A stable isotope aridity index for terrestrial environments

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We use the oxygen isotopic composition of tooth enamel from multiple mammalian taxa across eastern Africa to present a proxy for aridity. Here we report tooth enamel $\delta^{18}O$ values of 14 species from 18 locations and classify them according to their isotopic sensitivity to environmental aridity. The species are placed into two groups, evaporation sensitive (ES) and evaporation insensitive (EI). Tooth enamel $\delta^{18}O$ values of ES animals increase with aridity, whereas the tooth enamel $\delta^{18}O$ values of EI animals track local meteoric water $\delta^{18}O$ values, demonstrating that bioapatite $\delta^{18}O$ values of animals with different behaviors and physiologies record different aspects of the same environment. The enrichment between tooth enamel $\delta^{18}O$ values of ES and EI animals records the degree of ^{18}O enrichment between evaporated water (ingested water or body water) and source water, which increases with environmental aridity. Recognition of the ES–EI distinction creates the opportunity to use the ^{18}O composition of bioapatite as an index of terrestrial aridity.

bioapatite | East Africa | oxygen-18 | mammals | water use

Terrestrial responses to major climate changes, such as glaciations, orogenic events, and shifts in ocean circulation, are often characterized in terms of water availability or aridity (1–3). Although aridity proxies exist for different terrestrial settings (4–6), they are not applicable in every circumstance and additional proxies must be developed for further study of terrestrial environmental change. The ^{18}O composition of bioapatite has been used as a proxy for rainfall $\delta^{18}O$ and seasonality of past environments (7, 8), but its utility in paleoenvironmental problems is limited by the complexity of climatic, environmental, physiological, and behavioral variables that influence bioapatite $\delta^{18}O$ values. The correlation between bioapatite $\delta^{18}O$ values and both meteoric water $\delta^{18}O$ values and relative humidity demonstrate that animals have different isotopic responses to environmental change (7, 9, 10). In this study, we present tooth enamel $\delta^{18}O$ data of 14 mammal species sampled from 18 locations in eastern Africa, which represent a gradient in environmental aridity. This data set shows that the tooth enamel $\delta^{18}O$ values of some species vary with aridity whereas those of other species track the ^{18}O composition of meteoric water. We use the different isotopic responses of the sampled species as the empirical basis for an aridity proxy.

Results

There is a marked increase in water deficit (WD) from the closed canopy Ituri Forest in the Democratic Republic of Congo to the arid shrublands of Lake Turkana in northern Kenya (Table 1, which is published as supporting information on the PNAS web site). WD in these regions negatively correlates to mean annual relative humidity (RH) ($P < 0.01$) and is used as a measure of aridity. Although previous studies compare bioapatite $\delta^{18}O$ values with RH (6, 9, 10), RH data are not used here because they are not available for all study locations. Meteoric water $\delta^{18}O$ ($\delta^{18}O_{mw}$) values at these localities average $-3.1‰$ (SE, $1.2‰$) and do not vary significantly with WD ($P = 0.476$). We use this average $\delta^{18}O_{mw}$ value for localities where water isotope data are not available.

The isotopic enrichment between tooth enamel and meteoric water ($\varepsilon_{enamel\text{-}mw}$), where $\varepsilon_{A\text{-}B} = ((R_A/R_B) - 1) \times 1{,}000$ is plotted as a function of WD (Figs. 1 and 2). The use of an enrichment factor, ε, enables tooth enamel $\delta^{18}O$ ($\delta^{18}O_{enamel}$) values to be compared between sites, regardless of changes in $\delta^{18}O_{mw}$ values. $\varepsilon_{enamel\text{-}mw}$ for hippopotamus (*Hippopotamus amphibius*), bush pig (*Potomochoerus larvatus*), elephant (*Loxodonta africana*), rhinoceros (*Diceros bicornis*), warthog (*Phacochoerus africanus*), zebra (*Equus burchelli*), impala (*Aepyceros melampus*), and baboon (*Papio anubis*), is not sensitive to changes in WD (*P* values range from 0.14 to 0.91) (Fig. 1). Baboons from Mpala are the only mixed (C_3–C_4) feeding baboons (otherwise C_3 browsers), and when not considered the baboon regression has a *P* value <0.001 instead of 0.14. $\varepsilon_{enamel\text{-}mw}$ of a second group, giraffids (*Giraffa camelopardalis* and *Okapia johnstoni*), oryx (*Oryx beisa*), dikdik (*Madoqua kirkii*), Grant's gazelle (*Gazella granti*), and buffalo (*Syncerus caffer*), increases with WD (*P* values range from <0.001 to 0.08) (Fig. 2 *a–e*). The regression coefficients (slopes) of the second group do not vary significantly from each other ($P > 0.1$, *F* test).

Discussion

The Evaporation Sensitive (ES)–Evaporation Insensitive (EI) Distinction. The regressions between WD and $\varepsilon_{enamel\text{-}mw}$ show that aridity affects $\delta^{18}O_{enamel}$ values of some animals and not others. We assign the ES classification to those animals (giraffids, dikdik, and oryx) for which $\varepsilon_{enamel\text{-}mw}$ is sensitive to WD ($P < 0.05$). $\varepsilon_{enamel\text{-}mw}$ values of EI animals (hippopotamus, warthog, elephant, rhinoceros, and zebra) do not vary with aridity ($P > 0.10$). Although high regression coefficients ($>10^{-3}$) suggest that the response of $\varepsilon_{enamel\text{-}mw}$ values to WD for baboon, impala, Grant's gazelle, and buffalo, is similar to that for ES taxa, these animals are placed in neither category because the regressions are not significant at the 0.05 level. ANOVA comparisons using a post hoc Scheffé test show that, within a single location, $\delta^{18}O_{enamel}$ values of EI animals (hippopotamus, warthog, elephant/rhinoceros, and zebra) form significantly different groups ($P < 0.05$), whereas $\delta^{18}O_{enamel}$ values of the ES animals (giraffids, dikdik, and oryx) do not vary significantly from each other ($P > 0.05$). Bushpig $\delta^{18}O_{enamel}$ values are not included in the ES–EI classification because the data represent a very small range in WD.

The evaporative enrichment of ^{18}O in ingested water and in body water has a strong influence on $\delta^{18}O$ and δD values of both mammalian and avian body tissues, and it is a likely explanation for the sensitivity of ES $\varepsilon_{enamel\text{-}mw}$ values to aridity (9–12). The ^{18}O composition of leaf, surface, and body waters increase with greater evaporation, but the specific isotopic response of waters

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Abbreviations: WD, water deficit; RH, relative humidity; ES, evaporation sensitive; EI, evaporation insensitive.

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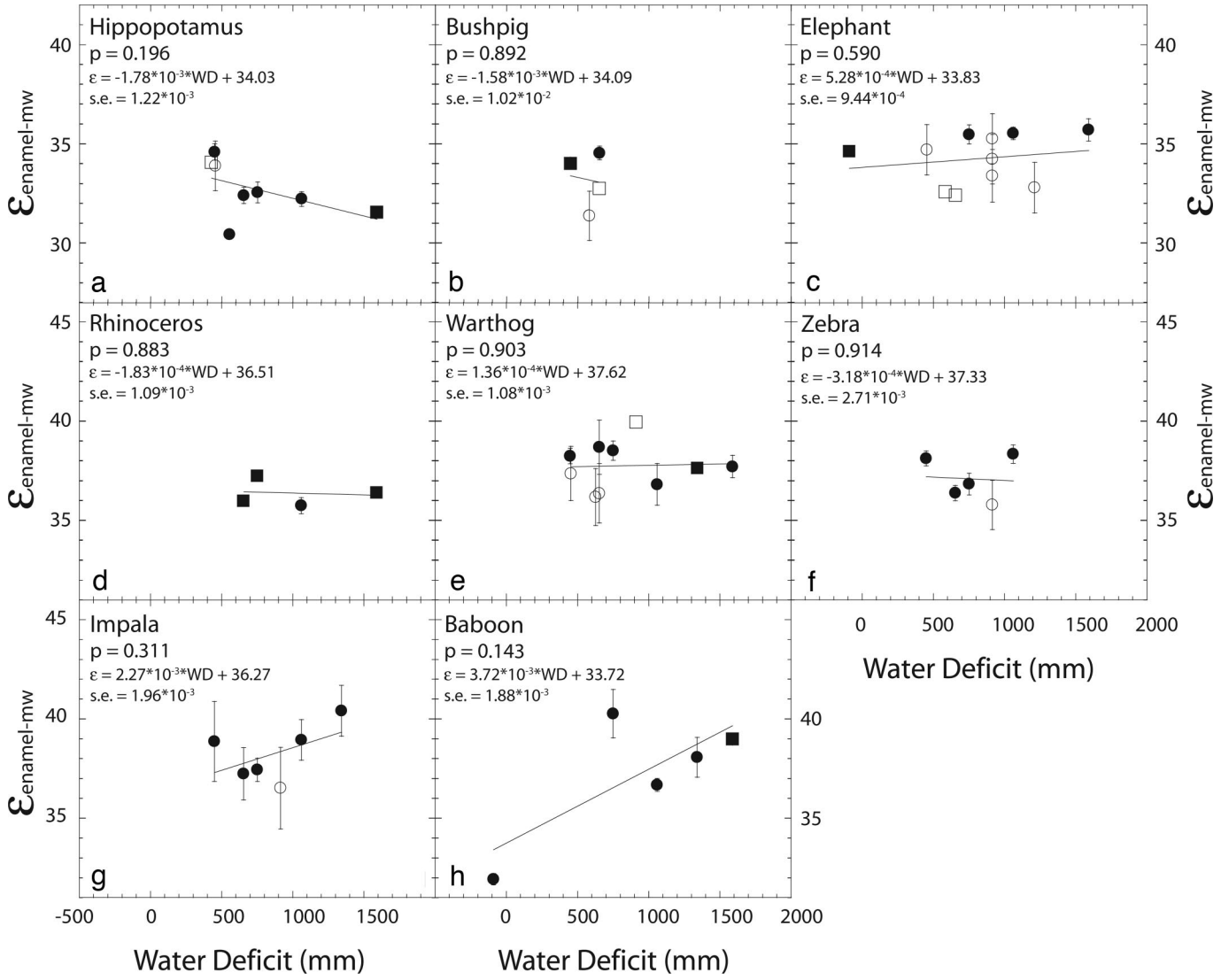

Fig. 1. $\varepsilon_{\text{enamel-mw}}$ values for hippopotamus, bushpig, elephant, rhinoceros, warthog, zebra, impala, and baboon, plotted against water deficit (WD) (*a–h*). Circles represent $\varepsilon_{\text{enamel-mw}}$ values calculated from average $\delta^{18}O_{\text{enamel}}$ values at a location, whereas squares represent $\varepsilon_{\text{enamel-mw}}$ values calculated from sites where the $\delta^{18}O_{\text{enamel}}$ value from only one tooth was available. Error bars report the standard error associated with each ε value. No error bars are reported for $\varepsilon_{\text{enamel-mw}}$ represented by squares. Filled symbols (●, ■) represent $\varepsilon_{\text{enamel-mw}}$ values calculated from measured $\delta^{18}O_{\text{mw}}$ values, whereas open symbols (○, □) represent $\varepsilon_{\text{enamel-mw}}$ values calculated from a regional $\delta^{18}O_{\text{mw}}$ average, −3.1‰. The linear regression, *P* value, and the standard error (s.e.) on the regression coefficient are reported for each plot.

varies with boundary layer thickness, geometry of the evaporating water body, and the ^{18}O composition of atmospheric water vapor (13). Of these waters, leaf water is most sensitive to evaporative enrichment in ^{18}O (14, 15) and its relationship with WD represents a theoretical maximum slope between $\varepsilon_{\text{enamel-mw}}$ and WD. To demonstrate the isotopic effect of evaporation on residual waters, we calculated leaf water $\delta^{18}O$ ($\delta^{18}O_{\text{lw}}$) values and $\varepsilon_{\text{lw-mw}}$ by using a modified version of Craig and Gordon's 1965 model (16). $\varepsilon_{\text{lw-mw}}$ increases with WD ($P = 0.02$) (Fig. 2*f*).

We do not have the behavioral and physiological data that are necessary to calculate the flux of $^{18}O/^{16}O$ through the sampled animals and the relative effects of drinking, leaf and body waters on $\delta^{18}O_{\text{enamel}}$ values of ES and EI animals, but we can use known behaviors and physiologies of these species to evaluate the contributions of these different waters to the $\delta^{18}O_{\text{enamel}}$ values of the animals included in this study. The sensitivity of ES $\varepsilon_{\text{enamel-mw}}$ values to aridity cannot be explained by evaporated drinking waters because (*i*) it is unlikely that ES animals consistently drink more water from evaporated sources than EI animals, and (*ii*) samples from animals known to drink evaporated waters were excluded from the data set (see *Materials and Methods*). Physiology and the evaporative enrichment of ^{18}O in body water may have an important role in determining the sensitivity of $\delta^{18}O_{\text{enamel}}$ values to aridity, but the diversity of ES animals, which are both large and small, panters and sweaters, and grazers and browsers (17–19), eliminates a single physiological explanation for the ES grouping.

The ingestion of evaporated leaf water is a viable explanation for increased $\varepsilon_{\text{enamel-mw}}$ values of ES animals in water limited environments. ES animals identified in this study, oryx, giraffids, and dikdik, can survive with little or no drinking water and often obtain a substantial proportion of their water from leaves (17, 18, 20). In contrast, EI animals are associated with water, drink water daily, or consume nonleafy (nonevaporated) and moist portions of plants when water is less available (21–24). All of these behaviors would reduce the intake of evaporated relative

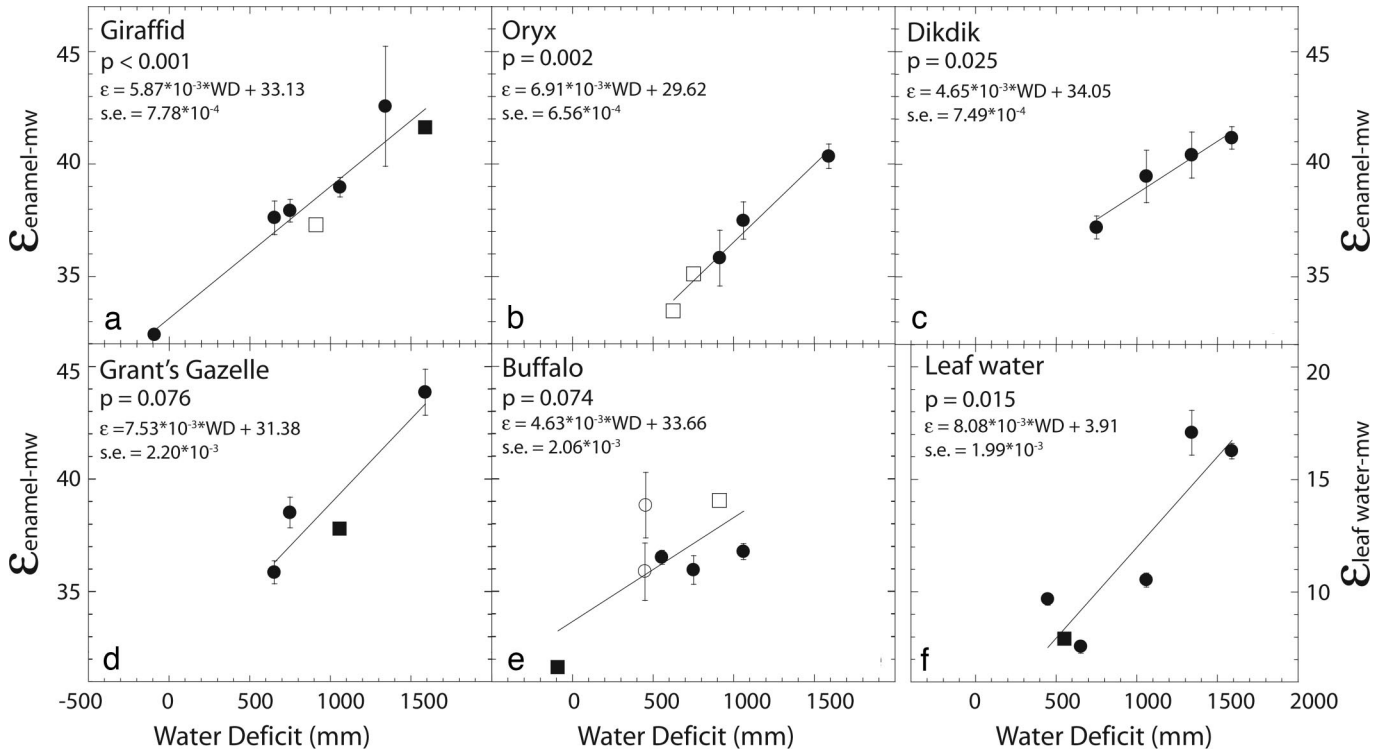

Fig. 2. $\varepsilon_{\text{enamel-mw}}$ values (*a–e*) for giraffids, oryx, dikdik, Grant's gazelle, and buffalo, and $\varepsilon_{\text{lw-mw}}$ values (*f*), plotted against water deficit. $\varepsilon_{\text{lw-mw}}$ values are only plotted for sites where $\delta^{18}O_{mw}$ and RH data are available. Symbols are the same as Fig. 1. The linear regression, *P* value, and the standard error (s.e.) on the regression coefficient are reported for each plot.

to unevaporated waters. The proximity to water sources, large body size, skin that adsorbs water and mud, and wallowing behaviors of many EI animals may provide additional explanations for the isotopic buffering of EI animals from increased aridity (23, 25).

A combination of variables that affect the ^{18}O evaporative enrichment of both leaf and body water is likely responsible for the ES and EI groupings. Fortunately, a specific mechanism for the groupings is not needed to develop $\delta^{18}O_{enamel}$ values as indicators of aridity. The consistent slope for the ES $\varepsilon_{\text{enamel-mw}}$–WD regressions reflects a general relationship between evaporated waters and aridity that can be used to estimate aridity from $\delta^{18}O_{enamel}$ values.

Aridity Index. $\delta^{18}O_{enamel}$ values of ES taxa track evaporative enrichment with respect to source water, whereas $\delta^{18}O_{enamel}$ values of EI taxa track the ^{18}O composition of source water. The enrichment between $\delta^{18}O_{enamel}$ values from ES and EI animals ($\varepsilon_{ES–EI}$) represents the degree of ^{18}O enrichment between leaf or body water and meteoric water ($\varepsilon_{\text{lw-mw}}$ or $\varepsilon_{\text{bw-mw}}$), which increases with aridity. Integrating two isotopic records to develop a proxy for aridity that controls for the isotopic composition of meteoric water is not a new concept; Cormie *et al.* (6) estimated RH by comparing bone collagen δD and $\delta^{18}O$ values of bone collagen. Here, we modify the concept for application to $\delta^{18}O_{enamel}$ values.

When $\delta^{18}O_{enamel}$ values of ES animals are pooled, the isotopic enrichment between ES and EI $\delta^{18}O_{enamel}$ values ($\varepsilon_{ES–EI}$) varies significantly with WD for most EI animals such as hippopotamus, elephant/rhinoceros (pooled), and warthogs (*P* values <0.02) (Figs. 3 *a–c*). The linear regression between $\varepsilon_{ES–EI}$ and WD is insignificant (*P* value = 0.2) for zebra, which may be explained by few $\varepsilon_{ES–EI}$ data points that lie within a small WD range. Among the significant WD-$\varepsilon_{ES–EI}$ regressions, the slopes do not significantly differ from each other ($P > 0.1$, *F* test), nor do they differ significantly from the slopes of regressions between WD and $\varepsilon_{\text{ES-mw}}$ or $\varepsilon_{\text{lw-mw}}$ ($P > 0.1$, *F* test). We propose that the pooled or common slope of the WD-$\varepsilon_{ES–EI}$ regressions, 5.01E-03 (SE, 1.98E-03), is an aridity index that can be used to compare water deficit values within modern or fossil ecosystems. Application of the aridity index to the fossil record requires attention to the following three issues: identification of ES and EI taxa, sample size, and geologic context.

1. Identification of ES and EI species in the fossil record should be guided by behavioral information, morphology, carbon isotopic data, water dependency, and the ES–EI status of animals in modern analog systems. ES or EI status can be confirmed by using $\delta^{18}O_{enamel}$ values themselves: $\varepsilon_{ES–EI}$ changes between sites that differ in water deficit, whereas $\varepsilon_{EI–EI}$ and $\varepsilon_{ES–ES}$ do not.
2. Minimum sample size required for the aridity index is determined by a power analysis [significance (α) = 0.05, power level ($1 - \beta$) = 0.2, two-tailed test], wherein 10 samples are needed to determine a difference of 1.5‰ between populations of $\delta^{18}O_{enamel}$ values with a standard deviation of 1.3‰, which is the average standard deviation of $\delta^{18}O_{enamel}$ values for samples size >10. For the aridity index, this means that 10 ES and 10 EI specimens at one site are needed to determine water deficit values within 140 mm (using an aridity index slope of 5.01E-03 and determining the error for $\varepsilon_{ES–EI}$ by propagating the standard errors on mean $\delta^{18}O_{enamel}$ values). Fewer samples are needed if less precision is acceptable. Clementz and Koch (26) calculated a similar sample size necessary to determine differences of 1‰ between mean $\delta^{18}O_{enamel}$ values of terrestrial animals, also using a power analysis.
3. Establishing proper geological context is critical for application of the aridity index. $\varepsilon_{ES–EI}$ must be calculated from fossil teeth found within well constrained time intervals, preferably

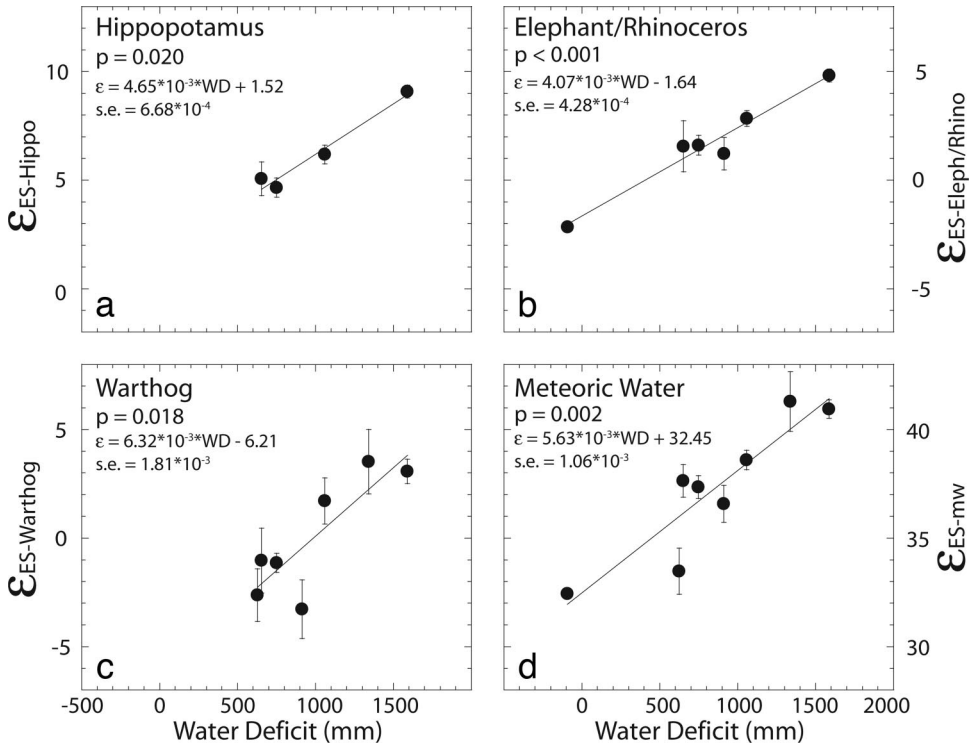

Fig. 3. The isotopic enrichment (ε) between ES $\delta^{18}O_{enamel}$ values (averaged values of all ES specimens at each site) and $\delta^{18}O_{enamel}$ values of hippopotamus (*a*), elephant/rhinoceros (*b*), warthog (*c*), and meteoric water (*d*) is plotted with water deficit. ES and EI taxa are grouped based on ANOVA results presented in text. Linear regressions, *P* values, and the standard error (s.e.) on the regression coefficient are reported for each plot. Error bars represent the standard error associated with each ε value.

within one sedimentological stratum or well correlated unit. To reduce the possibility that animals accessed evaporated surface waters, fossils from lacustrine sediments should be avoided unless it can be demonstrated that the lake water was not enriched in ^{18}O relative to meteoric water.

With the above criteria met, the aridity index can be used to convert differences in $\varepsilon_{ES–EI}$ between sites to differences in WD. The use of $\varepsilon_{ES–EI}$ and not absolute $\delta^{18}O_{enamel}$ values eliminates the need to know taxon-specific fractionation factors between environmental water and body water $\delta^{18}O$ values, and it controls for changes in $\delta^{18}O_{mw}$ values that are independent of aridity.

Conclusion

The empirical relationships presented here lay the foundation for a simple approach that relates $\delta^{18}O_{enamel}$ values to aridity when it is not practical to model the flux of $^{18}O/^{16}O$ through an animal. The aridity index is a general relationship between aridity and $\delta^{18}O_{enamel}$ values that is built on the distinction between ES and EI animals, wherein ES $\delta^{18}O_{enamel}$ values track evaporation of leaf or body water and EI $\delta^{18}O_{enamel}$ values track the ^{18}O composition of meteoric water (drinking, stem, and root water). The isotopic enrichment between $\delta^{18}O_{enamel}$ values of ES and EI animals, $\varepsilon_{ES–EI}$, increases linearly with water deficit along a consistent slope, the aridity index, which can be used to calculate relative differences in water deficit through time or across space. The development of the aridity index with data from modern eastern African mammals makes the aridity index most immediately applicable to East African paleoenvironments, where aridity is considered to have influenced human evolution (27). However, the ES–EI distinction is a broadly applicable concept that can be used to evaluate animal water-use strategies in terrestrial systems where the ^{18}O composition of bioapatite is unaltered.

Materials and Methods

In the past 10 years, modern mammal teeth from animals living within the past 45 years have been sampled from museum collections and field locations across eastern Africa for stable isotopic analysis. For this study, a subset of the larger tooth enamel isotopic data set (493 teeth of 1,000) was used to place better control on water sources accessed by animals (Table 2, which is published as supporting information on the PNAS web site). Tooth enamel $\delta^{18}O$ ($\delta^{18}O_{enamel}$) values were only included in this subset if they were from animals with $^{18}O_{enamel}$ results from four or more locations. Of these animals, $^{18}O_{enamel}$ data were excluded if (*i*) they were from locations with large isotopic gradients (e.g., mountainous regions) or (*ii*) they were from animals known to drink evaporated water enriched in ^{18}O with respect to local meteoric water.

Waters used to approximate $\delta^{18}O_{mw}$ values were sampled from sources with little evaporation (e.g., rivers with small catchments, springs, and shallow wells). Environmental aridity is characterized in terms of WD, the difference between potential evapotranspiration (PET) and mean annual precipitation (MAP), (WD = PET − MAP) using available data from references (28–31) and from N. Georgiadis (personal communication). PET was calculated from mean annual temperature and latitude (32).

Enamel was separated from dentine and powdered with a diamond or carbide bit following the long-axis of the tooth. Where multiple samples from each tooth were taken, the average value was used and considered as one sample. Enamel powders were pretreated by standard procedures in preparation for stable isotope analysis of the carbonate component of tooth enamel (33, 34). Waters were prepared for isotopic analysis by equilibration with CO_2. Isotope ratios were measured on a Finnigan MAT 252 mass spectrometer at Stable Isotope Ratio Facility for Environmental Research, University of Utah. All isotopic ratios

are reported in δ-notation, where $\delta = ((R_{sample}/R_{standard}) - 1) \times 1{,}000$ and R is $^{18}O/^{16}O$. δ values of tooth enamel are reported in reference to Vienna PeeDee Belemnite (VPDB), and waters are reported in reference to Vienna standard mean ocean water (VSMOW). For calculations in which it was necessary to convert values in the VPDB scale to the VSMOW scale, the following relationship was used: $\delta^{18}O_{VSMOW} = 1.3086 \times \delta^{18}O_{VPDB} + 30.86$ (35). The enrichment between related isotopic values is reported as ε, where $\varepsilon_{A\text{-}B} = ((R_A/R_B) - 1) \times 1{,}000$.

For sites where data were available, $\delta^{18}O_{lw}$ values were calculated by using a modified version of the 1965 Craig and Gordon evaporative enrichment model (13, 16, 36). To calculate $\delta^{18}O_{lw}$ values, mean annual temperature, mean annual RH (0600 h), and water $\delta^{18}O$ values were used, and it was assumed that atmospheric humidity ^{18}O composition is 9‰ lower than measured $\delta^{18}O_{water}$ values, there is no temperature difference between the leaf and air, stomatal conductance = 0.3 $m^{-2}\cdot s^{-1}$, and boundary layer conductance = 1 $m^{-2}\cdot s^{-1}$. Statistics were computed with the statistical program JMP 4 (SAS Institute) and SYSTAT 10 (SPSS), or using methods described in Sokal and Rohlf (37).

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