

# Diet to tissue discrimination factors for the blood and feathers of the monk parakeet (*Myiopsitta monachus*) and the ring-necked parakeet (*Psittacula krameri*)

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## Abstract

*Diet to tissue discrimination factors for the blood and feathers of the monk parakeet (Myiopsitta monachus) and the ring-necked parakeet (Psittacula krameri).* Stable isotope analyses (SIAs) have been widely used in recent years to infer the diet of many species. This isotopic approach requires using diet to tissue discrimination factors (DTDFs) for each prey type and predator tissue, i.e., to determine the difference between the isotopic composition of the predator tissues and the different prey that conform its diet. Information on DTDF values in Psittaciformes is scarce. The aim of this study was to assess DTDF values for the carbon and nitrogen isotopes of the monk parakeet (*Myiopsitta monachus*) and the ring-necked parakeet (*Psittacula krameri*), two invasive alien species of concern. We fed captive birds of the two parakeet species on a single-species diet based on sunflower seeds to establish the DTDFs for the blood and feathers. In the monk parakeet (N = 9) DTDFs were  $\Delta\delta^{13}\text{C}$  2.14‰ ± 0.90 and  $\Delta\delta^{15}\text{N}$  3.21‰ ± 0.75 for the blood, and  $\Delta\delta^{13}\text{C}$  3.97‰ ± 0.90 and  $\Delta\delta^{15}\text{N}$  3.67‰ ± 0.74 for the feathers. In the ring-necked parakeet (N = 9), the DTDFs were  $\Delta\delta^{13}\text{C}$  (‰) 2.58 ± 0.90 and  $\Delta\delta^{15}\text{N}$  (‰) 2.35 ± 0.78 for the blood, and  $\Delta\delta^{13}\text{C}$  3.64‰ ± 0.98 and  $\Delta\delta^{15}\text{N}$  4.10‰ ± 1.84 for the feathers. DTDF values for the ring-necked parakeet blood were significantly higher than those for the monk parakeet blood. No difference was found between the two species in the DTDF for feathers. Our findings provide the first values of DTDFs for blood and feathers in these parakeets, factors that are key to infer the diet of these species based on SIA.

Key words: Stable isotopes, Parrots, Captive birds, Control diet, Invasive species

## Resumen

*Factores de discriminación entre la dieta y los tejidos para la sangre y las plumas de la cotorra argentina (Myiopsitta monachus) y la cotorra de Kramer (Psittacula krameri).* Los análisis de isótopos estables se han venido utilizando de forma generalizada en los últimos años para inferir la dieta de numerosas especies. El método isotópico consiste en utilizar factores de discriminación entre la dieta y los tejidos para cada tipo de presa y tejido de depredador, es decir, determinar la diferencia entre la composición isotópica de los tejidos del depredador y la de las distintas presas que integran su dieta. Hay muy poca información sobre los valores de los factores de discriminación entre la dieta y los tejidos relativa a los psitaciformes. La finalidad de este estudio fue evaluar los valores de los factores de discriminación de los isótopos de carbono y nitrógeno de la cotorra argentina (*Myiopsitta monachus*) y la cotorra de Kramer (*Psittacula krameri*), que son dos especies exóticas invasivas motivo de preocupación. Alimentamos a aves en cautividad de las dos especies de cotorra con una dieta exclusivamente a base de semillas de girasol para establecer los factores de discriminación relativos a la sangre y las plumas. En la cotorra argentina (N = 9), los factores de discriminación para la sangre fueron:  $\Delta\delta^{13}\text{C}$  2,14‰ ± 0,90 y  $\Delta\delta^{15}\text{N}$  3,21‰ ± 0,75 y para las plumas:  $\Delta\delta^{13}\text{C}$  3,97‰ ± 0,90 y  $\Delta\delta^{15}\text{N}$  3,67‰ ± 0,74. Los factores de discriminación de la cotorra de Kramer (N = 9) para la sangre fueron:  $\Delta\delta^{13}\text{C}$  (‰) 2,58 ± 0,90 y  $\Delta\delta^{15}\text{N}$  (‰) 2,35 ± 0,78 y para las plumas:  $\Delta\delta^{13}\text{C}$  3,64‰ ± 0,98 y  $\Delta\delta^{15}\text{N}$  4,10‰ ± 1,84. Los valores de los factores de discriminación entre la dieta y la sangre de la cotorra de Kramer fueron significativamente más elevados que los de la cotorra argentina. Con respecto a las plumas, no se observaron diferencias entre las dos especies. Nuestros

resultados proporcionan los primeros valores de los factores de discriminación para la sangre y las plumas en cotorras, que son fundamentales para inferir la dieta de estas especies mediante el análisis de isótopos estables.

Palabras clave: Isótopos estables, Cotorras, Aves en cautividad, Dieta de control, Especies invasoras

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## Introduction

Diet is a key aspect in ecological studies (Newton and Brockie, 1998). In the last few decades, analyses of stable isotopes of carbon and nitrogen have been widely used as ecological tracers to assess the diet of several species (Tieszen et al., 1983; Hobson and Clark, 1992b; Becker et al., 2007; Bearhop and Inger, 2008). Nevertheless, deriving quantitative estimates of dietary contributions needs precise estimates of the difference in isotopic composition between predator tissues and preys (diet to tissue discrimination factor, DTDF) (Bond and Diamond, 2011; Parnell et al., 2013).

Recently, researchers have documented considerable variation in DTDF computations. Some reviews and meta-analyses summarized this variation as a function of a number of environmental and physiological factors that included environment (terrestrial, freshwater, marine), trophic level, taxon, tissue, metabolic rate (poikilotherm, homeotherm), nitrogenous excretion (ammonia, urea, uric acid), and sample treatment procedures (Dalerum and Angerbjörn, 2005; Caut et al., 2008; Phillips et al., 2014). As the DTDF is highly sensitive to these factors and is species-specific, it needs to be estimated for various taxa (McCutchan et al., 2003; Caut et al., 2009). However, there is a lack of information for the *Psittaciformes* order, to date computed only for the kea *Nestor notabilis* (Greer et al., 2015).

The aim of this study was to estimate the DTDF of  $^{13}\text{C}$  and  $^{15}\text{N}$ , the two most common stable isotopes used to estimate the diet of a species, for the blood and feathers of the monk parakeet (*Myiopsitta monachus*) and the ring-necked parakeet (*Psittacula krameri*). These two parakeets are currently widespread in Europe and other parts of the world and are considered to cause severe damage to agriculture, ornamental vegetation, and human facilities (Menchetti and Mori, 2014; Senar et al., 2016). DTDFs obtained from this study will allow the development of mixing models to investigate the diet of the species. Such information may be fundamental in controlling their populations.

## Material and methods

The birds used in this study were sampled in the city of Barcelona. The two species have been present in the area since the early 1970s (Batllori and Nos, 1985). The population of the monk parakeet reached 5,000 individuals in 2015, while the population of the rose-ringed parakeet reached 340 individuals in 2014 (Senar et al., 2017a, 2017b).

### Avian samples

We collected nine monk parakeets and nine rose-ringed parakeets of different ages and both sexes at the Ciutadella Park (Barcelona city) during autumn (September) 2017 and winter (January) 2018, after the end of their reproductive period. The birds were captured with a modified Yunnick trap located on a terrace of the Museu de Ciències Naturals de Barcelona. Monk parakeets were housed in captivity at the museum for

90 days and rose-ringed parakeets for 75 days. As DTDFs differ among food sources, throughout the entire captivity period we fed parakeets with a single-species diet based on sunflower seeds. We provided this single food source for the entire study period to allow the isotope ratios in the parakeet tissues to equilibrate with their control diet (Kelly et al., 2012). Blood sampling was conducted biweekly by puncturing the brachial vein and collecting blood in an Eppendorf tube. This should allow the verification of whether the feathers grown after the acclimatization period reflect the isotope composition of sunflower seeds and whether they allow a reliable approximation of DTDF. We verified this by analyzing the stabilization of isotopes in the blood throughout the experiment. Breast feathers were removed at day 45 (monk parakeet) and 30 (rose-ringed parakeet) of the experiment, and hence were forced to grow under the same conditions for all individuals. We chose to remove the feathers after six weeks because complete blood turnover occurs generally in about 30 days (Pearson et al., 2003). New induced feathers were again removed after six weeks when they were fully grown and while they were still on the sunflower seed diet. To calculate the DTDF of the blood in both species, we used samples of the last biweekly puncturing, at the end of the experiment, for both species.

### Stable isotope analyses

As the lipid component of the diet can affect  $\delta^{13}\text{C}$  values (Tarroux et al., 2010; Perkins et al., 2013), five samples of sunflower seeds were previously washed of lipids using successive rinses in a 2:1 chloroform:methanol solvent, and were later oven-dried at 50°C for 24 h. Feather samples were cleaned in a 0.1M NaOH solution to prevent contamination, oven-dried at 50°C for 24 hours and ground into a fine powder. Blood samples were lyophilized and ground to a fine powder, and then directly analyzed isotopically. We did not perform lipid extraction on whole-blood or feather samples because of the typically low proportion of lipids in bird blood and feathers (Bearhop et al., 2002). We did not apply any linear normalization equation (LNE) to the lipid content of the blood. In fact, when the ratio C:N is less or around 4, as we found in our samples, it is not necessary to take the lipid content of the samples for terrestrial animals into account (Post et al., 2007). The C:N ratios for the blood were  $3.31 \pm 0.03$  and  $3.40 \pm 0.17$  for the monk parakeet and the ringed-necked parakeet respectively.

The stable isotope analyses were performed at the 'Serveis Científic-Tècnics' at the University of Barcelona. Stable carbon and nitrogen isotope assays for all samples were weighed using a micro-balance Mettler Toledo MX5 and placed in tin cups and crimped for combustion, using an elemental analysis-isotope ratio mass spectrometry (EA-IRMS). Stable isotope abundances are expressed in  $\delta$  notation in parts per thousand (‰) according to:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] * 1,000$$

where X is  $^{13}\text{C}$  or  $^{15}\text{N}$  and R is the corresponding

Table 1. Mean isotopic values ( $\pm$  SD) of  $\delta^{13}\text{C}$  (‰) and  $\delta^{15}\text{N}$  (‰) for the diet item ( $N = 5$ ), and for blood samples ( $N = 9$ ) and regrown feathers ( $N = 9$ ) for monk and rose-ringed parakeets after being fed in captivity with a single-source diet based on sunflower seeds over 90 and 75 days, respectively.

Tabla 1. Promedio de los valores isotópicos ( $\pm$  DE) de  $\delta^{13}\text{C}$  (‰) y  $\delta^{15}\text{N}$  (‰) relativos a la dieta ( $N = 5$ ) y a las muestras de sangre ( $N = 9$ ) y las plumas nuevas ( $N = 9$ ) de las cotorras argentina y de Kramer tras haber sido alimentadas en cautividad con una fuente de alimentación exclusivamente a base de semillas de girasol durante 90 y 75 días, respectivamente.

|                           | Sunflower seeds   | Monk parakeet     |                   | Rose-ringed parakeet |                   |
|---------------------------|-------------------|-------------------|-------------------|----------------------|-------------------|
|                           |                   | Blood             | Feathers          | Blood                | Feathers          |
| $\delta^{13}\text{C}$ (‰) | $-27.04 \pm 0.89$ | $-24.90 \pm 0.12$ | $-23.07 \pm 0.16$ | $-24.46 \pm 0.11$    | $-23.40 \pm 0.40$ |
| $\delta^{15}\text{N}$ (‰) | $4.06 \pm 0.73$   | $7.27 \pm 0.18$   | $7.73 \pm 0.13$   | $6.41 \pm 0.28$      | $8.16 \pm 1.69$   |

$^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  ratio. The standard values for  $^{13}\text{C}$  and  $^{15}\text{N}$  were Pee Dee Belemnite (PBD) and atmospheric nitrogen (AIR), respectively. Measurement errors (SD) were  $\pm 0.15\text{‰}$  for  $\delta^{13}\text{C}$  and  $\pm 0.25\text{‰}$  for  $\delta^{15}\text{N}$ .

After allowing sufficient time for equilibration between the tissue and the control diet, the DTDF can be estimated by a calculation:

$$\Delta\delta X_{\text{tissue}} = \delta X_{\text{tissue}} - \delta X_{\text{diet}}$$

i.e. the average isotopic value from the tissue minus the average isotopic value from the food. We estimate the variability of this calculation (standard deviation, SD) as the square root of summed variances for diet and tissues samples. (Giménez et al., 2016). We carried out Kruskal–Wallis tests in JMP (SAS Institute Inc., Cary, North Carolina) to assess whether there were any statistically significant differences in the DTDFs of the tissues between the two species as well as between tissues within the same species.

To estimate the patterns of carbon and nitrogen turnovers, we applied the exponential model:

$$y = a + be^{cT}$$

where  $y$  represents the isotope value of the tissue in question,  $a$  and  $b$  are parameters determined by initial and asymptotic conditions,  $c$  is the turnover rate of carbon in the tissue, and  $T$  is time (d) since the diet switch (Tieszen et al., 1983; Hobson and Clark, 1992a; Suzuki et al., 2005) in JMP (SAS Institute Inc., Cary, North Carolina).

## Results

Table 1 shows the results of our isotopic analyses for the samples of sunflower seed, blood, and regrown feathers. The analysis of the stabilization of isotopes in the blood throughout the experiment provided a low fit to the exponential model ( $R^2 < 0.20$  in all cases). Half-life turnover rates for ringed-necked parakeets were estimated in  $25.5 \pm 20.1$  days for  $\delta^{13}\text{C}$  and  $21.3 \pm 17.7$  days for  $\delta^{15}\text{N}$ , whereas those

for monk parakeets were  $52.2 \pm 35.3$  days for  $\delta^{13}\text{C}$  and  $10.4 \pm 4.31$  days for  $\delta^{15}\text{N}$ . However, the curves approached an asymptote before the feathers were removed (fig. 1). Birds of both species maintained on the sunflower seed diet showed remarkably consistent isotope values for blood after this stabilization (fig. 1). Since we obtained suitable isotopic dietary shifts and stabilization was reached before inducing regrowth, for both the monk and the rose-ringed parakeets, we proceeded to estimate DTDF for each species (table 2). The two species differed in blood DTDF values for both isotopes (Kruskal–Wallis test:  $\delta^{13}\text{C}$   $\chi^2_{(1)} = 12.79$ ,  $p < 0.001$ ;  $\delta^{15}\text{N}$   $\chi^2_{(1)} = 12.79$ ,  $p < 0.001$ ). However, the DTDF values of the feathers did not differ significantly between the two species (Kruskal–Wallis test:  $\delta^{13}\text{C}$   $\chi^2_{(1)} = 2.38$ ,  $p = 0.12$ ;  $\delta^{15}\text{N}$   $\chi^2_{(1)} = 1.22$ ,  $p = 0.26$ ). The comparison of DTDF values between the two tissues within the same species resulted in statistically significant differences for both isotopes (monk parakeet blood vs. feathers:  $\delta^{13}\text{C}$   $\chi^2_{(1)} = 12.79$ ,  $p < 0.001$ ;  $\delta^{15}\text{N}$   $\chi^2_{(1)} = 12.79$ ,  $p < 0.001$ ; rose-ringed parakeet blood vs. feathers:  $\delta^{13}\text{C}$   $\chi^2_{(1)} = 12.79$ ,  $p < 0.001$ ;  $\delta^{15}\text{N}$   $\chi^2_{(1)} = 5.48$ ,  $p = 0.02$ ).

## Discussion

The stabilization of both isotopes in monk parakeet blood seems to be reached earlier than in ring-necked parakeets. This suggests that the diet of the individuals in the wild was similar to the diet provided in captivity for monk parakeets but not so similar for rose-ringed parakeets. As a consequence, stabilization in isotope blood values followed a flat trend, and hence, the exponential model was not the most suitable model to explain the turnover patterns. This is not surprising since the 'exponential-fit' approach overemphasizes data at the extremes (Cerling et al., 2007).

The DTDFs for  $\delta^{15}\text{N}$  of the feathers in *M. monachus* ( $3.67 \pm 0.74\text{‰}$ ) and in *P. krameri* ( $4.10 \pm 1.84$ ) are close to the frequently adopted DTDF value of  $3.40\text{‰}$  per trophic level (Post, 2002). Comparisons of  $\delta^{13}\text{C}$  DTDF values obtained in this work with that of other

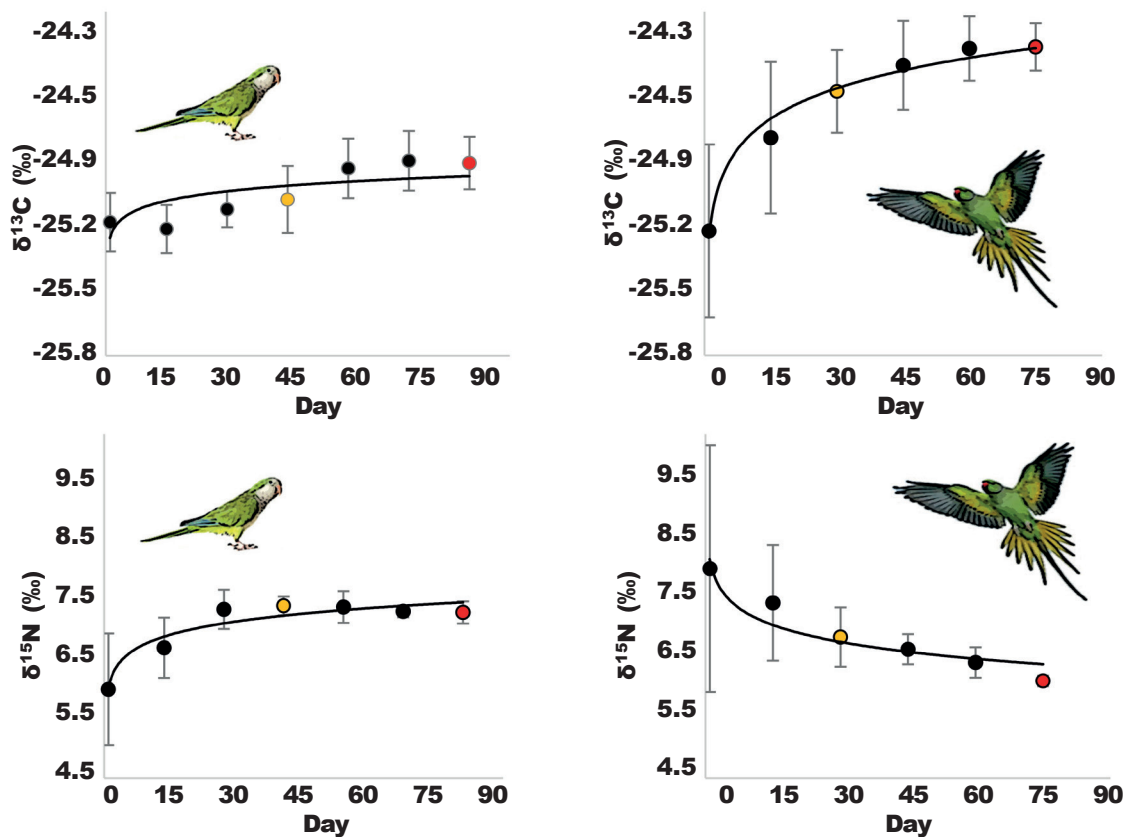


Fig. 1. Change in isotopic values for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the blood of captive monk (*Myiopsitta monachus*) (left) and ring-necked parakeets (*Psittacula krameri*) (right) switched on day 1 from the wild diet to a sunflower seed diet. The yellow dots represent the day on which we removed the feathers. The red dots represent the day we took the last blood samples, and the regrown feathers. We present element-turnover patterns for carbon and nitrogen by fitting a single value to the means of the combined data for all individuals for the diet on each extraction. Error bars represent the standard deviation of the mean for all experimental birds. (N = 9 for each species).

Fig. 1. Cambio en los valores isotópicos de  $\delta^{13}\text{C}$  y  $\delta^{15}\text{N}$  en la sangre de la cotorra argentina (*Myiopsitta monachus*) (izquierda) y la cotorra de Kramer (*Psittacula krameri*) (derecha) en cautividad en el primer día posterior al cambio de la dieta natural por la dieta a base de semillas de girasol. Los puntos amarillos representan el día en que se extrajeron las plumas. Los puntos rojos representan el día en que se tomaron las muestras de sangre y las plumas nuevas. Presentamos los patrones de recambio del carbono y el nitrógeno ajustando un único valor al promedio de los datos combinados de todos los individuos para la dieta en cada extracción. Las barras de error representan la desviación estándar de la media de todas las aves del experimento. (N = 9 para cada especie).

species is obscured by the fact that some studies do not present the  $\delta^{13}\text{C}$  values with prior lipid extraction (Hobson and Bairlein, 2003). The need to extract lipids from the food source derives from the fact that the carbon from the lipid fraction of the diet may not contribute to the carbon content of proteins in blood or keratin in feathers, and therefore the differential lipid contents of food can contribute to variation in DTDF as well as inflate its value, since isotopic values of the lipid fraction are typically isotopically depleted (Perkins et al., 2013). Regardless, we found that the DTDFs for  $\delta^{13}\text{C}$  that we obtained for both species

(monk parakeet:  $3.97 \pm 0.90\text{‰}$  and the rose-ringed parakeet:  $3.64 \pm 0.98\text{‰}$ ) are among the highest values reported for this isotope (Caut et al., 2009), considerably exceeding the commonly assumed 0–1‰ DTDF value per trophic level (Post, 2002). Nevertheless, the high DTDF values for  $\delta^{13}\text{C}$  that we found was similar to that found by Greer et al. (2015) in the Kea parrot. This may result from the fact that, parrots generally have higher basal metabolic rates (BMRs) than other bird species (McNab, 2012). DTDF values are positively correlated to the respiration rate (De Niro and Epstein, 1978). The higher blood DTDF



Table 2. Diet to tissue discrimination factors ( $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$ ) for feathers and blood for monk ( $N = 9$ ) and rose-ringed parakeets ( $N = 9$ ) derived from a single-source diet based on sunflower seeds over 90 and 75 days, respectively.

Tabla 2. Factores de discriminación ( $\Delta^{13}\text{C}$  y  $\Delta^{15}\text{N}$ ) entre la dieta y las plumas y la sangre de la cotorra argentina ( $N = 9$ ) y la cotorra de Kramer ( $N = 9$ ) obtenidos a partir de una sola fuente de alimentación a base de semillas de girasol durante 90 y 75 días, respectivamente.

|                      | $\Delta\delta^{13}\text{C}$ (‰) | $\Delta\delta^{15}\text{N}$ (‰) |
|----------------------|---------------------------------|---------------------------------|
| Feathers             |                                 |                                 |
| Monk parakeet        | $3.97 \pm 0.90$                 | $3.67 \pm 0.74$                 |
| Rose-ringed parakeet | $3.64 \pm 0.98$                 | $4.10 \pm 1.84$                 |
| Blood                |                                 |                                 |
| Monk parakeet        | $2.14 \pm 0.90$                 | $3.21 \pm 0.75$                 |
| Rose-ringed parakeet | $2.58 \pm 0.90$                 | $2.35 \pm 0.78$                 |

in the rose-ringed parakeet compared to that of the monk parakeet could, therefore, be a consequence of ring-necked parakeets displaying a higher BMR than monk parakeets.

The  $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$  values from the feathers of the two species were higher than those from their blood (table 2). The differences between  $\Delta^{13}\text{C}$  values from blood and feathers are probably due to the fact that the two tissues differ in biochemical composition (Martínez del Río et al., 2009). Feathers typically have more positive  $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$  values than blood or muscle (Caut et al., 2009).

In conclusion, our study provides the first values of DTDFs for the monk and the rose-ringed parakeets and contributes to the scarce knowledge of DTDF values for the order Psittaciformes.

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