

**ONTOGENETIC DIETARY SHIFTS AND FOOD SELECTION OF ENDEMIC
SQUALIUS CAROLITERTII (ACTINOPTERYGII: CYPRINIFORMES: CYPRINIDAE)
IN RIVER TORMES, CENTRAL SPAIN, IN SUMMER**

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Background. The northern Iberian chub *Squalius carolitertii* (Doadrio, 1988) is a small endemic cyprinid inhabiting the rivers of the Iberian Peninsula. The knowledge of feeding patterns is essential to understand the ecological role of fish populations, helping to the development of conservation and management plans. The aim of the present study was to analyze the ontogenetic dietary shifts and food selection of *S. carolitertii*, contributing to knowledge of the feeding behaviour of this fish species.

Materials and methods. Diet composition of *S. carolitertii* was compared to benthos and drift composition in a river of Central Spain (Ávila, River Tormes) using selectivity indices of Ivlev and Savage. The age of 57 *S. carolitertii* collected in August 2010 was determined by scale reading and by length frequency analyses (LFA) with the Petersen method. Maximum length of benthos, drift and prey invertebrates was measured for each item to establish whether prey-size selection depends upon the size-frequency distribution of available prey.

Results. Detritus were found in 33 fish (57.9% of occurrence). Nymphs of *Baetis* spp. were the most abundant prey (46.6%) and were identified in the 49.1% of the stomachs. Moreover, *Baetis* spp. was selected positively from the benthos and drift by all age classes. Abundant potential prey items such as *Epeorus* spp. in the benthos and Simuliidae in the drift were negatively selected. Individuals without detritus in the gut contained more animal prey items than individuals with a dominance of detritus, and the frequency of occurrence of detritus decreased with the age. Mean prey size increased with fish size ($r = 0.646$, $P < 0.001$).

Conclusion. Age-related diet shifts occur at three different levels: (1) frequency of occurrence of detritus decreases with fish age; (2) prey selection varied with fish age; and (3) mean prey size increased as fish size increased. The rejection of *Epeorus* spp. and Simuliidae suggests that other factors, apart of prey abundance, including site-specific prey accessibility, prey size, energetic selection criteria and prey preference of fishes play an important role in feeding behaviour of *S. carolitertii*. Prey-size selection is probably dependent on the size-frequency distribution of the available prey.

Keywords: diet, selection, *Squalius carolitertii*, prey, drift, summer

INTRODUCTION

In the Iberian Peninsula, the freshwater fish fauna is dominated by cyprinids and is characterized by a high level of endemism (Doadrio 2001), as around 45% of Iberia's native fish species are endemic (Gómez and Lunt 2007). Recently, Iberian populations of the genus *Leuciscus* were transferred into the genus *Squalius* (see Sanjur et al. 2003, Kottelat and Freyhof 2007), and the majority of species are endemic at drainage level (Leunda et al. 2009). The development of effective conservation programmes for endemic fish species requires a clear understanding of the

ecological requirements of these species, and a better knowledge of their feeding habits is essential for this objective.

The Northern Iberian chub, *Squalius carolitertii* (Doadrio, 1988), is a small endemic cyprinid inhabiting the rivers of the Iberian Peninsula across a large area, including the Douro, Mondego, Lima, Minho, and Lézé basins (Doadrio 1988, 2001, Carmona and Doadrio 2000). Recently Perea et al. (2011) reported this species for the first time from the upper reaches of the Alberche River (a tributary of the Tagus basin in central Spain) and in the

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Oitavén River (a tributary of the Verdugo River in north-western Spain). Except for the populations in the Tajo basin, this species is listed as vulnerable (VU) in the Spanish Red Data Book (Doadrio 2001) and as Least Concern (LC) in the IUCN Red List of Threatened Species (Crivelli 2006). Although some studies of *S. carolitertii* have been undertaken in recent years, the biology of this species remains largely unknown. Its general habitat requirements (Carmona and Doadrio 2000, Santos et al. 2004, Maia et al. 2006) are well known, and data on population parameters, growth and reproduction have been reported by Maia et al. (2006). Genetic, morphologic and phylogenetic studies have also focused on this fish species (Coelho et al. 1995, Zardoya and Doadrio 1998, Gómez and Lunt 2007, Cunha et al. 2009). However, diet composition of *S. carolitertii* remains poorly documented and the limited information available on feeding behaviour comes from Sánchez-Hernández and Cobo (2011). On the other hand, information concerning the feeding behaviour of other species of *Squalius* in the Iberian Peninsula is more freely available, especially for *Squalius pyrenaicus* (Günther, 1868) (see Rodríguez-Jiménez 1987, Magalhães 1993a, b, Coelho et al. 1997, Blanco-Garrido et al. 2003).

In particular, although Sánchez-Hernández and Cobo (2011) found that *S. carolitertii* was an omnivorous fish feeding predominantly on aquatic invertebrates, other aspects of its feeding ecology, including food selection, ontogenetic diet shifts and seasonal differences in diet composition remain unknown. A better knowledge of the feeding habits of *S. carolitertii* would provide important information to help understand the trophic requirements of this fish. Several researchers have demonstrated that studies based on food selection provide insight into factors involved in prey choice in freshwater fish species (Rincón and Lobón-Cerviá 1999, Johnson et al. 2007, Sánchez-Hernández et al. 2011a, b). Hence, the goal of this research was to study the ontogenetic dietary shifts and food selection of *S. carolitertii* in an Iberian river during summer, contributing to the knowledge of the feeding behaviour of this fish species.

MATERIAL AND METHODS

Individuals of *Squalius carolitertii* were collected from the River Tormes in Ávila (Central Spain, UTM: 30T 288707 4466342). This is a tributary of the River Duero (897 km total length). Environmental characteristics of the study site are detailed in Sánchez-Hernández and Cobo (2011).

The study was conducted in a wadeable riffle section of the river, and samples were collected in August 2010. Prior to electrofishing, samples of potential prey species (benthic and drifting invertebrates) were collected to study prey selection in *S. carolitertii*. Benthic invertebrates were collected using a 0.1 m² Surber sampler ($n = 9$). Brundin nets (250 µm mesh size, 1 m length, 30 cm mouth diameter) were used to collect drifting aquatic and terrestrial invertebrates between 1000 and 1400 h. After collection, both benthic and drift samples were fixed using 4% formalin

and stored for later processing. In the laboratory, macroinvertebrates were identified to the lowest taxonomic level possible and the abundance of each item was calculated.

Fish were collected using pulsed direct-current backpack electrofishing equipment (Hans Grassl GmbH, ELT60II). For the purpose of the study 57 *S. carolitertii* captured were killed immediately by an overdose of anaesthetic (benzocaine), and transported in coolboxes (approx. 4°C) to the laboratory, where they were frozen at -30°C until processed. Fork length of *S. carolitertii* ranged from 4.4 to 14 cm (mean fork length ± standard error = 6.5 cm ± 0.28). The age of fishes was determined by scale reading and by length frequency analyses (LFA) with the Petersen's method. Thus, the sample includes specimens from one to five years: $n_{age 1} = 35$, $n_{age 2} = 13$, $n_{age 3} = 7$ and $n_{age 5} = 2$. No *S. carolitertii* of age 4 were collected.

In the laboratory the fish were dissected and their gastrointestinal tracts removed. No empty gastrointestinal tracts were observed. Prey items were allocated to diet categories as follows: aquatic invertebrates, terrestrial invertebrates, and other prey items. The abundance of detritus was not quantified because it was impossible to count individual items, but the number of gastrointestinal tracts in which it was found was noted. A visual evaluation of detritus volume was made according to the method of Collares-Pereira et al. (1996). Three categories were established: absence (0%), presence (<50%), and dominance (>50%). To describe the diet, data are presented on the relative abundance of prey items (A_i):

$$A_i = 100 \times \Sigma S_i \times \Sigma S_i^{-1}$$

where: S_i = gastrointestinal tract content (number) composed by prey i , and S_i = the total gastrointestinal tract content of all gastrointestinal tracts in the entire sample, and frequency of occurrence of preys (F_i):

$$F_i = 100 \times N_i \times N^{-1}$$

where N_i is the number of fish with prey i in their gastrointestinal tract and N is the total number of fish with gastrointestinal tract contents of any kind.

In order to study prey selection of Northern Iberian chub, feeding selectivity was measured using Ivlev's selectivity index (Ivlev 1961) and the Savage index (Savage 1931). Possible values of Ivlev's selectivity index range from -1 to +1, with negative values indicating rejection or inaccessibility of the prey, zero indicating random feeding, and positive values indicating active selection. The Savage index varies from zero (maximum negative selection) to infinity (maximum positive selection).

Finally, maximum length of benthos, drift and prey invertebrates was measured for each item with a digital calliper (Mitutoyo Absolute, 0.01-mm precision, Japan) to study whether prey-size selection is dependent upon the size-frequency distribution of available prey. Invertebrates were grouped into 2-mm length classes. When invertebrates were fragmented or partially digested, the prey length was estimated from the width of the cephalic capsule (see Rincón and Lobón-Cerviá 1999), which was normally the best preserved part.

Statistical analyses were conducted using the PASW Statistics 18 software. Kruskal–Wallis tests for non-normal data were used for detecting differences among detritus categories and age classes, and Mann–Whitney U test were used for *a posteriori* comparisons. The Spearman correlation was used to examine correlations between the feeding variables (number of prey items, percentage of aquatic invertebrates, percentage of terrestrial invertebrates and mean prey size) and fish size. All tests were considered statistically significant at $P < 0.05$.

RESULTS

A total of 4381 specimens of the benthic invertebrate fauna were collected, and could be grouped into 31 taxa. *Epeorus* spp. was the most abundant, and represented 29.6% of the total number of individuals. *Baetis* spp. and *Simuliidae* contributed 19% and 12.4%, respectively to total abundance (Table 1). The drift was composed of both aquatic and terrestrial invertebrates (Table 1). A total of 225 invertebrates representing 27 taxa were collected from the drift, dominated by simuliid larvae (66.2%).

Detritus were found in 33 fish (57.9% of occurrence). A total of 371 prey items were identified in the gastrointestinal tracts of Northern Iberian chub, including 23 types of prey (Table 1). Sixteen of these 23 categories were aquatic invertebrates, and only six corresponded to terrestrial invertebrates. In general, nymphs of *Baetis* spp. were the most abundant prey (46.6%) and were identified in 49.1% of the gastrointestinal tracts (Table 1). Terrestrial invertebrates were also present (7.4% of total prey). Piscivory was observed in only one *S. carolitertii* (age 3, 10.8 cm fork length, FL). The diet varies with fish age (Table 2), with the most abundant prey item differing in each age class: Simuliidae (42.3%) in age 1, Hydracarina (33.3%) in age 2, *Baetis* spp. (79.2%) in age 3, and *Baetis* spp. and *Allogamus* sp. (both 28.6%) in age 5.

The number of prey items tended to decrease as the amount of detritus increased in Northern Iberian chub (Fig. 1; Kruskal–Wallis test; $P < 0.001$), as can be seen in Fig. 1 the number of prey items was higher in absence category than presence category (Mann–Whitney U test, $P = 0.022$) and dominance category (Mann–Whitney U test, $P < 0.001$). On the other hand, the number of prey items consumed by Northern Iberian chub ranged from 0 to 126 (mean \pm standard error = 6.5 ± 2.23), with the relation between the number of prey items consumed and fish size positive but not significant ($r = 0.234$; $P = 0.080$). Only one fish had as many as 126 prey individuals in the gastrointestinal tract, but 73.7% of the total *S. carolitertii* sample analysed in the present study had consumed between 1 and 18 prey items.

Fourteen chub specimens (24.6% of the total) had only detritus in their gastrointestinal tract. These included nine specimens of age 1 and five fish of age 2. Table 2 shows that the frequency of occurrence of detritus decreases with age (range: 65.7% in age 1 to 0% in age 5). On the other hand, no differences were found in the number of animal prey items (Kruskal–Wallis test; $P = 0.293$), the percentage

of aquatic invertebrates (Kruskal–Wallis test; $P = 0.238$) or the percentage of terrestrial invertebrates (Kruskal–Wallis test; $P = 0.736$) among age classes. Moreover, the present study shows that the correlation between fish length and the percentage of aquatic invertebrates was positive but not significant ($r = 0.208$; $P = 0.12$), and also the percentage of terrestrial invertebrates was not related to fish size ($r = -0.038$; $P = 0.777$).

A comparison of macroinvertebrate availability in the environment (drift and benthos) and prey selectivity using both Ivlev's selectivity and Savage indices shows that *S. carolitertii* selected positively for different items (Tables 1 and 2 and Fig. 2). Despite the high abundance of *Epeorus* spp. and *Hydropsyche* spp. in the benthos and Simuliidae in the drift, these items, according to Ivlev's selectivity index, were selected negatively (Fig. 2). On the other hand, *Baetis* spp. were selected positively in both benthos and drift by all ages, making up a large component of the diet for all age classes (20.4% in age 2 to 79.2% in age 3). Despite this preference for *Baetis* spp., according to both selectivity indices, prey selection varies with fish age as can be seen in Table 2.

Mean prey size was correlated with fish size ($r = 0.646$; $P < 0.001$). Furthermore, there were differences in the average size of consumed among age classes (Kruskal–Wallis test; $P = 0.003$). Thus the average prey size (mean \pm standard error) was larger for age 3 (8.2 ± 1.601 mm) fish than for age 2 (3.5 ± 0.677 mm) and age 1 fish (3.6 ± 0.304 mm) (Mann–Whitney U test, $P = 0.018$ and Mann–Whitney U test, $P = 0.02$, respectively). No differences were found between age 1 and age 2 (Mann–Whitney U test, $P = 0.839$) or between age 5 (9.6 ± 0.350 mm) and age 3 (Mann–Whitney U test, $P = 0.317$).

Organisms 2–4 mm long were generally the most numerous size class in drift and benthos samples (42% and 46.9% of the total, respectively). The 4–6 mm size category was also abundant in both drift and benthos samples (37% and 35.8%, respectively, see Table 3 and Fig. 3). *S. carolitertii* fed mainly on prey within the 1–15 mm size range (3.72 ± 0.275 mm), with prey of 2–4 mm being the most commonly consumed (55.6% of total). Less numerous in the gastrointestinal tracts, but very important in *S. carolitertii* diet, was the < 2 mm size category (Table 3 and Fig. 3). As can be seen in Fig. 3, the size-frequency distributions of diet, benthos and drift for 6–8, 8–10, and > 10 mm size categories were similar. On the other hand, < 2 and 2–4 mm size categories were more frequently encountered in the diet than in the environment (benthos and drift), and 4–6 mm size category was more frequently in the environment (benthos and drift) than in the diet (Fig. 3). Moreover, although the most abundant prey size category in benthos, drift and prey invertebrates was the same (2–4 mm), significant differences in the mean length among samples were found (Kruskal–Wallis test; $P < 0.001$), being higher in the drift (5.3 ± 0.309 mm) than in the gastrointestinal tracts (Mann–Whitney U test, $P < 0.001$) and higher in the benthos (4.99 ± 0.117 mm) than in the gastrointestinal tracts (Mann–Whitney U test, $P < 0.001$),

Table 1

Principal parameters of the food of *Squalius carolitertii* from the Tormes River, Central Spain

Prey item	DS	Benthos A_i [%]	Drift A_i [%]	Diet		Ivlev index		Savage index	
				A_i [%]	F_i [%]	Benthos	Drift	Benthos	Drift
<i>Dugesia</i> spp.	A	1.86	—	—	—	-1	—	0	—
Lumbriculidae	A	0.08	—	—	—	-1	—	0	—
<i>Ancyclus fluviatilis</i>	A	0.76	0.89	—	—	-1	-1	0	0
Hydracarina gen. sp.	A	0.08	—	6.7	8.8	1	1	84.2	IF
Copepoda gen. sp.	A	—	—	0.3	1.8	1	1	IF	IF
<i>Baetis</i> spp.	N	18.97	7.56	46.6	49.1	0.4	0.7	2.5	6.2
<i>Ephemerella</i> spp.	N	3.39	1.33	—	—	-1	-1	0	0
<i>Ecdyonurus</i> spp.	N	3.3	1.78	—	—	-1	-1	0	0
<i>Epeorus</i> spp.	N	29.55	1.78	2.7	15.8	-0.8	0.2	0.1	1.5
<i>Habrophlebia</i> sp.	N	0.08	—	0.5	1.8	0.7	1	6.7	IF
<i>Leuctra geniculata</i>	N	2.03	1.78	0.3	1.8	-0.8	-0.7	0.1	0.2
<i>Perla</i> sp.	N	0.08	—	—	—	-1	—	0	—
<i>Boyeria irene</i>	N	0.08	—	—	—	-1	—	0	—
<i>Ophiogomphus</i> sp.	N	0.42	0.89	—	—	-1	-1	0	0
<i>Aphelocheirus aestivalis</i>	A	1.19	0.44	0.8	5.3	-0.2	0.3	0.7	1.8
<i>Micronecta</i> sp.	A	—	0.44	—	—	—	-1	—	0
<i>Elmis</i> sp.	L	0.34	0.44	0.5	3.5	0.2	0.1	1.6	1.2
<i>Macronychus</i> sp.	L	1.19	0.44	—	—	-1	-1	0	0
<i>Oulimnius</i> sp.	A	—	0.44	—	—	—	-1	—	0
<i>Esolus</i> spp.	A	0.85	—	—	—	-1	—	0	—
<i>Orectochilus</i> sp.	L	—	0.44	—	—	—	-1	—	0
<i>Hydraena</i> sp.	A	0.17	—	—	—	-1	—	0	—
<i>Micrasema</i> sp.	L	0.59	—	—	—	-1	—	0	—
<i>Hydropsyche</i> spp.	L	11.18	4	2.4	14	-0.6	-0.2	0.2	0.6
<i>Lepidostoma</i> sp.	L	0.08	—	—	—	-1	—	0	—
Leptoceridae	L	—	—	0.3	1.8	1	1	IF	IF
<i>Allogamus</i> sp.	L	0.17	0.44	2.2	7	0.9	0.7	12.7	4.9
<i>Chimarra marginata</i>	L	3.3	0.89	0.3	1.8	-0.8	-0.5	0.1	0.3
<i>Polycentropus</i> sp.	L	0.25	—	1.1	7	0.6	1	4.3	IF
<i>Rhyacophila</i> spp.	P	1.1	0.89	—	—	-1	-1	0	0
<i>Rhyacophila</i> spp.	L	1.78	0.44	1.6	10.5	-0.05	0.6	0.9	3.7
<i>Atherix</i> sp.	L	0.68	0.89	—	—	-1	-1	0	0
Tanypodinae	L	3.05	3.56	3	14	-0.01	-0.1	0.97	0.8
Orthoclaudiinae	L	0.93	—	—	—	-1	—	0	—
Empididae	L	0.08	—	—	—	-1	—	0	—
Tipulidae	L	—	0.44	—	—	—	-1	—	0
Simuliidae	L	12.36	66.22	22.9	40.4	0.3	-0.5	1.9	0.3
Brachycentridae	A	—	0.44	—	—	—	-1	—	0
Trichoptera gen. sp.	A	—	—	1.9	8.8	—	1	—	IF
Ephemeroptera gen. sp.	A	—	—	0.5	3.5	—	1	—	IF
Asilidae	A	—	0.44	—	—	—	-1	—	0
Chironomidae	A	—	0.44	—	—	—	-1	—	0
Simuliidae	A	—	1.78	—	—	—	-1	—	0
Diptera gen. sp.	A	—	—	0.5	3.5	—	1	—	IF
Formicidae	A	—	0.44	3.2	17.5	—	0.8	—	7.4
Coleoptera gen. sp.	A	—	—	0.5	3.5	—	1	—	IF
Arachnida gen. sp.	A	—	—	0.8	5.3	—	1	—	IF
OP Fish		—	—	0.3	1.8	—	—	—	—
OP Detritus		—	—	—	57.9	—	—	—	—

DS = developmental stage; A = adult; N = nymph; L = larvae; P = pupae; A_i = relative abundance; F_i = frequency of occurrence; IF = Savage index tended to infinity; OP = other prey items.

Table 2
Diet composition and feeding parameters in age groups of *Squalius carolitertii* from the Tormes River, Central Spain

Prey item	Age 1						Age 2						Age 3						Age 5					
	Diet		Savage		Ivlev		Diet		Savage		Ivlev		Diet		Savage		Ivlev		Diet		Savage		Ivlev	
	A_i	F_i	B	D	B	D	A_i	F_i	B	D	B	D	A_i	F_i	B	D	B	D	A_i	F_i	B	D	B	D
<i>Dugesia</i> spp.	—	—	-1	—	0	—	—	—	—	0	—	—	—	—	-1	—	0	—	—	—	—	-1	—	0
Lumbriculidae	—	—	-1	—	0	—	—	-1	—	0	—	—	—	-1	—	0	—	—	—	—	—	-1	—	0
<i>Ancyclus fluvialtilis</i>	—	—	-1	-1	0	0	—	—	-1	0	0	—	—	-1	-1	0	0	—	—	—	-1	-1	0	0
Hydracarina gen. sp.	4.5	5.7	1	1	56.1	IF	33.3	23.1	0.995	1	416.7	IF	—	—	-1	1	0	IF	—	—	-1	1	0	IF
Copepoda gen. sp.	0.6	2.9	1	1	IF	IF	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Baetis</i> spp.	24.4	42.9	0.1	0.5	1.3	3.2	20.4	53.8	0.04	0.5	1.1	2.7	79.2	57.1	0.6	0.8	4.2	10.5	28.6	100	0.2	0.6	1.5	3.8
<i>Ephemera</i> spp.	—	—	-1	-1	0	0	—	—	-1	-1	0	0	—	—	-1	-1	0	0	—	—	-1	-1	0	0
<i>Ecdyonurus</i> spp.	—	—	-1	-1	0	0	—	—	-1	-1	0	0	—	—	-1	-1	0	0	—	—	-1	-1	0	0
<i>Epeorus</i> spp.	2.6	11.4	-0.8	0.2	0.1	1.4	3.7	15.4	-0.8	0.4	0.1	2.1	2.6	42.9	-0.8	0.2	0.1	1.5	—	—	-1	-1	0	0
<i>Habrophlebia</i> sp.	1.3	5.7	0.9	1	16.0	IF	—	—	-1	—	0	—	—	-1	—	0	—	—	—	—	-1	-1	0	—
<i>Leuctra geniculata</i>	—	—	-1	-1	0	—	—	—	-1	-1	0	—	—	0.6	14.3	-0.5	0.3	0.4	—	—	-1	-1	0	0
<i>Pera</i> sp.	—	—	-1	—	0	—	—	—	-1	—	0	—	—	—	-1	—	0	—	—	—	-1	-1	0	—
<i>Boyeria irene</i>	—	—	-1	—	0	—	—	—	-1	—	0	—	—	—	-1	—	0	—	—	—	-1	-1	0	—
<i>Ophiogomphus</i> sp.	—	—	-1	-1	0	0	—	—	-1	-1	0	0	—	—	-1	-1	0	0	—	—	-1	-1	0	0
<i>Aphelocheirus aestivalis</i>	1.3	5.7	0.04	0.5	1.1	2.9	—	—	-1	-1	0	0	—	—	-1	-1	0	0	14.3	50	0.8	0.9	12.0	32.5
<i>Micronecta</i> sp.	—	—	-1	-1	—	0	—	—	-1	-1	0	0	—	—	-1	-1	0	0	—	—	-1	-1	—	0
<i>Elmis</i> sp.	0.6	2.9	0.3	0.2	1.9	1.5	—	—	-1	-1	0	0	—	—	-1	-1	0	0	14.3	50	1	0.9	42.0	32.5
<i>Macrorhynchus</i> sp.	—	—	-1	-1	0	0	—	—	-1	-1	0	0	—	—	-1	-1	0	0	—	—	-1	-1	0	0
<i>Oulimnius</i> sp.	—	—	-1	-1	—	0	—	—	-1	-1	—	0	—	—	-1	-1	—	0	—	—	-1	-1	—	0
<i>Esolus</i> spp.	—	—	-1	—	0	—	—	—	-1	—	0	—	—	—	-1	—	0	—	—	—	-1	-1	—	0
<i>Orectochilus</i> sp.	—	—	-1	-1	—	0	—	—	-1	-1	—	0	—	—	-1	-1	—	0	—	—	-1	-1	—	0
<i>Hydraena</i> sp.	—	—	-1	—	0	—	—	—	-1	-1	0	—	—	—	-1	-1	0	—	—	—	-1	-1	—	0
<i>Micrasema</i> sp.	—	—	-1	—	0	—	—	—	-1	-1	0	—	—	—	-1	-1	0	—	—	—	-1	-1	—	0
<i>Hydropsyche</i> spp.	4.5	17.1	-0.4	0.06	0.4	1.1	3.7	15.4	-0.5	-0.04	0.3	0.9	—	—	-1	-1	0	0	—	—	-1	-1	0	0
<i>Lepidostoma</i> sp.	—	—	-1	—	0	—	—	—	-1	—	0	—	—	—	-1	—	0	—	—	—	-1	-1	—	0
Leptoceridae	—	—	-1	—	—	—	—	—	—	—	—	—	0.6	14.3	1	1	IF	IF	—	—	—	—	—	—
<i>Allogamus</i> sp.	—	—	-1	-1	0	0	—	—	-1	-1	0	0	3.9	57.1	0.9	0.8	22.9	8.9	28.6	50	0.99	0.97	168.1	64.9
<i>Chimarra marginata</i>	0.6	2.9	-0.7	-0.2	0.2	0.7	—	—	-1	-1	0	0	—	—	-1	-1	0	0	—	—	-1	-1	0	0
<i>Polycentropus</i> sp.	—	—	-1	—	0	—	—	—	-1	—	0	—	—	1.9	42.9	0.8	1	7.8	IF	14.3	50	0.97	1	57.1
<i>Rhyacophila</i> spp.	—	—	-1	-1	0	0	—	—	-1	-1	0	0	—	—	-1	-1	0	0	—	—	-1	-1	0	0
<i>Rhyacophila</i> spp.	2.6	11.4	0.2	0.7	1.4	5.8	1.9	7.7	0.02	0.6	1.04	4.2	0.6	14.3	-0.5	0.2	0.4	1.5	—	—	-1	-1	0	0

Aquatic Invertebrates

cont. Table 2

<i>Atherix</i> sp.	—	—	-1	-1	0	0	—	—	-1	-1	0	0	—	-1	-1	0	0	
Tanypodinae	3.8	14.3	0.1	0.04	1.3	1.1	1.9	7.7	-0.2	-0.3	0.6	0.5	2.6	42.9	-0.1	-0.2	0.9	0.7
Orthocladinae	—	—	-1	—	0	—	—	—	-1	—	0	—	—	—	-1	—	—	0
Empididae	—	—	-1	—	0	—	—	—	-1	—	0	—	—	—	-1	—	—	0
Tipulidae	—	—	—	-1	—	0	—	—	—	-1	—	0	—	—	—	-1	—	—
Simuliidae	42.3	51.4	0.5	-0.2	3.4	0.6	25.9	15.4	0.4	-0.4	2.1	0.4	3.2	42.9	-0.6	-0.9	0.3	0.05
Brachycentridae	—	—	—	-1	—	0	—	—	—	-1	—	0	—	—	—	-1	—	0
Trichoptera gen. sp.	1.9	8.6	—	1	—	IF	1.9	7.7	—	1	—	IF	1.9	14.3	—	1	—	IF
Ephemeroptera gen. sp.	1.3	5.7	—	1	—	IF	—	—	—	—	—	—	—	—	—	—	—	—
Asilidae	—	—	—	-1	—	0	—	—	—	-1	—	0	—	—	—	-1	—	0
Chironomidae	—	—	—	-1	—	0	—	—	—	-1	—	0	—	—	—	-1	—	0
Simuliidae	—	—	—	-1	—	0	—	—	—	-1	—	0	—	—	—	-1	—	0
Diptera gen. sp.	0.6	2.9	—	1	—	IF	—	—	—	—	—	—	0.6	14.3	—	1	—	IF
Formicidae	5.1	17.1	—	0.8	—	11.7	5.6	23.1	—	0.9	—	12.6	0.6	14.3	—	0.2	—	1.5
Coleoptera gen. sp.	0.6	2.9	—	1	—	IF	—	—	—	—	—	—	0.6	14.3	—	1	—	IF
Arachnida gen. sp.	1.3	5.7	—	1	—	IF	1.9	7.7	—	1	—	IF	—	—	—	—	—	—
Fish	—	—	—	—	—	—	—	—	—	—	—	—	0.6	14.3	—	—	—	—
Detritus	—	65.7	—	—	—	—	—	53.8	—	—	—	—	—	28.6	—	—	—	—
OP	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

A_i = relative abundance; F_i = frequency of occurrence; Ivlev = Ivlev's selectivity index; Savage = Savage selectivity index; IF = Savage index tended to infinity; OP = other prey items; B = Benthos; D = Drift; Benthos and drift composition are shown in Table 1.

but no differences were found between benthos and drift (Mann–Whitney U test, $P = 0.45$).

DISCUSSION

Previously, the dietary composition of *S. carolitertii* has been described by Sánchez-Hernández and Cobo (2011). In contrast with Sánchez-Hernández and Cobo (2011), who studied summer food resource partitioning between four sympatric fish species in the River Tormes (Central Spain), in our case the diet of *S. carolitertii* was compared with samples of potential prey (benthic and drifting invertebrates) to study prey selection and ontogenetic dietary shifts, to help understand the trophic requirements of this fish species.

The relative orientation of the mouth indicates the water depth at which feeding normally occurs (Winemiller 1991). Generally, the mouth position in species of the genus *Squalius* is subterminal. According to this characteristic, Blanco-Garrido et al. (2003) stated that mouth position in *S. pyrenaicus* allows them to capture prey inhabiting benthic-, water-surface-, or pelagic habitats. It is also interesting to note that typically benthonic items living under rocks or

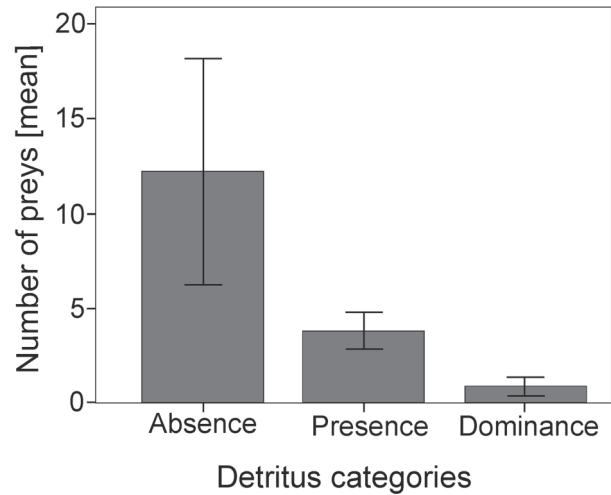


Fig. 1. Mean number of preys in the gastrointestinal tracts of *Squalius carolitertii* from the Tormes River, Central Spain in relation to the amount of detritus remaining; Error bars represent 95% confidence intervals

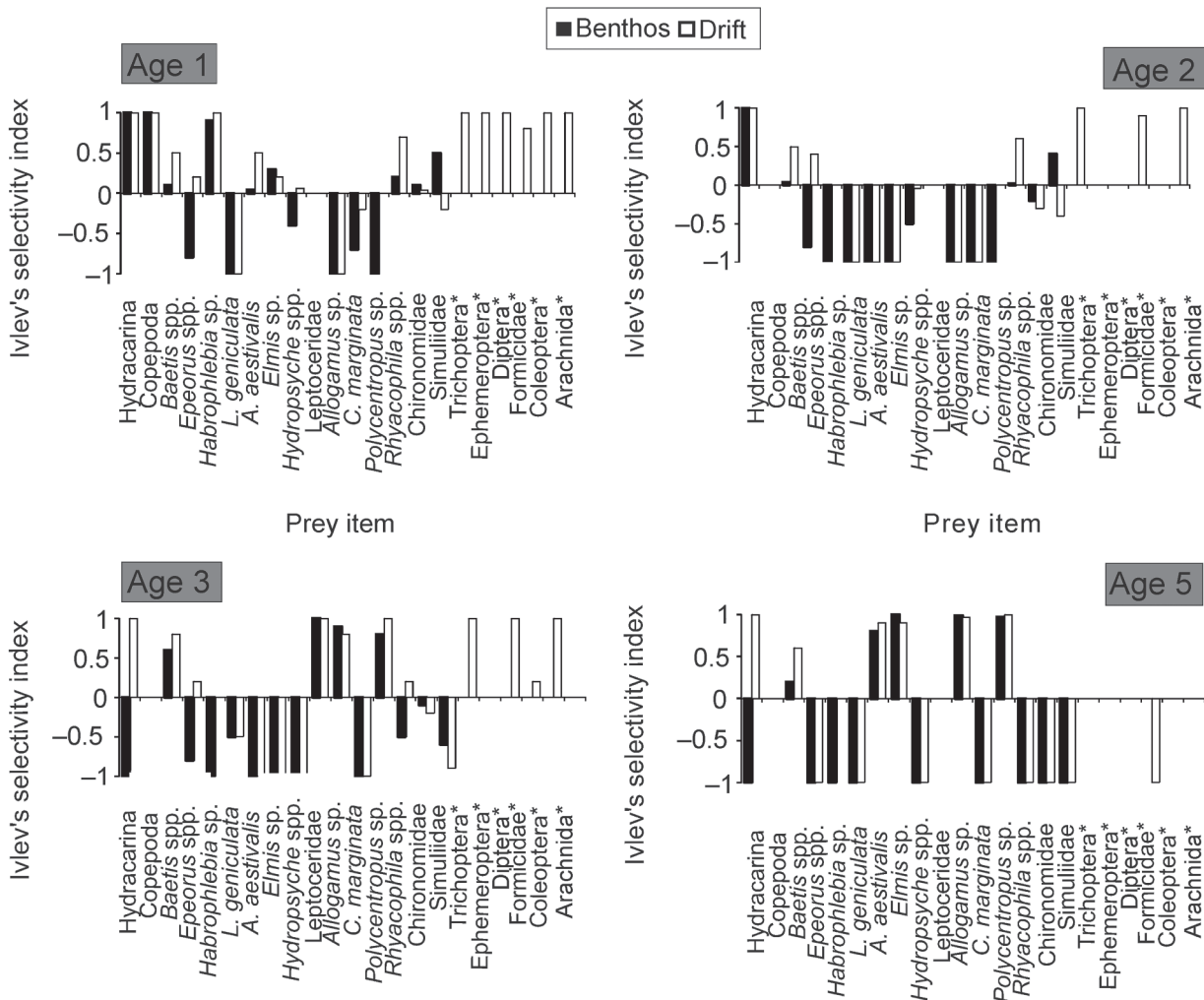


Fig. 2. Ivlev's selectivity index of *Squalius carolitertii* from the Tormes River, Central Spain (*terrestrial prey); Data are presented for each age class

on the surface of substrata, with low mobility and drift such as *Dugesia* spp. or *Ancylus fluviatilis* (see Tachet et al. 2002, Oscoz et al. 2011) were negatively selected. Moreover, Rodríguez-Jiménez (1987) found that the absence of sand and detritus in the gastrointestinal tracts of cyprinid fish species could indicate that the fish do not feed strictly on the bottom as in the present study. Thus, our findings show that *S. carolitertii* has the ability to feed at different depths of the water column as, previously, Blanco-Garrido et al. (2003) found for *S. pyrenaicus*.

The species of the genus *Squalius* have been considered as typically omnivorous (Coelho et al. 1997, Blanco-Garrido et al. 2003, Sánchez-Hernández and Cobo 2011). Our results show that the utilization of detritus could be linked to the ability to ingest animal prey items, and individuals with an absence of detritus in the gut had more animal prey items than individuals with a dominance of detritus. Thus, the feeding strategy development by *S. carolitertii* could be the result of an energetic selection criteria, since animal prey items are more profitable than detritus or plant material (Bowen 1979, 1987), being an important component of the diet of cyprinids (Magalhães 1993a, b, Encina and Granado-Lorencio 1994, Blanco-Garrido et al. 2003, Sánchez-Hernández et al. 2011b).

As predicted by optimal foraging theory (OFT), the fish should select those prey items that maximize their net rate of energy gain (Pyke et al. 1977, Gerking 1994). Thus, different researchers have demonstrated that prey selection in fishes is related to prey characteristics (e.g., size, locomotor abilities, accessibility, or anti-predator behaviour), fish characteristics (e.g., prior experience, locomotor abilities, stomach fullness, mouth gape, sensory capabilities, and fish size) and physical habitat characteristics (e.g., flow patterns and structural complexity of habitat) (Gerking 1994 and references therein). Concerning food selection, our results are in good agreement with those obtained in other studies in different fish species, and fishes do not always consume the most abundant taxa available in the environment (de Crespín de Billy and Usseglio-Polatera 2002, Sánchez-Hernández et al. 2011a, b). Despite the high abundance of *Epeorus* spp. and *Hydropsyche* spp. in the benthos and Simuliidae in the drift, these items were selected negatively. Previously, Sánchez-Hernández and Cobo (2011), using Amundsen's method and Tokeshi's graphical model, stated that Northern Iberian chub shows a generalist feeding strategy with *Baetis* spp. and Simuliidae dominating as prevalent food. This could explain the positive selection of *Baetis*

spp. in the benthos and drift, demonstrating that all age classes choose this item for feeding. Moreover, the preference for *Baetis* spp. found in this study could also be related to site-specific prey accessibility as demonstrated by other researchers (Oscoz et al. 2006, Johnson et al. 2007, Leunda et al. 2008, Sánchez-Hernández et al. 2011a).

On the other hand, according to several authors prey size and other factors related to fish size including, for example, handling ability of fishes are important variables that determine food selection (Cunha and Planas 1999, de Crespín de Billy et al. 2002, Sánchez-Hernández et al. 2011a). This study shows that the size-frequency distribution of gastrointestinal tracts was not identical to that in the benthos and drift samples. This supports the findings of Sánchez-Hernández et al. (2011a) who found that the size-frequency distribution of potential prey items in the benthos was different to that of prey in the stomachs of brown trout. Our findings are in concordance with the observations of brown trout by Rincón and Lobón-Cerviá (1999), and prey-size selection is probably dependent of the characteristics of the size-frequency distribution of the available prey. The high abundance of items with length less than four millimetres could be related to the small mouth gape of Northern Iberian chub. In this context, Blanco-Garrido et al. (2003) have found that in *S. pyrenaicus* the mean prey size consumed was positively correlated with mouth size. Thus, active choice by prey-size selection in *S. carolitertii* appeared to be important criteria implying in food selection.

During their life history fish undergo ontogenetic dietary shifts (Magalhães 1993b, Blanco-Garrido et al. 2003, Fochetti et al. 2008). These shifts during life stage transitions may be accompanied by a marked reduction in intra-specific competition within the fish population, facilitating the partitioning of resources (Elliott 1967, Amundsen et al. 2003, Oscoz et al. 2006). In the present study, age-related shifts in the diet of *S. carolitertii* occurred at three different levels. Firstly, the diet composition shifts during ontogeny (Magalhães 1993b, Blanco-Garrido et al. 2003). Magalhães (1993b) found that throughout ontogeny *S. pyrenaicus* shifts from soft-bodied to hard-shelled prey and decreased animal prey breadth. In our case, the diet varied with fish age, with the most frequently consumed prey item being Simuliidae in age 1, Hydracarina in age 2, *Baetis* spp. in age 3 and *Baetis* spp. and *Allogamus* sp. in age 5. Moreover, the presently reported study shows that the frequency of occurrence of detritus in the gut decreases with age. Secondly, prey selection vary with fish age, these findings emphasize the observations of other researchers

Table 3

Benthos, drift, and diet of *Squalius carolitertii* from the Tormes River, Central Spain, in individual 2-mm prey size categories

Frequency	Prey size category [mm]					
	0–2	2–4	4–6	6–8	8–10	>10
Benthos frequency [%]	0.7	46.9	35.8	10.3	2.6	3.7
Drift frequency [%]	1	42	37	8	5	7
Diet frequency [%]	26.7	55.6	6.7	3.7	0.7	6.7

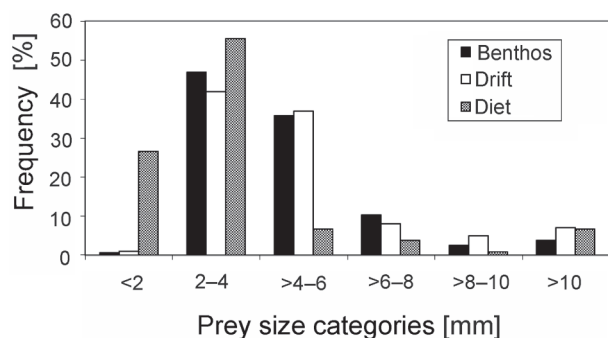


Fig. 3. Size-frequency of the benthos, drift and diet of *Squalius carolitertii* from the Tormes River, Central Spain

(Lukoschek and McCormick 2001, Fochetti et al. 2008) and are broadly in accordance with Magalhães (1993b), who stated that morphological constraints, prey handling costs and habitat partitioning are responsible for size-related changes in diet, since *Squalius* species shows size-dependent microhabitat use (Santos and Ferreira 2008). Thirdly, ontogenetic dietary shifts may also occur at the level of prey size. Several researchers have found that mean prey size increases as predator size increases (Magalhães 1993b, Blanco-Garrido et al. 2003, Montori et al. 2006) and this may also be the case in our study.

Finally, it is important to note that the fish in this study were captured during daylight and all parts of the gastrointestinal tract of each fish were analysed. Thus gut contents could also include prey items from the night drift. This could affect study results since drift composition varies throughout the day (Rieradevall and Prat 1986). Nevertheless, despite this problem, our findings show that other factors apart of prey abundance, including site-specific prey accessibility, prey size, energetic selection criteria and prey preference of fish play an important role in feeding behaviour of *S. carolitertii*.

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