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ARTICLE

Diet of the Bobolink (*Dolichonyx oryzivorus*) in rice fields on its wintering grounds in Argentina

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ABSTRACT: Winter diet of Bobolinks (*Dolichonyx oryzivorus*), a bird considered a pest of rice fields, is known to consist primarily of seeds. However, it is not yet possible to establish the extent to which non-rice plants and animal components contribute to its diet. To contribute to these issues, we studied the diet of the Bobolink found in rice fields on its wintering grounds in Santa Fe, Argentina, to provide information on (*i*) the composition of the diet and (*ii*) the relative importance of plant and animal components in the diet and of the different prey categories. We captured Bobolinks with mist nets and obtained samples of stomach contents by warm water and emetic-based regurgitation to determine the composition of the diet ($n = 46$ samples) and the importance of the different prey ($n = 25$ samples), mainly during March, just prior to northbound migration. We confirmed that the Bobolink's diet in this region during this period is predominantly herbivorous (97%) and rice-based (55%), although it also consumes a large number of seeds of non-cultivated plants that represented 42% of the diet. Invertebrates, although of less importance than plant components (3%), had been consumed by 97% of captured individuals. Our results document the importance of non-cultivated plants and animal prey in the diet of Bobolinks in addition to rice.

KEY-WORDS: agroecosystems, birds, emetic, foraging, pests.

INTRODUCTION

Bobolink (*Dolichonyx oryzivorus*), an obligate-grassland species, has one of the longest annual migrations of any New World passerine (≈ 20,000 km round trip), with breeding and wintering grounds located in North America and South America, respectively (Renfrew *et al.* 2015). Reports of population declines on the breeding grounds (Sauer *et al.* 2004) highlighted the need for studies on the natural history and ecology of the species, in order to identify threats to the development of conservation strategies, mainly on the wintering grounds where studies have been more limited (Renfrew *et al.* 2015).

Knowledge of Bobolink ecology on its wintering grounds has increased significantly in the last decade (López-Lanús *et al.* 2007, Renfrew & Saavedra 2007). Additional research needed to guide conservation of Bobolinks includes investigating its trophic ecology on wintering grounds (Renfrew & Saavedra 2007, Blanco & López-Lanús 2008), because the Bobolink is considered a pest of rice fields (López-Lanús *et al.* 2007, Renfrew & Saavedra 2007), although the extent to which the species relies on rice during austral summer remains largely unknown (Renfrew *et al.* 2017). Although the diet of Bobolink on its wintering grounds is known to consist primarily of plant material (Renfrew & Saavedra 2007), no empirical studies have established the importance of rice and animal parts (invertebrates) in relation to other items in the diet. Recently, Renfrew *et al.* (2017) used stable isotopes to demonstrate that Bobolinks rely on rice for approximately one-third of their diet and that importance of rice in the diet is higher in rice fields than in natural grasslands within their wintering grounds.

Beyond these contributions, however, yet there is no quantitative information on the diet of the Bobolink on different parts of its wintering grounds, information necessary for a more precise quantification of damages and benefits that the species contributed to rice-field agroecosystems, based on the relative importance of rice, non-cultivated plants and invertebrates in the Bobolink diet. Here, we studied the diet of Bobolinks found on rice fields in Santa Fe, Argentina, an area where a large concentration > 100,000 individuals has been documented during several seasons (López-Lanús *et al.* 2007, López-

Lanús & Marino 2010). Our objectives were to provide information on (*i*) the diet of the species and (*ii*) the relative importance of plant and animal components of the diet and of the different prey categories.

METHODS

Study area

The study area included the rice zone located in the eastern part of Santa Fe province, Argentina (Fig. 1). This zone covers a north-south band, approximately 10–20 km wide, west of the San Javier River, part of the Paraná River system, from approximately Romang (29°29'S; 59°45'W) in the north to Cayasta (31°11'S; 60°9'W) in the south. This area is characterized by xerophilous forests of the Espinal ecoregion at higher elevations, and by marshes and flooded grasslands in lower elevations (López-Lanús & Marino 2010).

Bird captures and samples of stomach contents

We captured Bobolinks (n = 70 individuals) with mist nets to obtain samples of stomach contents in three rice fields from 14 January to 12 March 2016 (Fig. 1). We placed one to three mist nets in foraging sites and near day roosts. We opened the mist nets only after we had observed foraging behavior of flocks for at least 2 h to obtain captures after individuals have foraged to increase the regurgitation rate (Durães & Marini 2003).

We assessed the diet with a combination of techniques. First, we used warm water to force regurgitation because this technique is considered less harmful to the bird than emetic-based regurgitation, although also less effective (Major 1990, Poulin *et al.* 1994). Warm water was administered orally into the beak and down the esophagus by means of a 1 cm^3 syringe connected to a 1.5 mm diameter silicone tube that had been moistened in saline solution or vaseline. We inverted the bird over a plastic cup so that, as fluid was forced into its stomach, the excess fluid plus the stomach contents flowed into the cup (Hess 1997). We obtained 13 samples using this technique. Individuals that did not regurgitate on the first attempt were released to minimize stress. Preliminary inspection in the laboratory revealed few food items, represented by highly disaggregated seeds and arthropods. Given the low effectiveness of this method because very little food was regurgitated, we chose to use an emetic (potassium antimony tartrate) to obtain additional samples of stomach contents. Following Poulin *et al.* (1994), we gave birds 0.8 cm^3 of a 1.5% solution of antimony potassium

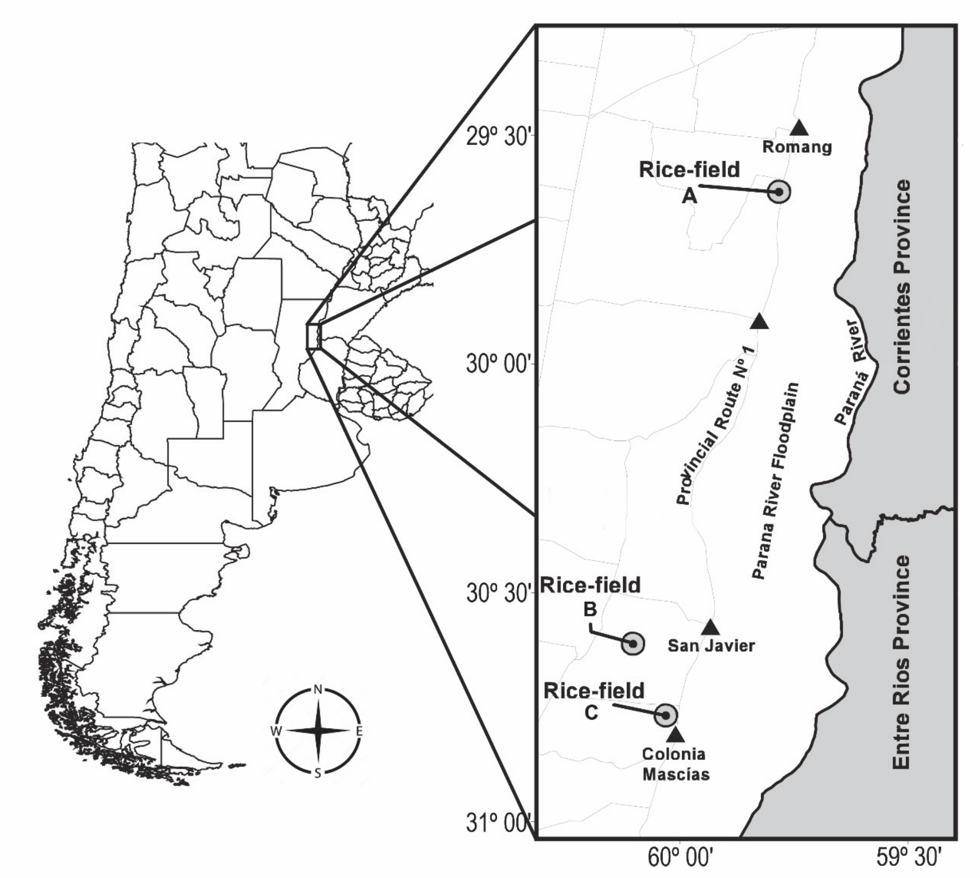

Figure 1. Location of the three rice fields (**A**, **B** and **C**) where Bobolinks (*Dolichonyx oryzivorus*) were captured in mist nets and sampled for gut contents.

tartrate per 100 g of body mass. After the solution was given orally through a 1.5-mm diameter flexible plastic tube attached to a l cm^3 syringe, we placed the bird in a small box lined with absorbent paper for 15 to 20 min to allow them to regurgitate and also to recover after regurgitation (Poulin *et al.* 1994, Johnson *et al.* 2002, Carlisle & Holberton 2006). We obtained 23 samples using this technique after which we discontinued its use because mortality rate was high (33%, *n* = 10 individuals). Two additional samples were from the stomach contents analysis of two individuals that did not regurgitate and died. Dead individuals were collected and deposited in the collection of the National Institute of Limnology (INALI: CONICET-UNL). Subsequently, we resumed using the saline solution regurgitation method obtaining eight more samples, and all of these birds were released successfully after samples were obtained. All gut contents were preserved in 70% ethanol.

Analysis of digestive tract contents

We examined contents of digestive tracts under a Nikon® stereoscope binocular. We counted, measured and classified prey categories to the lowest possible taxonomic level. We considered all samples, regardless of the collection technique to inventory the total number of prey items found in the diet of Bobolink (*n* = 46 samples). These samples were obtained from 14 January to 12 March 2016. However, to assess relative importance of prey categories, we did not use samples obtained by water-based forced regurgitation because this technique likely under sampled vegetal fraction of stomach contents (26.4 *vs.* 2.6 plants by sample in emetic- and water-based forced regurgitation samples, respectively) affecting the comparison with the animal fraction, which was affected, but to a lesser extent, by the technique (1.4 *vs.* 0.5 animal prey by sample in emetic- and water-based forced regurgitation samples, respectively). Thus, we calculated the number and frequency of prey types only from 25 samples to assess relative importance of prey categories, 15 represented only by emetic samples, two only by digestive tract dissection, and eight represented by both emetic and digestive tract samples. In this way, the samples considered in this analysis were obtained only at the beginning of March (between 08–11 March 2016). Given that we could only identify mature seeds in the digestive tract, the importance of rice in the diet was underestimated because we could not quantify immature liquid or "milky" stages of rice. However, because estimation of relative importance of rice was based on samples obtained in the final stage of rice production during March, it is unlikely that the immature liquid or "milk" stage of rice represented a high proportion of the rice consumed at this stage of the crop. We quantified the abundance (N%), frequency of occurrence (F%), volume (V%) and index of relative importance (IRI%) of each category of prey to determine the contribution of each category to the diet of the species. We calculated the biovolume of ingested categories by approximation to regular geometric shapes. The volume of fractionated and disarticulated prey was estimated by comparison with reference prey. However, this was implemented mostly for animal prey since seeds were found intact in most cases. Index of relative importance of prey (Pinkas *et al.* 1970) was used to determine the importance of each prey category in the diet.

RESULTS

General composition of the diet

We recorded 1597 items from all samples (*n* = 46 samples), corresponding to 17 food item categories (Table 1). Of the total items, 1521 (95%) were from plants, represented by 6 families and 10 plant species (in the case of rice, *Oryza sativa*, two varieties were recorded and treated separately: Rice and Red Rice, cultivated and weed, respectively) and 76 items (5%) were from invertebrates, represented by 6 orders (Table 1). Plant items were mainly Cyperaceae (48%) and Poaceae (43%). The high degree of digestion did not allow finer taxonomic resolution of invertebrate samples, with the exception of nymphs of Hemiptera assigned to the family Pyrrhocoridae and a Hemipteran assigned to the family Lygaeidae.

Relative importance of prey categories

Among the 25 individuals represented by complete (or mostly complete) stomach contents, plants represented almost the entire diet (IRI% = 97%), while animals were a minor component (IRI% = 3%), reflecting the greater number and volume of seeds in the diet (Table 1). However, although only 4% of the total number of prey items was invertebrates, 92% of the samples had at least one item from this category, indicating that most Bobolink individuals consumed at least some animal prey. The highest number and frequency of invertebrate in the diet corresponded to the orders Hemiptera and Coleoptera (Table 1).

Rice was the most important component of the diet because it was the most frequent prey category (F% = 73%) and represented the largest volume (V% = 59%) of items in the diet (Table 1). The most abundant component of the diet was a species of the family Cyperaceae (N% = 48% *vs.* N% = 15% for rice) that, although it also had a high frequency among samples (F% = 58%), had a minor relative importance in the diet than rice due to the

Table 1. Total number (*n* = 46 samples) and importance (*n* = 25 samples) of the prey categories found in diet samples of Bobolinks (*Dolichonyx oryzivorus*) on its wintering grounds in Argentina. Prey categories within the plant and animal fractions are arranged in decreasing order according to the number of categories of prey recorded. Values in bold indicate the totals for the plant and animal fractions. The script indicates that the item was not found in this group of samples. Total number of prey categories is based on all samples. Abbreviations: percentages of number (N%), frequency (F%), volume (V%) and index of relative importance (IRI%); NI: not identified.

Prey category	Total number (n)	Importance			
		N%	F%	V%	IRI%
Plant fraction	**1521**	**96**	**96**	**95**	**97**
Echinochloa sp.	235	15	15	6	3
Oryza sativa (Rice)	232	15	73	59	55
Paspalum sp.	96	6	35	3	3
Oryza sativa (Red Rice)	94	5	15	20	4
Cyperaceae NI	733	48	58	4	30
Polygonaceae	63	4	42	1	2
Solanum sp.	30	2	8	0	0
Conyza bonariensis	20	1	31	0	0
Amaranthus sp.	8	0	8	0	0
Sp. NI (Asteraceae?)	5	0	12	0	0
Sp. NI	5	0	4	0	0
Animal fraction	**76**	**4**	**92**	**5**	**3**
Coleoptera	25	1	42	3	1
Hemiptera	18	1	23	1	1
Diptera	4	0	12	0	0
Hymenoptera	3	0	4	0	0
Psocoptera	2	0	8	0	0
Araneae	1	-	-	-	-
Undetermined	23	1	50	0	0

smaller size and volume of the seeds (Table 1). Overall, seeds and fruits of non-rice species were more abundant and frequent than rice among the individuals analyzed, but rice accounted for a greater volume of the diet due to the larger size of the grains (Table 1, Fig. 2).

DISCUSSION

Bobolinks are persecuted by rice producers in their wintering grounds because they are considered pests of rice crops (López-Lanús *et al.* 2007, Renfrew & Saavedra 2007). However, knowledge of its trophic ecology on the wintering grounds is limited because, although Renfrew *et al.* (2017) estimated the importance of rice, no previous study has assessed quantitatively the importance of non-cultivated plants and animal prey in their diet. In this study, in agreement with previous studies of the species (*e.g.*, Martin & Gavin 1995, Renfrew & Saavedra 2007), we confirmed that Bobolinks have a diet that is

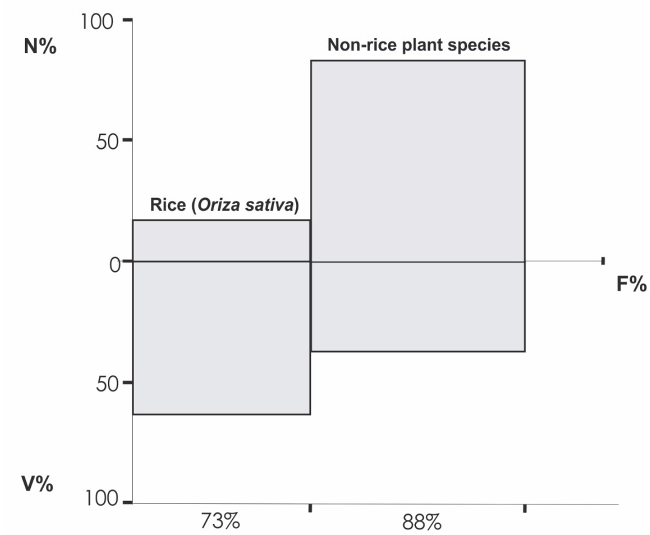

Figure 2. Relative importance index (IRI%) of rice (*Oryza sativa*) *vs.* other plant species in the Bobolink (*Dolichonyx oryzivorus*) diet in Santa Fe, Argentina. Abbreviations: percentages of number (N%), volume (V%) and frequency (F%).

predominantly herbivorous and rice-based during March, just prior to northbound migration (*i.e.*, emetic-based samples, on which the calculations of relative importance of the prey were based, were obtained at the beginning of this month) in areas of intensive rice production. However, our results also indicate that Bobolinks consume more seeds of non-cultivated plants. Invertebrates, although of minor importance in relation to plants, were present in the diet of most individuals, possibly as a result of occasional intake of invertebrates found while birds were searching for seeds (*e.g.*, arthropods associated with seeds of plants).

General composition of the diet

Grains of mature Rice (*O. sativa*) represented an important fraction of the diet in this study, which is in agreement with previous studies that have examined diet composition of Bobolinks in rice fields within their wintering area (*e.g.*, López-Lanús *et al.* 2007, Renfrew & Saavedra 2007, Blanco & López-Lanús 2008). Rice was more important in the diet because it was abundant, frequent, and comprised the greatest volume of the plant component in the diet. The observed relative importance of rice in the Bobolink diet (55%) was lower than that reported by Renfrew *et al.* (2017), who found that the relative proportion of rice was approximately 69% in a rice-producing region in Bolivia. Our results about the relative importance of the rice correspond only to the days before harvest, *i.e.* we only used samples obtained by emetics and stomach analysis obtained between 08–11 March 2016 to determine the relative importance of the different prey, when the rice is already in a mature and hard stage. Under these conditions, Bobolinks could include a greater proportion of other plants in their diet because rice is no longer found in the milky and soft stages preferred by the species in the studied rice fields.

Echinochloa sp. and a Cyperaceae species were among the most abundant food items. However, because these seeds and fruits are small, these were lower in volume of diet and relative importance than rice. Seeds of *Echinochloa* sp. probably corresponded to *E. colona*, a naturalized grass introduced from Europe (Pensiero & Gutiérrez 2005) that previously was reported by López-Lanús *et al.* (2008) as part of the diet of Bobolinks in the same area. *Cyperus* sp. is among the probable Cyperaceae genera corresponding to the species found in the rice field A, because this also has been reported as part of the diet of Bobolink in the area (López-Lanús *et al.* 2008). The same study also mentioned that Bobolinks feed on *Conyza bonariensis*, a grass species that was also found during the present study, mainly in samples from field A, where grasslands composed of this plant were observed. Other plant species present in the area that have been mentioned as part of the Bobolink diet (*e.g.*, *Echinochloa crus-galli*, *Echinochloa polystachya*, *Sorghum halepense*, *Hymenachne amplexicaulis*) were not found in samples during our study.

Remaining plant items corresponded to native or naturalized non-cultivated herbs and mostly coincided with those documented in previous studies. However, fruits of Polygonaceae and seeds of *Solanum* sp. (Solanaceae) and *Amaranthus* sp. (Amaranthaceae), although of little importance, had not previously been mentioned as part of the Bobolink diet in the area. Presence of Polygonaceae species has been reported in the Bobolink diet from breeding and stopover sites in the United States (Beal 1900, Meanley & Neff 1953). Presence of *Solanum* sp. seeds suggests that unless Bobolinks extract the seeds without ingesting the fruit, Bobolinks could also consume fleshy fruits such as those of these plant species. Solanaceae species have not been mentioned as part of the Bobolink diet in any previous study and, thus, this could simply correspond to an occasional intake, although it was found in samples from two individuals in rice field B.

Results of the current study also have expanded our knowledge of invertebrates consumed by Bobolinks. For example, although Hemiptera have been mentioned as present in the diet of the Bobolinks in the wintering area (López-Lanús & Marino 2010), the present study provides the first documentation of Hemiptera in diet samples; eight Hemiptera nymphs were found in the sample from a single individual Bobolink. Similarly, although some of the recorded invertebrate prey have been documented in samples from breeding and stopover sites *(e.g.*, Coleoptera, Hemiptera, Hymenoptera and Araneae; Beal 1900, Meanley & Neff 1953), documentation of invertebrate prey in the diet of Bobolinks on their wintering grounds was previously limited to caterpillars (Lepidoptera; Renfrew & Saavedra 2007, López-Lanús *et al.* 2008).

Considerations on the techniques used to study Bobolink diet

Our study showed that water-based forced regurgitation is a technique much less effective than emetic-based technique to study the Bobolink's diet. However, use of emetic implied a relatively high mortality rate that limited the number of samples obtained. This contrasts with studies that have shown that mortality caused by emetics is relatively low in icterids (Poulin *et al.* 1994, Poulin & Lefebvre 1995). However, responsiveness to emetics is highly species-specific (Durães & Marini 2003). Although the manipulation of birds mainly during the oral administration into the beak and down the esophagus by means of a syringe connected to a silicone tube can cause damage to the birds if not implemented correctly, all the birds that received the same treatment, but with warm water were not damaged and were successfully released. This indicates that the mortality was directly related to the emetic and not to the manipulation. Emetic solution was prepared in laboratory by a professional chemist to assure a correct preparation. Characteristics that differentiate the Bobolink from other icterids like the high energetic cost of migration and the use of agrochemicals in rice fields where they feed could be related to this sensitivity to the emetic. These aspects could be evaluated by studying the health conditions of these populations. Beyond this, we do not recommend the use of emetics for the study of the diet of Bobolink. Alternatively, the use of water-based forced regurgitation can be used to establish qualitative lists of ingested prey, and not of their relative importance, because of the little food regurgitated by this technique in relation to the use of emetic. Other techniques such as the use of stable isotopes and the genetic analysis of feces could improve our knowledge of the Bobolink diet on its wintering grounds.

Conservation implications

Our results show that Bobolinks consume a great quantity of seeds and grains of non-cultivated plant species. These results are remarkable because we captured Bobolinks within rice fields where the rice crop is the predominant land-cover. Thus, these results agreed with the idea that damage to rice crops could be lower in fields that maintain the presence of non-cultivated patches and roadsides with the presence of naturally occurring grass, which provide an alternative food source to the Bobolink.

Our results also showed that Bobolinks could contribute to the control of pest insects in rice fields. Although the insect component of the diet was of relatively minor importance, the documentation and confirmation of the presence of invertebrates in the Bobolink diet suggest that Bobolinks can potentially have a beneficial role in agroecosystems based on their contribution to the regulation of populations of these insects. This function can be important considering that the number of Bobolinks counted in the region has exceeded 100,000 individuals (López-Lanús *et al.* 2007, López-Lanús & Marino 2010). Insects consumed by Bobolinks included members of the orders Hemiptera and Coleoptera, both of which include species that can damage rice crops (Kruger & Burdyn 2015). The opportunistic behavior shown by the Bobolink to capture their animal prey can be important when these groups of insects become abundant.

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