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Use of saguaro fruit by white-winged doves: isotopic evidence of a tight ecological association

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Abstract We report the use of stable isotope and crop content analyses to quantify the use of saguaro (*Carnegiea gigantea*) nectar and fruit by migratory desert white-winged doves (*Zenaida asiatica mearsnii*). Saguaro resources had characteristically ^{13}C-enriched CAM values (δ^{13}C=–12.8±0.7‰ SD VPDB and –13.1±0.5‰ SD VPDB for nectar and fruit, respectively) relative to other food plants used by doves ($\delta^{13}C_{C3}$=–24.9±3.3‰ SD VPDB). The water contained in saguaro nectar and fruit was deuterium enriched (δD=19.6±2.0‰ SD VSMOW and 48.4±1.6‰ SD VSMOW for nectar and fruit, respectively) relative to other water sources (ranging from –41 to –19‰ VSMOW). During the fruiting season, there was a positive correlation between δ^{13}C in dove liver tissues and percent of saguaro in crop contents. A two-point mixing model indicated that during the peak of saguaro fruit use, most of the carbon incorporated in dove tissues was from saguaro. Desert white-winged doves appear to be saguaro specialists. Averaged over the period when doves were resident, saguaro comprised about 60% of the total carbon incorporated into dove tissues. Tissue δ^{13}C and δD of body water showed a significant positive correlation, indicating that doves were using saguaro as a source of both nutrients and water. However, at the peak of saguaro utilization, the doves' body-water δD was more positive (by about 20‰) than saguaro fruit water. We hypothesize that this enrichment is due to fractionated evaporative water losses by doves. Using dove carbon isotope data and a two end-point mixing model we estimate that, on average, doves consume the equivalent of 128 saguaro fruits per season; each fruit contains on average 26.0±14.8 g SD of pulp (wet mass) of which 19.4 g is water. Stable isotopes have been used to produce qualitative re-constructions of animal diets. Our study shows that they can be used to provide quantitative estimates of the flow of nutrients from resources into consumers as well.

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Introduction

To achieve pollination and seed dispersal, plants often provide animals with floral nectar, pollen and fruit pulp (Howe and Westley 1986). An important component of understanding these resource-consumer interactions includes an estimation of resources transferred from plants to the animals that visit their flowers and consume their fruits (Heinrich 1975). Here, we describe the use of stable isotopes to quantify the importance of floral and fruit resources offered by a specific resource, the saguaro cactus (*Carnegiea gigantea*) to an animal consumer, the western white-winged dove (*Zenaida asiatica mearnsii*).

The saguaro is a defining floristic feature of the Sonoran Desert (Shreve 1945, 1951). These massive, long-lived (125- to 175-years) columnar cacti occur throughout much of southwestern Arizona in the United States and western Sonora in Mexico (Turner et al. 1995). Reproductive plants typically produce hundreds of flowers and fruits each season (Schmidt and Buchmann 1986) and long periods of drought appear to have little effect on saguaro reproductive output (Thackery and Leding 1929; Steenbergh and Lowe 1977). Because saguaros bloom and produce fruit during the hottest and driest periods in the Sonoran Desert (May to mid-July, Azmet 1999) and because the saguaro's reproductive output is predictable from year to year, they probably represent a crucial source of energy and water for many desert animals. Indeed, Steenbergh and Lowe (1977) asserted that saguaros are "keystone" elements of the Sonoran Desert flora. Following Hurlbert (1997), we interpret the notion of the "keystoneness" of saguaro as meaning that these plants transfer an important amount of nutrients and water to the animals that use them, and that in the absence of saguaros, species diversity as well as the productivity and abundance of consumers would be diminished (Mills et al. 1993). Surprisingly, the quantitative importance of the saguaro as a water and nutrient resource for the animal community is unknown (Steenbergh and Lowe 1977; Haughey 1986).

In Sonora, México, the vernacular name of the western white-winged dove is "palomas pitayeras" ("dove from the columnar cactus fruit"). This name recognizes their close association with columnar cacti. White-winged doves are the most frequent avian visitor to the saguaro's flowers and fruit (Haughey 1986; Fleming et al. 1996). Their presence in the Sonoran Desert closely corresponds with the period of flowering and fruiting of the saguaro (Shreve and Wiggins 1964). Furthermore, their breeding range broadly overlaps the saguaro's distribution in Arizona and Sonora (Cottam and Trefethen 1968; Turner et al. 1995).

The reliance of animals on different plant species is usually estimated indirectly from pollen loads, faecal samples, analysis of crop contents, or foraging observations (Collins et al. 1990). Increasingly, however, stable isotopes have been used to quantify the importance of different resources to animal consumers (Gannes et al. 1997, 1998). In this investigation, we combine stable isotope-based analysis of white-winged dove tissues, which provides an estimate of the incorporation of saguaro resources into the consumer, with an analysis based on dove crop contents, which provides an independent analysis of saguaro use, as well as a temporal overview of the plants species used by doves. Our study relies on the presence of unique, well-defined, stable carbon and hydrogen isotope values in the nutrients and water of saguaro resources. These characteristics allow the tracking of the incorporation of saguaro nutrients and water into the tissues of doves. A previous study using crop analyses established that saguaro was the only plant resource with the C4/CAM photosynthetic pathway that was extensively (>0.5% of diet) used by white-winged doves in this region (Haughey 1986). Our analyses of crop contents, as a supplement to the stable isotope analyses, provided a unique opportunity to see how isotope methods compared with more traditional methods of diet analysis. We also examine the use of distinctive deuterium values found in the water of saguaro fruit and floral nectar as potential tracers to estimate consumer use of these resources.

Materials and methods

Study site

Research was conducted on lands within the U.S. Air Force Barry M. Goldwater Bombing Range (32°49′N; 112°26′W) in southern Arizona. This 10,797-km^2 area contains large undisturbed tracts of land isolated from agricultural areas. We chose a study site isolated from these areas because they could potentially provide doves with food resources (C4 agricultural crops) that have carbon isotope values similar to those of saguaro (CAM). The nearest agricultural area was approximately 50 km away. Our analyses of dove crop contents indicate that these distant agricultural areas were not used by white-winged doves. The plant community on the study site is characterized as relatively undisturbed upland Sonoran Desert scrub (Turner et al. 1995), and is described by Felger (1998).

Although many species of cacti are present within the study area (e.g. *Echinocereus* spp., *Ferocactus* spp., *Mammillaria* spp. and *Opuntia* spp.), only saguaro was used by white-winged doves (Haughey 1986; B.O. Wolf and C. Martinez del Rio, unpublished work). Floral and fruit resources of other cactus species were either not available during the same period as the saguaro, were not eaten by birds, or produced very small amounts of nectar and fruit compared to the saguaro (B.O. Wolf and C. Martinez del Rio, unpublished data). Saguaro densities typically averaged >10 plants ha^{-1} and ranged from 1 to >60 plants ha^{-1} on the immediate study area (B.O. Wolf and C. Martinez del Rio, unpublished data). To obtain a relative estimate of flower and fruit availability, the proportion of saguaros with flowers or ripe fruit was estimated at every dove-collecting date by counting the number of plants with and without flowers or ripe fruit along a single 0.5-km transect within the study area.

Spring and early summer in this region are characterized by extremely high air temperatures and little rainfall. Precipitation for each year from 1993 to 1997, between 1 May and 15 July averaged 8±3 mm SD and 23±13 mm SD for weather stations immediately to the east (Maricopa) and west (Gila Bend) of the study area. Average daily maximum air temperatures for May, June and July were 34, 38 and 42°C, respectively, with maximum shade air temperatures ranging between 38 and 50°C (Azmet 1999).

Bird collection and analysis of pollen loads and crop contents

We collected white-winged doves every week to 10 days, from 30 April to 9 September 1998. Doves were collected by shooting as authorized under permits issued by the US Fish and Wildlife Service and Arizona Department of Game and Fish. Four to 7 birds were collected between 0700 and 1000 hours MST on each sampling date, for a total of 91 birds. White-winged doves typically feed immediately after leaving the roost site about sunrise and then fly to water (where they were collected) (Cottam and Trefethen 1968). Immediately after collection, birds were placed in plastic bags and transported in ice to the University of Arizona laboratory freezer and held at –20°C. Within 10 days, carcasses were thawed and tissue samples (liver and pectoralis muscle) and crop contents were extracted. Crop contents were freeze-dried to a constant mass. Crop materials were sorted by plant species, and samples were weighed to calculate the percentage contribution of each plant seed to the total crop contents. Where possible, seeds were identified to genus and species by comparison with plant material in the University of Arizona Herbarium.

A qualitative estimate of the saguaro pollen load carried by each dove was obtained by swabbing the bill, crown, cheeks and chin of each bird with a clean strip of transparent adhesive tape. The tape was placed on a glass slide, and saguaro pollen was quantified under a dissecting stereo-microscope at ×40 magnification. The abundance of pollen grains in each sample was ranked using a qualitative score (0, no pollen grains; 1, less than 25; 2, from 25 to 50 pollen grains; 3, from 50 to 100 pollen grains; 4, more than 100 pollen grains). Only pollen from the saguaro was found on white-winged doves.

Sample preparation for isotope analyses

Water for δD analysis was extracted from dove pectoralis tissue by cryogenic vacuum distillation (Ehleringer 1989). Liver tissues (2–4 g) for carbon isotope analysis were freeze-dried to constant mass (Labconco Freezone 4.5 freeze drying system) and lipids were removed by ether extraction; liver samples were then ground into a fine powder.

Saguaro floral nectar was collected for isotope ratio measurements of $\delta^{13}C$ of sugars and δD of water. Flowers were covered with wedding veil material the evening before anthesis, and nectar was collected with Pasteur pipettes the following morning. Water was extracted from nectar samples by cryogenic vacuum distillation, and a sample of nectar solids (primarily sugars, Baker and Baker 1982; Scogin 1985) was obtained for carbon isotopic analyses after freeze-drying.

Because white-winged doves grind and digest the seeds of saguaro (Steenbergh and Lowe 1977), we homogenized and analyzed fruit pulp and seeds together in the proportion that they are ingested by doves. Water for δD analyses was extracted from fruit pulp by cryogenic vacuum distillation and dried samples were ground to a fine powder for carbon isotopic analyses (Ehleringer 1989). We also measured the carbon isotopic composition of seeds from plant species other than saguaro that were most frequently ingested by white-winged doves. Seeds were collected from plants in the field or obtained from dove crops. We measured the $\delta^{13}C$ of seeds from six individual plants or several (four to six) dove crops. Seeds were dried and ground into a fine powder for carbon analyses.

Stable isotope analyses

Powdered plant and animal tissues (ca. 0.15 mg) were loaded into pre-cleaned tin capsules for isotopic analysis. Carbon isotope ratios of plant and animal tissues were measured on a continuous flow isotope ratio mass spectrometer (VG Isotech, Optima) with samples combusted in a Carlo Erba NA 1500 elemental analyzer at the Columbia University Biosphere 2 stable isotope facility. The precision of these analyses was ±0.3‰ SD. Laboratory standards, vacuum oil ($\delta^{13}C$=–27.5‰ VPDB) and ANU sucrose ($\delta^{13}C$= –10.5‰ VPDB, NAST 8542), were included in each run in order to make corrections of raw values obtained from the mass spectrometer.

Hydrogen isotope ratios were measured using a dual inlet isotope ratio mass spectrometer (Delta S, Finnigan MAT, San Jose, Calif.) fitted with a gas preparation (Finnigan HD) auto-sampler device at the stable isotope facility in the Geosciences Department at the University of Arizona. The precision of these analyses was better than ±2.0‰ SD. Standard laboratory waters were calibrated against the international standards GISP and VSMOW and run with samples to provide corrections.

Stable isotope ratios were expressed using standard delta notation (δ) in parts per thousand (‰) as:

$$\delta X=(R_{sample}/R_{standard}-1)\times 1000$$

where R_{sample} and $R_{standard}$ are the molar ratios of $^{13}C/^{12}C$ or $^{2}H/H$ of the sample and reference, respectively. Samples were referenced against international standards, VPDB for carbon, and VSMOW for water.

Estimation of the incorporation of saguaro carbon into dove tissues

We estimated the proportion of a dove's diet that was derived from saguaro by using a two end-point mixing model (Kline et al. 1990; Gannes et al. 1997):

$$\delta^{13}C_{liver}=p(\delta^{13}C_{saguaro})+1\text{-}p)(\delta^{13}C_{C3})+\alpha$$

in which p is the fraction of saguaro in the diet that is incorporated into the focal tissue and α is a fractionation factor (estimated at 0.3‰; Hobson and Clark 1992b). The fractionation factor is defined as the difference in isotopic composition between tissues and diet when animals are feeding on a pure diet (DeNiro and Epstein 1978; Hobson and Clark 1992b; Cerling and Harris 1999). The carbon isotope ratio of saguaro fruit used in these calculations was $\delta^{13}C_{saguaro}$=–13.1‰ VPDB. The average $\delta^{13}C$ value used for C3 plants was $\delta^{13}C_{C3}$=–24.9‰ VPDB. The carbon isotopic composition of C3 seeds consumed by doves was remarkably constant (coefficient of variation=3.3%, Table 1) and allowed us to use a single C3 value in our two point mixing model.

Table 1 $\delta^{13}C$‰ VPDB of saguaro and C3 plant resources (seeds or fruit) utilized by white-winged doves with sampling dates. Means±SE and sample sizes (n) are presented. With the exception of saguaro and *Lycium berlandieri*, in which fruit pulp and seeds were analyzed together, the isotopic composition data presented are for seeds

Plant species	$\delta^{13}C$‰ VPDB
Carnegiea gigantea	
Floral nectar:	
5/24/1998	–13.0±0.3 (8)
6/11/1998	–12.9±0.2 (11)
6/29/1998	–12.3±0.1 (9)
Fruit pulp:	
6/20/1998	–12.9±0.1 (10)
7/20/1998	–13.0±0.2 (9)
8/03/1998	–13.4±0.2 (10)
Acacia constricta	–25.2±0.4 (6)
Amsinckia tesselata	–25.8±0.3 (6)
Eschscholtzia minutiflora	–25.9±0.3 (4)
Fouquieria splendens	–23.9±0.4 (6)
Jatropha cardiophylla	–24.5±0.8 (4)
Lycium berlandieri (pulp and seeds)	–24.3±0.2 (6)
Ditaxis neomexicana	–24.6±0.2 (7)
Lupinus concinnus	–24.7±0.3 (4)

Two factors critically affect the ability of our analyses to predict saguaro utilization by doves through time. One is an understanding of how stable carbon isotope ratios of diet change or "fractionate" as they are incorporated into consumer tissues (DeNiro and Epstein 1978; Tieszen et al. 1983; Hobson and Clark 1992b). Fractionation factors may vary among species with different dietary habits, as well as among different tissue types within a single individual. In addition, different tissue types within a single individual vary in their carbon turnover rates, thus affecting the period over which each tissue integrates the dietary history of the animal. In this study, we chose to analyze liver tissue because previous work on birds (Hobson and Clark 1992b) suggests that fractionation in this tissue is minimal and because it provides a period of integration that is relatively short. In another granivorous bird, the Japanese quail, Hobson and Clark (1992b) found that diet to tissue fractionation was small (ca. 0.3‰) and liver carbon had a half-life of approximately 2.5 days (Hobson and Clark 1992a).

Results

Availability of flowers and fruit

Saguaro flowers were present at our plant census site from mid-April to the end of June; saguaro flower production peaked at the end of May (Fig. 1). Ripe fruit was available from the middle of June to the beginning of August, but saguaro fruit availability was maximal in July. Because our census described the availability of flowers and fruits at a very local scale, it probably does not reflect the availability of flowers and fruits for white-winged doves accurately. These birds are extremely strong fliers and on the broad scale that they sample (i.e., within 15 km of the census point; Cottam and Trefethen

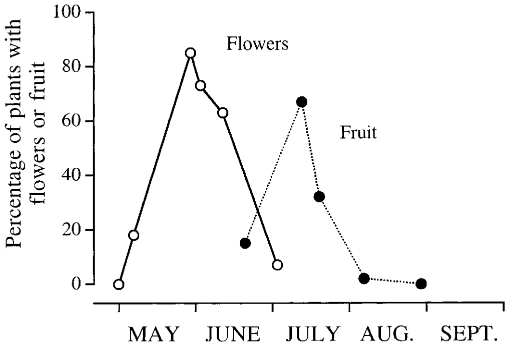

Fig. 1 Phenology of saguaro flower and fruit production along a 0.5-km transect on the Barry Goldwater Air Force Range. Data are based on approximately 100 cacti

1968), saguaro flowers could be found throughout the months of May and June. Similarly, saguaro fruits were observed on a broad scale from June until the beginning of September.

Use of saguaro resources as revealed by pollen loads and crop contents

Pollen loads indicated that birds visited saguaro flowers intensely from the beginning of May until the 1st week of June. Indeed, all birds collected in May and the 1st week of June had saguaro pollen on their bills and head feathers. Pollen loads indicated that some individuals continued to visit saguaro flowers throughout the summer even when floral abundance was low (Fig. 2a). Saguaro fruit was first found in dove crops on 11 June and was still present on 9 September in the last birds collected, even though we were rarely able to find saguaro fruit by searching visually over a very large area. Saguaro fruit accounted for 70±42% SD (n=27), 91±15% SD (n=17), 64±34% SD (n=16) and 6±14% SD (n=5) of crop contents by mass during June, July, August, and the 1st week of September, respectively. Saguaro fruit accounted for 55±44% SD (n=81) of dove crop contents, averaged over the entire season. Seeds or fruit from eight species of plants with the C3 photosynthetic pathway made up the balance of the white-winged dove's diet. Saguaro and these eight plant species accounted for 91% of the dove's total mass of food recovered in crop contents (Table 1).

Use of saguaro resources as revealed by stable isotope analyses

The carbon isotope values of saguaro floral nectar and fruit pulp were temporally stable and distinct from other plant resources used by white-winged doves (Table 1). Except for saguaro, the seeds and fruit found in the crops of white-winged doves were from plants species with C3 photosynthesis (37 species). The average δ^{13}C of C3

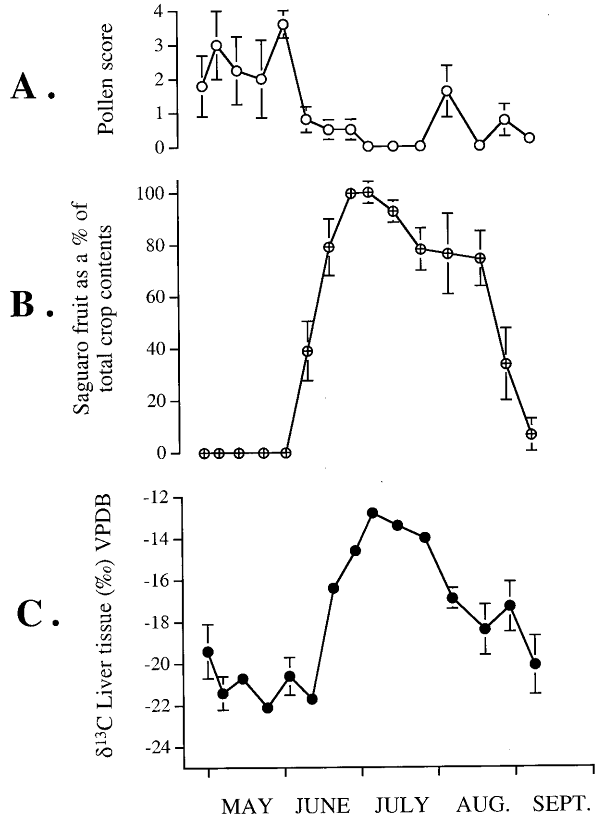

Fig. 2 Use of saguaro flowers and fruit by white-winged doves as estimated by: **A** pollen load scores, **B** proportion of crop contents by dry mass that was composed of saguaro fruit, and **C** δ^{13}C of liver tissue. *Points* are means and *error bars* are standard errors

plants (n=8) making up the majority of non-saguaro items in the dove's diet was –24.9±0.3‰ VPDB.

From the end of April to the middle of June, the average δ^{13}C of liver tissue in white-winged doves was relatively constant, but individual values varied widely and ranged from –15.3 to –23.8‰ VPDB. The onset of saguaro fruit availability in mid-June was accompanied by a rapid increase in the δ^{13}C of dove liver tissues, which remained high (>50% incorporation) to the end of August (Fig. 2a). Because the percentage of saguaro in crop contents and the δ^{13}C of liver tissue were positively correlated (r=0.75, P<0.001, n=81), the carbon incorporation curve roughly tracked that of the percentage of saguaro in crop contents (Fig. 2b). The carbon isotopic values of dove liver tissues provided a similar picture of saguaro utilization to that delineated by crop contents. During June, after the onset of saguaro fruit availability, stable isotope analyses revealed that 84±11% SD (n=18) of the carbon in dove livers was derived from saguaro fruit. During July, August, and September, stable isotope measurements indicated that saguaro comprised 92±24% SD (n=17), 64±21% SD (n=18) and 42±28% SD (n=6) of white-winged doves' incorporated carbon. Overall, from the onset of fruit availability in mid-June through to August, saguaro fruit accounted for 80±22.4% SD (n=53) of incorporated carbon.

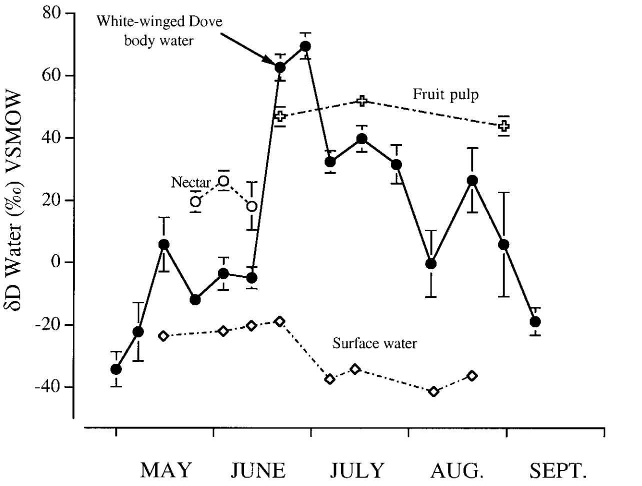

Fig. 3 Temporal changes in the hydrogen isotopic composition of the body water of white-winged doves (*filled circles*). The δD of water from an Arizona Game and Fish Department water catchment (*diamonds*), and of the water contained in saguaro nectar (*unfilled circles*) and fruit pulp (*crosses*) are also presented for reference

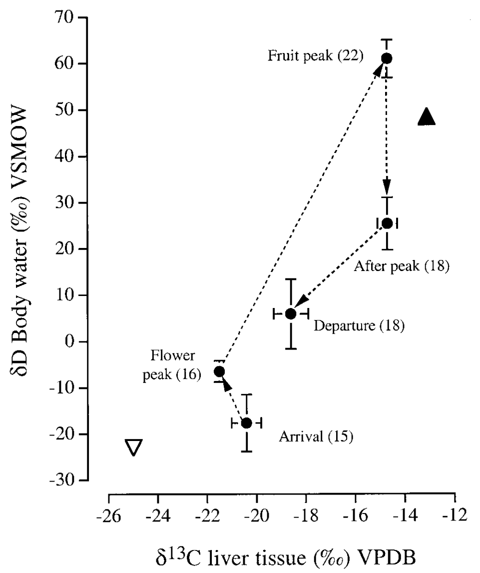

Fig. 4 Temporal trajectory of the carbon and hydrogen isotopic composition of tissues and body water of white-winged doves. Intervals were chosen to summarize significant events in the period during which doves stay in the Sonoran Desert. Birds arrive at the end of April and the beginning of May and depart at the beginning of September. For reference, the average carbon and hydrogen isotopic composition of C3 food plants and catchment water (*unfilled triangle at left corner*) and of saguaro fruit (*filled triangle in upper right corner*) are shown. *Points* are means±SE. Sample sizes are shown in *parentheses*

The water contained in saguaro floral nectar and fruit pulp had distinctive hydrogen isotope ratios relative to that of available surface water. Between 8 May and 29 June, the δD of floral nectar averaged 19.6±2.0‰ VSMOW (n=36) and that of saguaro fruit δD averaged 48.4±1.6‰ VSMOW (n=82, Fig. 3). The isotopic composition of the water in saguaro nectar and fruit pulp did not vary significantly with time (ANOVA, nectar, $F_{1,22}$=3.52, P=0.07, n=23; fruit, $F_{2,75}$=1.02, P=0.37, n=77). In contrast, evaporative enrichment (Gonfiantini 1986) resulted in an increase in deuterium isotopic composition of water contained in the Arizona Game and Fish water catchment used by doves; water contained in this catchment averaged –29.8±2.6‰ VSMOW (n=10, range –41 to –19 from 4/30/1998–8/20/1998). Monsoon rains, starting in July, periodically refilled catchments with water that was depleted in deuterium compared to catchment water (Fig. 3). Although available surface water showed some variation over time, the water provided by saguaro nectar and fruit was very distinct from this water resource.

The δD of white-winged dove body water appeared to track saguaro use (Fig. 3). Birds collected in early May had deuterium values that were close to those of water catchments. δD in dove body water was significantly higher than catchment water throughout the period when saguaro flowers were available (t>3.4, P<0.05, for the three periods in which we had concurrent catchment and body-water measurements). A sharp increase in body-water δD coincided with the increase in fruit consumption that occurred with the onset of fruit ripening starting in the middle of June. During the period of fruit availability, there was a tight correlation between body-water δD and the $\delta^{13}C$ of liver tissue (r=0.72, P<0.001, n=91, Fig. 4) and the percent of saguaro in crop contents (r=0.75, P<0.001, n=81, Fig. 2).

Discussion

Both crop contents and $\delta^{13}C$ measurements of liver tissue revealed a very strong reliance of white-winged doves on saguaro fruit. Pollen-load analyses indicated saguaro flower visitation by virtually all doves and crop-content data revealed that saguaro fruit was the most abundant component in the doves' diet. Complementing these data, the temporal variation in carbon stable isotope ratios of tissues demonstrated that saguaro fruit was not only ingested, but also that its nutrients were extensively incorporated into white-winged dove tissues. Indeed, at the peak of fruit availability, the isotopic composition of dove livers was almost indistinguishable from that of saguaro fruit. White-winged doves breeding in the Sonoran Desert appear to be ecological saguaro specialists (sensu Sherry 1990). Saguaro fruit consumption led, not only to a ^{13}C enrichment of dove tissues, but also resulted in a significant deuterium enrichment of their body-water pool. The correlation between liver $\delta^{13}C$ and body-water δD provided evidence of the importance of saguaro, not only as a source of nutrients and energy but also as a source of water.

Figure 4 summarizes in schematic form the temporal variation in the carbon and hydrogen isotopic composition of the liver and body water of white-winged doves. For clarity, we divided the time during which doves are resident in the Sonoran Desert into five periods. These periods correspond to more or less distinctive biological events. This figure highlights our most significant findings and it also points to some perplexing features of our study. In the first part of our discussion, we examine the temporal variation in the carbon isotopic composition of liver tissues and its covariation with the hydrogen composition of body water (Fig. 4). Next, we discuss the most enigmatic finding from this study: that during the peak period of saguaro fruit use, the body-water pool of white-winged doves was enriched in deuterium above source water, the saguaro fruit (Figs. 3, 4). We examine this apparent anomaly, point out the current limitations of using hydrogen isotope ratios to trace water sources in animals, and outline how these limitations may be overcome. In a final section, we use liver carbon isotope data and crop content data to estimate the amount of saguaro fruit ingested by white-winged doves during their stay in the Sonoran Desert.

Temporal trends in carbon isotopic composition

Pollen-load data indicated that nearly all doves fed at saguaro flowers. However, concurrent liver carbon and body-water deuterium isotope data do not allow an isotopically based assessment of saguaro nectar use by white-winged doves. Later in the season, when fruit became available, the incorporation of saguaro carbon into dove tissues is unquestionable. These differences in incorporation of saguaro carbon into dove tissues between the periods of nectar and fruit use has several potential (and non-exclusive) interpretations: (1) the consumption of nectar by doves may have been insufficient for the nectar value to be detected in liver tissues; (2) although doves consumed significant quantities of floral nectar, they routed the carbon of nectar constituents into metabolism and not into tissue synthesis (Gannes et al. 1998); or (3) doves arrived with a C4/CAM carbon signal from the wintering grounds, and during the flowering period this signal was replaced by that of a mixture of saguaro nectar and C3 plants.

Saguaro nectar and fruit have very different nutritional compositions. The nutrients in nectar are primarily sugars (glucose, fructose, and glucose; Scogin 1985), whereas saguaro fruit pulp contains significant quantities of protein and lipid, in addition to sugars (Greenhouse 1979). White-winged doves grind and digest saguaro seeds and thus obtain the nutrients contained in their endosperm and embryos (Steenbergh and Lowe 1977). If doves allocate the carbohydrates in saguaro nectar into metabolism or energy storage (lipid synthesis), the incorporation of saguaro carbon into liver measured during the early part of the season probably underestimates the importance of saguaro nectar in their diet and in their energy balance. In contrast, doves acquire protein (a source of amino acids for tissue synthesis) in addition to sugars and lipid (sources of energy) from saguaro fruit (Greenhouse 1979). The carbon in saguaro fruit is more likely to become incorporated into liver tissues than the carbon in nectar because dietary protein is likely to be routed into tissue protein (Schwarcz 1991; Tieszen and Fagre 1993), whereas sugars are more likely to be catabolized and/or deposited as lipid (Schwarcz 1991; Ambrose and Norr 1993). To obtain a more accurate picture of the importance of saguaro nectar to the energy balance of doves, we will need isotopic measurements of exhaled CO_2 and/or other body components (e.g., lipid stores; Ambrose and Norr 1993). As a consequence of the potential for isotopic routing, we only estimate saguaro use from our two end-point mixing model when doves were feeding on saguaro fruit.

Temporal trends in hydrogen isotopic composition

The correlation between liver $\delta^{13}C$ and body-water δD suggested that doves incorporated water and nutrients from saguaro into their bodies simultaneously. The incorporation of the carbon isotopic value of diet into liver tissues probably occurs without a large isotopic fractionation (Hobson and Clark 1992b). Thus, we used a two end-point mixing model to estimate the contribution of saguaro to the carbon budget of doves. In contrast, body water appears to show significant fractionation. For example, in late June and early July, the δD in the doves' body water was 15–20‰ higher than the δD of water in saguaro fruit (Figs. 3, 4). This phenomenon was also observed during May when the body-water pool of doves was enriched in deuterium about 20‰ above the value of the available surface water resource. Similar deuterium enrichments of body water over source water have been reported in humans (Luz et al. 1984; Schoeller et al. 1986).

Schoeller et al. (1986) demonstrated that the degree of body-water deuterium enrichment increases with the rate of fractionated evaporative water losses (see also Gonfiantini 1986). Similarly, the body water (and hence the enamel hydroxyapatite) of aquatic mammals and mammals that are frequent drinkers appears to be depleted in ^{18}O compared to that of drought-adapted animals that rely more on metabolic water or endure extensive periods of heat stress (Bocherens et al. 1996; Kohn 1996). A significant fraction of this difference is the result of ^{18}O fractionation in transcutaneous and breath water vapor (Schoeller et al. 1986). Considering the high evaporative water losses experienced by birds living in hot deserts, our finding of substantial deuterium enrichment in the body water of white-winged doves is not surprising.

Our data provided a clear finding: they indicated that water from saguaro fruit was incorporated into the body water of doves (Figs. 3, 4). However, the large and possibly variable fractionations observed with deuterium prevented a quantitative estimate of the contribution of saguaro nectar and fruit to the water balance of white-winged doves. The dependence of deuterium fractionation on the relative proportion of total water losses that are evaporative, and thus fractionated, poses a significant challenge to the interpretation of the relative contribution of different source waters to an animal's body water. Fortunately, because the mechanisms of deuterium enrichment are understood, this challenge can be met. Field isotopic deuterium fractionations can be predicted from laboratory measurements of isotopic enrichment, as a function of evaporative water losses relative to total water flux under varying thermal regimes. These measurements can be combined with field measurements of water flux and estimates of evaporative water loss under natural conditions (Kohn 1996). In plant ecology, research directed at understanding the deuterium composition in the water contained in xylem, branches, and leaves has played an important role in clarifying water sources and water dynamics in plants (Dawson 1993). Disentangling the determinants of deuterium composition of body water can lead to a better understanding of the water economy of animals as well.

How much saguaro fruit do white-winged doves eat?

Our data unambiguously demonstrate that saguaro fruit is the single most important item in the diet of desert-dwelling white-winged doves. The purpose of this section is to provide a preliminary quantitative estimate of the amount of fruit consumed by a dove during its stay in the Sonoran Desert. Fruit allows separate estimation of resource use through crop contents and carbon isotope incorporation data. We emphasize that our estimates are preliminary and based on a series of informed assumptions (see below). However, we believe that this exercise is useful because it allows us to identify the measurements needed to make a better estimate of saguaro use by doves.

The amount of energy provided by saguaro to a dove during a season lasting from day t_0 to day t_f equals:

$$\int_{t_0}^{tf} (F_s(t)E(t))dt$$

where $F_s(t)$ is the fraction of the dove's field metabolic rate [$E(t)$] contributed by saguaro. We estimated the field metabolic rate of a 143-g desert bird such as a white-winged dove as 133 kJ per day (Nagy 1987). If we use the fraction of saguaro carbon in liver as an estimate of $F_s(t)$, and interpolate linearly between weekly values to integrate the estimated amount of energy derived from saguaro from 11 June to 9 September by a dove equals 9333 kJ. To estimate the mass of saguaro fruit consumed, we require two more estimates: (1) the energetic content of saguaro fruit (14.65 kJ/g; Greenhouse 1979), and (2) the metabolizable energy coefficient (MEC*) of saguaro pulp (i.e. the fraction of energy contained in ingested fruit pulp that was retained by doves). Because the relative amount of energy available from seeds and pulp in saguaro fruit is about the same, and because the average MEC* for seeds and pulp is 0.6 and 0.9, respectively (Karasov 1990), we assumed that the MEC* for saguaro fruit ingested by doves was 0.75 [(0.6+0.9)/2]. With these estimates, the dry mass of saguaro fruit ingested by a dove can be estimated as 849 g ($0.75^{-1}\times14.65^{-1}\times9333$). An average ripe saguaro fruit has a wet pulp mass of 26.0±14.8 g SD (range 8.0–88.6, n=79) and a dry pulp mass of 6.6±3.6 g SD (range 1.9–21.3, n=79). Thus, we estimate that an average dove ingested 3337 g of fruit pulp and seeds, which is equivalent to 128 fruits. Using the fraction of saguaro fruit in crop contents as an estimate of $F_s(t)$ produces a very similar value for seasonal fruit consumption (122 fruits per season). With estimates of the population density of doves, these values can be used to calculate the flow of fruit from saguaro into dove populations. The appropriate equation needed to estimate the energy flow from saguaro into white-winged dove populations is:

$$\int_{t_0}^{tf} [F_s(t)E(t)N(t)]dt$$

where $N(t)$ equals the population density of doves as a function of time.

Because our estimate of individual saguaro fruit consumption is based on a number of assumptions, its accuracy is uncertain. However, the preliminary calculations described above are useful because they identify the measurements that are required to obtain a sound estimate of the use of saguaro by doves. All these measurements (i.e., field metabolic rates, MEC*, population density) can be obtained with currently available techniques (i.e., doubly labelled water and laboratory feeding trials; Robbins 1993). This study is a initial step towards a community level analysis of the role of the saguaro as a nutrient and water source for desert animals. Stable isotopes have proven to be important tools that allow broad qualitative reconstruction of animal diets (Gannes et al. 1997), and here our analyses have shown that they can closely match estimates of resource use derived using other methods. In conjunction with other techniques, stable isotopes may prove to be pivotal in animal ecology by providing quantitative estimates of the flow of nutrients from resources into consumers.

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References

Ambrose SH, Norr L (1993) Carbon isotope evidence for routing of dietary protein to bone collagen, and whole diet to bone apatite carbonate: purified diet growth experiments. In: Lambert J, Grupe G (eds) Molecular archaeology of prehistoric human bone. Springer, Berlin Heidelberg New York, pp 1–37

Azmet (1999) (Arizona meteorological online archive and database) http://ag.arizona.edu/azmet

Baker G, Baker I (1982) Chemical constituents of nectar in relation to pollination mechanisms and phylogeny. In: Nitecki H (ed) Biochemical aspects of evolutionary biology. University of Chicago Press, Chicago, pp 131–171

Bocherens H, Koch PL, Mariotti A, Geraads D, Jaeger J (1996) Isotopic biogeochemistry (^{13}C, ^{18}O) of mammalian enamel from african pleistocene hominid sites. Palaios 11:306–318

Cerling TE, Harris JM (1999) Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. Oecologia 120:347–363

Collins BG, Grey GJ, McNee S (1990) Foraging and nectar use in nectarivorous bird communities. In: Morrison ML, Ralph CJ, Verner J, Jehl JR (eds) Avian foraging: theory, methodology, and applications, vol 13. Allen Press, Lawrence, pp 110–122

Cottam C, Trefethen JB (1968) Whitewings; the life history, status, and management of the white-winged dove. Van Nostrand, Princeton

Dawson TE (1993) Water sources of plants as determined from xylem-water isotopic composition: perspectives on plant competition, distribution and water relations. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant carbon-water relations. Academic Press, San Diego, pp 465–496

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506

Ehleringer JR (1989) Carbon isotope ratios and physiological processes in aridland plants. In: Rundel PW, Ehleringer JR, Nagy KA (eds) Stable isotopes in ecological research. Springer, Berlin Heidelberg New York, pp 41–54

Felger RS (1998) Checklist of plants of Cabeza Prieta National Wildlife Refuge Arizona. Drylands Institute, Tucson

Fleming TH, Tuttle MD, Horner MA (1996) Pollination biology and the relative importance of nocturnal and diurnal pollinators in three species of Sonoran Desert columnar cacti. Southwest Nat 41:257–269

Gannes LZ, O'Brien DM, Martinez del Rio C (1997) Stable isotopes in animal ecology: assumptions, caveats and a call for more laboratory experiments. Ecology 78:1271–1276

Gannes LZ, Martinez del Rio C, Koch P (1998) Natural abundance variations in stable isotopes and their uses in animal physiological ecology. Comp Biochem Physiol 119A:725–737

Gonfiantini R (1986) Environmental isotopes in lake studies. In: Fritz P, Fontes J-C (eds) Handbook of environmental isotope geochemistry, vol 2. The terrestrial environment. Elsevier, Amsterdam, pp 113–168

Greenhouse R (1979) The iron and calcium content of some traditional Pima foods and the effects of preparation methods. MSc Thesis, Arizona State University

Haughey RA (1986) Diet of desert-nesting western white-winged doves, *Zenaida Asiatica mearnsii*. MSc Thesis, Arizona State University

Heinrich B (1975) Energetics of pollination. Annu Rev Ecol Syst 6:139–169

Hobson KA, Clark RG (1992a) Assessing avian diets using stable isotopes. I. Turnover of ^{13}C in tissues. Condor 94:181–188

Hobson KA, Clark RG (1992b) Assessing avian diets using stable isotopes. II. Factors influencing diet-tissue fractionation. Condor 94:189–197

Howe HF, Westley LC (1986) Ecology of pollination and seed dispersal. In: Crawley MJ (ed) Plant ecology. Blackwell, London, pp 185–215

Hurlbert S (1997) Functional importance vs keystoneness: reformulating some questions in theoretical biocenology. Aust J Ecol 22:369–382

Karasov WH (1990) Digestion in birds: chemical and physiological determinants and ecological implications. In: Morrison ML, Ralph CJ, Verner J, Jehl JR (eds) Avian foraging: theory, methodology, and applications, vol 13. Allen Press, Lawrence, pp 391–415

Kline TC, Goering JJ, Mathisen OA, Poe PH, Parker PL (1990) Recycling of elements transported upstream by runs of Pacific Salmon. I. ^{15}N and ^{13}C evidence in Sashin Creek, Southeastern Alaska. Can J Fish Aquat Sci 47:136–144

Kohn MJ (1996) Predicting animal $\delta^{18}O$: accounting for diet and physiological adaptation. Geochim Cosmochim Acta 60: 4811–4829

Luz B, Kolodny Y, Horowitz M (1984) Fractionation of oxygen isotopes between mammalian bone-phosphate and environmental drinking water. Geochim Cosmochim Acta 48: 1689–1693

Mills LS, Soule' ME, Doak DF (1993) The keystone-species concept in ecology and conservation. Bioscience 43:219–224

Nagy KA (1987) Field metabolic rate and food requirement scaling in mammals and birds. Ecol Monogr 57:111–128

Robbins CT (1993) Wildlife feeding and nutrition. Academic Press, San Diego

Schmidt, JO, Buchmann SL (1986) Floral biology of the saguaro, *Cereus giganteus*. Oecologia 69:491–498

Schoeller DA, Leitch CA, Brown C (1986) Doubly labelled water method: in vivo oxygen and hydrogen isotope fractionation. Am J Physiol 251:R1137–R1143

Schwarcz HP (1991) Some theoretical aspects of isotope paleodiet studies. J Archaeol Sci 18:261–275

Scogin R (1985) Nectar constituents of the Cactaceae. Southwest Nat 30:77–82

Sherry TW (1990) When are birds dietarily specialized? Distinguishing ecological from evolutionary approaches. In: Morrison ML, Ralph CJ, Verner J, Jehl JR (eds) Avian foraging: theory, methodology, and applications, vol 13. Allen Press, Lawrence, pp 337–352

Shreve F (1945) The saguaro, cactus camel of Arizona. Natl Geogr 88:69–704

Shreve F (1951) Vegetation of the Sonoran Desert. Carnegie Inst Washington Publ 591:192

Shreve F, Wiggins IL (1964) Vegetation and flora of the Sonoran Desert. Stanford University Press, Stanford

Steenbergh WF, Lowe CH (1977) Ecology of the saguaro. II. Reproduction, germination, establishment, growth, and survival of the young plant. US Government Printing Office, Washington, DC

Thackery FA, Leding AR (1929) The giant cactus of arizona: the use of its fruit and other cactus fruits by the indians. J Hered 20:400–414

Tieszen LL, Fagre T (1993) Effect of diet quality and composition on the isotopic composition of respiratory CO_2, bone collagen, bioapatite, and soft tissues. In: Lambert J, Grupe G (eds) Molecular archaeology of prehistoric human bone. Springer, Berlin Heidelberg New York, pp 123–135

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32–37

Turner RM, Bowers JE, Burgess TL (1995) Sonoran desert plants: an ecological atlas. University of Arizona Press, Tucson