# ORIGINAL ARTICLE

# Stable isotope analysis of diet confirms niche separation of two sympatric species of Namib Desert lizard

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## Abstract

We used stable isotopes of carbon and nitrogen to study the trophic niche of two species of insectivorous lizards, the Husab sand lizard *Pedioplanis husabensis* and Bradfield's Namib day gecko living sympatrically in the Namib Desert. We measured the $\delta^{13}C$ and $\delta^{15}N$ ratios in lizard blood tissues with different turnover times (whole blood, red blood cells and plasma) to investigate lizard diet in different seasons. We also measured the $\delta^{13}C$ and $\delta^{15}N$ ratios in available arthropod prey and plant tissues on the site, to identify the avenues of nutrient movement between lizards and their prey. Through the use of stable isotope mixing models, we found that the two lizard species relied on a largely non-overlapping but seasonally variable array of arthropods: *P. husabensis* primarily fed on termites, beetles and wasps, while *R. bradfieldi* fed mainly on ants, wasps and hemipterans. Nutrients originating from $C_3$ plants were proportionally higher for *R. bradfieldi* than for *P. husabensis* during autumn and late autumn/early winter, although not summer. Contrary to the few available data estimating the trophic transfer of nutrients in ectotherms in mixed $C_3$ and $C_4$/crassulacean acid metabolism (CAM) plant landscapes, we found that our lizard species primarily acquired nutrients that originated from $C_4$/CAM plants. This work adds an important dimension to the general lack of studies using stable isotope analyses to estimate lizard niche partitioning and resource use.

**Key words:** Namib Desert, niche partitioning, *Pedioplanis*, *Rhoptropus*, stable isotopes

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## INTRODUCTION

For all species there exists a particular set of biotic and abiotic conditions that bound their existence, which may be thought of as the species' niche (Hutchinson 1957). Species niches can be characterized by habitat

requirements, geographical distribution, thermal niches or other dimensions, which may not be independent of each other. Resource use is one niche dimension that is widely studied because the resources that organisms use play an important role in determining species diversity, and may allow different species within a similar feeding guild to coexist in the same habitat (Simberloff & Dayan 1991). Within the same feeding guild, a species may be a resource specialist or a resource generalist (Futuyma & Moreno 1988). Habitats usually will be able to support a greater number of specialist species that consume non-overlapping resources than generalist species that overlap in their resource consumption (Roughgarden 1974). This is due to competitive exclusion, the theory that two similar species are unable to coexist with one another unless there is some level of divergence in how they use resources (Hardin 1960; Pianka 1974). Indeed, a basic premise of community ecology is that the coexistence of otherwise similar species within a feeding guild may be accomplished by the use of distinct resources (MacArthur 1958; Bowers & Brown 1982).

Research on lizard community structure and function has been important for characterizing the concept of the species niche, as well as understanding how different species coexist (e.g., Schoener 1977; Pianka 1986). Arid ecosystems in particular may be ideal places to examine species niche partitioning because in such environments lizard diversity is often high, it can be relatively easy to secure large samples of individual lizards (Pianka 1986), and limited resources have the potential to intensify competition (MacArthur & Levins 1967). For example, despite the low availability of plant resources, the Namib Desert is home to a diverse lizard fauna with high levels of endemism (Robinson & Cunningham 1978; Murray & Schramm 1987; Herrmann & Branch 2013), and, as with other hot deserts, high lizard biomass may represent an important component of the food web in this ecosystem (Pianka 1986). However, few studies to date have examined resource partitioning and trophic dynamics within the Namib lizard fauna (Robinson & Cunningham 1978; Murray & Schramm 1987; Murray et al. 2016).

The use of stable isotopes, particularly carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) stable isotopes, is an effective and minimally invasive way to quantify the spatial and temporal patterns of consumer resource partitioning (Gannes et al. 1997; Boecklen et al. 2011). Because tissue $\delta^{15}$N increases by approximately 3.0‰, on average, across each trophic level within a food web,

$\delta^{15}$N may be used as an indication of an organism's trophic level (DeNiro & Epstein 1981; Peterson & Fry 1987). Conversely, tissue $\delta^{13}$C changes very little (approximately 0–1.0‰), on average, across trophic levels within a food web; consequently, $\delta^{13}$C may be used to trace carbon sources (DeNiro & Epstein 1978; Peterson & Fry 1987). During photosynthesis plants discriminate against carbon dioxide molecules containing the $^{13}$C isotope. However, due to differences in the enzymes responsible for carboxylation, plants which use the $C_3$ photosynthetic pathway (e.g. trees and most forbs) have significantly lower $^{13}$C values compared to plants that use either the $C_4$ (e.g. many grasses) or crassulacean acid metabolism (CAM; many succulents) photosynthetic pathway. These differences lead to $C_3$ plants having a lower $\delta^{13}$C ratio compared to $C_4$/CAM plants (Ehleringer et al. 1986, 1997). $C_3$ and $C_4$/CAM plants also have important structural differences (e.g. $C_4$ plants are characterized by Kranz anatomy, which includes the presence of thick-walled bundle sheath cells), which influence their nutritional profitability to consumers, and may have distinct growth responses to seasonal patterns of precipitation and climate (Ode et al. 1980; Schulze et al. 1996; Barbehenn et al. 2004a,b; Muldavin et al. 2008). Consequently, ecosystem primary productivity can be divided into distinct resource compartments based on plant photosynthetic pathways.

Importantly, the physiological differences between $C_3$ and $C_4$/CAM plants mean that they are likely to be affected differently under current projections of climate change and enhanced atmospheric $CO_2$ levels (IPCC 2014). For example, higher $CO_2$ levels may improve $C_3$ plant nutrient and water use efficiency, and favor plants with high demands for woody structural tissue, such as trees, compared to herbaceous plants, such as grasses (most of which are $C_4$ in arid regions; Drake et al. 1997; Bond et al. 2003). However, warmer and drier climatic conditions would tend to favor $C_4$/CAM plants (Bond et al. 2003). From a consumer's perspective these differences matter because many animals selectively forage on either $C_3$ or $C_4$/CAM plants, and the nutritional quality of these plant groups is not the same (Ehleringer et al. 2002; Barbehenn et al. 2004a,b). Furthermore, enhanced $CO_2$ levels may translate into negative consumer effects due to lower plant tissue nitrogen content and higher carbon to nitrogen ratios (Ehleringer et al. 2002).

Here we investigate and compare the resource partitioning of 2 sympatric and similarly-sized species

of insectivorous Namib lizards. The Husab sand lizard, *Pedioplanis husabensis* Berger-Dell'Mour & Mayer, 1989, is a 2.5–3.0-g lacertid lizard endemic to rocky substrates in the west-central Namib Desert between the ephemeral Swakop and Khan Rivers (Berger-Dell'Mour & Mayer 1989). Bradfield's Namib day gecko, *Rhoptropus bradfieldi* Hewitt, 1935, is a 3.0–4.0-g rock-dwelling diurnal gecko endemic to the Namib Desert (Branch 1998). We examine the trophic niches for each of these lizard species by analyzing the carbon and nitrogen stable isotope ratios in plant tissues, available arthropod prey and lizard tissues. Because *P. husabensis* and *R. bradfieldi* differ in their foraging strategy and habitat use (Murray *et al.* 2014, 2015), we predict that there will be significant differences between their trophic niches, evidenced by different tissue isotope values.

## MATERIAL AND METHODS

### Study site

Our study site is along the dry Swakop River, Namibia, at Hildenhof, approximately 40 km east of Swakopmund (22°42.049′S, 14°54.890′E; 210 m; see Murray *et al.* [2014, 2015] for further details) in the Namib Desert. The dry riverbed vegetation is characterized by a riparian woodland consisting of scattered trees and shrubs including *Vachellia erioloba* (camelthorn), *Tamarix usneoides* (tamarisk), *Faidherbia albida* (ana tree), *Euclea pseudebenus* (wild ebony) and *Salvadora persica* (mustard bush), growing in and along the edges of the sandy riverbed (Cowlishaw & Davies 1997). Adjacent to the riverbed are bare rocky slopes sparsely-covered with small shrubs such as *Arthraerua leubnitziae* (pencil bush) and *Sesuvium sesuvoides* (desert pink). A narrow zone of more densely-spaced shrubs such as *Zygophyllum stapffii* (dollar bush), *Lycium* sp. and *Salsola* sp. (salt bush) is situated on the silty substrates where the rocky slopes meet the river channel. Perennial grasses make up a small proportion of plant cover and generally are restricted to the edges of the river channel (I. Murray, personal observation). The study site is in a hyper-arid system with mean annual precipitation of approximately 25 mm, and 25–50 fog days per year may be expected based on data from other similar sites (Olivier 1995; Haensler *et al.* 2011; Eckardt *et al.* 2013). After sporadic precipitation events, such as one during April 2013, annual grasses such as *Stipagrostis* sp. were also evident.

### Lizard tissue collection

We captured lizards during austral summer (December 2012–January 2013) and austral autumn (May 2013) using noose poles. We took blood samples (approximately 50 µL) from the infraorbital sinus with heparinized capillary tubes before releasing the lizards unharmed (Murray *et al.* 2014). All procedures were approved by the University of the Witwatersrand's Animal Ethics Screening Committee (clearance certificate number 2012/50/03) and were in accordance with the Namibian Ministry of Environment and Tourism Research/Collecting Permit 1744/2012.

We collected whole blood from adult lizards of both species between December 2012 and January 2013 (austral summer), and in May 2013 (austral autumn/early winter). Blood was sampled from 21 male and 5 female *P. husabensis* and 13 male and 8 female *R. bradfieldi* during austral summer, and 17 male and 26 female *P. husabensis* and 7 male and 11 female *R. bradfieldi* in austral autumn.

We centrifuged the blood samples collected in autumn to separate out the plasma and red blood cells (RBC). Plasma was not available for the blood that we collected during summer because we used the plasma water for the determination of field metabolic rates (Murray *et al.* 2014, 2015). We air-dried RBC and loaded approximately 0.4 mg of RBC and dried whole blood into 4 × 6-mm tin cups (Costech Analytical Technologies, California, USA; #041070). In addition, we pipetted approximately 15 µL of plasma into 4 × 6-mm tin cups immediately after centrifuging and air-dried the samples before folding the tin cups for analysis. We did not extract lipids from the blood samples because blood contains too little lipid to confound analyses (Bearhop *et al.* 2000). Due to small blood volumes, several of the *R. bradfieldi* samples did not yield large enough nitrogen peaks to be analyzed by isotope ratio mass spectrometry, resulting in fewer nitrogen isotope ratios being reported than carbon ratios. For several additional samples we did not have sufficient RBC sample masses to run either carbon or nitrogen. We lost several plasma samples in the mass spectrometer.

In small insectivorous lizards, plasma has a carbon retention time (the average amount of time a carbon atom is retained in tissue and a means to estimate tissue-specific turnover times; Martínez del Rio & Anderson-Sprecher 2008) of 25 days while RBC have a carbon retention time of 61 days (Warne *et al.* 2010b). Because

plasma and RBC have different biological turnover rates, their isotope ratios reflect dietary history over both short (plasma) and long (RBC) periods (Boecklen *et al*. 2011). Consequently, plasma from blood collected in May reflected diet in late autumn/early winter, and RBC from blood collected in May reflected diet during autumn. Although plasma was unavailable for blood collected in summer, the use of whole blood is well established in the published literature (e.g. Boecklen *et al*. 2011) for estimating diet. The carbon retention time for whole blood is unknown for lizards, but because it is likely to have a retention time between that of plasma (25 days) and RBC (61 days), with RBC largely driving whole blood carbon retention times (Flaherty *et al*. 2010; Warne *et al*. 2010b), we can confidently make the assumption that isotope ratios in whole blood collected in summer reflect early summer diet.

## Characterization of plant and arthropod resources

We collected tissue from 30 plant species on the site during May 2013, which represented a majority of the species growing during our lizard sampling activity. We sampled multiple leaves and stems from 3 to 5 randomly selected plants of each species and stored them in paper envelopes. The plant tissues were dried in an oven at 55°C and samples were homogenized with a clean mortar and pestle to create a homogenate for each species. We analyzed the carbon and nitrogen stable isotope ratios for each species using aliquots (approximately 1 mg) of the dried homogenate.

We sampled arthropods from areas where lizards were active and foraging during May 2013 by walking through the habitat and hand capturing arthropods (beetles, ants, termites and spiders) and sweeping vegetation with a net (flies, bees, wasps, true bugs, as well as some beetles and spiders). We made a concerted effort to sample ants and termites (identified as key components of lizard diet; Murray *et al*. 2016) in the same microhabitats where we saw lizards. Arthropods were kept cool (approximately 15°C) in vials for 1–3 days, a period in which we assumed that all gut contents were metabolized, and then frozen (approximately −4°C) for storage. We acknowledge the potential difficulties involved with inferring lizard consumption of arthropods in summer based on the tissue isotope ratios of arthropods collected during late autumn/early winter. However, for the primary prey items that lizards feed on, such as termites and ants, the long periods of

time required for growth and development means that any diet switches in those arthropods could take several months to be reflected in the arthropod tissue isotope ratios (termites [Watson 1973]; ants [Mooney & Tillberg 2005; Straka & Feldhaar 2007; Menke *et al*. 2010]). We identified arthropods to the species level where possible, and otherwise to the order, family or genus level, using references for southern African arthropods (Scholtz & Holm 1985; Marsh 1986; Uys 2002; Picker *et al*. 2004). As with plant samples, we dried arthropods in an oven and homogenized individuals before loading them in 4 × 6-mm tin capsules.

## Stable isotope analyses

We analyzed all of our tissue samples for carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) stable isotope ratios using a continuous flow isotope ratio mass spectrometer (Delta V Plus, ThermoFinnigan, Bremen, Germany) connected to an Elemental Analyzer (Flash EA 1112 series, ThermoFinnigan, Bremen, Germany) in the University of Pretoria Isotope Ratio Mass Spectrometry Laboratory. The instrumental precision of these measurements was ± 0.1‰ SD based on repeated measurements of internal laboratory standards. All sample runs included a laboratory standard (Merck Gel $\delta^{13}$C = −20.57‰; $\delta^{15}$N = 6.8‰) and blank after each set of 12 unknowns. Isotope concentrations are reported in delta notation ($\delta$) in parts per thousand (‰): $\delta X = (R_{sample}/R_{standard} - 1) \times 1000$. $R_{sample}$ and $R_{standard}$ represent the ratio of heavy to light isotopes ($^{13}$C/$^{12}$C or $^{15}$N/$^{14}$N) for the sample and standard. The results are normalized to the international standards air for $\delta^{15}$N and Vienna Pee Dee Belemnite for $\delta^{13}$C.

When carbon and nitrogen stable isotope ratios in consumer tissue are analyzed, there is often an offset between the diet and the tissue termed the diet-to-tissue discrimination factor ($\Delta$). Diet-to-tissue discrimination factors may significantly differ according to diet quality, growth rates, tissue or species (Caut *et al*. 2008; Caut *et al*. 2009; Boecklen *et al*. 2011). Determining discrimination factors requires time and labor-intensive feeding trials, which have not been carried out for all species (Gannes *et al*. 1997; Martínez del Rio & Carleton 2012). Consequently, we used the mean $\Delta\delta^{13}$C (0.4‰, 91 studies) and the mean $\Delta\delta^{15}$N (2.3‰, 65 studies) determined for poikilotherm tissue diet-to-tissue discrimination factors during controlled feeding trials to adjust our lizard tissue $\delta^{13}$C and $\delta^{15}$N values (McCutchan *et al*. 2003). We assumed that all lizard tissues analyzed would have similar discrimination factors.

## Data analyses: Tissue stable isotope ratios

We tested for sex-related differences in tissue stable isotope ratios for both species using 2-sample *t*-tests. Plasma and RBC samples from individual lizards are not independent, so we used repeated measures linear mixed effects models to compare the carbon and nitrogen isotope ratios between species across seasons. We used an unstructured repeated covariance type, and modeled species, season and the species*season interaction as fixed effects and individual lizard as a random effect. We conducted post hoc comparisons using a Bonferroni correction. We used 2-sample *t*-tests to compare the seasonal incorporation of arthropods feeding on $C_4$/CAM plant resources by the two lizard species.

## Data analyses: Isotopic niche metrics

To compare seasonal changes in lizard dietary niches (using different tissues to estimate seasonal dietary changes), we used the Stable Isotope Bayesian Ellipses in R (SIBER) package to calculate the standard ellipse area corrected for small sample sizes ($SEA_c$) as well as the area of overlap for the summer, autumn and late autumn/early winter dietary niches (Jackson *et al.* 2011). $SEA_c$ is a proxy for the trophic niche, and is the bivariate standard deviation of the stable isotope ratios (e.g. carbon and nitrogen) characterizing a group of consumers; $SEA_c$ thus represents the core isotopic niche for each lizard species. We also describe the area of the convex hull (TA), and the associated Layman niche metrics estimating additional measurements of species niche structure calculated using the package Stable Isotope Analyses in R (SIAR), for comparative purposes. The TA (the smallest surface that encompasses all of the carbon and nitrogen stable isotope ratios for individuals of a species in a bivariate plot) is a geometric approach that may be used to estimate consumer dietary niche breadth, although TA is more sensitive to sample size than $SEA_c$, and fails to take into account uncertainty within a dataset (Layman *et al.* 2007; Parnell *et al.* 2010; Jackson *et al.* 2011). Layman niche metrics further characterize diet spacing patterns between individuals in a population, and include the mean distance to centroid (CD), the mean nearest neighbor distance (MNND) and the standard deviation of the mean nearest neighbor distance (SDNND; Layman *et al.* 2007). For example, high values of MNND would indicate a more diverse trophic niche, while high SDNND indicates a high degree of unevenness in the spacing of the individual lizards in bivariate isotopic space (Layman *et al.* 2007).

## Data analyses: Mixing models

We estimated the extent to which lizards used arthropods dependent on $C_4$/CAM versus $C_3$ plant resources with a 2-end-point mixing model (Martínez del Rio & Wolf 2005):

$$\delta^{13}C_{(tissue)} = p(\delta^{13}C_{(C4/CAM)}) + (1-p)(\delta^{13}C_{(C3)}) + \Delta;$$

where "*tissue*" is either lizard plasma, whole blood or RBC; $p$ is the fraction of $C_4$/CAM plant resources assimilated in lizard tissue; and $\Delta$ is the carbon discrimination factor (0.4‰, McCutchan *et al.* 2003). The subscripts "$C_3$" and "$C_4/CAM$" represent the carbon isotope ratios of $C_3$ and $C_4$/CAM plant photosynthetic pathways, respectively.

We estimated the proportional contribution of arthropod prey groups to lizard tissues using the Bayesian Stable Isotope Sourcing Using Sampling (SISUS; Erhardt & Bedrick 2013) software which provides a significant advantage over other stable isotope mixing models because SISUS allows for the variability of stable isotope ratios in diet categories, as well as accounts for uncertainty in stable isotope discrimination factors (Erhardt & Bedrick 2013). We identified potential prey based on those groups that we have found previously in lizard fecal pellets (Murray *et al.* 2016). We used SigmaPlot 8.0 (Systat Software, San Jose, CA, USA), Microsoft Excel 2007 (Microsoft, Redmond, WA, USA), IBM SPSS 21.0 (SPSS, Chicago, IL, USA) and R 2.15.2 (R Development Core Team 2009) for all analyses. For all analyses, significance was accepted at $P < 0.05$ and values are reported as mean ± SD.

## RESULTS

$C_3$ plants and $C_4$/CAM plants growing on the site had non-overlapping carbon isotope ratios, a critical observation allowing the sources of the nutrients assimilated by insectivorous lizards to be traced back to the plant functional groups consumed by their prey (Fig. 1). Mean carbon isotope ratios were −26.2‰ ± 0.4‰ (range, −30.3‰ to −23.7‰; $n = 16$ species) in $C_3$ plant tissues and −14.5‰ ± 0.3‰ (range, −16.4‰ to −13.0‰; $n = 14$ species) in $C_4$/CAM plant tissues. Plant tissue nitrogen ratios were 12.0‰ ± 1.0‰ in $C_3$ plants (range, 7.4‰ to 18.4‰) and 10.4‰ ± 0.9‰ in $C_4$/CAM plants (range, 6.8‰ to 19.3‰).

**Figure 1** Mean (± SD) $\delta^{15}N$ and $\delta^{13}C$ ratios for seasonal diet as estimated from plasma (late autumn/early winter), whole blood (WB; summer) and RBC (autumn) from the Husab sand lizard (Pehu, *Pedioplanis husabensis*) and Bradfield's Namib day gecko (Rhbr, *Rhoptropus bradfieldi*). Blood tissue carbon and nitrogen isotope ratios are plotted relative to the $\delta^{15}N$ and $\delta^{13}C$ tissue values for individual species of plants belonging to different functional groups (30 species; $C_3$ shrubs/trees, $C_4$ grasses, $C_4$ shrubs and crassulacean acid metabolism [CAM] succulents) available on the site. Lizard blood tissue $\delta^{15}N$ (2.3‰) and $\delta^{13}C$ (0.4‰) ratios have been adjusted by subtracting the appropriate diet-tissue-discrimination factors determined for poikilotherms (McCutchan *et al*. 2003).
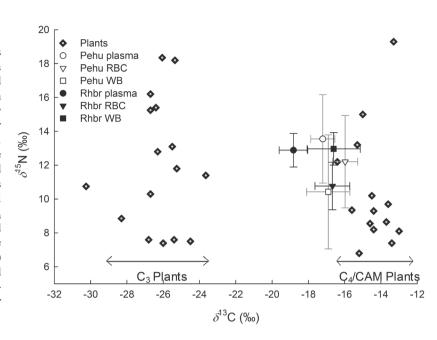
**Table 1** Mean (± SD) $\delta^{15}N$ and $\delta^{13}C$ ratios of potential prey items collected during May 2013 for the Husab sand lizard (*Pedioplanis husabensis*) and Bradfield's Namib day gecko (*Rhoptropus bradfieldi*) along the dry Swakop riverbed in the Namib Desert, Namibia

| Prey category | *n* | Mean $\delta^{13}C$ (‰) | Mean $\delta^{15}N$ (‰) |
|---|---|---|---|
| Arachnida | | | |
| Araneae | 12 | −17.9 ± 2.3 | 18.3 ± 2.4 |
| Insecta | | | |
| Coleoptera | | | |
| *Psammodes/Physosterna/Zophosis/Scarabidae* (beetles1) | 14 | −14.9 ± 1.9 | 17.2 ± 3.8 |
| *Somaticus/Gonocephalum/Stenocara* (beetles2) | 19 | −20.1 ± 3.2 | 15.2 ± 3.4 |
| Hemiptera | 2 | −21.9 ± 0.9 | 13.4 ± 3.3 |
| Hymenoptera | | | |
| Ants | | | |
| *Lepisiota capensis* | 4 | −19.5 ± 2.2 | 16.7 ± 1.6 |
| *Pheidole* sp. | 3 | −15.3 ± 0.3 | 14.9 ± 0.1 |
| *Camponotus* sp. | 2 | −13.3 ± 0.3 | 14.5 ± 1.5 |
| Bees | 4 | −17.8 ± 4.6 | 14.8 ± 3.9 |
| Wasps | 3 | −18.7 ± 5.0 | 8.8 ± 3.6 |
| Isoptera | | | |
| *Trinervitermes* sp. | 6 | −17.1 ± 0.3 | 5.8 ± 0.2 |
| *Hodotermes mossambicus* | 8 | −18.6 ± 0.5 | 7.3 ± 0.6 |
| *Psammotermes allocerus/Amitermes* sp. | 16 | −16.1 ± 0.8 | 11.8 ± 1.0 |

Based on similar tissue isotope ratios the beetle genera *Somaticus*, *Gonocephalum* and *Stenocara* were combined into the category "beetles2," and the genera *Psammodes*, *Physosterna*, *Zophosis* and Scarabidae were combined into the category "beetles1." Sample sizes (*n*) indicate the numbers of individuals sampled with the exception of the small ant *Lepisiota capensis* in which case each sample was a homogenate of 4 individual ants from a single nest.

The potential arthropod prey groups of lizards occupied largely non-overlapping domains in carbon and nitrogen isotope niche space (Table 1). For example, mean $\delta^{13}$C ranged from $-21.9$‰ ± 0.9‰ in hemipteran insects to $-13.3$‰ ± 0.3‰ in ants in the genus *Camponotus* (Table 1). Arthropod prey groups also occupied a diversity of trophic levels, as evidenced by their tissue $\delta^{15}$N, which ranged from 18.3‰ ± 2.4‰ in spiders to 5.8‰ ± 0.2‰ in termites of the genus *Trinervitermes*. There also was significant variation in tissue nitrogen and carbon isotope ratios for different arthropod genera within the same order, apparent, for example, in the distinct and non-overlapping $\delta^{13}$C and $\delta^{15}$N ratios for genera of termites and ants (Table 1). The distinct isotope ratios of the lizards' potential prey allowed unambiguous identification of their diets.

There were no sex-related differences in blood carbon and nitrogen isotope ratios for both *P. husabensis* (RBC $\delta^{15}$N, $t_{41} = -0.22$; $P = 0.829$; RBC $\delta^{13}$C, $t_{41} = -0.55$; $P = 0.582$; plasma $\delta^{15}$N, $t_{21} = 0.32$; $P = 0.752$; plasma $\delta^{13}$C, $t_{23} = 0.49$; $P = 0.631$; whole blood $\delta^{15}$N, $t_5 = 1.98$; $P = 0.105$; whole blood $\delta^{13}$C, $t_6 = 1.39$; $P = 0.215$) and *R. bradfieldi* (RBC $\delta^{15}$N, $t_{12} = -0.01$; $P = 0.996$; RBC $\delta^{13}$C, $t_{14} = -1.89$; $P = 0.08$; plasma $\delta^{15}$N, $t_7 = -1.63$; $P = 0.148$; plasma $\delta^{13}$C, $t_{15} = 0.10$; $P = 0.920$; whole blood $\delta^{15}$N, $t_{18} = -0.3$; $P = 0.767$; whole blood $\delta^{13}$C, $t_{10} = -1.25$; $P = 0.238$), so we combined male and female values for both species (Table 2). On average blood $\delta^{15}$N did not differ between lizard species ($F_{1,82.091} = 0.009$; $P = 0.925$), but there was a significant difference between seasons ($F_{2,67.392} = 21.170$; $P = 0.000$) with the $\delta^{15}$N ratio reflecting the late autumn/early winter dietary niche (plasma) significantly higher than those reflecting

autumn (RBC) but not summer (whole blood) dietary niches. The interaction between species and season also was significant ($F_{2,67.392} = 6.554$; $P = 0.003$; Table 2). Across seasons the tissue $\delta^{15}$N ratios increased similarly for *P. husabensis*, while for *R. bradfieldi* the $\delta^{15}$N ratios were similar in summer and late autumn/early winter but declined in the autumn dietary niche (Table 2).
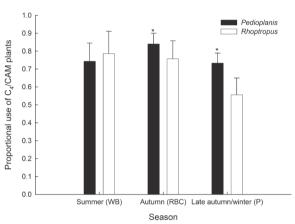


**Figure 2** Mean (± SD) proportional use of C$_4$/crassulacean acid metabolism [CAM] plant-derived resources, relative to C$_3$ resources, as estimated from the $\delta^{13}$C ratios for the summer (WB, whole blood), autumn (RBC, red blood cells) and late autumn/early winter (P, plasma) tissue $\delta^{13}$C ratios from the Husab sand lizard (*Pedioplanis husabensis*) and Bradfield's Namib day gecko (*Rhoptropus bradfieldi*). *$P < 0.001$; 2-sample *t*-test for seasonal species differences in resource use.

**Table 2** Mean (± SD) $\delta^{15}$N and $\delta^{13}$C ratios for austral summer dietary niches (November–January as estimated from whole blood), autumn dietary niches (March–May as estimated from red blood cells), and late autumn/early winter dietary niches (April–May, as estimated from plasma) for the Husab sand lizard (*Pedioplanis husabensis*) and Bradfield's Namib day gecko (*Rhoptropus bradfieldi*)

| Isotope ratio | Summer | | Autumn | | Late autumn/early winter | |
|---|---|---|---|---|---|---|
| | *Pedioplanis husabensis* | *Rhoptropus bradfieldi* | *Pedioplanis husabensis* | *Rhoptropus bradfieldi* | *Pedioplanis husabensis* | *Rhoptropus bradfieldi* |
| Mean $\delta^{13}$C (‰) | $-17.0 \pm 1.2$ ($n = 27$) | $-16.7 \pm 1.5$ ($n = 21$) | $-16.0 \pm 0.7$ ($n = 43$) | $-16.9 \pm 1.2$* ($n = 16$) | $-17.3 \pm 0.7$ ($n = 39$) | $-19.4 \pm 1.1$* ($n = 17$) |
| Mean $\delta^{15}$N (‰) | $10.8 \pm 2.7$ ($n = 26$) | $13.0 \pm 1.0$* ($n = 21$) | $12.2 \pm 2.7$ ($n = 43$) | $10.9 \pm 1.4$ ($n = 14$) | $13.5 \pm 2.7$ ($n = 39$) | $12.6 \pm 1.0$ ($n = 12$) |

Sample sizes ($n$) indicate the number of individual lizards from which samples were analyzed. *Significant inter-species difference (95% confidence interval estimates; $P < 0.05$).

Blood $\delta^{13}C$ ratios were on average significantly lower in *R. bradfieldi* than in *P. husabensis* ($F_{1,103.760} = 21.494$; $P < 0.001$). In addition, $\delta^{13}C$ ratios reflecting late autumn/early winter diet (plasma) were significantly lower than the $\delta^{13}C$ ratios reflecting both autumn (RBC) and summer (whole blood) diet ($F_{2,60.412} = 161.763$; $P < 0.001$). The interaction between species and season again was significant ($F_{2,60.412} = 23.812$; $P < 0.001$; Table 2). For *P. husabensis*, tissue $\delta^{13}C$ ratios reflecting both the summer and late autumn/early winter dietary niches were lower than those reflecting the autumn dietary niche (Table 2). In *R. bradfieldi* the $\delta^{13}C$ ratios remained the same for both the summer and autumn dietary niches, but were significantly lower for the late autumn/ early winter dietary niche (Table 2).

In addition to the species differences in seasonal dietary niches, *P. husabensis* assimilated significantly more nutrients from arthropods that fed primarily upon $C_4$ or CAM plants than *R. bradfieldi* during autumn (2 sample *t*-test; $t_{57} = 3.91$; $P = 0.000$) and late autumn/ early winter (2 sample *t*-test; $t_{54} = 8.78$; $P = 0.000$) but not summer (2 sample *t*-test; $t_{46} = -1.30$; $P = 0.201$; Fig. 2). During summer *R. bradfieldi* and *P. husabensis* both derived approximately 75% of their diet from arthropods that consumed $C_4$/CAM plants. However, compared to *P. husabensis*, during autumn and late autumn/early winter *R. bradfieldi* sourced 10–20% fewer resources from arthropods feeding on $C_4$/CAM plants (Fig. 2).

More evidence for the significant differences in the isotopic niches between *R. bradfieldi* and *P. husabensis* was that both the $SEA_c$ and TA of *P. husabensis's* dietary niche were larger than those of *R. bradfieldi* across all seasons (Table 3, Fig. 3). During the summer and late autumn/early winter, the dietary niche $SEA_c$ was more than twice that for *P. husabensis* than for *R. bradfieldi*, while during autumn *P. husabensis's* dietary niche was only slightly greater than that of *R. bradfieldi* (Table 3). There was also considerable seasonal overlap in the summer, autumn and late autumn/early winter $SEA_c$s for *P. husabensis*, while the dietary niche for *R. bradfieldi* was spatially distinct across these seasons (Fig. 3). The niches of the two species overlapped in summer such that the area of that overlap occupied approximately half of the total niche area in *R. bradfieldi*, but only one-fifth of *P. husabensis's* summer niche area (Table 3). During autumn, the overlap in the lizards' dietary niches took up a similar proportion of the total area of the autumn dietary niche in both species (Table 3). However, during late autumn/early winter there was almost no overlap in the dietary niches of *R. bradfieldi* and *P. husabensis*
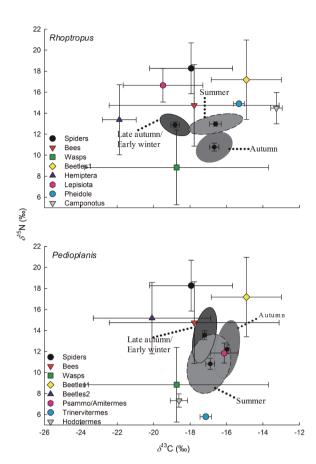


**Figure 3** The distribution of the mean (± SD) $\delta^{15}N$ and $\delta^{13}C$ ratios for potential arthropod prey categories relative to the mean (± SD) and the seasonal standard ellipse areas corrected for small sample sizes ($SEA_c$) during summer (whole blood), autumn (red blood cells) and late autumn/early winter (plasma) for sympatric Bradfield's Namib day geckos, *Rhoptropus bradfieldi* (upper panel) and Husab sand lizards, *Pedioplanis husabensis* (lower panel). Lizard blood tissue $\delta^{15}N$ (2.3‰) and $\delta^{13}C$ (0.4‰) ratios have been adjusted by subtracting the appropriate diet–tissue–discrimination factors determined for poikilotherms in a recent meta-analysis (McCutchan *et al.* 2003).

(Table 3). Furthermore, in all seasons the CD was greater for *P. husabensis* relative to *R. bradfieldi*, while the MNND and SDNND were either similar in size or greater for *R. bradfieldi* compared to *P. husabensis* (Table 3).

Analysis of the whole blood isotope ratios indicated that the summer diet of *P. husabensis* was 63% termites,

**Table 3** Calculated niche metrics based on the $\delta^{15}$N and $\delta^{13}$C ratios during austral summer as estimated from whole blood, austral autumn as estimated from red blood cells and austral late autumn/early winter as estimated from plasma in the Husab sand lizard (*Pedioplanis husabensis*) and Bradfield's Namib day gecko (*Rhoptropus bradfieldi*)

| Niche metric | Summer | | Autumn | | Late autumn/Early winter | |
|---|---|---|---|---|---|---|
| | *Pedioplanis husabensis* | *Rhoptropus bradfieldi* | *Pedioplanis husabensis* | *Rhoptropus bradfieldi* | *Pedioplanis husabensis* | *Rhoptropus bradfieldi* |
| Standard ellipse area | | | | | | |
| SEAc | 10.4 | 4.0 | 5.8 | 4.4 | 5.1 | 2.4 |
| Area of overlap | 2.3 | | 2.0 | | 0 | |
| Layman niche metrics | | | | | | |
| TA | 27.0 | 12.1 | 21.7 | 7.9 | 15.9 | 5.1 |
| CD | 2.6 | 1.5 | 2.4 | 1.5 | 2.3 | 1.0 |
| MNND | 0.73 | 0.63 | 0.44 | 0.59 | 0.35 | 0.58 |
| SDNND | 0.43 | 0.54 | 0.29 | 0.38 | 0.18 | 0.38 |

Niche metrics are based on standard ellipse areas corrected for small sample sizes (SEA$_c$) and the inter-species overlap between seasonal SEA$_c$, as well as the Layman niche metrics area of the convex hull (TA), distance to centroid (CD), mean nearest neighbor distance (MNND), and the standard deviation of the mean nearest neighbor distance (SDNND).

**Table 4** The relative contribution of arthropod prey groups to the diet of sympatric Bradfield's Namib day geckos (*Rhoptropus bradfieldi*) and Husab sand lizards (*Pedioplanis husabensis*), as calculated (mean ± SD) by the software Stable Isotope Sourcing Using Sampling (SISUS) using carbon and nitrogen isotopes

| Prey category | Summer diet | | Autumn diet | | Late autumn/early winter diet | |
|---|---|---|---|---|---|---|
| | *Pedioplanis husabensis* | *Rhoptropus bradfieldi* | *Pedioplanis husabensis* | *Rhoptropus bradfieldi* | *Pedioplanis husabensis* | *Rhoptropus bradfieldi* |
| Arachnida | | | | | | |
| Araneae | 0.05 ± 0.04 | 0.04 ± 0.03 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.16 ± 0.10 | 0.05 ± 0.04 |
| Insecta | | | | | | |
| Coleoptera | | | | | | |
| *Psammodes/Physosterna/Zophosis/Scarabidae* (beetles1) | 0.15 ± 0.08 | 0.07 ± 0.06 | 0.44 ± 0.07 | 0.01 ± 0.01 | 0.19 ± 0.08 | 0.04 ± 0.03 |
| *Somaticus/Gonocephalum/Stenocara* (beetles2) | 0.08 ± 0.06 | — | 0.01 ± 0.01 | — | 0.09 ± 0.07 | — |
| Hemiptera | — | 0.05 ± 0.04 | — | — | — | 0.40 ± 0.07 |
| Hymenoptera | | | | | | |
| Ants | | | | | | |
| *Camponotus* sp. | — | 0.21 ± 0.10 | — | 0.29 ± 0.01 | — | 0.04 ± 0.03 |
| *Pheidole* sp. | — | 0.13 ± 0.10 | — | 0.01 ± 0.01 | — | 0.05 ± 0.04 |
| *Lepisiota capensis* | — | 0.04 ± 0.03 | — | — | — | 0.08 ± 0.06 |
| Bees | 0.06 ± 0.05 | 0.07 ± 0.06 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.16 ± 0.13 | 0.07 ± 0.06 |
| Wasps | 0.08 ± 0.06 | 0.35 ± 0.04 | 0.01 ± 0.01 | 0.67 ± 0.01 | 0.08 ± 0.06 | 0.29 ± 0.04 |
| Isoptera | | | | | | |
| *Trinervitermes* sp. | 0.29 ± 0.10 | — | 0.32 ± 0.07 | — | 0.08 ± 0.06 | — |
| *Psammotermes allocerus/Amitermes* sp. | 0.25 ± 0.16 | — | 0.18 ± 0.16 | — | 0.15 ± 0.10 | — |
| *Hodotermes mossambicus* | 0.09 ± 0.07 | — | 0.02 ± 0.01 | — | 0.08 ± 0.06 | — |

—, not included in the diet, based upon prior gut content analyses (Murray *et al*. 2016).

wasps, 17% ants and 7% bees) but with considerable contributions from hemipterans (40%) and a low proportion of spiders and beetles (Table 4).

## DISCUSSION

The insectivorous lizards *P. husabensis* and *R. bradfieldi* occurred within an isotopically-diverse landscape of $C_3$ and $C_4$/CAM plants (Fig. 1), and, consequently, had an isotopically-distinct prey base of arthropods available to them (Table 1). That isotopic diversity allowed us to assess changes in lizard resource use over time. There was considerable variation between the two species in arthropod resource use (Fig. 3, Table 4). Although both lizard species showed some degree of seasonal variation in arthropod prey use, the dietary composition of the two lizard species did not overlap in its major constituents (Table 4). *Pedioplanis husabensis* fed predominantly on termites and beetles, while *R. bradfieldi* fed predominantly on ants, wasps and hemipteran insects. Furthermore, despite the presence of considerable $C_3$ plant biomass in their immediate habitat, these two insectivorous lizard species showed a preference for arthropods dependent on $C_4$/CAM plants (Fig. 2).

Overall, the $\delta^{15}N$ ratios for lizard tissues were similar, implying that *R. bradfieldi* and *P. husabensis* fed at the same trophic level, although the lower $\delta^{15}N$ ratios for *P. husabensis* in summer may reflect its high consumption of termites with their relatively low tissue $\delta^{15}N$ ratios at this time of year. However, when we examined $\delta^{15}N$ values in conjunction with $\delta^{13}C$ values seasonally, we found notable differences between *P. husabensis's* and *R. bradfieldi's* dietary niche (Table 2). For example, *P. husabensis* always had a larger dietary niche (TA and $SEA_c$) than did *R. bradfieldi* occupying the same habitat (Fig. 3; Table 3). Depending on the season, the TA of *P. husabensis* was 2.2–3.1 times larger than the corresponding TA in *R. bradfieldi*. The TA incorporates data from all individuals, including outliers that may be critical to capturing the population's or species' complete trophic spectrum (Layman *et al*. 2012); however, it is a metric that is sensitive to sample size (Jackson *et al*. 2011), and we sampled fewer *R. bradfieldi* than *P. husabensis* (Table 2). The $SEA_c$ is a metric which characterizes the niche far more robustly given a limited sample size, and the $SEA_c$ results echoed those yielded from the TA analysis: $SEA_c$ for *P. husabensis* was 1.3–2.6 times larger than that of *R. bradfieldi* (Table 3). In addition to having a larger isotopic niche, there was a

higher degree of trophic diversity among individual *P. husabensis* relative to *R. bradfieldi*, as evidenced by the higher CD (Table 3), but the higher SDNND and MNND for *R. bradfieldi* in autumn and late autumn/early winter indicated that individuals of this species had less redundancy in the trophic niche than did individuals of *P. husabensis* (Layman *et al*. 2007; Table 3). These niche differences reflect the consumption of distinct arthropod prey items and probably result from differences in foraging strategies between the two lizard species.

*Pedioplanis husabensis* uses an active foraging strategy and moves widely through its habitat (Murray *et al*. 2014). In contrast, *R. bradfieldi* uses a sit-and-wait foraging strategy in which it ambushes its prey from an immobile and exposed position (Murray *et al*. 2015). Relative to sit-and-wait foraging lizards, actively-foraging lizards are likely to have larger territories and move over greater distances through a diversity of habitats (Pianka 1986; Vitt *et al*. 2003). While we lack data on home range size and the spatial length of daily movements in *P. husabensis* and *R. bradfieldi*, data from other communities of insectivorous desert lizards indicate that the hourly distances moved by active foraging lizards are 4 to 4.5 times the distances (Anderson & Karasov 1981; Huey & Pianka 1981) and the home ranges 4 times larger (Anderson & Karasov 1981) than those of sympatric sit-and-wait foraging lizards. As lizards forage over greater distances they are likely to encounter a greater degree of habitat heterogeneity. Because landscape heterogeneity is positively correlated with arthropod diversity (Liu *et al*. 2013), it is likely that more widely foraging lizards may come into contact with a more diverse assortment of prey and, thus, have a larger trophic niche, as we found for *P. husabensis*. Because actively-foraging lizards use visual and chemosensory means to locate prey above and below ground, they are also capable of feeding on a greater array of potential prey, such as subterranean insect larvae and immobile insect pupae that are not available to sit-and-wait foraging lizards (Pianka 1986; Vitt *et al*. 2003). Therefore, the consequences of foraging actively may contribute to the larger trophic niche of *P. husabensis*.

Compared to *P. husabensis*, the variable and non-overlapping seasonal $SEA_c$s for *R. bradfieldi* may be related to a sit-and-wait predator foraging opportunistically during a particular time of year (Fig. 3). The reduced trophic redundancy and increased "unevenness" characterizing *R. bradfieldi* in isotopic

space during autumn and late autumn/early winter (high MNND and SDNND; Table 3) implies that *R. bradfieldi* show a less uniform pattern of resource use. This pattern could be due to individual geckos encountering a relatively heterogeneous variety of arthropods during sit-and-wait foraging bouts. In contrast, individuals of the actively-foraging *P. husabensis* can target distinct prey resources, specifically making its dietary niche more uniform. We acknowledge that we cannot be sure that the isotopically-distinct SEA$_c$ found seasonally for *R. bradfieldi* are the result of the inclusion of different types of arthropod prey in the diet; the diets of the arthropods themselves may have varied seasonally, and we did not collect arthropod samples during summer. Further data collection would be required to better address this question.

From the perspective of individual consumers, the relative importance of the C$_3$ versus C$_4$ components of plant primary productivity varies by species, season and habitat (Magnusson *et al*. 1999; Warne *et al*. 2010a). In addition, C$_4$ plant production represents an important component of food web nutrients, particularly in arid ecosystems (Ehleringer *et al*. 1997; Still *et al*. 2003). There are relatively few studies estimating the transfer of C$_3$ versus C$_4$-derived nutrients to higher-level consumers, such as lizards in a nutritional landscape containing both C$_3$ and C$_4$ plants (Magnusson *et al*. 1999, 2001; Warne *et al*. 2010a). However, the available data indicate that lizards continue to acquire considerable amounts of nutrients from prey that feed on C$_3$ plant resources even when appreciable proportions of total primary productivity stem from C$_4$ plants (Magnusson *et al*. 1999, 2001; Smith *et al*. 2002; Warne *et al*. 2010a). However, contrary to other published studies, our lizards included a large proportion of arthropods that consumed resources derived from C$_4$/CAM plants.

While we cannot distinguish between the carbon isotope ratios of C$_4$ and CAM plants on our study site, CAM plants were a minor component of the landscape and were represented chiefly by scattered and isolated succulents, while perennial and annual C$_4$ grasses and the C$_4$ shrub *Salsola* sp. were conspicuous and regular components of the landscape (I Murray, personal observation). Some Namib Desert plants are capable of facultatively switching between C$_3$ and CAM photosynthesis depending on water stress (e.g. Winter *et al*. 1978), and insect use of these resources could lead to a misinterpretation of insectivore use of plant resources based on tissue stable isotope ratios. However, these plants were relatively minor components of the local flora (e.g. *Mesembryanthemum guericheanum*; represented by several widely scattered small individuals). Consequently, an enriched carbon isotope ratio in insectivores here is likely to represent significant use of C$_4$ plants.

*Pedioplanis husabensis* obtained more than 70% of its nutrients from arthropods that sourced most of their carbon from C$_4$ plants in all seasons that we studied, as did *R. bradfieldi* in summer and autumn. During late autumn/early winter, however, *R. bradfieldi* preyed almost equally on arthropods dependent on C$_3$ plants (Fig. 2). For *P. husabensis*, we believe that its consumption of termites brought about its tight linkage to C$_4$ plants. The termite genera that it fed upon are known to feed largely on C$_4$ grasses (e.g. *Hodotermes*, *Psammotermes* and *Trinervitermes*; De Visser *et al*. 2008; Symes & Woodborne 2011), and our carbon isotope analyses of individual termites supported this assertion (Table 1). However, we are unable to distinguish isotopically between termites feeding on C$_4$ grasses and on woody C$_4$ shrubs such *Salsola* sp. that occurred on the site, which means that the importance of C$_4$ grasses to the arthropods making up *P. husabensis's* dietary niche may be overestimated. In an entirely different system, consumption of termites also was considered to underlie flow of C$_4$ grass-derived nutrients into lizard and frog tissues (Magnusson *et al*. 1999, 2001).

Compared to *P. husabensis*, *R. bradfieldi* fed to a greater extent on arthropods that used C$_3$ plant biomass (Figs. 2 and 3). During late autumn/early winter in particular, *R. bradfieldi* acquired nutrients from arthropods that used significantly more C$_3$ plant-derived resources than did *P. husabensis*, incorporating up to 54% of its carbon from C$_3$ plant resources (Fig. 2). We surmise that the late autumn/early winter dietary niche of *R. bradfieldi* reflected incorporation of arthropods using C$_3$ plant production available after recent precipitation (Noy-Meir 1973, 1974; Polis 1997). As a sit-and-wait forager *R. bradfieldi* is likely to feed largely on more mobile arthropods that are active during its diurnal activity period (Pianka 1986). Indeed, the SISUS mixing model results showed its diet to be made up of mobile and diurnally-active insects like ants, wasps and hemipterans (Table 4). The ecology of these arthropod groups also enables them to transfer this C$_3$ plant biomass to *R. bradfieldi* effectively. For example,

hemipterans make up a large part of the total available arthropod biomass after rare desert precipitation events (Polis 1991), and many small wasps feed on $C_3$ flower pollen or are predators on insects that feed on $C_3$ plant production (Scholtz & Holm 1985; Picker *et al*. 2004).

Our previous work documenting the diet of *P. husabensis* and *R. bradfieldi* during May of 2013 using fecal pellet analyses generally supports our estimates of diet composition based on the SISUS mixing model results (Murray *et al*. 2016). These fecal pellet analyses showed that the diet of *P. husabensis* was dominated numerically by termites (71%) and that of *R. bradfieldi* by ants (87%; Murray *et al*. 2016). However, we note that the mixing model results and the fecal pellet analyses do not align perfectly such that, in some seasons, *P. husabensis* and *R. bradfieldi* incorporated fewer nutrients from termites and ants than the fecal pellet analysis implied (Table 4). These contrasting results are perhaps not surprising given the very short periods over which fecal pellet analyses survey diet (days) relative to the period over which the mixing model results based on body tissues do (1–2 months), as well as the fact that the fecal pellet diet analyses estimated prey items and not proportional contribution to diet. We further acknowledge that diet reconstructions estimated from isotope mixing models may give false-positive results even if the items are not included in the diet and it may be difficult to include coverage of all possible dietary items. In addition, here we have employed blood only, and left unexplored the differential routing of prey macronutrients and their associated stable isotope ratios to different tissues (Podlesak *et al*. 2006; Voigt *et al*. 2008).

Recent models imply that climate change in the Namib Desert could result in a mean annual increase of up to 3°C and a reduction in annual precipitation by up to 22%, with coastal regions of the Namib Desert likely experiencing less pronounced change (Thuiller *et al*. 2006). In this xeric environment the potential benefits that increased atmospheric $CO_2$ levels may have for plant photosynthetic efficiency elsewhere are not likely to be capable of compensating for warmer and drier conditions. $C_3$ plant biomass is projected to decrease significantly in parts of the Namib Desert, while $C_4$ plant biomass is likely to change to a much lesser extent in the Namib Desert (Thuiller *et al*. 2006). However, modeling the impacts of climate change in the Namib Desert is made more complex due to the significant role that fog-derived moisture plays in this system (Henschel & Seely 2008). The number of days that fog occurs may increase slightly in the coastal Namib Desert, but decrease by 23–39% further inland (Haensler *et al*. 2011).

While we do not know how reliant on fog moisture versus precipitation the $C_4$/CAM plants are that fed the arthropods that the lizards preyed on, most of the $C_3$ plants in the dry riverbed are reliant upon ground water, and evidence exists suggesting that some riverbed trees (e.g. *Vachellia erioloba* and *Faidherbia albida*) may already be experiencing significant water stress from reduced ground water availability (Schachtschneider & February 2010). Furthermore, most of the primary productivity on the gravel plains of the Namib Desert is from annual $C_4$ grasses that grow in response to rainfall (Henschel *et al*. 2005). While the effects of reduced precipitation, warmer temperatures and higher atmospheric $CO_2$ levels could potentially lead to losses of $C_4$/CAM plant biomass and reductions in plant nutritional quality, lizards in this study occupy dry riverbed habitat at the juxtaposition of a $C_3$ riparian woodland plant community and a $C_4$/CAM desert plant community. Their potential resource use flexibility, coupled with this habitat juxtaposition, may allow enhanced consumer resilience despite negative climate change impacts to particular plant groups.

We describe and compare the movement of nutrients from the $C_3$ and $C_4$/CAM photosynthetic pathways of primary productivity into two secondary consumers (lizards) in the Namib Desert. We show that two sympatric species of insectivores consume isotopically distinct arthropod resources, and that despite the very high available biomass of $C_3$ plants in the adjacent riparian plant community, these two lizard species both rely heavily on a food web based on $C_4$/CAM plant resources. Although the amount of flexibility that these lizards and their arthropod prey have in their dietary ecology is unknown, we think it possible that any potential negative impacts that climate change may have on the availability or nutritional quality of $C_3$ versus $C_4$/CAM plants in this system may be partially buffered by the food web flexibility provided by the adjacent plant community types. Our findings highlight the importance of understanding how environmental change may impact different plant functional groups when considering ecosystem-level implications of climate change for consumer populations. Expanding the temporal, spatial and consumer scope of tissue stable isotope analyses may be particularly useful for better understanding food web dynamics in the Namib Desert.

## ACKNOWLEDGMENTS

## REFERENCES

Anderson RA, Karasov WH (1981). Contrasts in energy-intake and expenditure in sit-and-wait and widely foraging lizards. *Oecologia* **49**, 67–72.

Barbehenn RV, Karowe DN, Chen Z (2004a). Performance of a generalist grasshopper on a C-3 and a C-4 grass: Compensation for the effects of elevated $CO_2$ on plant nutritional quality. *Oecologia* **140**, 96–103.

Barbehenn RV, Karowe DN, Spickard A (2004b). Effects of elevated atmospheric $CO_2$ on the nutritional ecology of C-3 and C-4 grass-feeding caterpillars. *Oecologia* **140**, 86–95.

Bearhop S, Teece MA, Waldron S, Furness RW (2000). Influence of lipid and uric acid on $\delta^{13}C$ and $\delta^{15}N$ values of avian blood: Implications for trophic studies. *The Auk* **117**, 504–7.

Berger-Dell'Mour HAE, Mayer W (1989). On the parapatric existence of two species of the *Pedioplanis undata* group (Reptilia: Sauria: Lacertidae) in the central Namib Desert (Southwest Africa) with description of the new species *Pedioplanis husabensis*. *Herpetozoa* **1**, 83–95.

Boecklen WJ, Yarnes CT, Cook BA, James AC (2011). On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution, and Systematics* **42**, 411–40.

Bond WJ, Midgley GF, Woodward FI (2003). The importance of low atmospheric $CO_2$ and fire in promoting the spread of grasslands and savannas. *Global Change Biology* **9**, 973–82.

Bowers MA, Brown JH (1982). Body size and coexistence in desert rodents: Chance or community structure? *Ecology* **63**, 391–400.

Branch WR (1998). *Field Guide to Snakes and Other Reptiles of Southern Africa*. Struik, Cape Town.

Caut S, Angulo E, Courchamp F (2008). Caution on isotopic model use for analyses of consumer diet. *Canadian Journal of Zoology* **86**, 438–45.

Caut S, Angulo E, Courchamp F (2009). Variation in discrimination factors ($\delta^{15}N$ and $\delta^{13}C$): The effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology* **46**, 443–53.

Cowlishaw G, Davies JG (1997). Flora of the Pro-Namib Desert Swakop River catchment, Namibia: Community classification and implications for desert vegetation sampling. *Journal of Arid Environments* **36**, 271–90.

DeNiro MJ, Epstein S (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* **42**, 495–506.

DeNiro MJ, Epstein S (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* **45**, 341–51.

De Visser SA, Freymann BP, Schnyder H (2008). Trophic interactions among invertebrates in termitaria in the African savanna: A stable isotope approach. *Ecological Entomology* **33**, 758–64.

Drake BG, Gonzàlez-Meler MA, Long SP (1997). More efficient plants: A consequence of rising atmospheric $CO_2$? *Annual Review of Plant Biology* **48**, 609–39.

Eckardt FD, Soderberg K, Coop LJ *et al.* (2013). The nature of moisture at Gobabeb, in the central Namib Desert. *Journal of Arid Environments* **93**, 7–19.

Ehleringer JR, Rundel PW, Nagy KA (1986). Stable isotopes in physiological ecology and food web research. *Trends in Ecology and Evolution* **1**, 42–5.

Ehleringer JR, Cerling TE, Helliker BR (1997). $C_4$ photosynthesis, atmospheric $CO_2$ and climate. *Oecologia* **112**, 285–99.

Ehleringer JR, Cerling TE, Dearing MD (2002). Atmospheric $CO_2$ as a global change driver influencing plant-animal interactions. *Integrative and Comparative Biology* **42**, 424–30.

Erhardt EB, Bedrick EJ (2013). A Bayesian framework for stable isotope mixing models. *Environmental and Ecological Statistics* **20**, 377–97.

Flaherty EA, Ben-David M, Smith WP (2010). Diet and food availability: Implications for foraging and dispersal of Prince of Wales northern flying squirrels across managed landscapes. *Journal of Mammalogy* **91**, 79–91.

Futuyma DJ, Moreno G (1988). The evolution of ecological specialization. *Annual Review of Ecology, Evolution, and Systematics* **19**, 207–33.

Gannes LZ, O'Brien DM, Martínez del Rio C (1997). Stable isotopes in animal ecology: Assumptions, caveats, and a call for more laboratory experiments. *Ecology* **78**, 1271–6.

Haensler A, Cermak J, Hagemann S, Jacob D (2011). Will the southern African west coast fog be affected by future climate change? Results of an initial fog projection using a regional climate model. *Erdkunde* **2011**, 261–75.

Hardin G (1960). The competitive exclusion principle. *Science* **131**, 1292–7.

Henschel JR, Seely MK (2008). Ecophysiology of atmospheric moisture in the Namib Desert. *Atmospheric Research* **87**, 362–8.

Henschel JR, Burke A, Seely M (2005). Temporal and spatial variability of grass productivity in the central Namib Desert. *African Study Monographs* **30**, 43–56.

Herrmann HW, Branch WR (2013). Fifty years of herpetological research in the Namib Desert and Namibia with an updated and annotated species checklist. *Journal of Arid Environments* **93**, 94–115.

Huey RB, Pianka ER (1981). Ecological consequences of foraging mode. *Ecology* **62**, 991–9.

Hutchinson GE (1957). Population studies – animal ecology and demography – Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology* **22**, 415–27.

IPCC (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Pachauri RK, Meyer LA, eds. *IPCC*, Geneva, Switzerland, 151 pp.

Jackson AL, Inger R, Parnell AC, Bearhop S (2011). Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* **80**, 595–602.

Layman CA, Arrington DA, Montana CG, Post DM (2007). Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology* **88**, 42–8.

Layman CA, Araújo MS, Boucek R *et al.* (2012). Applying stable isotopes to examine food web structure: an overview of analytical tools. *Biological Reviews* **87**, 542–62.

Liu R, Zhu F, Song N, Yang X, Chai Y (2013). Seasonal distribution and diversity of ground arthropods in microhabitats following a shrub plantation age sequence in desertified steppe. *PLoS ONE* **8**, e77962.

MacArthur RH (1958). Population ecology of some warblers of northeastern coniferous forests. *Ecology* **39**, 599–619.

MacArthur R, Levins R (1967). The limiting similarity, convergence, and divergence of coexisting species. *American naturalist* **101**, 377–85.

Magnusson WE, de Araújo MC, Cintra R *et al.* (1999). Contributions of $C_3$ and $C_4$ plants to higher trophic levels in an Amazonian savanna. *Oecologia* **119**, 91–6.

Magnusson WE, Lima AP, Faria AS, Victoria RL, Martinelli LA (2001). Size and carbon acquisition in lizards from Amazonian savanna: evidence from isotope analysis. *Ecology* **82**, 1772–80.

Marsh AC (1986). Checklist, biological notes and distribution of ants in the central Namib Desert. M*adoqua* **14**, 333–44.

Martínez del Rio C, Anderson-Sprecher R (2008). Beyond the reaction progress variable: the meaning and significance of isotopic incorporation data. *Oecologia* **156**, 765–72.

Martínez del Rio C, Carleton SA (2012). How fast and how faithful – The dynamics of isotopic incorporation into animal tissues. *Journal of Mammalogy* **93**, 353–9.

Martínez del Rio C, Wolf BO (2005). Mass balance models for animal isotopic ecology. In: Starck JM, Wang T, eds. *Physiological and Ecological Adaptations to Feeding in Vertebrates*. Science Publishers, Enfield, pp. 141–74.

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* **102**, 378–90.

Menke SB, Suarez AV, Tillberg CV, Chou CT, Holway DA (2010). Trophic ecology of the invasive argentine ant: Spatio-temporal variation in resource assimilation and isotopic enrichment. *Oecologia* **164**, 763–71.

Mooney KA, Tillberg CV (2005). Temporal and spatial variation to ant omnivory in pine forests. *Ecology* **86**, 1225–35.

Muldavin EH, Moore DI, Collins SL, Wetherill KR, Lightfoot DC (2008). Above ground net primary production dynamics in a northern Chihuahuan Desert ecosystem. *Oecologia* **155**, 123–32.

Murray GW, Schramm D (1987). A comparative study of the diet of the wedge-snouted sand lizard, *Meroles cuneirostris* (Strauch), and the sand diving lizard, *Aporosaura anchietae* (Bocage), in the Namib Desert. *Madoqua* **15**, 55–61.

Murray IW, Fuller A, Lease HM, Mitchell D, Wolf BO, Hetem RS (2014). The actively foraging desert lizard *Pedioplanis husabensis* behaviorally optimizes its energetic economy. *Canadian Journal of Zoology* **92**, 905–13.

Murray IW, Fuller A, Lease HM, Mitchell D, Wolf BO, Hetem RS (2015). Low field metabolic rates for geckos of the genus *Rhoptropus* may not be surprising. *Journal of Arid Environments* **113**, 35–42.

Murray IW, Fuller A, Lease HM, Mitchell D, Hetem RS (2016). Ecological niche separation of two sympatric insectivorous lizard species in the Namib Desert. *Journal of Arid Environments* **124**, 225–32.

Noy-Meir I (1973). Desert ecosystems: Environment and producers. *Annual Review of Ecology, Evolution, and Systematics* **4**, 25–51.

Noy-Meir I (1974). Desert ecosystems: Higher trophic levels. *Annual Review of Ecology, Evolution, and Systematics* **5**, 195–214.

Ode DJ, Tieszen LL, Lerman JC (1980). The seasonal contribution of $C_3$ and $C_4$ plant species to primary production in a mixed prairie. *Ecology* **61**, 1304–11.

Olivier J (1995). Spatial distribution of fog in the Namib. *Journal of Arid Environments* **29**, 129–38.

Parnell AC, Inger R, Bearhop S, Jackson AL (2010). Source partitioning using stable isotopes: Coping with too much variation. *PLoS ONE* **5**, e9672.

Peterson B, Fry B (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology, Evolution, and Systematics* **18**, 293–320.

Pianka ER (1974). Niche overlap and diffuse competition. *PNAS* **71**, 2141–5.

Pianka ER (1986). *Ecology and Natural History of Desert Lizards*. Princeton University Press, Princeton.

Picker M, Griffiths C, Weaving A (2004). *Field Guide to Insects of South Africa*. Struik, Cape Town.

Podlesak DW, McWilliams SR (2006). Metabolic routing of dietary nutrients in birds: Effects of diet quality and macronutrient composition revealed using stable isotopes. *Physiological and Biochemical Zoology* **79**, 534–49.

Polis GA (1991). Complex trophic interactions in deserts: An empirical critique of food-web theory. *American Naturalist* **138**, 123–55.

Polis GA, Hurd SD, Jackson CT, Piñero FS (1997). El Niño effects on the dynamics and control of an island ecosystem in the Gulf of California. *Ecology* **78**, 1884–97.

Robinson MD, Cunningham AB (1978). Comparative diet of two Namib Desert sand lizards (Lacertidae). *Madoqua* **11**, 41–53.

Roughgarden J (1974). Niche width: Biogeographic patterns among Anolis lizard populations. *American Naturalist* **108**, 429–42.

Schachtschneider K, February EC (2010). The relationship between fog, floods, groundwater and tree growth along the lower Kuiseb River in the hyperarid Namib. *Journal of Arid Environments* **74**, 1632–7.

Schoener TW (1977). Competition and the niche. In: Tinkle DW, Gans C, eds. *Biology of the Reptilia*. Academic Press, New York, pp. 35–136.

Scholtz CH, Holm E (1985). *Insects of Southern Africa*. Butterworths, Durban.

Schulze ED, Ellis R, Schulze W, Trimborn P, Ziegler H (1996). Diversity, metabolic types and δ13C carbon isotope ratios in the grass flora of Namibia in relation to growth form, precipitation and habitat conditions. *Oecologia* **106**, 352–69.

Simberloff D, Dayan T (1991). The guild concept and the structure of ecological communities. *Annual Review of Ecology, Evolution, and Systematics* **22**, 115–43.

Smith KF, Sharp ZD, Brown JH (2002). Isotopic composition of carbon and oxygen in desert fauna: investigations into the effects of diet, physiology, and seasonality. *Journal of Arid Environments* **52**, 419–30.

Still CJ, Berry JA, Collatz GJ, DeFries RS (2003). Global distribution of $C_3$ and $C_4$ vegetation: Carbon cycle implications. *Global Biogeochemical Cycles* **17**, 1–14.

Straka J, Feldhaar H (2007). Development of a chemically defined diet for ants. *Insectes sociaux* **54**, 100–4.

Symes CT, Woodborne S (2011). Estimation of food composition of *Hodotermes mossambicus* (Isoptera: Hodotermitidae) based on observations and stable carbon isotope ratios. *Insect Science* **18**, 175–80.

Thuiller W, Midgley GF, Hughes GO *et al.* (2006). Endemic species and ecosystem sensitivity to climate change in Namibia. *Global Change Biology* **12**, 759–76.

Uys V (2002). *A Guide to the Termite Genera of Southern Africa*. Plant Protection Research Institute Handbook No. 15. Agricultural Research Council, Pretoria.

Vitt LJ, Pianka ER, Cooper WE Jr, Schwenk K (2003). History and the global ecology of squamate reptiles. *American Naturalist* **162**, 44–60.

Voigt CC, Rex K, Michener RH, Speakman JR (2008). Nutrient routing in omnivorous animals tracked by stable carbon isotopes in tissue and exhaled breath. *Oecologia* **157**, 31–40.

Warne RW, Pershall AD, Wolf BO (2010a). Linking precipitation and $C_3$–$C_4$ plant production to resource dynamics in higher-trophic-level consumers. *Ecology* **91**, 1628–38.

Warne RW, Gilman CA, Wolf BO (2010b). Tissue carbon incorporation rates in lizards: Implications for studies using stable isotopes in terrestrial ectotherms. *Physiological and Biochemical Zoology* **83**, 608–17.

Watson JAL (1973). The worker caste of the hodotermitid harvester termites. *Insectes Sociaux* **20**, 1–20.

Winter K, Lüttge U, Winter E, Troughton JH (1978). Seasonal shift from $C_3$ photosynthesis to crassulacean acid metabolism in *Mesembryanthemum crystallinum* growing in its natural environment. *Oecologia* **34**, 225–37.

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