

Identifying the water sources consumed by bison: implications for large mammalian grazers worldwide

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Abstract. The sources of drinking water consumed by grazers vary over time and may be highly selective, similar to choices in diet. Water sources consumed by large grazers in natural populations are not typically measured directly. Instead, consumption is inferred based on animal proximity to water sources. Here, we analysed the stable isotopic signature of water ($\delta^{18}\text{O}$ and δD) extracted from fecal samples from a herd of bison in mesic grassland as a direct estimation of the water sources consumed over time. Bison at this site have their choice of a range of habitats and drinking water sources. Potential source-water samples measured had a large range of isotopic signatures, allowing the isotopic composition of water from bison fecal samples to be proportionally estimated based on varying sources. Results indicate bison have low reliance on multiple streams on site; rather, the majority of water consumed was from rainfall-fed sources (puddles and wallows) and from forage. Our research suggests that source-water analysis from fecal samples is a robust technique when samples from large grazers can be collected soon after production. These results have implications for analyses of the foraging patterns and landscape utilization by this and other large grazers, because hotter and drier future conditions are likely to reduce the frequency and amount of rainfall-fed puddles available for consumption in many grassland systems worldwide.

Key words: *Bison bison*; δD ; $\delta^{18}\text{O}$; grassland; isoscape; mixing models; Special Feature: Isoscapes; water.

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INTRODUCTION

Large herbivore diversity and density are key drivers of ecosystem structure and function in grasslands and savannas worldwide (Collins et al. 1998, Holdo et al. 2007, Riginos and Grace 2008). At the landscape-scale, the often patchy distribution of resource availability over time drives distribution patterns of large herbivores

(Fryxell 1991, Bailey et al. 1996, Wilmshurst et al. 1999, Allred et al. 2011, Smit 2011). Forage quality for large herbivores can be constrained by access to reliable water sources (Bailey et al. 1996, Redfern et al. 2003). As such, access to water imposes a limitation on herd distributions across the landscape and regulates an animal's ability to access both high quality and quantities of forage (Redfern et al. 2003). Limitations on

grazing distances from water sources vary among ungulate species according to digestive throughput rate, fecal moisture content, urine volume and osmolality and other physiological and morphological characteristics associated with the grazer's ability to maintain an optimal temperature and water balance (Cain et al. 2012). Some ruminants (e.g., African buffalo, *Syncerus caffer*) may be especially sensitive to the foraging constraints set by access to surface water availability, because trade-offs between forage quality and water availability are manifest during periods when forage quantity is low (Redfern et al. 2003, Smit et al. 2007, Smit 2011) and when the moisture content of grasses decreases following senescence (Western 1975).

Most evidence for the drinking-water sources consumed by large grazers has been anecdotal or inferential based on proximity to water sources. For example, presumed drinking water sources have been reported using visual observations of herd habitat selection (Coppock et al. 1983, Bergstrom and Skarpe 1999, de Leeuw et al. 2001), camera traps (Whiting et al. 2010), aerial census (Western 1975, Redfern et al. 2003), and distances to water calculated using GPS collars (Fortin et al. 2003, Allred et al. 2011, Dancose et al. 2011, Cain et al. 2012). These techniques provide a template for presumed water-use by grazers based on the assumption that the water consumed comes from either the most abundant or most proximal water source to the animal's location. Fewer studies have linked drinking water sources using the stable isotopic signature of body water and final deposition in animal tissues (Kohn 1996, McKechnie et al. 2004, Podlesak et al. 2008, Kirsanow and Tuross 2011). Using rodents, Podlesak et al. (2008) showed that the half-life turnover of body-water was 3–6 days, but only ~50% of the stable isotopic signature of oxygen in blood samples originated from drinking water (the other ~50% was from atmospheric oxygen and food). The stable isotopic signature of water from fecal samples has been measured using the doubly-labelled water method to quantify the free-living energy exchange of reindeer (*Rangifer tarandus*) (Gotaas et al. 1997, Haggarty 1999, Gotaas et al. 2000). Water extracted from fecal samples may provide direct evidence of drinking-water sources selected by free-ranging species without the

need for an introduced isotopic tracer if the potential drinking-water sources in an environment have a range of distinct isotopic signatures. Compared to body water analyses using blood or plasma, water from fecal samples should directly reflect drinking water sources because the digestive system is anaerobic, and thus reduces the potential for isotopic exchange of water with atmospheric sources of O₂ following consumption. Additionally, the residence time of water in the digestive system can be relatively short (~10 hours in cattle) before being metabolized or excreted (Seo et al. 2006).

To identify how the drinking-water sources may vary for large grazers, we studied the American bison (*Bison bison*), a species with a historic continental-wide distribution in North America (Hall and Kelson 1959, Shaw 2000, Anderson 2006, List et al. 2007), similar to that seen in the large grassland and savanna landscapes still available for grazers in Africa. Bison currently occur within constrained and managed geographic ranges, yet seasonal patterns of resource availability are likely to drive resource selection, consumption, and energetics (Rutley and Hudson 2000, Bergman et al. 2001, Allred et al. 2011). Bison movements across the landscape commonly reflect selective grazing preferences to maximize forage quality (Coppock et al. 1983, Coppedge et al. 1998, Coppedge and Shaw 1998, Knapp et al. 1999). Similar to selective grazing preferences, seasonal and spatial dynamics of water availability impact bison movements on the landscape (Coppedge and Shaw 1998). For example, bison in Prince Albert National Park, Saskatchewan were more likely to graze in meadows within 2 km of water during the summer than areas at greater distances from water sources (Fortin et al. 2003). Dancose et al. (2011) reported varying seasonal habitat selection by bison, corresponding with avoidance of water holes during the summer and selection of meadow habitat with water available during winter and spring. Similarly, stream corridors can modify travel routes and habitat accessibility through changes in landscape connectivity, influencing bison movements on the landscape (Bruggeman et al. 2007). While water availability can influence patterns of bison movements, many landscapes with bison either have uniform distributions of water sources (natural or arti-

cial), are of insufficient size to detect an effect of distance to water, or methods of source-water detection are ineffective, expensive, or labor-intensive.

In this study, we measured the sources of water consumed using bison fecal samples collected for 14 months (January 2010–March 2011) in mesic grassland (tallgrass prairie) in eastern Kansas, USA. Water sources included several grassland streams (Gray and Dodds 1998), rainfall collected in puddles and wallows, and water consumed from forage. We hypothesized that (1) water extracted from fecal samples of bison would provide a reliable proxy of the water consumed, and this technique may be advantageous compared to inferential studies of drinking-water sources, because selection may be influenced by behavior, demography and herd social structure rather than proximity alone, (2) while stream-water is the most reliable and abundant source of water available for bison consumption at this site, seasonal rainfall patterns are likely to impact the sources of water consumed, and (3) sources of water consumed would vary based on herd demography. Bison maintain mixed-herd groups with females, calves, and immature males forming large herds, and mature males forming separate small groups (Schuler et al. 2006). For these reasons, source-water consumption was presumed to vary based on gender and age-class.

MATERIALS AND METHODS

Site description

Research was performed at the Konza Prairie Biological Station (KPBS), a 3,487-ha native tallgrass prairie located in the Flint Hills of northeast Kansas, USA (40° N, 99.5° W). Regional climate for KPBS is characterized as mid-continental, with warm-wet summers, and cool dry winters. Daily mean air temperature (1982–2011) varies between the coolest ($-1.4 \pm 0.5^\circ\text{C}$ in January) and warmest ($26.1 \pm 0.3^\circ\text{C}$ in July) months. From 1982–2011, mean annual rainfall (± 1 SE) for the site was 805 ± 35 mm, with 75% of the total amount occurring during the growing season (April–September). Total precipitation for 2010 was 843 mm, with a mean event size of 2.3 ± 0.92 mm. The mean and median number of days between rain events varied based on season. From

January–February 2010, the mean and median number of days between rain events was 7.1 and 5 days, respectively. The frequency of events increased from April–October, with mean and median number of days between events of 3.3 and 2 days. During the seasonal temperature transitions (March, November–December), the mean and median number of days between rainfall events was 4.7 and 2 days, respectively.

KPBS is divided into watershed units for management, with varying intervals of fire frequency (1, 2, 4, or 20 years) and the majority of burn treatments occurring in spring. KPBS maintains a bison herd within the central 1012 ha section of the site. The bison herd on KPBS was established in 1987, and has grown in size since that time to a herd size of 300–400 individuals, with a male to female ratio of 1:5 (Craine et al. 2013). Bison at KPBS are not managed for production, and thus animal movements on site are unrestricted and time spent in various locations is directed by the herd, and not site personnel.

Data collection

Starting in January, 2010, fecal samples were collected approximately every 10 to 14 days continuing to March, 2011. At each collection date, a herd of individuals on the landscape was monitored, and 2–5 fecal samples were collected within minutes of being produced. At this time, the animal ID was recorded, and samples were transferred into a 12 mL glass exetainer with a septum (Labco, UK). Samples were stored prior to analysis in a cold freezer (-7°C). In total, 90 bison samples were collected during the 14 mo. study. Of these samples, 48% were from adult females, 32% from adult males, 8% from juvenile females (less than 3 years old) and 12% from juvenile males. Fecal samples were collected in each of the watersheds available to bison on KPBS, reflecting the full-range of burn frequencies and landscape composition available on site (1, 2, 4, and 20-year).

Water samples were collected on site for analysis as potential drinking-water sources. Within the area available to bison at KPBS, over 3000 bison wallows exist (A. Joern, *unpublished data*). These wallows vary in size (from 2 to 10 m diameter), their frequency of utilization, and in the duration of time with standing water

following a rainfall event (typically 1–5 days). To best characterize recent rainfall-fed sources available to the entire herd, precipitation was collected at KPBS following each rain event (>5 mm) from January, 2010 through March, 2011 as part of the NADP sample collection protocol (National Atmospheric Deposition Program; <http://nadp.sws.uiuc.edu>). Streamwater samples were collected weekly from the lower reach of King's Creek (the major stream that flows through the grazed section of KPBS) over the same time period (n = 38). Water samples were filtered at 0.2 micron using a Whatman Puradisc PES filter.

Isotopic analysis

Water was extracted from fecal samples using cryogenic vacuum distillation (Nippert and Knapp 2007) using an extraction line operated in the Stable Isotope Mass Spectrometry Laboratory at KSU. Water samples from bison, rainfall, and stream-water were analyzed for their stable isotopic composition using a Picarro WS-CRDS isotopic water analyzer using ChemCorrect software to identify possible interference or sample contamination. The stable isotopic ratios of water samples ($\delta^{18}\text{O}$ and δD) are reported as deviations from international standards using δ -notation in parts per thousand (‰):

$$\delta = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \right] \quad (1)$$

where R is the absolute ratio of the rare to common isotope, respectively. Working in-house standards were calibrated to the recommended primary water standards: GISP, SLAP, V-SMOW (Coplen 1995). Repeated measurements of working standards within each run were <0.2‰ and 0.5‰ (for $\delta^{18}\text{O}$ and δD) and analysis of QA/QC standards were within 0.1‰ of calibrated value.

Leaf-water modelling

Leaf water enrichment was predicted using the Craig-Gordon model (Craig and Gordon 1965) modified for plant water loss (Flanagan et al. 1991):

$$R_L = \alpha^* \left[\alpha^K R_S \left(\frac{w_i - w_s}{w_i} \right) + \alpha^{KB} R_S \left(\frac{w_s - w_a}{w_i} \right) + R_a \left(\frac{w_a}{w_i} \right) \right] \quad (2)$$

where R_s is the isotope ratio of water entering plant roots, R_a is atmospheric water vapor, α^* is the temperature-dependent equilibrium fractionation factor (Majoube 1971), α^K and α^{KB} are the fractionation factors for diffusion through stomata and the leaf-boundary layer, respectively. For the diffusional fractionation factors, the values of Merlivat were used (Merlivat 1978, Luz et al. 2009). w is the mixing ratio of water vapor in air (a), the substomatal cavity (i) and the leaf surface (s). R_a was assumed to be in equilibrium with R_s at air temperature, leaf temperature was assumed to be equal to air temperature. w_a and w_i were calculated using relative humidity measurements and w_s was determined iteratively (Ball 1987, Roden and Ehleringer 1999) using a constant stomatal conductance of $300 \text{ mmol m}^{-2} \text{ s}^{-1}$ and a boundary layer conductance of $2000 \text{ mmol m}^{-2} \text{ s}^{-1}$. The model was run each growing-season day and parameterized with daily mean air temperature and relative humidity. R_s was assumed to be equal to the most recent precipitation event and updated for each new event. We made no adjustments for potential differences in enrichment between C_3 grasses or forbs and C_4 grasses, because seasonal differences in VPD and precipitation at KPBS have a much greater impact on leaf water enrichment than differences in leaf water $\delta^{18}\text{O}$ by photosynthetic pathway. Similarly, differences in leaf water $\delta^{18}\text{O}$ between photosynthetic pathways are less distinct at sites (e.g., KPBS) with high growing-season relative humidity (Helliker and Ehleringer 2002).

Statistical analysis

All statistical analyses were performed using the R statistical package (R Development Core Team 2007). A mixed-effects model ANOVA (from the 'nlme' library in R) was used to identify statistical differences in bison $\delta^{18}\text{O}$ and δD as a function of bison age and gender, location sampled, time since last rainfall, and sample date as fixed effects. Bison are weaned between ages 2–3 years, so individuals were classified as 'adults' (+3 years) or 'juvenile' (≤ 3 years) in the analysis.

To quantify the proportional contribution of multiple drinking water sources in the bison's diet, a Bayesian isotopic mixing model analysis was performed using the SIAR package in R

(Parnell et al. 2010). This type of analysis produced similar mean source proportions as a simple linear mixing model approach (Phillips and Gregg 2001), but allows for the incorporation of greater uncertainty in all parameters and the generation of true probability distributions. Data were classified into 3 temporal periods for analysis, based on observed seasonal phenology during 2010: herbaceous growing season, April–October; winter, January–February; and seasonal transition, March, November–December. Analysis of water-source data in winter included only two sources (precipitation and streamwater), because live grass tissue was not available for consumption during this period.

RESULTS

The stable isotopic signature of precipitation at KPBS from January 2010 to March 2011 ranged from 2.3 to -19.9‰ for $\delta^{18}\text{O}$ and 19 to -150.8‰ for δD (Fig. 1A). Precipitation samples fell on the meteoric water line (MWL), an equation describing the relationship between hydrogen and oxygen isotope ratios in terrestrial water samples (Fig. 1A). Changes in precipitation $\delta^{18}\text{O}$ over the time period studied reflect changes in rainfall amount and seasonal air temperature recorded at KPBS (Fig. 2). The range of measured values for stream-water samples was lower (-4.5 to -6.8‰ for $\delta^{18}\text{O}$, and -32 to -39‰ for δD) compared to precipitation samples (Fig. 1A, inset). Stream-water isotopic samples had values near the MWL, but also showed evaporative enrichment during warm periods when values fell below the MWL. Water extracted from bison fecal samples spanned a broad range of $\delta^{18}\text{O}$ and δD values, similar to precipitation samples (Fig. 1B). Fecal samples fell on the MWL up to values of -4 – -25‰ for $\delta^{18}\text{O}$ and δD , respectively, at which point water from fecal samples were evaporatively enriched.

When analyzed for demographic differences in bison $\delta^{18}\text{O}$ or δD , no statistically significant differences ($P > 0.05$) were present for gender, age class or their interaction (Table 1). However, the date of sample collection was a statistically-significant ($P < 0.05$) predictor of $\delta^{18}\text{O}$ and δD from fecal water samples (Table 1).

The $\delta^{18}\text{O}$ composition from bison fecal samples, stream-water and precipitation were compared over time (Fig. 3). Stream-water samples

showed very little seasonal trend, with values near -5‰ for the entire period of study. Fecal samples and precipitation samples showed similar seasonal patterns with the lowest values in the coolest portions of the year and the highest $\delta^{18}\text{O}$ values in the growing season (April–October). Modeled values of leaf-water $\delta^{18}\text{O}$ show considerable temporal variation, but no seasonal trends.

The mean monthly $\delta^{18}\text{O}$ from precipitation and fecal samples were statistically correlated ($r^2 = 0.83$) across the year (Fig. 4). Fecal samples were consistently enriched (higher $\delta^{18}\text{O}$) compared to the corresponding mean precipitation $\delta^{18}\text{O}$ value, but the magnitude of enrichment varied over an annual time course with the mean $\delta^{18}\text{O}$ from fecal samples from January–May at -2.5‰ below the trendline compared to $+1.8\text{‰}$ above the trendline from June–November (Fig. 4). Mean monthly values in precipitation and fecal sample $\delta^{18}\text{O}$ grouped according to the observed seasonal transitions in phenology during the time sampled: April–October, March and November–December, and January–February (Fig. 4).

The mixing-model analysis partitioning sources of water consumed by bison showed distinct differences in $\delta^{18}\text{O}$ over an annual timeframe (Fig. 5). During the time period with live vegetation (April–October), precipitation sources had the highest contribution to the water consumed (77.1%), compared to 18.9% from leaf-water and 4% from streams (Fig. 5A). Estimates of source consumption during this period had minimal overlap for the 95% credibility intervals of the posterior density estimates (Fig. 5A). During seasonal transitions (March, November–December), bison had the greatest reliance on streamwater (62.7%) compared to precipitation (11.9%) or leaf-water (25.4% , Fig. 5B). During the coldest period of the year (January–February), drinking-water sources by bison were similar between stream (52.5%) and precipitation (47.5%), with considerable overlap in the 95% credibility intervals of the posterior density estimates (Fig. 5C).

DISCUSSION

Access to drinking water is an important constraint on grazer distribution and landscape

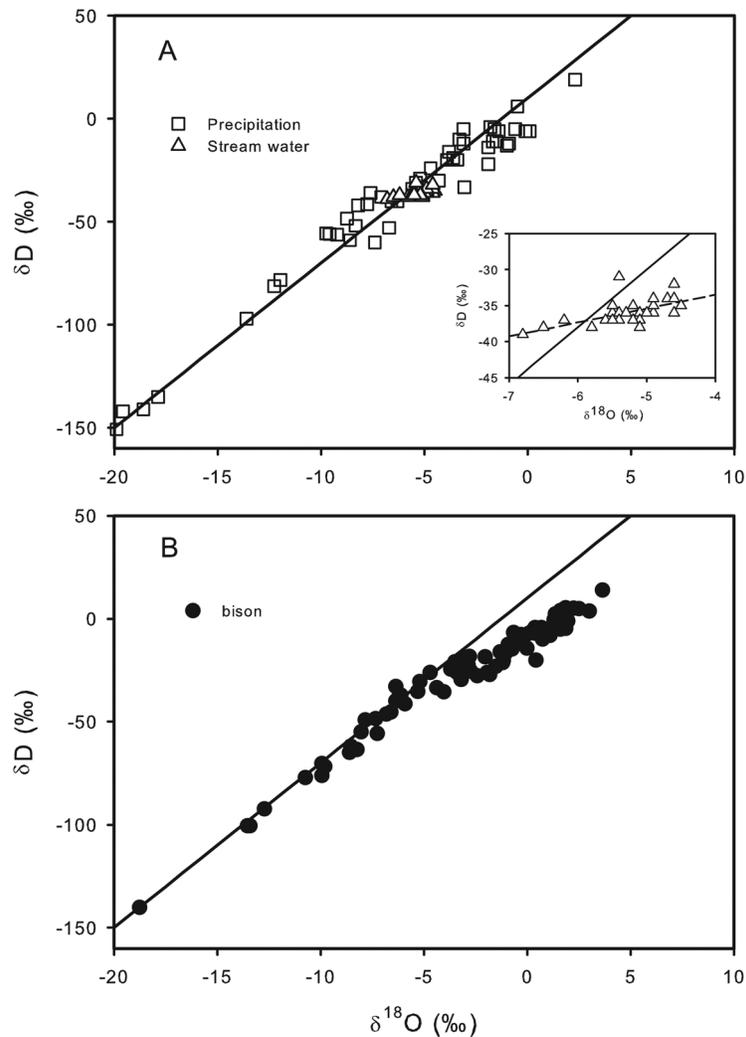


Fig. 1. Sample distribution on the meteoric water line. (A) Measured source-water samples across the entire study period. The solid black line is the global MWL ($\delta D = 8.0 \times \delta^{18}O + 10$). Inset panel has a constrained range of values on both axes to show stream water samples. (B) Bison fecal-water samples measured.

utilization in grassland and savanna ecosystems (Redfern et al. 2003). In this study, we used the stable isotopic composition of water from bison fecal samples as an indicator of the temporal pattern of water-sources consumed over time. At KPBS, the stream network represents the most permanent and accessible source of water for the bison herd, and yet these data show that the primary source of water consumed originated from rainfall-fed sources including puddles and bison wallows. Thus, proximity of this bison herd to semi-permanent water sources (stream-water) was not a reliable predictor of water consump-

tion for a large portion of the year.

Within this bison herd, the isotopic composition of water from fecal samples varied significantly over time (Fig. 3) reflecting changes in the source-water consumed by bison (Figs. 3, 5). Of the water sources available for consumption at KPBS, bison showed the greatest reliance on recent rainfall for the majority of the year (Fig. 5A). This is surprising, given the stochastic nature of rainfall events and the uncertainty of finding these ephemeral water sources compared to the greater permanence of water in streams. Bison on KPBS utilize the entire area available, so

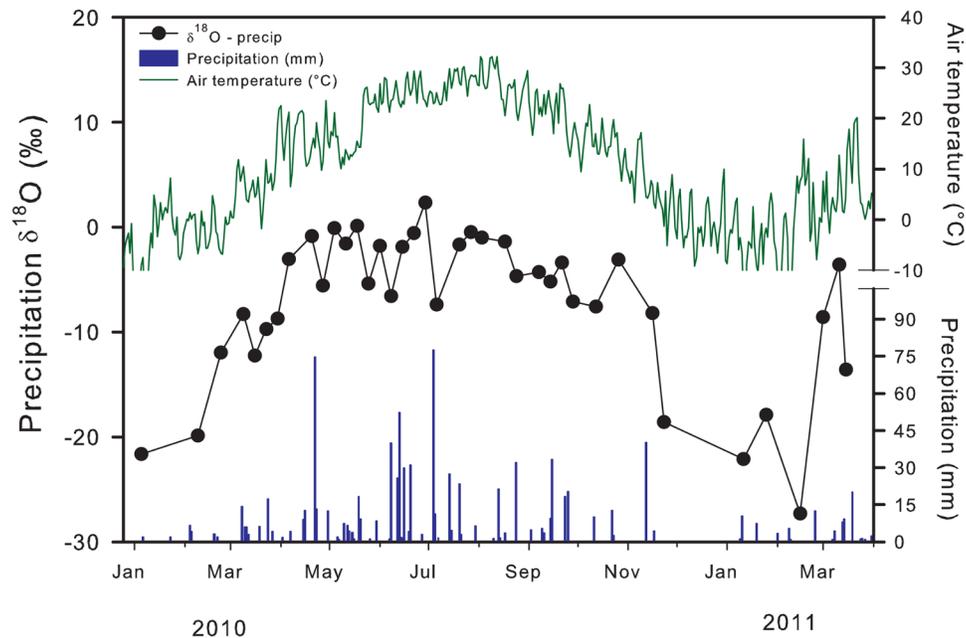


Fig. 2. Measured $\delta^{18}\text{O}$ in precipitation samples collected at KPBS from January 2010 to March 2011 (left y-axis). Rainfall event sizes (mm) are described on the lower right y-axis, while changes in mean daily air temperature ($^{\circ}\text{C}$) are described on the upper right y-axis.

choices for water were not restricted by accessibility or long-travel times to water sources. The reliance of these bison on wallows, puddles and ephemeral water sources likely reflects the greater proximity of these sources to the locations where bison spend the majority of time grazing. In a study focused on African grazers, infrequent rainfall events and low-water content in forage during the dry season resulted in increased reliance by grazers on permanent water sources (Smit 2011). Indeed, we found the greatest reliance on stream-water during seasonal transitions (Fig. 5B) as well as the coldest periods of winter (Fig. 5C) when precipitation-based sources are less available and water content of forage would be low. Thus, for this bison population, the source of water consumed varied seasonally, with the isotopic composition of water from bison fecal samples matching trends for precipitation (Fig. 4). The water source with the greatest permanence (stream-water) was used predominantly when grasses do not have living aboveground tissue (November–March).

The isotopic composition of water consumed did not vary according to demography (gender

or age class; Table 1). We hypothesized that the source of water consumed would vary based on known differences in herd structure and age, because gender by season differences in water use have been shown for other grazing species (Whiting et al. 2010), and bison calves typically nurse for the first 7–12 months (Green et al. 1993). In addition, it has been previously shown that lactation has the potential for body water isotopic

Table 1. Results from a mixed-effects model ANOVA assessing differences in bison sample $\delta^{18}\text{O}$ and $\delta^2\text{H}$ as a function of sample date, animal gender and age class.

Variable	df	F	P
$\delta^{18}\text{O}$			
Sample date	32, 47	5.60	<0.0001
Gender	1, 76	0.27	0.61
Age class	1, 76	0.69	0.40
Gender \times Age	1, 76	0.08	0.77
$\delta^2\text{H}$			
Sample date	32, 47	5.30	<0.0001
Gender	1, 76	0.08	0.77
Age class	1, 76	0.30	0.58
Gender \times Age	1, 76	0.21	0.65

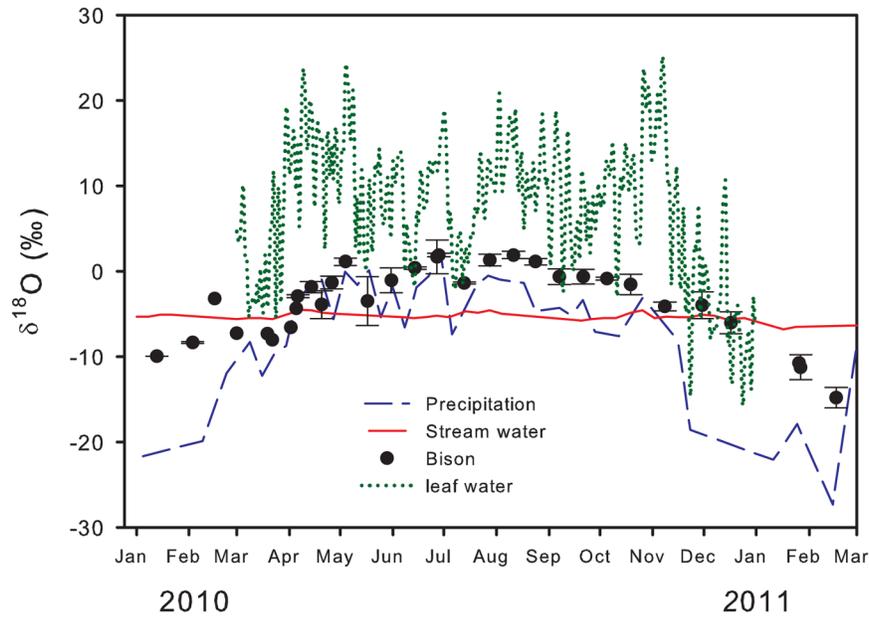


Fig. 3. Changes in fecal water samples and source-water $\delta^{18}\text{O}$ values over the time period studied. Fecal water samples (filled circle) are means \pm 1 SE. Source water samples (precipitation, short dash; stream, solid line; modeled leaf water, long dash) are fit using a smoothed spline to measured observations.

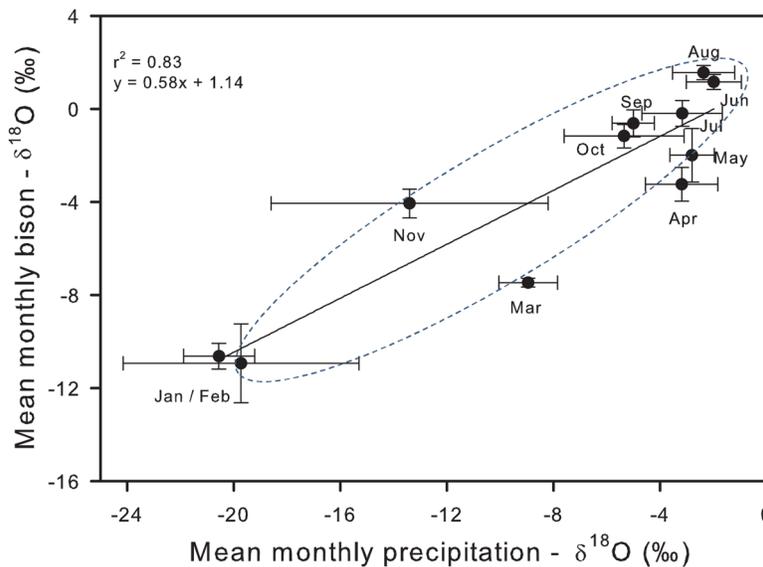


Fig. 4. Simple linear regression fit to the relationship between mean monthly precipitation $\delta^{18}\text{O}$ and mean monthly bison fecal water $\delta^{18}\text{O}$. Error bars represent \pm 1 SE. Data points are identified according to month observed. No precipitation events occurred in December 2010. The dashed blue ellipse shows the monthly temporal sequence for this relationship.

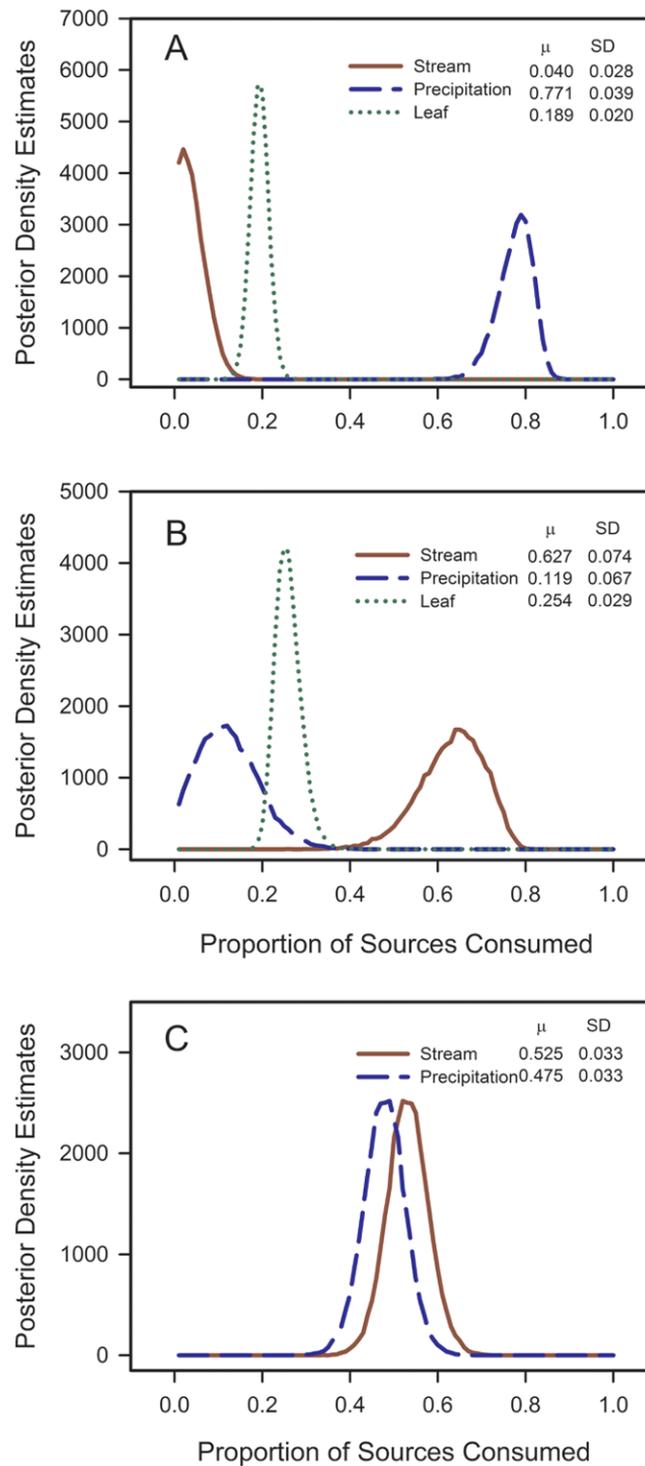


Fig. 5. Bayesian isotopic mixing-model results for drinking-water sources used by bison during 3 annual periods: (A) peak biomass (April–October) (B) seasonal transitions (March, November–December); (C) winter (January–February). For each model, the posterior density estimate is on the y-axis, source proportion on the x-axis. The mean source proportion and standard deviation are shown in inset tables.

fractionation (Haggarty 1999). Demographic differences in either stable isotope of water were not statistically different (Table 1), suggesting similar sources of water consumed within the herd, when assessed infrequently (~ 2 weeks) over the entire space available to this herd at KPBS. Thus, if within-population dynamics of water-use exist, it will require a greater temporal and spatial sampling frequency for identification.

These results show that water from bison fecal samples provide a robust indicator for partitioning drinking-water sources over time. This study utilized ambient temporal variability in the isotopic signature of water sources, rather than a tracer technique as originally described by Gotaas et al. (1997), using doubly-labeled water to estimate energy expenditure in reindeer. Utilizing the natural variability in the isotopic signatures of water is preferred over tracer techniques, which are typically more time-consuming and expensive to employ at the landscape-scale. Here, no evidence exists to suggest isotopic fractionation in water from bison fecal samples compared to potential drinking water sources. The distribution of fecal samples fall upon the meteoric water line (MWL) up to the local hydrologic inflow flux signature (at KPBS inflow represents groundwater recharged by precipitation: -6‰ for $\delta^{18}\text{O}$, -37‰ for δD [Nippert and Knapp 2007]). At this point on the MWL, the isotopic composition of water from fecal samples deviated from the MWL (Fig. 1B). If fractionation had occurred between water consumption and fecal deposition by bison, it is unlikely that the data in Fig. 1B would fall upon the MWL up to the inflow flux signature, and then deviate from the MWL with a similar slope as measured in local stream water samples (inset panel, Fig. 1A).

The fecal sampling technique has promise for discerning water sources among natural populations of other large grazers, especially when coupled with data collected using inferential techniques (observed, remotely-sensed, or GPS collars) when water-use is based on proximity to water sources (Redfern et al. 2003, Cain et al. 2012). Inferential analyses cannot discriminate between the possibility that resource-proximity may not equate to resource-use. Combining this isotopic approach with an inferential approach has the potential for even greater insight into

subtle population-level decisions (e.g., herd choices) that may vary in time and space. In addition, this technique using water from fecal samples is more suitable for ecological field studies than other isotopic techniques using blood or plasma samples (Podlesak et al. 2008) because the influence of atmospheric oxygen on fecal-derived $\delta^{18}\text{O}$ is low, and the use of fecal samples does not require animal capture to acquire samples for analysis. For this approach to be utilized in other locations and other grazing species requires prompt collection of fecal samples following production to minimize evaporative enrichment, as well as using locations where drinking-water sources have distinct isotopic composition. For example, the monthly values of bison/precipitation $\delta^{18}\text{O}$ form an elliptical temporal sequence (Fig. 4), a pattern that most likely reflects seasonal evaporatively-enriched sources (e.g., forage or rain-fed puddles), with greater enrichment during the hottest/driest portion of the year (June–November).

The amount of water required for bison and seasonal changes in water demand by bison remains to be determined. Previously, estimates of the annual water supply were calculated for cattle in the Flint Hills region of Kansas considering seasonal evaporation and through-flow (Duesterhaus et al. 2008). For an animal with a mean weight of 250 kg, cattle require on average 30 L per day per individual, but this amount varied over time (Duesterhaus et al. 2008). In a study of the comparative ecology of cattle and bison in tallgrass prairie in Oklahoma, USA, Allred et al. (2011) showed cattle foraging close to permanent water sources, while bison spent more time at greater distances from permanent water sources. These authors concluded that ephemeral water sources may constitute an important fraction of the total drinking water consumed by bison (Allred et al. 2011). Our results support Allred et al. (2011) and highlight the significance of bison wallows as rainfall catchments. Wallow creation and use distributed across the landscape enhances the availability of drinking water sources in the locations where bison spend the majority of their time grazing. In addition, our results also suggest water from forage may constitute a significant fraction of the water budget for bison. On KPBS, $\sim 85\text{--}98\%$ of the fresh weight of grasses is water

when soil moisture is readily available (T. Ocheltree, *unpublished data*), and live grass tissue is generally available for consumption during the grassland growing season (April to October). Results from the mixing model analysis (Fig. 5) suggest that water from forage was ~19–25% of the total consumed depending on month. The increased use of streamwater in the winter and during seasonal transitions by bison may reflect the low water content of forage during cold periods and lower availability of rainfall-fed ephemeral water sources.

Our analysis demonstrates that ephemeral water sources on the landscape and water from forage are the key components of the water budget for this grazer in the year studied. In addition, the sources of drinking-water for this population of bison were seasonally-dependent with shifting reliance on multiple sources according to seasonal availability. The year of research for this study (2010) had similar rainfall to the long-term mean precipitation measured at KPBS. Forecasted climate changes for the Great Plains region of North America include increased air temperatures and increased variability in precipitation patterns (Christensen et al. 2007), increasing the likelihood of droughts during the vegetative growing season (Easterling et al. 2000, Meehl et al. 2005). With increased drought frequency and severity, ephemeral water sources are likely to be less available for consumption by bison during the vegetative growing season, the impacts of which are likely to alter decisions of resource-use and landscape utilization by bison in this system. With fewer puddles and wallows containing water in the areas grazed, bison are likely to spend more time travelling to permanent water sources (rivers) at the expense of time spent foraging and resting, similar to results linking access to drinking-water sources and forage for sable antelope (*Hippotragus niger*) and zebra (*Equus grevyi*) in South Africa (Cain et al. 2012). The consequences of modified landscape utilization by large grazers, driven by reduced water availability and climate change, have the potential to impact conservation efforts (Sander-son et al. 2008), predator-prey dynamics (McCluney et al. 2012), forage quality and availability (Craine et al. 2009), migratory behavior (Fryxell and Sinclair 1988) and overall grassland structure and function (Collins et al. 1998).

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SUPPLEMENTAL MATERIAL

SUPPLEMENT

Water isotope data collected from bison fecal samples, streamwater samples, and precipitation samples from January 2010 to March 2011 on the Konza Prairie, Kansas USA (*Ecological Archives* C004-001-S1).