Calibrating $\delta^{18}\text{O}$ in *Dromaius novaehollandiae* (emu) eggshell calcite as a paleo-aridity proxy for the Quaternary of Australia

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Abstract

The large, flightless Australian bird, *Dromaius novaehollandiae* (emu), breeds at about the same time of year throughout its range, and fragments of its eggshells are commonly preserved in geological and archaeological archives spanning the Late Quaternary. The $\delta^{18}\text{O}$ of eggshell calcite is set primarily by the $\delta^{18}\text{O}$ of body water in the days prior to egg calcification. Changes in the $\delta^{18}\text{O}$ of body water are dominantly determined by the $\delta^{18}\text{O}$ of ingested water. We provide a calibration dataset of 334 $\delta^{18}\text{O}$ analyses on late Holocene *Dromaius* eggshell from 21 sites across a wide range of environmental conditions and climate states. In regions where interannual rainfall variability is high, the average of 20 samples from different years is required to derive a $\delta^{18}\text{O}$ estimate within $\pm 1\%$ of the millennial mean; fewer than 10 different-year samples is sufficient for less variable rainfall regions. Many climate variables in Australia are highly correlated with $\delta^{18}\text{O}$ in eggshell, but the strongest correlation is with the average site-specific point-potential evapotranspiration (PPET) for February plus March, two summer months prior to egg calcification, which explains 93% of the variance in the mean $\delta^{18}\text{O}$ of our calibration dataset. Eggshell $\delta^{18}\text{O}$ can be used as a PPET (paleo-aridity) proxy in older samples providing four assumptions are closely approximated: 1) no difference in season of breeding over time, 2) breeding in most years, 3) little change in $\delta^{18}\text{O}$ of precipitation over time, and 4) the bird's source water is exposed to evaporative processes. The reconstructed PPET derived from 150 $\delta^{18}\text{O}$ measurements in an 80 ka time-series of eggshell from south-central Australia where these assumptions are satisfied is consistent with independent estimates of environmental water balance derived from changes in lake level and dustiness. The tight correlation between eggshell $\delta^{18}\text{O}$ and PPET indicates that the average $\delta^{18}\text{O}$ in time-series of *Dromaius* eggshell is a robust proxy to reconstruct changes in moisture balance through the Late Quaternary of Australia.

1. Introduction

The stable isotopes of H, C, N, and O in biogenic minerals that are preserved in geological archives offer tantalizing clues of past environments, diets, and climates (Koch, 1998). For

many fossil types, studies of modern organisms through controlled feeding experiments or large datasets provide key calibration series, allowing the isotopic composition of fossil samples to be converted to quantitative measures of past ecological and environmental conditions. An extensive literature has emerged utilizing stable isotopes preserved in the bones and teeth of mammalian fossils for such purposes. Changes in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ have been used to reconstruct paleodiets or to infer changes in past climates (DeNiro and Epstein, 1978; Hedges et al., 2006). The stable isotopes of oxygen ($\delta^{18}\text{O}$) provide information about the source of an animal's drinking water and the contribution of metabolic water to its mineralized hard parts, i.e. bones and teeth (Blumenthal et al., 2014), and references therein).

Avian eggshell is a relatively underutilized fossil medium that records changes in past climates and ecosystems. Eggshell consists of a multi-layered structure of calcite crystals, nucleated on an inner protein membrane and secreted around a honeycomb pattern of cross-bonded organic sheets that account for ~3% of the eggshell mass. The organic matter is largely protein (~70%) with the balance made up of carbohydrate and lipid (Burley and Vadehra, 1989). Studies on eggshells of ostrich and seabirds confirm the potential for the isotopes in both the calcite crystals ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) and the intracrystalline organic molecules ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) to track the birds' diet, local rainfall, and aridity (Folinsbee et al., 1970; Johnson et al., 1993; Johnson et al., 1998; Johnson et al., 1999; Miller et al., 2005; Newsome et al., 2011; Stern et al., 1994; von Schirnding et al., 1982). The goal of this paper is to evaluate the potential for $\delta^{18}\text{O}$ in eggshell calcite of the Australian emu, *Dromaius novaehollandiae*, to serve as a proxy for paleo-aridity across Australia.

2. *Dromaius novaehollandiae*, the Australian emu

The emu is a member of the flightless palaeognath birds commonly called ratites, and is the world's second largest extant bird, after the African ostrich. Female *Dromaius* are the larger sex, reaching up to 1.8 meters tall and weighing ~50 kg; males are smaller, usually about 1.5 meters tall and 35-40 kg (Fowler, 1991). *Dromaius* inhabits all of Australia, although their numbers vary considerably, depending on availability of food and water. In the NE of the country, their close cousin, the cassowary, occupies relict rainforest habitat.

Dromaius are generalist feeders and modestly omnivorous. Their diet consists of almost anything palatable. Throughout Australia the birds eat a wide variety of fruits, seeds, green herbage of annual and perennial plants, including grasses, and when available insects and small lizards or snakes (Davies, 1978). Although they have low moisture requirements, water makes up 65 to 70% of their body mass (Dawson et al., 1984). To maintain water balance they drink free water in significant quantities daily when it is available, although during extreme drought they can satisfy a significant fraction of their required water from plant leaves. The $\delta^{18}\text{O}$ of ingested water equilibrates with the $\delta^{18}\text{O}$ of body water within a week (Folinsbee et al., 1970; Schaffner and Swart, 1991), and eggshell calcification occurs over 1 to 2 days. Consequently, differences in $\delta^{18}\text{O}$ of eggshell calcite represent an integration of the isotopic composition of ingested water in the preceding days to week. Despite large regional variations in climate over the entirety of their breeding range, *Dromaius* breed everywhere at about the same time of year. The birds pair for breeding in December and January, and the female typically lays 8 to 12 large (130 x 90 mm) dark green

eggs in a crude nest as early as April, but most commonly between May and July (Malecki et al., 1998). Eggs are laid about every third day. The male exclusively tends the nest, with incubation lasting 55 to 65 days, and he also rears the chicks from hatching until they are able to live on their own. *Dromaius* eggs have been an important food source for humans, and the eggshell of consumed eggs are commonly found in archaeological sites, including eggshell with diagnostic variable burn patterns caused by humans discarding eggshell fragments in and around cooking fires (Miller et al., 2016).

Preservation of *Dromaius* eggshell in the natural environment has low probability. Once the eggs hatch, the abandoned nest offers little physical protection. Eggshell fragments left after hatching are subject to rapid degradation by windblown quartz sand, and animals often consume eggshell for the calcium it provides. Rapid burial appears to be the key to preservation. Optimal preservation potential occurs when the birds nest among sand dunes, where mobile sand subsequently buries eggshell fragments shortly after the eggs hatch. As a consequence, the frequency of eggshell in the fossil record is more closely aligned with the frequency of sand movement during the hatching period than with the abundance of nesting birds. Because rainfall and soil waters are typically slightly acidic, eggshell buried in sand lacking other carbonates is slowly dissolved and rarely survives on millennial timescales. Optimal preservation settings includes aeolian systems either in limestone terrain, or where sand is sourced from sea-shell-rich ocean beaches, or in regions where carbonate minerals are precipitated in and around salt lakes, a situation common across much of the Australian interior.

Finding archived eggshell is greatly enhanced by recent aeolian deflation. Because eggshell is heavier than sand grains, fragments are concentrated as a lag across the floors of deflationary hollows in dune systems. We target carbonate-bearing sand dunes where recent deflation has occurred. Eggshell found as a lag may include fragments of different ages. We use AAR to provide age constraints on all eggshell fragments for which stable isotope ratios will be determined. Eggshell also is found *in situ* in stratigraphic sections created by wind, wave, or stream erosion.

3. Oxygen isotopic composition of *Dromaius* eggshell

The processes that determine the oxygen isotopic composition of eggshell calcite (CaCO_3) are summarized by (Koch, 1998) and (Nagy, 1989). Oxygen in eggshell calcite is in isotopic equilibrium with body fluids, with the fractionation of oxygen isotopes during calcification determined by the internal temperature of the female bird. Birds, like mammals, are endotherms; female *Dromaius* maintain an internal body temperature at about 38.5°C (Maloney and Dawson, 1993). Hence, isotopic fractionation can be considered a constant, with observed variability in our samples reflecting differences in *Dromaius* body water $\delta^{18}\text{O}$.

Body water is derived primarily from ingested water, which for *Dromaius* includes surface drinking-water sources and plant leaf-water, with additional input from metabolic water produced by oxidation of food (Luz et al., 1984). The oxidation process introduces atmospheric oxygen ($\sim +23\text{‰}$) into the body water pool. Johnson et al. (1998) found that the $\delta^{18}\text{O}$ of ostrich eggshell raised in a controlled setting tracked the $\delta^{18}\text{O}$ of drinking water linearly, suggesting that the proportion of highly enriched isotopes of atmospheric oxygen is

either small or a constant proportion. Studies of other large animals (body mass >100 kg) found that the $\delta^{18}\text{O}$ of body water closely follows that of ingested water (Ayliffe and Chivas, 1990; Bryant and Froelich, 1995; Levin et al., 2006). Although Johnson et al. (1998) found that the eggs of free-range ostrich exhibited highly variable $\delta^{18}\text{O}$, that variability most likely reflects the high percentage of their ingested water being derived from highly variable pre-formed water in leaves, rather than free-water sources.

The $\delta^{18}\text{O}$ values of drinking water sources are set by the $\delta^{18}\text{O}$ of precipitation, modified by evaporation that occurs before the birds consume the water. Across the semi-arid zone weighted annual mean $\delta^{18}\text{O}$ of precipitation exhibits little variability, averaging $-5 \pm 1\text{‰}$ (SMOW). Because calcification of the *Dromaius* egg occurs in autumn, free-standing water sources (e.g. small pools) available at that time are likely to be isotopically enriched in ^{18}O by evaporative processes in the preceding weeks to months. If leaves provide a significant portion of the bird's required water, then body water will be even more enriched in ^{18}O , because the $\delta^{18}\text{O}$ of leaf-water is usually significantly more positive relative to the $\delta^{18}\text{O}$ of precipitation; enrichment increases with decreasing relative humidity and with increasing temperature (Cernusak et al., 2003; Förstel, 1978; Gonfiantini et al., 1965).

From this understanding the $\delta^{18}\text{O}$ of contemporary *Dromaius* eggshell is expected to reflect primarily the $\delta^{18}\text{O}$ of ingested water, both drinking and plant water, averaged over the week prior to calcification. The average time that freestanding water sources have been subjected to evaporative processes is highly variable, depending primarily on the frequency of rain events and the transport distance from rain event to the water source. Drier and hotter regions are expected to have more enriched and more variable source-water $\delta^{18}\text{O}$ relative to drinking-water sources in maritime regions.

4. Materials and Methods

Calibration datasets inherently assume that environmental variables are constant. We targeted late Holocene (<3 ka) eggshell during field campaigns across Australia because little change in rainfall is likely over this interval. Although "modern" eggshell is often available, European pastoral activities have changed water source and availability. Birds in many arid regions now have near-continuous access to bore water from deep aquifers, suggesting, that eggshell older than 100 years is likely more reliable for calibration purposes. We collected eggshell from as wide a range of climate conditions as possible. Most samples are from naturally occurring surface finds, almost exclusively in regions where recent aeolian deflation has exposed buried eggshell. Some samples are from archaeological sites collected during permitted excavations, and a few samples are from recently hatched *Dromaius* nests. The $\delta^{18}\text{O}$ in more than 330 eggshell fragments from 21 sites scattered across central, southern, and western Australia comprise our calibration dataset (Fig. 1; Table 1).

Nearly all eggshell in the calibration dataset have been analyzed for the extent of isoleucine epimerization, the ratio of the protein amino acid isoleucine, to its non-protein diastereomer alloisoleucine (A/I), following procedures outlined in Miller et al. (2000). We use A/I as a measure of sample age, restricting our samples for this study to those eggshells with A/I suggesting they were laid <3 ka ago. Because of the temperature dependency of the racemization reaction, A/I for 3-ka-old samples increases with increasing mean annual

temperature (Fig. 2).

Dromaius eggshell has a tripartite structure, with different amino acid composition in each. To minimize possible isotopic variability between structural layers, we remove the two outer layers mechanically, and then further reduce the remaining sample by dissolving 30% of the mass with the stoichiometric addition of 2N HCl, ensuring the reaction is complete by placing the samples in a vacuum centrifuge. Once cleaned, the sample consists exclusively of the palisade layer.

From the clean eggshell fragment a subsample of 0.1 to 0.2 mg of powdered eggshell is placed in an Exetainer[®] vial and flushed with helium to remove air in a Thermo Fisher Gas Bench. Six drops of 100% phosphoric acid were added to each vial manually, then placed into a heating block kept at 70°C. For each sample run of 40 to 80 eggshell powders we analyzed several blank vials and multiple standards to calculate $\delta^{18}\text{O}$ of the eggshells. Internal lab standards along with NBS-18, NBS-19, and RO-22 (Isoanalytical) were used during each run. Evolved CO_2 was analyzed in a Thermo Finnigan Delta XL Plus or Delta V Plus mass spectrometers at the Geophysical Laboratory of the Carnegie Institution of Washington, Washington, DC or the University of California, Merced. The $\delta^{18}\text{O}$ of an internal lab carbonate standard analyzed six times was $\pm 0.15\text{‰}$. Eggshell calcite $\delta^{18}\text{O}$ is expressed in ‰ with respect to the PDB standard; for water, $\delta^{18}\text{O}$ is reported in ‰ with respect to the SMOW standard. Replicate analyses of the same eggshell fragment agree within better than 0.3‰ (Table 3).

Metrics for the mean climate state across Australia are available through the Australian Government Bureau of Meteorology as both site-specific measurements and in the form of contoured maps, including monthly and annual averages of rainfall, rainfall variability, and temperature (<http://www.bom.gov.au/climate/data/>), as well as point-potential, areal-potential, and areal-average evapotranspiration (http://www.bom.gov.au/jsp/ncc/climate_averages/evapotranspiration/). We consider point-potential evapotranspiration (PPET) as the most reliable proxy for the isotopic enrichment of freestanding water that *Dromaius* would have access to in each region. PPET is the evaporation and transpiration that would occur from a small water body assuming unlimited water supply and no other large moisture sources in the vicinity. To derive estimates of potential evaporation where our calibration samples were collected we use contoured mean monthly maps; with an estimated uncertainty on PPET of ± 15 mm water equivalent, about half a contour interval.

5. Results

Arid and semi-arid regions of Australia are well known for their unusually high interannual variability in rainfall. Interannual changes in rainfall amount and seasonality under invariant temperature will produce very different average $\delta^{18}\text{O}$ of freestanding water from year to year. Although the average state of source-water $\delta^{18}\text{O}$ is required to reconstruct the climate of the past, individual eggshell $\delta^{18}\text{O}$ provide only a single breeding season's record of source water $\delta^{18}\text{O}$.

5a. How many eggshell are required to derive a reliable mean $\delta^{18}\text{O}$ of *Dromaius* eggshell for a given climate state? To reconstruct the average evaporative state it is

necessary to determine the minimum number of different-year eggshell required to derive a reasonable estimate of the mean $\delta^{18}\text{O}$ of contemporary eggshell. The large suite of samples from Lake Eyre (Fig. 1), the most arid region in Australia, provides a representative dataset for regions with low rainfall totals and high interannual variability; a smaller series of *Dromaius* eggshell from the central south coast represents less variable rainfall regions (Fowlers Bay; Fig. 1). From 126 Holocene *Dromaius* eggshell collected around Lake Eyre, eggshell $\delta^{18}\text{O}$ averages $6.7 \pm 4.0\text{‰}$, with a variance of 16.7‰ (range: -4.0 to +15.5‰; Table 1). We set as our target a reliable prediction of the average $\delta^{18}\text{O}$ to within $\pm 1\text{‰}$ of the mean value (+6.7‰). To determine the number of samples required to reach that precision we randomize the full series, then track the average $\delta^{18}\text{O}$ value as an ever-increasing number of samples are utilized, eventually reaching 30 samples. The data series is then randomized again and the process repeated 35 times. We find that the resultant mean value reaches within $\pm 1\text{‰}$ of the group mean in nearly all cases by the 20th sample (Fig. 3).

For the Fowlers Bay Holocene series ($\delta^{18}\text{O}$ mean: $0.5 \pm 2.0\text{‰}$, variance: 1.1‰, range: -3.4 to +6.5‰, $n = 34$) the mean value is within 1‰ of the group mean after averaging only a few samples, and after 10 samples it is within $\pm 0.5\text{‰}$ of the group mean (Fig. 3). Based on these results we estimate an uncertainty on the calibration data sets with variance >5 to be $\pm 1\text{‰}$ using 20 different-year samples, and for sites with variance <5 the uncertainty is estimated to be $\pm 0.5\text{‰}$ using at least 10 different-year samples. Table 1 summarizes the average values of $\delta^{18}\text{O}$ in *Dromaius* eggshell in our calibration dataset. Larger numbers of eggshell are required for samples from the arid and semi-arid zones than for maritime regions, due to the inherent differences in rainfall variability. For sites in the semi-arid zone (variance $>6\text{‰}$) at least 10 samples were analyzed per site, and for many, more than 20 samples. From the more maritime sites (variance $<5\text{‰}$) at least 7 samples have been analyzed and >10 samples for most sites.

5b. $\delta^{18}\text{O}$ in *Dromaius* eggshell as a climate predictor The average $\delta^{18}\text{O}$ in *Dromaius* eggshells primarily reflects the $\delta^{18}\text{O}$ of ingested water averaged over the week prior to calcification (e.g., Johnson et al., 1998). During hot summers with infrequent rainfall, drinking water sources are likely to be more enriched in the heavier ^{18}O relative to ^{16}O than years with regular rainfall. As freestanding water sources become increasingly scarce during drought years, *Dromaius* will depend more on leaf-water to meet their water needs. Both changes imply that the $\delta^{18}\text{O}$ of *Dromaius* eggshell in any given year will be more enriched in ^{18}O as local conditions become increasingly arid. During the most extreme drought years *Dromaius* will not breed and are known to travel relatively long distances to more favorable habitats. Hence, periods of extreme aridity may not be recorded by *Dromaius* eggshell.

A cross-correlation matrix between a range of climate parameters and $\delta^{18}\text{O}$ of *Dromaius* eggshell (Table 2) indicates strong correlations between eggshell $\delta^{18}\text{O}$ and climate terms linked to aridity; F-ratios are calculated for the most highly correlated variables. Mean eggshell $\delta^{18}\text{O}$ is most highly correlated with PPET for specific months, especially Feb ($r^2=0.97$; F ratio=460) and March ($r^2=0.94$; F ratio=167), but also highly correlated with annual PPET ($r^2=0.79$; F ratio=73). Eggshell $\delta^{18}\text{O}$ also shows significant correlations with

mean maximum temperature ($r^2=0.85$; F ratio=84), precipitation minus evaporation ($r^2=0.79$; F ratio=72) and mean annual temperature (MAT: $r^2=0.75$; F ratio=30), but little correlation with mean minimum temperatures ($r^2=0.33$; F ratio=7). The correlation of $\delta^{18}\text{O}$ with average annual rainfall ($r^2=0.48$; F ratio=16) is not as strong as MAT, but the correlation improves when comparing to rainfall variability ($r^2=0.73$; F ratio=38). These results are consistent with significant co-variation of climate terms related to temperature, evaporation, and rainfall variability across arid/semi-arid Australia.

The least squares linear regression between eggshell $\delta^{18}\text{O}$ and Feb+Mar PPET (Table 2) has a correlation coefficient of 0.93 (Fig. 4). Uncertainty on the estimated site-specific PPET is ± 15 mm w.e. assuming no more than one-half contour interval uncertainty on site-specific interpolations from contoured monthly PPET maps (<http://www.bom.gov.au>). *Dromaius* egg calcification occurs commonly in May and June, but including PPET for April ($r^2=0.55$; F ratio=18) in the correlation results in a reduction in the correlation coefficient ($r^2=0.89$; F ratio=141). This suggests that most of the evaporative enrichment of *Dromaius* source water occurs in late summer, February and March, and less during April as temperatures decline. The strong linear correlation between mean eggshell $\delta^{18}\text{O}$ and Feb+Mar PPET ($r^2=0.93$; F ratio=294; Fig. 4A) suggests that potential evaporation in the months leading up to the time of egg formation exerts the strongest control on the $\delta^{18}\text{O}$ in eggshell calcite, with the year-to-year variability dominated by changes in precipitation amount in the 2 or 3 months prior to eggshell calcification. The correlation between mean eggshell $\delta^{18}\text{O}$ and annual PPTE is strong (Fig. 4B), and would be much stronger if we eliminated the two NW Cape samples in karst topography that limits PPET.

6. Reconstructing paleo-aridity from $\delta^{18}\text{O}$ in dated *Dromaius* eggshell

Our calibration dataset is based on egg calcification during the late Holocene (<3 ka), a time interval that has seen little overall climate change compared to the large changes in climate and ecosystems that occurred over the past 100,000 years. However, to apply the modern relationship between $\delta^{18}\text{O}$ of eggshell calcite and indices of aridity as a paleoaridity proxy through the late Quaternary, during which time the global climate system varied significantly, four assumptions must be met:

1) The birds do not change their seasonality of breeding. *Dromaius* currently breed at the same time across the continent despite strong climate contrasts, and breeding time is hormonally driven and unlikely to change on millennial timescales. Hence we assume this condition is met for all regions.

2) The birds breed every year. During extreme drought female *Dromaius* may remain in an area but not lay eggs. This would reduce the magnitude of an isotope-derived aridity signal. Hence our most arid state, as defined by eggshell $\delta^{18}\text{O}$, may underestimate the magnitude of extreme aridity.

3) Changes in $\delta^{18}\text{O}$ of precipitation are small relative to the changes in evaporative enrichment of *Dromaius* source water over the past ~ 100 ka. This assumption is difficult to quantify. Except for a portion of Western Australia $\delta^{18}\text{O}$ of precipitation currently exhibits little variability across Australia. However, during MIS 2, model simulations suggest $\delta^{18}\text{O}$ of precipitation in Australia may have been as much as 2‰ more negative than currently (Lee et

al., 2008). A secondary potential source of variation is related to air mass trajectories, with both maritime air masses and monsoon incursions delivering more negative $\delta^{18}\text{O}$ of their rain than is associated with westerly circulation (Risi et al., 2013; Srivastava et al., 2015).

During the LGM oceanic surface waters around Australia were 1 to 2‰ depleted relative to modern values, and this depletion would have been incorporated in some manner into $\delta^{18}\text{O}$ of precipitation. At the same time, oceanic SST were also the lowest of the past 120 ka (Barrows and Juggins, 2005), and, by inference, LGM rainfall over Australia is predicted to have been reduced, on average. Less rainfall reduces the influence of the “amount effect” on the $\delta^{18}\text{O}$ of precipitation. The relative importance of these two opposing influences on the $\delta^{18}\text{O}$ of precipitation is uncertain, which adds an increment of uncertainty to estimated PPET derived from changes in eggshell $\delta^{18}\text{O}$.

4) The bird’s drinking water is dominantly from surface-water sources. In terrain where water is efficiently removed from the ground surface (high relief or karst terrain), the potential for evaporative isotopic enrichment is limited. In karst terrain there may be no standing water on the land surface, but birds may access water in caves where the cave floor intercepts groundwater that has experienced little surface exposure, hence little isotopic enrichment even during a highly arid climate state. In these situations variations in eggshell $\delta^{18}\text{O}$ may be dominated by changes in $\delta^{18}\text{O}$ of precipitation. If a significant proportion of the bird’s water requirements were met by leaf water (which only happens during drought), it would amplify the aridity signal, although breeding is less likely in such times.

In the Lake Eyre region the birds may utilize mound springs, artesian flow of Great Artesian Basin (GAB) groundwater, which has a $\delta^{18}\text{O}$ (SMOW) ranging between -6.5 and -5.0‰ (Harrington et al., 2013; Lehmann et al., 2003), similar to the weighted annual mean $\delta^{18}\text{O}$ of precipitation (-6‰), although the distribution of mound springs is sparse. However, the expansion of European pastoral activities was associated with greatly increased delivery of GAB water to distant stock-watering points that are also accessible to emu. The impact of isotopically different source water is born out by our samples. From Lake Eyre 39 collections contain eggshell <3 ka, of which 13 are <50 years old, post-dating pastoral expansion of GAB watering points, with the remaining 26 collections predating European settlement of the region. The $\delta^{18}\text{O}$ of the younger group (5.5 ± 2.1 ‰) is less enriched relative to the $\delta^{18}\text{O}$ of the older group (7.04 ± 3.9 ‰), which we interpret to reflect increased access to GAB water for the birds in recent decades. To reduce the bias resulting from pastoral activities, we utilize only the 26 pre-European-settlement, >3 ka samples in our calibration series.

Dromaius eggshell from the same region as our calibration samples but that calcified in the more distant past are expected to have a different mean $\delta^{18}\text{O}$ if the primary determinants on *Dromaius* source-water $\delta^{18}\text{O}$ were different then. For example, samples from the Port Augusta region calcified during the LGM (30-15 ka) have a mean $\delta^{18}\text{O}$ of 5.4 ± 4.2 ‰ ($n=21$), indicative of more arid conditions than during the middle Holocene (8-3 ka), when $\delta^{18}\text{O}$ was less enriched and less variable (0.9 ± 2.6 ‰; $n=34$). Based on the correlation between eggshell $\delta^{18}\text{O}$ and Feb+Mar PPET, the climate around Port Augusta during the

LGM (Feb+Mar PPET: 570 ± 150 mm w.e.) was similar to the modern climate around Lake Eyre, 300 km to the north.

7. The changing water balance of western NSW over the past 80 ka derived from $\delta^{18}\text{O}$ in *Dromaius* eggshell

The strong correlation between eggshell $\delta^{18}\text{O}$ and Feb+Mar PPTE (Fig. 4A) suggests that the evolution of $\delta^{18}\text{O}$ in a dated time series of *Dromaius* eggshell could provide a quantitative index of paleoaridity through time. The lower Darling River and nearby Willandra Lakes region (Fig. 1), likely meets the assumptions above. It is a continental interior region, over which there is no strong seasonality of precipitation, and where surface water accessible to birds occurs in pools and shallow lakes. From this region 680 individual fragments of *Dromaius* eggshell have been collected, primarily from sandy lunettes on the down-wind margins of normally dry lakes, and each has been analyzed for the extent of amino acid racemization in organic residues contained within the calcite crystals. An age model derived from 47 calibrated radiocarbon dates on eggshell carbonate constrain ages back to ~40 ka, and 2 OSL dates extend the control to 50 ka, allowing A/I to be converted to age. Eggshell $\delta^{18}\text{O}$ has been determined on a subset of 146 samples with an uneven distribution over the past 80 ka (Fig. 5 and SI). To convert the $\delta^{18}\text{O}$ time-series to an estimate of paleoaridity requires averaging the individual $\delta^{18}\text{O}$ values to adequately define changes in the mean climate state (rather than yearly weather conditions) through time. The late Holocene calibration series from the Darling River area exhibits high year-to-year variability (variance = 10.9 ‰; Table 1). For a region with high variance we use a 20-point running mean to capture the central tendency of the $\delta^{18}\text{O}$ data, and convert that value to an estimate of Feb+Mar PPET using the equation derived in Fig. 4.

Our eggshell-derived reconstruction of paleo-aridity over the past 80 ka shows coherent changes in climate state (Fig. 5). Relatively moist conditions prevailed from ~80 ka until shortly before 40 ka, after which a steady increase in aridity is reconstructed. Strongly arid conditions were reached by ~35 ka, with peak aridity at ~20 ka, coinciding with the peak of the last glacial maximum (LGM) in marine isotope stage 2 (MIS 2). Aridity began to lessen in the reconstruction about 14 ka, with increasing effective moisture peaking between 9 and 7 ka, after which the region gradually became more arid. The increase in reconstructed Feb+Mar PPET from a mean value of ~400 mm w.e. prior to 40 ka, to a peak of 540 mm w.e. at the height of the LGM (Fig. 5) is equivalent to moving the lower Darling/Willandra region ~600 km north in modern climate space. Similarly, the decrease in Feb+Mar PPET between the LGM and peak mid-Holocene wetness corresponds to a 300 km southward shift.

The paleo-aridity index derived from the integrated $\delta^{18}\text{O}$ in eggshell calcite bears strong similarities to an independent reconstruction of water balance changes in the lower Darling River and the Willandra Lakes over the same time interval. The Willandra Lakes are a series of small basins filled by overflow from the Lachlan River with headwaters farther east. Because the lunettes on the downwind sides of these lakes contain abundant evidence of early human inhabitants, the region has experienced intensive research over the past 50 years. Recent improvements in geochronology now provide reliable ages on changes in moisture balance over the region for the past 60 ka. The hydrologic evolution of this region

suggests relatively high, although slowly weakening, river discharge from 60 ka through to about 40 ka, after which river discharge rapidly declined, with maximum aridity occurring during the LGM (30 to 15 ka) (Bowler et al., 2005). During the transition from the LGM to the Holocene, river discharge increased again, with the highest flows during the early- to middle- Holocene, and more arid conditions with reduced river flows after 5 ka (Copper, 2005), although the water balance was never as positive after 40 ka as it was immediately prior to 40 ka. This pattern of relative changes in the moisture balance reconstructed from dated geomorphic and stratigraphic evidence is closely replicated by the evolution of Feb+Mar PPET reconstructed from the running mean $\delta^{18}\text{O}$ in *Dromaius* eggshell (Fig. 5) and provides support for our assertion that $\delta^{18}\text{O}$ values archived in eggshells of *Dromaius* provide a season-specific assessment of regional aridity, providing the key assumption outlined above are met. The 30 ka highstand in the Bowler et al. (2003) reconstruction occurs while dustiness remains high, suggesting this brief wet interval may be linked to rare heavy rainfall event(s), and is not likely a significant climate shift. In the caption to that figure, Bowler et al (2003) write: “These two independent lines of evidence [lake level and dustiness] indicate a major climatic change of regional extent at 40 kyr.”, which is what the eggshell $\delta^{18}\text{O}$ also indicates.

8. Discussion

The two Ningaloo sites calibration sites (Fig. 1) fall far from the linear regressions describing the dependency of $\delta^{18}\text{O}$ on PPET (Figs. 4A and 4B). For both sites the mean $\delta^{18}\text{O}$ and its variance are unexpectedly low, indicating less evaporative enrichment and interannual variability is recorded by eggshell $\delta^{18}\text{O}$ than recorded at other sites with similar climates. The hydrological system of the Ningaloo region is dominated by the underlying karst topography; there is virtually no surface water. Rainwater rapidly drains through cracks in the underlying limestone until it reaches the boundary of the Tulki and older Mandu Limestone layers where it pools above higher density salt water (Allen, 1993). The few semi-permanent standing water bodies are limited to caves located over isolated perched water tables (Humphreys and Adams, 1991). It is likely that emu in the Ningaloo region utilize fresh-water sources that have had less exposure to evaporation than in other regions.

The isotopic composition of precipitation in the Ningaloo region, which originates from clouds forming over the Indian Ocean, may differ from rainwater elsewhere in our calibration region, which is derived largely from subtropical, continental storms (Denniston et al., 2013). Summer rains associated with tropical cyclones may also deliver heavy rain events during some years. Furthermore, an analysis of $\delta^{18}\text{O}$ and δD of water vapor has shown that kinetic isotope fractionations related to relative humidity, sea surface temperature, and cloud cover are important factors in determining precipitation $\delta^{18}\text{O}$ values (Liu et al., 2014). Ningaloo MAP averages ~280 mm w.e., with the $\delta^{18}\text{O}$ of precipitation for the two wettest summer months (Feb, Mar) averaging ~4‰ less negative than most of our other calibration sites; winter rains (May, June) have less different $\delta^{18}\text{O}$ of precipitation, but occur outside the breeding season (http://wateriso.utah.edu/waterisotopes/pages/data_access/figures.html). Removing the two

anomalously situated Ningaloo sites from the regressions in Figure 4 increases the correlation coefficient for Feb+Mar PPET to 0.97 and for Annual PPET to 0.93.

Eggshell offer one of the few paleoenvironmental archives from which both climate and diet can be derived from the same medium, reducing uncertainties that arise when comparing proxy-derived climate and ecosystem reconstructions from different sources, each with its own chronological uncertainties. The $\delta^{13}\text{C}$ in eggshell calcite and in its intracrystalline protein is set by the isotopic composition of the food consumed by the female bird in the weeks to months before egg calcification, offset by predictable fraction factors (Johnson et al., 1999; Miller et al., 2005). Changes in the average egg-season $\delta^{13}\text{C}$ value of *Dromaius* diet are derived from the same samples in which $\delta^{18}\text{O}$ is used to reconstruct paleo-aridity. The similarities between our 80 ka reconstruction of moisture stress (derived from eggshell $\delta^{18}\text{O}$) and ecosystem status (derived from eggshell dietary $\delta^{13}\text{C}$) with reconstructions from lake level and fluvial evidence suggests that similar eggshell-isotope time series from elsewhere may separate the impacts of climate vs. humans on the Australian continent over the Late Quaternary.

9. Conclusions

The $\delta^{18}\text{O}$ in eggshell calcite of the Australian emu, *Dromaius novaehollandiae*, is determined by the $\delta^{18}\text{O}$ of their body water, which is dominated by the $\delta^{18}\text{O}$ of the birds' source water in the week prior to egg calcification. Source water is primarily freestanding water in pools, lakes or streams, but also may include pre-formed leaf water. Because temperature of calcification and contributions from metabolic water are constants, changes in eggshell $\delta^{18}\text{O}$ reflect changes in the $\delta^{18}\text{O}$ of the birds' source water. Source water $\delta^{18}\text{O}$ largely reflects the balance between precipitation and evapotranspiration over the birds' breeding region in the months prior to breeding. Each eggshell provides a measure of the state of the water balance in the lead-up to the breeding season within a single year. Because high interannual rainfall variability characterizes much of Australia, at least 10, and, in the more variable regions, 20 eggshell fragments from closely spaced years are required to characterize the average state of the local moisture balance. A training set of over 330 eggshell calcified <~3000 years ago from 21 sites across Australia show moderate to strong correlations with climate parameters related to temperature and rainfall, but are most highly correlated with point-potential evapotranspiration (PPET) during late summer, and to annual PPET. Based on the strong correlation between PPET and $\delta^{18}\text{O}$ we suggest that the evolution of regional paleo-aridity through the Late Quaternary can be reconstructed from the $\delta^{18}\text{O}$ in dense time-series of dated *Dromaius* eggshell collected within a limited geographic range, provided the four primary assumptions are reasonably satisfied.

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Table 1

Location	Fig. 1	Mean $d^{18}\text{O}$	StDev	Var	n	StdEr	Feb PPET	Mar PPET	Feb-Mar PPET	Annual PPET	Annual precip. (mm w.e.)	Mean Max Temp (°C)
Lake Eyre, SA	LE	7.04	3.90	15.20	26	0.76	315	285	600	2550	250	31.0
Arcoona Stn, SA	AR	5.01	3.54	13.84	11	1.07	295	250	545	2400	180	27.6
Woomera, SA	WO	4.54	2.43	5.91	11	0.73	285	260	545	2420	180	27.6
Lake Frome, SA	LF	4.79	2.90	8.41	28	0.55	280	265	545	2300	275	28.0
Darling Lakes NSW	DM	3.51	3.19	10.14	12	0.92	260	225	485	1950	580	26.2
Ningaloo North, WA	NN	3.10	1.72	4.47	18	0.41	285	275	560	2750	250	31.8
Ningaloo South, WA	NS	2.80	2.62	6.88	23	0.55	282	270	552	2650	250	31.0
Cervantes, WA	CE	2.68	3.24	10.53	14	0.87	240	215	455	2000	150	26.5
Wilcatana, SA	WC	3.19	3.79	14.38	9	1.26	255	235	490	2150	225	26.0
Port Augusta, SA	PA	2.92	3.80	14.45	31	0.68	250	230	480	2100	250	25.6
Dongara, WA	DO	2.08	0.02	0.84	11	0.01	240	240	480	2150	500	26.8
Eucla, WA	EU	0.51	1.29	1.67	21	0.28	200	180	380	1800	240	24.9
Fowlers Bay, SA	FB	0.35	1.01	1.01	11	0.30	200	170	370	1750	290	23.1
Lake Albacutya, Vic	LA	0.26	2.00	4.01	11	0.60	225	190	415	1650	500	24.5
YorkePen, SA	YP	-0.89	1.56	2.43	21	0.34	190	150	340	1450	500	22.3
Nullarbor, SA	NU	-0.90	1.28	1.64	9	0.43	195	170	365	1700	250	25.0
Coffin Bay, EyrePen, SA	CB	-1.00	1.22	1.48	7	0.46	170	150	320	1400	550	22.7
Cactus Beach, SA	CA	-0.87	1.56	2.42	20	0.35	190	165	355	1700	300	25.2
Southwest WA	SW	-1.68	1.72	2.95	14	0.46	180	140	320	1450	1048	22.4
Kangaroo Is., SA	KI	-2.92	0.95	0.89	13	0.26	160	135	295	1400	600	24.5
King Is., Bass Strait	BS	-3.30	1.21	1.46	13	0.34	130	120	250	1100	1000	16.7

Table 1 Primary climate and isotopic data from Dromaius eggshell (<3 ka) for the 21 regions (Fig. 1) that comprise our calibration series. Two-letter abbreviations refer to site locations shown on Figure 1.

Table 2

	$\delta^{18}\text{O}$	StDev	Var	MAT	MaxT	MinT	Ppt	Ppt Var	PPET Ann.	PPET Feb	PPET Mar	PPET Apr	PPET F+M	PPET F+M+A	P-E	E/P
$\delta^{18}\text{O}$	1.00	0.49	0.38	0.75	0.85	0.33	0.48	0.73	0.79	0.97	0.94	0.55	0.96	0.93	0.79	0.67
StDev	0.49	1.00	0.94	0.22	0.30	0.04	0.17	0.25	0.24	0.47	0.39	0.04	0.43	0.33	0.24	0.32
Var	0.38	0.94	1.00	0.23	0.33	0.03	0.19	0.22	0.25	0.49	0.44	0.06	0.47	0.37	0.26	0.26
MAT	0.75	0.22	0.23	1.00	0.91	0.66	0.33	0.74	0.89	0.76	0.78	0.84	0.78	0.85	0.75	0.55
MaxT	0.85	0.30	0.33	0.91	1.00	0.37	0.49	0.82	0.96	0.91	0.92	0.83	0.92	0.96	0.88	0.67
MinT	0.33	0.04	0.03	0.66	0.37	1.00	0.03	0.28	0.39	0.23	0.23	0.46	0.23	0.30	0.23	0.14
Ppt	0.48	0.17	0.19	0.33	0.49	0.03	1.00	0.61	0.50	0.52	0.50	0.39	0.52	0.52	0.78	0.59
Ppt Var	0.83	0.24	0.25	0.89	0.96	0.39	0.50	1.00	0.88	0.80	0.81	0.68	0.81	0.84	0.88	0.90
PPET Ann.	0.83	0.24	0.25	0.89	0.96	0.39	0.50	0.88	1.00	0.89	0.91	0.82	0.91	0.95	0.92	0.76
PPET Feb	0.97	0.47	0.49	0.76	0.91	0.23	0.52	0.80	0.89	1.00	0.96	0.62	0.99	0.96	0.85	0.76
PPET Mar	0.94	0.39	0.44	0.78	0.92	0.23	0.50	0.81	0.91	0.96	1.00	0.68	0.99	0.98	0.72	0.42
PPET Apr	0.55	0.04	0.06	0.84	0.83	0.46	0.39	0.68	0.82	0.62	0.68	1.00	0.66	0.78	0.74	0.44
PPET F, M	0.96	0.43	.047	.078	0.92	0.23	0.52	0.81	0.91	0.99	0.99	0.66	1.00	0.98	0.86	0.74
PPET F,M,A	0.93	0.33	0.37	0.85	0.96	0.30	0.52	0.84	0.95	0.96	0.98	0.78	0.98	1.00	0.89	0.72
P-E	0.79	0.24	0.26	0.75	0.88	0.23	0.78	0.88	0.92	0.85	0.72	0.74	0.86	0.89	1.00	0.80
E/P	0.67	0.32	0.26	0.55	0.67	0.14	0.59	0.90	0.76	0.76	0.42	0.44	0.74	0.72	0.80	1.00

Table 2. Cross-correlation matrix between mean $\delta^{18}\text{O}$ and its uncertainties for each of our calibration sites and a range of climate variables for each calibration site derived from the Government of Australia Bureau of Meteorology. MAT = mean annual temperature; MaxT = mean maximum temperature; MinT = mean minimum temperature; Ppt = mean annual precipitation; PptVar = variance about Ppt; PPET = point-potential evapotranspiration (mm water equivalent); P-E = mean annual precipitation - mean annual evaporation; E/P = mean annual evaporation/mean annual precipitation. Correlation coefficients >0.85 are in bold.

Table 3

Lab ID	Taxon	Site	A/I	Age (cal)
6871A	Genyornis	Lake Eyre, Wm's Point, WPAU	0.58	55 ± 5 ka (OSL)
6871A	Genyornis	Lake Eyre, Wm's Point, WPAU	0.58	55 ± 5 ka (OSL)
6871A	Genyornis	Lake Eyre, Wm's Point, WPAU	0.58	55 ± 5 ka (OSL)
6871A	Genyornis	Lake Eyre, Wm's Point, WPAU	0.58	55 ± 5 ka (OSL)
6871A	Genyornis	Lake Eyre, Wm's Point, WPAU	0.58	55 ± 5 ka (OSL)
6871A	Genyornis	Lake Eyre, Wm's Point, WPAU	0.58	55 ± 5 ka (OSL)
				mean
				stdev
7136A	Genyornis	Lake Eyre, Wm's Point, WPAU	0.58	55 ± 5 ka (OSL)
7136A	Genyornis	Lake Eyre, Wm's Point, WPAU	0.58	55 ± 5 ka (OSL)
7136A	Genyornis	Lake Eyre, Wm's Point, WPAU	0.58	55 ± 5 ka (OSL)
7136A	Genyornis	Lake Eyre, Wm's Point, WPAU	0.58	55 ± 5 ka (OSL)
				mean
				stdev
7511A	Dromaius	Lake Eyre, Wm's Point, SIU	0.34	8490 ± 110 BP (14C)
7511A	Dromaius	Lake Eyre, Wm's Point, SIU	0.34	8490 ± 110 BP (14C)
7511A	Dromaius	Lake Eyre, Wm's Point, SIU	0.34	8490 ± 110 BP (14C)
7511A	Dromaius	Lake Eyre, Wm's Point, SIU	0.34	8490 ± 110 BP (14C)
7511A	Dromaius	Lake Eyre, Wm's Point, SIU	0.34	8490 ± 110 BP (14C)
				mean
				stdev

Table 3 Reproducibility of oxygen and carbon isotope measurements in differ

Figure 1

Figure 1 Location map showing all 21 calibration sites. Table 1 provides full names for each site and details of the isotopic data and climate terms for each site.

Figure 2

Figure 2 Theoretical relation between A/I and temperature mean annual site temperature for a 3 ka old *Dromaius* eggshell based on equations derived in Miller et al. (2000).

Figure 3

Figure 3 (A) To derive the minimum number of eggshell $\delta^{18}\text{O}$ required to achieve $\pm 1\%$ of the group mean a series of 126 Holocene *Dramalus* eggshell from LE (Fig. 1; mean $\delta^{18}\text{O}$: 6.7‰) were randomly added 30 times. In nearly all cases the mean $\delta^{18}\text{O}$ was within $\pm 1\%$ of the group mean by the 20th sample. (B) The evolution of the standard deviation for LE samples. (C) The evolution of the standard deviation for the more maritime FB samples.

Figure 4

Figure 4 The correlation between the mean $\delta^{18}\text{O}$ from each of the collection sites with the site-specific Feb + Mar PPET (Fig. 4A) and Annual PPET (Fig 4B). The two outliers are Ningaloo North and Ningaloo South (see Discussion for an explanation). The linear least-squares regression (Fig. 4A) is used to reconstruct paleo aridity over the past 80 ka in Fig. 5

Figure 5

Figure 5 Paleoaridity derived from the 20-point running mean of the $\delta^{18}\text{O}$ in individually dated *Dromaius* eggshell from the DM region (Fig. 1) using the equation from Figure 4A. Inset lower right shows the distribution of dated eggshell in the time domain. Top inset shows the change in local moisture balance derived from lake level and fluvial records (Bowler et al., 2005).