Stable Isotope and Dental Microwear Ecology of the Arid Adapted Red Kangaroo (*Macropus rufus*)

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BRIEF. Analysis of the relationship between climate, isotopes, and dental microwear for the red kangaroo.

ABSTRACT. The fossil record provides vital information about the response of taxa to anthropogenic climate change in Australia. Stable isotope analysis of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) can be used to infer local climatic conditions of fossil taxa over time, while dental microwear texture analysis (DMTA) can be used to infer diet. This study examines relationships between isotope and DMTA variables in the red kangaroo (*Macropus rufus*) and climate/geographic variables (mean annual temperature, relative humidity, $\delta^{18}O$ precipitation, latitude). $\delta^{18}O$ enamel values were significantly correlated with both relative humidity and $\delta^{18}O$ precipitation, similar to prior work. The best predictive model for $\delta^{18}O$ enamel values used mean annual temperature, relative humidity, and $\delta^{18}O$ precipitation together. For $\delta^{13}C$, a significant relationship was found between mean annual temperature and latitude, with the best predictive model including both relative humidity and mean annual temperature. *M. rufus* also exhibits a positive relationship between complexity and total annual precipitation; however, no other DMTA variables correlated with geographic or climatic variables. Collectively, these data demonstrate that kangaroos track their local environments today and are therefore model organisms for understanding the past.

INTRODUCTION.

Australia is currently experiencing dramatic anthropogenic climate change, characterized by a continuous rise in mean annual temperature and drought prevalence [1]. Since 1910, maximum annual surface temperature has increased by 0.6°C in Australia [2], and projections for the next century anticipate a further increase in mean annual temperature of 2.2 - 5°C [3]. Simultaneously, annual rainfall has declined by as much as 15% in some regions since the 1970s [4]. These changes will affect the seasonality of Australia's climatic regimes by altering the length of seasons as well as temperature and precipitation extremes [5]. These changes can also modify the physiology, ecology, and distributions of biotic organisms.

Understanding how taxa have historically responded to changing climate conditions is vital to anticipating the ecological ramifications of anthropogenic global warming. Analysis of fossils can reveal the long-term responses of taxa to global warming in the past. Specifically, stable isotope analysis of enamel can be used to infer ancient climates and environments. DMTA can be used to infer diet, but little is known about the potential relationship between DMTA and climate conditions.

Previous studies of stable isotopes have suggested that extant macropods record climate data in their tissues such as enamel and bone [6, 7]. However, variation in behavior, physiology, and geographic range in this genus may affect how specific species track local environments. To most effectively utilize the fossil record as a proxy for environmental conditions, we must first determine specifically how individual taxa are recording their environments using stable isotopes and dental microwear.

Today, the red kangaroo (*Macropus rufus*) is ubiquitous across the arid interior of Australia [8]. Distinct from other species of *Macropus*, *M. rufus* has adapted to this arid environment through the concentration of its urine to protect against dehydration [9], the process of which may impact how this species records climate conditions in its tissues. This study seeks to examine how *M. rufus* records climate data in comparison with the *Macropus* genus as a whole while assessing the potential additional relationship between DMTA values and climate variables.

Oxygen ($\delta^{18}O$) concentration in enamel is reflective of hydrologic conditions of the environment and is an indicator of environmental seasonality [10] and aridity [11]. Enamel $\delta^{18}O$ values reflect climatic conditions such as temperature, evaporation, and precipitation rate when taxa ingest water from free water sources [12]. For species that primarily intake water from plants, $\delta^{18}O$ concentrations reflect relative humidity and aridity [6, 11]. Moreover, the variations within enamel $\delta^{18}O$ composition can be used to infer climatic seasonality, with wet/cooler seasons generally exhibiting lower $\delta^{18}O$ values than drier/warmer seasons [13, 14].

Carbon [$\delta^{13}C$] concentration of enamel reflects the dietary preferences of ancient fauna. In herbivores, dietary changes are reflective of changes in plant availability, which is impacted by climate. Concentrations of $\delta^{13}C$ distinguish between the photosynthetic pathways C_3 (Calvin-Benson) and C_4 (Hatch-Slack) [15, 16]. Pure C_3 consumers would be expected to have a $\delta^{13}C$ value of < -8‰, and pure C_4 consumers would have a $\delta^{13}C$ value of >1‰ [15 - 17]. In Australia, C_3 plants (e.g., dicotyledonean trees and shrubs) are most abundant in cool, wet conditions, while C_4 grass (i.e., monocotyldeonean grass) is widespread in areas where summers are hot and dry [18, 19].

Dental microwear texture analysis (DMTA) can discriminate between grazers and browsers [e.g., 20, 21]. DMTA uses scanning white light confocal microscopy to record 3D surface data and characteristics: complexity (Asfc), anisotropy (epLsar), and textural fill volume (Tfv), which are quantified by scale sensitive fractal analysis (SSFA) [22 - 24]. However, little is understood about the potential relationships between DMTA characteristics and climate indicators.

When examined together, stable isotope and dental microwear texture analyses may be used to clarify the relationship between fossil tissues and climate indicators in *M. rufus*. These analyses may determine the validity of comparing fossil tissue data for extant and extinct species in relation to climate conditions. By identifying how taxa have responded to climate change in the past, it may be inferred how individual species or groups will respond to current anthropogenic climate change. This will improve the efficacy of conservation efforts by allowing researchers to anticipate future disruptions to vital ecosystems and provide more time to create and implement plans to mitigate the damages.

MATERIALS AND METHODS.

Stable Isotopes.

Tooth samples of extant *M. rufus* were collected from museum specimens at the Western Australian Museum (WAM) and the Australian Museum (AM). Lateerupting molars (third and fourth molars) were preferentially selected, because the stable isotope composition is not altered by the ingestion of mother's milk early in development [7]. Tooth enamel was removed using a Dremel Drill and ground into a fine powder. This powder was treated with 30% hydrogen peroxide to remove organics and 0.1 N acetic acid for 18 hours to remove secondary carbonates [25]. Isotope analyses were conducted at the Department of Geological Sciences at the University of Florida, using 1 mg of enamel per sample. Stable carbon and oxygen isotope ratios were measured using a VG Prism stable isotope ratio mass spectrometer with an in-line ISOCARB automatic sampler. Data were normalized by the Pee Dee Formation Belemnite (VPDB) standard conventions [25]:

$$\delta^{13}C\ (‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000;\ \text{where } R = \frac{^{13}C}{^{12}C} \qquad (1)$$

$$\delta^{18}O\ (‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000;\ \text{where } R = \frac{^{18}O}{^{16}O} \qquad (2)$$

Also included are isotope data from Murphy *et al.* (2007) [7] (n=153) and Prideaux *et al.* (2007) [26] (n=46).

Dental Microwear Texture Analysis.

Samples of extant *M. rufus* (n=17) were collected from AM and WAM. To remove contaminants, the shearing facet was cleaned with acetone. The tooth was molded with polyvinylsiloxane dental impression material and casts were prepared using Epotek 301 epoxy resin and hardener. The wear facet of the tooth replicas was scanned in 3D in four adjacent fields of view using a SensofarPlμ white-light scanning confocal microscope (204 x 276 μm^2) [22 - 24]. Scans were analyzed using SSFA software (ToothFrax and SFrax, Surfract Corp.) to quantify: complexity (Asfc), anisotropy (epLsar), and textural fill volume (Tfv) [22- 24]. Complexity reflects changes in surface roughness across changing scales and can be used to determine the brittleness of foods that taxa consume [22, 23]. Anisotropy is a measure of directional uniformity, and in herbivores, high epLsar is indicative of grass consumption [20 - 23]. Textural fill volume is a measure of the difference in volume filled by 2 μm^3 and 10 μm^3 cuboids, indicating features of scale between the cuboid resolutions [23].

Climate Data.

Temperature and precipitation data were acquired for each specimen from nearby climate stations, and data were averaged by month collected. For each specimen, the research station that was closest to the collection locality was selected. Data from each full year at a given station were averaged. Data are reported at the Australian Bureau of Meteorology website (http://www.bom.gov.au/climate/data/). Precipitation $\delta^{18}O$ (VSMOW‰) values were estimated for each specimen based on collection locality data using the online oxygen isotopes in precipitation calculator at www.waterisotopes.org. Relative humidity data were extracted from an ARC GIS 10 layer (http://en.openei.org/datasets/node/616) by the U.S. National Aeronautics and Space Administration (NASA) and Surface meteorology and Solar Energy (SSE). These data are regional averages of relative humidity at 10 m. elevation, calculated using data from July 1, 1983 to June 30, 2005.

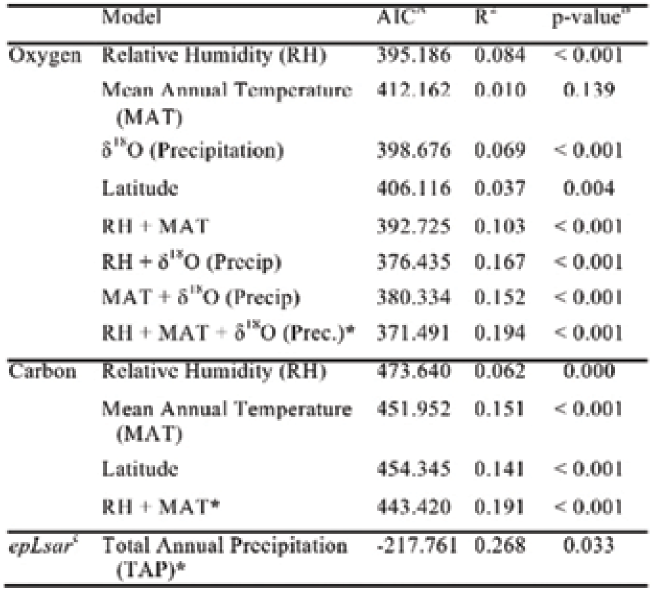
Figure 1. Location of all *M. rufus* isotope specimens sampled ([7], [26] and proprietary data). Samples for dental microwear texture analysis (DMTA) are delineated by black dots, while specimens sampled (including published data) for stable isotope analysis are indicated by gray triangles.

Statistical Analysis.

Spearman correlation analysis was run on all data to assess relationships between climate, isotopic, and DMTA variables for extant *M. rufus*. For all significant relationships ($p<0.05$), linear regression models were run to quantify the relationships using XLSTAT. As done by Murphy *et al.* (2007), Akaike's Information Criterion (AIC) values were utilized to analyze the relative efficacy of models. Any p-values <0.001 are hereafter considered significant.

RESULTS.

Table 1. Results of a linear regression model analysis for oxygen and carbon enamel isotopes and DMTA characteristics of *Macropus rufus*, with the best model for each variable indicated by an asterisk.



	Model	AIC[A]	R^2	p-value[B]
Oxygen	Relative Humidity (RH)	395.186	0.084	< 0.001
	Mean Annual Temperature (MAT)	412.162	0.010	0.139
	$\delta^{18}O$ (Precipitation)	398.676	0.069	< 0.001
	Latitude	406.116	0.037	0.004
	RH + MAT	392.725	0.103	< 0.001
	RH + $\delta^{18}O$ (Precip)	376.435	0.167	< 0.001
	MAT + $\delta^{18}O$ (Precip)	380.334	0.152	< 0.001
	RH + MAT + $\delta^{18}O$ (Prec.)*	371.491	0.194	< 0.001
Carbon	Relative Humidity (RH)	473.640	0.062	0.000
	Mean Annual Temperature (MAT)	451.952	0.151	< 0.001
	Latitude	454.345	0.141	< 0.001
	RH + MAT*	443.420	0.191	< 0.001
epLsar[C]	Total Annual Precipitation (TAP)*	-217.761	0.268	0.033

[A] AIC= Akaike's Information Coefficient; analysis of relative efficacy of models where a lower value indicates a better fit and a simpler model is given preference.

[B] p-value (alpha<0.05).

[C] epLsar= anisotropy, a measure of the directional uniformity of features

$\delta^{18}O$ values were -2.2 to 11.6 (‰ VPDB) with an average of 3.4±2.6. $\delta^{13}C$ values ranged from -1.1 to -17.8 (‰ VPDB) with an average of -8.4±3.0.

Correlations between $\delta^{18}O$ values and climatic conditions are significant for relative humidity (RH) as well as precipitation $\delta^{18}O$ (Table 1). For individual climate criteria, RH is a better predictive model for $\delta^{18}O$ (AIC= 395.2), although the relationship is weak (R^2= 0.084). This study found a greater model efficacy when considering multiple climate variables. Of the complex models, the best predictive model analyzed RH, mean annual temperature (MAT), and precipitation $\delta^{18}O$ together (AIC= 371.5). The least effective complex model explored relationships with RH and MAT (AIC=392.7), although this model remained more effective than analysis of any single variable. Although significant for all complex models, the correlation between $\delta^{18}O$ and climate variables was weak (R^2 <0.20) (Figure 2a-c).

Correlation between $\delta^{13}C$ values and climatic data was significant ($p<0.001$) for individual conditions MAT and latitude, with a stronger correlation with individual climate conditions than for $\delta^{18}O$. Of the single variable models, MAT was found to be the best predictor for $\delta^{13}C$ (AIC= 452.0) (Figure 2e-f). This study additionally explored a model that considers both RH and MAT, which was found to be the most effective overall for $\delta^{13}C$ (AIC= 443.4) and demonstrated the strongest correlation (R^2= 0.19).

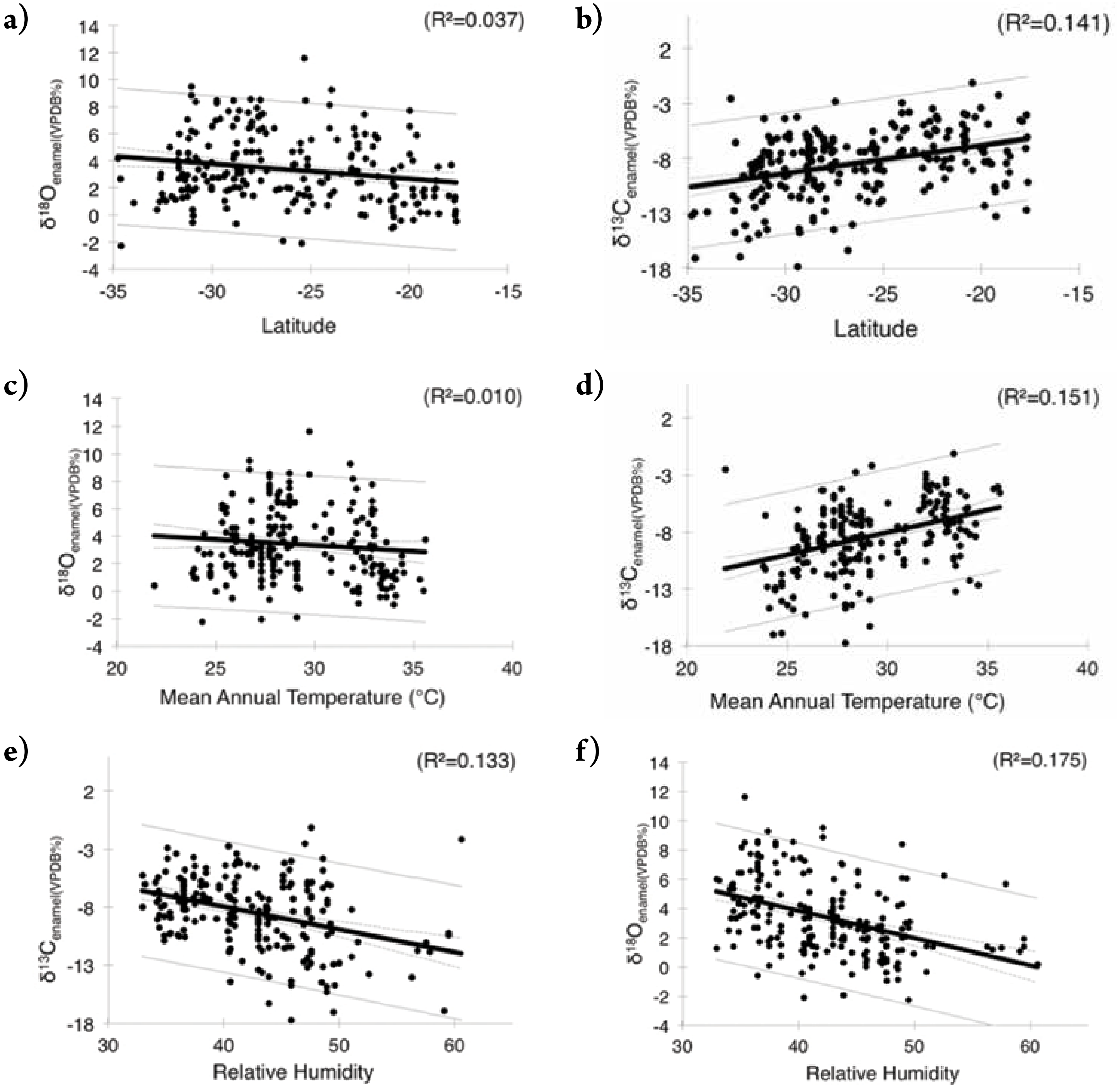


Figure 2. Scatterplots between enamel isotopes and latitude ((a) δ18O, (d) δ13C), mean annual temperature ((b) δ18O, (e) δ13C), and relative humidity ((c) δ18O, (f) δ13C) and versus for all *M. rufus* sampled. Each dot represents a single *M. rufus* molar specimen and relationships are indicated by a line of best fit (thick bold line) bounded by 95% confidence intervals (lighter gray lines).

Complexity (Asfc) values ranged from 0.88 to 4.57 with an average of 1.90±0.89. Anisotropy (epLsar) values ranged from 0.0013 to 0.0094 with an average value of 0.0054±0.0017. Textural fill volume (Tfv) ranged from 6410 to 15524 with a mean of 12437±2373. Spearman correlation analysis demonstrates a positive relationship between epLsar and total annual precipitation (TAP) to be the only significant correlation between DMTA characteristics and climate variables. The relationship between epLsar and TAP was stronger than the relationship between δ^{13}C or δ^{18}O for individual climate indicators (R^2= 0.27).

DISCUSSION.

This study found weak but significant correlations between oxygen isotopes of *M. rufus* and both precipitation δ^{18}O and RH (Figure 2a-f). These results are congruent with the findings of Murphy *et al.* (2007), which found a similar correlation between δ^{18}O and RH for the Macropus genus, although stronger (R^2= 0.57) than is presented here. However, the results of this study differ from the findings of Burgess and DeSantis (2013), which demonstrated moderate significant correlations between δ^{18}O and MAT (R^2=0.30) and latitude (R^2=0.25)

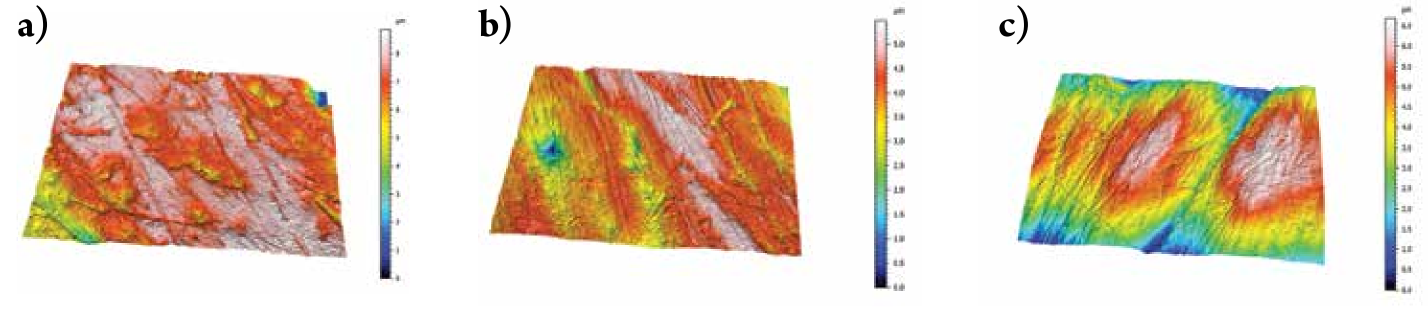


Figure 3. Three-dimensional color scans of Macropus rufus molars. Specimen AMM9331 (a) exhibits high complexity; specimen AMM12763 (b) exhibits moderate anisotropy and pitting; specimen AMM37200 (c) exhibits higher anisotropy.

for *Macropus rufogriseus* while no significant correlation between enamel δ^{18}O and RH or precipitation δ^{18}O was found in *M. rufogriseus* [27].

More variation exists between species for δ^{18}O than δ^{13}C, which may be largely attributable to differences in the evaporation sensitivity of taxa. This study found a significant correlation between δ^{18}O of *M. rufus* and both δ^{18}O (precipitation) and relative humidity, which suggests that *M. rufus* is ingesting water from plant sources and is evaporation sensitive (Table 1) [7, 11]. More research should be done to determine if there are physiological reasons for different species-climate relationships or if relative environmental variability contributes to the differing relationships between climate conditions and enamel isotope values.

Whereas the findings of this study suggest interspecies variation for relationships between δ^{18}O and climate conditions, less variation was found for δ^{13}C. For both *M. rufogriseus* [27] and *M. rufus*, δ^{13}C was not significantly correlated with RH, and was best modeled by MAT for analysis of a single variable. However, for each climate condition, the correlation with δ^{13}C was much stronger for *M. rufogriseus* ($R^2 = 0.41$) when compared with *M. rufus* (Table 1).

While carbon isotopes and DMTA are both proxies for diet, no significant relationship was found between the two indicators. Carbon isotope values showed a significant relationship between RH and MAT while dental microwear characteristics showed only a significant relationship between epLsar and TAP. These findings are concurrent with expectations that an increase in grass supply resulting from increased precipitation would correlate with increased epLsar. Stomach content analysis and observation has revealed that *M. rufus* primarily consumes grass with some shrubs where resources are scarce [28, 29]. This is confirmed here, where dental microwear of *M. rufus* was characterized by high epLsar and low Asfc. However, carbon isotope analysis suggests that *M. rufus* may primarily consume browse, as characterized by a mean δ^{13}C of -8.4%O. Because most grasses utilize the C_4 photosynthetic pathway, a C_3 carbon signature typically implies a browser, but coupled with high epLsar and low Asfc (Figure 3a-c), it can be inferred that *M. rufus* primarily consumes C3 grass.

These preliminary findings confirm that DMTA may be used in conjunction with carbon isotope analysis to clarify specific diet composition for kangaroo species in Australia, but more research can help clarify the accuracy of using DMTA characteristics as proxies for local climate conditions. These findings suggest that proxy data from fossil and historic kangaroos, including stable isotopes and dental microwear, can help infer past climates.

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REFERENCES

1. IPCC, *Report of Intn. Pannel for Climate Change*. (2014).
2. Della-Marta P, Collins D, Braganza K, Aust. *Meterologic. Mag*. 53 75-93 (2004).
3. Whetton, P, Climate change: *Science and Solutions for Aust*. 37 (2011).
4. Hope P, Timbal B, Fawcett R, *Intn. J. Climatology*. (2009).
5. Whetton P, Fowler A, Hylock M, Pittock A, *Climate Change*. 3-4 289-317 (1993).
6. Ayliffe A, Chivas A, *Geochimica*. 9 2603-2609 (1990).
7. Murphy B, Bowman D, Gagan M, *Geochimica*. 15 3847-3858 (2007).
8. Nowak R, *Walker's Marsupials of the World*. (2005).
9. Denny M, Dawson T, *Aust. J. Zoo*. 17 777-784 (1969).
10. Kohn M, Schoeninger M, Valley J, *Chem. Geo*. 1-2 97-112 (1998).
11. Levin N, Cerling T, Passey B, Harris J, Ehleringer J, *PNAS*. 30 11201-11205 (2006).
12. Dansgaard W, *Tellus*. 4 436-468 (1964).
13. DeSantis L, Feranec R, MacFadden B, *PLoS*. 6 (2009).
14. Fraser R, Grün R, Privat K, Gagan M, *Palaeogeography, Palaeoclimatology, Palaeoecology*. 269 66-77 (2008).
15. Bender M, *Phytochem*. 6 1239-1244 (1971).
16. O'Leary M, *BioScience*. 5 328-336 (1988).
17. Cerling T, Harris J, *Oecologia*. 3 347-363 (1999).
18. Hattersley P, *Oecologia*. 1-2 113-128 (1983).
19. Ehleringer J, Cerling T, Dearing M, *Comp. Bio*. 42 424-430 (2002).
20. Prideaux G *et al*., *PNAS*. 28 11646-11650 (2009).
21. DeSantis L, *Surf. Topogr*. 4 (2016).
22. Scott R *et al*., *Nature*. 436 (2005).
23. Scott R *et al*., *Hum. Evol*. 4 339-349 (2006).
24. Ungar P, Brown C, Bergstrom T, Walker A, *Scanning*. 4 185-193 (2003).
25. Coplen T, *Geothermics*. 5-6 707-712 (1995).
26. Prideaux *et al*., *Nature*. 7126 422-425 (2007).
27. Burgess C, DeSantis L, *Young Scientist*, 3 28-31 (2013).
28. Dawson T, Ellis B, *J. Arid Environ*. 3 257-271 (1994).
29. Edwards G, Dawson T, Croft D, *Aust. J. Ecology*. 20 324-334 (1995).

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