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Original Article

Diet and between-tissue isotope comparisons reveal different foraging strategies for age and sex of a Saffron Finch (*Sicalis flaveola* Linnaeus, 1766) population

A dieta e comparações isotópicas entre os tecidos revelam diferentes estratégias de forrageamento para idade e sexo de uma população de canário-da-terra (Sicalis flaveola Linnaeus, 1766)

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Abstract

Measuring stable isotopes in different tissues offers the opportunity to provide insight into the foraging ecology of a species. This study aimed to assess how diet varies between yellow females, yellow males, and dull individuals of a Saffron Finch (*Sicalis flaveola*) population. We measured $\delta^{13}C$ and $\delta^{15}N$ in blood over a year, and in different feathers, to estimate seasonal consistency of resource use for each category. We conducted this study in a private farm in the Central Brazilian savannas. We sampled 195 individuals in seven field samplings between January 2017 and March 2018. The mean blood $\delta^{13}C$ values were similar among yellow females, yellow males and dull individuals, indicating that this population of Saffron Finch predominantly accesses similar resources throughout the year, with a predominant C_4 signal. Although Saffron Finch is considered a granivorous species, the mean $\delta^{15}N$ values found indicate that both adults and juveniles also incorporate in their tissues some invertebrate. The slight isotope-tissue difference between feathers and blood is similar to the reported in previous studies and may reflect tissue-to-tissue discrimination. The isotopic space of yellow males was greater than that of yellow females and dull individuals, indicating greater dietary diversity due to greater inter-individual variation in diet. In Saffron Finch, which delays plumage maturation, competition-driven partitioning of food resources seems essential in driving carotenoid-based plumage coloration between age classes and sexes.

Keywords: animal tissues, Cerrado, isotopic space, stable isotopes, temporal variation.

Resumo

O uso de isótopos estáveis em diferentes tecidos oferece a oportunidade de conhecer a ecologia de forrageamento de uma espécie. O objetivo deste estudo foi avaliar a variação da dieta entre fêmeas amarelas, machos amarelos e indivíduos pardos de uma população de canário-da-terra (*Sicalis flaveola*). Foram analisados o $\delta^{13}C$ e $\delta^{15}N$ no sangue ao longo de um ano, e em diferentes penas, para estimar a consistência sazonal do uso de recursos nas três categorias de indivíduos. Este estudo foi conduzido em uma fazenda particular localizada no Cerrado do Brasil Central. Foram amostrados 195 indivíduos em sete campanhas de campo entre janeiro de 2017 e março de 2018. Os valores médios de $\delta^{13}C$ no sangue foram semelhantes entre fêmeas amarelas, machos amarelos e indivíduos pardos, indicando que esta população de canário-da-terra acessa predominantemente recursos semelhantes ao longo do ano, com um sinal predominante C_4. Embora o canário-da-terra seja considerada uma espécie granívora, os valores médios de $\delta^{15}N$ encontrados indicam que tanto os adultos quanto os juvenis também incorporam em seus tecidos fontes alimentares de alto nível trófico. A pequena diferença isotópica entre penas e sangue é semelhante à registada em estudos anteriores e pode ser explicada pela discriminação tecido-tecido. O espaço isotópico dos machos amarelos foi maior em comparação às fêmeas amarelas e aos indivíduos pardos, o que indica uma maior diversidade alimentar devido a uma maior variação intra-individual em sua dieta. Em canário-da-terra, espécie com característico atraso na maturação da plumagem, o acesso aos recursos alimentares, motivado pela competição, parece desempenhar um papel essencial na coloração da plumagem baseada em carotenóides entre classes etárias e sexos.

Palavras-chave: tecidos animais, Cerrado, espaço isotópico, razões isotópicas, variação temporal.

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1. Introduction

Environments, such as Neotropical savannas, characterized by heterogeneous resource availability, might afford multiple features of a species' ecological niche, resulting in generalized resource and habitat choice. Given the importance of species' food web roles for ecosystem functioning, there is a need to understand relationships between environmental heterogeneity and the niches that different sexes and ages of the same species are able to use.

Stable isotope analysis provides complementary information to traditional measurements of foraging ecology (Buelow et al., 2018). The combined use of $\delta^{13}C$ and $\delta^{15}N$ has proved to be efficient in unraveling the trophic dimension of the ecological niche, helping to describe food webs and trace the flow of energy in an ecosystem. Several studies have used stable isotopes as an important tool to infer the trophic position of consumers and their ecological niches as a function of the habitat structure (Newsome et al., 2007; Layman et al., 2012).

The $\delta^{13}C$ values in animal tissues allow inferences to be made about the basal food source, which is related to the C_3 or C_4 plant resource incorporated into the diet. The $\delta^{15}N$ values indicate the trophic level since it increases along the food chain (Boecklen et al., 2011). Combining $\delta^{13}C$ and $\delta^{15}N$ values allow inferences regarding the use of resources on multiple time scales in the same individual when analyzing tissues with different turnover periods (Martínez del Rio et al., 2009; Guaraldo et al., 2019; Pereira et al., 2023). In addition, such combination can infer some important niche properties, such as niche width, trophic position, diversity of assimilated resources and degree of overlap between sex and age in a population (Newsome et al., 2007; Newsome et al., 2012; Yeakel et al., 2016).

Newsome et al. (2007) calculated niche in multivariate δ space, calling it isotopic space, indicating whether individuals from the same group are similar or different in terms of trophic position and use of basal resources. Isotopic space has been used to inform about spatiotemporal patterns of niche change, acting as a strong predictor of the ability to exploit food resources and species' responses to habitat changes (Newsome et al., 2007; Newsome et al., 2012; Yeakel et al., 2016). Changes in the width of isotopic space may be related to habitat fragmentation (Carvalho et al., 2017; Pereira et al., 2023), urbanization (Navarro et al., 2021), changes in the availability of food resources (Bosenbecker and Bugoni, 2020; Pompermaier et al., 2022) and seasonal changes in essential food resources (Camargo et al., 2021).

The species chosen here as a study model was the Saffron Finch (*Sicalis flaveola*). The Saffron Finch is a resident bird, and it is widely ditribuited in central Brazil, which is dominated by the Cerrado biome (a Neotropical savanna in South America) (Rising and Jaramillo, 2024). Adults develop a definitive and conspicuous yellow plumage only after the second reproductive period. Although Saffron Finch has been classified as granivorous, it seems to also incorporate arthropods in its diet (Rising and Jaramillo, 2024). According to the literature, the concentration of carotenoids in grains and seeds is low compared to fruits and could therefore limit the expression of the plumage of the Saffron Finch (McGraw et al., 2001; Mahler et al., 2003), so it is likely that carotenoids are incorporated mainly from arthopods rich in carotenoids (Maoka et al., 2021).

The Cerrado is marked by a rainy season when there is an increase in the abundance and variety of food resources. At this season, several plant species begin to flower and there is an increase in the supply of seeds and fruits, and an abundance of arthropods, which are coordinated to an annual cycle linked to the molting and reproductive period (Pereira, 2011). During the dry season, the availability of food resources tends to decrease and the competition for high quality food items tends to increase. Therefore, temporal variation in resource supply might provide relevant information on foraging capacity in such seasonal ecosystems.

Multiple tissues with different turnover rates in the same individual reflect the diet on different time scales. The period of integration of the isotopic values does not immediately reflect the isotopic composition of the diet in the tissues but is integrated over time. Metabolically active tissues, such as blood, provide information about diet in the short-term depending on the metabolism of the group of interest, while inert tissues, such as feather, provide information about diet in the long term, although it depends on the time of formation of such specific tissue when molt occurs (Vander Zanden et al., 2015). Guaraldo et al. (2019) investigated the variation in the width of the isotopic space of resident individuals of *Elaenia cristata* throughout the year (breeding, moulting and non-breeding periods) using different tissues. During the non-breeding period, the isotopic space analyzed isotopes values from claw (integrating 4-5 months period) suggested these individuals rely on a plant-based diet. In the reproductive period, the isotopic space analyzed with blood isotopic values showed intermediate dietary patterns compared to the other periods (Guaraldo et al., 2019).

Isotopic compositions of bird tissues should reflect isotopic changes in their environment throughout the annual cycle. Although this leads to questions about the trophic ecology of the species, the main focus of the article, however, is how different classes within the species track these environmental changes differently. This approach allows us to observe intraspecific variations without delving into the detailed dynamics of trophic ecology.

This study aimed to assess how diet varies between different categories (females, males, and juveniles) of a Saffron Finch population from Central Brazil, inferred by the $\delta^{13}C$ and $\delta^{15}N$ blood values over time, and how the isotopic values between different tissues with different turnover periods (blood *versus* feather) to estimate the seasonal consistency of resource use for each category.

We first evaluated the relative contribution of plants and invertebrates to the diet of the Saffron Finch. We tested the hypothesis that expansion of the isotopic space during rich resource periods occurs and consequent reduction in isotopic space overlap. We, then, expected that expansion of dietary variability during rrich resource periods would occur. Conversely, during low resource periods the population isotopic space would retract.

2. Material and Methods

2.1. Studied site

The study was conducted at Fazenda Tabapuã dos Pirineus (15º46'40" S, 48º49'22" W, elev. 1100m), an 800ha rural property in the Alto Corumbá basin located in the municipality of Cocalzinho de Goiás, Goiás, Brazil. It is part of a large project assessing different features of the Saffron Finch population in the region (Costa et al., 2022).

In the region, there is a predominance of grassland and savanna phytophysiognomies, with riparian forest along the preserved streams. An important part of the basin is the Serra dos Pirineus State Park, which corresponds to a total area of 2822 ha (Cordeiro et al., 2020). In the managed areas of the basin, the predominant area is pasture with exotic grasses, followed by agricultural cultivation such as corn and soybeans (Cordeiro et al., 2020). During the studied period, the average annual rainfall was 1415 mm, and the average annual temperature was 21.9 ºC (INMET, 2024). The local climate is classified as Aw by Köppen, characterized by two distinct, well-marked seasons: a dry season from May to September, with a monthly average of less than 50 mm and a rainy season from October to April, reaching 250 mm in December and January (ANA, 2024).

2.2. Studied specie

The studied species was the Saffron Finch (*Sicalis flaveola*), that prefers open environments such as forest edges and pastures (Palmerio and Massoni, 2009), forraging mainly on the ground. According to Rising and Jaramillo (2024), Saffron Finch's diet is predominantly granivorous, but also includes small arthropods. It is a social species that forms flocks when it is not breeding (Silveira and Méndez, 1999). During the breeding season, they form socially monogamous pairs (Benítez-Saldivar et al., 2022), which successfully nest in tree trunks, human constructions like roofs, and abandoned nests of other species (Palmerio and Massoni, 2009).

When young, they have a brown plumage shared with first-year sub-adults. Only after the second breeding season, males develop a definitive yellow plumage with a characteristic orange forehead (Marques-Santos et al., 2018). Some individuals of both sexes may present delayed plumage maturation (Marques-Santos et al., 2018). The individuals of Saffron Finch collected here were classified as yellow females (adult females displaying yellow plumage), yellow males (adult males displaying yellow plumage), and dulls (juveniles or sub-adults of both sexes displaying brown plumage).

From our knowledge, there are no studies testing for differences in $\delta^{13}C$ and $\delta^{15}N$ in feathers regarding the influence of carotenoid pigments in bulk isotopic values. The highest levels of carotenoids in feathers have been reported to be less than 0.001% (Koch et al., 2016), indicating that such pigments play a minor role influencing the bulk isotopic compositions of feathers.

2.3. Captures of birds and tissues sampled

In total, 195 (18 yellow females, 44 yellow males, and 133 dulls) individuals of *S.f brasiliensis* were caught using mist nets (12x3m) every two months between January 2017 and March 2018. The mist nets were opened in the early morning and remained open until late afternoon in each campaign. No co-specific playback was used to attract the birds to the mist nets. The individuals that were captured received metal rings provided by CEMAVE (National Center for Research and Conservation of Wild Birds). Afterward, around 100 µL of blood was collected via brachial venipuncture and deposited on a glass slide to be air-dried. Whole feathers were collected from the wing (outermost primary feather - P10), tail (second outermost feather - R2), and a pool of feathers from the breast and a pool of feathers from the forehead, with the number of tissues sampled from the same individual varying during the field campaigns.

The procedures described here were approved by the Ethics and Animal Use Committee - University of Brasilia (approval No. 55712/2016). The field collection licenses were permitted by the Chico Mendes Institute for Biodiversity Conservation (SISBIO Nº 8745-1) and genetic use by the National System for the Management of Genetic Heritage and Traditional Knowledge Association License (SISGEN NºA018ECD).

2.4. Stable isotope analysis

The feather samples (breast, forehead, tail and wing) were previously cleaned with distilled water and then with a 2:1 ratio of chloroform and methanol solutium. The feather samples were then dried in an oven at 50º C for 24 hours. After this process, each feather sample was cut into small pieces and then stored in plastic cryotubes until being analyzed.

All the samples (blood and feathers) were weighed to match the minimum mass for the analysis (0.5 mg), then encapsulated in a tin capsule. Carbon and nitrogen isotope ratios were determined by combustion using an elemental analyzer (Carlo Erba, CHN-1100) coupled to a Thermo Finnigan DELTA Plus mass spectrophotometer at the Isotope Ecology Laboratory of the Center for Nuclear Energy in Agriculture (CENA), University of São Paulo, Piracicaba, SP, Brazil.

The international standard reference used for carbon analysis was the Vienna Pee Dee Belemite (Vienna PDB), and for nitrogen analysis was the atmospheric air. All bird tissue samples were interspersed with internal standards: sugar cane leaf ($\delta^{13}C$ = -13.1‰ and $\delta^{15}N$ = 5.1‰) and tropical soil ($\delta^{13}C$ = -26.5‰ and $\delta^{15}N$ = 12.1‰) during the analyses.

2.5. Statistical analysis

All statistical analyses were carried out after checking the assumptions of normality (Shapiro-Wilk test) and homogeneity of variances (Levene test).

As the blood of Saffron Finch individuals was collected every two months over a period of one year and the blood isotopic turnover ratio is estimated to be around one month for passerines (Hobson and Clark, 1992), each month sampled could be independently analyzed. The monthly isotopic values of the blood did not follow a normal distribution, so the Kruskal Wallis test was applied to check for differences in the isotopic values of each category over the months of sampling and to compare the categories in each month sampled. The Wilcoxon test was applied for paired comparisons.

With a similar approach, the isotopic values of the feathers were used since they correspond to the diet during growth, thus providing information on how the categories use the resources at the time of molting. The different feathers (breast, forehead, tail, and wing) did not follow a normal distribution, and the Kruskal Wallis test was used to identify the differences in the isotopic values of the different feathers in each category, and between the categories for each type of feather with the Wilcoxon test used for paired comparisons. The Wilcoxon test for paired comparisons was also used to check for differences in diet between feathers and blood, comparing the isotopic values of each type of feather with the blood of the same individual.

The "SIBER" package (Jackson et al., 2011) was used to estimate the differences in resource use and niche width between the plumage color classes of Saffron Finch. 95% of the $\delta^{13}C$ and $\delta^{15}N$ values of the wing feathers were considered to calculate the Standard Ellipse Area (SEA), a measure of the isotopic space that considers the niche position occupied in a two-dimensional space. In addition, the SEA was compared between plumage color classes using a Bayesian inference approach (SEA.B; in ‰^{2}), an estimate of the extent of the niche, which reflects the variability of the diet between the categories. It provided information on how generalized or specialized the diet of each plumage color category of Saffron Finch might be. SEA.B was calculated on 10,000 interactions using the MCMC method to generate Bayesian credibility intervals of 50, 75 and 95%. The comparison of the width of the isotopic spaces between categories was carried out with paired tests using the SEA.B values through the probability of one group being larger (reference group) than another: SEA.B - group A > SEAB - group B. The probability of difference can vary from 0.5, as a probability of being the same or less certain, to 1 as a greater certainty of difference.

The "nicheROVER" package (Swanson et al., 2015) was used to calculate the probability of overlapping the isotopic space based on the isotopic values of the wing feathers between the categories. This package considers the likelihood of the niche extent of one group being incorporated in relation to another group (Swanson et al., 2015), allowing to assess the likelihood of diet similarity between categories and, therefore, the degree to which individuals may be competing for resources. Therefore, niche overlap was estimated by running 1,000 iterations using the MCMC method of isotopic values to calculate regions of niche overlap with a probability level of alpha = 0.95 (95% probability) and 95% confidence intervals.

The raw data that generated all figures and tables is available at "Data for: blood and feather carbon and nitrogen stable isotopes for the Saffron Finch", Mendeley Data, V1, doi: 10.17632/3v5jm2vkgy.1".

3. Results

3.1. Temporal variation of blood $\delta^{13}C$ and $\delta^{15}N$ in Saffron Finch individuals

Blood $\delta^{13}C$ values did not differ between individuals from different plumage color categories (Kruskal Wallis H = 0.007; df = 2; p = 0.99), with the $\delta^{13}C$ values of the blood of yellow males and dull individuals sharing a similar average with the yellow females over the months.

Yellow females did not differ between sampled months (Kruskal Wallis H = 8.27; df = 6; p = 0.21; Figure 1A), with an average of -12.1±1‰. The $\delta^{13}C$ values of the blood of yellow males differed between sampled months (Kruskal Wallis H = 18.5; df = 6; p = 0. 0004) with a range of -13.2‰ to -11.3‰ (Figure 1B), and similarly, dull individuals differed in $\delta^{13}C$ between months (Kruskal Wallis H = 36.35; df = 6, p < 0.0001) with a range of -13.7‰ to -11.5‰ (Figure 1C).

Blood $\delta^{15}N$ values did not differ among individuals from different plumage color categories (Kruskal Wallis H = 0.77; df = 2; p = 0.67). Blood $\delta^{15}N$ values did not differ between sampled months for yellow females (Kruskal Wallis H = 7.02; df = 6; p = 0.31; Figure 1D) and yellow males (Kruskal Wallis H = 11.95; df = 6; p = 0.06; Figure 1E). Both categories shared similar mean values (yellow females = 5.8±0.6‰, yellow males = 5.8±0.8‰). The blood $\delta^{15}N$ of dull individuals differed significantly between months (Kruskal Wallis H = 11, 95, df = 6; p < 0.0001) and showed a mean of 5.6±1‰ and a range of 3.8‰ to 6.7‰. The paired comparisons showed that the blood $\delta^{15}N$ values in October were 2.2‰ lower compared to the other months, while the variation in $\delta^{15}N$ values varied between the other months was 1.2‰ (Figure 1F).

Blood $\delta^{13}C$ and $\delta^{15}N$ values in October varied significantly between categories (Table 1). For blood $\delta^{13}C$, yellow females and yellow males did not differ (p = 0.5) and with dull individuals (p=0.16), but yellow males had, on average, 1‰ higher blood $\delta^{13}C$ values compared to dull individuals (p = 0.005; Table 1). For blood $\delta^{15}N$, yellow females and yellow males did not differ either (p=1 and with dull individuals (p=0.16), but yellow males showed values 2.1‰ higher compared to the dull individuals (p = 0.005).

3.2. Tissue-isotope comparisons for Saffron Finch individuals

The $\delta^{13}C$ values did not differ between different feathers for yellow females (Kruskal Wallis H = 1.84; df = 3; p = 0.6) with an average of -10.4±0.7‰; for yellow males (Kruskal Wallis H = 3.6; df = 3; p = 0.30) with a mean of -10.8±1.4‰; and for dull individuals (Kruskal Wallis H = 7.5; df = 3; p = 0. 056) with a mean of -10.3±1.13‰.

The $\delta^{15}N$ values did not differ between different feathers of yellow females (Kruskal-Wallis H = 1.84; df = 3, p = 0.6) with a mean of 5.8±0.6‰, but they did differ for the feathers of yellow males (Kruskal Wallis H = 10.8; df = 3, p = 0.01). Pairwise comparisons showed that forehead feathers had lower values compared to wing (p = 0.1), tail (p = 0.002) and breast (p = 0.02) feathers. The different feathers of dull individuals did not differ in their $\delta^{15}N$ values (Kruskal Wallis H = 3.45; df = 3; p = 0.32) with an average of 6.2±0.9‰ (Table 2).

The average variation in isotopic values between each type of feather and blood was similar between categories but varied for each type of feather (Table 2). The $\delta^{13}C$ values of the tail feathers varied significantly between categories. Yellow females and dull individuals did not differ in their

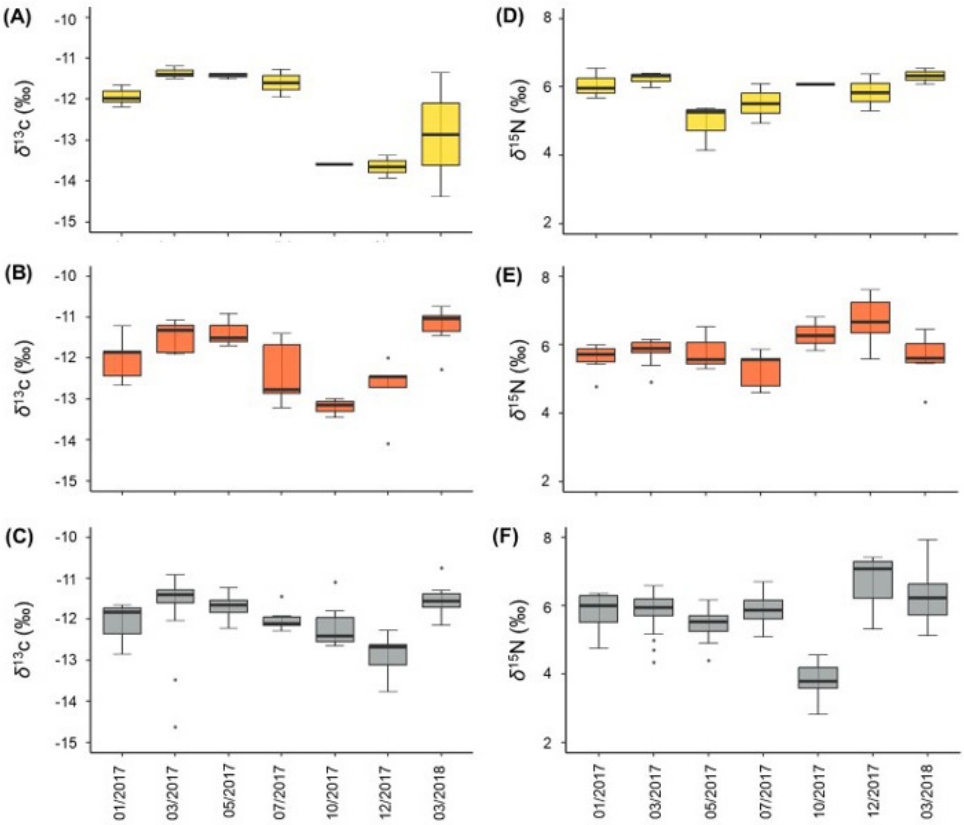

Figure 1. Variation in blood $\delta^{13}C$ over the months for yellow females (in yellow; A), yellow males (in orange; B), and dull individuals (in gray; C). Variation of blood $\delta^{15}N$ over the months for yellow females (in yellow; D), yellow males (in orange; E), and dulls (in gray; F). The central line shows the median, and the bottom and top of the box are the first and fourth quartiles.

Table 1. Variation in $\delta^{13}C$ and $\delta^{15}N$ (mean ± SE) in blood for yellow females, yellow males and dull individuals. Each line of the table represents the comparisons made using the Kruskall-wallis test.

Sampling months	Isotope	Category			Kruskall-wallis		
		Yellow female	Yellow male	Dull	H	df	P
January 2017	$\delta^{13}C$	-11.9±0.3	-12±0.5	-12±0.4	0.56	2	0.97
	$\delta^{15}N$	6.1±0.4	5.6±0.4	5.8±0.6	2.06	2	0.35
March 2017	$\delta^{13}C$	-11.3±0.1	-12.3±2.5	-11.6±0.8	0.04	2	0.97
	$\delta^{15}N$	6.2±0.2	5.8±0.4	5.8±0.5	3.06	2	0.21
May 2017	$\delta^{13}C$	-11.4±0.1	-11.3±0.4	-11.7±0.3	2.94	2	0.22
	$\delta^{15}N$	4,9±0.7	5.8±0.6	5.5±0.5	3.41	2	0.18
July 2017	$\delta^{13}C$	-11.6±0.5	-12.4±0.8	-12±0.3	2.2	2	0.32
	$\delta^{15}N$	5.5±0.8	5.3±0.5	5.9±0.5	2.4	2	0.29
October 2017	$\delta^{13}C$	-13.6±NA	-13.2±0.2	-12.2±0.5	8.4	2	0.01
	$\delta^{15}N$	6.1±NA	6.3±0.5	3.8±0.5	8.26	2	0.01
December 2017	$\delta^{13}C$	-13.6±0.4	-12.7±0.8	-13.7±2.3	2.62	2	0.26
	$\delta^{15}N$	5.8±0.7	6.7±0.8	6.7±0.8	1.89	2	0.38
March 2018	$\delta^{13}C$	-12.9±2.1	-11.2±0.5	-11.5±0.4	3.37	2	0.18
	$\delta^{15}N$	6.3±0.3	5.6±0.7	6.3±0.9	3.53	2	0.17

$\delta^{13}C$ values, but yellow males had, on average, 0.8‰ lower values compared to dull individuals ($p = 0.001$).

The distribution of $\delta^{13}C$ and $\delta^{15}N$ values with the position of each individual sampled from the category of females and yellow males, and dull individuals with the mean values and standard deviation for each feather type (breast, forehead, tail and wing) is reported in Figure 2. The $\delta^{13}C$ values of yellow females and yellow males varied between 1.5 and 1.8‰ and between 1.3 and 1.5, while the $\delta^{15}N$ values varied between 0.1 and 1.1‰ and between 0.2 and

Table 2. Variation in $\delta^{13}C$ and $\delta^{15}N$ (mean ± standard deviation) of blood and different feathers (breast, forehead, tail, and wing) for yellow males, yellow females, and dull individuals. For each line in the table, the paired difference indicates the difference in isotopic values between blood and feathers from the same individuals in each category, and the paired comparison indicates the significance of this respective difference.

Category	Feather types (N° of samples)	Isotope	Blood	Feather	Paired differences	Pair-wise test
Yellow Female	Breast (13)	$\delta^{13}C$	-12.1±1.2	-10.2±0.5	1.9±1.3	P<0.0001
		$\delta^{15}N$	5.7±0.7	6.3±1	0.6±1.2	P<0.03
	Forehead (6)	$\delta^{13}C$	- 12.2±1.1	- 10.7±0.6	1.5±1.3	P = 0.02
		$\delta^{15}N$	5.8±0.6	5.7±0.9	0.1±1	P = 1
	Tail (9)	$\delta^{13}C$	- 11.6±0.3	-10.5±0.6	1.1±0.7	P = 0.01
		$\delta^{15}N$	5.5±0.7	6.6±0.9	1.1±1.1	P = 0.001
	Wing (13)	$\delta^{13}C$	- 12.1±1.2	-10.2±0.5	1.8±1.3	P < 0.0001
		$\delta^{15}N$	5.7±0.7	6.3±1	0.5±1.7	P = 0.056
Yellow Male	Breast (27)	$\delta^{13}C$	- 12.2±1.6	-10.6±1	1.6±1.1	P<0.0001
		$\delta^{15}N$	5.8±0.7	6.5±0.8	0.7±0.1	P = 0.004
	Forehead (27)	$\delta^{13}C$	- 12.3±1.6	-10.9±1.1	1.4±1.9	P<0.0001
		$\delta^{15}N$	5.8±0.1	6±0.7	0.2±1	P=0.5
	Tail (22)	$\delta^{13}C$	-12.2±1.6	-10.9±1.1	1.3±2.2	P<0.0001
		$\delta^{15}N$	5.7±0.1	6±0.7	0.9±1.1	P<0.0001
	Wing (30)	$\delta^{13}C$	- 12.1±1.5	-10.7±1.8	1.4±2.3	P<0.0001
		$\delta^{15}N$	5.9±0.7	6.5±1.1	0.6±1.3	P=0.002
Dull	Breast (37)	$\delta^{13}C$	- 11.7±0.7	-10.4±0.5	1.3±1.4	P<0.0001
		$\delta^{15}N$	5.7±0.1	6.4±0.1	0.7±1	P<0.0001
	Forehead (22)	$\delta^{13}C$	-11.7±0.5	-10.7±0.5	1,0±1.4	P<0.0001
		$\delta^{15}N$	5.9±0.5	6.3±1	0.4±1	P=0.06
	Tail (42)	$\delta^{13}C$	-11.7±0.1	-10.1±0.1	1.6±1.8	P<0.0001
		$\delta^{15}N$	5.8±0.1	6.2±0.1	0.3±1.1	P=0.01
	Wing (55)	$\delta^{13}C$	- 12±1.1	- 10.4±1.3	1.8±0.2	P<0.0001
		$\delta^{15}N$	5.6±0.7	6±0.7	0.4±1.5	P=0.02

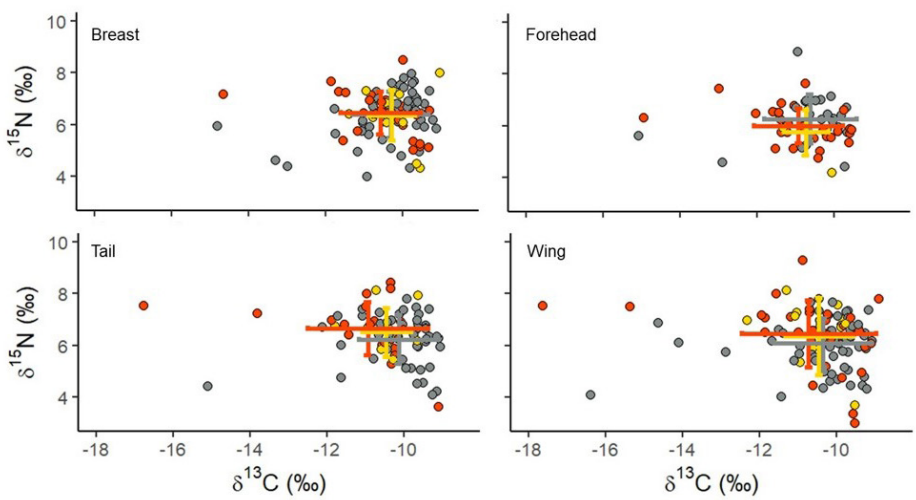

Figure 2. Distribution of $\delta^{13}C$ and $\delta^{15}N$ values with the position of each individual sampled from the category of females (in yellow) and yellow males (in orange), and dull (in gray), and the mean values and standard deviation for each feather type (breast, forehead, tail, and wing).

0.9‰, respectively. For the dull individuals, the $\delta^{13}C$ varied between 1 and 1.8‰ and the $\delta^{15}N$ between 0.3 and 0.7‰.

3.3. Isotopic space

The width of the isotopic space of yellow males showed a high probability of difference with yellow females (probability = 0.96; Figure 3) and with dull individuals (probability = 0.97; Figure 3). The width of the isotopic space of yellow males (6.5‰^{2}) was 49% greater than that of yellow females (3.3‰^{2}) and 43.5% greater than that of dull individuals (3.7‰^{2}) (Figure 3).

The posterior probability distribution of the niche region metric isotopic space of yellow males showed a percentage overlap of 91.4% with the isotopic space of yellow females (Figure 4). However, there was a lower percentage of overlap between the niche region metric isotopic space of yellow females and dull individuals compared to yellow males (Figure 4). Yellow females showed an overlap percentage of 58.5% and dull individuals of 75.6% in relation to the niche region metric isotopic space of yellow males (Figure 4). The overlap of the niche region metric isotopic space was greater in dull individuals than in yellow females, with an overlap percentage of 78.1%, while yellow females showed an overlap of 68.9% in relation to dull individuals (Figure 4).

4. Discussion

The mean blood $\delta^{13}C$ values were similar between yellow females, yellow males, and dull individuals, indicating that Saffron Finch predominantly accesses similar resources throughout the year, with a predominant C_4 signal. The mean blood $\delta^{15}N$ values were also similar between yellow females, yellow males, and dull individuals. However, the average $\delta^{15}N$ values for each category were higher than for other similar granivorous species reported in the literature (Navarro et al., 2023). Although Saffron Finch is considered a granivorous species, such results indicate that they also rely on invertebrates, such as small arthropods, as also reported by Dzielski et al. (2021).

When comparing the $\delta^{15}N$ values between categories over the months, the yellow males had a higher average of 2.2‰ compared to the dull individuals. The dull individuals, on the other hand, showed a large variation in $\delta^{15}N$ values throughout the year. During the transition between the dry and rainy seasons, dull individuals presented the lowest blood $\delta^{15}N$ values compared to the other months, indicating a shift in their diet to a more plant-based food item. A hierarchical segregation in foraging among the categories is presumed since it corresponds to the end of the dry season. Therefore, the availability of food resources might be more limited. These observations suggest a hierarchical segregation in foraging between the categories in this period of the year. The same pattern was found for other Cerrado species (Camargo et al., 2021). Competition probably increases, and the yellow males should show social dominance and higher foraging efficiency (see Dey et al., 2014).

Considering that Saffron Finch develops a definitive yellow plumage after the second reproductive period (Marques-Santos et al., 2018), it is possible that in juvenile birds, the carotenoids incorporated into the diet are preferentially allocated to physiological demands. Thus, only sub-adults in good nutritional and health conditions should be able to invest considerable amounts of carotenoids to change plumage and signal sexual maturity, which could explain delayed plumage maturation in this species.

The different feathers showed higher isotopic values compared to blood. However, the difference in isotopic values between the different feathers and blood was less than 2‰ for $\delta^{13}C$ and 1‰ for $\delta^{15}N$ in all categories. The $\delta^{15}N$ values of the wing feathers of yellow females and the feathers of the foreheads of all categories were similar to blood. Greer et al. (2015) reported for the kea parrot (*Nestor notabilis)* discrimination factors between blood and feather of 1.94‰ for $\delta^{13}C$ and 0.77 ‰ for $\delta^{15}N$. They explain that lipid $\delta^{13}C$ values are lower than those of proteins, and lipids are present in greater quantities in blood than in feathers. Furthermore, the isotopic ratios

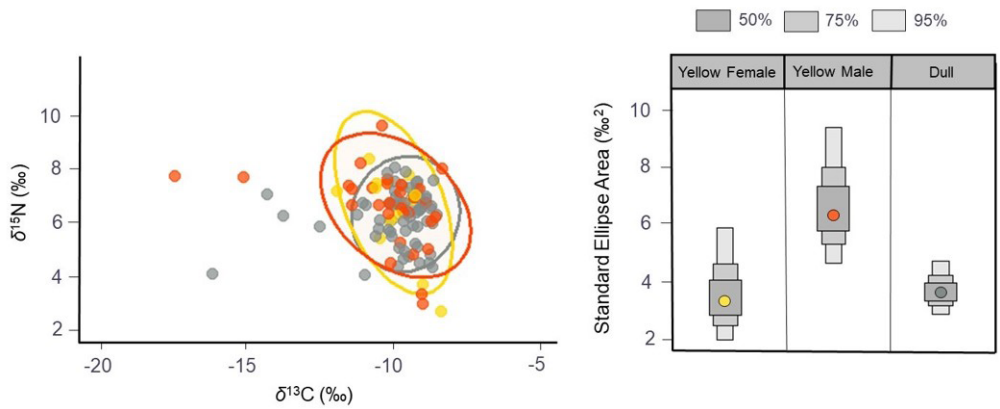

Figure 3. Estimation of the width of the isotopic niche space of yellow females (yellow dots), yellow males (orange dots), and dull individuals (gray dots) of Saffron Finch with the isotopic values of wing feathers. The boxes on the right indicate credibility intervals of 95, 75 and 50% and the dots represent the SEA.B value.

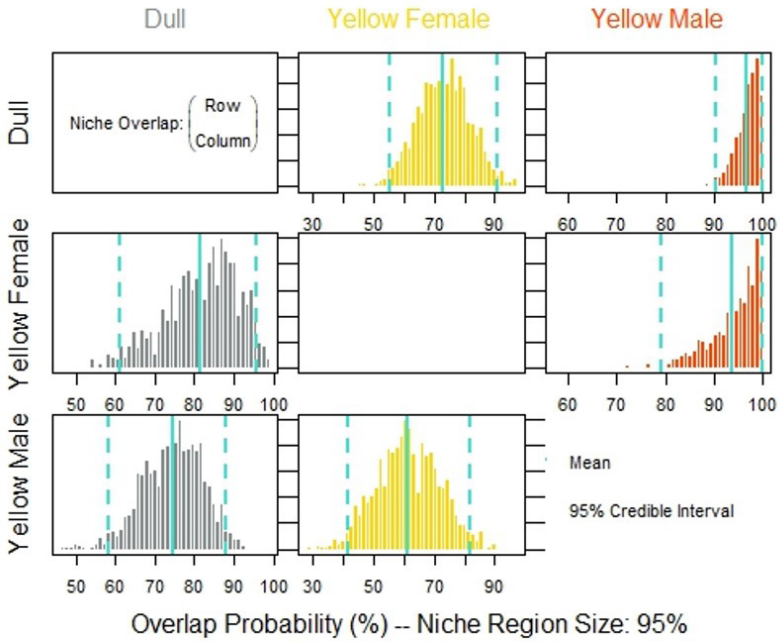

Figure 4. Bayesian plot of the posterior probability distribution of niche region metric (%) for yellow females (in yellow), yellow males (in orange) and dull individuals (in gray) of Saffron Finch. The posterior means are represented by solid lines, and the 95% credibility intervals by dotted lines; both are displayed in turquoise color.

of different amino acids vary, and the distinct amino acid composition of these tissues can also contribute to variations in $\delta^{13}C$ values.

The results did not evidence a notable shift in isotopic space comparing tissues of different times of integration of the diet. The isotopic space of yellow males was greater than yellow females and dull individuals, indicating greater dietary diversity due to greater intra-individual variation in diet. The isotopic space of yellow females and males showed greater variation in $\delta^{15}N$. However, yellow females had the lowest isotopic space among the categories. While males are more dominant in the exploitation of food resources, females must invest in resources more related to reproduction (Walker et al., 2014). Competition-driven partitioning of food resources plays an important role in driving carotenoid-based plumage coloration among age classes and sexes in species, such as Saffron Finch, that delay plumage maturation (Dale et al., 2015; Marques-Santos et al., 2018; Pagani-Nuñez et al., 2019).

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