Ardeola 56(1), 2009, 71-84

FERN FEEDING ECOLOGY OF THE AZORES BULLFINCH *PYRRHULA MURINA*: THE SELECTION OF FERN SPECIES AND THE INFLUENCE OF NUTRITIONAL COMPOSITION IN FERN CHOICE

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SUMMARY.—*Fern feeding ecology of the Azores bullfinch* Pyrrhula murina*: the selection of fern species and the influence of nutritional composition in fern choice.*

Aims: Ferns are an important component of many ecosystems and potentially provide an abundant food resource for consumers, but there are very few studies on the ecology of fern feeding by vertebrates. We describe the selection of sporangia and leaves among fern species by the Azores bullfinch (*Pyrrhula murina*), and addressed the importance of nutritional composition in fern choice.

Location: Serra da Tronqueira (37º 47' N, 25º 13' W), a mountainous district on the east of São Miguel Island, Azores.

Methods: We established transects throughout the range of the Azores bullfinch to record: i) the abundance of sporangia (= number of fertile leaves), and of each leaf phenological stage (crozier, expanding leaves and recently expanded leaves) for each fern species, and ii) fern consumption, as the number of leaves with beak marks (fern stripping) for each fern species. The composition of both mature sporangia and young fern leaves (lipids, proteins, phenolics and caloric content) was compared between consumed and non-consumed fern species.

Results: In winter and early spring the Azores bullfinch foraged on sporangia of *Woodwardia radicans*, *Culcita macrocarpa* and *Pteris incompleta*, and in spring/early summer, it took leaves of *Osmunda regalis* and *Pteridium aquilinum* and sporangia of *O. regalis.* From the three leaf phenological stages, expanding leaves and recently expanded leaves were preferred over croziers. The spores of consumed fern species were significantly higher in lipids than leaves of consumed species, but leaves had a higher content in protein and phenolics than spores. The lipid content of spores was nearly significantly different between consumed and non-consumed species, but the nutritional composition of leaves was similar between consumed and non consumed fern species.

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Conclusions: The Azores bullfinch was selective when foraging on fern sporangia and leaves. Fern species with sporangia of higher lipid content appeared to be preferred. When compared to seeds, fern sporangia possess similar caloric content and, therefore, should be an important source of energy during the winter. The consumption of leaves in early spring, possessing high amounts of proteins and reasonable high values of calories, may enable birds to survive until other foods became available. This study suggests that overall energy may be the most limiting factor for the Azores bullfinch when selecting foraging fern species in winter.

Key words: Azores bullfinch, fern feeding, fern nutritional composition, food preference.

RESUMEN.—*Ecología alimentaria de helechos para el camachuelo de las Azores* Pyrrhula murina*: selección de las especies de helechos e influencia de la composición nutricional en su elección.*

Objetivos: Los helechos son un componente importante en los diferentes ecosistemas y potencialmente proporcionan una abundante fuente de recursos para los consumidores, aunque existan pocos estudios sobre la ecología alimentaria de helechos en vertebrados. Se describe la selección de esporangios y hojas de helechos que realiza el camachuelo de las Azores (*Pyrrhula murina*), un ave endémica de la Isla de San Miguel, Azores, y evaluamos la importancia de la composición nutricional en esta selección.

Localización: Sierra da Tronqueira (37º 47' N, 25º 13' W), área montañosa al este de la Isla de San Miguel, Azores.

Métodos: Se establecieron transectos a lo largo del rango de distribución del camachuelo de las Azores para registrar: (i) la abundancia de esporangios (número de hojas fértiles), y el estado fenológico de cada hoja (crozier, hojas en expansión y recientemente expandidas) para cada especie de helecho y, (ii) consumo de helechos, entendido como el número de hojas con marcas dejadas por el pico del ave (*fern stripping)* en cada una de las especies. La composición de los esporangios maduros y de las hojas jóvenes (lípidos, proteínas, fenoles y contenido calórico) fue comparada entre especies consumidas y no consumidas.

Resultados: En invierno y principios de primavera el camachuelo de las Azores se alimenta de esporangios de *Woodwardia radicans*, *Culcita macrocarpa* y *Pteris incompleta,* y en primavera y a comienzos de verano se alimenta de hojas de *Osmunda regalis* y *Pteridium aquilinum* y esporangios de *O. regalis*. De los tres estados fenológicos, las hojas en expansión y las recientemente expandidas son preferidas frente a los croziers. Las esporas de las especies consumidas tuvieron un contenido significativamente más alto en lípidos que las hojas de especies consumidas, pero las hojas tienen un contenido mayor en proteínas y fenoles que las esporas. El contenido de lípidos de las esporas fue casi significantemente diferente entre especies consumidas y no consumidas, pero la composición nutricional de las hojas fue similar entre especies consumidas y no consumidas.

Conclusiones: El camachuelo de las Azores fue selectivo al alimentarse de los esporangios y de las hojas de helecho. Los preferidos parecen ser los esporangios con mayor contenido en lípidos. Cuando se comparan con semillas, los esporangios tienen un contenido similar de calorías y, como consecuencia deben ser una importante fuente de energía durante el invierno. El consumo de hojas a comienzos de la primavera, con altas cantidades de proteínas y un razonablemente alto contenido de calorías, puede permitir a estas aves sobrevivir hasta que otros alimentos estén disponibles. El estudio sugiere que la energía debe ser el factor más limitante para el camachuelo de las Azores en la selección de las especies de helecho en invierno.

Palabras clave: alimentación de helechos, camachuelo de las Azores, composición nutricional de los helechos, preferencias alimentarias.

INTRODUCTION

The selection of food resources by herbivorous-granivorous animals is influenced by a variety of factors. Preference determines which available foods are consumed and is mainly influenced by food size and handling time (Diaz, 1994), nutritional value (Schaefer *et al*., 2003) and secondary compounds such as phenolics. Ferns (Pteridophyta) are an important component of many ecosystems (Tryon, 1986) and potentially provide an abundant food resource for consumers. Invertebrates such as gastropods and insects consume ferns to some extend but very few vertebrate species are known to consume ferns regularly. Some exceptions are found amongst birds such as the Takahē (*Notornis mantelli*) and the Kaka (*Nestor meridionalis*) in New Zealand (Mills *et al*., 1980; O´Donell and Dilks, 1994), the cantabrian capercaillie (*Tetrao urogallus cantabricus*) in Spain (Rodríguez and Obeso, 2000) and the Azores bullfinch or priolo (*Pyrrhula murina*) in the Azores, Portugal (Ramos, 1994). The reasons behind the ubiquitous low consumption of ferns by vertebrates are not clear but it has been suggested to be related with the high concentration of diverse biochemical defences on ferns (Seigler, 1991; Moran, 2004; Marrs and Watt, 2006) and lignin contents (Cornelissen *et al*., 2004).

The critically endangered Azores bullfinch (IUCN, 2005) is an endemic bird from the laurel forest of São Miguel Island, Azores, where ferns, both sporangia and leaves (Ramos, 1995), form an important part of its diet. Ramos (1995) described the seasonal pattern of fern feeding by the Azores bullfinch and showed that sporangia of three species are consumed: *Woodwardia radicans*, from October to March, with a peak in November-January, *Culcita macrocarpa*, from November to April, with a peak in January-March, and *Osmunda regalis*, in May-July. In relation to leaves, only young ones were consumed, especially from *Pteridium aquilinum* and *O. regalis*, in April-June, but *O. regalis* seemed highly preferred over *P. aquilinum* because birds switched from *P. aquilinum* to *O. regalis* as soon as this last species became available (Ramos, 1994). Ramos (1994, 1995) described these patterns of fern consumption but he neither examined seasonal variations in the consumption of fern leaves in relation to leaf developmental stage nor nutritional composition of ferns, which is likely to influence fern choice. In winter, the Azores bullfinch (Ramos, 1996b) and other bullfinches (Matthews, 1983) seem to select foods on the basis of their energetic content. Therefore, more nutritional fern leaves and sporangia [i.e. with higher caloric, protein and lipid contents (Sih and Christensen, 2001)] should be preferred and those with higher content in phenolics should be avoided (Snyder, 1992). Several studies revealed a negative correlation between phenolic content and herbivory (Jakubas *et al*., 1989; Snyder, 1992), including the European bullfinch (*Pyrrhula pyrrhula*) (Wilson, 1984; Greig-Smith and Wilson, 1985). To assess this point we made a very extensive survey of fern sporangia and leaf feeding by the Azores bullfinch and analysed the variation in caloric, lipid, protein and phenolic content of consumed and non-consumed species.

This study provides the first complete overview of fern feeding by a vertebrate species. We present the foraging preference patterns for fern species and shifts in fern choice by the Azores bullfinch. The specific objectives were: (i) to determine which fern species and which parts of these species, fertile leaves with sporangia and unfertile leaves, are selected by the Azores bullfinch on different periods, (ii) to assess which developmental stages of fern leaves are preferred, and (iii) to compare the caloric, lipid, protein and phenolic content of sporangia and leaves between consumed and non consumed fern species.

METHODS

Study area

The Azores bullfinch has always been confined to the eastern part of the Island of São Miguel, Azores, Portugal. Its distribution is highly associated with the native laurel forest, composed of evergreen trees (e.g. *Ilex perado spp. azorica, Laurus azorica, Vaccinium cylindraceum*) between 350 - 900 m (Ramos, 1996a). This forest has 947 vascular plant species, 71 (7.5 %) of which are ferns (Dias, 1996). The understory is dominated by evergreen ferns (e.g. *C. macrocarpa*, *Dryopteris affinis*, *Dryopteris aemula*, *Dryopteris azorica*, *Pteris incompleta*, *W. radicans*) and winter deciduous ferns (e.g. *Blechnum spicant*, *O. regalis*, *P. aquilinum*) (Dias, 1996). The area has been reduced and altered by plantations of japanese red cedar (*Cryptomeria japonica)* and by the invasion of exotic plants, mainly australian cheesewood (*Pittosporum undulatum)*, kahili ginger (*Hedychium gardneranum)* and lily-of-the-valley tree (*Clethra arborea)*. It is classified as a Special Protection Area (SPA) under the Natura 2000 network.

Sporangia consumption

To determine which fern species are preferred, 259 linear transects of 50 x 2 m were performed, covering the whole distribution area of the Azores bullfinch. All fern fertile leaves i.e. those with sori (clusters of sporangia) were counted and all obvious beak marks of the Azores bullfinch (hereafter named fern stripping) were recorded. These are very characteristic as no other bird species in the area feed on ferns (Ramos, 1994; 1995). These transects were made between 7 January and 23 March 2007, which includes the main consumption period of fern sporangia (Ramos, 1995). We plotted the number of fertile leaves per transect for the following fern species/groups (*W. radicans*, *C. macrocarpa*, *Dryopteris sp*., *B. spicant*, *P. incompleta* and other fern species: *Diplazium caudatum*, *Christella dentata*, *Stegnogramma pozoi*, *Adiantum hispidulum*, *Cyathea cooperi*, *Pityrogramma ebenea* and *Dicksonia antarctida*), and compared these with the number of fertile stripped leaves per transect.

Leaf consumption

To evaluate the abundance and consumption of fern leaves, the appearance of new leaves and the developmental stage to document shifts in preferences by birds, we marked 30 transects of 8 x 4 m on 15 March 2007. All ferns within each transect were identified, individually marked and their developmental stage recorded. All transects were revisited and new individuals were marked every 15 days until 15 May 2007. In order to investigate which developmental stages were preferred, we recorded the developmental stage of all marked individuals and the presence of fern stripping on each visit. Only young leaves (i.e. developing or recently developed) were considered, as the Azores bullfinch does not feed on old leaves (Ramos, 1994). Four different leaf developing stages were defined: (i) crozier: the initial uncoiling leaf with the petiole elongation. This stage has a characteristic “fiddle-head” shape due to faster growth of leaf base compared to the apex; (ii) expanding leaf: the stage after crozier during rachis elongation and leaflet expansion; (iii) recently expanded leaf: the final overall expansion phase with all leaflets expanded; (iv) fertile leaf: this stage was recorded for *O. regalis* as this is the only fern species producing mature sporangia between March and May. In this species, sporangia form a tassel-like outgrowth at the apex of the leaf that matures after leaf expansion; mature sporangia can be recognized by turning green as the mature spores inside contain chlorophyll.

Nutritional determinations of sporangia and leaves

Laboratorial determinations of caloric, lipid, protein and phenolic content of spores and leaves were carried out. Analyses were conducted for spores of *W. radicans, C. macrocarpa*, *P. incompleta, Dryopteris sp.*, *B. spicant* and *O. regalis* (unique spring spore), and for leaves of *P. aquilinum, O. regalis, C. macrocarpa*, *W. radicans*, *P. incompleta* and *Dryopteris sp*. Only mature sporangia and young leaves (expanding or recently expanded) were sampled as the Azores bullfinch only feeds on these parts of the ferns (Ramos, 1994). Young leaves could be recognized by their soft lamina texture, due to the thin cuticle. Leaves and sporangia were frozen at -80 ºC until analysis (1 - 4 months). Prior to analyses they were oven dried at 60 ºC until weight stabilization (approx. 4 days). To determine the caloric content of spores and leaves, a small quantity of the samples (50 to 200 mg) was converted into pastilles. Each item was crushed to dust in a mortar, and the heat of combustion was determined using a Parr 1425 Semimicro bomb calorimeter (Parr Instrument Company). Three pastilles of each sample were used to calculate the mean caloric content. Lipids were extracted and determined with a chloroform : methanol mixture according to Folch *et al.* (1957). Soluble proteins were measured with the Folin- Ciocalteu reagent (Folin and Ciocalteu, 1927) with the method developed by Lowry *et al.* (1951). For free phenolic content, samples were analysed using the method described by Julkunen-Titto (1985), based on the reduction of the phosphotungstic-phosphomolybdic (Folin and Denis, 1912) present in the Folin-Ciocalteu reagent (Folin and Ciocalteu, 1927). Both protein and phenolic concentrations in all samples were determined from calibration curves of standards of bovine albumine (Armour and Company, Chicago) and gallic acid (Hagerman and Butler, 1989) respectively. For each lipid, protein and phenol assay, 100 mg dry weight samples were used and results are given in mg/g dry weight of spores or leaves.

Statistical analyses

A Logistic Regression Model was built to predict the probability of observing: (i) stripped fertile leaves (i.e. with sporangia) of consumed fern species from January to March. The predicted variable was consumption (1) or no consumption (0) of sporangia and the explanatory variables were fern species (*W. radicans*, *C. macrocarpa*, *Dryopteris sp*, and *P. incompleta*) and abundance of each fern species, measured as the number of fertile leaves of each species present in each transect. (ii) Stripped leaves of *W. radicans*, *C. macrocarpa*, *P. aquilinum* and *O. regalis* after 30 April, when leaves of all developmental stages of these four fern species were available. The predicted variable was consumption (1) or no consumption (0) of a specific fern leaf and the predictors were fern species, phenological stage and abundance of each phenological stage, measured as the number of leaves of each stage present in each transect.

For both models, Hosmer and Lemeshow´s (2000) methods and model-building strategy were followed. Selection began with a univariate analyses to assess the relationship between each predictor variable and the dependent variable. Any variable whose univariate test had a *P*-value smaller than 0.25 was a candidate for the multivariate model. All categorical variables were included in the model using reference cell coding which consists of defining a reference category for each variable, with which the other categories were compared. The importance of each variable in the multivariate model was verified using the Wald statistic for each variable. Finally, all interactions among the variables incorporated in the main effects model were included into the model one at a

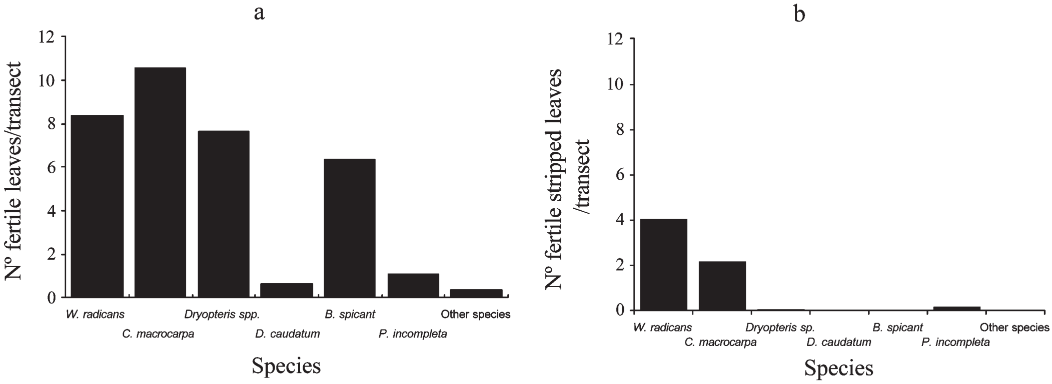

FIG. 1.—Nº of fertile leaves / transect (a) and Nº of stripped fertile leaves transect (b). Fertile leaves were measured as number of leaves with sori and consumption as the number of leaves with stripping marks. *[Nº de hojas fértiles / transecto (a) y Nº de hojas fértiles consumidas transecto (b). Las hojas fértiles fueron medidas como número de hojas con soros y la alimentación como número de hojas con marcas de consumo.]*

time and their significance assessed with the Likelihood Ratio test and the Wald statistic ($p < 0.05$). After obtaining a definitive model, using only significant variables, the intercept coefficients of the predictors in the model, together with the Odds Ratio, were analysed to understand the nature of the relationship between each predictor and the outcome variable. The relative contribution of each variable to explain fern stripping was addressed using the Odds Ratio to compare the probability of having a fern stripping at each category with the reference level.

A Chi-square test was used to assess differences in consumption among the four phenological stages of *O. regalis* between 30 April and 15 May, when these four stages were available. Mann-Whitney tests were used to compare caloric content, lipids, proteins and phenolics of both leaves and spores between consumed and non-consumed fern species. Fern species with a very marginal consumption (Spores of *Dryopteris sp*. and leaves of *C. macrocarpas*) were included in the non-consumed group. The mean of 2 to 10 determinations of each nutrient (median = 3) per fern species was used in the analyses.

Statistical analyses were performed with Statistica 6.0 (StatSoft, Inc. 1984 - 2001) and SPSS. Data is presented as mean ± SD.

RESULTS

Sporangia consumption

Fern stripping was recorded on four species: *W. radicans*, *C. macrocarpa, P. incompleta* and *Dryopteris sp*. (fig. 1), but the last species, although abundant, had very few beak marks. The Logistic Regression Model for sporangia fern stripping predicted 73 % of the responses correctly and fitted the data well (Hosmer and Lemeshow test: $P = 0.76$, table 1). As expected, sporangia consumption differed among fern species: when compared to *W. radicans* (the reference species) the birds consumed *C. macrocarpa* approximately in the same proportion (odds ratio = 0.98) and consumed significantly less *Dryopteris sp*. (odds ratio = 0.01, table 1). *P. incompleta* was less consumed than *W. radicans* but the difference was not significant (table 1). Abundance had a significant positive influence on sporangia consumption, presumably be-

TABLE 1

Logistic Regression model to predict the probability of fern stripping on sporangia of *Woodwardia radicans*, *Culcita macrocarpa*, *Dryopteris sp*. and *Pteris incompleta* using two explanatory variables: fern species and abundance (= number of fertile leaves) of each species present in each transect (n = 259 transects). Given are the coefficients of the variables ± SE, the Wald statistic and its P-values, and estimated Odds ratio (OR). *Woodwardia radicans* is the reference category for fern species.
[Modelo de regresión logística para predecir la probabilidad de consumo de esporangios de Woodwardia radicans, Culcita macrocarpa, Dryopteris sp. *y* Pteris incompleta *utilizando dos variables explicativas: especie de helecho y abundancia (= número de hojas fértiles) de cada una de las especies presentes en cada transecto (n = 259 transectos). Se dan los coeficientes de las variables ± SE, el estadístico Wald y sus valores de P, y las estimaciones de Odds ratio (OR).* Woodwardia radicans *es la categoría de referencia en las especies de helechos.]*

	Coefficient	Wald	P	OR
Fern species				
Woodwardia radicans		16.61	0.001	
Culcita macrocarpa	-0.23 ± 0.42	0.32	0.573	0.98
Dryopteris sp.	-6.91 ± 1.84	14.15	< 0.001	0.01
Pteris incompleta	-1.37 ± 0.77	3.15	0.076	0.25
Abundance	0.10 ± 0.03	11.73	0.001	1.10
Abundance * fern species		9.94	0.019	
Abundance * *C. macrocarpa*	-0.74 ± 032	5.48	0.019	0.93
Abundance * *Dryoperis sp.*	-0.01 ± 042	0.01	0.990	1.00
Abundance * *P. incompleta*	0.29 ± 079	0.13	0.717	1.03
Constant	-0.03 ± 0.32	0.01	0.922	0.97
Hosmer & Lemeshow test	$\chi^2_8 = 4.95$, P = 0.763			
% Cases classified correctly	73.1 %			

cause *W. radicans* and *C. macrocarpa*, two of the most abundant fern species, were the most consumed, (table 1; fig. 1). There was an interaction between fern species and abundance: when compared to the abundance of *W. radicans*, the abundance of *C. macrocarpa* had a significantly negative influence on consumption (table 1). In fact, *C. macrocarpa* was more abundant, but less consumed, than *W. radicans* (fig. 1).

Leaf consumption

Figure 2 compares the abundance, as number of sterile leaves per species, with the number of leaves exhibiting stripping marks, from mid March to mid May. From all species present in transects only *P. aquilinum* and *O. regalis* exhibited conspicuous fern stripping by the Azores bullfinch in this period (figure 2). *W. radicans* and *C. macrocarpa* were very little consumed as they exhibited stripping signs only on up to four and seven leaves, respectively. Between 20 and 75 leaves of *Dryopteris sp*., *P. incompleta* and *B. spicant* were marked until 15 April. As there were no fern stripping on these species not more leaves were marked after this visit; however marked leaves were checked until mid May but there was no evidence of consumption.

New *P. aquilinum* leaves become available in late March when the Azores bullfinch was ob-

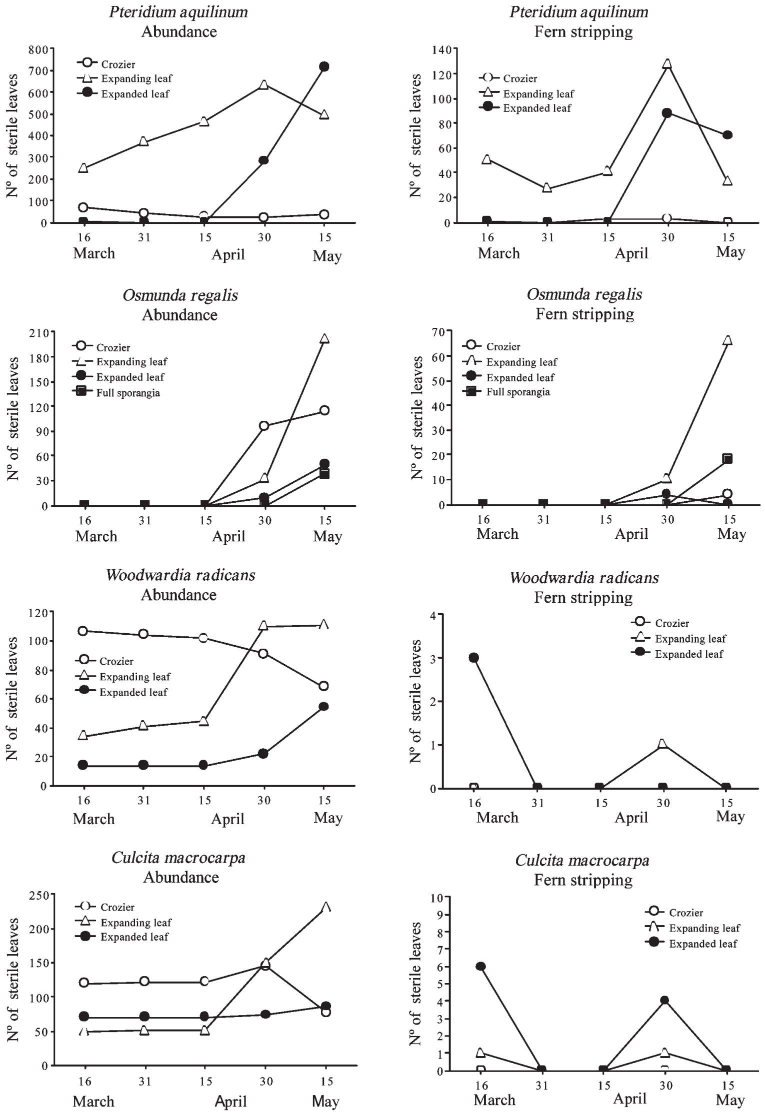

FIG. 2.—Abundance and fern stripping on leaves from 16 March to 15 May 2007 along 30 transects repeated every two weeks. Abundance was measured as the number of leaves and fern stripping as the number of leaves with beak marks for the first visit or leaves that had new beak marks, i.e. in the previous 15 days, for next visits. Three phenological stages were differentiated, crozier, expanding leaf, recently expanded leaf and, for *Osmunda regalis* there was a fourth category, full sporangia (see methods for definitions). Note different scales on Y axis.

[Abundancia de marcas de consumo en las hojas desde el 16 de marzo al 15 de mayo de 2007 a lo largo de 30 transectos, repetidos cada dos semanas. La abundancia fue medida como el número de hojas y el consumo como el número de hojas con marcas del pico en la primera visita u hojas con nuevas marcas, es decir, en los 15 días anteriores, en las próximas visitas. Fueron diferenciados tres estados fenológicos, crozier, hojas en expansión, recientemente expandidas y, para Osmunda regalis *había una cuarta categoría, esporangios completamente llenos (véanse métodos con las definiciones). Notar las diferentes escalas en el eje Y.]*

TABLE 2

Logistic Regression model to predict the probability of fern stripping on leaves of *Woodwardia radicans*, *Culcita macrocarpa*, *Pteridium aquilinum* and *Osmunda regalis* using three explanatory variables: fern species, phenological stage and abundance (= number of leaves of each phenological stage) present in each transects (N = 30 transects). Given are the coefficients of the variables ± SE, the Wald statistic and its P-values, and estimated Odds ratio (OR). *Woodwardia radicans* is the reference category for fern species and crozier for phenological stage.
[Modelo de regresión logística para predecir la probabilidad de consumo de hojas de Woodwardia radicans*,* Culcita macrocarpa*,* Pteridium aquilinum *y* Osmunda regalis *utilizando tres variables explicativas: especie de helecho, estado fenológico y abundancia (= número de hojas de cada estado fenológico) presentes en cada transecto (N = 30 transectos). Se dan los coeficientes de las variables ± SE, el estadístico Wald y sus valores de P, y las estimaciones de Odds ratio (OR).* Woodwardia radicans *es la categoría de referencia en las especies de helechos y los croziers para el estado fenológico.]*

	Coefficient	**Wald**	**P**	**OR**
Fern species				
Woodwardia radicans		41.29	<0.001	
Culcita macrocarpa	0.70 ± 1.13	0.38	0.536	2.02
Pteridium aquilinum	3.67 ± 1.04	12.48	<0.001	39.40
Osmunda regalis	3.83 ± 1.06	13.04	<0.001	45.97
Phenological stage				
Crozier		20.97	<0.001	
Expanding leaf	2.08 ± 0.58	12.76	<0.001	8.04
Recently expanded leaf	2.76 ± 0.60	20.90	<0.001	15.86
Abundance	-0.61 ± 0.16	14.47	<0.001	1.06
Constant	-6.72 ± 1.15	34.10	<0.001	0.01
Hosmer & Lemeshow test	χ^2_8 = 5.11, P = 0.75			
% Cases classified correctly	87.3 %			

served feeding on them. The birds consumed very young leaves, leaving conspicuous bill marks in the lamina of expanding leaves; petioles of croziers were also broken and chewed (*pers. obs*.). Despite the high availability of *P. aquilinum* leaves in mid May, there was a marked decrease in the proportion of leaves with fern stripping at this time (figure 2). This decrease coincided with a noticeable increase in fern stripping on leaves of *O. regalis*, clearly suggesting a diet shift by the Azores bullfinch (figure 2).

The Azores bullfinch started to feed on sporangia of *O. regalis* as soon as these became available (early May; figure 2). A Chi-square analysis showed significant differences in the consumption of the four phenological stages (crozier, expanding leaf, recently expanded leaf and full sporangia) of *O. regalis* from 30 April to 15 May (= 93.64, *P* < 0.001). Sporangia were clearly preferred (observed vs expected value = 18 vs 7.2) over leaves and expanding leaves were preferred (observed vs expected value = 76, vs 44.1) over recently expanded leaves and crozier (figure 2).

The Logistic Regression Model for the presence of leaf fern stripping on *C. macrocarpa*, *W. radicans*, *P. aquilinum* and *O. regalis* predicted 87.3 % of the responses correctly and fitted the data well (Hosmer and Lemeshow test: *P* = 0.75, table 2) with

TABLE 3

Chemical determinations for available and consumed ferns in winter and early spring. Available ferns were only abundant species for each of the main period of sporangia feeding (November-March) and leaf feeding (April-June). Results are mean ± SD. For each chemical component and date 2 to 10 analyses were made (median = 3). Sporangia of *Dryopteris sp*. were marginally consumed and included in the non-consumed group.

	Species		**Calories**	
			Sampling date	**KJ/g**
Sporangia	**Consumed**	*Woodwardia radicans*	13 April 06	21.86 ± 0.41
		Culcita macrocarpa	13 Ago 06	19.09 ± 0.56
		Pteris incompleta	10 Nov 07	27.86 ± 0.13
	Non-consumed	*Dryopteris sp.*	29 Oct 06	16.88 ± 1.93
		Blechnum spicant	10 Nov 07	20.36 ± 0.07
Leaves	**Consumed**	*Pteridium aquilinum*	12 April 06	18.37 ± 0.62
		Osmunda regalis	08 May 07	21.49 ± 0.34
	Non-consumed	*Woodwardia radicans*	12 April 07	19.48 ± 0.40
		Culcita macrocarpa	12 April 07	20.74 ± 0.50
		Pteris incompleta	12 April 06	18.28 ± 0.31
		Dryopteris sp.	12 April 06	20.15 ± 0.28

three significant variables, fern species, phenological stage and abundance (table 2). The sign of the coefficients and the Odds Ratio for each of these variables show that: (i) fern stripping in *P. aquilinum* and especially in *O. regalis* (the highest odds ratio) were much higher than that in *W. radicans* (the reference species). (ii) Compared to fern stripping on croziers (the reference category) those on expanding and recently expanded leaves were 8 and 16 times higher (table II). (iii) Fern abundance had a significantly negative influence on leaf consumption, presumably because highly abundant fern species (*W. radicans* and *C. macrocarpa*) showed very few signs of consumption. The interaction between phenological stage and fern species was not included in the final model because it did not contribute to explain fern stripping.

Nutritional determinations of sporangia and leaves

The caloric content of consumed fern species was highest for sporangia of *P. incompleta* and lowest for *P. aquilinum* leaves (table 3). The sporangia of consumed species were significantly higher in lipids ($U = 0.0$, $P = 0.049$), but not in caloric content ($U = 2.0$, $P = 0.275$, both $n = 3$), than leaves of non-consumed species. The protein and phenolic content of consumed species were significantly higher in leaves than in sporangia (both $U = 0.0$, $P = 0.049$ and $n = 3$, table 3). From the comparison of caloric content, lipids, proteins and phenolics between sporangia of consumed and non-consumed species, the lipid content was almost significantly higher for spores of consumed species and the opposite occurred for phenolics (both $U = 0.0$, $P = 0.08$, $n_1 = 3$ and $n_2 = 2$). No nu-

TABLE 3

[Determinaciones químicas en helechos disponibles y consumidos en invierno y principios de primavera. Los helechos disponibles eran sólo especies abundantes en cada uno de los principales períodos de consumo de esporangios (noviembre-marzo) y consumo de hojas (abril-junio). Los resultados son la media ± SD. Para cada componente químico y fecha se realizaron entre 2 y 10 análisis (media = 3). Los esporangios de Dryopteris sp. *fueron marginalmente consumidos e incluidos en el grupo de no consumidos.]*

Lipids		**Proteins**		**Phenolics**	
Sampling date	**mg/g**	**Sampling date**	**mg/g**	**Sampling date**	**mg/g**
12 Oct 07	185.00 ± 8.49	07 Dec 06	1.65 ± 0.02	07 Dec 06	1.21 ± 0.03
12 Oct 07	274.25 ± 26.06	07 Dec 06	2.27 ± 0.02	07 Dec 06	0.66 ± 0.02
16 Mar 07	257.50 ± 7.78	16 Mar 07	0.72 ± 0,08	16 Mar 07	1.10 ± 0.03
10 Nov 07	174.50 ± 10.61	10 Nov 07	0.74 ± 0.04	10 Nov 07	0.63 ± 0.04
10 Nov 07	167.50 ± 21.92	10 Nov 07	1.41 ± 0.27	10 Nov 07	0.39 ± 0.05
12 April 07	36.50 ± 13.44	12 April 06	9.03 ± 0.41	12 April 07	1.92 ± 0.04
08 May 07	36.00 ± 10.15	08 May 07	5.45 ± 0.33	08 May 07	1.96 ± 0.05
12 April 07	16.00 ± 18.38	12 April 07	42.87 ± 0.79	12 April 07	2.19 ± 0.21
12 April 07	34.00 ± 1.41	12 April 07	37.57 ± 1.38	12 April 07	2.17 ± 0.06
12 April 07	31.00 ± 5.66	12 April 07	2.37 ± 0.16	12 April 07	2.18 ± 0.05
12 April 07	12.00 ± 5.66	12 April 07	1.37 ± 0.10	12 April 07	1.92 ± 0.16

tritional determinations in leaves differed significantly between consumed and non consumed fern species (Mann-Whitney test, $P > 0.05$; table 3).

From the end of April until July the only fern species with spores was *O. regalis*. Nutritional composition of samples collected on the 8 May 2007 was (mean ± SD per g of dry weight) 19.46 ± 0.05 KJ/g for caloric content, 42.0 ± 7.07 mg/g for lipids, 3.69 ± 0.05 mg/g for proteins and 1.89 ± 0.02 mg/g for phenolics.

DISCUSSION

In this study fern stripping was evaluated within the whole distribution area of the Azores bullfinch and showed that: (i) during winter, sporangia of *W. radicans*, *C. macrocarpa* and *P. incompleta* were highly preferred over those of all other fern species available. (ii) In spring, leaves of *O. regalis* and *P. aquilinum* were preferred over those of *W. radicans* (although leaves of *O. regalis* appeared later than those of other species which may introduce some bias in this conclusion). (iii) Abundance influenced positively and negatively the consumption of fern sporangia and leaves, respectively, as the most abundant species with sporangia were the most consumed but the opposite occurred for fern leaves. Regarding the consumption of the main fern species this study confirms earlier observations by Ramos (1994; 1996 b). Obvious beak marks of Azores bullfinch were observed on fertile leaves of *P. incompleta* which was not detected in foraging observations made in the 1990s. *P. incompleta* increased as a consequence of the removal of exotic plants, which began in 2004 as part of an ongoing habitat restoration project. This increase in abundance is likely to have translated in an increase of consumption by the Azores bullfinch. However, it was much less abundant than *W. radicans* and *C. macrocarpa*, and occurred mainly in dense forest, which may explain the lack of records of observations of birds foraging in *P. incompleta* in the 1990's. When young leaves of *P. aquilinum* and *O. regalis* become available, in mid March and mid April respectively, the Azores bullfinch began feeding on them as most sporangia of *W. radicans* and *C. macrocarpa* had already released their spores and were no longer available for feeding (Ramos, 1995). Sporangia of *O. regalis*, had a higher lipid content (42.0 mg/g) that leaves (36.0 mg/g), which may contribute to explain preference for sporangia over leaves in this species.

After comparing the mean caloric content of sporangia from fern species important in the diet of the Azores bullfinch (*C. macrocarpa*, *W. radicans*, and *P. incompleta*: mean = 22.94 KJ/g, range = 19.09 to 27.86 KJ/g) with the caloric content of seeds eaten by the Azores bullfinch in other seasons (unpublished data), with values ranging from 15.58 KJ/g (*Rumex conglomeratus*) to 27.12 KJ/g (*Leicesteria formosa*), the conclusion is that the caloric content of spores is relatively high. The caloric content of leaves varied from 18.37 KJ/g (*P. aquilinum*) to 21.49 KJ/g (*O. regalis*), which are comparatively higher than the 17.10 KJ/g for the fern *Polystichum sp.*, consumed by cantabrian capercaillie (Rodríguez and Obeso, 2000). The caloric content of leaves was lower than that of sporangia but relatively similar to that of other foods highly consumed by the Azores bullfinch such as flower buds of *Ilex perado* (21.78 KJ/g). Therefore, although seeds should be more rewarding per unit handling time because of their larger mass, this comparison shows that fern sporangia can be an important source of energy.

Regarding sporangia, the most consumed species (*W. radicans*, *C. macrocarpa* and *P. incompleta*) were higher in lipid content than non consumed fern species. The importance of high energetic spores for the Azores bullfinch may be explained by the fact that birds will easily meet their metabolic requirements of thermoregulation using foods higher in lipids (Mills *et al.*, 1980). Also, the fact that birds switched rapidly from *P. aquilinum* to *O. regalis* leaves as soon as the latter became available in May is likely to be a consequence of a higher caloric content in *O. regalis* than in *P. aquilinum*. Our data suggest that during winter and early spring the Azores bullfinch are likely to select sporangia of fern species that are higher in lipids, which must be important to maximize energy intake.

Phenolics in spores did not differ between consumed and non consumed fern species but, contrarily to expectations, phenolics in leaves of consumed species were significantly higher than those in non-consumed species. It should be noted that our drying temperature was too high to ensure the stability of phenols, and this may have biased our phenolic determinations. However, Ramos (1996b) showed also that seed phenolics of the most important tree species consumed in winter, *C. arborea*, did not differ between used and unused individual trees, which suggests that phenolics are of little importance in explaining the selection of foods by the Azores bullfinch in winter. The low number of fern stripping marks on croziers may be due to high concentration of phenolics in this leaf stage (Marrs and Watt, 2006), but this was not examined in our study. Proteins, however, should be important for herbivorous birds such as bullfinches (Summers and Jones, 1976) to maintain body weight and for the growth of muscles and tissues of first year birds (Brice and Grau, 1991; Zanotto and Bicudo, 2005). Moreover, the carcinogenicity of the vegetative tissues of *P. aquilinum* (Moran, 2004) may also contribute to explain why the Azores bullfinch significantly decreased feeding on leaves of *P. aquilinum* as soon as *O. regalis* leaves became available

(Ramos, 1994). Both vegetative tissues and spores of *P. aquilinum* can damage the DNA of consumers, whereas those of *O. regalis* cannot (Simán *et al*., 2000).

The amount of lignin, an important component of the plant cell wall, determines to a great extent leaf digestibility (Cornelissen *et al*., 2004). In most cases, digestibility decreases as leaves age as a consequence of lignification (Lowman and Box, 1983; Hill and Lucas, 1996), which may contribute to explain why old fern leaves were never consumed by the Azores bullfinch. Digestibility of fern leaves from several species consumed by the roosevelt elk (*Cervus elaphus roosevelti*) ranged from 23 to 46 %, whereas that of grasses ranged from 55 to 76 % (Hutchins, 2006), meaning that fern leaves are likely to be less suitable for herbivores than grasses.

In summary, this is the first study examining in detail the selection of fern sporangia and leaves by a vertebrate species and describes how important ferns are for the Azores bullfinch. The conclusions were that during winter sporangia of *W. radicans*, *C. macrocarpa* and *P. incompleta* were preferred than those of other species. In early spring *P. aquilinum* and *O. regalis* leaves were consumed. In spring, expanding and recently expanded leaves were preferred over croziers except for *O. regalis*, in which sporangia were available and preferred over leaves. The consumption of leaves in early spring, possessing high amounts of proteins and reasonable high values of lipids and calories, may enable birds to survive until better foods became available. The selection of sporangia from fern species containing the highest lipid content indicates that Azores bullfinches are actively selecting the most energetic food items. This study suggests that chemical composition of fern sporangia and leaves is involved in explaining particular shifts in fern preference by the Azores bullfinch throughout the winter, with lipids playing an important role in the selection of fern (and parts of fern) species.

ACKNOWLEDGEMENTS.—We thank the Portuguese Society for the Study of Birds (Birdlife in Portugal) and the team of the Priolo Life Project for help with field work. Two anonymous referees and Prof. Chris Feare improved an earlier version.

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[Recibido: 18-12-2008]
[Aceptado: 30-04-09]