

Summer feeding relationships of the co-occurring *Phoxinus phoxinus* and *Gobio lozanoi* (Cyprinidae) in an Iberian river

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Abstract. The alimentary tract content composition of 106 European minnow (*Phoxinus phoxinus*, 25–75 mm TL) and 49 gudgeon (*Gobio lozanoi*, 31–123 mm TL) captured in August 1996 in the river Larraun (Northern Spain) was investigated in order to analyse their diet composition and interspecific diet overlap. Both species fed mainly on Chironomidae larvae, Trichoptera larvae and terrestrial invertebrates, although trophic diversity was significantly higher for European minnows. The diet composition and the feeding strategy plots revealed a similar ontogenetic shift of the relative contribution of these prey items to a more pronounced generalist feeding strategy as fish of both species grew larger. Additionally, comparisons between diet composition and the benthic macroinvertebrate community revealed that *P. phoxinus* and *G. lozanoi* refused Caenidae, showing a preference for Chironomidae and Psychomyiidae. The simplified Morisita index proved that the diet overlap between the two species was indeed very high and significant. We discuss how morphological constraints –i.e. mouth size–, prey handling costs and habitat partitioning could be responsible for the observed inter- and intra-specific (size-related) changes in diet preferences. However, we suggest that the combination of differential microhabitat use, diel feeding rhythms and diet plasticity could minimize the interspecific competition, allowing co-occurrence of these Cyprinidae species in relatively high densities in this reach of the river Larraun.

Key words: *Gobio lozanoi*, *Phoxinus phoxinus*, diet composition, diet overlap, feeding strategy, ontogenetic diet shift, river Larraun

Introduction

The European minnow, *Phoxinus phoxinus* (Linnaeus, 1758), and the gudgeon, *Gobio gobio* (Linnaeus, 1758) are widely distributed in Europe (B a n a r e s c u 1990). Until recently, the Iberian populations of gudgeon were considered to be descendants of introduced populations of *G. gobio*. However, recent studies (D o a d r i o & M a d e i r a 2004, M a d e i r a et al. 2005) have assigned the Iberian and southwestern French (Adour River Basin) populations of gudgeon to a new species –*Gobio lozanoi* n. sp.– based on genetic and morphological characters.

Different authors have studied the diet of these species separately in Europe (e.g., K e n n e d y & F i t z m a u r i c e 1972, M y l l y l a et al. 1983, P r z y b y l s k i & B a n b u r a 1989) and in the Iberian Peninsula (M a g a l h a e s 1993a, V a l l a d o l i d & P r z y b y l s k i 1996, O s c o z et al. 2001, 2003). In spite of their wide distribution and co-occurrence, little has been studied about the trophic interaction between *P. phoxinus* and *G. lozanoi*, or even between *P. phoxinus* and *G. gobio*. Only N e v e u (1981) carried out a similar, but mainly descriptive, study on the diet and feeding rhythms of several fish species co-occurring in the river Nivelle (Southwestern France). Amongst the species that N e v e u

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(1981) studied were the European minnow and the gudgeon, but at present the latter might be considered a *G. lozanoi* population. Therefore, to the authors' knowledge, our study is the only paper that aims to summarize an approach to the summer trophic interaction between *P. phoxinus* and the recently described *G. lozanoi*.

Being the trophic interaction between species –competition and predation– an important mechanism in determining the distribution of aquatic communities (Declercq et al. 2002), diet composition and diet overlap studies constitute an important tool to improve aquatic management. Therefore, the aim of this study was to analyse these two variables

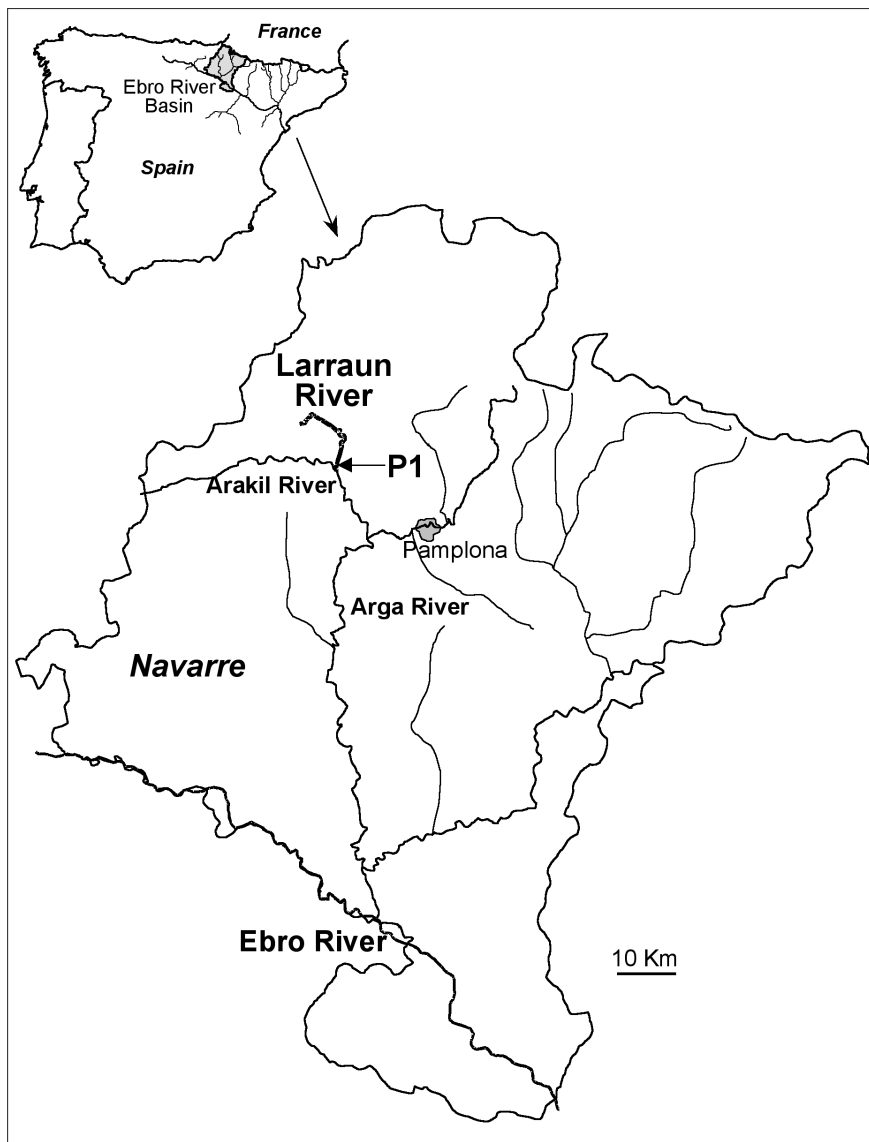


Fig. 1. Location of the sampled reach (P1) in the river Larraun, a tributary of the Ebro River (Navarre, Northern Spain).

–diet composition and dietary overlap– and prey selection on these Cyprinidae species in an Iberian river during summer, contributing to the knowledge of the feeding ecology of *P. phoxinus* and *G. lozanoi*.

Study Area

The study was carried out in the river Larraun (Navarre, Northern Spain), a tributary of the river Arakil (Ebro River Basin) (Fig. 1). The river Larraun has a catchment area of 221 km² and a total length of 21 km. Its altitude ranges from 640 m at the source to 430 m at its confluence with the river Arakil, flowing mainly over limestone. Bank vegetation consists mainly of alders (*Alnus glutinosa*), willows (*Salix* spp.), black poplars (*Populus nigra*) and different species of *Quercus*. The river reach sampled (P1) is located on the lower course of the river Larraun, close to the locality of Irurtzun (Fig. 1). The study area combined lentic and lotic habitats, and had an average depth of 42 cm and a mean channel width of 14 m. The substratum was dominated by bedrock, small boulders and cobbles, interspersed with gravel.

In summer 1996, the fish community was dominated by the French nase *Chondrostoma miegii* Steindachner, 1866 (62.96 fish/100 m²) along with *P. phoxinus* (26.54 fish/100 m²), *G. lozanoi* (22.12 fish/100 m²) and Graells barbel *Barbus graellsii* Steindachner, 1866 (16.07 fish/100 m²). Other fish species such as brown trout *Salmo trutta* Linnaeus, 1758 and stone loach *Barbatula barbatula* (Linnaeus, 1758) also occur in relatively low densities (0.44 fish/100 m²). A more detailed description of the river reach characteristics and the fish community can be found elsewhere (O s c o z 2003).

Materials and Methods

Analyzed specimens of *P. phoxinus* and *G. lozanoi* were captured by means of an electrofishing survey carried out in a 100-m-length river stretch in August 1996. Fishes were preserved in ice, but not frozen. Once in the laboratory, the alimentary tracts were removed and preserved in a 4% formaldehyde solution. The alimentary tracts of 106 *P. phoxinus* (25–75 mm total length –TL) and 49 *G. lozanoi* (31–123 mm TL) were analyzed. The analyzed specimens were arbitrarily assigned to three size classes for each species as follows: <50 mm TL, 50–60 mm TL, >60 mm TL for *P. phoxinus*, and <70 mm TL, 70–80 mm TL, >80 mm TL for *G. lozanoi*.

The digestive content items of the first one-third of the alimentary tracts were identified under a magnifying microscope (x7–45). Three prey item groups were identified: plant material, terrestrial invertebrates and aquatic invertebrates. When possible, aquatic invertebrates were identified to family level and terrestrial invertebrates to order level. The semi-quantitatively abundance of plant material was visually quantified, and the number of alimentary tracts in which it appeared was also recorded. The Vacuity index (%VI) was calculated as a percentage of the ratio of empty alimentary tracts and the total number of alimentary tracts examined (H y s l o p 1980).

In animal prey items, the frequency of occurrence (%F) of a given prey type is defined as the number of alimentary tracts in which that prey occurs (*N*), expressed as a frequency of the total number of alimentary tracts in which prey are present (*N*). According to C a i l l i e t (1977) this index represents population-wide food habits. The relative abundance of

a given prey ($\%A_i$) is defined as the percentage of total alimentary tract contents in all predators comprised by that given prey, and it is informative about the feeding behaviour (M a c d o n a l d & G r e e n 1983). In mathematical terms, these can be described by the equations:

$$\%F_i = (N_i/N).100 \qquad \%A_i = (\sum S_i/\sum S_i).100$$

where S_i is the alimentary tract content (number) composed by prey i , and S_i the total alimentary tract content of all alimentary tract in the entire sample.

In addition, the use of the Shannon-Weaver diversity index $H' = -\sum(S_i/S_i) \cdot \ln(S_i/S_i)$ provides a relatively objective indication of niche breadth (M a r s h a l l & E l l i o t t 1997). Therefore, the calculation of the H' index and the subsequent t -test (P o o l e 1974) between two given samples was performed with the PAST data analysis package (H a m m e r et al. 2001).

In order to determine the diet overlap between *P. phoxinus* and *G. lozanoi* the Simplified Morisita index or Morisita-Horn index (C_H) (M o r i s i t a 1959, H o r n 1966) was used, as different studies suggest that this index is the least biased measure (S m i t h & Z a r e t 1982). The simplified Morisita index was calculated as:

$$C_H = \frac{2(\sum S_{ij}/\sum S_i)}{\sum S_i^2 + \sum S_j^2}$$

where S_{ij} is the proportion of the prey item i of the total preys consumed by species j , and S_{ik} is the proportion of the prey item i of the total preys consumed by species k . Moreover, results obtained with this index are very homogeneous and relatively unaffected by the number of food categories considered and by decimal precision (C o r t é s 1997). Overlap coefficients higher than 0.60 are considered to be indicative of a biologically significant overlap (Z a r e t & R a n d 1971, P u s e y & B r a d s h a w 1996).

In order to determine the feeding strategy of both species, feeding strategy diagrams were constructed following C o s t e l l o ' s (1990) method with the modifications suggested by A m u n d s e n et al. (1996). These diagrams are based on a two-dimensional representation, where each point represents the frequency of occurrence ($\%F_i$) and the prey-specific abundance ($\%P_i$), calculated according to the following formula:

$$\%P_i = (\sum S_i/\sum S_i).100$$

where S_{ii} is the total alimentary tract content only in those predators with prey i in their alimentary tracts. Information about prey importance and feeding strategy of the predator can be obtained by examining the distribution of points along the diagonals and the axes of the diagram: (1) the prey importance is represented in the diagonal from lower left (rare prey) to upper right (dominant prey); (2) the feeding strategy is represented in the vertical axis from bottom (generalization) to top (specialization); (3) and the relationship between feeding strategy and the between- or within-phenotype contributions to the niche width is represented in the diagonal from lower right (high within-phenotype component, WPC) to upper left (high between-phenotype component, BPC).

Furthermore, a Tokeshi plot (T o k e s h i 1991) was constructed to graphically examine differences in individual *versus* population feeding habits, plotting mean individual diet diversity (H'_{ind}) against group diet diversity.

Prey selection was also analyzed, comparing the diet composition with the composition of the benthic macroinvertebrate assemblage present in the sampling point. A benthos sample was collected on the same day as the electrofishing survey using a handnet (opening 210x260 mm; 0.1-mm mesh size) following the European standard methods ISO 7828:1985, by kicking in all the different habitats found in the river reach proportionally to their abundance. The sample was preserved at the capture site with 4% formalin solution. Once in the laboratory the formalin was replaced by 70% ethanol, and all the macroinvertebrates were counted and classified (T a c h e t et al. 1984, 2000). Detailed data on macroinvertebrate assemblage are available elsewhere (O s c o z 2003). Prey selection was quantified using the Savage's index (S a v a g e 1931) $W_i = A_i/D_i$, where A_i is the relative abundance of prey i in the alimentary tract content, and D_i is the relative availability of this resource in the river. The values of W_i vary between 0 and ∞ , where 1 means no selection of prey i , whereas values lower and higher than 1 show avoidance (negative preference) and selection (positive

Table 1. Diet composition of the *Phoxinus phoxinus* specimens from the river Larraun. Data are presented for each size class and for the pooled data (Total) as the percentage of occurrence (% F_i) and the relative abundance (% A_i). Trophic diversity (H') and Vacuity Index values (%VI) are also shown. (Unid.: Undetermined).

Taxon	<50 mm		50–60 mm		>60 mm		Total	
	% F_i	% A_i	% F_i	% A_i	% F_i	% A_i	% F_i	% A_i
Aquatic invertebrates								
Nematoda	-	-	6.38	6.05	5.00	1.12	4.60	3.88
Hydracarina	-	-	4.26	0.93	15.00	4.49	5.75	1.66
Ancyliidae	5.00	1.75	6.38	1.40	5.00	1.12	5.75	1.39
Gammaridae	10.00	3.51	4.26	1.40	35.00	7.87	12.64	3.32
Elmidae	-	-	2.13	0.47	5.00	1.12	2.30	0.55
Chironomidae	80.00	73.68	68.09	62.79	55.00	42.70	67.82	59.56
Limoniidae	10.00	3.51	6.38	1.86	5.00	1.12	6.90	1.94
Simuliidae	5.00	1.75	-	-	-	-	1.15	0.28
Baetidae	-	-	4.26	0.93	-	-	2.30	0.55
Caenidae	-	-	-	-	5.00	2.25	1.15	0.55
Heptageniidae	-	-	2.13	0.47	-	-	1.15	0.28
Ephemeroptera (Unid.)	-	-	4.26	0.93	-	-	2.30	0.55
Hydropsychidae	5.00	1.75	8.51	1.86	15.00	3.37	9.20	2.22
Psychomyidae	20.00	8.77	25.53	7.91	45.00	13.48	28.74	9.42
Rhyacophilidae	-	-	2.13	0.47	-	-	1.15	0.28
Trichoptera (Unid.)	5.00	1.75	2.13	0.47	-	-	2.30	0.55
Arthropoda (Unid.)	5.00	1.75	10.64	2.33	10.00	2.25	9.20	2.22
Total	95.00	98.25	82.98	90.23	85.00	80.90	98.85	89.20
Terrestrial invertebrates								
Arachnida	-	-	-	-	5.00	1.12	1.15	0.28
Diptera	-	-	17.02	6.51	30.00	14.61	16.09	7.48
Trichoptera	-	-	6.38	1.40	10.00	2.25	5.75	1.39
Insecta (Unid.)	5.00	1.75	6.38	1.86	5.00	1.12	5.75	1.66
Total	5.00	1.75	27.66	9.77	40.00	19.10	25.29	10.80
Algae and plants	15.00	-	44.68	-	75.00	-	44.83	-
H'	1.10		1.56		1.93		1.67	
% VI	28.57		12.96		16.67		17.92	

Table 2. Diet composition of the *Gobio lozanoi* specimens from the river Larraun. Data are presented for each size class and for the pooled data (Total) as the percentage of occurrence (%F_i) and the relative abundance (%A_i). Trophic diversity (H') and Vacuity Index values (%VI) are also shown. (Unid.: Undetermined).

Taxon	<70 mm		70-80 mm		>80 mm		Total	
	%F _i	%A _i	%F _i	%A _i	%F _i	%A _i	%F _i	%A _i
Aquatic invertebrates								
Nematoda	9.09	1.09	6.67	5.02	12.50	0.65	9.52	2.27
Hydracarina	-	-	6.67	0.46	12.50	0.65	7.14	0.49
Ancylidae	-	-	-	-	18.75	0.98	7.14	0.49
Anomopoda	9.09	2.17	-	-	-	-	2.38	0.32
<i>Pacifastacus leniusculus</i>	-	-	-	-	31.25	1.63	11.90	0.81
Gammaridae	9.09	1.09	13.33	0.91	37.50	3.27	21.43	2.11
Ostracoda	-	-	-	-	6.25	0.33	2.38	0.16
Elmidae	-	-	-	-	6.25	0.33	2.38	0.16
Chironomidae	90.91	79.35	100.00	72.60	100.00	65.36	97.62	70.02
Limoniidae	-	-	20.00	1.37	18.75	0.98	14.29	0.97
Simuliidae	-	-	-	-	6.25	0.33	2.38	0.16
Baetidae	-	-	20.00	1.37	12.50	0.65	11.90	0.81
Caenidae	-	-	-	-	12.50	0.65	4.76	0.32
Leuctridae	-	-	6.67	0.46	-	-	2.38	0.16
Hydropsychidae	-	-	6.67	0.46	18.75	1.63	9.52	0.97
Psychomyidae	18.18	3.26	60.00	10.50	75.00	12.09	54.76	10.21
Trichoptera (Unid.)	18.18	2.17	-	-	-	-	4.76	0.32
Arthropoda (Unid.)	18.18	2.17	-	-	6.25	0.33	7.14	0.49
Total	100.00	91.30	100.00	93.15	100.00	89.87	100.00	91.25
Terrestrial invertebrates								
Coleoptera	-	-	-	-	6.25	0.33	2.38	0.16
Formicidae	-	-	-	-	6.25	0.33	2.38	0.16
Diptera	63.64	7.61	53.33	6.39	50.00	5.56	54.76	6.16
Trichoptera	9.09	1.09	6.67	0.46	37.50	2.29	19.05	1.46
Insecta (Unid.)	-	-	-	-	31.25	1.63	11.90	0.81
Total	72.73	8.70	53.33	6.85	87.50	10.13	71.43	8.75
Algae and plants	27.27	-	66.67	-	56.25	-	52.38	-
H'	0.89		1.05		1.43		1.29	
% VI	31.25		6.25		5.88		14.29	

preference), respectively. This index was chosen because it is more objective than similar ones, and it is possible to verify its statistical significance with a χ^2 test (Manly et al. 1993) after applying Bonferroni adjustment (α /number of categories).

Results

From the total number of fish examined, the alimentary tracts of 19 *P. phoxinus* (%VI= 17.92) and seven *G. lozanoi* (%VI= 14.29) were found empty, and therefore were not further analyzed. The remaining alimentary tracts were then analyzed: 361 preys were found in *P. phoxinus* and 617 in *G. lozanoi*. Aquatic preys, especially Chironomidae, were the main prey item found in the two species, both with respect to their occurrence as well as their relative abundance

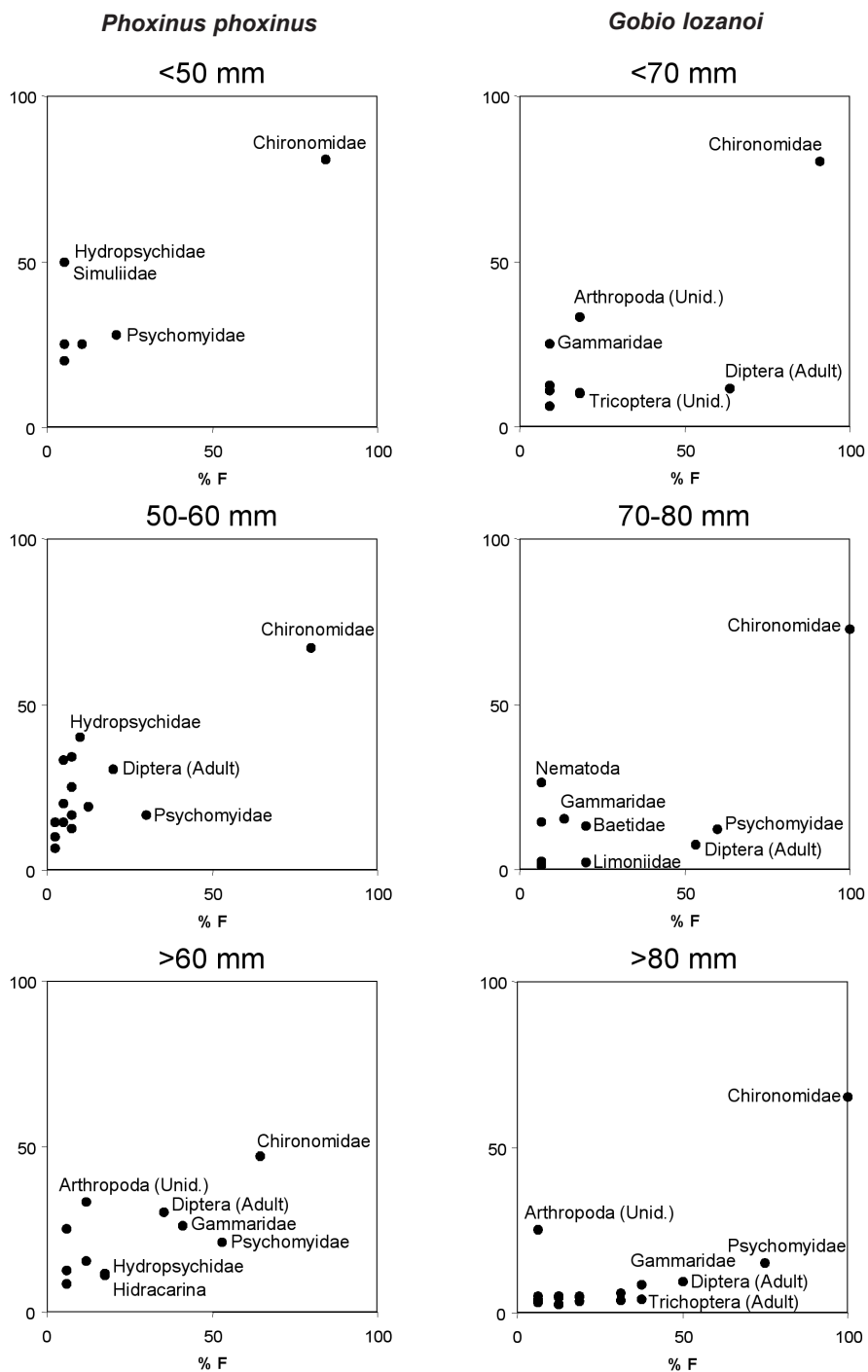


Fig. 2. Feeding strategy plots for each size class of *Phoxinus phoxinus* and *Gobio lozanoi* from the river Larraun. (%F; percentage of occurrence of prey *i*; %P; prey specific abundance; Points represent different prey items).

(Table 1 and Table 2), although *P. phoxinus* proportionally consumed fewer Diptera larvae than *G. lozanoi*. In the latter species, terrestrial prey ($\%F_i = 71.43$) (mainly Diptera) appeared more frequently consumed than in the case of European minnow ($\%F_i = 25.29$), although the relative abundance was similar for both species and close to 10%. As both species based their diet on Diptera, mainly Chironomidae, as well as terrestrial prey and Trichoptera, the value of the simplified Morisita index was high ($C_H = 0.987$) indicating a biologically significant dietary overlap between these two cyprinid species. Algae and plant material were found in more than a half ($\%F_i = 52.38$) of the analyzed *G. lozanoi* alimentary tracts, and in 12.64% of *P. phoxinus*, but always in relatively small quantities and frequently along with substrate remains. Diet diversity index value (Table 1 and Table 2) was significantly higher for European minnow ($P < 0.05$).

Taking into account the different size classes, the Vacuity index ($\%VI$) was always higher in the smaller fish of both species (Table 1 and Table 2). Although there was an evident ontogenetic dietary shift throughout the different size classes of both species, the diet remained composed mainly by aquatic preys. The simplified Morisita index values were high, indicating a biologically significant dietary overlap between all size classes in both *P. phoxinus* (<50 mm vs. 50–60 mm $C_H = 0.998$; 50–60 mm vs. >60 mm $C_H = 0.908$; <50 mm vs. >60 mm $C_H = 0.837$) and *G. lozanoi* (<70 mm vs. 70–80 mm $C_H = 0.989$; 70–80

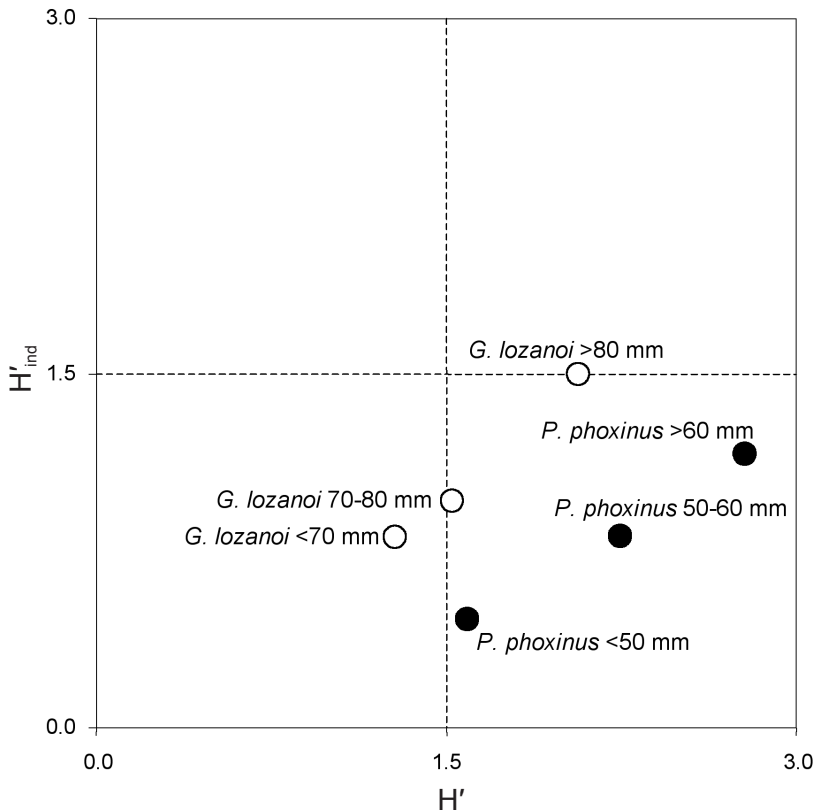


Fig. 3. Tokeshi plot of the feeding strategy of each size class of *Phoxinus phoxinus* and *Gobio lozanoi* from the river Larraun.

mm vs. >80 mm $C_H=0.991$; <70 mm vs. >80 mm $C_H=0.972$). Chironomidae was the most consumed item in all European minnow size classes (Table 1) but its abundance decreased as the fish grew older, whilst the consumption of terrestrial invertebrates, Trichoptera larvae and other aquatic preys increased in larger specimens. A similar pattern was observed in *G. lozanoi* (Table 2), the consumption of Chironomidae decreased whilst predation on Trichoptera larvae, Crustacea and terrestrial preys increased as fish grew larger. Both species showed a higher number of prey items, an increase of the diet diversity and a higher occurrence of algae and plant material as fish grew larger. The only non-significant *t*-test comparison between size classes diversity values was the one between <70 mm TL vs. 70–80 mm TL *G. lozanoi*.

According to the interpretation guidelines of the feeding strategy plots pointed out in the Material and Methods section, Fig. 2 shows that both species had similar generalist feeding strategies. Most of the prey points are located in the lower left corner corresponding to an important number of rare preys, whilst Chironomidae is the only dominant prey item. Even if this was the dominant prey item in every size class, both species consumed fewer Chironomidae as fish grew larger, resulting in an even more pronounced generalist feeding strategy. When values of H'_{ind} were plotted against H' from both species in a Tokeshi plot (Fig. 3), they tended to cluster in the lower right region corresponding to a generalist feeding habit with a heterogeneous diet. In both species there was a tendency to increase group diversity and mean individual diet diversity values as they grew larger.

In accordance with the Savage index (W_i) for the benthic prey selection, both species exhibited similar preferences at least for some prey items in the different size classes (Table 3 and Table 4). Both species positively selected Chironomidae and Psychomyidae, whereas Caenidae were avoided. Furthermore, smaller European minnow positively selected Limoniidae whereas larger specimens positively selected Hydropsychidae (Table 3). Besides, 50–60 mm TL European minnows positively selected Ancyliidae and Nematoda, avoiding Hydracarina, Gammaridae and Leuctridae. All *G. lozanoi* size classes avoided

Table 3. Prey selection made by different size classes of *Phoxinus phoxinus* from the river Larraun. The relative prey item (Taxon) availability (D_i) in the benthos sample, its relative use (S_i) and the Savage index (W_i), along with its statistical significance (after Bonferroni' correction) are presented. (ns: $p>0.05$; *: $p<0.05$; **: $p<0.01$).

Taxon	Benthos	<50 mm			50-60 mm			>60 mm		
	D_i	S_i	W_i		S_i	W_i		S_i	W_i	
Hydracarina	0.103	0.000	0.000	ns	0.010	0.100	**	0.056	0.542	ns
Elmidae (Adults)	0.044	0.000	0.000	ns	0.005	0.117	ns	0.014	0.315	ns
Gammaridae	0.113	0.036	0.316	ns	0.015	0.137	**	0.097	0.861	ns
Chironomidae	0.278	0.750	2.702	**	0.696	2.507	**	0.528	1.901	**
Limoniidae	0.002	0.036	19.726	**	0.021	11.388	**	0.014	7.671	ns
Baetidae	0.050	0.000	0.000	ns	0.010	0.206	ns	0.000	0.000	ns
Caenidae	0.246	0.000	0.000	**	0.000	0.000	**	0.028	0.113	**
Ancyliidae	0.002	0.018	9.863	ns	0.015	8.541	**	0.014	7.671	ns
Nematoda	0.002	0.000	0.000	ns	0.067	37.012	**	0.014	7.671	ns
Leuctridae	0.065	0.000	0.000	ns	0.000	0.000	**	0.000	0.000	ns
Hydropsychidae	0.006	0.018	2.959	ns	0.021	3.416	ns	0.042	6.904	**
Psychomyidae	0.002	0.089	49.315	**	0.088	48.400	**	0.167	92.056	**
Other preys	0.089	0.054	0.600	ns	0.052	0.577	ns	0.028	0.311	ns

Table 4. Prey selection made by different size classes of *Gobio lozanoi* from the river Larraun. The relative prey item (Taxon) availability (D_i) in the benthos sample, its relative use (S_i) and the Savage index (W_i), along with its statistical significance (after Bonferroni' correction) are presented. (ns: $p>0.05$; *: $p<0.05$; **: $p<0.01$).

Taxon	Benthos	<70 mm			70-80 mm			>80 mm		
	D_i	S_i	W_i		S_i	W_i		S_i	W_i	
Hydracarina	0.103	0.000	0.000	*	0.005	0.048	**	0.007	0.071	**
Elmidae (Adults)	0.044	0.000	0.000	ns	0.000	0.000	*	0.004	0.083	*
Gammaridae	0.113	0.012	0.105	*	0.010	0.087	**	0.036	0.322	**
Chironomidae	0.278	0.869	3.130	**	0.779	2.808	**	0.727	2.620	**
Limoniidae	0.002	0.000	0.000	ns	0.015	8.123	**	0.011	6.025	**
Baetidae	0.050	0.000	0.000	ns	0.015	0.294	ns	0.007	0.145	*
Caenidae	0.246	0.000	0.000	**	0.000	0.000	**	0.007	0.030	**
Ancylidae	0.002	0.000	0.000	ns	0.000	0.000	ns	0.011	6.025	**
Nematoda	0.002	0.012	6.575	ns	0.054	29.783	**	0.007	4.017	ns
Leuctridae	0.065	0.000	0.000	ns	0.005	0.076	**	0.000	0.000	**
Hydropsychidae	0.006	0.000	0.000	ns	0.005	0.812	ns	0.018	3.013	ns
Psychomyidae	0.002	0.036	19.726	**	0.113	62.273	**	0.135	74.314	**
Other preys	0.089	0.071	0.800	ns	0.000	0.000	**	0.029	0.326	**

Hydracarina and Gammaridae, whilst specimens larger than 70 mm TL also avoided Elmidae and Leuctridae, positively selecting Limoniidae. Furthermore, the largest *G. lozanoi* specimens positively selected Ancylidae, whilst 70–80 mm TL size class positively selected Nematoda.

Discussion

The recent assignation of the Iberian and southwestern French gudgeon populations to a new species (*Gobio lozanoi*) (Doadrio & Madeira 2004, Madeira et al. 2005) based on genetic and morphological characters, partially constrains the comparison of our results with previous works. Nevertheless, *P. phoxinus* and *G. lozanoi* from the river Larraun fed mainly on aquatic invertebrates, something already underlined by different authors in previous works in both the Iberian and non-Iberian rivers (Nevéu 1981, Mullyla et al. 1983, Magalhães 1993a, Oscoz et al. 2001, Declercq et al. 2002, Oscoz et al. 2003).

The lower percentage of empty guts and the higher number of prey items found in *G. lozanoi* might be biased due to the larger average body size of the analysed specimens of this species in contrast to *P. phoxinus*. However, this could be more likely related to the diel feeding activity of these species and to the fact that they were captured at first morning hours. The probability to find empty guts in *G. lozanoi* was lower since it has its maximum feeding activity at dawn, whereas *P. phoxinus* feed more actively at midday and night (Nevéu 1981). Nonetheless, the Vacuity Index was low in both Cyprinidae species as a direct consequence of the greater food availability and the higher energy requirements for growth and reproduction during spring and summer months (Caiola et al. 2001). In southern populations of *G. lozanoi* growth and spawning have been reported in August (Bernet 1960, Lobón-Cerviá & Torres 1983, Lobón-Cerviá et al. 1991), whereas some populations of *P. phoxinus* in pre-Pyrenean streams are still spawning in August (P. M. Lenda, pers. obs.).

Chironomidae were the most consumed prey item in both species, as has been found in some other European rivers (S t r a š k r a b a et al. 1966, M a g a l h a e s 1993a). However, as *P. phoxinus* consumed lower quantities of Chironomidae and more of other available resources than *G. lozanoi*, the trophic diversity index of the former was higher. The higher consumption of Chironomidae by *G. lozanoi* is presumably related to the fact that this species occupies a more benthic position in the water column (G r o s s m a n et al. 1987, M a s t r o r i l l o et al. 1996), where Chironomidae larvae mostly occur (P i n d e r 1986).

The low consumption of plant material was probably due to its low absorption rate and energy content (L i e n 1981), therefore suggesting that animal prey items would be energetically more profitable for both species (P e n c z a k et al. 1984). However, another two factors must be taken into account in order to explain the observed low consumption of plant material. First, the location of the studied reach in an upstream area within the Ebro River drainage, where a low primary productivity is expected (V a n o t t e et al. 1980) and consequently a lower algae and/or plant material abundance. Second, the relatively short gut and its isometric growth in both species result in a less efficient processing of plant diets (H o f e r 1991).

Our results suggest an ontogenetic diet shift, a fact largely reported in many freshwater fishes' feeding patterns (e.g. K e n n e d y & F r i t z m a u r i c e 1972, M y l l y l a et al. 1983, L o b ó n - C e r v i á & R i n c ó n 1994, O s c o z et al. 2005a). In both species, smaller specimens consumed mainly benthic preys (mostly Diptera larvae) and fewer terrestrial invertebrates, probably because feeding at the river bed implies a lower predation risk than feeding at the surface (V o l l e s t a d & A n d e r s e n 1985, H a u g e n & R y g g 1996). However, this could also be due to the different habitat use made by smaller fishes, as juveniles of many cyprinid species occur mostly in the shallower areas (M a g a l h a e s 1993b). The larger specimens of both species accounted for a higher consumption of terrestrial invertebrates, Trichoptera and Gammaridae. It is quite certain that the harder and larger body of these prey items require a bigger mouth-size. Additionally, the higher prey handling costs and the greater swimming ability in the case of terrestrial preys (E a s t o n & O r t h 1992), prevent the smaller individuals from over-exploiting these prey items. These differences in the diet composition between size classes –ontogenetic diet shift– could reduce the trophic competition, facilitated to some extent by the habitat segregation. Furthermore, it also allows the larger fish to have a higher number of potential prey items available and a wider niche breadth, as was indicated by their higher trophic diversity index values.

In accordance with the interpretation of the feeding strategy plots, the position of the prey points mainly in the lower left corner of the diagrams indicates a generalist feeding strategy in both species (A m u n d s e n et al. 1996). However, some prey items, such as Chironomidae, seemed to contribute more to the alimentary tract contents since they were positioned in the upper right corner. The Tokeshi plot also indicates a generalist feeding strategy in both species with a heterogeneous diet, since they tended to cluster in the lower right region of the diagram. In both species there was a similar ontogenetic shift to a more generalist feeding strategy as fish grew larger. This pattern is in accordance with the observed higher use of Chironomidae larvae by smaller size classes, and so the smaller fish would be more specialised in this prey item even if they still have a generalist feeding strategy. An apparent generalist population may be made up of individual generalists or, equally plausible, of a variety of individual specialists. The first explanation seems to be more consistent with our analysed population, according to the interpretation of the feeding strategy plots.

Diet variation in fish is related to prey availability, the accessibility to that prey and the risk of predation (S t r a š k r a b a et al. 1966). As a result of constraints of these trade-offs, preys that are easier to capture or with higher energetic value are consumed more (O s c o z et al. 2005a). This could explain the positive selection of Diptera and Psychomyidae, because both are highly energetic preys easy to capture by their low mobility (P e n c z a k et al. 1984, E a s t o n & O r t h 1992). Larger specimens of European minnow also showed preference for Hydropsychidae and Ancyliidae, which are highly energetic preys usually found on stones and macrophytes, where this species captures its preys (S t r a š k r a b a et al. 1966). Although all three size-classes in both fish species showed a positive preference for Chironomidae and Psychomyidae, the selection of these prey items also suggested an ontogenetic diet shift. In both species, the Savage index value decreased for Chironomidae whilst it increased for Psychomyidae as fish grew larger. The apparently high consumption and preference of nematodes may not be real since some could be parasites of the Diptera larvae eaten by *P. phoxinus* or *G. lozanoi*. Moreover, some other nematodes could also be fish gut parasites (B r o t h e r i d g e et al. 1998, B y r n e et al. 2002). Further studies are necessary to determine whether their presence is due to real active consumption or to parasitism.

On the other hand, the negative selection observed in *G. lozanoi* and medium-sized *P. phoxinus* with respect to Gammaridae and Elmidae is in accordance with that observed by N e v e u (1981) in the river Nivelle (France). The intense sclerotization of these preys could probably lead to a lower energetic value and their refusal, somewhat similar to that already observed in salmonids (P o w e r 1992, O s c o z et al. 2005b). In the same way, smaller preys (like Hydracarina) or those that camouflage themselves or hide under stones (like some Caenidae and Plecoptera) would be difficult to detect (R a j a s i l t a & V u o r i n e n 1983) and as a result, would be consumed less.

The great similarity between the diet composition of the *P. phoxinus* and *G. lozanoi* resulted in a high diet overlap, and suggests the existence of an intense competition for the trophic resources between these two species as well as between different size classes. However, the intensity of these inter- and intra-specific competitions could be reduced by means of the predator-prey density influence, as well as the segregation of microhabitats (S t r a š k r a b a et al. 1966, P r z y b y l s k i & B a n b u r a 1989) and diel activity patterns (N e v e u 1981). Microhabitat segregation between species and differential diel activity might be working to allow the co-occurrence of these species in the same area, as gudgeon occupy a more benthic habitat (G r o s s m a n et al. 1987, M a s t r o r i l l o et al. 1996) and feed mainly at dawn, while the European minnow does so throughout the day (N e v e u 1981). Similarly, the competition between size classes within each species would be reduced by microhabitat segregation, as smaller cyprinids occur mostly in the shallower areas (M a g a l h a e s 1993b). Furthermore, the heterogeneous generalist feeding strategy in both species could result in lower interspecific competition. Subsequently, our results suggest that the combination of differential microhabitat use, diel feeding activity and diet plasticity could account for the co-occurrence of the *P. phoxinus* and *G. lozanoi* in relatively high densities in this reach of the river Larraun.

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