The Diet of Monk Parakeet Nestlings (*Myiopsitta monachus*) in an Urban Area: a Study Using Stable Isotopes

D. Mazzoni[1*], J. Pascual[1,2], L. Arroyo[1], T. Montalvo[2], J. González-Solís[3] & J.C. Senar[1]

[1] Evolutionary and Behavioural Ecology Research Unit, Museu de Ciències Naturals de Barcelona, Pº Picasso s/n, Parc Ciutadella, 08003 Barcelona, Spain

[2] Agencia de Salut Publica de Barcelona, Spain, and CIBER de Epidemiología y Salud Pública, Barcelona, Spain

[3] Institut de Recerca de la Biodiversitat (IRBio) and Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 643, Barcelona 08028, Spain.

*daniele.mazzoni.bio@gmail.com

Abstract

Capsule

Previous observations in Barcelona and other cities showed that ca. 40% of the food ingested by adult Monk Parakeets is of anthropogenic origin, here we show that this type of food source is also used for feeding nestlings.

Aims

To quantify the proportion of anthropogenic food within the nestlings' diet.

Methods

We analysed stable isotopes of ^{13}C and ^{15}N in feathers of Monk Parakeet nestlings collected in the Barcelona city area. We also sampled potential food sources ingested by Monk Parakeets to reconstruct the nestling diet using Bayesian mixing models with MixSIAR.

Results

Almost 30% of the nestlings' diet was composed of anthropogenic food.

Conclusions

Since food availability is a major factor regulating population growth, we propose educating the general public to reduce the food supply for the species and ultimately limit its population growth.

Key Words: Monk Parakeet, stable isotopes, diet, nestlings, invasive species.

Introduction

Invasive alien species introduced by humans, deliberately or accidentally, outside of their natural geographic range, is a major concern threatening biodiversity. Birds are among the most successful invasive species (Blackburn *et al.* 2009), and among them, parrots (Psittaciformes) have been particularly successful in establishing new populations after accidental escapes or deliberate releases (Cassey *et al.* 2004). The Monk Parakeet (*Myiopsitta monachus*) is one of the most successful parrot invasive species worldwide (Strubbe & Matthysen 2009; Postigo *et al.* 2017). It was widely introduced in Europe and its population has increased, reaching more than 20.000 Monk Parakeets in the wild across 179 municipalities in eight EU countries (Postigo *et al.* 2019). The first record of free-living Monk Parakeets breeding in Spain dates back to 1975 in the city of Barcelona (Batllori & Nos 1985) and in 2015 its population reached 5,000 individuals (Senar *et al.* 2017a).

The Monk Parakeet is causing agricultural losses, injuring ornamental trees by picking off small branches for nest-building, damaging electric lines and other human-made structures during nesting, producing noise pollution (Bucher; E. H. Bucher *et al.* 1991; Aramburú & Bucher 1999; Conroy & Senar 2009; Avery & Shiels 2018). Moreover, Monk Parakeets carry ectoparasites from their native range, increasing the spread of parasites and the risk of transmission to native species (Mori *et al.* 2015).

Monk Parakeets show a generalist diet, which includes a wide range of wild and cultivated seeds, fruits and vegetable matter, including grass seeds and grain, cactus stems, root vegetables, and tree fruits, and sometimes insects and their larvae (Juniper & Parr 1998). In the countries of introduction, the Monk Parakeet also shows a generalist feeding behaviour (Schaetzen & Jacop 1985; Weiserbs & Jacob 1999; South & Pruett-Jones 2000; Avery *et al.* 2012). Furthermore, Monk Parakeets have a propensity for living in urban and suburban areas (Avery & Shiels 2018), where Monk Parakeets heavily use backyard feeders, eating sunflower seeds, bread and any other food provided by humans (Bull 1973; Schaetzen & Jacop 1985; South & Pruett-Jones 2000). Thus, anthropogenic food plays an important role in the diet of the introduced populations of this invasive species, but little is known about its relevance in the diet of nestlings. To date, all of the studies on the diet of nestlings have been carried out in the native range (Aramburu & Corbalán 2000; Pezzoni *et al.* 2009). The first study in a wild population showed that the diet of Monk Parakeet nestlings was entirely based on wild plants and was composed of fruits and seeds (Aramburu & Corbalán

2000). However, in a rural area, while the diet was mostly represented by wild species (89%), 11% of food remains were corn (Pezzoni *et al.* 2009).

Stable isotope analyses (SIA) of ^{13}C and ^{15}N has become a common tool to study feeding ecology in a wide range of animals (Tieszen *et al.* 1983; Hobson & Clark 1992; Becker *et al.* 2007; Bearhop & Inger 2008). As animals 'are what they eat', the isotope ratios in their tissues, indicated by the symbol "δ" meant as the ratio value of ^{13}C in relation to ^{12}C and ^{15}N to ^{14}N, can be used to make inferences about their diets and the type of habitats in which they live (Bearhop & Inger 2008).

Indeed, isotope ratios of the "consumer" tissues constantly change as diet "prey" also change reflecting the diet in which they were formed. The rate of these changes depends on the tissue and its metabolic turnover rate. For istance, plasma gives information of the diet over days, blood and muscle over months and bone collagen over years (Dalerum & Angerbjörn 2005). Since feathers are metabolically inert once grown, the isotope ratio does not change and thus reflects the diet during their formation (Hobson & Clark 1992).

In the SIAs a crucia role has been played by the diet-tissue discrimination factors (DTDF) denoted by "Δ". The DTDF represent the difference in $\delta^{13}C$ (or $\delta^{15}N$) values between the dietary isotope value and the resulting tissue of the consumer ($\Delta^{13}C = \delta^{13}C_{consumer} - \delta^{13}C_{diet}$; $\Delta^{15}N = \delta^{15}N_{consumer} - \delta^{15}N_{diet}$) (Hussey *et al.* 2010).

To reconstruct the assimilated diet, knowing the isotope ratios and DTDFs, we can use stable isotope mixing models (SIMMs) to estimate the proportion of each food source (prey) contributing to a mixture (consumer) based on the stable isotope values of predators and their prey. Recent advances in mixing models using Bayesian statistics, such as MixSIAR, improved estimates based upon simpler linear mixing models by explicitly taking into account uncertainty in source values, categorical and continuous covariates, and prior information (Parnell *et al.* 2010; Stock *et al.* 2018).

The aim of this study was to quantify the diet composition of Monk Parakeet nestlings using novel diet reconstruction techniques (MixSIAR) in a Bayesian framework. Given that food availability is one of the major factors regulating population growth (Newton & Brockie 2003), understanding the importance of anthropogenic food, is an interesting data to inform whether or not it is appropriate to carry out public campaigns for reducing the quantity of food available to Monk Parakeets, and possibly reduce the population growth of this invasive species.

Materials & Methods

Study area

The study was conducted in Barcelona, Spain, where the population of the Monk Parakeet reached 5.000 individuals in 2015 (Senar *et al.* 2017a). We collected feather samples from a total of 86 Monk Parakeet nestlings from 30 trees at 28 colonies located throughout Barcelona (see figure 1 in Mori et al. (2019)). We selected at random a subsample of available colonies spread apart, so that they were representative for the distribution of the MP in Barcelona.

Sampling protocols

We used feathers to perform SIAs since this tissue provides data spanning the period over which they were grown (Bearhop & Inger 2008). All the samples were collected from May to July 2016. Breast feather samples were collected from nestlings in the nest. The age of the sampled nestlings ranged from 3 to 5 weeks, a period in which the plumage had already appeared. In cases where there was more than one nest per tree, we collected only one nestling per nest. In cases where a nest had more than one chamber, we only collected one nestling per chamber, to avoid pseudoreplication.

The food samples presumably ingested by the chicks (N = 45) were collected either from the interior of the sampled nests, from the plants that we observed were consumed by the parakeets around the sampled colonies, or from food provided by the citizens that we observed was consumed by parakeets. This sampling lasted for 2 months. All the diet samples were stored in a freezer at -40°C to avoid deterioration.

Food samples collected included flowers of *Catalpa bignonioides, Jacaranda mimosifolia* and *Tipuana tipu*; fruits of *Celtis australis, Cercis siliquastrum, Gleditsia triacanthos* and *Melia azedarach;* herbaceous plants such as Poaceae (grass) and *Trifolium spp.*; leaves of *Melia azedarach* and *Tilia cordata*; and seeds of *Jacaranda mimosifolia* and *Platanus spp*. Some of the sampled plants were allochthonous and were anthropogenically introduced for ornamental purposes. We also collected samples of food of anthropogenic origin, including peanuts (*Arachis hypogaea*), sunflower seeds (*Helianthus annuus*), rice (*Oryza sativa*), and bread (*Triticum spp.*). Three samples of each source were collected and frozen at -40°C until their use in the lab.

Isotope analyses

Feathers were washed in 0.1 M NaOH solution and distilled water to remove particles that could cause contamination and change the isotopic signature. We shredded the vane of the feathers and food using a scissor to obtain a fine powder. As the lipidic component of the diet can affect $\delta^{13}C$ values (Sweeting *et al.* 2006; Tarroux *et al.* 2010; Perkins *et al.* 2013), flowers, fruits, seeds, and anthropogenic food were previously washed of lipids using successive rinses in a 2:1 chloroform:methanol solvent, and were later oven-dried at 50°C for 24h along with leaves and herbaceous plant samples.

We performed the SIAs at the Serveis Científico-Tècnics at the University of Barcelona. Subsamples of 0.3 mg ± 0.03 (weighed using a microbalance Mettler Toledo MX5) of homogenised materials were loaded into tin cups pre-washed on toluene and crimped for combustion. Isotopic ratios of carbon and nitrogen were calculated using an elemental analysis-isotope ratio mass spectrometry (EA-IRMS). The standard values for $\delta^{13}C$ and $\delta^{15}N$ were Vienna Pee Dee Belemnite (VPBD) and atmospheric nitrogen (AIR), respectively. Measurement errors (SD) were ±0.15‰ for $\delta^{13}C$ and ±0.25‰ for $\delta^{15}N$. The laboratory of isotopic ratio mass spectrometry applies international standards, generally run for every 12 samples: IAEA CH7 (86% of C), IAEA CH6 (42.1% of C), IAEA N1, IAEA N2 (21.2% of N), IAEA NO3 (13.9% of N), IAEA 600 (49.5% of C and 28.9% of N), USGS 40 (40.8% of C and 9.5% of N), UREA (20.2% of C and 46.8% of N) and ACETANILIDE (71.1% of C and 10.4% of N).

Data analysis

We used a Bayesian Mixing Model analysis MixSIAR (Stock *et al.* 2018) in R studio 3.4.2 which runs the models through the JAGS software (Plummer 2003), to reconstruct the diet using the isotopic ratios from feathers and prey items. Model reliability was tested with two diagnostics: 1) missing a source and 2) using an incorrect discrimination factor. A violation of these mixing model assumptions would result in the model being rejected. Based on these criteria, the model was found to be reliable. This allowed us to quantify the percentage contribution of each dietary source to the consumer's overall diet. Isotopic values of food sources were corrected by DTDF (diet-tissue discrimination factor): $\Delta^{13}C=3.97\pm0.90‰$ and $\Delta^{15}N=3.67\pm0.74‰$ evaluated for the adults of the Monk Parakeet (Mazzoni *et al.* 2019). The uncertainty of the solutions of a mixing model will depend on the number of tracers and sources included. If the isotopic ratios of the sources are very large, the solutions will have a wide distribution for each source, making the results difficult to interpret (Parnell *et al.* 2010). Alternatively, combining related sources may produce a much narrower solution and lead to easier interpretation (Parnell *et al.* 2010; Phillips *et al.* 2014). For these reasons, we combined food sources a posteriori based on their distribution in the isotopic space (pooling food sources with similar signatures), similarly to Borray-Escalante et al. (2020). We combined the food samples into three groups of natural food sources including flowers and fruits (Flo/Fru), herbaceous plants (Herb.) and leaves and seeds (Lea/See); and two groups of anthropogenic food sources including Bread/Rice/Sunflower seeds (Bre/Rice/Sunf.) and peanuts (Pean.).

Results

Stable isotopic composition of the feathers and potential food sources of Monk Parakeet nestlings are displayed in Table 1. The isospace plot generated from the model showed that flowers and fruits displayed the highest isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$, whereas "herbaceous plants" displayed low isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ and "Peanuts" the lowest (Fig. 1).

The Bayesian mixing models implemented in MixSIAR produced posterior probabilities of the contribution of each food source to the diet of consumers. We represented the median values and the 95% credibility interval of these posterior distributions (Fig. 2). Our results showed that nestlings were largely fed with food provided by humans (bread, rice, sunflower seeds, peanuts) (31%), followed by natural food sources, such as herbaceous plants (31%) and leaves and seeds obtained from arboreal vegetation (25%). Flowers and fruits were found in a low proportion (7%). The isotopic signatures of the nestlings were well included in the resource polygon (Fig. 1), suggesting that we had sampled most of the preferred food items.

Discussion

The use of stable isotope mixing models with a Bayesian approach enabled us to infer the diet of Monk Parakeet nestlings in an urban locality in an invaded area for the first time. The diet provided to nestlings was similar to that consumed normally by adults. According to both visual data and data from isotope analyses, food of anthropic origin can represent in Barcelona up to 40% of the diet of adult birds, followed by herbaceous plants (27%) and leaves and seeds (22%) (Carrillo-Ortiz 2008; Borray-Escalante *et al.* 2019). Therefore, our results suggest that adults do not select food items specifically for nestling provisioning and that, where it is available and utilised, anthropogenic food is not only for adult birds but also forms a relevant part of the nestling diet.

Anthropogenic food sources in South America may also be important and reach an overall value of 50% in the diet of adults (Aramburu 1997; Aramburú & Bucher 1999). This food mostly refers to maize and sunflower seeds and is thus obtained from cultivated areas. This contrasts with invaded urban areas, where anthropogenic food is abundant, but is mostly provided by the local human population (Borray-Escalante et al. 2019). The prominent role of anthropogenic food has been demonstrated for Barcelona but also for other localities in Europe and North America (Bull 1973; Schaetzen & Jacop 1985; South & Pruett-Jones 2000). Indeed, in the northern areas of the United States, Monk Parakeet populations during winter months seem to be completely dependent on anthropogenic foods, such as sunflower seeds (South & Pruett-Jones 2000).

Within their natural range, adult Monk Parakeets spend a lot of time feeding on grass, consuming several plant families, such as Poaceae, Asteraceae, Fabaceae, which may represent up to 50% of the diet (Aramburu 1997). These plants are also provided by the adults to nestlings (Aramburu & Corbalán 2000; Pezzoni *et al.* 2009). In Barcelona, our category “herbaceous plants” also included species of the families Poaceae and Fabaceae (Ferrer 2017), indicating that these plants play an important role in the diet of nestlings even outside of their native range and in urban areas. Here we would like to point out that part of the differences found between areas could potentially be due to the fact that the type of study locations were rural in the native range and urban in our study in non-native range, so that the two factors (nativeness and urbaness) are interlinked in complex ways.

From a population perspective, the direct resource subsidization of urban consumers can lead to population growth, driven by enhanced fecundity and survival (Newsome *et al.* 2015). Generally, birds adjust their clutch size to environmental conditions, showing larger clutches and greater breeding success when food is abundant (Martin 1987; Stock *et al.* 2016). Consequently, the high breeding success of Monk Parakeets in invaded areas (Senar *et al.* 2019) can partly be explained by human food supplementation. The fact that the abundance of Monk Parakeets in the different districts of Barcelona was positively correlated to the abundance of retirees, which is typically the social sector that most often provides food to parakeets (Rodríguez-Pastor *et al.* 2012), supports this view. Nevertheless, this can also be seen as an opportunity. Previous work on Feral Pigeon

Columba livia has shown that public education can decrease food supplementation and is an effective approach in reducing population size (Haag-Wackernagel 1993; Haag-Wackernagel 1995; Senar *et al.* 2017b). Even though these results showed that anthropogenic food has an important role in the nestlings' diet, these cannot be generalized in all the urban areas outside their native range, as our study refers to the urban context of Barcelona and probably this proportion could fluctuate among different urban areas in European countries. In any case, we advocate for public education programs to reduce citizing feeding and hence population growth of Monk Parakeets in urban areas similar to Barcelona. Future work should test the impact of this approach for controlling the parakeet population.

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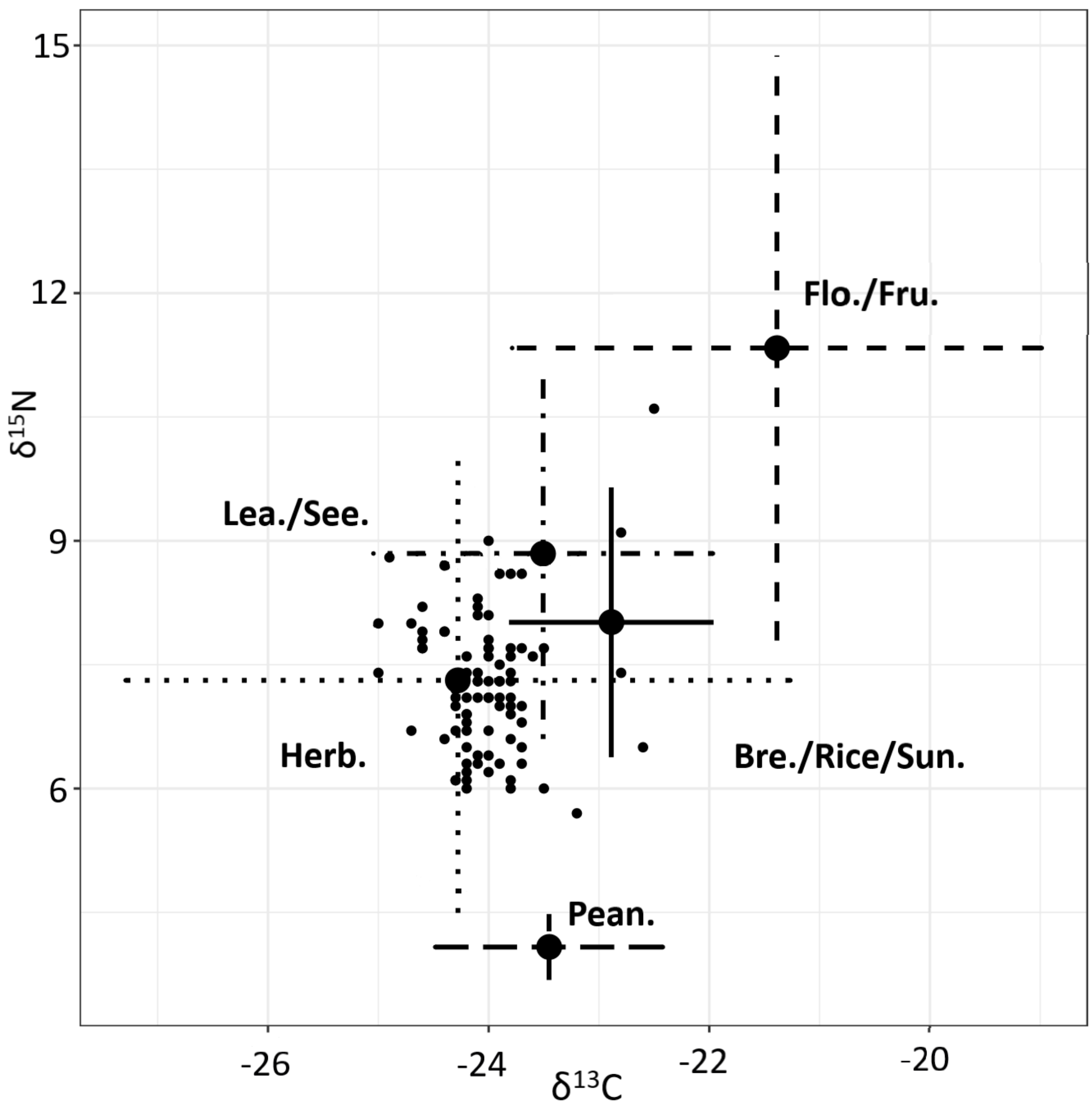

Figure 1. Isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of feathers of Monk parakeet nestlings (*Myiopsitta* monachus) and their main dietary source types. Large circles represent food source mean values, and bars represent SD after DTDF (diet-tissue discrimination factor) was applied for both isotopes. Natural food sources: flowers and fruits (Flo/Fru), herbaceous plants (Herb.) and leaves and seeds (Lea/See). Anthropogenic food sources: Bread/Rice/Sunflower seeds (Bre/Rice/Sunf.) and peanuts (Pean.). Small circles represent individual values of nestlings (N = 86).

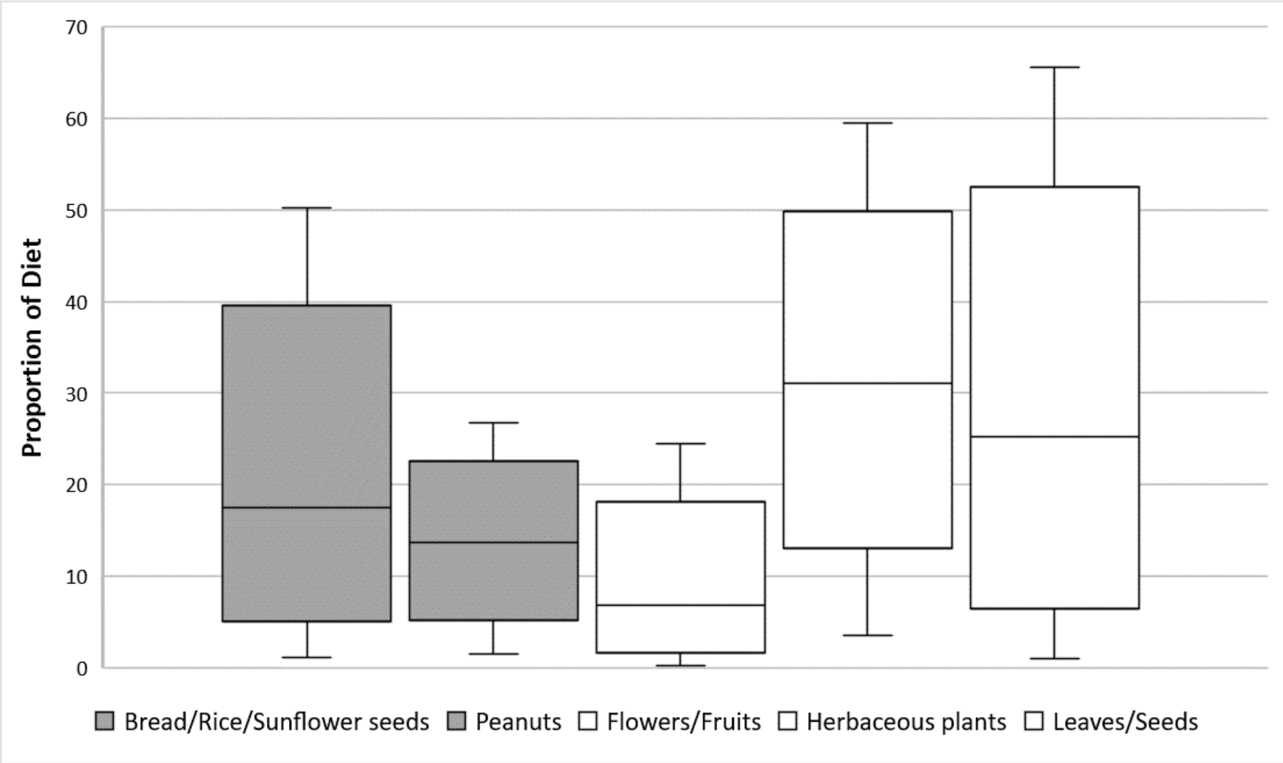

Figure 2. Dietary proportion % estimates for all sampled nestlings of Monk parakeet. The two groups are represented with two different colours: anthropogenic food sources (grey) and natural food sources (white). For each food source group, the central line represents the median value, the box shows the 50% credibility interval and the whiskers represent the 95% credibility interval.

Table 1: Mean isotopic signatures (±S.D.) of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) for nestling monk parakeet feathers and for diet items.

Monk parakeet	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**N**
Nestling feathers	-24.14 ± 0.36	7.1 ± 0.99	86
Food Sources			
Bread / Rice / Sunflower seeds	-26.85 ± 0.91	4.34 ± 1.63	9
Flowers / Fruits	-25.35 ± 2.39	7.66 ± 3.54	18
Herbaceous plants	-28.24 ± 3.01	3.64 ± 2.81	6
Leaves / Seeds	-27.47 ± 1.53	5.17 ± 2.24	9

Peanuts	-27.43 ± 1.01	0.41 ± 0.38	3