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Published in:
Journal of African Earth Sciences

DOI:
10.1016/j.jafrearsci.2023.104999

Publication date:
2023

Citation for published version (APA):
Rey, K., Amiot, R., Fourel, F., Luyt, J., Fluteau, F., & Lécuyer, C. (2023). Paleoenvironmental implications of Permo-Triassic geographic shift in oxygen stable isotope (δ18Op) from tetrapod bone in the South African Karoo Basin. *Journal of African Earth Sciences*, *205*, 1-10. Article 104999.
https://doi.org/10.1016/j.jafrearsci.2023.104999

Paleoenvironmental implications of Permo-Triassic geographic shift in oxygen stable isotope ($\delta^{18}O_p$) from tetrapod bone in the South African Karoo Basin.

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Abstract

The Beaufort Group of the Main Karoo Basin of South Africa provides an uninterrupted record of fluvio-lacustrine sedimentation from the Middle Permian to the Middle Triassic and covers an area of approximately 200,000 km², about 20% of the country's surface area. A diverse range of vertebrate taxa including fish, therapsids, amphibians, archosauromorphs and parareptiles, has been recovered from this rock succession and provides an ideal sampleset to investigate temporal and geographical variations of local hydrology using stable isotopes. Questions relating to temporal and biological variations in oxygen stable isotope ratios of apatite phosphate ($\delta^{18}O_p$) in Beaufort tetrapod taxa have previously been addressed, but geographic variation of bioapatite-recorded $\delta^{18}O_p$ across the Permo-Triassic of South Africa has not yet been studied. Here we highlight variations in $\delta^{18}O_p$ recorded over a large geographic spread in the Karoo basin for four Permo-Triassic vertebrate assemblage zones (AZ). Tetrapods, mainly therapsids, were sampled from two Permian (*Cistecephalus* AZ and *Daptocephalus* AZ) and two Triassic biozones (*Lystrosaurus declivis* AZ and *Cynognathus* AZ). For each of these biozones, fossils were sampled from several localities and their $\delta^{18}O_p$ values compared. Results from the Permian data showed no isotopic difference in apatite from localities divided by longitude 24°. Isotopic differences seem to appear further east in the Basin. Most of the Permian localities (restricted to the south-western part of the Basin) were under the influence of a water system originating from the southern mountainous source terrain. Oxygen isotope compositions of vertebrates from the *Lystrosaurus declivis* AZ show a significant latitudinal gradient interpreted as an evaporation effect from a southern source in the mountains running toward the north. The Basin during the *Cynognathus* AZ was under a similar pattern with an evaporation effect highlighted with lower $\delta^{18}O$ values in the south and higher in the north.

Key-words: Late Permian, Middle Triassic, stable isotopes, climate, therapsids

1. Introduction

1.1 Geological setting of the Karoo Basin

The evolution of life is punctuated by mass extinction events impacting the dynamics of ecosystems of both marine and continental realms. On land, the Main Karoo Basin of South Africa was filled over a long period of sediment deposition from the Late Carboniferous to the early Jurassic. The rocks of the Karoo thus record three mass extinctions: the end-Capitanian mass extinction (Day et al., 2015; Day and Rubidge, 2021) around 260Ma, followed by two of the "Big Five" extinction events: the end-Permian one, 10Ma later (Botha-Brink et al., 2014; Smith and Botha-Brink, 2014; Viglietti et al., 2018; Botha et al., 2020; Stanley and Yang, 1994; Bond and Grasby, 2017) and the end-Triassic mass extinction events, around 200Ma ago (Bordy et al., 2020).

Within the Karoo Supergroup, the continuous sedimentary record of the Beaufort Group, (Middle Permian to Middle Triassic) comprises seven tetrapod assemblage zones (Smith et al., 2020) which has facilitated research focused on both Permian mass extinction events (Smith, 1995; Smith and Ward, 2001; Smith and Botha-Brink, 2014; Day et al., 2015; Viglietti et al., 2018, 2021; Day and Rubidge, 2021). The abundance of fossil fish, therapsids, amphibians, archosauromorphs and parareptiles recovered from these sedimentary strata has enabled geochemical studies on vertebrate bone from numerous localities across the entire Karoo Basin (Rey et al., 2016), which are temporally and geographically separated from each other by tens of kilometres (Botha et al., 2005; MacLeod et al., 2017; Rey et al., 2018). However, questions relating geographic variations of bioapatite-recorded $\delta^{18}O_p$ across the Permo-Triassic of South Africa has not yet been studied. Therefore, this study aims to understand the distribution of the $\delta^{18}O$ in the Main Karoo Basin and explains this distribution

1.2 Stable oxygen isotope trends in continental waters

Surface water isotopic values depend on the water cycle, which is rooted in the evaporation of surface oceanic waters, then followed by cloud motion towards higher latitudes and land regions during which “Rayleigh type” distillation and air temperature control the isotopic compositions of rainfall and snow.

Oxygen isotopic values of meteoric water ($\delta^{18}O_{mw}$) are lower than oceanic values (average $\delta^{18}O_{sea\ water} = 0‰$ today) as a result of fractionation processes which occur during initial evaporation and subsequent precipitation. The Rayleigh model represents those successive changes (Dansgaard, 1964; Ciais and Jouzel, 1994; Fricke and O’Neil, 1999; Grafenstein et al., 1996; Yoshimura et al., 2003) and shows that the $\delta^{18}O$ value of meteoric water decreases with successive precipitation events, thus creating a latitudinal gradient. This gradient is controlled by air temperature, the most important parameter influencing the $\delta^{18}O$ of meteoric water (Siegenthaler and Oeschger, 1980). Given that metabolic activity permissive of biomineralization is limited to a narrow range of temperatures, it follows that isotopic values of bioapatite have potential to be an important tool to interpret palaeoclimatic and palaeoenvironmental conditions during in-vivo biomineralization.

However, the effect of temperature on the air mass is not limited to its latitude but is also the result of altitude, which is referred as the ‘altitude effect’. When moisture-rich air masses rise along the flank of a mountain and gain altitude, the temperature decrease results in the condensation of moisture, and the resulting rain has lower $\delta^{18}O$ values (Dansgaard, 1964; Rozanski et al., 1993). This results in an isotopic lapse rate for precipitation. In the case of monsoon, most to all the water in the airmasses is poured on a local area. This results in precipitations with lower $\delta^{18}O$ values (Lee and Fung, 2008; Kurita et al., 2009; Wen et al., 2012) than surrounding areas of similar latitude and unaffected by this ‘monsoonal effect’.

Similarly, air masses transported for long distances into deep interiors of continent forms a $\delta^{18}O$ gradient with lower values the more inland the precipitation occurs. This phenomenon is independent of latitude (Rozanski et al., 1993; Winnick et al., 2014) and is often referred as the ‘continental effect’.

Accordingly, the $\delta^{18}O_{mw}$ reflects the combination of all these parameters (i.e. latitudinal, altitudinal and continental effects) (Dansgaard, 1964; Niewodnizański et al., 1981; Rozanski et al., 1982; Grafenstein et al., 1996; Fricke and O’Neil, 1999; Kern et al., 2014; Winnick et al., 2014). Once precipitation reaches the Earth’s surface, its isotopic compositions ($\delta^{18}O_{surf.\ water}$) can evolve before being consumed through the diet of animals. Evaporation and mixing with other water sources are factors ultimately controlling the $\delta^{18}O_{surf.\ water}$ values (Gat, 2008; Yde et al., 2016; Zhao et al., 2016).

1.3 Stable oxygen isotope in continental vertebrates

Water from precipitation is the main source of drinking water for continental vertebrates transferring the $\delta^{18}O_{surf.\ water}$ values to their mineralised tissues after a value shift depending on the animal body temperature and physiology (D'Angela and Longinelli, 1990; Kohn et al., 1996). Apatite is the main component of bones and teeth where it is divided between enamel and dentine. This is a mineral of calcium phosphate [$Ca_{10}(PO_4)_6(OH)_2$], which through precipitation can incorporate some carbonate in substitution of the phosphate group. The substitution can range from less than 1% to 13.4% in extant vertebrates (Brudefold and Soremark, 1967; Rink and Schwarcz, 1995; Vennemann et al., 2001; Tarnowski et al., 2002). Enamel has the highest apatite concentration, with approximately 97% of its structure composed by this mineral (Hillson, 1986) with high concentration of big inter-grown crystals (Mills, 1967). Dentine and bones are only constituted by 70% of apatite (Young et al., 2013) which is more porous than enamel, as it comprises smaller and less densely inter-grown crystals (Mills, 1967). As a result, alteration of the original isotope compositions is more likely to occur with secondary precipitation within or on the surface of the bioapatite crystals, adsorption of ions on the surface of those crystals, or even through dissolution and recrystallization along with isotopic exchange with sedimentary pore waters (Zazzo et al., 2004a). The enamel layer can reach up to 5mm of thickness in extant mammals (Lucas et al., 2008), but is very thin or absent on therapsids teeth (Sullivan et al., 2003).

This study focused on measuring and interpreting apatite $\delta^{18}O$ values of tetrapods recovered from localities spread over the entire Karoo Basin and covering several assemblage zones. Taking all the environmental and physiological factors modifying the $\delta^{18}O$ into account allows to highlight potential gradients of $\delta^{18}O$ values and discuss their probable causes.

It is an honour to present this paper to celebrate the huge multidisciplinary contribution of Roger Smith to Karoo palaeontology, sedimentology and stratigraphy. It is especially pertinent as Roger Smith and his collaborators pioneered isotope studies in the Karoo.

2. Material and Methods

2.1 Sampling

From across the basin, a total of 72 teeth and 119 bones from 35 tetrapod genera were selected and analysed for stable oxygen isotope composition of their phosphate and carbonate groups and the carbonate content of apatite (**Supplementary Table 1**). As tooth enamel of Permo-Triassic tetrapods is very thin, or absent (Sullivan et al., 2003), most of the tooth samples in this dataset consist of dentine. For some specimens, bones were sampled, as some of the species, such as *Oudenodon*, do not have teeth (Botha and Angielczyk, 2007) or are represented by only post-cranial remains.

The fossils, all curated in the Karoo Collection of the Evolutionary Studies Institute, University of the Witwatersrand, comprise 5 genera of temnospondyls, 1 of pareiasaurs, 1 of archosauriforms and 28 of therapsids (3 of gorgonopsians, 14 of dicynodonts, 4 of therocephalians and 7 of cynodonts). The samples were recovered from 30 different localities (**Fig. 1**) which occur over four of the seven Beaufort Group assemblage zones (**Supplementary Table 1;** Smith et al., 2020). A total of 126 specimens were selected for this study and to increase the sample size, 53 specimens previously published from Rey et al., (2016) are included in this study. Calculations of palaeogeographic coordinates of the sampling sites were undertaken using the global Apparent Polar Wander Path (APWP) in South African coordinates (Torsvik et al., 2012). The paleomagnetic Euler parameters (longitude, rotation – latitude is set to zero) were calculated by an iterative method to fit the paleolatitudes calculated with the global APWP in South African coordinates of Torsvik et al. (2012). The Euler parameters that have been obtained are quite similar to those obtained by

Torsvik et al. (2008). Uncertainties take into account those from the South African APWP and those from geological ages. Palaeolatitudes and associated uncertainties are shown in **Supplementary Table 1**.

2.2 Localities

The localities represented in this study were selected because they have all produced several identifiable fossils, at least to genus level, which are known to be restricted to a single assemblage zone and with at least one species occurring in several other localities. Of the seven Beaufort Group assemblage zones, the fossils are from two Permian assemblage zones (*Cistecephalus* AZ and *Daptocephalus* AZ) and from the two Triassic zones (*Lystrosaurus declivis* AZ and *Cynognathus* AZ). It is worth mentioning that the samples from the *Daptocephalus* AZ are all restricted to the lower subzone, *Dicynodon-Theriognathus*, while those from the *Cynognathus* AZ are all restricted to the middle *Trirachodon-Kannemeyeria* Subzone.

The Main Karoo Basin is divided into three different geographic sectors on the basis of differing lithostratigraphies (Smith et al., 2020) and each of the four assemblage zones are represented within two of the three sectors (**Fig. 2**). In this study, the different lithostratigraphic provenances are associated with area numbers (**Fig. 2**). Area 2 has been split into 2a and 2b. 2a represents the north-eastern part of the Beaufort Basin, and 2b includes localities close to 24° E longitude and closer to the localities in area 3 than those in area 2a (**Fig. 1, Supplementary Table 1**).

2.3 Analytical techniques

Phosphate ions were isolated from apatite using acid dissolution and anion exchange to measure their specific oxygen isotope composition (Lécuyer, 2004). Silver phosphate crystals were precipitated using a thermostatic bath set at a temperature of 70 °C, then filtered, washed with double deionized water, and dried at 50 °C. The crystals of Ag_3PO_4 were analysed using a high-temperature pyrolysis technique involving a VarioPYROcube™ elemental analyzer (EA) interfaced in continuous flow (CF) mode to an Isoprime™ isotope ratio mass spectrometer (IRMS) (EA-Py-CF-IRMS technique) at the Laboratoire de Géologie de Lyon (UMR 5276, Université Claude Bernard Lyon 1).

For each sample, 5 aliquots of 300 µg of Ag_3PO_4 were mixed with 300 µg of pure black carbon powder and loaded into silver foil capsules. Pyrolysis was performed at 1450 °C in order to produce carbon monoxide (CO). The resulting CO was analysed via continuous flow isotope measurements in a EA carrier gas (Fourel et al., 2011). Their measurements were calibrated against the NBS120c (natural Miocene phosphorite from Florida: $\delta^{18}O = 21.7‰$; Lécuyer et al., 1993) and the NBS127 (barium sulfate, $BaSO_4$: $\delta^{18}O = 9.3‰$; Hut, 1987). Alongside the samples derived from fossil bioapatites, silver phosphate samples precipitated from standard NBS120c were repeatedly analysed ($\delta^{18}O_p = 21.6‰$; $1\sigma = 0.4$; $n = 31$) to control the absence of isotopic fractionation that could have occurred during the wet chemistry. Data are reported as $\delta^{18}O_p$ values vs. V-SMOW in ‰ δ units, wherein $\delta^{18}O_{(V\text{-}SMOW)} = [(\delta^{18}O/\delta^{16}O) / (\delta^{18}O/\delta^{16}O) - 1] *1000$. Stable oxygen isotopes, and corresponding $\delta^{18}O_p$ (V-SMOW) values, from Rey et al. (2016) were at that time only calibrated with the NBS120c standard. They were later recalibrated using both NBS120c and NBS127 standards to improve the accuracy of the low $\delta^{18}O$ values. The new values are used in this study.

To measure the stable oxygen isotope composition of the carbonate group, which is substituted into the hydroxyl and phosphate sites within the carbonated-hydroxyl bioapatite fossil vertebrates under consideration herein, about 10 mg of tooth or bone powder was pre-treated following the protocol of Koch et al. (1997). Organic matter was removed using 2% NaOCl solution before rinsing five times with double deionized water and air-dried at 40 °C for 24 h. Then, 0.1 M acetic acid was added to the powder and left overnight to remove potential secondary precipitated calcite. The powder was then rinsed five times with double deionized water and air-dried at 40 °C overnight. The powder/solution ratio was kept constant at 0.04 g mL^{-1} for both treatments.

Stable isotope ratios were determined using a Thermo Gasbench II at the Stable Light Isotope Laboratory of the Archaeology Department of the University of Cape Town. For each sample, an aliquot of 2 mg of pre-treated apatite was reacted with seven drops of supersaturated orthophosphoric acid at 72 °C for at least two hours under a He atmosphere before starting 9 measurement cycles of the isotopic composition of the CO_2 produced with a Thermo Finnigan Delta Plus XP continuous flow isotope ratio mass spectrometer. The measured oxygen isotopic compositions were normalized relative to the NBS-18 ($\delta^{18}O = -23.03$ ‰ V-PDB) and an internal calcite standard 'Cavendish Marble' ($\delta^{18}O = -8.95$ ‰ V-PDB). Reproducibility for the oxygen isotopic compositions of apatite carbonate is better than ± 0.30‰.

The oxygen isotopic compositions are expressed as δ values relative to V-PDB (in ‰ δ units) and $\delta^{18}O$ values were converted to V-SMOW following the equation of Coplen et al. (1983).

3 Results

3.1 Isotopic values of tooth and bone phosphate

Fossils were sampled for either their tooth or their bone, therefore no direct comparison between the two hard tissues was possible. However, data from Rey et al. (2016) allowed the comparison to show similar $\delta^{18}O$ values of bone and tooth from the same individuals.

Moreover, the $\delta^{18}O_p$ values of bone/tooth range from: 3.5/5.4‰ to 8.0/7.6‰ V-SMOW for specimens from the *Cistecephalus* AZ; 3.9/3.4‰ to 11.3/10.0‰ V-SMOW for the *Daptocephalus* AZ samples; 4.4/6.3‰ to 14.3/13.0‰ V-SMOW for the *Lystrosaurus declivis* AZ specimens; and 6.9/6.0‰ to 13.9/13.8‰ V-SMOW for the *Cynognathus* AZ samples. The comparison of the mean values between bone and tooth using the non-parametric test of Wilcoxon-Mann-Whitney test shows no significant difference for each assemblage zone (**Supplementary Table 2**). Following this absence of difference, the values from bones and teeth are not used separately for the interpretations.

3.2 Comparison between area 2b and area 3

The *Daptocephalus* AZ and the *Cistecephalus* AZ, localities are represented on both sides of the present-day 24°E longitude which divided the Beaufort basin based on differing lithostratigraphy (**Fig. 2**). The values of the *Daptocephalus* AZ from the seven localities in area 2b were compared to those from the four localities on area 3. The Wilcoxon-Mann-Whitney test demonstrates no significant difference between all localities, even when the localities with significantly different means (Ringfontein, Graaff Reinet Commonage and Krugers Kraal) were removed. In particular, the locality Klipfontein, presents some values several per mil higher than the remaining samples from the same time period. However, removing those values does not impact the results. For the *Cistecephalus* AZ, the test compared the values from four localities in area 2b and four in area 3 and no significant differences were identified. Removing the locality Uitspansfontein situated further west than the rest, or the locality Matjeshoek with a different median, did not change the result.

4 Discussion

The wealth of tetrapod fossils from the large Main Karoo Basin enables the sampling of fossils from localities of the same assemblage zone, but which may be geographically separated by several hundreds of kilometres. This facilitates the study of lateral geographic variability in stable isotopes. In this study, this phenomenon is best demonstrated by the sampled Triassic assemblage zones as the Permian localities available for sampling are all grouped in the southwestern part of the basin (**Fig. 1 map**) (Smith, 2020; Smith et al., 2020; Viglietti, 2020).

Palaeolatitudes and palaeolongitudes range from -58.9/-19.9° to-57.3/-18.0° for localities from the *Cistecephalus* AZ; -58.9/-18.6° to-58.4/-18.6° for the ones from the *Daptocephalus* AZ; -61.3/-16.6° to -59.4/-5.3° for the *L. declivis* localities; and -61.6/-12.3% to -59.7/-5.3° for the *Cynognathus* ones. The uncertainty on all values is 4.2° (Supplementary Table 1). Palaeogeography reconstructions from the Permian to the present day have considered Africa as a unified continental block (Torsvik et al., 2012). Thus, for this study the present-day map (**Fig. 1**) was rotated to obtain matching palaeolatitudes (λ) and palaeolongitudes (ϕ) of the localities for each sampled assemblage zone (**Fig. 5**). Mean locality $\delta^{18}O_p$ values per AZ were used to estimate latitudinal and longitudinal gradients to identify observable trends.

4.1 Robustness of the oxygen stable isotope signal

Preservation of the primary isotopic signal can be tested by comparing the $\delta^{18}O_p$ values of each sample with the $\delta^{18}O_c$ values (Iacumin et al., 1996; Zazzo et al., 2004a, b). Carbonate and phosphate oxygen isotopes in apatite of skeletal tissues of extant mammals are positively correlated, because mineral precipitation is close to equilibrium with body water for both chemical groups (Iacumin et al., 1996).

Re-equilibration of both of those compounds during diagenesis is not expected because isotopic exchange rates between carbonate-water and phosphate-water are radically different. Therefore, altered biogenic apatites should show isotopic shifts from the empirical $\delta^{18}O_p$–$\delta^{18}O_c$ line (Zazzo et al., 2004b) while pristine $\delta^{18}O$ values should display a positive regression line with a slope close to unity. Also, at low temperature, the phosphate of the apatite is hardly altered by inorganic effects (Tudge, 1960; Lécuyer et al., 1999) but can be altered by microbially mediated diagenesis (Zazzo et al., 2004a).

Data selected from Rey et al. (2016) all tested for diagenetic alteration of the oxygen stable isotope compositions of the phosphate group and were interpreted to have kept, at least partially, their pristine signal. The new $\delta^{18}O_p$ values obtained from this study were compared to their corresponding $\delta^{18}O_c$ values (**Fig. 3**) and with the regression line from Zazzo et al. (2004b). All data points below the grey area, representing the standard deviation on the equation, were considered to have lost their pristine oxygen isotope compositions of apatite phosphate. The origin is most likely from microbially mediated alteration (Zazzo et al., 2004a) and not from temperature increases caused by the Cape Fold Belt orogenesis that took place during the Late Permian (Blewett et al., 2019) as it affected only single individuals from several places and not complete localities or areas. The values were removed from our dataset and the rest of them are considered to have kept, at least partially, their pristine signal for further interpretation. Remaining data points were also screened by their apatite-bound carbonate content (in weight percent). In extant vertebrates, the carbonate content is often around 5% in bone and dentine and down to 3% in enamel (Bigi et al., 2016), but it can range from less than 1% and up to 13.4 % in shark teeth (Brudefold and Soremark, 1967; Rink and Schwarcz, 1995; Vennemann et al., 2001; Tarnowski et al., 2002). The sampled fossils with values higher than 13.4% were considered to contain additional inorganic carbonate precipitated from diagenetic fluids which would result in potentially biased $\delta^{18}O_c$ values of apatite carbonate (**Supplementary Table 1**).

To conclude, after removal of diagenetically altered samples, and without distinguishing between bone and tooth, the $\delta^{18}O_p$ values range from: 3.5‰ to 8.0‰ V-SMOW for specimens from the *Cistecephalus* AZ; 3.4‰ to 11.3‰ V-SMOW for the *Daptocephalus* AZ samples; 4.4‰ to 14.3‰ V-SMOW for the *Lystrosaurus declivis* AZ specimens; and 6.0‰ to 13.9‰ V-SMOW for the *Cynognathus* AZ samples.

The mean $\delta^{18}O_p$ value for each locality was calculated (**Supplementary Table 1**) and when an individual presented two values (bone and tooth), they were averaged together before being used to generate the mean value of the corresponding locality. Mean $\delta^{18}O_p$ values were also calculated using only values associated with a carbonate content below 6% (**Supplementary Table 1**), to reflect the majority of present day carbonate content (Bigi et al., 2016). Most localities do not have their mean $\delta^{18}O_p$ values affected, or only slightly, by this change of calculation and all interpretations are made using the upper limit of 13.4% of carbonate content. However, we emphasize that three localities do not record any values below 6% of carbonate content: Driefontein in the *Cynognathus* AZ, Stoffelton in the *L. declivis* AZ and Matjesfontein in the *Cistecephalus* AZ.

4.2 Permian isotopic gradients.

For both Permian assemblage zones, the palaeolatitudinal gradients were calculated using a least square linear regression on the mean $\delta^{18}O$ values of the localities. No statistical correlation between the mean $\delta^{18}O$ values of each locality and the corresponding palaeolatitude was found using the Spearman test (p-values of 0.54 and 0.77) **(Supplementary Table 2**).

Similarly, the statistical test of Spearman on the palaeolongitudinal gradients shows no significant correlation, with p-values of 0.67 and 0.96 **(Supplementary Table 2**). The gradient for the *Daptocephalus* AZ being -0.5‰/ϕ is because of the most western locality (Van Wyksfontein, Colesberg district) which is represented by only one value which is higher than the rest of the localities. Removing this locality, the gradient goes down to 0.0 ‰/ϕ.

4.3 Triassic isotopic gradients.

The sampled localities from both Triassic assemblage zones are distributed over a larger area than the Permian sites. Also, the sampled Triassic localities follow a palaeoaxis with localities running both north-south and east-west for the *Lystrosaurus declivis* AZ, while the localities for the *Cynognathus* AZ define a northeast-southwest palaeoaxis (**Fig. 5**).

In the case of the *Lystrosaurus declivis* AZ, the estimated gradients were each calculated using the localities with similar palaeolongitude or palaeolatitude. The three localities

Harrismith (Harrismith district), Donald (Bethulie district) and Klip Fonteyn (Middelburg district) were used to construct the palaeolongitudinal gradient with a value of -1.2 ‰/ϕ (R^2 = 0.99) from east to west. For the palaeolatitudinal gradient, the Donald and Klip Fonteyn localities were removed, for not being aligned with the rest of the localities, to obtain a value of -0.2 ‰/λ (R^2 = 0.90) from north to south (**Supplementary Table 2**). However, even with a remarkably high R^2, the Spearman tests consider these gradients to not be significant with respective p-values of 0.33 and 0.08. We can argue that the first p-value is probably the result of the low number of localities used for the gradient. When the remaining localities are added, R^2 decreases to 0.72, but the p-values for the Spearman test changes to 0.01, making the new gradient of -1.1 ‰/ϕ significant.

For the paleolatitudinal gradient, the p-values of 0.08 is slightly above the arbitrary limit of 0.05 for significance. In statistical terms, it would mean that the gradient of -0.2 ‰/λ could be considered significant if we accept to slightly increase the risk of error by 3%.

For the *Cynognathus* AZ, statistically significant gradients were calculated for both the palaeolongitude and palaeolatitude, but with a possible co-dependence of the results as the localities align on a northeast-southwest axis (**Fig. 5**). The calculated gradients have slopes of -0.6 ‰/ϕ (R^2 = 0.46) from east to west for the palaeolongitude and -0.2 ‰/λ (R^2 = 0.63) from north to south for the palaeolatitude. In these cases, the Spearman tests produced significant p-values, being respectively 0.002 and 0.04. It can be noted that the locality of Driefontein (Rouxville district) is situated far north in the Basin and may influence the gradient calculation. Recalculating the gradient without the Driefontein data results in a new slope of -0.3‰/ϕ (R^2 = 0.72) which maintains its statistical significance (p-value = 0.012). However, the associated palaeolatitudinal gradient does not change much (0.1‰/λ) but becomes non-significant (p-value = 0.16).

4.4 Significance of the gradients.

4.4.1 Permian assemblage zones.

Both the *Cistecephalus* AZ and the *Daptocephalus* AZ did not record significant isotopic gradients suggesting that the entire area (2b+3) hosted the same hydrographic system. The fact that no gradient could be detected is probably because the geographic distance of 100 km between all points is too spatially limited to record any substantial isotopic difference of meteoric waters across the paleolandscape, but one exception occurs for each Assemblage Zone (**Fig. 4**). For the *Cistecephalus* AZ, the locality Uitspansfontein (Beaufort West district), which has a mean value within the range of the other localities, is situated north-west of the rest of the localities (**Fig. 5**). The similar value indicates that this locality is probably under the same hydrographic system as the rest.
For the *Daptocephalus* AZ, the Van Wyksfontein (Colesberg) locality is situated further east (area 2a) and returned a higher value (9.2‰) compared to those of the rest of the localities (between 4.2‰ and 7.3‰).

A first hypothesis regarding this longitude isotopic difference between the areas 2b+3 and the area 2a is a difference in climate. In their study to reconstruct global paleoclimate in the late Permian and Early Triassic, Liu et al. (2021) used the presence of amphibian fossils as an indicator of a warm temperate climate as modern amphibians do not survive below 0°C (Bennett et al., 2018). They concluded that the boundary between warm and cool temperate climate zones roughly extended across South Africa during the Late Permian. This would agree with the low values from areas 2b+3 indicating water originating from a cold temperate climate and the high value from area 2a indicating water originating from the warm temperate one, despite the fact that the localities are on similar palaeolatitude. However, a change in climate is not an abrupt line but rather a gradient and the distance between Van Wyksfontein and the other localities might not be represented by such a difference in the stable isotope composition.

Another hypothesis explaining the isotopic difference could be a different water system affecting the area 2a, than areas 2b+3 as suggested in the simplified palaeogeographic model of the Beaufort Group (e.g., Smith et al., 1993). Indeed, a recent study on sedimentary megafan orientation and their extent (Bordy and Paiva, 2021) in our study area (2b and 3) during an earlier time period, highlights the provenance of sediments (and indirectly of water) from the south, which does not change through the few millions years studied. This study also shows that the megafans were affecting the sedimentology up to 100-200 km north from their source, in the Cape Fold Belt which was moving further south through time. Based on the work of Bordy and Paiva (2021), we propose that the water consumed by our studied animals originated mostly from the higher altitudes on these south-western mountains, which is in accordance with the low $\delta^{18}O$ values measured. The Van Wyksfontein locality was probably not affected by this hydrographic system, explaining the higher $\delta^{18}O$ value.

Evaporation could also account for this higher value if water was sourced from the same location as the other localities but was subject to evaporation before reaching the Van Wyksfontein locality. Such a hypothesis would imply an increase in the values of up to 5‰ over approximately 100-150km. In a modern climatic setting, such a difference has been observed for example in China (Li et al., 2015) with an increase in river $\delta^{18}O$ values from -10.3‰ to -3.0‰ along the Yellow River, and also from -9.5‰ to -6.2‰ along the Liao River.

These increases are interpreted as being the result of evaporation. The effect of evaporation is not isolated but is an important factor in introducing variability into the values measured.

Based on this variability in present-day data, it is difficult to validate the evaporation theory on our Permian data where a larger spread of localities needs to be sampled for a more precise view on the δ^{18}O variability and the environmental events affecting it in the Karoo Basin at this time. However, it can be added that two specimens (*Oudenodon* and *Dicynodon*) from the Klipfontein (Graaff-Reinet district) locality recorded higher values than the remaining sampled specimens from the area 2b and 3, but quite similar to the one from the Van Wyksfontein locality. These two species were also sampled from several other localities where their values are not different from the rest of the sampled species (**Supplementary Table 1**). These higher values might be an indication that these specimens consumed water further north than where they were excavated.

4.4.2 *Lystrosaurus declivis* AZ

The gradients are based on a selection of localities with either similar palaeolatitude or palaeolongitude. The palaeolatitudinal gradient of 0.2‰/λ could be explained by Rayleigh distillation, like the present-day latitudinal gradient. The stable oxygen isotope palaeogradient is flatter than that of the present-day (**Fig. 4**), estimated at 0.4‰/λ within the latitude of -61.5° to -59°. Adding to its debatable significance (p-value = 0.08), all the δ^{18}O mean values fit within the large range of present-day data. However, such a flatter gradient would indicate warmer climatic conditions, at a global scale which accords with the greenhouse climate of the Early Triassic inferred from a range of diverse proxies (Sun et al., 2012; Retallack, 2013; Rey et al., 2016; Nowak et al., 2020) and would support the conclusion as a meaningful latitudinal gradient. However, this is based on assuming that the main moisture-rich air mass is moving from north to south but little work was done to identify the winds direction during the Triassic of South Africa. Simulated climate for the Late Permian suggests that the atmospheric circulation in southern Africa is dominated by westerlies at that time (Fluteau et al., 2001). However, the low spatial resolution of the climate model used in this work did not allow to well account for the Gondwanides topography in the experiment. Therefore, it is difficult to conclude if the palaeolatiotudinal gradient originates from Rayleigh distillation.

Another hypothesis is the influence of evaporation. Nowadays, the Cape Fold Belt is limited to the western part of the country but is assumed to have been present south of the present shoreline during the Permo-Triassic (Hiller and Stavrakis, 1984; Veevers and Powell, 1994; Catuneanu and Elango, 2001). Studies on paleocurrents and sedimentology (Hiller and Stavrakis, 1984; Smith, 1995; Haycock et al., 1997) demonstrate that the main direction of rivers in the eastern part of the basin where oriented south to north, in accordance with the presence of a large mountain chain.This river flow, from south to north, associated with the hot climate of the Early Triassic (Rey et al., 2016), corresponds to the recorded mean $\delta^{18}O$ values with the lowest values in the south, originating from the mountains, and the highest in the north, after the effect of evaporation on the river water.

Concerning the palaeolongitudinal gradient, while focusing on middle to high latitudes, present-day data on ‘continental effect’ have reported values for Europe of 2‰/1000km (0.12‰/ϕ for 60° of paleolatitude) between Ireland and the Ural Mountains (Rozanski et al., 1993). Deininger et al. (2016) calculated similar gradients ranging from 0.26/100km to 0.16/km, highlighting the influence of the North Atlantic Ocean on the intensity of the gradient. Specifically focusing on the Adriatic-Pannonian region, Kern et al. (2020) proposed a ‘continental effect’ gradient for winter precipitation of 2.4‰/100km (1.4‰/ϕ). This higher gradient is probably due to a change of the main moisture, with the first 400km being mostly influenced by the Mediterranean Sea, then by moisture from the Atlantic Ocean. The Mediterranean Basin is characterized by a specific relationship between $\delta^{18}O$ and air temperature that differs from the rest of Europe (Gat and Carmi, 1970; Lécuyer et al., 2018, 2021).

Our Main Karoo Basin data gives a gradient of 1.2‰/ϕ, similar to present day Central Europe (Kern et al., 2020), suggesting a ‘continental effect’ that was probably influenced by a change to a main air mass with a different $\delta^{18}O_{mw}$. This ‘continental effect’ theory only works if the main moisture-rich air mass is coming from the east. Same as with the palaeolatitudes, it is difficult to define the wind direction at this time, especially if several main currents were involved in the process. Considering a westerly wind regime (Fluteau et al., 2001), a ‘continental effect’ would be expected in the opposite direction with the highest values in the western part of the Basin.

Another hypothesis is that the differences manifested by our calculated gradient could indicate that the Triassic localities do not record a continuous longitudinal gradient, but rather different rates of evaporation linked to their distance to the mountains. In the western part on the basin, the incoming water originated from the same source as during the late Permian (Smith, 1995; Bordy and Paiva, 2021). Therefore, the Klip Fonteyn (Middleburg district) locality in area 2b and Donald (Bethulie district) locality in area 2a recorded a similar signal as the eastern locality or Van Wyksfontein locality during the *Daptocephalus* AZ, which has values which increase with distance from the Cape Fold Belt. This theory goes against a possible longitudinal gradient from east to west but weighs in favour of an evaporation effect affecting the whole basin once when all localities are considered.

4.4.3 *Cynognathus* AZ

The localities present a gradient of values with the highest measured $\delta^{18}O_p$ values being in the northeast part of the basin while the lowest are situated on the southwestern part (**Fig. 4**). This value repartition seems to fit with the other mean $\delta^{18}O$ values obtained from the other assemblage zones. Therefore, a potential latitudinal gradient based on the Rayleigh distillation model and a longitudinal gradient generated from a ‘continental effect’ with east to west moisture-rich air mass is probably not the cause of the values recorded here. Instead, with a change in the $\delta^{18}O$ values in river systems but not their origins (Smith, 1995), the lowest values are interpreted to correspond to ingested water coming from the Cape Fold Belt, with little to no evaporation due to the proximity, while the other localities have recorded more evaporated values the furthest they are from the mountains.

5 Conclusion

The large study of the $\delta^{18}O_p$ of the apatite of Permo-Triassic vertebrates across the entire Main Karoo Karoo Basin of South Africa reveals that the localities in the Permian assemblage zones, in close proximity, produces a homogenous $\delta^{18}O_p$ signal. This signal, constituted of low values, reflect drinking water originating from a colder environment, most likely at high altitudes in the Cape Fold Belt to the southwest of the sampled localities. Further East at the time, the $\delta^{18}O_p$ at Van Wyksfontein locality from the *Daptocephalus* AZ suggests an evaporation effect on river water coming from the Cape Fold Belt. For the Triassic, the *Lystrosaurus declivis* AZ shows a significant latitudinal gradient which is more likely to reflect an evaporation effect in a river system originating from the mountain chain in the south and flowing toward north, rather than the Rayleigh distillation model in the opposite direction. The *Cynognathus* AZ recorded a similar signal with lowest values in the southwest and the highest in the northeast. This gradient is also interpreted as an evaporation effect.

The variability in $\delta^{18}O$ values documented in this study highlights the fact that the Main Karoo Basin was influenced by hydraulic systems all originating from the southern mountains, constituting the Cape Fold Belt today. The origin of the water did not change through the time range of the studied assemblage zones.

The ‘evaporation effect’ is highlighted throughout the Basin, suggesting that most of the available water for the species was from river systems rather than from precipitation directly in the Basin.

This variability in $\delta^{18}O$ values needs to be considered in future isotopic studies of this region, especially for those devoted to climate variation or vertebrate physiology as the choice of the localities would impact results. Further work is required to increase the spatial distribution of sampled areas, especially adding localities in the southeast part of the Basin. This would allow for further quantification of palaeogradients across the Main Karoo Basin.

Acknowledgments

The authors would greatly thank Prof. Bruce Rubidge for his expertise on the Permo-Triassic Main Karoo Basin and its fauna, which greatly improved this manuscript. Dr. Hannah F. James is acknowledged for language editing.

The authors thank the South African Heritage Resources Agency (SAHRA) for their authorization to sample the fossils, and export sub-samples for stable isotope analysis of phosphate (PermitID: 2790 and 2841). Marion Bamford, Bernhard Zipfel and Sifelani Jirah are acknowledged for access to the Karoo fossil collections of the ESI and for sanction to sample the tetrapod fossils. We also thank both anonymous reviewers for their constructive and helpful comments. This work was supported by the Palaeontological Scientific Trust (PAST) and its Scatterlings of Africa programmes, National Research Foundation (NRF) African Origins Platform of South Africa and DST/NRF Centre of Excellence in Palaeosciences.

References

Amiot, R., Lécuyer, C., Buffetaut, E., Fluteau, F., Legendre, S., Martineau, F., 2004. Latitudinal temperature gradient during the Cretaceous Upper Campanian–Middle Maastrichtian: $\delta^{18}O$ record of continental vertebrates. Earth and Planetary Science Letters 226, 255–272. https://doi.org/10.1016/j.epsl.2004.07.015

Bennett, J.M., Calosi, P., Clusella-Trullas, S., Martínez, B., Sunday, J., Algar, A.C., Araújo, M.B., Hawkins, B.A., Keith, S., Kühn, I., 2018. GlobTherm, a global database on thermal tolerances for aquatic and terrestrial organisms. Scientific Data 5, 1–7.

Bigi, A., Boanini, E., Gazzano, M., 2016. 7 - Ion substitution in biological and synthetic apatites, in: Aparicio, C., Ginebra, M.-P. (Eds.), Biomineralization and Biomaterials. Woodhead Publishing, Boston, pp. 235–266. https://doi.org/10.1016/B978-1-78242-338-6.00008-9

Blewett, S.C.J., Phillips, D., Matchan, E.L., 2019. Provenance of Cape Supergroup sediments and timing of Cape Fold Belt orogenesis: Constraints from high-precision 40Ar/39Ar dating of muscovite. Gondwana Research 70, 201–221. https://doi.org/10.1016/j.gr.2019.01.009

Bond, D.P.G., Grasby, S.E., 2017. On the causes of mass extinctions. Palaeogeography, Palaeoclimatology, Palaeoecology, Mass Extinction Causality: Records of Anoxia, Acidification, and Global Warming during Earth's Greatest Crises 478, 3–29. https://doi.org/10.1016/j.palaeo.2016.11.005

Bordy, E.M., Abrahams, M., Sharman, G.R., Viglietti, P.A., Benson, R.B.J., McPhee, B.W., Barrett, P.M., Sciscio, L., Condon, D., Mundil, R., Rademan, Z., Jinnah, Z., Clark, J.M., Suarez, C.A., Chapelle, K.E.J., Choiniere, J.N., 2020. A chronostratigraphic framework for the upper Stormberg Group: Implications for the Triassic-Jurassic boundary in southern Africa. Earth-Science Reviews 203, 103120. https://doi.org/10.1016/j.earscirev.2020.103120

Bordy, E.M., Paiva, F., 2021. Stratigraphic architecture of the Karoo river channels at the End-Capitanian. Frontiers in Earth Science 8, 701.

Botha, J., Angielczyk, K.D., 2007. An integrative approach to distinguishing the Late Permian dicynodont species *Oudenodon bainii* and *Tropidostoma microtrema* (Therapsida: Anomodontia). Palaeontology 50, 1175–1209.

Botha, J., Huttenlocker, A.K., Smith, R.M., Prevec, R., Viglietti, P., Modesto, S.P., 2020. New geochemical and palaeontological data from the Permian-Triassic boundary in the South African Karoo Basin test the synchronicity of terrestrial and marine extinctions. Palaeogeography, Palaeoclimatology, Palaeoecology 540, 109467.

Botha, J., Lee-Thorp, J., Chinsamy, A., 2005. The palaeoecology of the non-mammalian cynodonts Diademodon and Cynognathus from the Karoo Basin of South Africa, using stable light isotope analysis. Palaeogeography, Palaeoclimatology, Palaeoecology 223, 303–316.

Botha-Brink, J., Huttenlocker, A.K., Modesto, S.P., 2014. Vertebrate paleontology of Nooitgedacht 68: A *Lystrosaurus maccaigi*-Rich Permo-Triassic boundary locality in South Africa, in: Kammerer, C.F., Angielczyk, K.D., Fröbisch, J. (Eds.), Early Evolutionary History of the Synapsida. Springer Netherlands, pp. 289–304.

Brudefold, F., Soremark, R., 1967. Chemistry of the mineral phase of enamel—Crystalline organization of dental mineral, in: Structural and Chemical Organization of Teeth. Miles AED, San Diego, pp. 247–277.

Catuneanu, O., Elango, H.N., 2001. Tectonic control on fluvial styles: the Balfour Formation of the Karoo Basin, South Africa. Sedimentary Geology 140, 291–313.

Ciais, P., Jouzel, J., 1994. Deuterium and oxygen 18 in precipitation: Isotopic model, including mixed cloud processes. J. Geophys. Res. 99, 16793–16803. https://doi.org/10.1029/94JD00412

Coplen, T.B., Kendall, C., Hopple, J., 1983. Comparison of stable isotope reference samples. Nature 302, 236.

D'Angela, D., Longinelli, A., 1990. Oxygen isotopes in living mammal's bone phosphate: Further results. Chemical Geology: Isotope Geoscience section 86, 75–82. https://doi.org/10.1016/0168-9622(90)90007-Y

Dansgaard, W., 1964. Stable isotopes in precipitation. Tellus 16, 436–468. https://doi.org/10.1111/j.2153-3490.1964.tb00181.x

Day, M.O., Ramezani, J., Bowring, S.A., Sadler, P.M., Erwin, D.H., Abdala, F., Rubidge, B.S., 2015. When and how did the terrestrial mid-Permian mass extinction occur? Evidence from the tetrapod record of the Karoo Basin, South Africa. Proceedings of the Royal Society of London B: Biological Sciences 282, 20150834.

Day, M.O., Rubidge, B.S., 2021. The late Capitanian mass extinction of terrestrial vertebrates in the Karoo Basin of South Africa. Frontiers in Earth Science 9, 15.

Fluteau, F., Besse, J., Broutin, J., Ramstein, G., 2001. The Late Permian climate. What can be inferred from climate modelling concerning Pangea scenarios and Hercynian range altitude? Palaeogeography, Palaeoclimatology, Palaeoecology 167, 39–71.

Fourel, F., Martineau, F., Lécuyer, C., Kupka, H.-J., Lange, L., Ojeimi, C., Seed, M., 2011. $^{18}O/^{16}O$ ratio measurements of inorganic and organic materials by elemental analysis-pyrolysis-isotope ratio mass spectrometry continuous-flow techniques. Rapid communications in mass spectrometry: RCM 25, 2691–2696. https://doi.org/10.1002/rcm.5056

Fricke, H.C., O'Neil, J.R., 1999. The correlation between $^{18}O/^{16}O$ ratios of meteoric water and surface temperature: its use in investigating terrestrial climate change over geologic time. Earth and Planetary Science Letters 170, 181–196. https://doi.org/10.1016/S0012-821X(99)00105-3

Gastaldo, R.A., Kamo, S.L., Neveling, J., Geissman, J.W., Bamford, M., Looy, C.V., 2015. Is the vertebrate-defined Permian-Triassic boundary in the Karoo Basin, South Africa, the terrestrial expression of the end-Permian marine event? Geology 43, 939–942.

Gastaldo, R.A., Kamo, S.L., Neveling, J., Geissman, J.W., Looy, C.V., Martini, A.M., 2020. The base of the *Lystrosaurus* Assemblage Zone, Karoo Basin, predates the end-Permian marine extinction. Nature Communications 11, 1–8.

Gat, J.R., 2008. The isotopic composition of evaporating waters – review of the historical evolution leading up to the Craig–Gordon model. Isotopes in Environmental and Health Studies 44, 5–9. https://doi.org/10.1080/10256010801887067

Gat, J.R., Carmi, I., 1970. Evolution of the isotopic composition of atmospheric waters in the Mediterranean Sea area. Journal of Geophysical Research 75, 3039–3048.

Grafenstein, U.V., Bocherens, H., Müller, J., Trimborn, P., Alefs, J., 1996. A 200 year mid-European air temperature record preserved in lake sediments: An extension of the $\delta^{18}O_p$-air temperature relation into the past. Geochimica and Cosmochimica Acta 60, 4025–4036. https://doi.org/10.1016/S0016-7037(96)00230-X

Haycock, C.A., Mason, T.R., Watkeys, M.K., 1997. Early Triassic palaeoenvironments in the eastern Karoo foreland basin, South Africa. Journal of African Earth Sciences 24, 79–94.

Hiller, N., Stavrakis, N., 1984. Permo-Triassic fluvial systems in the southeastern Karoo basin, South Africa. Palaeogeography, Palaeoclimatology, Palaeoecology 45, 1–21.

Hillson, S., 1986. Teeth. Cambridge University Press, Cambridge.

Hut, G., 1987. Consultants’ group meeting on stable isotope reference samples for geochemical and hydrological investigations.

Iacumin, P., Bocherens, H., Mariotti, A., Longinelli, A., 1996. Oxygen isotope analyses of co-existing carbonate and phosphate in biogenic apatite: a way to monitor diagenetic alteration of bone phosphate? Earth and Planetary Science Letters 142, 1–6. https://doi.org/10.1016/0012-821X(96)00093-3

Kern, Z., Hatvani, I.G., Czuppon, G., Fórizs, I., Erdélyi, D., Kanduč, T., Palcsu, L., Vreča, P., 2020. Isotopic ‘altitude’and ‘continental’effects in modern precipitation across the Adriatic–Pannonian Region. Water 12, 1797.

Kern, Z., Kohán, B., Leuenberger, M., 2014. Precipitation isoscape of high reliefs: interpolation scheme designed and tested for monthly resolved precipitation oxygen isotope records of an Alpine domain. Atmospheric chemistry and physics 14, 1897–1907.

Koch, P.L., Tuross, N., Fogel, M.L., 1997. The effects of sample treatment and diagenesis on the isotopic integrity of carbonate in biogenic hydroxylapatite. Journal of Archaeological Science 24, 417–430.

Kohn, M.J., Schoeninger, M., Valley, J., 1996. Herbivore tooth oxygen isotope compositions: Effects of diet and physiology. Geochimica and Cosmochimica Acta 60, 3889–3896. https://doi.org/10.1016/0016-7037(96)00248-7

Kurita, N., Ichiyanagi, K., Matsumoto, J., Yamanaka, M.D., Ohata, T., 2009. The relationship between the isotopic content of precipitation and the precipitation amount in tropical regions. Journal of Geochemical Exploration 102, 113–122.

Lachniet, M.S., Patterson, W.P., 2009. Oxygen isotope values of precipitation and surface waters in northern Central America (Belize and Guatemala) are dominated by temperature and amount effects. Earth and Planetary Science Letters 284, 435–446.

Lécuyer, C., 2004. Oxygen isotope analysis of phosphate. Handbook of stable isotope analytical techniques 1, 482–499.

Lécuyer, C., Atrops, F., Amiot, R., Angst, D., Daux, V., Flandrois, J.-P., Fourel, F., Rey, K., Royer, A., Seris, M., 2018. Tsunami sedimentary deposits of Crete records climate during the 'Minoan Warming Period'(≈ 3350 yr BP). The Holocene 28, 914–929.

Lécuyer, C., Bojar, A.-V., Daux, V., Legendre, S., 2021. Geographic variations in the slope of the δ^2H–δ^{18}O meteoric water line over Europe: a record of increasing continentality. Geological Society, London, Special Publications 507, 5–17.

Lécuyer, C., Grandjean, P., Sheppard, S.M.F., 1999. Oxygen isotope exchange between dissolved phosphate and water at temperatures ≤135°C: inorganic versus biological fractionations. Geochimica and Cosmochimica Acta 63, 855–862. https://doi.org/10.1016/S0016-7037(99)00096-4

Lee, J.-E., Fung, I., 2008. "Amount effect" of water isotopes and quantitative analysis of post-condensation processes. Hydrological Processes 22, 1–8.

Li, S.-L., Yue, F.-J., Liu, C.-Q., Ding, H., Zhao, Z.-Q., Li, X., 2015. The O and H isotope characteristics of water from major rivers in China. Chinese Journal of Geochemistry 34, 28–37.

Liu, J., Angielczyk, K.D., Abdala, F., 2021. Permo-Triassic tetrapods and their climate implications. Global and Planetary Change 205, 103618.

Lucas, P., Constantino, P., Wood, B., Lawn, B., 2008. Dental enamel as a dietary indicator in mammals. BioEssays 30, 374–385.

MacLeod, K.G., Quinton, P.C., Bassett, D.J., 2017. Warming and increased aridity during the earliest Triassic in the Karoo Basin, South Africa. Geology 45, 483–486.

Mills, A., 1967. Structural and Chemical Organization of Teeth. Elsevier.

Niewodniczański, J., Grabczak, J., Barański, L., Rzepka, J., 1981. The altitude effect on the isotopic composition of snow in high mountains. Journal of Glaciology 27, 99–111.

Nowak, H., Vérard, C., Kustatscher, E., 2020. Palaeophytogeographical Patterns Across the Permian–Triassic Boundary. Frontiers in Earth Science 8.

Retallack, G.J., 2013. Permian and Triassic greenhouse crises. Gondwana Research 24, 90–103. https://doi.org/10.1016/j.gr.2012.03.003

Rey, K., Amiot, R., Fourel, F., Rigaudier, T., Abdala, F., Day, M.O., Fernandez, V., Fluteau, F., France-Lanord, C., Rubidge, B.S., Smith, R.M., Viglietti, P.A., Zipfel, B., Lécuyer, C., 2016. Global climate perturbations during the Permo-Triassic mass extinctions recorded by continental tetrapods from South Africa. Gondwana Research 37, 384–396. https://doi.org/10.1016/j.gr.2015.09.008

Rey, K., Day, M.O., Amiot, R., Goedert, J., Lécuyer, C., Sealy, J., Rubidge, B.S., 2018. Stable isotope record implicates aridification without warming during the late Capitanian mass extinction. Gondwana Research 59, 1–8.

Rink, W.J., Schwarcz, H.P., 1995. Tests for diagenesis in tooth enamel: ESR dating signals and carbonate contents. Journal of Archaeological Science 22, 251–255. https://doi.org/10.1006/jasc.1995.0026

Rozanski, K., Araguás-Araguás, L., Gonfiantini, R., 1993. Isotopic patterns in modern global precipitation. Climate change in continental isotopic records 1–36.

Rozanski, K., Sonntag, C., Münnich, K.O., 1982. Factors controlling stable isotope composition of European precipitation. Tellus 34, 142–150. https://doi.org/10.1111/j.2153-3490.1982.tb01801.x

Rubidge, B.S., Erwin, D.H., Ramezani, J., Bowring, S.A., Klerk, W.J. de, 2013. High-precision temporal calibration of Late Permian vertebrate biostratigraphy: U-Pb zircon constraints from the Karoo Supergroup, South Africa. Geology 41, 363–366. https://doi.org/10.1130/G33622.1

Siegenthaler, U., Oeschger, H., 1980. Correlation of ^{18}O in precipitation with temperature and altitude. Nature 285, 314–317. https://doi.org/10.1038/285314a0

Smith, R.M.H., 2020. Biostratigraphy of the *Cistecephalus* Assemblage Zone (Beaufort Group, Karoo Supergroup), South Africa. South African Journal of Geology 2020 123, 181–190.

Smith, R.M.H., 1995. Changing fluvial environments across the Permian-Triassic boundary in the Karoo Basin, South Africa and possible causes of tetrapod extinctions. Palaeogeography, Palaeoclimatology, Palaeoecology 117, 81–104. https://doi.org/10.1016/0031-0182(94)00119-S

Smith, R.M.H., Botha-Brink, J., 2014. Anatomy of a mass extinction: Sedimentological and taphonomic evidence for drought-induced die-offs at the Permo-Triassic boundary in the main Karoo Basin, South Africa. Palaeogeography, Palaeoclimatology, Palaeoecology 396, 99–118. https://doi.org/10.1016/j.palaeo.2014.01.002

Smith, R.M.H., Eriksson, P.G., Botha, W.J., 1993. A review of the stratigraphy and sedimentary environments of the Karoo-aged basins of Southern Africa. Journal of African Earth Sciences (and the Middle East), Geology and Development in Southern Africa 16, 143–169. https://doi.org/10.1016/0899-5362(93)90164-L

Smith, R.M.H., Rubidge, B.S., Day, M.O., Botha, J., 2020. Introduction to the tetrapod biozonation of the Karoo Supergroup. South African Journal of Geology 123, 131–140. https://doi.org/10.25131/sajg.123.0009

Smith, R.M.H., Ward, P.D., 2001. Pattern of vertebrate extinctions across an event bed at the Permian-Triassic boundary in the Karoo Basin of South Africa. Geology 29, 1147–1150. https://doi.org/10.1130/0091-7613(2001)029<1147:POVEAA>2.0.CO;2

Stanley, S.M., Yang, X., 1994. A double mass extinction at the end of the Paleozoic Era. Science 266, 1340–1344. https://doi.org/10.1126/science.266.5189.1340

Sullivan, C., Reisz, R.R., Smith, R.M., 2003. The Permian mammal-like herbivore *Diictodon*, the oldest known example of sexually dimorphic armament. Proceedings of the Royal Society of London. Series B: Biological Sciences 270, 173–178.

Sun, Y., Joachimski, M.M., Wignall, P.B., Yan, C., Chen, Y., Jiang, H., Wang, L., Lai, X., 2012. Lethally hot temperatures during the Early Triassic greenhouse. Science 338, 366–370. https://doi.org/10.1126/science.1224126

Tarnowski, C.P., Ignelzi, M.A., Morris, M.D., 2002. Mineralization of developing mouse calvaria as revealed by Raman microspectroscopy. Journal of Bone and Mineral Research 17, 1118–1126. https://doi.org/10.1359/jbmr.2002.17.6.1118

Torsvik, T.H., Müller, R.D., Van der Voo, R., Steinberger, B., Gaina, C., 2008. Global plate motion frames: toward a unified model. Reviews of geophysics 46.

Torsvik, T.H., Van der Voo, R., Preeden, U., Mac Niocaill, C., Steinberger, B., Doubrovine, P.V., van Hinsbergen, D.J.J., Domeier, M., Gaina, C., Tohver, E., Meert, J.G., McCausland, P.J.A., Cocks, L.R.M., 2012. Phanerozoic polar wander, palaeogeography and dynamics. Earth-Science Reviews 114, 325–368. https://doi.org/10.1016/j.earscirev.2012.06.007

Tudge, A.P., 1960. A method of analysis of oxygen isotopes in orthophosphate—its use in the measurement of paleotemperatures. Geochimica et Cosmochimica Acta 18, 81–93. https://doi.org/10.1016/0016-7037(60)90019-3

Veevers, J.J., Powell, C.M., 1994. Permian-Triassic Pangean Basins and Foldbelts Along the Panthalassan Margin of Gondwanaland. Geological Society of America.

Vennemann, T.W., Hegner, E., Cliff, G., Benz, G.W., 2001. Isotopic composition of recent shark teeth as a proxy for environmental conditions. Geochimica et Cosmochimica Acta 65, 1583–1599. https://doi.org/10.1016/S0016-7037(00)00629-3

Viglietti, P.A., 2020. Biostratigraphy of the *Daptocephalus* Assemblage Zone (Beaufort Group, Karoo Supergroup), South Africa. South African Journal of Geology 2020 123, 191–206.

Viglietti, P.A., Benson, R.B., Smith, R.M., Botha, J., Kammerer, C.F., Skosan, Z., Butler, E., Crean, A., Eloff, B., Kaal, S., 2021. Evidence from South Africa for a protracted end-Permian extinction on land. Proceedings of the National Academy of Sciences 118.

Viglietti, P.A., Smith, R.M., Rubidge, B.S., 2018. Changing palaeoenvironments and tetrapod populations in the Daptocephalus Assemblage Zone (Karoo Basin, South Africa) indicate early onset of the Permo-Triassic mass extinction. Journal of African Earth Sciences 138, 102–111.

Wen, R., Tian, L., Weng, Y., Liu, Z., Zhao, Z., 2012. The altitude effect of δ^{18}O in precipitation and river water in the Southern Himalayas. Chinese Science Bulletin 57. https://doi.org/10.1007/s11434-012-4992-7

Winnick, M.J., Chamberlain, C.P., Caves, J.K., Welker, J.M., 2014. Quantifying the isotopic 'continental effect.' Earth and Planetary Science Letters 406, 123–133. https://doi.org/10.1016/j.epsl.2014.09.005

Yde, J.C., Knudsen, N.T., Steffensen, J.P., Carrivick, J.L., Hasholt, B., Ingeman-Nielsen, T., Kronborg, C., Larsen, N.K., Mernild, S.H., Oerter, H., Roberts, D.H., Russell, A.J., 2016. Stable oxygen isotope variability in two contrasting glacier river catchments in Greenland. Hydrology and Earth System Sciences 20, 1197–1210. https://doi.org/10.5194/hess-20-1197-2016

Yoshimura, K., Oki, T., Ohte, N., Kanae, S., 2003. A quantitative analysis of short-term ^{18}O variability with a Rayleigh-type isotope circulation model. J. Geophys. Res. 108, 4647. https://doi.org/10.1029/2003JD003477

Young, B., Woodford, P., O'Dowd, G., 2013. Wheater's Functional Histology: A Text and Colour Atlas. Elsevier Health Sciences.

Zazzo, A., Lécuyer, C., Mariotti, A., 2004a. Experimentally-controlled carbon and oxygen isotope exchange between bioapatites and water under inorganic and microbially-mediated conditions. Geochimica et Cosmochimica Acta 68, 1–12. https://doi.org/10.1016/S0016-7037(03)00278-3

Zazzo, A., Lécuyer, C., Sheppard, S.M.F., Grandjean, P., Mariotti, A., 2004b. Diagenesis and the reconstruction of paleoenvironments: A method to restore original $\delta^{18}O$ values of carbonate and phosphate from fossil tooth enamel. Geochimica et Cosmochimica Acta 68, 2245–2258. https://doi.org/10.1016/j.gca.2003.11.009

Zhao, Y., Zheng, B., Wang, L., Qin, Y., Li, H., Cao, W., 2016. Characterization of Mixing Processes in the Confluence Zone between the Three Gorges Reservoir Mainstream and the Daning River Using Stable Isotope Analysis. Environ. Sci. Technol. 50, 9907–9914. https://doi.org/10.1021/acs.est.5b01132

Figures and Tables Captions

Figure 1: Localities in the Main Karoo Basin of studied Permian and Triassic tetrapod-bearing localities along with their assemblage zone provenance. Locality abbreviations are explained in Supplementary Table 1. Area numbers are explained in **Fig. 2**.

Figure 2: Integrated stratigraphic chart of the Beaufort Group of the Main Karoo Basin tentatively correlated to the Assemblage zones based on Smith et al. (2020). Assemblage zones of the processed samples are coloured. Locality abbreviations are explained in **Supplementary Table 1**. Sources for radiometric age: (1) Gastaldo et al. (2020); (2) Botha et al. (2020); (3) Gastaldo et al. (2015); (4) Rubidge et al. (2013).

Figure 3: Phosphate $\delta^{18}O_p$ values plotted against corresponding carbonate $\delta^{18}O_c$ values, with the empirical isotopic equilibrium line (Zazzo et al., 2004b), a slope close to unity (bold line), and the uncertainty on the intercept value (grey area). The altered samples are circumscribed by a black line. Circle symbols correspond to previously published data (Rey et al., 2016) while triangle symbols correspond to new data collected in this study.

Figure 4: Mean $\delta^{18}O_p$ values per locality plotted against their palaeolatitudes and palaeolongitudes. Black lines and grey areas represent the present-day modelled curve and range of $\delta^{18}O_p$ for terrestrial vertebrate calculated for each latitude from modern precipitation using the following relationship : $\delta^{18}O_p = \delta^{18}O_{mw} + 21.9$ (Amiot et al., 2004).

Figure 5: Present day map rotated to reflect the palaeopositions of the localities for each of the assemblage zones. Colours of the localities reflect their mean $\delta^{18}O$ values in ‰, V-SMOW.

Supplementary Table 1: Stable isotope compositions of phosphate ($\delta^{18}O_p$) and carbonate ($\delta^{18}O_c$) of apatite of all sampled tetrapods with locality, area number, assemblage zone and carbonate content for each specimen. Asterisks indicate samples considered diagenetically-altered. Crosses indicate potentially carbonate-altered sampled (over 6% carbonate content). Italic means $\delta^{18}O_p$ values are calculated with sampled containing less than 6% of carbonate content.

Supplementary Table 2: P-values from test of normality (Shapiro test) and the comparison of median (Wilcoxon-Mann-Whitney test) between bone and teeth values per period and between localities as well as calculated gradient between mean $\delta^{18}O_p$ values with palaeolongitudes and latitudes and the p-values of their significance (Spearman test).