

## Feeding ecology of the Ruivaco *Achondrostoma oligolepis*, a Portuguese endemic cyprinid fish

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### ABSTRACT

#### Feeding ecology of the Ruivaco *Achondrostoma oligolepis*, a Portuguese endemic cyprinid fish

This study assessed the feeding ecology of the ruivaco *Achondrostoma oligolepis*, a Portuguese endemic resident cyprinid fish whose dietary habits are virtually unknown. Samples were taken seasonally in three medium-sized rivers representing a gradient of temporality. The stomach contents of 97 individuals (42-126 mm total length, TL) were analysed. Although there was no significant overall variation in diet composition between rivers, differences were found among seasons. A broad range of food categories was identified, although a smaller subset of primarily detritus (77.6 %) and plant material (18.4 %) constituted the base diet. Of the animal prey, Coleoptera and Diptera were the most prevalent, occurring in 13.2 % and 9.8 % of the fish, respectively, and were consumed mainly in the spring. Based on the observed diet composition and feeding strategy, the ruivaco could be considered a generalist, foraging on the most abundant and available prey.

**Key words:** Feeding strategies, native species, diet composition, seasonal variability, Portugal.

### RESUMEN

#### Ecología alimentaria del Ruivaco *Achondrostoma oligolepis*, un pez ciprinideo endémico de Portugal

Este estudio evaluó la ecología alimentaria del ruivaco (*Achondrostoma oligolepis*), un ciprínido endémico de Portugal cuyos hábitos alimenticios son prácticamente desconocidos. Las muestras fueron recogidas estacionalmente, en tres ríos medianos representando un gradiente de temporalidad. Los contenidos estomacales de 97 individuos (42-126 mm de longitud total, LT) fueron analizados. Aunque en general no hubo variación significativa en la composición de la dieta entre ríos, sí se encontraron diferencias entre las estaciones. Se identificó una amplia gama de categorías de alimentos, aunque un subconjunto más pequeño conformó la base de la dieta, encontrándose principalmente dominada por detritus (77.6 %) y material vegetal (18.4 %). Los coleópteros y dípteros fueron los grupos más frecuentes entre las presas animales, siendo encontrados en el 13.2 % y el 9.8 % de los peces respectivamente, y consumidos principalmente en primavera. Con base en la composición de la dieta y en la representación de la estrategia trófica, el ruivaco puede ser considerado como generalista, alimentándose de las presas más abundantes y disponibles.

**Palabras clave:** Ecología trófica, *Achondrostoma oligolepis*, composición de la dieta, variabilidad estacional, Portugal.

## INTRODUCTION

Examining the feeding ecology of fishes is important for evaluating the ecological role that these species play in a broader context and understanding their position in the food web structure (Allan & Castillo, 2007). Additionally, assessing the diet of fishes is important for aquatic management. The European Water Framework Directive (European Union, 2000), a framework to assess ecological integrity of rivers and streams across Europe, has developed numerous indices of biological integrity for fish. These indices frequently include metrics for measuring the position of individual fish in the food chain, which consequently show significant responses to disturbance (e. g., Pont *et al.*, 2007). However, while the trophic ecology of some Iberian fish species is based on expert judgment, there is little support from ecological data, particularly for small-sized cyprinids. There is still a large gap in information about seasonal and size-related feeding habits. Thus, quantifying the diet composition of such species is extremely important, not only to improve knowledge of species ecology but also to obtain more accurate estimates of indices of biological integrity.

The ruivaco *Achondrostoma oligolepis* (Robalo, Doadrio, Almada & Kottelat, 2005) is a small-sized (< 150 mm TL) cyprinid endemic to Portuguese rivers. Its distribution covers the central and northern river basins of Portugal, occupying a great variety of lotic systems from cold-water and permanent streams to warm-water and intermittent watercourses (Ferreira *et al.*, 2007a). This species normally inhabits moderate to slow-flowing waterways with sandy or gravel substratum and aquatic vegetation and is generally considered tolerant to stream degradation (Santos *et al.*, 2004; Ferreira *et al.*, 2007b). It reaches sexual maturity in the second year of life and reproduces from April to June. There are studies that focus on the genetic variability (Robalo *et al.*, 2007) and spatial distribution of this species at different spatial scales (Ferreira *et al.*, 2007a), but none have addressed feeding ecology. This information could prove extremely useful for other small



**Figure 1.** Map of the study area, showing the location of the Arunca (1), Corvo (2) and Estorãos (3) rivers. *Mapa del área de estudio, mostrando la localización de los ríos Arunca (1), Corvo (2) y Estorãos (3).*

cyprinid species (*Achondrostoma spp.*), such as the endangered Western ruivaco *Achondrostoma occidentale* (Robalo, Almada, Sousa-Santos, Moreira & Doadrio, 2005) (Robalo *et al.*, 2008) whose feeding habitats are poorly understood.

This study aimed to address the seasonal feeding ecology of the ruivaco at sites with distinct environmental characteristics in central and northern Portugal. Specifically, the following questions were asked: (a) What are the main food items consumed by the ruivaco?; (b) Are there differences in the diet composition of the ruivaco among sites, seasons, and size-classes, and if so, what are the most important dietary items?; and (c) To what trophic guild could the ruivaco be assigned?

## METHODS

### Study area

The ruivaco individuals were captured at three sites in the Arunca, Corvo and Estorãos rivers (Fig. 1). These rivers were chosen to reflect a gradient of intermittency from seasonal to

perennial. The Arunca is a seasonally intermittent, medium-sized (length = 60 km) river in the Mondego River basin, W Portugal. The climate is Mediterranean pluviseasonal oceanic with most of the rainfall (mean annual value c. 800 mm) between October and March. There is virtually no flow in the warmest months of July and August when large sections of river turn into a series of unconnected temporary pools (intermittent reaches *sensu* Gasith & Resh, 1999). The Arunca is a shallow (mean depth = 24.7 cm), low-gradient river with an abundance of riffle and pool habitats and sparse aquatic vegetation. Stream-bed materials are heterogeneous, mainly dominated by sand, gravel and pebbles, with silting in pool habitats. Continuous riparian vegetation, mainly willow *Salix spp.* and common alder *Alnus glutinosa* gallery forest, border the river channel. The Corvo is a medium-sized (length = 40 km) tributary of the Mondego River and is intermediate in its degree of desiccation and mean annual precipitation (1100 mm). It is relatively deep (mean depth = 62.7 cm) with a predominantly silt-sandy substratum that lacks well-defined pool-riffle sequences. The river channel is bordered by semi-fragmented riparian vegetation with irrigated agriculture in the uplands. The availability of cover for fish along both rivers is generally high, mainly in the form of logs, twigs, undercut banks and root masses. Apart from the ruivaco, other species, such as the Iberian barbel *Barbus bocagei* (Steindachner), the Iberian straight-mouth nase *Pseudochondrostoma polylepis* (Steindachner) and the Northern Iberian chub *Squalius carolitertii* (Doadrio), also occur in these rivers. The Estorãos is a small-sized (length = 18 km) perennial river in the Lima River basin, NW Portugal. Climate is temperate oceanic with relatively high run-off due to a mean annual rainfall of 1800 mm. It is a lowland river with well-defined riffle sections that are interconnected with pool habitats. While the riffles are abundant, the pool habitats predominate. The substratum is mainly composed of silt and sand with abundant riparian and aquatic vegetation. Riparian vegetation is continuous on both banks and consists mainly of English oak (*Quercus robur*), common alder

(*Alnus glutinosa*), willow (*Salix spp.*) and poplar (*Populus spp.*). There are no major pollution sources in the river. Other fish species recorded at the study site included the Northern Iberian chub and the brown trout *Salmo trutta* (L.).

### Fish sampling

Fish sampling was conducted two (Estorãos River) to three (Arunca and Corvo rivers) times at each site during daylight hours in the spring, the beginning of the fish spawning season and seasonal plant growth (April 2007); the summer, the period of minimum flow (July 2007); and the autumn, the beginning of the wet season periods (October 2007). No sampling was conducting during floods in the winter because high water levels prevented efficient sampling. At each site, a 150 m-long reach was electrofished (DC, 200–400 V, Hans Grassl model IG-200) in accordance with CEN (2003) standards, encompassing repeating habitat types (riffles, pools). After sampling, all the collected individuals were identified, counted and measured (TL to the nearest mm). Most individuals were returned alive to the river. However, whenever possible, a sample of 15 individuals of the ruivaco that represented the size range in each collection was retained for diet analysis. They were euthanised immediately with an overdose of benzocaine and transported on ice to the laboratory. The specimens were fixed in 4 % formaldehyde solution for 48 h and then preserved in 70 % ethanol solution.

### Diet analysis

In the laboratory, individuals were dissected and contents of the gastrointestinal tract were removed. Only fish whose gastrointestinal tract was >75 % full were selected. When available, fish with empty guts were replaced randomly with other individuals. Diet items were initially identified to the lowest readily recognisable *taxon*, usually family, under a dissecting microscope (up to 50× magnification) or a high-power microscope (100–400× magnification), weighted and assigned to 14 food categories: detritus, plant material, Diptera, Trichoptera, Coleoptera,

Hymenoptera, Odonata, Ephemeroptera, Lepidoptera, Mollusca, Nematoda, zooplankton, fish eggs and clutches, and other prey (i.e., unidentified macroinvertebrate remains).

### Benthos Availability

To support data on the diet composition of the ruivaco, food availability was assessed in the same fish sampling sites by sampling benthic macroinvertebrate communities. Samples were taken along a 50 m-long stretch in accordance with the official Portuguese protocol for macroinvertebrate sampling (INAG, 2008). The sampling area was selected to cover the greatest possible diversity of habitats, including riffles and areas of deposition. Type and extent of habitats were visually estimated. Six 1 m-long sampling units of the most representative habitats (stones, sand and silt, boulders (> 256 mm), submerged plants and algae) were taken using a 0.25 m × 0.25 m handnet. The composite sample was placed in a labelled plastic flask and fixed in situ using 4 % formaldehyde. In the laboratory, samples were washed, sieved, sorted and identified to the lowest recognisable *taxon*, usually family, by a low-power stereo-microscope and later assigned to order level in accordance with resource-use data (i.e., diet analysis). Data are expressed as relative abundance or number of each food category.

### Data analysis

Three metrics for the description of stomach contents were used to evaluate the importance of each food category (*i*): frequency of occurrence ( $F_i$ , %), prey abundance ( $A_i$ , %), and prey-specific abundance ( $P_i$ , %) (Amundsen, 1995; Amundsen *et al.*, 1996), which were defined according to the following equations:

$$F_i = 100 (N_i N^{-1}) \quad (1)$$

$$A_i = 100 (\sum S_i \sum S_i^{-1}) \quad (2)$$

$$P_i = 100 (\sum S_i \sum S_{ii}^{-1}) \quad (3)$$

where  $N_i$  is the number of individuals with item *i* in gastrointestinal contents,  $N$  is the total

number of individuals,  $S_i$  is the stomach content (weight) composed of item *i*,  $S_i$  is the total stomach content (weight) of all stomachs in the entire sample, and  $S_{ii}$  is the total stomach content in only those fish with prey *i* in their stomach. To test for significant differences in the abundance ( $A_i$ ) of food categories among sites, a non-parametric Kruskal-Wallis test was employed. When an overall significant effect was detected, Tukey post hoc tests were then used for pairwise comparisons. In addition, the trophic diversity of the sampled populations was calculated according to the Shannon-Wiener diversity index ( $H' = -\sum p_i \cdot \log_2 p_i$ ,  $p_i$ -proportion of prey item *i* among the total weight of preys), which provides a relatively objective indication of niche breadth (Marshall & Elliott, 1997). Low values indicate diets dominated by few items, which we then define as specialists, and high values indicate diets dominated by many items, which we define as generalists. In the present study, diets with values greater than 2 were considered high, whilst values lower than 1 were considered low (Encina *et al.*, 2004). In order to evaluate diet specialisation, the evenness index ( $E = H' / (H'_{\max})^{-1}$ ) was computed. Values close to 0 indicate a stenophagous diet, whereas an index closer to one points to a euryphagous diet. The relative importance of each food category among sites and seasons was also assessed by calculating the Index of Relative Importance (IRI) (Windell, 1971), which is described by the equation:

$$IRI = (F_i A_i)^{0.5} \quad (4)$$

Significant differences in diet composition among seasons were investigated using a randomisation method the ANOSIM analysis (Clarke & Warwick, 1994). ANOSIM operates on a resemblance matrix and is similar to a standard univariate ANOVA but does not require normality or homoscedasticity of data. This method employs the *R* statistic to assess the existence of significant differences between the pre-determined groups for a given factor (e.g., season). *R* ranges between -1 and +1, where 0 indicates completely random grouping. For each season, a Similarity Percentages analysis

(SIMPER) was performed to determine which food categories contributed most to the differences among the seasons.

Feeding strategy was determined using Costello's (1990) graphical method, as modified by Amundsen *et al.* (1996). These diagrams are based on two-dimensional representation where each point represents the frequency of occurrence ( $F_i$ ) and the prey-specific abundance ( $P_i$ ). Prey importance (rare prey will be located near the lower left corner of the graph and dominant prey near the upper right corner) and feeding strategy (most points at the bottom of the graph reflect generalisation and most points at the top reflect specialisation) can thus be evaluated by examining the distribution of points along the diagonals and axes of the diagram. In addition, the relationship between feeding strategy and between- or within-phenotype contributions to

the niche width is also represented. Points located at the lower right corner represent a high within-phenotype component, whereas points located at the upper left corner represent a high between-phenotype component (Amundsen *et al.*, 1996). Statistical analyses were carried out with the STATISTICA (StatSoft Inc., 2000) and PRIMER packages (Clarke & Warwick 1994).

## RESULTS

Stomach contents of 97 ruivaco specimens (42–126 mm TL) were analysed. Although the species consumed a large spectrum of food categories, the diet base was primarily detritus ( $F_i = 77.6\%$ ) and plant material ( $F_i = 18.4\%$ ) (Table 1). Of all the animal prey items recorded, Coleoptera and Diptera were the most frequent, occurring in

**Table 1.** Frequency of occurrence ( $F_i$ , %) and abundance ( $A_i$ , %) (weight) of food categories found in the stomachs of the ruivaco in the Arunca, Corvo and Estorãos rivers. Significant differences among rivers were searched by Kruskal-Wallis tests ( $H$ ). The results of a posteriori Tukey test are indicated. Rivers (A-Arunca; C-Corvo; E-Estorãos) with the same lowercase letter are not significantly different; \*\*\*  $P < 0.001$ , ns-not significant. *Frecuencia de ocurrencia* ( $F_i$ , %) y *abundancia* ( $A_i$ , %) (peso) de los tipos de presa encontrados en los estómagos de los ruivacos en los ríos Arunca, Corvo y Estorãos. Las diferencias estadísticas entre los ríos fueron registradas por las pruebas de Kruskal-Wallis ( $H$ ). Resultados de una prueba a posteriori de Tukey se indican. Ríos (A-Arunca, C-Corvo, E-Estorãos) con la misma letra minúscula no son significativamente diferentes; \*\*\*  $P < 0.001$ , ns-no significativo.

Food category	Acronym	Arunca ( $N = 34$ )		Corvo ( $N = 38$ )		Estorãos ( $N = 25$ )		$H$	$P$	Tukey test
		$F_i$ (%)	$A_i$ (%)	$F_i$ (%)	$A_i$ (%)	$F_i$ (%)	$A_i$ (%)			
Detritus	DET	64.7	27.6	92.1	77.9	76.0	44.3	18.6	***	A <sup>a</sup> C <sup>b</sup> E <sup>ab</sup>
Plant material	VEG	20.6	27.2	17.9	9.1	16.8	11.1	0.7	ns	
		Vegetal debris, seed, filamentous and vascular algae, macrophytes								
Diptera	DIP	11.8	26.9	10.1	8.9	7.3	24.0	1.4	ns	
		Chironomidae, Ceratopogonidae, Limoniidae, Simuliidae								
Trichoptera	TRI	4.4	2.6	3.9	0.4	2.0	0.4	1.3	ns	
		Philopotamidae, Glossosomatidae, Rhyacophilidae, Hydropsychidae								
Coleoptera	COL	11.8	5.6	11.8	0.8	16.0	17.0	0.3	ns	
		Elmidae								
Hemynoptera	HYM	5.9	0.4	2.6	0.0	0.0	0.0	1.7	ns	
		Formicidae								
Odonata	ODO	4.4	1.6	1.3	0.2	2.0	0.0	1.5	ns	
		Gomphidae, Aeshnidae								
Ephemeroptera	EFE	4.9	4.5	7.9	0.8	1.3	0.0	3.6	ns	
		Baetidae, Heptageniidae, Ephemeridae								
Lepidoptera	LEP	0.0	0.0	2.6	0.1	0.0	0.0	1.6	ns	
Mollusca	MOL	8.8	3.2	5.3	0.7	0.0	0.0	3.7	ns	
		Hydrobiidae, Planorbidae								
Nematoda	NEM	0.0	0.0	0.0	0.0	8.0	0.0	5.8	ns	
Zooplankton	ZOO	0.0	0.0	3.9	1.0	0.0	0.0	3.1	ns	
		Cladocera, Cyclopoida								
Fish eggs and clutches	EGG	1.5	0.0	1.3	0.2	14.0	0.2	8.1	ns	
Other prey	OTH	38.2	0.4	18.4	0.1	28.0	3.0	3.0	ns	
		Unidentified macroinvertebrate remains								
Trophic diversity ( $H'$ )		2.42		1.21		2.01				
Evenness diversity ( $E$ )		0.70		0.33		0.60				

13.2 % and 9.8 % of the fish and accounting for 7.8 % and 19.9 % of the abundance ( $A_i$ ), respectively. Trichoptera, Odonata and Ephemeroptera were also found in the stomachs of the ruivaco but were less important ( $A_i < 3$  %). Unidentified macroinvertebrate remains (categorised as “Other prey”) occurred in a fair percentage of stomachs ( $F_i = 28.2$  %) but were consumed in very low proportions (*c.*  $A_i = 1$  %).

Overall, there was no difference in diet composition among rivers as shown by non-significant spatial variation in abundance (Kruskal-Wallis test,  $p > 0.05$ ) in 13 out of 14 food categories (Table 1). Only detritus was more frequently consumed in the Corvo than the Arunca (Tukey test,  $p < 0.001$ ). The trophic diversity of the ruivaco was relatively high, particularly in the Arunca and the Estorãos ( $H' = 2.42$  and  $2.01$ , respectively), as was its evenness index ( $E = 0.70$  and  $0.60$ , respectively), which indicates a euryphagous diet. In the Corvo, diversity was lower ( $H' = 1.21$ ,  $E = 0.33$ ) as a result of a high proportion of consumed detritus ( $A_i = 77.9$  %).

Relative abundance of benthic macroinvertebrates in the Arunca and the Corvo varied across seasons, particularly in the case of Diptera and Ephemeroptera, which were the most abundant potential forms of prey available to the ruivaco in the spring (both categories  $> 75$  %), decreasing in following seasons (Table 2). The same groups

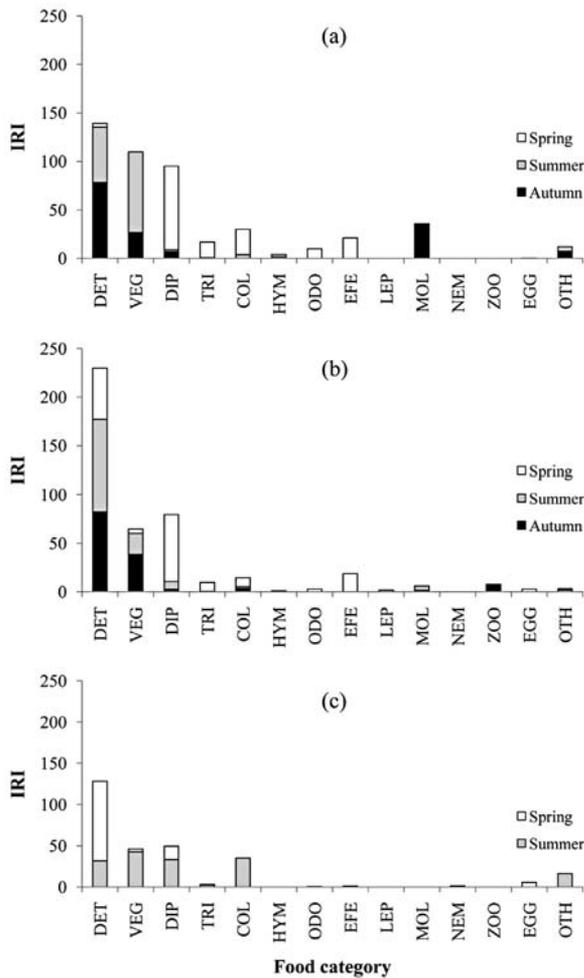
were also found to be the most abundant (55–65 %) in the Estorãos, although their availability did not change markedly between spring and summer.

The importance of different food categories was found to differ across seasons (Fig. 2). In the Arunca, diet was dominated by detritus and plant material in summer ( $IRI = 56.9$  % and  $83.1$  %, respectively) and autumn ( $78.3$  % and  $26.6$  %, respectively), with Mollusca consumption ( $35.9$  %) increasing in the latter. Conversely, animal prey items, such as Diptera ( $86.3$  %), Coleoptera ( $26.3$  %) and Ephemeroptera ( $21.2$  %), were mainly consumed in the spring, whereas detritus ( $4.0$  %) was eaten only marginally. In the Corvo, detritus consumption was important throughout all seasons ( $94.8$  % in summer and  $82.3$  % in autumn), although with a lower proportion was consumed in the spring ( $52.7$  %) when Diptera ( $68.9$  %) took the lead in this species' diet. Ephemeroptera ( $18.7$  %) and Coleoptera ( $9.4$  %) were also consumed during this period. In the Estorãos, the spring diet was highly dominated by detritus ( $96.3$  %), with Diptera ( $16.2$  %) and fish eggs ( $5.6$  %) of less importance. In the summer, detritus consumption ( $31.9$  %) was replaced by a more diverse diet of vegetation ( $42.5$  %), Coleoptera ( $34.6$  %) and Diptera ( $33.1$  %).

These results were confirmed by the pairwise analysis of similarity (ANOSIM) and SIMPER tests. These showed that differences in diet com-

**Table 2.** Relative abundance (number) of benthic macroinvertebrates found in the Arunca, Corvo and Estorãos rivers across the different sampling seasons. *Abundancia relativa (número) de macroinvertebrados bentónicos que se encontraron en los ríos Arunca, Corvo y Estorãos en diferentes estaciones de muestreo.*

Food category	Arunca			Corvo			Estorãos	
	spring	summer	autumn	spring	summer	autumn	spring	summer
Diptera	50.9	13.5	23.5	32.1	24.5	14.9	36.2	25.4
Trichoptera	1.8	10.6	17.3	4.9	46.4	29.4	6.7	7.7
Coleoptera	0.2	13.2	14.7	0.5	3.7	0.0	12.0	13.3
Hemiptera	0.2	0.0	0.3	0.0	1.8	0.6	0.0	3.2
Hemynoptera	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Odonata	0.2	0.8	5.9	0.9	1.8	4.6	11.5	3.9
Ephemeroptera	45.7	38.4	3.6	58.8	14.9	18.1	28.7	32.9
Lepidoptera	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6
Megaloptera	0.0	0.9	1.3	0.0	1.2	5.4	1.6	0.0
Mollusca	1.0	22.6	32.7	0.3	2.8	16.6	0.0	0.0
Nematoda	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Plecoptera	0.0	0.0	0.7	2.5	2.9	10.4	3.3	12.0



**Figure 2.** Index of relative importance (IRI) (%) presented in a stack of the different food categories in the diet of the ruivaco *Achondrostoma oligolepis*. (a) Arunca River; (b) Corvo River; (c) Estorãos River. *Índice de Importancia Relativa (IRI) (%)*, presentado en fichas, de las diferentes categorías de presas en la dieta del ruivaco *Achondrostoma oligolepis*. Ríos (a) Arunca; (b) Corvo; (c) Estorãos.

position among seasons were greatest in the Arunca, particularly between spring and the remaining seasons (ANOSIM,  $R > 0.75$ ,  $P < 0.01$ ) (Table 3). In the Estorãos and the Corvo, the degree of separation was also significant, but to a lesser extent ( $R < 0.40$ ,  $P < 0.01$ ) in the latter. In this case, only differences in diet composition between summer and autumn were found to be non-significant ( $R = 0.007$ ,  $P > 0.05$ ). The results of the SIMPER analysis comparing food categories consumed between seasons agree with patterns observed in previous analyses (Table 4).

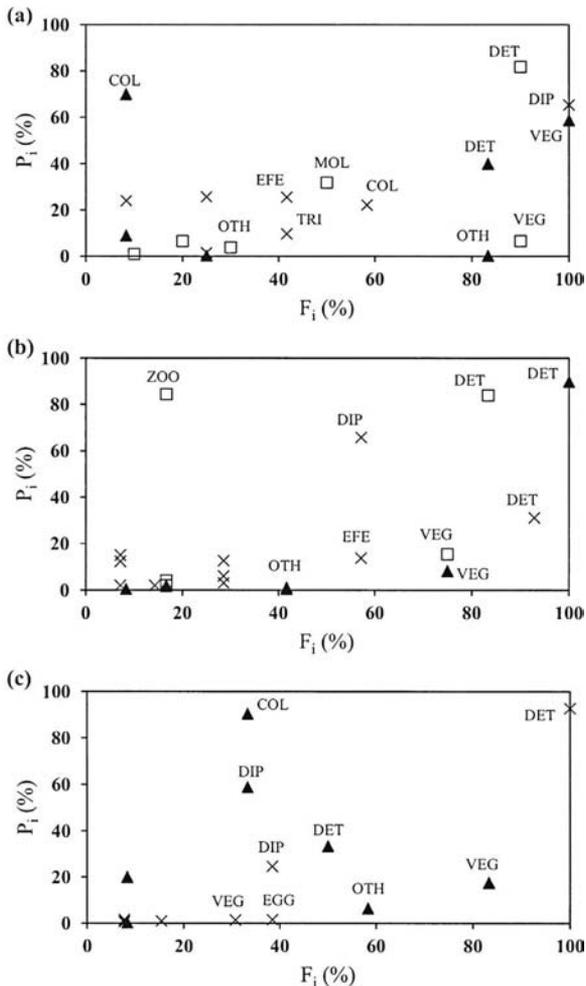
**Table 3.** ANOSIM pairwise R statistics for diet composition of the ruivaco among seasons in each of the three studied rivers. \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ . *Estadísticas R de ANOSIM para la composición de la dieta del ruivaco entre estaciones en cada uno de los tres ríos estudiados.* \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ .

River	Seasons	R	P
Arunca	spring vs summer	0.953	***
	spring vs autumn	0.919	***
	summer vs autumn	0.371	***
Corvo	spring vs summer	0.284	**
	spring vs autumn	0.337	***
	summer vs autumn	0.007	
Estorãos	spring vs summer	0.481	***

**Table 4.** Average similarity (%) on the diet composition of the ruivaco within different seasons for each of three studied rivers and corresponding breakdown contributions (%). Food acronyms are listed in Table 1. *Similitud media (%) en la composición de la dieta en las distintas estaciones para cada uno de los tres ríos estudiados y contribuciones correspondientes (%)*. Siglas de los elementos de la dieta están en la Tabla 1.

Season	Rivers	Similarity	
		Average (%)	Contribution (%)
Spring	Arunca	41.5	DIP (62.8), COL (23.6), EFE (6.6)
	Corvo	44.1	DET (67.0), DIP (22.1), EFE (8.0)
	Estorãos	71.9	DET (94.5)
Summer	Arunca	56.3	VEG (71.2), DET (27.9)
	Corvo	60.7	DET (88.7), VEG (9.8)
	Estorãos	26.7	VEG (48.4), COL (23.3), DET (18.4)
Autumn	Arunca	43.6	DET (79.4), VEG (16.0)
	Corvo	53.3	DET (77.6), VEG (20.2)
	Estorãos	—	—

In the Arunca, average similarity in diet among seasons ranged between 41.5 % and 56.3 %, with detritus and plant material contributing over 90 % of the similarity in summer and autumn. On the contrary, in the spring, Diptera consumption alone contributed over 60 % of the average similarity. The results were similar for the Corvo, with detritus and plant material both accounting for more than 95 % of the average similarity



**Figure 3.** Costello's graphic method modified by Amundsen (1996) for all food categories in the diet of the ruivaco in Arunca (a), Corvo (b) and Estorãos (c) rivers. (x) Spring; (▲) Summer; (□) Autumn. Note that acronyms for rare food categories (i.e., located in the lower left corner) were omitted for clarity. *Método gráfico de Costello modificado por Amundsen (1996) para todas las categorías de presas en la dieta del ruivaco en los ríos Arunca (a), Corvo (b) y Estorãos (c).* (x) Primavera; (▲) Verano; (□) Otoño.

(range: 44.1 %-60.7 %) in summer and autumn. As in the Arunca, Diptera was also an important component of diet composition during spring with a moderate contribution of 22.1 %. In the Estorãos, the maximum contribution of a single item was 94.5 % (detritus in the spring). This was the only item contributing to the average similarity of 71.9 %. During summer, a decrease in the proportion of detritus (18.4 %) in the diet was

observed along with an increase in the proportions of plant material (48.4 %) and Coleoptera (23.3 %) to the average similarity (26.7 %).

Analysis of feeding strategy plots, according to Costello's graphical method as modified by Amundsen, seems to indicate an overall generalist feeding strategy in the three studied rivers. Most points representing food categories are located at the bottom of the graph ( $P_i$  (%) < 40 and  $5 < F_i$  (%) < 90), thereby reflecting generalization (Fig. 3). Nonetheless, a frequent ( $F_i$  (%) > 80) and high ( $P_i$  (%) > 80) seasonal consumption of particular food items in some rivers could denote temporary differentiation in diet. As shown by previous results, detritus was the dominant food item in the Arunca and the Corvo in summer and autumn, whereas Diptera was dominant in spring. In the Estorãos, detritus was the most frequent and abundant food category consumed in the spring, but both prey, including Coleoptera and Diptera, and plant material were consumed in greater abundance in summer. The overall absence of points located at the upper left corner of the diagrams indicates that a high between-phenotypic contribution was unlikely to occur.

## DISCUSSION

This analysis of ruivaco feeding habits revealed a diet composed largely of detritus and plant material, although it also showed ingestion of a broad range of less frequently consumed items, such as benthic macroinvertebrates. The importance of detritus and plant material has been highlighted for other native Iberian species, particularly cyprinids (Encina *et al.*, 1999), as these materials often represent the only available food resources in fluctuating environments and periods of food shortage (Magalhães, 1992; Encina *et al.*, 2004). This is the case with the Arunca and the Corvo, which seasonally experience seasonal periods of extended water scarcity and where the highest contribution of detritus has been observed in this species' diet. The consumption of detritus and plant material permits a considerable drop in the cost of searching for food (Collares-Pereira *et al.*, 1996) despite the fact that their assimilation

by fish and nutritional value are low compared to animal items. However, with the exception of detritus, there was no significant variation in diet composition between rivers, although pronounced seasonal variations were detected and may denote an opportunistic behaviour involving the use of locally abundant food resources.

The significant variation in diet composition observed across seasons may reflect the availability of different items in the environment and their accessibility (Greenberg *et al.*, 1997). In the Arunca and the Corvo, the contribution of detritus and plant material to the diet of ruivaco was highest in summer and autumn with virtually no consumption of animal prey items. Animal prey items, particularly Diptera and Ephemeroptera, however, were consumed in quantity and composed the main forage base in spring in both rivers. These seasonal shifts in food consumption are likely related to seasonal fluctuations in abundance of aquatic macroinvertebrates (Ribeiro *et al.*, 2007). Accordingly, the relative abundance of Diptera – an order that is of great importance to fish because of its high caloric content and low mobility that facilitates its capture (Easton & Orth, 1992) – is greater in spring and decreases by early autumn (Magalhães, 1993). The results in this study conform to this pattern of abundance of benthic macroinvertebrates in the Arunca and the Corvo in spring (> 50 %), with subsequent decreases by autumn (< 25 %), lowering the likelihood of consumption by fish. During periods of flooding, biological resources are destroyed. The high torrential flow, lower temperature and scarce food availability are unfavourable to fish. At the end of winter, after living in harsh environmental conditions, trophic resources are used for somatic energy storage (Encina & Granado-Lorencio, 1997a). This energy can be used later in spring during gonad maturation and reproduction (Encina & Granado-Lorencio, 1997b). Similar results were found for a “sister” species, *A. arcasii*, in other Iberian rivers, where the importance of detritus in the diet decreased when availability of macroinvertebrate prey increased (Lobón-Cerviá & Rincón, 1994). This could be a feeding strategy for fish species that inhabit fluctuating environments. Conversely, in the Es-

torãos, the availability of the animal prey Diptera and Coleoptera was equally abundant throughout spring and summer, although their consumption was more important in summer. It is possible that the colder and wetter climate of the Estorãos may have induced a delay in the emergence of several groups of prey that have aerial adult stages, such as Ephemeroptera and Diptera, which preferentially occurs in the summer when environmental conditions are more favourable. Bonada *et al.* (2007) presented similar findings when they examined biological trait differences in benthic macroinvertebrates between Mediterranean and temperate rivers. Analysis of feeding strategy plots with food item points mainly located at the bottom of the diagrams suggests that the ruivaco adopts a generalist feeding strategy in all of the studied rivers (Noble *et al.*, 2007). This result is consistent with other studies in Iberia (Magalhães, 1993; Valladolid & Przybylski, 1996) and highlights the versatility of feeding habits of species that have evolved in seasonally fluctuating environments. However, some food categories, such as detritus, plant material and Diptera, seem to contribute more to diet, as they are positioned in the upper right corner of the diagram. At the same time, the overall absence of points in the upper left corner of the diagrams suggests the absence of a high between-phenotypic component, i.e., there was no evidence that some of prey have been consumed by a few specialised individuals. It is clear that further studies addressing the influence of sex and size-related microhabitat use on a seasonal and diet basis are needed to clarify and advance the knowledge about the mechanisms that regulate food-resource use by this species.

Taken together, results from the present study suggest that the ruivaco is a generalist fish that feeds opportunistically according to food availability. This plasticity allows the species to inhabit different environments with apparently no costs for its life history, particularly in harsh, seasonally fluctuating rivers. The assignment of the trophic guild to the target species, as presented in this study, is essential not only for an improved understanding of their feeding habits but also as a basis to assess food habits of other small-size cyprinids (*Achon-*

*drostoma* spp.), particularly the critically endangered Western ruivaco, for which knowledge of their trophic ecology remains poor and scarce.

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