Isotopic Niche Variation in a Higher Trophic Level Ectotherm: Highlighting the Role of Succulent Plants in Desert Food Webs

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Abstract

Stable isotope analysis of animal tissues allows description of isotopic niches, whose axes in an n-dimensional space are the isotopic ratios, compared to a standard, of different isotope systems (e.g. δ13C, δ15N). Isotopic niches are informative about where an animal, population or species lives and about what it consumes. Here we describe inter- and intrapopulation isotopic niche (bidimensional δ13C-δ15N space) of the Orange-throated whiptail (Aspidoscelis hyperythra), an arthropodivorous small lizard, in ten localities of Baja California Sur (Mexico). These localities range from extreme arid to subtropical conditions. Between 13 and 20 individuals were sampled at each locality and 1 cm of tail-tip was collected for isotope analysis. As expected, interpopulation niche width variation was much larger than intrapopulation one. Besides, isotopic variation was not related to age, sex or individual size of lizards. This suggests geographic variation of the isotopic niche was related to changes in the basal resources that fuel the trophic web at each locality. The position of Bayesian isotope ellipses in the δ-space indicated that whiptails in more arid localities were enriched in 13C, suggesting most of the carbon they ingested came from CAM succulent plants (cacti, agaves) and in minor degree in C4 grasses. Contrarily, whiptails in subtropical areas were depleted in 13C, as they received more carbon from C3 scrubs and trees. Localities closer to sea-level tended to be enriched in 15N, but a clear influence of marine subsidies was detected only at individual level. The study contributes to identify the origin and pathways through which energy flows across the trophic webs of North American deserts.
Introduction

The classical concept of ecological niche corresponds to a hypervolume in n-dimensional space, with the axes representing environmental variables (or resources) along which it is delimited the range where a species can survive (fundamental niche) or actually is present (realized niche) [1–2]. But historically practical difficulties to apply the niche concept to different species and circumstances [3] moved some ecologists to accept that, although the "niche is a central concept of ecology (…) we do not know exactly what it means" [4], reaching some of them (e.g. [5]) to recommend avoid the term niche in the scientific literature.

Recently, however, new methodological and technological approaches are renewing the concept of ecological niche and improving the ways to quantify it [6–7]. In particular, the use of stable isotope analysis (hereafter, SIA) on animal tissues is allowing describing isotopic niches, whose axes in a multidimensional space would be different isotopic signatures (e.g. δ¹³C or δ¹⁵N) [8]. Isotopic values can indicate what an animal had consumed (e.g. [9]) and some characteristics of the place or the habitat in which it lives or lived (e.g. [10]).

Isotopic signatures of primary producers are carried up to the highest levels in the food web with only minor changes. Thus, the geographic variation in the isotopic niche of generalist predators would reflect spatial changes in their food-webs [11]. For example, intraspecific variation in isotopic niche has been detected between predator populations of distant areas [12] and those relying on freshwater and marine food-webs [8]. At lower spatial scales, isotopic niche of particular populations will include individual variation in the ranges of prey consumed, but also intra-locality variation among food sources [13–14]. Consequently, interpopulation variation in isotopic niche across a species range can inform about the spatial patterns of isotopic variation in primary producers, while within-population variation can illustrate about individual patterns of resource use at local level.

We have compared the long-term isotopic niche of ten populations of Orange-throated whiptail (Aspidoscelis hyperythra; Sauria, Teiidae). A. hyperythra is an arthropodivorous small lizard that can be considered a good model of higher trophic-level consumer at the Baja California desert food-webs (see below). We used estimates of δ¹³C and δ¹⁵N in body tissues characterized by a low-turnover. Our main aim was to relate whiptail niche variation to geographic differences in the type of plants fuelling the communities and to individual (intrapopulation) variability in the use of resources.

The main condition to obtain informative results from SIA is to have enough variation in the isotopic composition of the primary resources [6]. In our study, we expected to find at least four sources of isotopic variation: a) at interpopulation scale, primary producers at different areas would be mainly succulent plants (many cacti and some agaves) or bushes and trees; both groups use different photosynthetic pathways and consequently their δ¹³C signatures are very different (see Methods and S1 Table); b) also, the food-webs of the areas closer to the sea could be more or less subsidized by nutrients from marine origin, which have contrasting δ¹⁵N signatures [15]; c) at intrapopulation scale, individuals can behave as specialist on different food sources at isotopic level ("individual niche") [16]; for instance, home ranges of individual lizards vary in vegetation, soil type, humidity, etc.; this will make some individuals to rely more on arthropods feeding on succulents, while others will rely on those feeding on bushes; d) finally, at the localities with less complex communities it could be expected to find shorter food-chains, which could be detected at the isotopic composition of predators (mainly δ¹⁵N, that increases about 3 points upwards each trophic level [17–18]; however, this discrimination factor could be lesser in ectotherms, making difficult the assignation of trophic levels [19–20]). Based on these potential sources of variation we predict:
1. Interpopulation variation in the isotopic niche of our high-level predator will be greater than intrapopulation variation, mainly (but not only) due to the contrasting vegetation characteristics in our sampled localities.

2. The isotopic niche of each population will reflect the biotic and abiotic environmental conditions of the area. An increased importance of succulents as primary producers fuelling the food-web, detected as an enrichment of $\delta^{13}C$ (i.e. less-negative values of $\delta^{13}C$) and maybe higher $\delta^{15}N$, was expected at drier, more desert localities. Also, the probability of detect some influence of marine subsidies through an enrichment of $\delta^{15}N$, should be higher at low altitudes and closer to the sea.

3. Individual (intrapopulation) variation in the isotopic niche should be high, as whiptails have reduced home ranges and thus each of them is dependent on the resources of a limited area in the “mosaic nature of the environment” [1]. Thus, they could behave as specialist although the species or population was generalist [14, 21]. However, we do not expect isotopic variation related to the sex or size of lizards, because they are very generalist arthropodivores, lack of apparent sexual dimorphism and use the same habitats all along their life (see below).

Until now, few studies have used small terrestrial ectotherms as model organisms for isotopic studies (but see [22–24]). Also, SIA have been relatively scarce in desert biomes [25]. To our knowledge, this is the first study evaluating geographical variation in the isotopic niche of a desert high-trophic level ectotherm. The results should be informative about the less-known relative importance of different plant types as producers fuelling animal communities in North American deserts.

**Study System, Material and Methods**

**Model species**

Orange-throated whiptails (herein whiptails) are small lizards (4–7 g in weight) native to Southwestern California (USA) and the peninsula and some islands of Baja California (Mexico). Their sexual dimorphism is scarce, being not possible to distinguish males from females at field conditions unless you catch them. They are non-territorial and forage actively during the day, searching in the leaf litter, the shallow soil and the low branches for a large variety of animal foods ([26–30], authors pers.obs.). Following [31], they should be considered generalist arthropodivores. Their individual hunting range is small, about 445 m² [32], while the mean distance between recaptures of the same individual approached 11 m [28]. Because of their foraging behaviour (they are active searchers and not ambushers [30]) and their wide food-spectrum, they can be considered suitable “samplers” of the local community of arthropods, which includes different trophic roles (herbivores, detritivores, predators, parasitoids). Therefore, the species is a good model of higher-level consumer in the desert community.

**Study area**

The study has been carried out in the southernmost quarter of the Baja California peninsula, approximately between 23°N-26°N in latitude and 109°W-112°W in longitude. Baja California is a volcanic fringe of land running from north-west to south-east, 1300 km long and, on the average, about 100 km wide. Most of the study area is considered “thermotropical” [33], with annual mean temperature of about 23°C. Rains, concentrated mainly in the summer, are very scarce, increasing towards the south and in altitude (we did not sampled whiptails above 500 m; see Table 1 for characteristics of each locality).
Very arid desert conditions predominate in the north of the area, while subtropical conditions influence the south [34]. Many species of cacti are present in the desert, especially giant cardons (*Pachycereus pringlei*), which frequently characterize the landscape, and *Opuntia* spp., *Stenocereus* spp., *Ferocactus* spp., *Mammillaria* spp., etc.; different agaves (*Agave* spp.) and Joshua trees (*Yucca* spp.) are common too; trees are scarce (some legume trees such as *Prosopis* sp. and *Lysiloma candida* at the usually dry riverbeds) but there are shrubs, such as *Larrea divaricata* and *Fouquieria* spp. On the other hand, at the subtropical thorny forest of the south, succulents are also abundant, mainly at low altitude, but woody shrubs, large bushes and deciduous trees, including *Bursera* spp., *Lysiloma divaricata*, *Tecoma stans*, *Cyrtocarpa edulis*, *Jatropha cinerea*, *Cercidium* spp., *Caesalpinia* spp., *Mimosa xantii*, etc., predominate [35]. Near the shore there are some mangrove species (*Rhizophora mangle*, *Laguncularia racemosa*, *Avicennia germinans*).

Succulents, trees, shrubs and grasses use several photosynthetic pathways, which have an important effect on their isotopic signatures [36]: C3 plants fix CO2 using the enzyme ribulose bisphosphate carboxylase and typically have δ13C values rounding -28‰ (-26.43 ± 1.75, mean ± SD, in our samples; S1 Table); C4 plants take up initially CO2 by carboxylation of phosphoenolpyruvate, having δ13C signatures around -14‰ (-14.86 in our sample; S1 Table); CAM plants refer to Crassulacean Acid Metabolism and have δ13C signatures rather similar to C4 plants (-13.91 ± 1.24 in our sample; S1 Table). While cacti and agaves (and at least one succulent Euphorbiaceae species) are photosynthetic CAM plants in Baja California, trees are obligate C3 and, according our data (see S1 Table), all woody shrubs would be C3 plants. Most grasses are potential C4 plants (S1 Table), but they are spatially and temporally very scarce in the area, except locally following summer rains, which do not reach all areas every year.

### Whiptail capture and sampling

In different periods from September 2005 to February 2010, between 13 and 20 whiptails were captured and sampled in each of ten localities well-spaced in the area (Fig 1). These localities vary widely in vegetation type, annual rainfall, altitude and distance to the sea (see Table 1). To avoid killing whiptails, we snared them with nooses across their head, by a fishing rod with a thread loop at the tip. Immediately after capture, each individual was sampled by cutting about 1 cm of the tail tip using a scalpel disinfected with ethanol, and released within a few minutes, of

### Table 1. Main characteristics of the ten sampled localities (ordered from South to North).

| Locality | Coord. (N, W) | Coastline dist. (km) | Altitude (m.a.s.l) | Rainfall (mm) | Veget.type | δ 13C‰(n) | δ 15N‰(n) |
|----------|--------------|---------------------|-------------------|--------------|------------|------------|------------|
| CSL      | 22.92360109.9774 | 4.22                | 173               | 239          | 1          | -17.87 ± 0.36 (16) | 10.32 ± 0.26 (16) |
| MIG      | 23.06735110.0984 | 0.98                | 45                | 202          | 2          | -21.29 ± 0.32 (19) | 11.16 ± 0.33 (19) |
| CNAR     | 23.24340109.7461 | 21.74               | 209               | 414          | 1          | -25.22 ± 0.70 (19) | 10.73 ± 0.13 (20) |
| RIB      | 23.56870109.5598 | 2.72                | 27                | 217          | 1          | -20.33 ± 0.14 (20) | 12.81 ± 0.48 (20) |
| BART     | 23.74020109.8694 | 15.97               | 447               | 397          | 1          | -21.89 ± 0.13 (19) | 7.83 ± 0.27 (19) |
| INO      | 23.77451105.6611 | 1.98                | 13                | 93           | 4          | -17.89 ± 0.27 (19) | 14.31 ± 0.21 (19) |
| KM 83    | 24.26863110.9555 | 19.69               | 153               | 129          | 2          | -18.26 ± 0.33 (20) | 12.58 ± 0.21 (20) |
| TECO     | 24.34629110.2862 | 0.53                | 31                | 217          | 3          | -18.47 ± 0.39 (15) | 7.74 ± 0.30 (17) |
| IHIU     | 24.97434111.4075 | 51.63               | 134               | 160          | 2          | -17.03 ± 0.43 (13) | 12.09 ± 0.40 (13) |
| BAJO     | 26.08441113.3253 | 0.09                | 8                 | 130          | 2          | -21.53 ± 0.28 (20) | 13.13 ± 0.34 (20) |

Sample sizes (n) and levels of δ13C and δ15N (mean ± SE) correspond to lizard tail tips for each locality. Shortest distance to the coastline and average altitude were obtained from a GIS of the area, average annual rainfall was estimated from WORLDCLIM Ver. 1.2 (http://www.worldclim.org) and vegetation type was assigned from 1:250000 charts of [77]. Vegetation types: 1, deciduous dry forest; 2, desert scrubs (mostly Cactaceae); 3, halophyte scrubs; 4, fog desert shrub (mostly Cactaceae, but with humidity from the sea and lichens).

doi:10.1371/journal.pone.0126814.t001
all them run immediately. Individuals with obviously regenerating tail were overlooked. Tail-clipping is frequently used in the study of reptiles, as it is considered a nondestructive sampling technique (e.g. [37]). Besides, at field conditions it is an efficient method for preventing re-sampling. Whiptails have the ability for tail autotomy and posterior regeneration, so the loss of just the tail tip should not be too stressful for them (see [38]). Tail tip clips were used for isotopic analyses.

All animals were captured on open public land and close to roads or villages, where free access and transit was allowed. Because the localities CNAR, MIG, BART and RIB are in the buffer zone of the Biosphere Reserve "Sierra de la Laguna" and Aspidoscelis hyperythra is an endemic species catalogued in Mexico as threatened, to obtain the permit from the authorities we send them previously a detailed protocol. Permission for the complete protocol (capture, sampling of tail, and releasing) was obtained from the environmental authority in Mexico (D.G. Vida Silvestre, Secretaría de Medio Ambiente y Recursos Naturales, México, permit # 11311; see Acknowledgments section), who is in charge of the ethical considerations. There is not a formal Institutional Animal Care and Use Committee.

Stable isotopic analyses
Isotope measurements were carried out at the Stable Isotope Laboratory of the Instituto Andaluz de Ciencias de la Tierra (CSIC-UGR, Granada, Spain). Organic matter (lizard tail tips and plant tissues) was homogenized and later analyzed for the isotopic composition of nitrogen
and carbon by means of a Carlo Elba NC1500 (Milan, Italy) elemental analyzer on line with a Delta Plus XL (ThermoQuest, Bremen, Germany) mass spectrometer (EA-IRMS). Because practically lizard tail tip does not content lipids [39] and little is known in detail about isotopic routing in Lacertidae, we did not extract lipid from our samples (but see [40]). The stable composition of samples is reported as δ values per mil:

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

Where \( R = \frac{13C}{12C} \) for δ\(^{13}\)C values and \( R = \frac{15N}{14N} \) for δ\(^{15}\)N values.

Commercial CO₂ and N₂ were used as the internal standard for the carbon and nitrogen isotopic analyses. For carbon, two internal standards of -30.63 ‰ and -11.65 ‰ (Vienna Pee Dee Belemnite; VPDB) were analyzed every 10 samples. For nitrogen, two internal standards of -1.02 ‰ and +16.01 ‰ (AIR) were used. Precision calculated, after correction of the mass spectrometer daily drift, from standards systematically interspersed in analytical batches was better than ± 0.1 ‰ for both δ\(^{13}\)C and δ\(^{15}\)N.

All the individual isotopic values can be consulted in additional supporting data (S2 Table)

**Isotopic niche metrics**

Isotopic niche characteristics were described with quantitative metrics developed by [41], which used mean δ\(^{13}\)C and mean δ\(^{15}\)N of all species in a given community. In order to adapt their community level metrics to a population level, able to encompass intra and interpopulation variation, we did not use means, but the values of δ\(^{13}\)C and δ\(^{15}\)N of all individuals sampled in each population. For each population we calculated:

1. Total Area (TA) of the convex hull encompassing all points, which can be considered a measure of population niche width. Nevertheless, because this estimator is very dependent of sample size (e.g. [42]), we also calculated:

2. Bayesian Standard Ellipse Area (SEA\(_B\)), bootstrapping data (n = 10000). Standard ellipse (SEA) contains approximately 40% of the points and it is a measure of the mean core population niche, being to bivariate data as standard deviation is to univariate data [43]. It provides a good estimate of the population niche area, but with tendency to underestimate at small size samples. SEA\(_B\) nullify this bias, allowing comparisons among populations widely differing in size sample. Mean areas and the low and upper 95% credible limits will be shown. Additionally, we calculated:

3. Carbon range (CR) and Nitrogen range (NR), corresponding to the distance between the two individuals with the highest and the lowest δ\(^{13}\)C and δ\(^{15}\)N values within each population; they estimate the total carbon and nitrogen range exploited by each population.

4. Mean Distance to Centroid (CD), calculated as the mean Euclidean distance of each individual of a population to the δ\(^{13}\)C-δ\(^{15}\)N centroid for that population; it is an estimator of the population isotopic diversity.

5. Mean Nearest Neighbor Distance (MNND) that reflects the packing of the individuals in the two-dimensional isotopic space.

All calculations were made in R using the SIAR package [44].

**Geographic variation analysis**

Besides the graphical representation of the ellipses characterizing isotopic niches, differences among localities in δ\(^{13}\)C and δ\(^{15}\)N levels were analyzed fitting general linear mixed models
using Proc Glimmix in SAS [45], including the sampling date as a random factor. To evaluate our prediction that δ13C local levels would relate to aridity and those of δ15N to coastline distance, altitude, and aridity, we regressed their mean values across localities. In addition to evaluating our predictions, we also evaluated the effect of other five ecological correlates or potentially confounding factors: season (i.e. proportion of individuals sampled in summer-autumn), age (proportion of sampled adults), sex (proportion of adult females when juveniles were not considered), size (snow-went length) and occurrence of regenerated tail (proportion of sampled individuals with apparently regenerated tail). Because of departures from normality and the presence of outliers, we used robust regression [46] rather than least-squares regression. Robust fits are minimally influenced by outliers in the independent variable space, in the response space, or in both. Analyses were performed by using robust MM-regression procedure available in S-Plus 6 [47], and the significance of each variable was assessed by using robust F-tests (FR).

Given that spatial autocorrelation could inflate Type I statistical error, we evaluated whether residuals from significant robust fits for mean population values were spatially autocorrelated. To this end, we used Moran’s I mark-correlation function [48] which allowed us to investigate how the residuals of two populations separated by distance r differ from their expected value under a null model of not spatial autocorrelation. It ranges from −1 (indicating perfect dispersion) to +1 (perfect correlation). Spatial autocorrelation analyses were conducted with the software Programita [48] available at www.Programita.com.

Results

Isotopic niche descriptors

Our localities differ widely in biotic and abiotic conditions, as well as in the average isotopic values of their whiptail tissues (Table 1). Also, there is a substantial geographic variation in the isotopic metrics (Table 2) and the position of population ellipses in the bi-dimensional δ13C-δ15N space (Fig 2).

As expected, niche area was much broader at the species than at the population level, both for Total Area (TA) and for Bayesian Standard Ellipse Area (SEAB) (Table 2). Specifically, SEAB at the species level was, on average, five times larger than at the population level, and up

| Populations | TA    | SEAB (95% limits) | CR   | NR   | CD   | MNND  | n  |
|-------------|-------|-------------------|------|------|------|-------|----|
| CSL         | 11.15 | 4.40 (4.38–4.42)  | 5.36 | 3.53 | 1.51 | 0.64  | 16 |
| MIG         | 17.42 | 6.26 (6.23–6.29)  | 5.93 | 5.47 | 1.73 | 0.63  | 19 |
| CNAR        | 12.96 | 6.35 (6.32–6.38)  | 8.52 | 2.01 | 2.75 | 0.62  | 19 |
| RIB         | 13.08 | 4.71 (4.69–4.73)  | 2.71 | 7.26 | 1.70 | 0.44  | 20 |
| BART        | 4.69  | 2.09 (2.08–2.10)  | 2.78 | 4.45 | 1.03 | 0.30  | 19 |
| INO         | 10.13 | 3.63 (3.61–3.64)  | 6.51 | 2.77 | 1.14 | 0.54  | 19 |
| KM 83       | 12.74 | 4.50 (4.48–4.52)  | 6.51 | 3.68 | 1.41 | 0.66  | 20 |
| TECO        | 13.22 | 5.78 (5.75–5.81)  | 4.68 | 3.64 | 1.72 | 0.76  | 15 |
| IHU         | 19.28 | 7.58 (7.54–7.62)  | 6.29 | 4.66 | 1.92 | 1.01  | 13 |
| BAJO        | 18.66 | 5.76 (5.75–5.79)  | 5.19 | 6.88 | 1.57 | 0.70  | 20 |
| TOTAL       | 112.0 | 21.03 (20.99–21.06)| 14.2 | 12.1 | 3.28 | 0.39  | 180|

TA = Total area of convex hull; SEAB = Bayesian standard ellipse area; CR = δ13C range; NR = δ15N range; CR = Mean distance to centroid; MNND = Mean nearest neighbour distance; n = bivariate sample size.

doi:10.1371/journal.pone.0126814.t002
to ten times larger as compared with the smallest population value (BART). That suggests the
wide whiptail species isotopic niche results in fact from the sum of thinner, more specialized,
local niches. At population level, large niches as estimated by TA (e.g. IHU, MIG) seemed to
the more related to simultaneously high values in Carbon Range (CR) and Nitrogen Range
(NR) than to extreme values in any of them (e.g. CNAR, RIB).

Considering all individuals, CR and NR were rather large, indicating that whiptails use a
large range of isotopic resources. $\delta^{13}$C ranged from -28.67‰ to -14.44‰ (coefficient of varia-
tion [CV] = 14.1%), encompassing the whole range of the plants sampled in the area (in fact,
some vegetal source in CNAR, at least, must be more depleted in $\delta^{13}$C than any of the plant
species represented in S1 Table). $\delta^{15}$N ranged between 5.7‰ and 17.8‰ (CV = 21.6%), inside
the range of plants ($C_3$ plants, mean $\pm$ SD = 8.68 $\pm$ 3.64; $C_4$-CAM plants, 11.22 $\pm$ 3.16). CR and
NR varied widely among populations. The highest CR corresponded to CNAR, just the popula-
tion more depleted in $^{13}$C on the average. Also, the highest NR corresponded to RIB, the sec-
ond population more enriched on the average in $^{15}$N.

Although no precisely, at population level, the metrics related to isotopic diversity (Mean
Distance to Centroid, CD) and packaging (Mean Nearest Neighbor Distance, MNND) were

**Fig 2. Stable isotope standard ellipses of whiptails for each of ten studied localities in southern Baja California (Mexico).** Ranges and mean values of $\delta^{15}$N and $\delta^{13}$C of $C_3$ y $C_4$-CAM plants sampled in the whole area are also shown. Localities are named according to Table 1.

doi:10.1371/journal.pone.0126814.g002
related to estimators of niche width. For instance, the highest CD corresponded to CNAR, the population having a largest CR, and the lowest one to BART, the population having smallest TA, SEA_B and MNND. Also, the highest MNND corresponded to IHU, the population having largest TA and SEA_B.

With respect to the position of the ellipses in relation to δ^{13}C axis (Fig 2), CNAR ellipse had the lowest central value (-25.22), while the more enriched was IHU ellipse (-17.03). En relation to δ^{15}N, the lowest central value corresponded to TECO population (7.74) and the highest to INO (14.31). δ^{13}C and δ^{15}N tended to be positively related (see below).

**Geographical variation**

We did not find any relationship of δ^{15}N with non-geographical factors such as sampling season, age, sex, size, or the occurrence of a regenerated tail (P > 0.166; see Table 3). In the case of δ^{13}C, sampling season, age, size, or the occurrence of a regenerated tail did not have any significant effect (P > 0.074; Table 3). However, δ^{13}C was negatively related with the proportion of females in population samples (P < 0.001; Fig 3E). This effect, again, was not revealed by standard least-square regression (F_1, 8 = 0.096, P = 0.419).

Contrarily, whiptails in the studied localities strongly differed in δ^{13}C value (F_9, 163 = 34.65, P < 0.0001). Average local values of δ^{13}C tended to be higher in more desert areas, i.e. there was a significant negative correlation between average δ^{13}C in each locality and annual average rainfall (F_R = 4.72, d.f. = 1, P < 0.05; Fig 3A). We also found strong differences in δ^{15}N among localities (F_9, 165 = 62.17, P < 0.0001). There was a significant negative relationship between local elevations and δ^{15}N average values (F_R = 11.97, d.f. = 1, P < 0.001; Fig 3B), suggesting that lizards living closer to sea level (RIB, BAJO, INO) tended to include a higher proportion of heavier N in their tissues. Interestingly, this relationship turned no-significant (F_1,8 = 3.57, P = 0.096) when using standard least-squared regression, likely because in contrast with robust regression it does not account for the effect of outliers such as the locality TECO (Fig 3B). Indeed, this locality shows the lowest δ^{15}N value, although it is located close to the sea (also, it was the only one with halophyte vegetation; Table 1).

As expected, average δ^{15}N values were negatively related to average annual rainfall (F_R = 11.43, d.f. = 1, P < 0.001; Fig 3C), suggesting that increased local aridity enhanced δ^{15}N levels (INO, IHU, Km 83). Contrary to expectations, no relationship was found between local levels of δ^{15}N and distance to coastline (P = 0.959).

There was not any significant relationship between average values of δ^{13}C and δ^{15}N across localities (F_R = 0.32, d.f. = 1, P = 0.564). However, when considering individual whiptails as

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**Table 3. Main results from robust regressions for the relationships of δ^{15}N and δ^{13}C in *Aspidoscelis hyperythra* tissue and different ecological correlates.**

|          | d.f. | F_R    | P     | d.f. | F_R    | P     |
|----------|------|--------|-------|------|--------|-------|
| **δ^{15}N** |      |        |       |      |        |       |
| Season   | 1    | 1.405  | 0.225 | 1    | 1.871  | 0.161 |
| Age      | 1    | 0.166  | 0.676 | 1    | 1.250  | 0.252 |
| Sex      | 1    | 0.002  | 0.967 | 1    | 13.19  | **0.001** |
| SVL      | 1    | 0.065  | 0.794 | 1    | 0.327  | 0.558 |
| Regenerated tail | 1    | 1.830  | 0.166 | 1    | 3.033  | 0.074 |

| **δ^{13}C** |      |        |       |      |        |       |
| Season   | 1    | 1.405  | 0.225 | 1    | 1.871  | 0.161 |
| Age      | 1    | 0.166  | 0.676 | 1    | 1.250  | 0.252 |
| Sex      | 1    | 0.002  | 0.967 | 1    | 13.19  | **0.001** |
| SVL      | 1    | 0.065  | 0.794 | 1    | 0.327  | 0.558 |
| Regenerated tail | 1    | 1.830  | 0.166 | 1    | 3.033  | 0.074 |

Season: proportion of individuals sampled in summer; Age: proportion of sampled adults; Sex: proportion of adult females when juveniles were not considered; SVL, Snout-vent length; Regenerated tail: proportion of sampled individuals with clear sign of regenerated tail. Significant result (P < 0.05) in boldface.

doi:10.1371/journal.pone.0126814.t003

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Isotopic Niche in a Desert Higher-Trophic Level Ectotherm

PLOS ONE | DOI:10.1371/journal.pone.0126814 May 14, 2015 9/17
Fig 3. Relationships between isotopic values and several variables across the ten sampled localities in southern Baja California (Mexico). (A) Negative relationship between average values of $\delta^{13}$C (a) or $\delta^{15}$N (C) in whiptail tissues and the estimated annual rainfall (B) Negative relationship between average $\delta^{15}$N values and local altitude. (D) Positive relationship of $\delta^{13}$C and $\delta^{15}$N values across individual lizards ($n = 180$). Note that robust regression (continuous line) often differs somewhat from least-squares regression (dotted line). The arrows in charts b and c point to TECO sampling site. (E) Negative relationship between average values of $\delta^{13}$C in whiptail tissues and the proportion of females in the ten sampled localities. Note that robust regression (continuous line) has even different sign (i.e. negative) than that estimated by standard least-squares regression (i.e. positive; dotted line).

doi:10.1371/journal.pone.0126814.g003
sampling unit \(n = 180\), there was a significant positive linear relationship between \(\delta^{13}C\) and \(\delta^{15}N\) \(\left(F_R = 5.47, \text{d.f.} = 1, P < 0.05; \text{Fig 3D}\right)\).

To evaluate a potential spatial autocorrelation in our data, we calculated Moran’s I at a range of spatial scales for the residuals of the three significant relationships concerning mean population values (i.e. \(\delta^{15}N\) vs. rainfall, \(\delta^{15}N\) vs. altitude, and \(\delta^{13}C\) vs. rainfall; Fig 3). In the three cases, observed Moran’s I values were always within the simulation envelopes (S1 Fig), indicating a lack of spatial autocorrelation in the residuals.

**Discussion**

**Use of tail tip clips as samples**

Tail tip clips from ectotherm vertebrates are frequently used as non-lethal sources of material for genetic (e.g. [49]), toxicological (e.g. [50]) and isotopic (e.g. [37,51]) studies. However, it must be considered that, besides diet, a large number of physiological, ecological and methodological factors generate variation in the isotopic signature of animal tissues [52, 25]. In particular, replacement rates of nutrient pools in each particular animal tissue do vary [53–54]. We have sampled the distal 1 cm of tail tip, which is made mainly by scales, skin, and bone, presumably tissues with low turnover; in addition, carbon incorporation rates in ectotherms are slower than those in endothermic vertebrates [55]. All this should minimize the effect of potential short-term variations in diet in our study (e.g. [24]). Lack of differences between pre- and post-summer whiptail samples in both \(\delta^{13}C\) \((P = 0.378)\) and \(\delta^{15}N\) \((P = 0.083)\) values supports this idea. However, some uncertainty regarding exact whiptail tail tip turnover time does exists, although it should not change our conclusions. Besides, in aquatic snakes it has been proved that scale clips and tail clips of the same individual do not differ substantially in isotopic composition ([56]). We assume tail tip stable isotope composition integrates the diet of whiptails along a time-span of several months.

**The wide isotopic niche of Orange-throated whiptail**

For all-data pooled, the values of metrics related to isotope niche width, such as TA, SEA\(_B\), CRy NR, indicate a large niche area for the species. Practically the whole range of isotope variation in plants of the area was encompassed by CR and NR at species level, confirming Orange-throated whiptails are able to cope with a large variety of resources. However, as we expected, this large niche at the level of species seems to be due to the sum of smaller niches at population and subpopulation levels [7, 14].

Hutchinson [2] suggested the niche of a species has sceno-poetic and bionomic dimensions. The sceno-poetic axes refer mainly to the bioclimatic stage in which a species performs (i.e. where it lives), whereas the bionomic axes refer to the resources it uses (i.e. how it behaves). The distinction is particularly relevant in the analysis of isotopic niches, as isotopic signatures of resources otherwise similar change geographically [6]. Our results strongly suggest that the broad isotopic niche of *Aspidoscelis hyperythra* is mainly due to its sceno-poetic dimensions, i.e. to changes related to the large distribution range of the species. To begin, we have discarded a possible influence of most of non-geographical factors, such as season and whiptail size, age and length (although our results indicate that the specific sex composition of samples could bias results and thus caution is needed in interpreting trends from samples with different gender proportions).

Geographical variation in predator or competitor pressure could generate geographic changes into the realized isotopic niche of a species, as proved by [57]. However, the spectrum of predators and competitors of *Aspidoscelis hyperythra* must be rather similar in all our localities. Hence, we suspect the variation we found was most related to geographical changes in
plant communities. Reinforcing this idea is the fact that the position of the population ellipses in the isotopic δ space can be related in a predictable way to geographical and environmental factors affecting plants (see below).

Whiptails are high-level generalist consumers, depredating on phytophagous invertebrates such as termites and on predators such as spiders. Unfortunately, we have not isotope data for arthropods at each locality. Thus, we cannot transform the isotopic niche in a trophic niche (see [6]) by applying the mixing models frequently used in the literature (see revisions in [25, 54]). However, by analyzing the position of population ellipses in the δ space we can infer the relative importance in the trophic web of producers with different isotopic signatures. For instance, according our predictions more arid and drier localities showed enriched average δ13C values, while subtropical localities showed depleted values. These results strongly suggests that in more desert localities (e.g., INO, KM83, IHU, TECO; Table 1, Fig 2) succulent plants and/or C4 grasses contribute most of the carbon circulating in the trophic web, while in more humid localities (e.g., BAR, RIB, MIG; Table 1) carbon would come both from succulents and C3 plants; only in CNAR, the locality receiving more rainfall, most of the carbon in the trophic web seemed to come from C3 scrubs and trees (Table 1, Fig 2).

**Variation in δ13C: the role of succulents in Baja California desert food webs**

Isotopic signatures of C4 and CAM plants are very similar, although on the average CAM plants were enriched in 15N (S1 Table). Would be C4 grasses or CAM succulents the plants supporting high-level consumers in our desert food webs? Perhaps because most previous studies in dry areas took place in grasslands, many authors usually ascribed the nutrients labelled by less negative δ13C values to C4 grasses, neglecting the potential role of succulents (e.g. [58–60, 24]). However, as stated by [61], cacti and agaves can reach high densities and relative biomass in North America arid areas (e.g. until ~56% of seasonal above-ground biomass [62]) and several studies unequivocally showed that heavier carbon used by some desert consumers derived almost exclusively from cacti [61, 63–64]. In our study area, several evidences suggest that most of the nutrients enriched in δ13C had their origin in cacti rather than in C4 grasses. Succulents are dominant at the landscape (the study area includes the region of North America with more Cactaceae [65]), ground among bushes is usually barren, and frequently we saw no grasses in many of our ten sampling localities (S2 Fig). Besides, cacti feed a lot of arthropods [66] and we captured many lizards while foraging around and under dead cacti. In fact, a study in different localities at the centre of our study area showed that whiptails foraged mainly under the tree Jatropha cinerea and the cacti Stenocereus gummosus and Cylindropuntia cholla [67]. Also, the high δ15N values detected in the tissues of most whiptails seemed to be closer to those of succulents than to those of C4 gramineae in the area (S1 Table). Thus, our study suggests, as previously suspected, that the production by succulents is essential for animal communities in the Baja California desert.

**Variation in δ15N: Are there marine subsidies?**

The interpretation of isotopic nitrogen levels in lizard tissues is more complicated, as many factors influence them [68–69, 18]. Variability of δ15N in plants may be high, mainly due to soil nitrogen cycling and plant physiology (see S1 Table). Besides, δ15N increases with the trophic level, and thus animals higher at the food chain tend to have higher δ15N values. Also, body condition affects the levels of isotopic nitrogen, as δ15N increases in starving animals [70] (but see [71]). Finally, at least in endotherms water stress does increase the proportion of δ15N in animal tissues [72, 18].
However, most of these factors influencing $\delta^{15}$N can be considered minor ones when compared with the influence of marine subsidies at the food web. For instance, working with *Uta stansburiana* (another arthropodivorous lizard) at the north of the Gulf of California, it was found that $\delta^{15}$N reached about 28.8‰ in individuals captured on islands subsidized by seabird guano, 17.9‰ in those of coastal areas without birds, and only 13.0‰ in lizards of inland areas [15]. Considering these values and those of the plants at our study area (S1 Table), a clear influence of marine subsidies cannot be recognized in none or our localities. This was expected, because the area of marine influence is rather thin (about 50 m; [15]) and we extended our capture grounds several hundreds meters inland, with the exception of BAJO. However, we have found a significant relation between the average levels of $\delta^{15}$N and the altitude above sea level (Fig 2), suggesting some marine influence would be possible at lower localities (via insects feeding on marine products landed by people, mainly shells and fish remains, or on shore wrack, or receiving dust and aerosols [73]).

Our results in relation to $\delta^{15}$N could be also partially explained by the isotopic composition of plants and the effects of water stress on the nitrogen-isotope ratios in animal tissues. Thus, the negative relationship between $\delta^{15}$N and average rain (Fig 3C) could be expected. Also, the positive weak linear relationship between $\delta^{13}$C and $\delta^{15}$N values across individuals (Fig 3D) had been previously found for different species of endotherms, including humans [74, 18], and across species guilds [75].

**Intrapopulation isotopic variation**

We have discarded an influence of age, size and other individual conditions. However, we have detected a rather high intra-local variation (individual niche [16, 76]) at least in some populations. This was reflected in the values of Total Area of the convex hull (TA), Carbon and Nitrogen ranges (CR and NR), Mean Distance to Centroid (CD) and other metrics (Table 2). We were not able to detect any general trend among localities, but it seems that the populations where more diversity of resources was expected (i.e. those having simultaneously high abundances of C₃ and CAM plants), were those showing larger variation. For example, CNAR is the population more influenced by subtropical C₃ plants and also it is the only locality covering more than 50% of the total CR (Table 2); high CR in CNAR can be explained because most (13 of 19; values not shown) whiptails were very depleted in $\delta^{13}$C, suggesting they relied on C₃ plants derived resources, while 6 individuals enriched in $\delta^{13}$C apparently relied more on C₄-CAM plants derived resources. In the same way, the two localities with higher variation in $\delta^{15}$N (RIB and BAJO, each of them covering more than 50% of the total NR; Table 2) were those placed lowest on sea level, where some influence of marine subsidies would be possible; in RIB, 17 individual whiptails had $\delta^{15}$N values under 13.0‰, but three others surpassed 17.0‰ (values not shown), suggesting this individuals had an atypical diet more influenced by the sea. This intralocality variation was predictable because microhabitat features (e.g. presence of a dead cardon or a C₃ tree, availability of fishermen waste, etc.) can influence severely the isotopic signatures of available arthropods and thus whiptails.

**Concluding notes**

This is the first study concerning the geographic variation in the isotopic composition of body tissues of a desert higher-level-trophic ectotherm. Trophic webs in North American desert ecosystems are rather complex [31], but using stable isotopes as technical tool and an arthropodivorous lizard as model, we showed that in Baja California Sur cacti and agaves (CAM plants), and likely to a lower extent C₄ grasses, have a large importance in the supply of nutrients to the higher levels of the food webs, particularly in the more arid localities. Instead, the contribution
of C₃ shrubs and trees was important in southern localities with more rain and subtropical vegetation. A clear influence of marine subsidies has not been detected in any locality. Our study contributes to identify the origin and pathways through which energy flows in desert ecosystems, revealing overlooked food resources (i.e. cacti) with potential chief effects on the structure and dynamic of animal populations.

Supporting Information

S1 Fig. Spatial autocorrelation (measured as Moran’s I) for the residual of the three significant robust fits. A), δ¹⁵N and rainfall; (B), δ¹⁵N and altitude; (C), δ¹³C and rainfall. (TIF)

S2 Fig. The study area and lizards. (A) Landscape at a subtropical shrubland in the Southern of the study area; (B) Landscape at the dry desert in the North of the study area, with abundance of succulents; (C) a Whiptail capturing an Orthoptera in the South; (D) a whiptail on the Northern desert. All the pictures by M. Delibes. (TIF)

S1 Table. Values of δ¹⁵N and δ¹³C in sampled C3, CAM and C4 plants collected at different localities of the study area in southern Baja California (Mexico). (DOC)

S2 Table. Values of δ¹⁵N and δ¹³C in each individual lizard sampled. The identification label corresponds to each population. (XLS)

Acknowledgments

Capture permits were granted by D.G. Vida Silvestre, Secretaría de Medio Ambiente y Recursos Naturales, México, permit # 11311. We are indebted to Abelino Cota and Dr. Godoy for their invaluable help in fieldwork and to Sofia Conradi for her technical assistance. Dr. Gonzalez-Forero and Dr. Martinez del Río critically read a previous version of the manuscript, suggesting new interesting approaches. Dr. Moreno-Rueda and two anonymous reviewers helped to improve the paper. Mr C. Swann revised the English.

Author Contributions

Conceived and designed the experiments: MD MCB AD. Performed the experiments: MD MCB LS. Analyzed the data: MD MCB JMF AG LS AD. Contributed reagents/materials/analysis tools: AG AD. Wrote the paper: MD MCB JMF AG LS AD.

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