



Diet and habitat aridity affect osmoregulatory physiology: An intraspecific field study along environmental gradients in the Rufous-collared sparrow

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ABSTRACT

The urine field osmolality in *Zonotrichia capensis* along a latitudinal gradient in rainfall and temperature in Chile was examined. We also investigated latitudinal variation in the renal traits that mediate how these birds cope with dehydration. We used the $\delta^{15}\text{N}$ of this species' tissue to investigate whether the reliance on animals and seeds varied among birds and if it had any effect on excretion and renal traits. We found a significant latitudinal variation in urine osmolality, a variable that was correlated with habitat aridity. We also found that the kidney size and proportion of kidney devoted to medullary tissue differed between birds from arid and mesic localities, but not in a linear fashion with aridity. The increment in the position in the food web, as measured by $\delta^{15}\text{N}$, led to an increment in urine osmolality, without changes in kidney features. Our data suggested that differences in dietary habits in the field could be not extended enough to cause changes in the kidney structure in Rufous-collared sparrows.

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1. Introduction

The water efflux in birds comprises the sum of evaporative water loss and the urinary and fecal water losses. Although in birds the evaporative water loss is greater than the urinary losses, excretory water loss still is a substantial proportion of total water loss (ca. 10–40% of total water loss; Moldenhauer and Wiens, 1970; Giladi and Pinshow, 1999). The ability to conserve water has been related to the ability to produce more concentrated urine and with an increase in the medullary tissue in avian kidneys (McNabb, 1969; Johnson, 1974; Johnson and Skadhauge, 1975; Casotti and Richardson, 1992; Casotti and Braun, 2000). Accordingly, species that live in an arid environment can excrete more concentrated urine and contain a larger percentage of medulla in their kidneys compared with species from mesic areas (Johnson, 1974; Casotti and Richardson, 1993; Casotti and Braun, 2000). Although some studies have shown that the ability to concentrate the urine is related to differences in renal structure (Poulson, 1965; Johnson and Ohmart, 1973a,b; Johnson and Skadhauge, 1975; Skadhauge, 1976; Goldstein and Braun, 1986; Casotti et al., 1998), others have failed to find any correlation between urinary concentrating abilities, kidney structure and with the habitat of the species (Johnson, 1972; Goldstein and Braun, 1989). As pointed out by Goldstein and Braun (1989), these comparisons suggest that there are broad scale influences, such as body size and phylogeny. With this in mind, studies in closely related birds would be more useful

in determining differences in the renal structure and function in birds with broader ecological differences. For instance, Casotti and Richardson (1992) studied Honeyeaters (Meliphagidae), and found that species from arid zones had a higher percentage of medulla in their kidneys, whereas species from wet zones had a higher percentage of renal cortex (see also Johnson and Mugaas, 1970; McNabb, 1969; Goldstein et al., 1990). To date, attempts to correlate osmoregulatory traits in birds with climate, in general remain equivocal.

On the other hand, several studies have shown that dietary composition can affect kidney structure and function in birds (McNabb et al., 1972; Ward et al., 1975a,b; Singer, 2003; Sabat et al., 2006a,b). For example, changes in dietary protein levels elicit a renal response, such as an elevated urine flow and medulla hypertrophy associated with an increase in the amount of nitrogenous waste produced (Goldstein et al., 2001; Sabat et al., 2004b; Aldea and Sabat, 2007). This introduces an additional variable that could confound any association between habitat and renal function. Thus, it is possible that kidney features could be shaped by the interaction of climatic and dietary characteristics. It has been reported that kidney mass and percentage of medullary tissue seems to be greater in birds inhabiting an arid environment and having a largely insectivorous diet than birds inhabiting mesic environments and eating a nectarivorous diet (Casotti and Richardson, 1992, 1993; Casotti et al., 1998).

Although several studies have examined renal function in birds at the interspecific level, relatively few have sought to understand renal function at the intraspecific level (Goldstein and Zahedi, 1990; Goldstein et al., 1990; Sabat et al., 2006b). Intraspecific differences in the physiology of geographically separated populations can provide insight into the evolutionary processes that permit species to cope with

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environmental variability. These studies are, therefore, important for understanding the origin of physiological diversity and the evolution of physiological tolerance (Garland and Adolph, 1991; Spicer and Gaston, 1999; Goldstein and Pinshow, 2006). In order to understand the proximal mechanism that lead to those differences, and also to increase the range of species for which osmoregulatory traits have been investigated in the field, we analyzed osmoregulatory features in four population of the Rufous-collared sparrow, *Zonotrichia capensis* (Passeriformes: Emberiziidae) in Chile. Chile experiences many changes in the availability of water and environmental temperatures. We hypothesized that *Z. capensis* might exhibit physiological diversity in renal traits in the field, along a geographical scale. Specifically we predicted that renal traits related with the ability to concentrate the urine and the concentration of body fluids might be positively correlated with the habitat aridity and also with their relative position in the food web. However different environments can often show temporary variations in food availability in both quality and quantity, and this may induce dietary shifts (Sabat et al., 1998). We also assessed if dietary habits influence kidney function. Due to the fact that dietary switches are often coupled with changes in the protein content of the food supply (Bell, 1990), we can use the nitrogen isotopic analysis of pectoralis to estimate their relative trophic position in the food web and therefore as an indirect index of the protein consumption of birds (Schondube et al., 2001). Using the nitrogen isotope ratio of a consumer's tissues to assess the relative position in the food web relies on two observations: 1) tissues often reflect the isotopic composition of an animal's diet (Hobson and Clark, 1992), and 2) although there can be significant inter-habitat variation, food sources are significantly depleted in ^{14}N relative to consumers (Gannes et al., 1997; Robbins et al., 2005). Besides, the isotopic signature of different tissues will reflect diet at different temporary scales. Thus, the choice of tissue type for isotopic analysis will depend, on the ecological question of interest. In this study, because *Z. capensis* often switch diets seasonally (Sabat et al., 1998), an isotopic signal of a tissue which integrates the trophic signal faster, would be an adequate indicator of the present relative position in the food web. Here we used isotopic signature of pectoralis muscle, which have an isotopic turnover of days to weeks (Hobson and Clark, 1992). Thus, the analyses of this tissue can provide short term dietary information.

2. Methods

2.1. Field methods and study site

Rufous-collared sparrow, *Z. capensis* (Passeriformes: Emberiziidae) is opportunistic, feeding on seeds and insects and showing seasonal dietary shifts according to food availability. The proportion of insects in the diet of sparrows is higher in winter than in summer in the same locality (López-Calleja, 1995; Sabat et al., 1998). In addition to this temporal variability, *Z. capensis* differ in their feeding habits among populations (López-Calleja, 1995; Novoa et al., 1996). Consequently, *Z. capensis* is a well-suited model to study population differences in physiological and ecological variables. This species have 22 races along its wide distribution range and, although we have no information about the mobility of the studied populations, the race we studied here, *Z. capensis chilensis*, has been reported as non migratory (Chapman, 1940). The only recognized migratory race is *Z. c. australis*, which is distributed southern than our range of study. This fact might ensure enough isolation among populations to promote population phenotypic differences.

We collected birds at sites along an approximately 1600 km, north to south, latitudinal gradient. A total of 38 birds were mist-netted during 2005 and 2006 from four localities in Chile, differing in more than three orders of magnitude of precipitation (Di Castri and Hajek, 1976). The localities were 1) Copiapo (27° 21' S, 70° 24' W), 2) La Serena (29° 54' S, 71° 15' W), 3) Quebrada de la Plata (33° 31' S, 70° 50' W) and 4) Valdivia (39° 48' S, 73° 14' W). Copiapó and La Serena are semi arid zones. Quebrada de la Plata has a Mediterranean climate and

Valdivia is a humid temperate oceanic area. We calculated an index of aridity (Q_i) of the month of capture among the different localities, which account for climatic variation (Table 1). The aridity index (Q) for each month was calculated as:

$$1000 \times \text{pp} / (T_{\text{max}} + T_{\text{min}})(T_{\text{max}} - T_{\text{min}})$$

where pp is the total monthly precipitation (mm) and T_{max} (°C) and T_{min} (°C) are the mean maximum and minimum temperature, respectively, for each month (Bozinovic et al., 2007). Q tends to be low in hot, dry areas and increases rapidly as the habitat becomes more mesic. After capture, we collected a sample of ureteral urine by inserting a small closed-ended cannula into the birds' cloaca (Goldstein and Braun, 1989; Sabat and Martinez del Rio, 2002). Urine drained into the cannula via a window placed dorsally over the ureteral orifices (Goldstein and Braun, 1989). Urine samples were centrifuged (5000 g, 5 min) cold stored (4 °C) for later osmometry analysis. The osmolality of the supernatant was measured (Wescor 5130B).

2.2. Laboratory measurements

Birds were transported to the laboratory at the University of Chile, Santiago, Chile (33° 27' S, 70° 42' W) and killed by CO₂ exposure. Birds were dissected and kidneys were removed from the synsacrum, weighed (± 0.001 g) and preserved in paraformaldehyde-glutaraldehyde. We estimated the area of medulla by point counting using the Cavalieri Principle on the right kidney, which was processed for routine light microscopy (Gundersen et al., 1988; Wauri, 1989). The percentage of renal medulla was determined as the area of medullary tissue divided by the total area of the kidney section. Medullary cones were dissected from the left kidney using a dissecting microscope and the entire collection was weighed (± 0.001 g) and counted.

Samples of pectoralis muscle were dried to constant mass at 80 °C and then de-fatted by ether extraction. Nitrogen isotope ratios 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 Columbia University Biosphere 2 stable isotope facility. Stable isotope ratios were expressed using standard delta notation (δ) in parts per thousand (‰) as:

$$\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

where R_{sample} and R_{standard} are the molar ratios of $^{15}\text{N}/^{14}\text{N}$ of the sample and reference, respectively. Samples were referenced against the international standard, atmospheric nitrogen.

2.3. Data analysis

As body mass differed significantly among populations ($F_{3,34} = 3.16$; $P = 0.04$) (see Table 1), we analyzed kidney mass by an analysis of covariance (using body mass as a covariate, ANCOVA). Data are presented as a mean \pm 1 SD. Characteristics of renal morphology were

Table 1

Geographical origin, and climatic data for four populations of Rufous collared sparrows in Chile

Locality	Month	T_{max}	T_{min}	pp	Q_i
Copiapo	July	20.8	4.8	6.9	4.3
La Serena	July	15.8	8.2	28.5	9.0
Quebrada	August	16.2	4.2	56.5	33.2
Valdivia	January	23.3	10.9	66.1	23.9

The aridity index (Q_i) was calculated using pp (the total monthly precipitation, mm), T_{max} and T_{min} (the mean maximum and minimum temperature, respectively, for each month). This index has high values when there is high precipitation, and lower temperatures; and low values when there are low precipitation and higher temperature).

examined by ANOVA (number of medullary cones by gram of kidney, percentage of kidney comprised by medullary tissue) and by ANCOVA (using kidney mass as a covariate) for medullary tissue. As the exponent of the allometric relationship between body and kidney mass was not significantly different from 1 ($t=1.22$, $P=0.14$), it would be appropriate to compare the percentage of body mass attributed to the kidney among the populations. Because $\delta^{15}\text{N}$ of primary producers could be affected by the climate of the capture sites (Robinson, 2001) and thus the isotopic signature of the consumers, we performed a series of multiple forward stepwise regression analysis using morphological and physiological variables as dependent variables and Qi and $\delta^{15}\text{N}$ as independent variables. This analysis allowed us to test whether climatic variables (integrated in Qi) and $\delta^{15}\text{N}$ (after removing the effect of Qi) may explain the variability of osmoregulatory parameters and morphology in *Z. capensis*. To satisfy normality assumptions of parametric statistical tests (Zar 1996), we arcsin square root transformed percentage data before analysis. As we could not obtain enough sample volume to measure the osmolality of body fluids in all individuals, the number of individuals used for each analysis may differ.

3. Results

The nitrogen signature of pectoralis ($\delta^{15}\text{N}$) differed significantly among localities (ANOVA; $F_{3,34}=13.112$; $P<0.001$, Table 2). Kidney mass also differed significantly among localities (ANOVA; $F_{3,33}=5.51$; $P=0.04$). The results of the *a posteriori* Tukey test revealed that birds from Valdivia had heavier kidneys than birds from Copiapo and La Serena, whereas those from Quebrada de la Plata differed from those from Copiapo (Table 2). The mass of medullary tissue was different among populations (ANOVA; $F_{3,33}=2.94$, $P=0.047$), and the *a posteriori* analysis revealed that birds from Copiapo had higher values than birds from Quebrada de la Plata (Table 2). Also, the number of cones per kidney mass is different among populations ($F_{3,34}=11.49$; $P<0.01$). The *a posteriori* Tukey test showed that birds from Copiapo, have more cones per gram of kidney than birds from the other three populations (Table 2). The percentage of kidney mass composed by medullary tissue differed significantly between Copiapo and Quebrada de la Plata and Valdivia (ANOVA; $F_{3,34}=3.21$; $P=0.035$, Table 2). Urine concentration seemed to increase higher up the food chain. We found a positive and significant link between urine concentration in the field and the $\delta^{15}\text{N}$ of pectoralis ($r^2=0.16$, $F_{1,32}=6.07$, $P=0.01$; Fig. 1). Results of the multiple regression analysis showed that urine osmolality was explained by Qi and $\delta^{15}\text{N}$ (multiple $r^2=0.46$, $P=0.001$). The magnitude of Beta coefficients in Table 3 allows us to compare the relative contribution of each independent variable in the prediction of the urine osmolality. The aridity index, Qi, explained 23% of the variance in urine osmolality. None of the independent variables explained a significant amount of the variance of the percentage of kidney mass, percentage of medulla, mass of medulla or

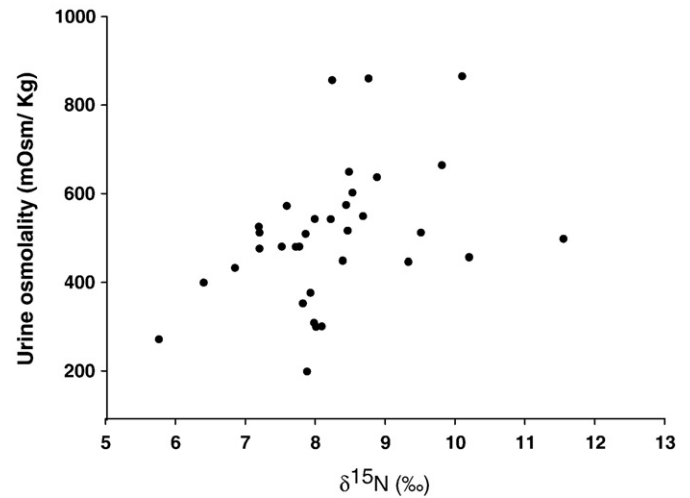


Fig. 1. Urine osmolality of freshly captured *Zonotrichia capensis* as a function of the $\delta^{15}\text{N}$ nitrogen isotopic signature of pectoralis.

the number of medullary cones by gram of kidney (Kidney mass: multiple $r^2=0.50$, $P=0.006$; % of Medulla: multiple $r^2=0.11$, $P=0.22$; Medulla mass of $r^2=0.12$, $P=0.19$; Cones by kidney mass: multiple $r^2=0.09$, $P=0.31$).

4. Discussion

In this study we assessed the putative effect of climate (i.e., aridity index, Qi) and dietary preferences (i.e., relative position in the food web, $\delta^{15}\text{N}$) on some kidney features and urine concentration in the field at the intraspecific level in birds. Kidney mass, the standardized number of medullary cones and the percentage of medullae of kidney differ among populations of *Z. capensis*. Also, *Z. capensis* varied in urine osmolality and kidney morphology among populations in an aridity gradient in Chile. Field urine concentrations were higher in the region with low water availability and higher temperatures, than in those characterized by humid and colder climates. In addition, we found a significant association between urine osmolality and the position in the food web, as revealed by the nitrogen isotopic signature of pectoral muscle.

Also, we found a higher variation in the nitrogen isotopic signature among bird populations (Table 2). Differences in isotopic signatures among birds may be caused when birds feed on a single and different type of resource, and also when birds consumes for extended periods of time different mixture resources. When the stable isotope analysis is combined with previous dietary studies of *Z. capensis* (López-Calleja, 1995; Sabat et al., 1998) it seems that some individuals were probably mainly seed-eaters, whereas others were omnivorous feeding on a mixed diet of seeds and invertebrates, and mainly invertebrates. A high value of $\delta^{15}\text{N}$ indicates that birds were in average, in a high trophic position and that they were probably consuming a larger proportion of animals. Thus, at least for the period of isotopic incorporation of the pectoralis muscle (i.e., days to a few weeks), birds with higher values of $\delta^{15}\text{N}$ in pectoralis were probably ingesting high amounts of protein.

Table 2

Body mass, isotopic signature of pectoralis and kidney measurements in *Zonotrichia capensis* from four localities in Chile

Locality	Copiapó (8)	La Serena (5)	Quebrada de la Plata (14)	Valdivia (11)
Body mass (g)	19.38±1.15 ^b	19.70±1.37 ^b	21.37±1.10 ^a	19.15±3.19 ^b
$\delta^{15}\text{N}$	9.07±0.75 ^{a,c}	11.78±2.84 ^a	7.90±0.67 ^b	7.33±1.29 ^{b,c}
Kidney mass (g)	0.200±0.038 ^b	0.224±0.016 ^{b,c}	0.247±0.038 ^{a,c}	0.261±0.030 ^a
Medullary cones	83.3±21.2 ^a	59.9±14.3 ^b	54.9±16.9 ^b	78.4±13.7 ^a
Medullary cones (n°/g kidney)	425±121 ^a	264±63 ^b	224±67 ^b	303±57 ^b
Medullary mass (g)	12.5±5.7 ^a	9.7±7.2 ^{a,b}	9.0±4.2 ^b	9.8±2.6 ^{a,b}
% Medullary tissue	6.07±2.31 ^a	4.53±3.07 ^{a,b}	3.67±1.55 ^b	3.98±0.82 ^b

Number of birds in parenthesis. Different superscripts indicate significant differences between populations after the Tukey test.

Table 3

Results of multiple regression analysis of urine osmolality, aridity indexes and nitrogen isotopic signature ($\delta^{15}\text{N}$) of *Zonotrichia capensis* along a latitudinal gradient in Chile

	r^2	Beta	% Explained	B	SE of B
Urine	0.46				
Qi		-0.48	23.0	-6.7	2.3
$\delta^{15}\text{N}$		0.36	12.9	33.0	15.2

Beta is the standardized regression coefficient, and B is the regression coefficient.

4.1. Are aridity and/or reliance on an invertebrate diet linked to differences in urine concentration?

Birds dwelling in dry and hot environments are osmotically stressed due to the necessity to conserve water (Goldstein and Braun, 1986; Casotti and Richardson, 1993; Tieleman and Williams, 1999; Tieleman and Williams, 2000). Our results suggest that *Z. capensis* are challenged by different levels of hydric stress along the latitudinal gradient (see Cavieres and Sabat, 2008). The significant effect of Qi on urine osmolality supports the notion that the dry north represents an environment where birds are challenged by both the absence of fresh water and high temperatures. This is confirmed by the significant and positive relationship between urine osmolality in the field and the Qi (Table 3). Our results agree with previous reports in which osmoregulatory parameters have been studied (Goldstein et al., 1990; Goldstein and Zahedi, 1990; Sabat et al., 2006a; Aldea and Sabat 2007), and also demonstrate that osmoregulatory features of birds may be challenged in a continuous fashion by temperature and water availability along climatic gradients. In addition, in this study urine osmolality appears to be related to the position of bird in the food web in *Z. capensis* (Fig. 1). A higher value of $\delta^{15}\text{N}$ indicates that birds are in a high position in the food web and they are probably consuming more animal prey and thus more protein. Thus, urine osmolality of *Z. capensis* is mainly influenced by water availability and temperature along the aridity gradient in Chile, and to lesser extent by the consumption of animal prey.

4.2. Are aridity and/or reliance on invertebrate diet associated with relative kidney size and the proportion of kidney devoted to medullary tissue?

The renal response of birds to environmental conditions can be separated into two categories. First, birds may exhibit an immediate physiological regulation in urine production and concentration, depending on their actual hydric state (Braun, 1978). On the other hand, modifications of kidney size and structure may be produced by acclimation or acclimatization to environmental conditions and also by adaptive adjustments in an evolutionary scale (Sabat et al., 2006a). In the present study, the comparison among populations supports the idea that the kidney of *Z. capensis* differ along the aridity gradient. However, the multiple regression analysis did not reveal that nitrogen signature and Qi had a significant effect on kidney size. Because Qi reflects the climatic condition of a region on a monthly basis, the modifications of kidney mass appears to respond to long-term variations and not to intra-annual variations in abiotic factors. This fact suggests that long-term changes in climatic conditions have a more pronounced effect on kidney size, than fluctuations occurring on a seasonal scale (see Casotti, 2001). Our results also support common observations on the kidney mass difference at interspecific level. Birds had relatively larger kidneys in the mesic south than in the arid north. This result is concordant with the observation that animals that face antidiuresis chronically in dry environments tend to have smaller kidneys than those that live in wetter environments (reviewed by McNab, 2002, see also Sabat et al., 2006b).

In addition, the percentage of kidney devoted to medullary tissue and the relative number of medullary cones were not affected by Qi, even though both variables were higher in the more arid locality of Copiapo, than in the southern localities. However, the analysis of total mass of medulla and the total number of medullary cones revealed an unexpected result: both variables were higher in the semi arid Copiapo and the humid Valdivia than in central populations. Although we have no explanation for this pattern, we hypothesize that the total mass of medulla and the total number of medullary cones *per se* might not be related to arid conditions. Instead, the medullary tissue and the number of medullary cones relative to kidney mass do, as demonstrated previously in birds at inter and intra-specific scale (Casotti and Braun, 2000; Sabat et al., 2006b). Nevertheless, the fact that the variation in the

percentage of medulla and the relative number of medullary cones is not consistent with aridity of the month of capture suggests that the internal structure of kidney (i.e., medullary tissue and cones) does not respond to gradual and immediate changes in environmental conditions in *Z. capensis*, whilst the urine osmolality does.

Kidney structure and size appears to be unaffected by the relative position in the food web. This contrasts with previous laboratory studies in *Z. capensis*, which have shown that kidney structure and function can be modified by dietary protein (Sabat et al., 2004b). This apparent contradiction could be explained by at least by two nonexclusive explanations. Firstly, it can be argued that differences in protein consumption do not differ between birds, even though they are probably consuming different proportions of animal prey. However, assuming that differences in $\delta^{15}\text{N}$ reflect different position in the food web and dietary habits, this hypothesis should be ruled out because insects and seeds (the main items consumed by *Z. capensis*) differ considerably in protein content (Karasov, 1990). On the other hand, the putative effect of diet on kidney structure relies on an assumption which has not been tested. It assumes that kidney structure can respond rapidly to changes in diet. However, if the time required for kidneys to respond to a diet is long relative to the rate at which diet composition changes at the field, the acclimation in kidney structure and function may not occur. Thus, the response of kidney would lag behind a relatively rapidly changing diet composition. Regarding this possibility, changes in kidneys elicited by an acclimation to protein can be produced in three or four weeks in *Z. capensis* (Sabat et al., 2004a; Aldea and Sabat, 2007), whereas changes in dietary habits could be faster. In this vein, the isotopic composition of a tissue is the result of the integration of isotopic inputs over time, and the time window of isotopic incorporation integrated by the composition of a tissue depends on the turnover rate of its constituent elements (Tieszen et al., 1983). The isotopic composition of tissues with high turnover, such as muscle may reflect an integration of recent inputs (e.g., 12 days, Hobson and Clark, 1992), which does not reflect the temporal scale at which acclimation occurs. However, this may also explain why physiological parameters that can undergo rapid modifications, such as, urine concentration, are correlated with $\delta^{15}\text{N}$. Because the isotopic composition of pectoralis should be responsive to recent temporal changes in diet isotopic composition, our data suggests that field differences in dietary habits are not extended enough to lead changes in the kidney structure in Rufous-collared sparrows. Instead, the response of *Z. capensis* to an increase in the position of the food web is through physiological regulation, e.g., elevating urine concentration, without any further modification of the kidney.

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