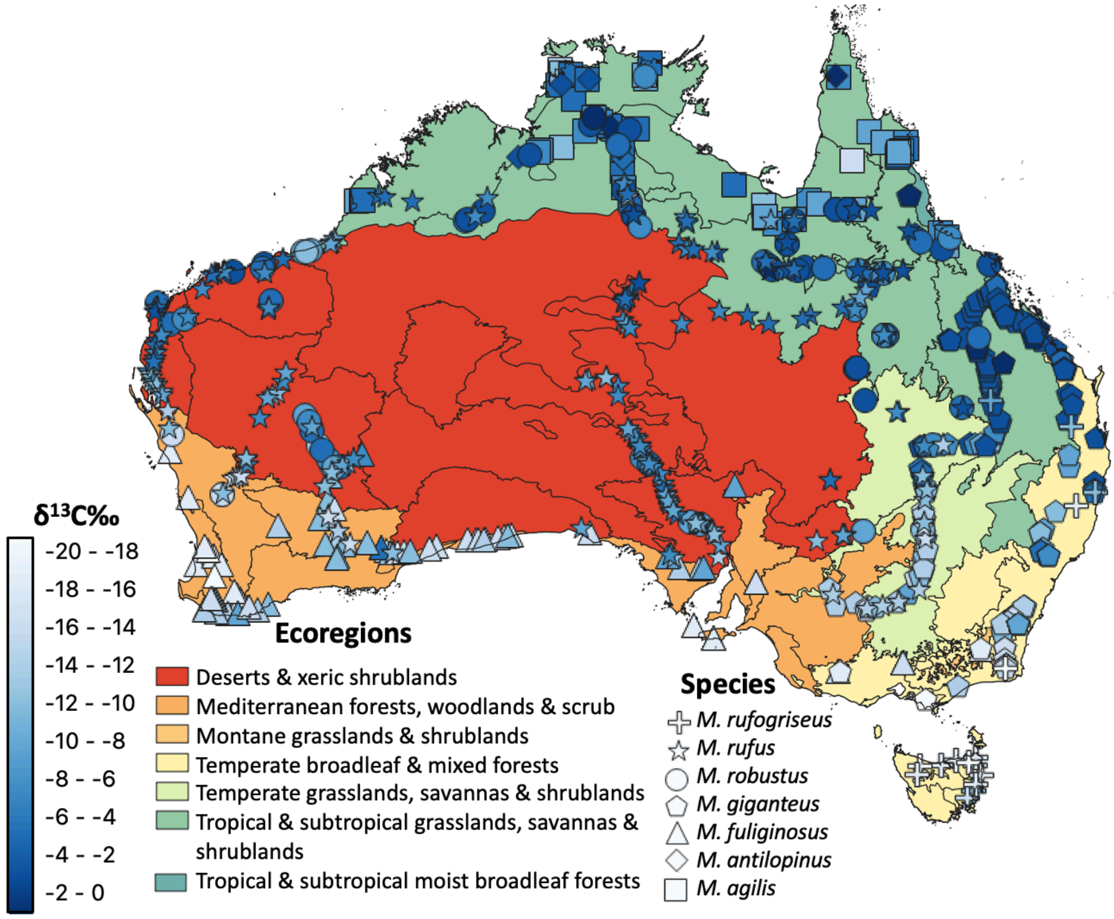

Figure 9: $\delta^{13}C$ ratios of major kangaroo species compared to the ecoregions of Australia. Australia's ecoregions grid data was sourced from Resolve (n.d.), based on models produced by the WWF.

The kangaroo $\delta^{18}O$ ratios were compared to Australia's aridity index (Figure 10). The majority of species inhabited humid regions with only two species, *M. rufus* and *M. robustus*, abundant in the arid-defined regions of Australia. *M. fuliginosus, M. giganteus* and *M. agilis* all ventured out into the semi-arid areas and their tooth enamel reflects an enrichment gradient of towards heavier oxygen isotopes with increased distance from the coast.

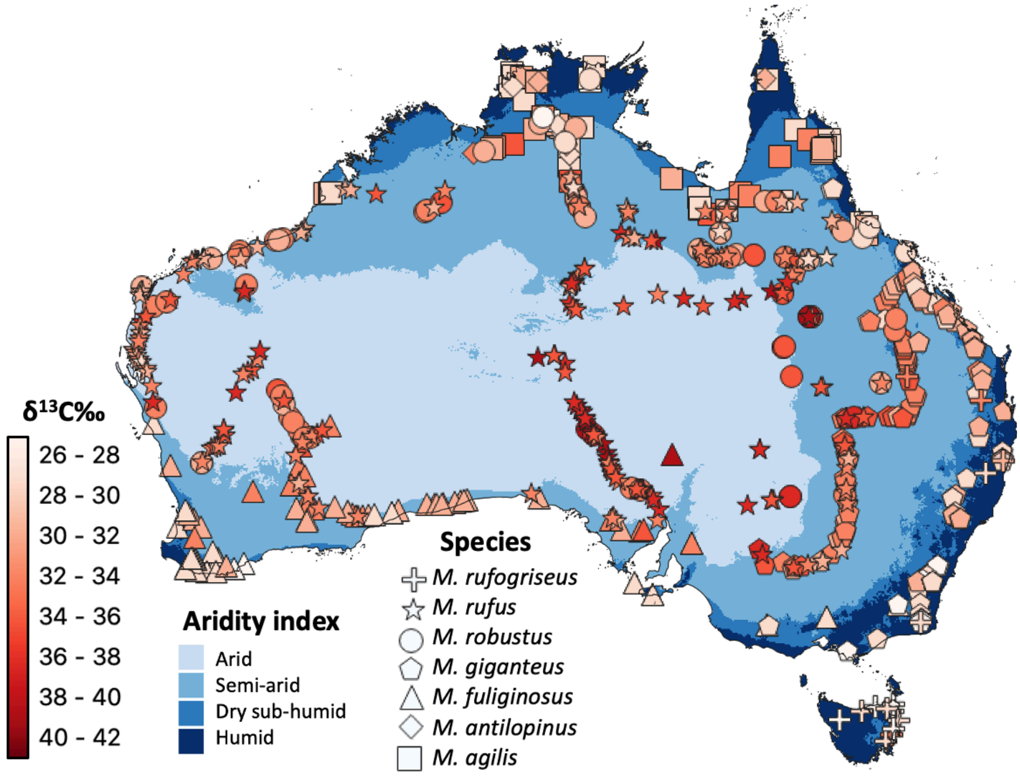

Figure 10: $\delta^{18}O$ ratios of major kangaroo species compared to Australia's aridity index (Harwood et al., 2019).

4.2. Statistically analysing isotope trends

4.2.1. HOLLINS AND ISOMAP VERSUS KANGAROO OXYGEN ISOTOPE RATIOS

There is a weak relationship between kangaroo $\delta^{18}O$ ratios and precipitation isoscapes, with an R^2-value of 0.20 for IsoMAP and 0.020 for Hollins et al. (2018) isoscape (Table

4). Both leaf water isoscapes show the same significant correlation with the kangaroo $\delta^{18}O$ ratios, with *p*-values <0.05 and R^2-values of 0.40 (Figure 11).

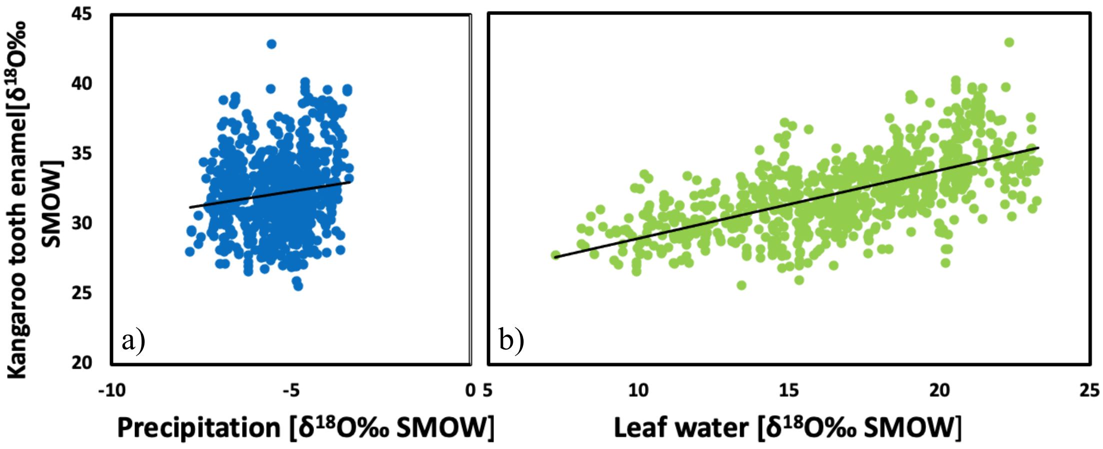

Figure 11: Linear regression models comparing the $\delta^{18}O$ ratios of kangaroo tooth enamel with (a) Hollins precipitation and (b) Hollins leaf water isoscapes.

4.2.2. Kangaroo values versus climate data

Kangaroo and leaf water $\delta^{18}O$ ratios decrease with increasing relative humidity, aridity and MAP, and increase with MAT (Figure 12, Table 4). All plots show strong similar relationships between $\delta^{18}O$ ratios of kangaroo tooth enamel and leaf water $\delta^{18}O$ ratios for all climate variables compared to precipitation $\delta^{18}O$ ratios (Figure 12, Table 4). Out of the four climate variables, relative humidity correlates the strongest with the kangaroo $\delta^{18}O$ ratios (R^2 = 0.46), then MAP (R^2 = 0.38), aridity (R^2 = 0.37), and MAT (R^2 = 0.037) (Table 4). $\delta^{18}O$ ratios of individual kangaroo species were also compared to the aridity index of Australia and species that inhabited the same aridity zone had similar slopes and R^2-values, such as *M. giganteus*, *M. robustus* and *M. rufogriseus* ($slope_{avg}$ = -7.6), as well as *M. fuliginous* ($slope_{avg}$ = -1.6). *M. rufus* was the only species with a significantly different slope to the other species (slope = -17).

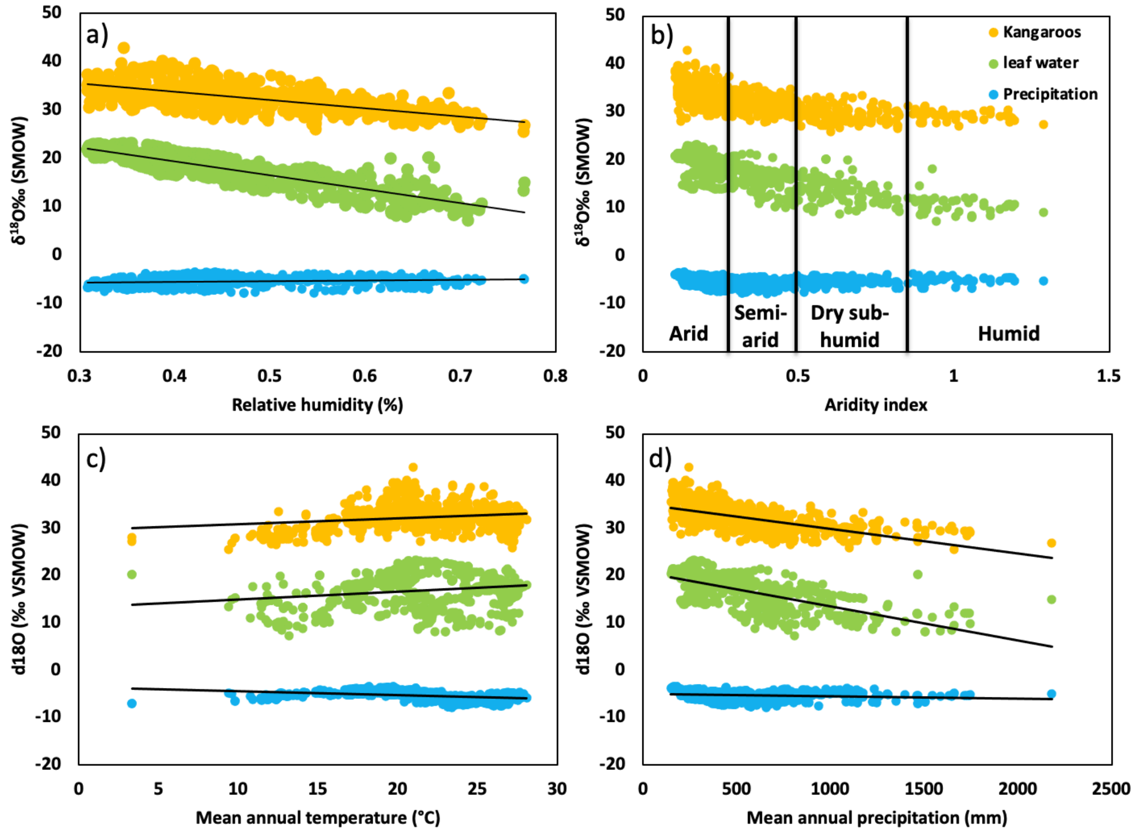

Figure 12: Scatter plots comparing kangaroo tooth enamel δ^{18}O ratios, Hollins leaf water and precipitation δ^{18}O ratios with (a) relative humidity (b) aridity index separated into climate regions (c) MAT and (d) MAP. Four outliers were excluded from (b) to compact the x-axis range.

Table 4: Correlation coefficients between the δ^{18}O ratios of kangaroo tooth enamel and climate data.

Variable compared with kangaroo δ^{18}O ratios tooth enamel	Slope coefficient	R^2	*p*-value
Relative humidity (%)	-17.27	0.46	5.83E-107
Aridity index	-5.60	0.37	1.32E-80
MAP (mm)	-0.0052	0.38	1.70E-81
MAT (°C)	0.12	0.037	6.33E-08
Hollins precipitation (δ^{18}O‰)	0.049	0.020	8.7403e-05
Hollins leaf water (δ^{18}O‰)	0.82	0.40	1.8778e-88
IsoMAP precipitation (δ^{18}O‰)	2.0251	0.20	3.131e-39
IsoMAP leaf water (δ^{18}O‰)	0.4568	0.40	4.0794e-88

4.2.3. KANGAROO VALUES VERSUS CLIMATE DATA BY SPECIES

As relative humidity correlated the strongest with kangaroo δ^{18}O ratios, it was used to investigate interspecies differences. Only two species, *M. giganteus* and *M. rufogriseus* had slopes lower than the group slope of -17.3, while the remaining all had higher slopes (Table 5, Figure 13). The similarities between species' slopes and the group slope is supported by the 95% confidence interval, as all species slope ranges overlap with the group slope's confidence interval. *M. agilis* and *M. antilopinus* did not have significant slopes (*p*-value >0.05), most likely due to small sampling sizes and so are not further analysed in this project.

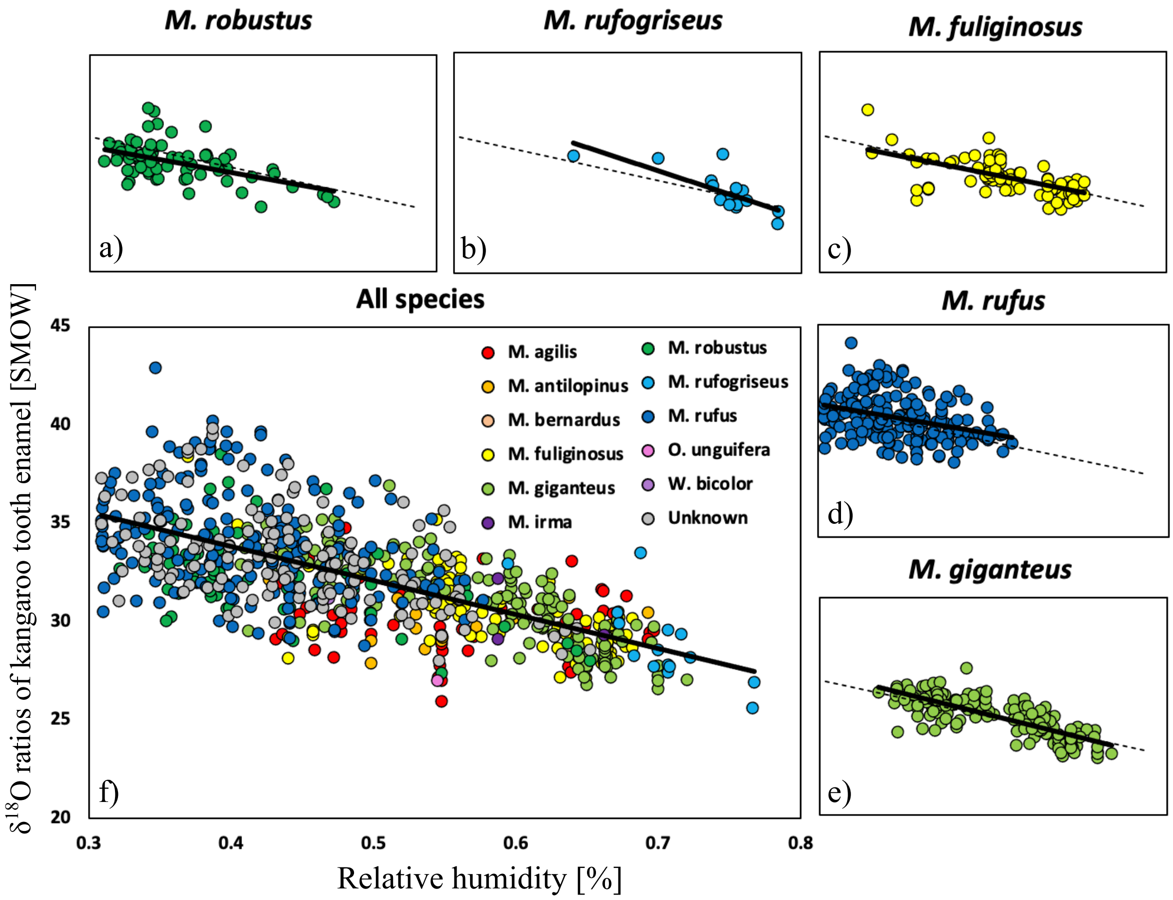

Figure 13: Modelled leaf water δ^{18}O ratios compared to relative humidity with (a) *M. robustus* (b) *M. rufogriseus* (c) *M. fuliginosus* (d) *M. rufus* (e) *M. rufus* and (f) the group data with the trend as a solid line. Individual species regression lines for subplots a-e are solid strokes, with the group trendline as dashed lines.

Table 5: Correlation coefficients between the δ^{18}O ratios of kangaroo tooth enamel for each species with relative humidity.

Kangaroo species	Slope coefficient	R^2-value	*p*-value	Sample size
All species	-17.27	0.4624	5.83e-107	777
Macropus fuliginosus	-15.68	0.4225	3.83e-12	92
Macropus giganteus	-20.12	0.6241	1.18e-37	169
Macropus robustus	-14.54	0.2601	2.71e-06	77
Macropus rufogriseus	-25.91	0.5929	0.0005	16
Macropus rufus	-14.12	0.1225	2.90e-07	199

5. DISCUSSION

5.1. Precipitation and leaf water isoscapes

5.1.1. CREATION OF THE ISOMAP PRECIPITATION ISOSCAPE

The precipitation isoscape generated in IsoMAP shows a relatively small variation in $\delta^{18}O$ ratios across the extent of Australia, with the largest changes occurring in the south-eastern mountain ranges of New South Wales and northern Tasmania due to the effects of elevation (Figure 3c) (Hollins et al., 2018). Latitudinal effects do not appear to be strongly reflected, with no change in precipitation $\delta^{18}O$ ratios to distinguish between the northern and southern rainfall patterns, similar to Liu et al. (2010).

One explanation for why the precipitation isoscape produced in IsoMAP appears so flat is the positioning of the isotope measuring stations. The stations available in IsoMAP are from the GNIP database, which include a maximum of 8 locations for Australia that have uneven sampling patterns over the 42-year period that was chosen, and all stations are located in coastal cities excluding one in Alice Springs (Figure 3a). As precipitation near the coast usually reflects the first rainout, which is close to ocean water $\delta^{18}O$ ratios, this could explain why the precipitation $\delta^{18}O$ ratios for each sampling station are relatively the same across Australia for the IsoMAP isoscape (Liu et al., 2010). The source of the precipitation is also an important factor to consider, as rainfall in the southern regions, such as Tasmania, is sourced more from the Southern Ocean, while northern regions receive input from oceans near the equator, which differ mainly in seasonal temperature variations (Barras & Simmonds, 2008).

5.1.2. COMPARISON OF THE HOLLINS AND ISOMAP PRECIPITATION ISOSCAPE

When the IsoMAP and Hollins et al. (2018) precipitation isoscapes were compared, the areas with the strongest similarities were the southern regions of Australia, particularly the south-eastern mountain ranges of NSW and the eastern coast of Tasmania (Figure 5). These similarities are related to the shared elevation parameter between the two models. The major differences were mainly in the northern regions and thought to be related to the increased number of inland data points and the additional climate considerations in the Hollins et al. (2018) model (Figure 4b). Hollins precipitation isoscape (Figure 4d) had more negative ratios in the northern regions from heavy tropical rainouts and less negative ratios in the south from stronger seasonal temperature effects, however, these latitudinal trends are mainly absent in the IsoMAP isoscape. This could be because the IsoMAP precipitation isoscape includes climate parameters without allowing for any additional regional considerations to be incorporated (Figure 2). On the other hand, Hollins et al. (2018) divided Australia up into five regions (north, south, east, west and inland) and calculated the relationship between MAT and MAP before incorporating the elevation, latitude and the distance from the coast parameters for each value. Overall, due to the extensive data collection and incorporation of additional climatic considerations in the precipitation isoscape, the Hollins et al. (2018) model was chosen to compare with the kangaroo tooth enamel $\delta^{18}O$ ratios.

5.1.3. HOLLINS AND ISOMAP LEAF WATER ISOSCAPE COMPARATIVE ANALYSIS

The leaf water isoscape generated in IsoMAP incorporates temperature and vapour pressure climate grids and shows a general enrichment in heavy oxygen isotopes towards the centre of Australia (Figure 4e). Relative humidity is a major driver of leaf water δ^{18}O ratios by controlling the amount of enrichment in heavy oxygen isotopes in leaves (Craig & Gordon, 1963; West et al., 2008). For example, in environments with lower relative humidity (such as central Australia) there is a larger vapour pressure difference between the leaf and surrounding atmosphere, which means more enrichment in ^{18}O occurs, producing higher δ^{18}O ratios (Cernusak et al., 2016). The decrease in δ^{18}O ratios in the south-eastern regions of NSW and the northern areas of Tasmania reflect major elevation and temperature changes, causing the modelled leaf water isotope ratios to vary on either side of the mountains with vapour pressure deficit (Barbour & Farquhar, 2000). The leaf water isoscape generated using the Hollins et al. (2018) model also showed similar trends, suggesting that differences in precipitation δ^{18}O ratios do not have a major impact on modelled leaf water δ^{18}O ratios (Figure 4f & 5b).

5.2.1. COMPARISON OF KANGAROO DATA WITH HOLLINS PRECIPITATION AND LEAF WATER ISOSCAPES AND THE RELATIVE HUMIDITY CONNECTION

The δ^{18}O ratios of kangaroo tooth enamel were compared with Hollins precipitation and leaf water isoscapes to identify whether they reflect local source water or ingested leaf water (Figure 11). Leaf water was found to have a much stronger correlation ($R^2 = 0.40$) with the kangaroo δ^{18}O ratios compared to precipitation ($R^2 = 0.020$), which is due to kangaroos having a lower water requirement and ingesting more water from food, namely, grasses (Figure 11, Table 4) (Murphy, 2006). Therefore, instead of reflecting local meteoric waters like other herbivores (e.g. bison in Hoppe, 2006), δ^{18}O ratios from kangaroo tooth enamel more strongly reflect modelled leaf water δ^{18}O ratios.

Relative humidity is a major control on $\delta^{18}O$ ratios of leaf water, which is supported by a linear relationship and an R^2-value of 0.85 (Cernusak et al., 2016). The $\delta^{18}O$ values of kangaroo tooth enamel also reflect a strong relationship with relative humidity ($R^2 = 0.40$) (Figure 12), while precipitation $\delta^{18}O$ ratios only weakly correlate with relative humidity ($R^2 = 0.028$). There are, however, other complex climatic factors and interactions, such as elevation, latitude and distance from the coast, that appear to have more significant effects on the precipitation $\delta^{18}O$ values (Gat, 1996). The strong relationship between the $\delta^{18}O$ values of kangaroos and relative humidity noted previously (Ayliffe & Chivas, 1990; Murphy & Bowman, 2006) was hypothesised to relate to the influence of relative humidity on leaf water $\delta^{18}O$ values. However, this is the first time a leaf water isoscape has been generated for Australia and used to show the relationship between modelled leaf water and kangaroo tooth enamel $\delta^{18}O$ values, and relative humidity. Slopes between the $\delta^{18}O$ ratios of kangaroo tooth enamel and relative humidity for each species appear similar to the group slope (Figure 13). This suggests that all species reflect similar proportions of leaf water $\delta^{18}O$ values.

5.2.2. OXYGEN ISOTOPE RATIOS OF KANGAROO SPECIES AND ARIDITY

Comparative analysis of the relationship between aridity and $\delta^{18}O$ values for all kangaroos and individual species also showed similarities in slopes, which suggests that $\delta^{18}O$ ratios reflect differences in habitats and diets. The $\delta^{18}O$ ratios of kangaroos most strongly reflect leaf water $\delta^{18}O$ values, which in turn are sensitive to relative humidity and aridity. Therefore, the $\delta^{18}O$ ratios of kangaroo tooth enamel also reflect aridity (Faith, 2018; Reid et al., 2019). In general, the aridity index for Australia shows a progression from a central arid zone towards humid climates in the northern and coastal regions (Figure 10).

Slopes between $\delta^{18}O$ ratios of kangaroo tooth enamel and aridity were found to vary the most between species inhabiting different aridity zones. *M. rufus* had the most different slope, which could be because it was the sole species analysed that primarily inhabited the arid zone of Australia (Figure 10). The results of this comparison to the Australian aridity index suggests similar conclusions to relative humidity as kangaroo species reflect differences in aridity depending on habitat. This means that regional grouping of kangaroo $\delta^{18}O$ ratios by aridity could be used to reconstruct past aridity zones, as suggested by Blumenthal et al. (2017) and Brookman and Ambrose (2013).

5.3. Kangaroo carbon isotope ratios reflect C3 and C4 distributions

5.3.1. THE RELATIVE ABUNDANCE OF C3 AND C4 GRASSES BASED ON THE CARBON ISOTOPE RATIOS OF KANGAROO TOOTH ENAMEL

The $\delta^{13}C$ ratios of kangaroo tooth enamel reflect relative distributions of C3 and C4 grasses in Australia. A shift from C4 to C3 plants is seen from the north to the south of Australia and is best reflected in *M. giganteus* as this kangaroo species spans almost the entire eastern coast and covers both C3 and C4 ranges (Figure 6b & 8f). The kangaroo $\delta^{13}C$ ratios correlate well with the C3 and C4 diversity map produced by Hattersley (1963) based on vegetation estimates, and with Murphy (2006) who used $\delta^{13}C$ ratios from kangaroo bone collagen to estimate C4 grass abundances. The relative distribution of C3 and C4 grasses found in this project correlates well with similar findings in other studies but are supported here with a larger dataset, broader spatial range and isotope ratios analysed from only M3 and M4 molars (Forbes et al., 2010; Fraser, 2005; Murphy, 2006).

5.3.2. DIETS OF GRAZERS AND BROWSERS

The $\delta^{13}C$ ratios of kangaroo tooth enamel also reflect variations among species' diet, particularly the proportion of C3 and C4 grasses ingested. Kangaroos are generally considered grazers consuming up to 100% grass diets however, many of the smaller species also browse on shrubs, fruits and roots (Table 3) (Brookman & Ambrose, 2012; Arman & Prideaux, 2015; Skippington et al., 2018). Most of *M. fuliginosus* data was close to average C3 plant $\delta^{13}C$ values, while *M. giganteus* spanned the whole C4 to C3 spectrum, reflecting its broad latitudinal habitat range (Figure 6b). *M. giganteus* individuals in the south have more negative ratios, comparable to the C3 diets of *M. fuliginosus*, while individuals in the north have similar values to *M. robustus* who have more C4 diets. However, the relative abundances of C3 and C4 grasses in kangaroo diet may vary more than the estimates suggests due to influence from water stress (Brookman & Ambrose, 2013). The clear shift from C4 plants in the north to C3 vegetation in the southern regions of Australia shows that bulk $\delta^{13}C$ ratios in kangaroo tooth enamel reflect relative distributions of C3 and C4 grasses in the environment they inhabit.

5.4. Implications for palaeoecology and palaeoclimatology

The $\delta^{18}O$ and $\delta^{13}C$ ratios of kangaroo tooth enamel were compared with various climate data to analyse the relationships between them and understand how the isotope ratios could be used to calibrate palaeo proxies. The $\delta^{18}O$ ratios of kangaroo tooth enamel were found to reflect modelled leaf water $\delta^{18}O$ values, while the $\delta^{13}C$ ratios reflected the relative abundance of C3 and C4 grasses. The offset between the $\delta^{18}O$ ratios of kangaroo tooth enamel and modelled leaf water was found to vary both between species and across Australia, with averages ranging from 14.2‰ for *M. robustus* to 17.5‰ for *M. rufogriseus* (Figure 11b). However, there was a general weak increase in the difference between modelled leaf water and kangaroo $\delta^{18}O$ ratios towards the coastal regions of Australia, again weakly following relative humidity gradients. If the offset between modern kangaroos and leaf water could be properly calculated, then reconstructions of leaf water $\delta^{18}O$ ratios in animal remains could be produced and used to estimate relative humidity levels. This in turn could be used to differentiate between environmental settings and reflect variations in Australia's climate.

The $\delta^{18}O$ ratios of kangaroo tooth enamel also has important connotations for reconstructing past aridity indexes and calculating water deficit (Levin et al., 2006; Faith, 2018). However, this method requires widespread obligate drinkers in addition to non-obligate drinkers, which Australia does not currently have. The $\delta^{13}C$ ratios of kangaroo tooth enamel are more clearly understood with a modern offset already calibrated by Murphy (2006). Differences in diet and kangaroo species ecological traits are reflected in the $\delta^{13}C$ ratios of tooth enamel (Figure 9). This shows that consideration for these differences need to be taken into account when using $\delta^{13}C$ ratios to reconstruct past C3 and C4 grass distributions.

6. CONCLUSIONS

This project generated a modelled leaf water isoscape for Australia based on precipitation $\delta^{18}O$ ratios in IsoMAP, as well as extrapolating another modelled leaf water isoscape based on precipitation $\delta^{18}O$ values from Hollins et al. (2018) model. Comparisons with these isoscapes showed that $\delta^{18}O$ ratios of kangaroo tooth enamel correlated stronger with the modelled $\delta^{18}O$ ratios of leaf water than those of precipitation. Relative humidity, aridity and modelled leaf water values showed similar trends with kangaroo $\delta^{18}O$ ratios, reflecting species diets and differences in habitat. The $\delta^{13}C$ ratios of kangaroo tooth enamel were found to reflect relative C3 and C4 grass distributions as a whole and while also showing variations among species due to differences in diet and habitats. The following conclusions can be drawn from these results.

1. As kangaroos are mainly non-obligate drinkers, the $\delta^{18}O$ ratios of tooth enamel reflect modelled leaf water $\delta^{18}O$ values and not local precipitation $\delta^{18}O$ values.
2. The modelled leaf water values are strong enough that differences in diet preferences and ecological traits among species do not have a measurable effect on the $\delta^{18}O$ ratios of kangaroo tooth enamel.
3. Relative humidity is a major influencing factor on $\delta^{18}O$ ratios of modelled leaf water and thus, also has a strong relationship with kangaroo tooth enamel.
4. The $\delta^{18}O$ ratios of kangaroo tooth enamel show a strong relationship with aridity, which means regional analysis of kangaroo $\delta^{18}O$ ratios could be used to reconstruct past aridity indexes.
5. Analysis of the $\delta^{13}C$ ratios of all kangaroo species' tooth enamel reflect relative distributions of C3 and C4 grasses while variations among species reflect diet and habitat differences.

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APPENDIX A: CLIMATE RASTERS

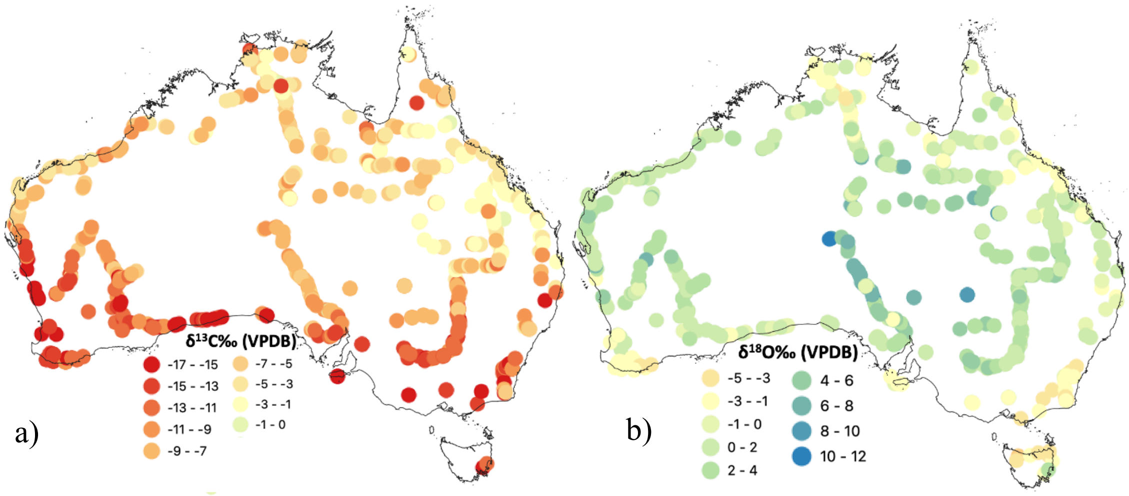

Figure 14: Spatially plotted (a) $\delta^{13}C$ and (b) $\delta^{18}O$ isotope ratios of kangaroo tooth enamel in QGIS compiled from Murphy (2006), Fraser (2005), Prideaux et al. (2007) and Prideaux et al (2009).

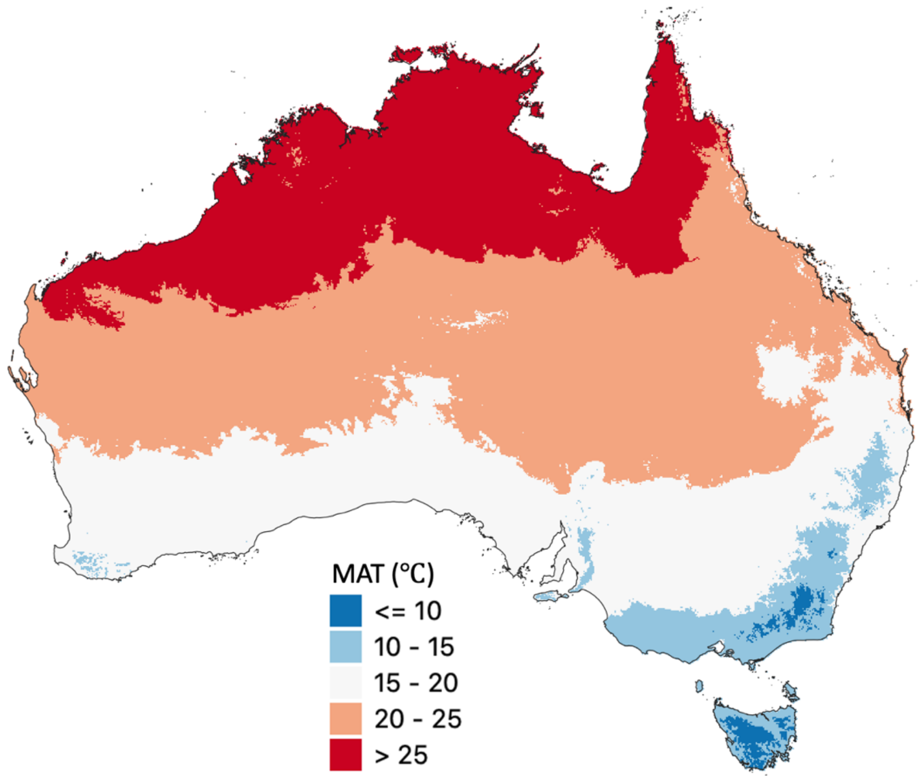

Figure 15: Raster of mean annual temperatures in Australia based on grid data downloaded from the Bureau of Meteorology (2005).

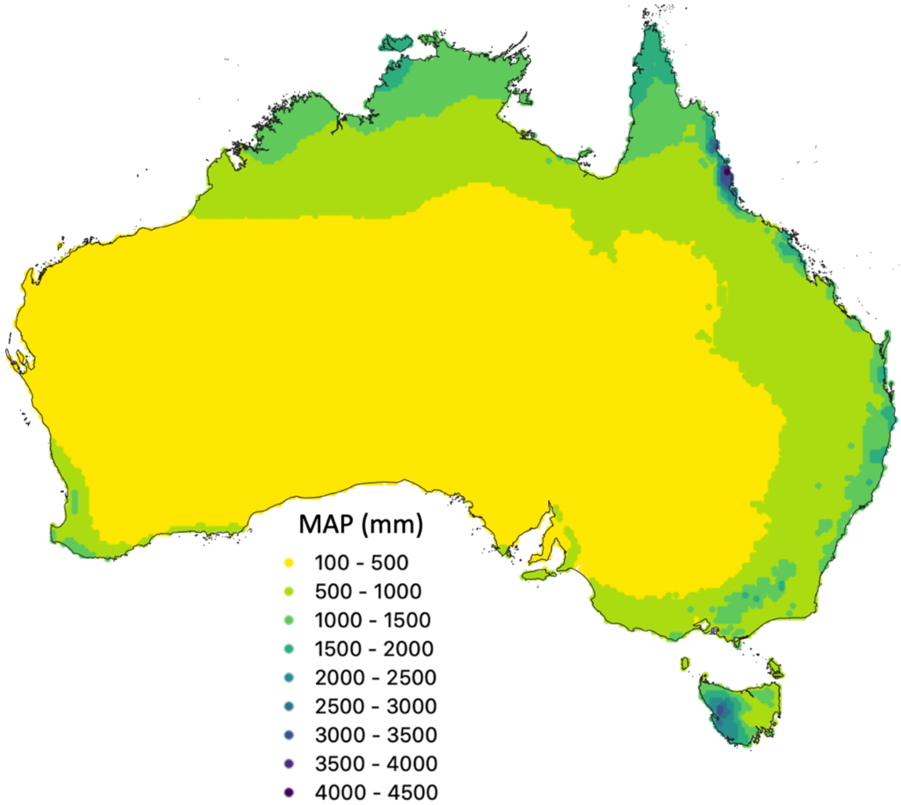

Figure 16: Raster of mean annual precipitation in Australia based on grid data downloaded from the Bureau of Meteorology (2020).

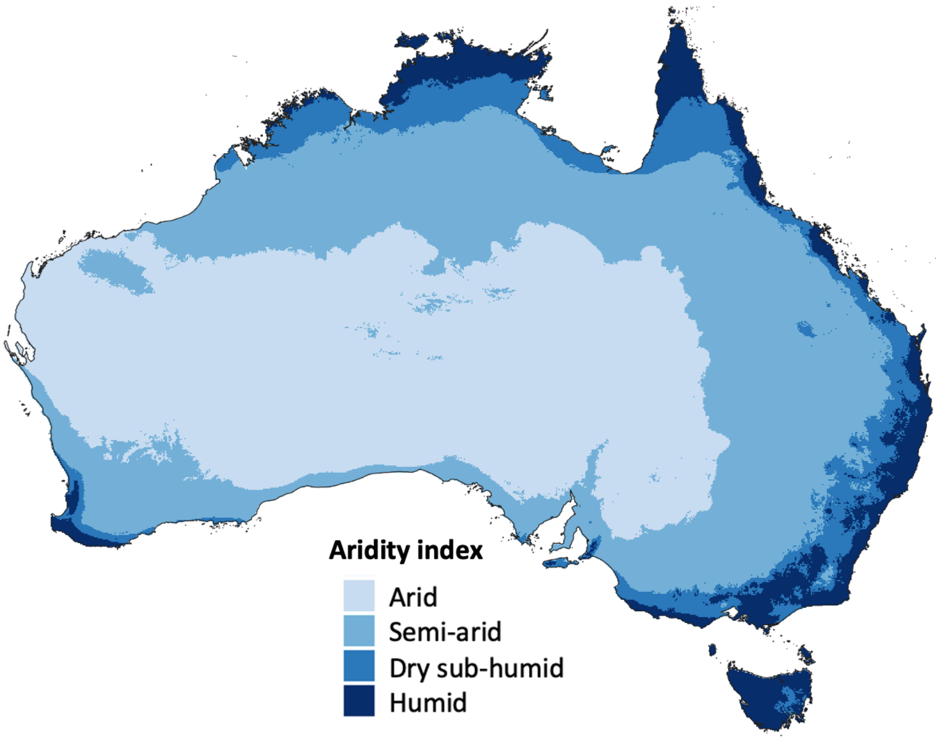

Figure 17: Raster of aridity index in Australia based on grid data downloaded from CSIRO Data portal (Harwood et al., 2019).