Differences in the use of surface water resources by desert birds are revealed using isotopic tracers

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PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3167v3 | CC BY 4.0 Open Access | rec: 21 Nov 2018, publ: 21 Nov 2018

ABSTRACT

The scarcity of free-standing water sources is a key determinant of animal and plant community structure in arid environments, and an understanding of the extent to which particular species use surface water is vital for modelling the effects of climate change on desert avifauna. We investigated interspecific variation in the use of artificial water sources among birds in the Kalahari Desert, South Africa, by 1) observations at waterholes and 2) tracing spatial water-use patterns during summer by isotopically-labelled water sources and blood sampling. More than 50% of the avian community (primarily insectivores and omnivores) were not observed to drink. The majority (53%) of species drinking at waterholes were granivorous, and their use of surface water was best predicted by their relative abundance in the community. Species representing the remaining dietary guilds drank significantly more on hot days. Blood samples revealed that only 11 of 42 species (mostly granivores and a few omnivores) showed evidence of drinking at a waterhole with enriched deuterium values; on average, in the latter birds, water from the enriched waterhole accounted for ~38% of their body water pool. These findings illustrate that two methods employed in this study provide different, but complementary data on the relative importance of a water source for an avian community. Although our results suggest that most avian species are independent of surface water, drinking patterns on the hottest days during our study period suggest that free-standing water might become more important for some of the non-drinking species under hotter climatic conditions.

Keywords: *water balance, stable isotopes, drinking behaviors*

INTRODUCTION

Small animals have limited capacities to store body water and consequently need to regulate water intake over time scales of minutes to hours, particularly in hot, arid terrestrial environments where high environmental temperatures and high vapor pressure deficits result in rapid evaporative water loss (Hill et al. 2004). Daily water demands are particularly high in small endotherms such as birds, because of their high mass-specific metabolic and evaporative water loss (EWL) rates (Wolf 2000, Williams and Tieleman 2005); for example, in small desert birds daily water fluxes may exceed 50% of body mass (Webster and Weathers 2000).

Birds gain water through metabolic water (i.e., oxidative water), drinking, and/or water rich food sources (i.e., preformed water) (Bartholomew and Cade 1963, MacMillen 1990). Although metabolic water production can sometimes balance total water losses at air temperatures (T_{air}) < 25 °C, birds need to supplement intake by drinking surface water or eating water-rich foods at higher T_{air} when rates of EWL exceed rates of metabolic water production (MacMillen 1990, MacMillen and Baudinette 1993, Williams 1999, 2001). Species relying on food with low water content (e.g., dry seeds) generally need to drink daily in hot, arid habitats (Bartholomew and Cade 1963, Cade and Maclean 1967, MacMillen 1990, Berry et al. 2001). Moreover, daily drinkers must sometimes allocate time to visit water sources that are distant from their foraging grounds and nesting sites. During daily trips to water, birds potentially incur costs related to metabolic heat production while flying (Aulie 1971, Hudson and Bernstein 1981, Engel et al. 2006a), and increased water requirements because EWL is elevated when flying during hot weather (Tucker 1968, Torre-Bueno 1978, Engel et al. 2006b). An additional cost is related to predation risk, which is often very high around isolated water sources on account of greater concentrations of predators (Cade 1965, Fisher et al. 1972, Rosenstock et al. 1999).

Birds that feed on diets with high water content (e.g. carnivores, insectivores and frugivores) can obtain most, if not all, of their required water intake while foraging (Wolf and Martinez del Rio 2000, Wolf et al. 2002). By being independent of surface water, species can therefore occupy habitats where surface water is unavailable (e.g. sandy deserts). However, one potential disadvantage of this mode of water acquisition is that foraging efficiency could greatly affect hydration state. If these birds experience reduced foraging efficiency on hot days, they are likely to face hydration bottlenecks when evaporative water loss demands exceed water intake (du Plessis et al. 2012; Smit and McKechnie 2015). Several studies of arid-zone birds have revealed that during hot weather individuals do not maintain body mass, despite maintaining foraging efforts during hot periods of the day, and the authors linked these patterns to high water efflux rates and increased evaporative cooling demands (du Plessis et al. 2012, Smit and McKechnie 2015, van de Ven 2017).

In many arid environments, fresh water sources are scarce and unpredictable and the availability of water-rich foods such as fruits and insects is generally low and/or restricted to only short seasons (Wolf and Martinez del Rio 2000, 2003). Artificial water sources are often provided by conservation managers or livestock farmers for large mammals, and may represent the only drinking water sources in many areas. Although artificial water sources may be beneficial to terrestrial organisms, including birds (McKechnie et al. 2012, Abdu et al. 2018) and large mammals (Hayward and Hayward 2012) under hot conditions, it has been shown that the presence of these artificial sources can greatly contribute to habitat degradation (Dean and Macdonald 1994, Jeltsch et al. 1997, Landman et al. 2012). An improved understanding of the dependence of terrestrial communities on freestanding water sources, and how this changes under hot conditions, is needed to inform conservation management decisions about the availability of artificial water sources. For example, Kearney et al. (2016) modelled thermal heat load and EWL rates in the endangered Night Parrot (*Pezoporus occidentalis*), and showed that reliance on drinking surface water will increase under climate warming scenarios.

The Kalahari Desert of southern Africa provides an ideal model system for testing predictions about the use of artificial surface water by birds. Environmental temperatures in the Kalahari during the summer are high and surface water sources scarce (even during high rainfall periods), and yet the region supports a diverse avian community. Previous studies in the southern African arid zone have demonstrated how the avian communities are strongly shaped by water resources (Simmons et al. 2002), and that some resident birds (e.g. sandgrouse, *Pterocles* spp.) will cover large distances to drink water daily at isolated water sources, and even transport water to their young, (e.g. *Pterocles* species) (Willoughby and Cade 1967, Cade and Maclean 1967). Yet, many other resident species in these deserts do not appear to make use of surface water sources (Serventy 1971, Maclean 1996). Quantifying surface water use has traditionally required many hours of observations to record birds drinking at surface water sources, in addition to determining the background avian community composition. In this study, we quantify interspecific variation in use of surface water in an arid-zone bird community using both direct observations of birds at waterholes and an isotopic labelling approach (Rosenstock et al. 1999, Hyde 2011). We propose that these two methods provide complementary insights into the use of surface water. We predicted that species whose diets are characterized by low water content (granivores) are more reliant on free-standing water sources compared to insectivores, frugivores and nectarivores. We also predicted that birds increase their use of free-standing water during hot and/or dry weather.

METHODS AND MATERIALS

Study Site

We examined interspecific variation in drinking dependence within a Kalahari Desert bird community at Tswalu Kalahari Reserve (TKR; ~1100 m a.s.l. 27.197°**S**, 22.439°E), Northern Cape Province, South Africa during the austral summers of 2009/2010 and 2011/2012. TKR consists of approximately 100,000 ha of semi-arid Kalahari savanna on vegetated red sand dunes. Mean annual rainfall over a 30-year period at TKR was 295 ± 172 mm (coefficient of variation, CV = 58.3%) (unpublished data, Tswalu Kalahari Reserve). With the exception of seasonal dams at the foothills of the Koranna Mountain range (1400-1600 m a.s.l., forming the eastern border of TKR), and a few isolated calcareous pans, no natural surface water occurs in the dune habitat (even during the rainy season). Artificial waterholes have been established throughout the reserve (generally more than 4 km apart), primarily to supply drinking water to large ungulates.

We assessed avian surface water use using two methods: first, by monitoring drinking patterns at four artificial waterholes directly, and second, by investigating the importance of surface water by enriching a waterhole with a stable isotope label and then sampling body water of the bird community at distances of 0–2.5 km from the labelled water source. Many previous studies have investigated the potential benefits of artificial water developments to desert avian communities using direct observations (Smyth and Coulombe 1971, Williams and Koenig 1980, Broyles 1995, Brien et al. 2006, Lynn et al. 2006, 2008; Simpson et al. 2011, Lee et al. 2017, Abdu et al. 2018). However, whereas direct observations provide qualitative data on which species drink and their drinking frequency, isotopic data provide quantitative insights into the importance of a water source to individuals' body water pools [see (Hyde 2011)].

Weather Data

Air temperature data were obtained every 10 min during the study period using a portable weather station (Vantage Pro2, Davis Instruments, Hayward, CA), set 2 m above the ground at a central location in the study site. This weather station had an aspirator fan to ensure air movement over the temperature and humidity sensors. We checked the factory calibration of the weather station against a mercury thermometer (resolution 0.1 °C, accuracy traceable to the South African Bureau of Standards) at a range of temperatures (5 to 50 °C).

Observations of Drinking Patterns

Avian drinking patterns were assessed using video cameras (Sony Handycam, DCR-SR45E, Sony Corporation, Tokyo, Japan) to record behavior continuously from sunrise to sunset during mid-summer (November to December 2009), at four waterholes in TKR. All four waterholes were in the dunes, each > 3 km away from its nearest neighbor and artificially maintained by reservoirs supplied with groundwater. All waterholes were fully exposed to the sun and the nearest vegetation was > 5 m away. We placed video cameras in custom-made rain- and baboon-proof steel boxes next to waterholes or in nearby trees. Video cameras were positioned to cover the maximum area over the waterhole to ensure birds would be visible when drinking at any edge of the water. Recordings commenced between dawn and sunrise, i.e. 0500 and 0600 hours (UTC + 2 hr), respectively (variation due to the 45-min travel time between the first and fourth cameras). The cameras were set to record continuously throughout the day, and in most cases batteries lasted until sunset. We downloaded video footage and recharged batteries on days following recordings and therefore video recordings were repeated on alternate days. We obtained video recordings over a total of 9, 13, 13 and 16 days at the four waterholes, respectively (~ 660 hr of video recordings). We subsequently viewed play-backs of each waterhole's recording during a focal period of 10min, at 30-min intervals (i.e. two 10 min focal periods per hr per waterhole). We recorded species and estimated the number of individuals utilizing the waterhole during each focal period. An independent assessment of numbers of individual birds per species drinking was not feasible and therefore we report statistics on presence/absence of drinking per species instead. From the recordings we generated a data set where we computed the presence or absence (as a binomial response) of individual species drinking at each waterhole over four consecutive focal periods (hereafter *drinking_int*). We further compared *drinking_int* at one waterhole obtained from sub-sampled video footage (18 x 10 min intervals) with continuous observations (video or manual) at one waterhole for an entire day, and found that only two species (generally rare species) were missed during interval counts compared to a full day of observation. To quantify the composition of bird species drinking at waterholes relative to the background avian community composition at TKR (hereafter referred to as relative abundance), we conducted 62 point counts along eight transects radiating up to 2.5 km of the four waterholes (see supplementary materials, Table S1). Point counts were on approximately 500 m apart and ranged from the habitat surrounding a waterhole up to 4 km from the nearest water source. During each point count (lasting 10 minutes), we identified and recorded all species seen or heard within a radius of ~ 200 m. We assumed that detectability did not vary with distance from the observer (as typically happens in more closed habitats such as forests (Reynolds et al. 1980). This assumption was reasonable at TKR because of the open nature of the habitat.

Water Resource Use Inferred from Stable Isotope Label

The relationship between ratios of stable isotope ratios of hydrogen and oxygen, namely δ^2H and δ^{18}O, measured in precipitation is typically described by the local meteorological water line (LMWL) (Craig 1961, Turner 1987, Tyler 2011). The δ^2H and δ^{18}O of body water of organisms vary collinearly—depending on the organisms' prime water source (McCluney and Sabo 2010). Where organisms obtain water from the environment (ultimately from precipitation) it is necessary to account for water fractionation at each trophic transition (Hobson 1999, Wolf and Hatch 2011): for example the leaves of plants lose water through transpiration and many herbivorous invertebrates ingest water with δ^2H and δ^{18}O values that deviate from the LMWL because of theses evaporative process in leaves (see Brooks et al. 2009). Insects also lose water evaporatively and so insectivorous birds assimilate water with an additive evaporative enrichment of both vegetation and insects, and so they will have a greater evaporative deviation from the LMWL. The kinetics of evaporation yield a linear relationship between δ^2H and δ^{18}O that differs from the LMWL (Brooks et al. 2009). The intersection between the linear δ^2H vs. δ^{18}O relationships for evaporated water (in plants and animals), and precipitation water (originating from a point along the LMWL) is an indication of the water source before evaporation (Gibson et al. 2008). Therefore δ^2H and δ^{18}O of body water of organisms (at the community level) will also vary collinearly—depending on whether the organisms obtain water directly or indirectly from precipitation (McCluney and Sabo 2010). We define this “community level” relationship of δ^2H and δ^{18}O in body water” as a physiological evaporative water line (PEWL). We made use of these principles to quantify water resource use of the bird community in the Kalahari Desert where natural surface water is extremely rare, but surface water obtained from groundwater is provided in artificial waterholes to serve livestock and wildlife. The δ^2H and δ^{18}O values of ground water are typically depleted compared to precipitation and we expect these values to be slightly evaporated and lie along the lower end of the LMWL. Birds that drink provisioned water regularly should show δ^2H and δ^{18}O values close to that of groundwater. For the remainder of the community, we expect δ^2H and δ^{18}O values to fall close to the LMWL if they obtain water directly from precipitation, or along the PEWL if they obtained water indirectly by feeding on other organisms.

The mode of water intake at individual level may be difficult to establish if an organism obtains water from a mixed resource base. Water resource use can be more directly assessed if the hydrogen or oxygen isotope ratios of a prominent surface water source is enriched above natural levels (McKechnie 2004, McKechnie et al. 2004). The presence of enriched isotope ratios in the body water pool of an individual can then be used to infer the percentage of an animal's body water pool derived from a particular water source, thereby giving a quantitative estimate of dependence on a free-standing water source (McCluney and Sabo 2010).

We collected blood samples from birds trapped (1) within 2 km of a non-enriched waterhole, and (2) within 2.5 km of a waterhole where we artificially enriched δ^2H levels (enrichment process described below). Blood samples obtained from the bird community before artificial enrichment allowed us to estimate the PEWL from background δ^2H and δ^{18}O values of body water.

We used mist nets (10 per trapping site) and spring traps baited with tenebrionid larvae to trap birds. Trapping sites around focal waterholes were selected along a radius from the waterhole in a direction devoid of waterholes/surface water sources for at least 6 km. We selected trapping sites starting at the first shrubs and trees from waterhole (10 m) at intervals of ~500 m to a distance of 2 km (non-enriched waterhole) or 2.5 km (enriched). Although we had no way to verify *in situ* that birds trapped used the waterholes we studied, the latter site was the furthest we could trap from the enriched waterhole without being nearer to neighboring waterholes. Most waterholes in the 100 000 ha section of the reserve where we caught birds receive water from one or two boreholes at the foothills of the Koranna range. We therefore expected low variation in background levels of all species obtaining water from non-enriched waterholes. Trapping started two days following enrichment and we spent at least one day at each trapping site and carried out 132 trapping hours (80 during February and 52 during October 2011), trapping a total of 333 birds (196 and 137 in February and October, respectively). We trapped birds during the morning, from 0600 hours (sunrise) until about 1100 hours, and in the afternoon (depending on temperature) from 1500 hours till 1900 hours (sunset). We obtained blood from each bird shortly after capture, or held trapped birds in cotton bags suspended in full shade until they could be processed. No birds were held for longer than 3 hours.

We obtained blood samples (10-150µl) by brachial venipuncture, using a sterile 27-gauge hypodermic needle and heparinized microcapillary tubes. We transferred each blood sample immediately to a 150-µl micropipette and flame-sealed both ends of the pipette (Speakman

1997). We stored the blood samples at 2-5 °C in a domestic refrigerator prior to further analyses. We separated pure water from the blood samples by cryogenic vacuum distillation, and measured δ^2H and δ^{18}O of the water samples obtained from bird blood and waterholes, using a PAL autosampler and DLT-100 liquid water isotope analyzer (Los Gatos Research, Mountain View, CA, USA). Samples were bracketed with laboratory standards referenced against Vienna Standard Mean Ocean Water samples (VSMOW, an international water standard produced by the International Atomic Energy Agency) for δ^2H and δ^{18}O, and calibrated enriched standards spanning the ranges of -79 to 978 $‰_{\mathrm{VSMOW}}$ (δ^2H) and -11.54 to 260.82 $‰_{\mathrm{VSMOW}}$ (δ^{18}O). A minimum of ten 1-µL replicates were analyzed per sample or standard, and values for the first five replicates were typically discarded in order to avoid isotopic memory effects.

In total, we obtained 68 samples (17 species) during the non-enrichment sampling periods, and 247 species during the enrichment sampling period (for samples per species see Supplementary material Table S1). All experimental procedures were approved by the University of Pretoria's Animal Use and Care Committee (clearance #EC058-09), permits for animal sample collection were obtained from Department of Environment and Nature Conservation (permit #FAUNA 721/2010).

Enriching the waterhole

We enriched the Donderkamp waterhole at TKR from February 21, to March 6, 2011, and again from October 21, to November 3, 2011, following Hyde (2011) by enriching the stable hydrogen isotope ratio (δ^2H). We chose Donderkamp waterhole as it was a fairly isolated surface water source (> 5 km from nearest neighboring water source) in our study area within TKR. The waterhole system consisted of a shallow (~20 cm depth) waterhole with a diameter of 3 m, with water supplied by a ~ 22,800-L reservoir (diameter = 4.3 m, depth = 1.57 m). Although large mammals frequently made use of the water source, we estimated that the reservoir took more than four weeks to run completely dry when the input of freshwater was closed. We therefore emptied the reservoir to approximately 14,000 L (1 m deep) and closed the main inlet to prevent the labelled water from being diluted by inflow ground water. We then added 585 mL of 99.99 ‰ deuterium oxide to the reservoir, estimated to enrich the waterhole by 200-300 $‰_{\mathrm{VSMOW}}$ above background levels, and stirred the water for at least two minutes. We then emptied the shallow waterhole to allow labelled water from the reservoir to enter the waterhole. We collected water samples before the experimental enrichment and subsequently every 1-2 days for two weeks after enrichment. The enriched levels showed no trends during each two week enrichment period, and we also confirmed that the δ^2H and δ^{18}O returned to “normal” groundwater levels between the two enrichments periods (seven months).

During February 2011 (wet season) we enriched the δ^2H levels in Donderkamp waterhole from -41.7 $‰_{\mathrm{VSMOW}}$ to 254 $‰_{\mathrm{VSMOW}}$ (mean of values obtained over 14 days after enrichment,). During October 2011 (dry season) we enriched the δ^2H levels in Donderkamp waterhole from -31.7 $‰_{\mathrm{VSMOW}}$ to 238 $‰_{\mathrm{VSMOW}}$. During both sampling periods there were no trends in δ^2H levels at the waterhole over the 14 days blood sampling period, providing us with a reliable ‘background drinking water” estimate.).

Data Analyses

Observations

We only included species in the behavioral analyses if they were observed more than three times during community relative abundance surveys, and observed on more than three occasions at one of the waterholes (Supplementary material Table S1). We fitted generalized mixed effects models (logistic regression), using the lme4 package in R, to determine if *drinking_int* (a binomial response) was related to fixed effects of (1) maximum T_{air}, (2) time of day, (3) relative abundance of the background community and (4) dietary guild (as described above), including the interaction effect of (3) and (4). Species and waterholes (four sites) were included as a random factor to account for the probability of repeated samples of same species at a waterhole. We selected family as “binomial” and used the “logit” link function. Maximum T_{air}, represented the highest air temperature recorded on the day of observation at TKR. Time of day was categorized as “morning” and “afternoon” for observations obtained before and after 1200 hours, respectively. We realized that temporal drinking patterns may vary at a finer scale that this, but we opted to reduce the number of levels per factor by testing whether birds drink before or during the hottest period of the day. Abundance was calculated as a proportional vector of the total number of point counts where a species was present *n*, over the total over the sum of *n* for all species. Abundance values, when treated as a predictor, were logit-transformed for analyses.

We fitted a global model to all the above factors, and removed non-significant terms (computed from the Type II Wald chi-square test, using the “Anova” function, package car) in a step-wise manner. In addition, we also ran models on each dietary guild and species separately, using the same fixed (excluding “diet”) and random terms; however, in individual species models we included “waterhole” only as a random factor. We computed Akaike Information Criteria (AIC) values of all models ran and used AIC score to validate the top two remaining models (in most cases the third model differed in AIC values >5), against a null model testing whether drinking is independent of any of the factors tested.

Stable isotopes

A two-endpoint mixing model (Gannes et al. 1997) was used to estimate the proportion of an individual's body water pool derived from an artificial water source enriched with δ^2H. This involved estimating the PEWL before enrichment, and then determining how δ^2H versus δ^{18}O values deviated from the expected values along the PEWL after enrichment. Artificially enriched δ^2H levels in a water source will result in δ^2H ratios falling significantly away from the PEWL (i.e. the baseline δ^2H versus δ^{18}O relationship).

Estimation of the natural physiological water line

We obtained 69 blood samples (18 species) at distances of 0 and 2 km around two non-enriched waterholes at times when no waterholes were artificially enriched.

We broadly categorized the dietary guild of each species following Smit et al. (2016) using available literature (Hockey et al. 2005). The estimation of the PEWL has to exclude birds that obtained water directly from precipitation as these values may follow the LMWL instead. We first plotted δ^2H and δ^{18}O of each sample, by dietary guild, and we then performed a chi-square distribution of fit for each guild. We established that all guilds showed δ^2H and δ^{18}O values that differed significantly from the LMWL ($p<0.01$) for the Kalahari region (Schachtschneider and February 2013). We performed an ANCOVA on δ^2H and δ^{18}O obtained during the non-enrichment period, including dietary guild as a factor, to establish if guilds had a separate PEWL. Although there was a significant difference in the PEWL of insectivores and granivores (see results for details), both these were not significantly different from the remaining dietary guilds and using separate PEWL lines did not change our findings on surface water dependency. We subsequently performed a linear regression by pooling δ^2H and δ^{18}O values of all guilds during the non-enrichment periods to estimate the PEWL; this line was described by the following function: $\delta^2H = 3.73\ \delta^{18}O + 10.0$, $r^2 = 0.77$. The slope of this equation falls within the expected range of evaporative meteoric water lines (Kendall et al. 1995). We subsequently used residual δ^2H values of samples obtained during the enriched period falling above of the 95 % prediction intervals of the respective baseline as evidence that individuals obtained water from the enriched waterhole; these were significantly different from the expected δ^2H and δ^{18}O relationship along the PEWL.

For all individuals showing evidence of drinking from the enriched waterhole we determined the proportion of each individual's body water derived from that source (hereafter referred to as P%) using δ^2H residuals from the PEWL predicted values for a given δ^{18}O value. P% was calculated using the following equation,

$$P\% = [\delta^2H_{bird} - \delta^2H_{baseline}]/[\delta^2H_{spike} - \delta^2H_{baseline}]$$

where δ^2H_{spike} is the δ^2H value of the enriched water hole, $\delta^2H_{baseline}$ is the expected δ^2H value of the sample based on the PEWL regression of $\delta^{18}O$ and δ^2H around the non-enriched source, and δ^2H_{bird} represents the measured δ^2H value for the bird sample.

We used a general linear model to test and the relationship between observed drinking pattern and P%; we regressed P% values of individuals against the proportional vector of *drinking_int* for that species at the Donderkamp waterhole. Proportional values were logit-transformed for analyses following Warton and Hui (2011). Data are available from figshare. https://doi.org/10.6084/m9.figshare.5313910.v1.

RESULTS

Weather during Study Periods

Mean daily maximum T_{air} on the 16 days for which we obtained video recordings was 32.1 °C, ranging from 26.4 °C to 37.4 °C (two days > 35 °C). Mean daily maximum T_{air} during the stable isotope study was 32.7 ± 2.7 °C (25–35 °C) during the wet season and 30.6 ± 4.2 °C (26–37 °C) during the dry season, respectively.

Drinking Patterns

Observational data

We recorded 69 species during the study (Supplementary material Table S1). The avian community at TKR was dominated by insectivores (49.3 % of species) and granivores (32.8%); birds of prey, omnivores, frugivores and nectarivores represented 10.4, 10.4, 9.0 and 1.5%, respectively. We observed a total of 36 species drinking at the waterholes (Supplementary material Table S1). The probability of observing birds drinking (*drinking_int*) was significantly related to maximum T_{air} (χ^2=22.37, $P < 0.001$, positive relationship), dietary guild (χ^2=13.22, $P < 0.01$; granivores showed a higher intercept), relative abundance in community (χ^2=6.29, $P < 0.05$; positive relationship), and the interaction between dietary guild and abundance (χ^2=9.28, $P < 0.05$); granivores were observed in relation to their abundance); time was not significant ($P > 0.10$). The above patterns largely remained when we analyzed dietary guilds separately. In granivores, drinking was significantly related to maximum T_{air} (Fig. 1) and abundance (species encountered more frequently were more likely to be observed drinking, Table 1, Fig. 2; summary of competing models provided in supplementary material Table S2), but not time. Abundance did not significantly predict drinking in the remaining dietary guilds (Table 1, Fig. 2), and instead hotter maximum T_{air} (for omnivores and insectivores, Fig. 2) and time of day (greater probability of afternoon drinking in all dietary guilds) significantly predicted drinking (Table 1). When species were analyzed individually we found that while the majority of species did not show temperature-dependent drinking, eight species drank significantly more on hot days, and three species (two doves and sandgrouse) drank significantly more on cooler days (Supplementary material Table S1).

Drinking inferred from isotopically labelled water sources

The δ^2H and δ^{18}O values of the two non-enriched water sources were -47.2‰$_{VSMOW}$ and -7.6 ‰$_{VSMOW}$ respectively (large reservoir), and -24.0 ‰$_{VSMOW}$ and -0.9 ‰$_{VSMOW}$ respectively (small shallow waterhole). These values fell very close the local meteoric water line (LMWL) established by (Schachtschneider and February 2013) for the Kalahari region Figure 3A.

Although neither of the dietary guilds showed δ^2H and δ^{18}O values that consistently matched the LMWL, some individual granivore samples followed the LMWL closely (Figure 3A). There was a significant difference in intercept and slope of δ^2H and δ^{18}O values among the different dietary guilds (Diet: F = 3.43, df = 3, $P < 0.05$; interaction of diet and δ^{18}O: F = 3.64, df = 3, $P < 0.05$). A post hoc analysis revealed that granivores showed a significantly lower intercept and steeper slope compared with insectivores (TukeyHSD, $P < 0.05$), although all remaining dietary guilds did not differ significantly from granivores or insectivores ($P > 0.05$). The δ^2H vs. δ^{18}O gradient is determined by the kinetics of water precipitation and evaporation (Brooks et al. 2009), and there appeared to be a continuum in the primary water source of the different dietery guild's; granivores were closest to the LMWL, while the other guilds were progressively more indicative of evaporated water (linked to a trophic cascade of water). Insectivores, frugivores and omnivores had higher δ^{18}O values (mean of 13.2, 12.9 and 11.0‰$_{\text{VSMOW}}$, respectively) compared with granivores (6.9 ‰$_{\text{VSMOW}}$), representing one/two water trophic intermediaries (vegetation and insects) in the former guilds (Figure 3B). These results on δ^{18}O values were evident even during the non-enrichment period.

Of the 247 individuals (35 species) for which we obtained isotope samples during the enrichment periods, 39 individuals (11 species) showed evidence of the enriched water source in their body water pools, by having δ^2H and δ^{18}O values that fell above the 95% predictive interval of the PEWL (Supplementary material Table S1). The δ^2H and δ^{18}O ratios for all insectivores (including a few carnivores) and frugivores fell within the 95% prediction intervals of the PEWL, which we interpret as evidence that these species did not use the labelled water source (Figure 3B). Some granivore and, to a lesser extent omnivore, samples represented the only guilds where δ^2H and δ^{18}O values fell above the LMWL, indicating use of the enriched water source (Figure 3B).

The number of individuals showing enriched body water pools (both total and proportion of trapped) were highest near the waterhole, and low at distances more than 500 m from the enriched waterhole (Fig. 4A). The samples sizes of P% estimates away from the waterhole (> 500m) were too small to conduct rigorous statistical analyses, but nevertheless there appeared to be no strong trends with increasing distance from waterhole (Fig. 4B). Two of the most commonly trapped species, Namaqua Doves (*Oena capensis*) and Violet-eared Waxbills (*Uraeginthus granatinus*), showed enriched P% values at most trapping sites, up to 2 km away from the enriched waterhole (Figure 4C-D). Although a number of samples were obtained from insectivores within 100-1000 m of the waterhole, none of these individuals used the waterhole (Table 2).

We found a significant effect of proportion of drinking for each species at Donderkamp and the P% values obtained in birds trapped when the waterhole was enriched ($F_{1,35} = 8.07$, $P < 0.01$, $r^2 = 0.19$). Restricting this analyses to granivores only, improved the fit substantially ($F_{1.28} = 16.72$, $P < 0.001$, $r^2 = 0.37$; Figure 5).

DISCUSSION

Our data, obtained using two complementary approaches, revealed that many species at our study site were largely independent of drinking freestanding water. Whereas our observations of avian drinking patterns provided ecologically relevant insights into drinking behavior (such as timing of drinking and temperature-dependence of drinking events), our stable isotope approach provided quantitative estimates of the importance of surface water for the water balance of birds in the surrounding area. Both techniques revealed that avian granivores are most dependent on surface water—with drinking frequency reflecting their relative abundance in the Kalahari community, and in many cases more that 30% or more of their body water pools were derived from the enriched source. In contrast, insectivores and most omnivores were largely independent of surface water as was evident from the stable isotope values of their body water and our observations that they were less frequently observed making use of surface water relative to their abundance estimates. Drinking probability increased on hot days for most birds when analyzed at guild level, but these patterns were most evident in insectivores. Only three species, all daily drinkers, used the waterhole less on hot days, suggesting that trips to waterholes became costly in terms of heat and water balance on the hottest days. These data corroborate previous findings based on observational data that relatively few species inhabiting the arid regions of Africa and Australia are dependent on drinking water (Willoughby and Cade 1967, Fisher et al. 1972).

Both of the approaches we used here revealed that granivores represent the guild with the greatest dependence on surface water. One advantage of the isotopic method over that of observations is that surface water dependence could be estimated without knowledge of the relative abundance of individual species in the surrounding avian community. Our drinking dependence estimates for individual species relied heavily on the assumption that we obtained an accurate estimate of the relative abundance of the avian community at TKR (Lee et al. 2017, Abdu et al. 2018). We believe this assumption will hold largely for resident bird species at TKR, but many species are visitors to the Kalahari region and their number may have fluctuated during our observation periods.

Natural variation in stable isotope ratios in the tissues of plants and animals has been widely employed to quantify the importance of a given resource at a landscape scale. For example, this technique has been used to show dependence of White-winged Doves (*Zenaida asiatica*) on saguaro fruit as a water source during summer in the Sonoran Desert (Wolf and Martinez del Rio 2000). Moreover, this method has also revealed how White-winged Doves obtain both water and nutrients from these fruits, whereas Mourning doves (*Zenaida macroura*) obtain mainly nutrients, but not water (Wolf et al. 2002). We are aware of only one previous study (Hyde 2011) using the technique of artificially enriching a water source to investigate drinking dependency in an avian community.

A few individuals of some granivorous species in our study, including Namaqua Doves (*Oena capensis*), Laughing Doves (*Spilopelia senegalensis*) and Yellow Canaries (*Crithagra flaviventris*), did not drink from the enriched waterhole based on their δ^2H values (Figure 3A). However, these individuals typically had δ^2H and δ^{18}O values closer to that of the non-enriched water-holes, suggesting they may have drank from other non-enriched water sources at TKR. The relatively depleted δ^{18}O values, in particular, suggest they were not taking in water from other trophic levels, as was the case in insectivores, frugivores and omnivores. Our isotopic assessment of the importance of surface water resources to the bird community was based on enrichment of a single waterhole. Therefore, we may have underestimated surface water dependency, as some of the regular drinking species almost certainly visited more than one waterhole thus diluting the label from our enriched source. Enriching more than one waterhole with δ^2H was not feasible in our study, because one of our aims were to establish how far individual birds would travel to the enriched source, and replicating the trapping and blood sampling of birds around more waterholes, simultaneously, was not logistically feasible.

Within the species where we found individuals with enriched isotope levels in their body water we found that the drinking frequency was significantly related to enrichment level. This pattern was largely driven by a few granivore species. The low P% values or absence of enriched isotope values in some omnivores and frugivores that have been observed to drink—both in the present study (e.g. mousebirds and some weavers) and previous studies (Willoughby and Cade 1967, Abdu et al. 2018), may be perhaps explained by drinking only on the hotter days, and relying largely on the abundant preformed water obtained from insects and fruits.

Insectivores were greatly under-represented at waterholes, despite this group being speciose in the avian community at TKR (Martin et al. 2015, Smit et al. 2016, Abdu et al. 2018). A few insectivore species, such as Ant-eating Chat, *Myrmecocichla formicivora*, and Common Fiscal, *Lanius collaris*, drank occasionally on hot days, yet their blood isotope values suggested that surface water did not contribute significantly to their water budget, even for those individuals trapped near the labeled water hole. Focal studies on individual birds will be required to test if free-standing water will become a greater component of their daily water budget when EWL exceeds preformed water intake under hotter conditions (Smit and McKechnie 2015).

Ecological Implications of Drinking

Previous studies in semi-arid and arid ecosystems of North America, using behavioral observations, have suggested a greater reliance on surface water sources by avifauna (Gubanich 1966, Williams and Koenig 1980, Lynn et al. 2008) compared with studies in southern Africa's Kalahari and Namib Deserts and Fynbos biome (present study; Willoughby and Cade 1967, Lee et al. 2017, Abdu et al. 2018). Drinking patterns in the Australian arid zone (Fisher et al. 1972) also suggest that comparatively few species (mostly granivores) make regular use of permanent freestanding water. These intriguing patterns suggest that African and Australian avifauna have been under greater selection to become independent of drinking water.

Spatial patterns in the use of surface water sources are of particular interest as these could structure animal communities when water sources are isolated in the landscape. Our isotopic labelling approach revealed that some granivores travelled in excess of 2 km to the enriched source, and that between 35 and 91% of their body water pools were derived from the experimentally enriched water hole. Small, granivorous species (e.g. Violet-eared Waxbill) were likely to be most strongly tied to water availability, and likely face strong trade-offs between elevated rates of EWL *versus* water gain when flying to water sources during hot weather. Future research could determine if an increase in the density of water points have a positive effect on water balance of small drinking dependent species, as well as species that rely more on water during hot weather.

Conclusions

Our study illustrates how two methods can be used to obtain complementary data on surface water dependency in a desert bird community. Information on the importance of surface water as a daily requirement, or as a limiting resource on hot days, will be important for developing water and energy balance models and time-activity budget models for specific species. For example, knowledge of whether species need to obtain their water through their food and by foraging, from metabolic water, or from drinking, will be important in understanding how much time and energy an individual need to spend to avoid dehydration on hot days. Our stable isotope analyses of the body water pools corroborate in a novel manner previous findings that desert birds taking in water-rich food rely less on surface water compared to species taking in food with lower water content. Our findings that the probability of drinking increases on hot days echo predictions that more species will rely on surface water, particularly artificial sources under future climate warming scenarios (McKechnie et al. 2012). Providing artificial water has been a contentious and highly debated issue, particularly when larger mammals degrade habitat around waterhole (Hayward and Hayward 2012, Landman et al. 2012). With these landscape-level changes we might expect increasing conflicts between provisioning of free-standing water to meet increasing evaporative cooling demands, and habitat protection where ecosystem function and microclimate availability are key objectives in semi-arid/ arid environments. Water provisioning as a global change conservation effort needs to be carefully evaluated and implemented.

ACKNOWLEDGEMENTS

We thank E Oppenheimer and Son, and the staff at Tswalu Kalahari Reserve. We are grateful to the Tswalu Foundation for sponsoring a field vehicle and accommodation at TKR. We are further grateful to O. Davies, C. Harding, R. Martin, S. Cunningham, R. Sutcliff, D. Nkosi, L. Barclay and M. Whitfield for their help in the field, and R. Lerm and P.J. Jacobs for help in the laboratory. All experimental procedures were approved by the University of Pretoria's Animal Use and Care Committee (clearance #EC058-09).

Funding statement: This research was supported by funding from National Research Foundation and the DST/NRF Centre of Excellence at the Percy FitzPatrick Institute to AEM. SW was employed by iThemba LABS, Johannesburg, during preparation of the manuscript.

Author contributions: BS, AEM, BOW conceived the study; BS performed the experiments, wrote the manuscript and analyzed the data; SW, AEM and BOW edited earlier drafts of the manuscript; AEM contribute resources and funding.

Data deposits: Data are deposited with figshare, and can be accessed at: https://doi.org/10.6084/m9.figshare.5313910.v1

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FIGURE CAPTIONS:

Figure 1: Drinking behavior (*drinking_int*) representing presence/absence of drinking during focal periods as a function of maximum air temperature recorded on the day of observations in (A) granivores, (B) omnivores, (C) insectivores, and (D) frugivores at Tswalu Kalahari Reserve during the summer (November to December 2009). The size of circles represent the number of observations at the different temperatures. The logistic regression represents the predicted proportion of drinking as determined by the outcome of mixed-effects models (Table 1); solid trendlines indicate significant regressions ($p < 0.05$) and dashed lines indicate regressions approaching significance ($p < 0.1$). The trend line for granivores was omitted for clarity as it approached the upper boundary of the predicted response across the air temperature range (granivores were observed drinking every day).

Figure 2: Drinking behavior (*drinking_int*, calculated as a proportional vector of the number instances where drinking behavior was observed) at the Donderkamp waterhole, Tswalu Kalahari Reserve, as a function of proportional abundance (calculated as a proportional vector of number of instances a species was observed during focals) of the species recorded in the reserve. Proportional values are logit transformed. The dotted line indicates the null model if drinking behavior is directly proportional to relative abundance. Carnivores (about three bird of prey species) were lumped with insectivores for simplicity. Granivores were the only guilds to drink in proportion to their abundance (see text and Table 1 for detailed statistics). The only granivore species that did not drink in proportion to its abundance was Scaly-feathered Finch (*Sporopipes squamifrons*, SFF), despite being one of the most frequently encountered species in the reserve.

Acronyms are defined as: GRA for granivore; FRU for frugivore; INS for insectivore; and OMN for omnivore.

Figure 3: A) δ^2H and δ^{18}O values of birds sampled around two non-enriched water sources in Tswalu Kalahari Reserve. B) δ^2H as a function of δ^{18}O of birds sampled within 2km of an enriched waterhole, Donderkamp, in Tswalu Kalahari Reserve (during February and October 2011). In both panels, the blue dashed line represent the local meteoric water line (LMWL) established by Schachtschneider and February (2013) for the Kalahari region. In both panels, the solid trendlines represent a linear regression model ($\delta^2H = 3.73*\delta^{18}O + 10.0$, $r^2 = 0.77$) fitted to δ^2H as a function of δ^{18}O from the samples obtained during the non-enrichment period with 95 % confidence (CI) and prediction intervals (PI) indicated by dashed and dotted bands along the regression, respectively. Data points falling above the upper 95 % prediction interval indicate that individuals used the enriched waterhole. The blue circles represent mean δ^{18}O and δ^2H values for the non-enriched waterholes, whereas patterned blue circles represent the mean enriched water samples during the two enrichment periods. Acronyms are defined as: GRA for granivore; FRU for frugivore; INS for insectivore; and OMN for omnivore; non-E WH for non-enriched waterhole; and E WH for enriched waterhole.

Figure 4: Spatial reliance of surface water as estimated through enriched δ^2H values in blood samples of birds trapped at increasing distances from an enriched waterhole at Tswalu Kalahari

Reserve (data were pooled for both sample seasons). (A) The number of individuals showing enriched δ^2H values and total number of individuals from which blood samples were obtained. (B) Boxplots showing median (solid line) and mean (dotted line) percentage of bird's body water pools derived from the enriched water source. Boxplots showing median percentage of bird's body water pools derived from the enriched water source for two species, Violet-eared Waxbill, *Uraeginthus granatinus* (C), and Namaqua Dove, *Oena capensis* (D), that showed highest number of individuals with enriched δ^2H values in blood, at sites within 1 km, and more that 1 km from the enriched waterhole. Sample sizes of distance categories are indicated above each box-plot.

Figure 5: Proportion of body water pool derived (P%) from the enriched waterhole increased significantly as a function drinking behavior (*drinking_int*, calculated as a proportional vector of the number instances where drinking behavior was observed) at Tswalu Kalahari Reserve. Proportional values were logit transformed following Warton and Hui (2011). The trendline shows the significant relationship in granivore dietary guild only, with 95 % confidence (CI) and prediction intervals (PI) indicated by dashed and dotted bands along the regression, respectively. See text for detailed statistics.

SUPPLEMENTARY MATERIAL

Additional results, including Table S1-2.

TABLES:

Table 1: Top performing models (based on Akaike Information Criteria; see methods for details) of *drinking_int*, which describes the probability of observing birds drinking, in four dietary guilds at Tswalu Kalahari Reserve, South Africa.

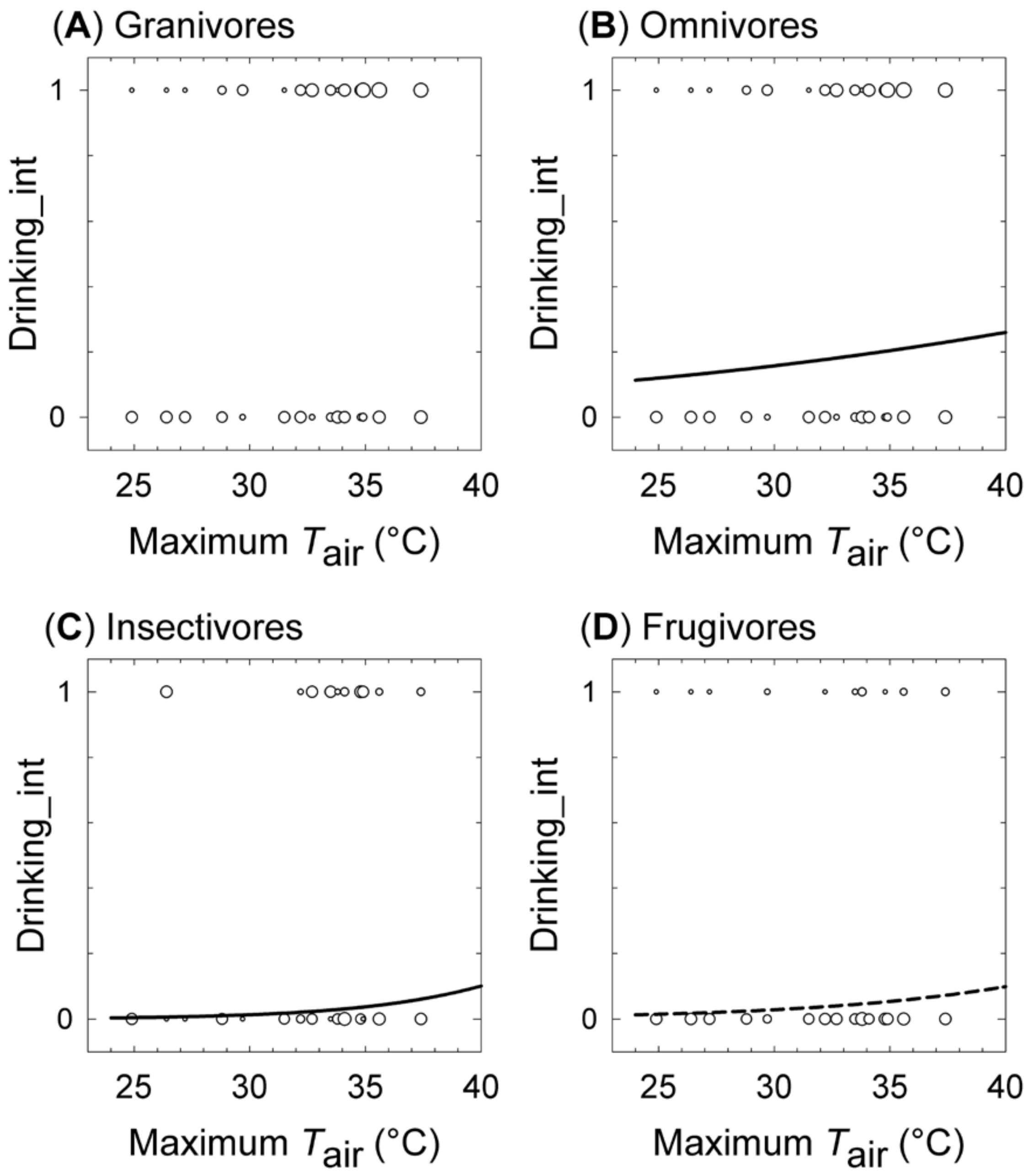
Granivores	*Drinking_int* = maximum Tair* + log (abundance)**
Omnivores	*Drinking_int* = maximum T_{air}** + Time**
Frugivores	*Drinking_int* = maximum T_{air} $^{\cdot}$ + Time** + log(abundance)$^{\cdot}$
Insectivores	*Drinking_int* = maximum T_{air} ** + Time*+ log(abundance)$^{\cdot}$

$^{\cdot}$ p<0.1; *p<0.05; **p<0.01

Table 2: Mean % body water pool (P% ±SD) derived from the enriched source at different distances from the sources in species that drank frequently and in a number of species not observed to drink.

	Distance to enriched waterhole (km)	
Species	**<1.0**	**>1.0**
Namaqua Dove *Oena capensis*	(9/10)	(2/3)
Violet-eared Waxbill *Uraeginthus granatinus*	(6/7)	(3/5)
Sociable Weaver *Philetairus socius*	(5/18)	(2/18)
Southern Masked-Weaver *Ploceus velatus*	(1/6)	(0/3)
Scaly-feathered Finch *Sporopipes squamifrons*	(6/30)	(2/18)
White-browed Sparrow-Weaver *Plocepasser mahali*	(2/11)	(0/14)
Yellow Canary *Crithagra flaviventris*	(1/4)	
Fawn-coloured Lark *Calendulauda africanoides*	(0/8)	(0/3)
Black-chested Prinia *Prinia flavicans*	(0/14)	(0/7)
Common Fiscal *Lanius collaris*	(0/4)	(0/2)
Marico Flycatcher *Bradornis mariquensis*	(0/4)	(0/1)
Acacia Pied Barbet *Tricholaema leucomelas*	(0/2)	(0/2)
Chestnut-vented Tit-Babbler *Parisoma subcaeruleum*	(0/5)	(0/3)
White-backed Mousebird *Colius colius*	(0/7)	(0/1)

Figure 1



772
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774 Figure 2

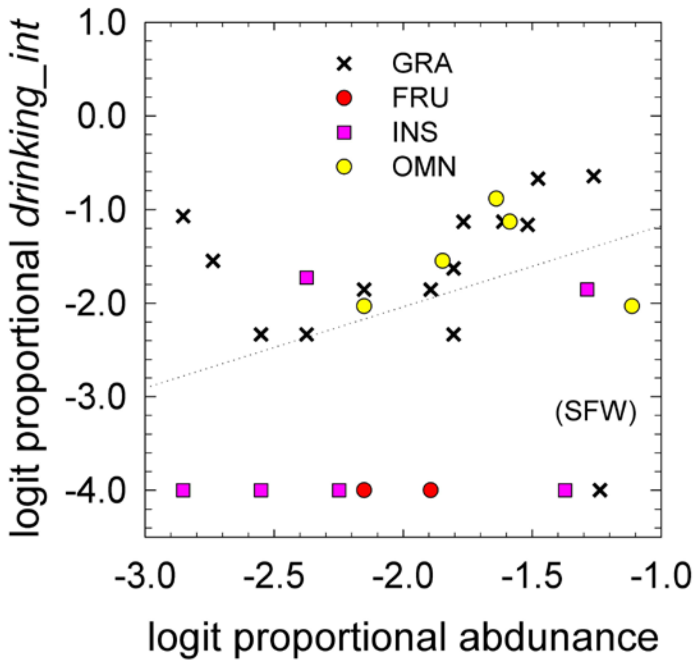

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Figure 3

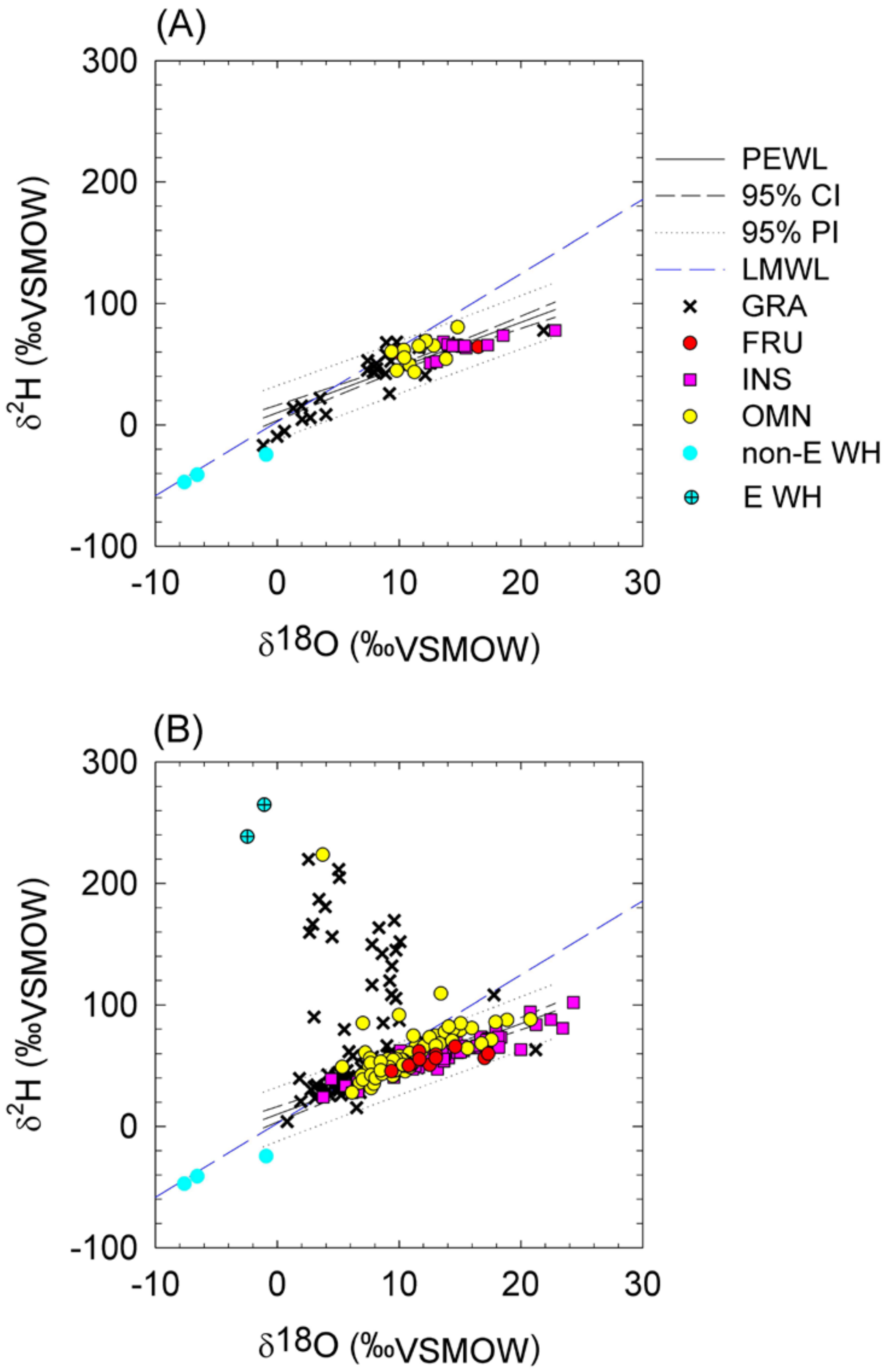
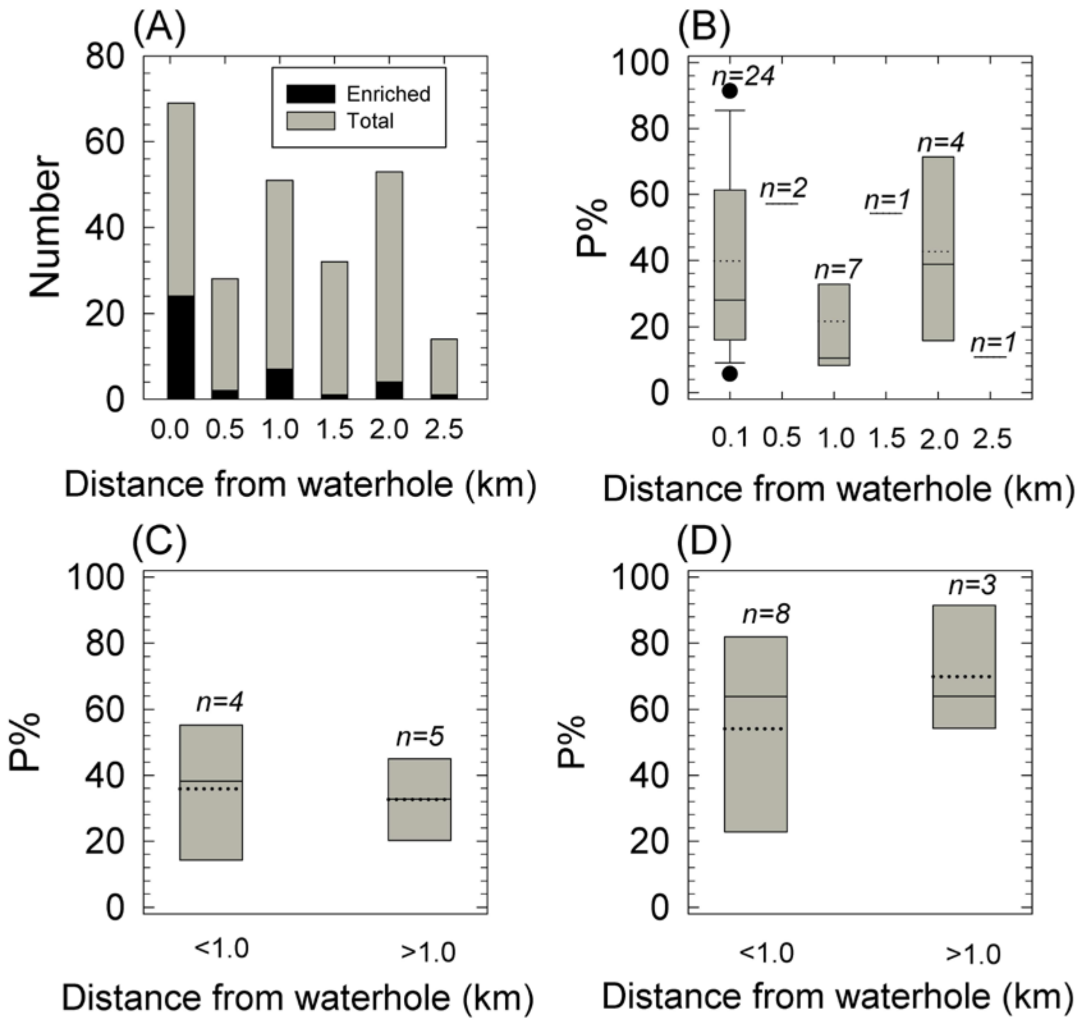
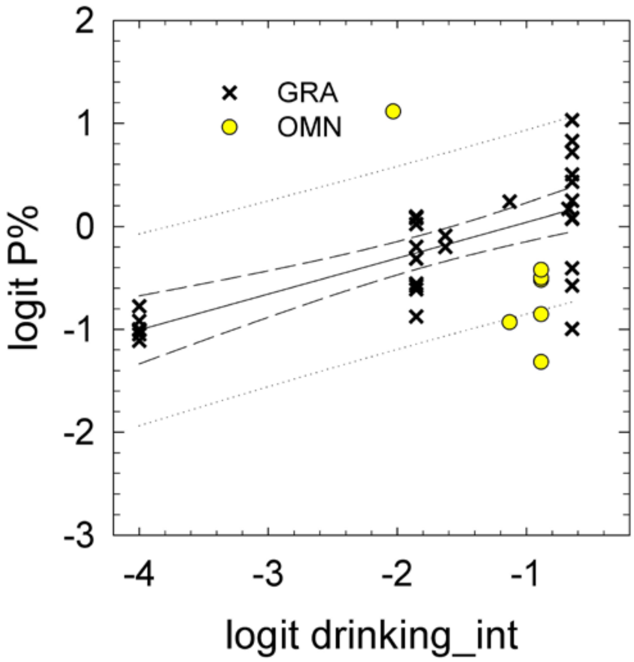

(A)
300
200
100
0
-100
-10
0
10
20
30
δ^{2}H (‰VSMOW)
δ^{18}O (‰VSMOW)
PEWL
95% CI
95% PI
LMWL
GRA
FRU
INS
OMN
non-E WH
E WH
(B)
300
200
100
0
-100
-10
0
10
20
30
δ^{2}H (‰VSMOW)
δ^{18}O (‰VSMOW)

Figure 4


(A)
Number
Enriched
Total
0
20
40
60
80
0.0
0.5
1.0
1.5
2.0
2.5
Distance from waterhole (km)
(B)
P%
0
20
40
60
80
100
n=24
n=2
n=7
n=1
n=4
n=1
0.1
0.5
1.0
1.5
2.0
2.5
Distance from waterhole (km)
(C)
P%
0
20
40
60
80
100
n=4
n=5
<1.0
>1.0
Distance from waterhole (km)
(D)
P%
0
20
40
60
80
100
n=8
n=3
<1.0
>1.0
Distance from waterhole (km)

783 Figure 5



784
785

Figure 6