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Microhabitat use by three endemic Iberian cyprinids in Mediterranean rivers (Tagus River Basin, Spain)

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Abstract Microhabitat use by three endemic Iberian cyprinids, *Barbus bocagei* (Steindachner), *Pseudochondrostoma polylepis* (Steindachner), and *Squalius pyrenaicus* (Günther) was studied in terms of depth, mean water column velocity, focal height, focal velocity, distance to shore and substrate. Data were obtained by snorkelling during spring and summer at nine sites of the Tagus River Basin, Spain. Habitat suitability criteria (HSC) were calculated, including fish position and focal velocity in the water column. Species comparison showed differences in depth and focal height (indicating a vertical segregation), and greater water velocities for *Pseudochondrostoma*. Size-class comparisons mainly showed differences in depth and focal height (correlated with fish size). The fish groups (3 species × 3 length classes) were assigned to microhabitat functional types. The results are essential for environmental flow assessments and allow 2- and 3-dimensional habitat simulations in Mediterranean rivers; they are also useful to define critical habitats for the conservation of native fish populations.

KEYWORDS: *Barbus, Chondrostoma,* habitat suitability criteria, microhabitat, *Squalius*.

Introduction

Since the European Water Framework Directive (WFD) was approved in 2000, perspectives for the evaluation of the ecological status of rivers have changed considerably. Moreover, environmental flow assessments have become a necessary tool on a European scale. In Spain, where great efforts have been put into place to determine environmental flows since the 1990s (García de Jalón 2003), the Spanish

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Water Authorities are applying physical habitat simulation extensively and are considering the incorporation of more ecological components into assessments (Garófano-Gómez, Martínez-Capel, Nebot, Mondéjar, Cavallé & Morillo 2008).

Studies on species-habitat relationships are not only a fundamental aspect of fish ecology, but also the basis for developing management tools such as habitat suitability indexes (Lamouroux, Capra, Pouilly & Souchon 1999; Martínez-Capel & García de Jalón 1999), necessary for environmental flow assessments. These habitat studies can also provide information to prioritise habitat improvement measures, and manage flow regimes to conserve native fish populations, which are being displaced by alien fishes in rivers with regulated flows (Bernardo, Ilhéu, Matono & Costa 2003). However, most studies do not provide habitat indexes; many are based on electric fishing and do not consider the position of the fish in the water column (focal height & focal velocity), which is essential to understand fish behaviour and to apply advanced river modelling techniques (Martínez-Capel, García de Jalón & Rodilla-Alamá 2004).

In the Iberian Peninsula, the majority of rivers experience Mediterranean climatic conditions; for this reason, river flows and aquatic habitat exhibit major inter-annual and inter-seasonal fluctuations (Vidal-Abarca, Suárez & Ramírez-Díaz 1992). This natural variability has been modified by many dams and hydroelectric power schemes (Baeza, Martínez-Capel and García de Jalón 2003). Fish communities are dominated by cyprinids (Ferreira, Oliveira, Caiola, De Sostoa, Casals, Cortes, Economou, Zogaris, Garcia-Jalon, Ilhéu, Martinez-Capel, Pont, Rogers & Prenda 2007) with a high number of endemic species that have a reduced distribution ranges compared with elsewhere in Europe (Granado-Lorencio 1996; Doadrio 2001). However, few studies have focused on the habitat use of such endemic species in the Iberian Peninsula (e.g. Grossman & De Sostoa 1994a, b; Magalhães, Beja, Canas & Collares-Pereira 2002).

In this study, the target species were three endemic fish that dominate the assemblages in many rivers: the Iberian barbel *Barbus bocagei* (Steindachner), hereafter referred to as barbel; the Iberian chub *Squalius pyrenaicus* (Günther), hereafter chub, and the Tagus nase *Pseudochondrostoma polylepis* (Steindachner) previously named *Chondrostoma polylepis* (Steindachner), hereafter nase. The objectives were to obtain habitat suitability criteria for environmental flow assessments, which allow consideration of the position of the fish in the water column, to describe and compare microhabitat use by the three Iberian species, and to classify fish species and length classes into functional types according to microhabitat variables.

Materials and methods

Study sites

Nine study sites were chosen from seven rivers of the Tagus River Basin in Spain (under Mediterranean-continental climate) on the basis of similar stream order, good underwater visibility, microhabitat variability, co-ocurrence of the three species, and minimal influence from human activity. Sampling took place between 1997 and 1999, during spring and summer, in the rivers Ambroz, Guadiela, Jarama (two sites), Lozoya (two sites), Sorbe, Tagus, and Tajuña (Fig. 1). Twenty four hydro-morphological units were sampled (15 pools, 8 riffles and 1 rapid) to obtain a general description of microhabitat use by the three species in hydraulically diverse habitats. Pools are very important habitats in Mediterranean rivers, because this is where a great proportion of the population is found (Granado-Lorencio 1996); therefore this habitat type was better represented than others. At some sites ($n = 4$) no riffles were present or impracticable to sample, thus all the sampling was performed in pools, resulting in a variable length and area between sites.

Habitat characteristics and sampling seasons are detailed in Table 1. All the sites were sampled once, with the exception of one pool that was sampled in 1997 and 1998 (R. Jarama); these two surveys were different (see Table 1) but they appear as 1 square in Fig. 1. The distance between the two sites in the River Sorbe was less than 1 km, thus in Fig. 1 they appear together. All sites were within well-forested basins, and the stream banks were vegetated with trees and shrubs (mainly of the Genus *Salix*, *Alnus*, and *Populus*). Composition of fish assemblages (Table 1) was obtained from previous studies made by electric fishing (unpublished) and during snorkelling. The assemblages at all sites contained the three target species and were dominated by cyprinids.

Microhabitat measurements

Microhabitat use was studied by snorkelling, following standard procedures (Heggenes, Brabrand & Saltveit 1990) during daylight hours because these species remain quiet for most of the night (A. De Sostoa, personal communication). Before the field work, each species was divided into length classes: small, medium, and large, based on previous studies (unpublished). The corresponding intervals of total length were < 7,

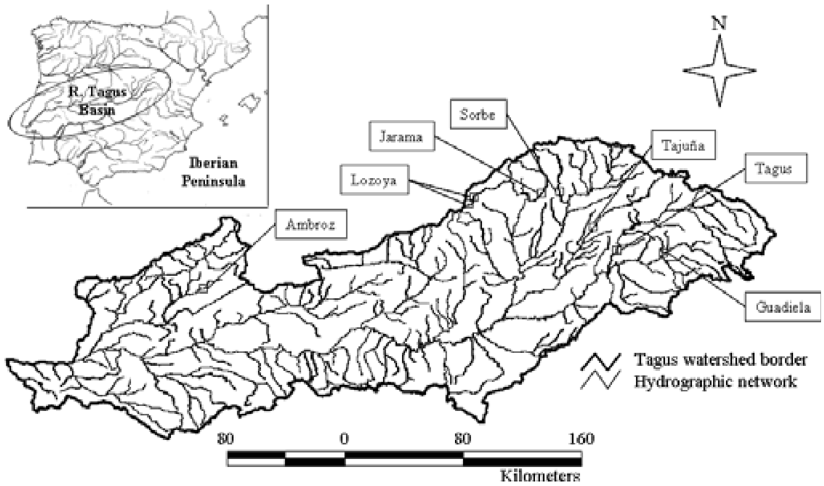

Figure 1. Map showing the Iberian Peninsula and the Tagus River Basin, with the nine study sites that were sampled once, with the exception of one pool that was sampled in the year 1997 and 1998 (R. Jarama, with a single square). In the R. Sorbe, distance between sites was less than 1 km (two squares together).

7–25, > 25 cm for barbel, < 7, 7–20, > 20 cm for nase, and < 5, 5–10, > 10 cm for chub. During the snorkelling, upon sighting a fish, a school, or a shoal of fish, the observer recorded the species, size class, number of fish, and focal height (F_h, distance to the bottom estimated as percentage of depth). Each species and length class was recorded independently. When a fish or shoal was disturbed by the diver, no data were recorded.

After the snorkelling was completed, water depth (D), mean water column velocity (V_m, m s^{-1}), velocity at the focal height (V_f, m s^{-1}), distance to the nearest shore (D_{ist}, at nearest 10 cm), and dominant substrate type (S) were measured. The following substrate types were used, bedrock, large boulders (∅ > 1024 mm), boulders (256–1024 mm), cobbles (64–256 mm), gravel (8–64 mm), fine gravel (2–8 mm), sand (62 μm–2 mm), and silt < 62 μm.

Microhabitat availability was randomly sampled along transects; a number of transects and points were selected in each site (minimum 70 points) to register the heterogeneity of habitat conditions in terms of the three variables measured, D, V_m, and S (Bovee, Lamb, Bartholow, Stalnaker, Taylor & Henriksen 1998). These data allowed comparison between microhabitat use and availability (non-random microhabitat use test).

Analysis of microhabitat use

Microhabitat use data were filtered before the analyses. For this purpose, the possibility of non-random microhabitat use by the fish was tested with the Kolmogorov-Smirnov two-sample test ($P < 0.05$), which compared the frequency distributions of use and availability (Groshens & Orth 1993) for each variable at each site. The test probabilities for each fish group were adjusted with the Bonferroni technique for multiple comparisons. Data were removed when there was a random use of habitat for all the microhabitat variables and when sample size was < 5 per species and length class at a site. Habitat suitability criteria (HSC) for D, F_h, V_m, V_f, D_{ist}, and S were calculated (for each species and size class) to facilitate habitat simulations and environmental flow studies. HSC represent the central window of 50% (optimal) and 95% (suitable) of the data distribution, respectively (Bovee *et al.* 1998). The distance to shore (D_{ist}) was considered as a percentage of the mean river width (D_{ist}).

Principal component analysis (hereafter PCA) was used to observe differences in microhabitat use among fish groups; for this analysis, the continuous variables were transformed ($\sqrt{X}$ or $\log_{10} X$) to approach normality; substrate and cover were discarded. The groups of fish (3 species × 3 length classes) were then assigned to functional types in accordance with their association with the principal components. Comparisons among the three species and among size classes, based on the scores on each principal component separately, were made using one-way ANOVA and the *post-hoc* test of Dunnett's C for multiple comparisons of means, which maintains the probability of type I error under 0.05 and does not assume variance homogeneity.

Table 1. Characterisation of the study sites. The site codes identify the stream name and survey year. Mesohabitats units (HMU), named as P - Pool, R - Riffle, Ra - Rapid, and fish species observed other than the target ones, (1) *Lepomis gibbosus* (L.), (2) *Esox lucius* (L.), (3) *Salmo trutta fario* (L.), (4) *Gobio gobio* (L.), and (5) *Cobitis calderoni* (Bacescu). The three target species were present in all the sites

	Ambroz	Guadiela	Jarama	Jarama	Lozoya	Lozoya	Sorbe	Tagus	Tajuña
Season	Summer 1999	Summer 1999	Summer 1997	Spring 1998	Spring 1998	Spring 1999	Spring 1998	Summer 1999	Spring 1999
Elevation m, asl	420	750	735	735	1090	1095	760	750	810
Total water surface area (m^2)	665	659	1229	1628	2003	2128	3944	3721	2621
Stream order	3	4	4	4	3	3	3	4	3
Mean annual flow (MAF, $m^3\ s^{-1}$)	3.76	5.04	5.03	5.03	1.41	1.41	2.62	14.43	2.21
Discharge per unit width (Q/W, $m^2\ s^{-1}$)	0.057	0.088	0.012	0.034	0.395	0.136	0.066	0.290	0.090
Water surface gradient (WSG, m km^{-1})	0.46	4.30	1.67	0.054	1.10	0.06	5.80	3.38	2.50
Mean depth ± SD (D, m)	0.61 ± 0.36	0.85 ± 0.37	0.32 ± 0.26	0.43 ± 0.24	0.70 ± 0.29	0.49 ± 0.20	0.58 ± 0.45	0.45 ± 0.21	0.48 ± 0.25
Maximum depth (maxD, m)	1.48	1.85	1.45	1.45	1.31	1.5	2.82	1.1	2
Mean water column velocity ± SD (Vm, m s^{-1})	0.140 ± 0.181	0.105 ± 0.107	0.050 ± 0.104	0.110 ± 0.130	0.468 ± 0.391	0.264 ± 0.167	0.172 ± 0.184	0.638 ± 0.339	0.199 ± 0.181
Maximum mean water column velocity (Vm, m s^{-1})	1.500	0.650	0.700	0.480	1.610	0.860	0.850	1.380	0.940
Dominant substrate (S)	cobbles	silt	cobbles	gravel	cobbles	cobbles	cobbles	gravel	silt
Mesohabitats units (HMU)	P	P	P,R,P,R	P	R,P	P	P,Ra,P,R,P,P	R,P,R	P,R,P,R,P
Fish	–	3	1,2,4	1,2,4	3,4,5	3,4,5	3,4,5	4	–

Since the surveys took place in different rivers, in two seasons and three consecutive years, the habitat use might have changed over time, in relation to the change in flow conditions. To measure this influence, correlation coefficients (Spearman's RHO) between the flow rate per unit width (one data per site) and the microhabitat variables for each species were calculated. Such variables were represented by the principal components extracted by PCA (mean scores of the species' data on each PC). The level of significance (0.05) was adjusted for each species using the Bonferroni technique.

Results

Microhabitat availability was recorded in 1517 points at the nine study sites (minimum 70 points per site) (Table 1). The average conditions of depth ranged from a site with a shallow mean depth, e.g. R. Jarama in 1997 (0.32 m) to another with approximately three times the former, R. Guadiela (0.85 m); maximum depth indicated a wide range of availability, varying from 1.1 to 2.82 m. The average conditions of water velocity varied from a site where slow habitats were dominant, e.g. R. Jarama in 1997 (0.050 m s^{-1}), to considerably larger velocities, e.g. R. Tagus (0.638 m s^{-1}); the maximum velocities registered were between 0.650 and 1.610 m s^{-1}.

A total of 912 independent fish microhabitat use observations were recorded at the nine study sites, but the minimum sample size requirement (five per fish group and site) and the test for non-random use of habitat reduced the independent samples to 870 (310 for barbel, 385 for nase, 175 for chub). These measurements corresponded to one or more fishes (schools), and the total number of fish observed was 5269 (1311 for barbel, 2991 for nase, 967 for chub). The three target species were interacting at the nine sites, but the sample size did not allow for analyses of the nine groups at the nine sites (Table 2). For the first objective, habitat suitability criteria were obtained for each fish group (Table 3); these data allowed observation of the wide range of habitat use data registered for the three species.

In the PCA, three components (hereafter PCs) were extracted with an accumulated variance of 83.9% (Fig. 2); only the variables with loadings $> |0.8|$ were considered to be associated with the PCs. PC_1 was positively correlated with water velocity (both V_m and V_f), PC_2 was positively correlated with deep microhabitats (D) and PC_3 was positively correlated with the focal height of the fish (F_h). The association of the fish groups with the PCs suggested the following classification into functional types:

Table 2. Summary of the sample size, after filtering the microhabitat use data, i.e. data from sites where non-random use of habitat was demonstrated for a fish group

	Number of sites	Sample size for each fish group
Barbel – small	2	27
Barbel – medium	6	180
Barbel – large	5	103
Nase – small	5	97
Nase – medium	6	144
Nase – large	5	144
Chub – small	2	62
Chub – medium	2	88
Chub – large	2	25
Total	–	870

- guild associated with deep water – large barbel (in deeper habitats than any other group) and large nase (in microhabitats with water velocity above the average);
- guild associated with shallow water – small fish of the three species and medium chub, small chub were associated with velocities below average.
- guild associated with low velocities – large chub.
- remaining fish groups: barbel and nase of medium size not associated to any variable.

The comparison among species (Table 4) indicated that barbel occupied deeper habitats and selected lower positions in the water column than the other two species; note barbel selected slower velocities than nase. The comparison among the three size classes of barbel showed differences in depth, with larger fish selecting deeper habitats (see Fig. 2). Small barbel selected lower focal height positions than the small fish of the other two species, but there was no difference between medium and large size (Table 4).

Nase occupied microhabitats with greater velocities than the other two species, in deeper habitats than chub and shallower habitats than barbel; they selected higher positions than barbel in the water column. A relationship between fish size and depth was observed because larger fish selected deeper habitats, with significant differences between the three classes ($P < 0.05$). Large and medium size nase were observed in higher positions in the water column than small nase; large nase also used faster velocities than small nase but there was no difference compared with medium size nase.

Chub selected shallower habitats than the other two species, and slower velocities than nase; focal height was larger for chub and nase than it was for barbel. Large chub occupied deeper habitats than the other

Table 3. Habitat suitability criteria for depth (D), focal height (F_h, distance from the bottom in percentage to depth), mean water column velocity (V_m), focal velocity (V), water velocity at the focal height of the fish), distance to shore (D_{ist}, in percentage to the mean river width), and substrate (S) for the nine fish groups. The optimal range [Opt] represents the central window of 50% in the data distribution, and suitable [Suit] is the central 95%

		D(m)	F_h (%)	V_m (m s^{-1})	V_f (m s^{-1})	D_{ist} (%)	S*
Barbel-small (*n* = 27)	Opt	0.31–0.54	4.0–10.0	0.066–0.397	0.026–0.236	10.7–23.7	3–5
	Suit	0.11–1.12	2.3–40.0	0.007–0.668	0.003–0.482	3.2–43.0	1–8
Barbel-medium (*n* = 180)	Opt	0.42–1.09	4.2–20.0	0.069–0.196	0.024–0.134	15.4–32.3	4–5
	Suit	0.22–1.95	1.5–50.0	0.004–0.466	0.000–0.302	3.1–53.8	1–8
Barbel-large (*n* = 103)	Opt	0.96–1.45	2.8–15.6	0.074–0.200	0.034–0.129	17.2–25.9	3–6
	Suit	0.55–2.24	0.4–44.0	0.005–0.591	0.000–0.401	1.5–49.5	1–8
Nase-small (*n* = 97)	Opt	0.34–0.61	5.0–40.0	0.039–0.303	0.039–0.234	5.8–21.5	1–4
	Suit	0.18–1.09	3.0–53.1	0.001–0.659	0.001–0.525	1.8–41.0	1–8
Nase-medium (*n* = 144)	Opt	0.42–0.96	4.0–30.0	0.08–0.284	0.045–0.213	12.9–32.3	3–5
	Suit	0.22–1.63	1.8–53.8	0.003–0.699	0.001–0.576	3.2–53.8	1–8
Nase-large (*n* = 144)	Opt	0.79–1.32	3.8–24.1	0.096–0.348	0.060–0.239	20.7–32.3	4–5
	Suit	0.31–1.94	1.9–63.8	0.004–0.770	0.007–0.531	6.1–59.2	1–8
Chub-small (*n* = 62)	Opt	0.26–0.51	9.8–38.4	0.016–0.190	0.010–0.157	10.7–32.3	4–5
	Suit	0.13–1.36	2.5–80.0	0.001–0.402	0.000–0.345	1.7–65.1	1–8
Chub-medium (*n* = 88)	Opt	0.30–0.50	8.8–23.5	0.089–0.211	0.054–0.156	16.1–32.3	4–5
	Suit	0.20–0.91	3.4–50.8	0.002–0.372	0.001–0.303	3.5–53.8	1–8
Chub-large (*n* = 25)	Opt	0.49–1.40	11.3–50.0	0.011–0.156	0.014–0.151	19.0–29.4	4–5
	Suit	0.23–1.68	2.9–70.0	0.004–0.288	0.005–0.203	0.0–58.8	1–8

*S, substrate types: 8 - bedrock; 7 - large boulders (∅ > 1024 mm); 6 - boulders (256–1024 mm); 5 - cobbles (64–256 mm); 4 - gravel (8–64 mm); 3 - fine gravel (2–8 mm); 2 - sand (62 μm–2 mm); 1 - silt (< 62 μm).

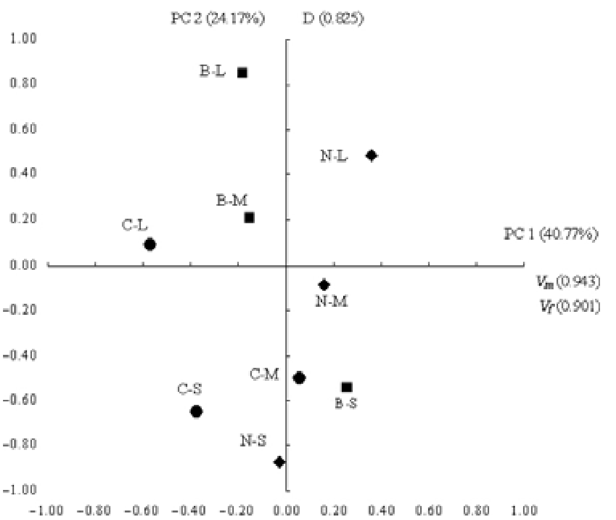

Figure 2. Mean scores on the factors extracted in the principal component analysis using microhabitat use data, for barbel (B, with squares), nase (N, with diamonds) and chub (C, with circles) in three size classes, small (S), medium (M) and large (L). Component loadings for each variable (with loadings > |0.8|) are indicated in each axis, as well as the percentage of the total variance explained.

two size classes and medium-sized chub were located in habitats with larger velocities compared with small and large chub.

Table 4. Results of the ANOVA *post-hoc* test of Dunnett's C, for multiple comparisons between species (table top) and size classes. Size classes are small, 1, medium, 2, and large, 3. For each comparison, the mean differences in order are mean(1)-mean(2), mean(1)-mean(3), and mean(2)-mean(3). The asterisk indicates differences in the multiple comparisons ($P < 0.05$). For clarity, instead of PCs, the original associated variables are presented with their accumulated variance

	V_m & V_f (40.77%)	D (24.17%)	F_h (18.94%)
Barbel-Nase	−0.313(*)	0.429(*)	−0.292(*)
Barbel-Chub	0.056	0.827(*)	−0.433(*)
Nase-Chub	0.370(*)	0.398(*)	−0.141
Barbel 1–2	0.412	−0.752(*)	−0.566(*)
Barbel 1–3	0.438	−1.397(*)	−0.447(*)
Barbel 2–3	0.026	−0.645(*)	0.118
Nase 1–2	−0.192	−0.785(*)	−0.410(*)
Nase 1–3	−0.387(*)	−1.361(*)	−0.549(*)
Nase 2–3	−0.195	−0.576(*)	−0.139
Chub 1–2	−0.432(*)	−0.148	0.033
Chub 1–3	0.199	−0.738(*)	−0.516
Chub 2–3	0.630(*)	−0.591(*)	−0.549

In general, the microhabitat use described for the three species was not correlated with variation of flow across sites (see Table 5). Most of the correlations (3 species × 3 PCs) were not significant, except in two cases, water velocity for barbel (PC_1) and focal height for chub (PC_3).

Table 5. Correlation coefficient (Spearman's RHO) and significance calculated for each species separately, between the flow rate per unit width (one datum per site) and the mean score by species on each principal component extracted by PCA. The level of significance (0.05) was adjusted for each species with the Bonferroni technique; significant correlations are marked with asterisks

	B-PC_1	B-PC_2	B-PC_3	*N*-PC_1	*N*-PC_2	*N*-PC_3	C-PC_1	C-PC_2	C-PC_3
Spearman's RHO	0.810*	−0.095	−0.452	0.714	−0.393	−0.500	−0.500	0.500	1.000**
P (Two-sided)	0.015	0.823	0.260	0.071	0.383	0.253	0.667	0.667	0.000

Discussion

The habitat sampling covered in this study was broader than used in previous microhabitat studies of Iberian species (Grossman & De Sostoa 1994a, b, in one river; Santos, Godinho & Ferreira 2004; in three rivers). The microhabitat use data showed that barbel occupied deeper microhabitats than the other two species; this finding could be related to its larger size relative to nase and chub (Doadrio 2001), as other studies demonstrated that larger fish tend to select deeper habitats (Heggenes & Traaen 1988; Hill & Grossman 1993; Lamouroux *et al*. 1999). Grossman & De Sostoa (1994a,b) did not find consistent differences in depth between *Barbus graellsii* (Steindachner) and *Chondrostoma toxostoma* (Vallot), which reach approximately the same length as the corresponding genera studied here. This could be a result of a narrower range of available conditions, because the maximum mean depth they recorded was 69 cm (spring 87, lower Matarraña).

Nase occupied locations with faster water velocities (V_m and V_f) than the other species, and the inter-classes comparison showed that high velocities were mainly selected by adults. A similar result was obtained for *C. polylepis* and *Squalius carolitertii* (Doadrio) in Iberian rivers (Santos *et al*. 2004). However, Grossman & De Sostoa (1994a,b), who analysed V_m and V_f, did not report differences between *B. graellsii* and *C. toxostoma*; this could be related to a smaller variability in the available microhabitats and the prolonged low water period during a relevant part of their study (> 1 year).

Chub occupied habitats with slower water velocities than barbel and shallower depths than the other two species, as reported elsewhere (Santos *et al*. 2004). Chub occupied the highest positions in the water column and nase was observed in lower positions (although the differences in F_h were not significant), whereas barbel was usually near the river bottom. Grossman & De Sostoa (1994a,b) found the same pattern between *C. toxostoma* and *Leuciscus cephalus* (L.) (no differences) and *B. graellsii* (lower in the water column). These results could support the concept that vertical segregation between species is linked to trophic adaptations, because barbel is a benthic feeder, therefore it is associated with the river bed; nase is mainly a herbivore, and chub uses a larger variety of food sources, including benthic plants, invertebrates and fishes (Lobón-Cerviá & De Diego 1988; Magalhães 1992; Valladolid & Przybylski 1996), therefore needing a wider range of positions to obtain food. In absolute terms, all species occupied the lower 50% of the water column, thus they can be considered mid-water to benthic dwellers (in agreement with Grossman, De Sostoa, Freeman & Lobón-Cerviá 1987).

Size-related differences in microhabitat use were consistent for the three species, i.e. smaller fishes occupied shallower microhabitats than larger ones. The small fish usually held positions in schools near the shore, with low and intermediate velocities (see Fig. 2), and their distance from shore was less than for larger fish ($P < 0.001$). The use of shallow habitats near the banks has been documented for small *B. bocagei* (Rincón, Barrachina & Bernat 1992), Iberian cyprinids (Grossman & De Sostoa 1994a, b) and other European cyprinids (Baras, Nindaba & Philippart 1995; Copp 1997; Lamouroux *et al*. 1999). Also, the small fish occupied lower positions in the water column than medium and large fish, although these differences were not significant for chub; these differences are also consistent with previous studies (Grossman & De Sostoa 1994a, b).

Some studies refer to changes in habitat use related to the potential effect of exotic predators (Schlosser 1987; Rincón, Velasco, González-Sánchez & Pollo 1990), especially for small fish. River Jarama was the only site where exotic predators [sunfish, *Lepomis gibbosus* (L.), and pike *Esox lucius* (L.)] were present, but their locations were not recorded; consequently, only a microhabitat comparison for small fish between sites with and without exotic predators was performed. In this river, *Barbus* and *Squalius* were present; small barbel selected microhabitats randomly, so this fish group was discarded from these considerations. For small chub, the use of depth was compared between the rivers Jarama and Ambroz, and the differences were not significant (ANOVA $P = 0.766$). This result suggests that the potential effect of exotic predators in the study could be of little relevance, although it was not completely discarded.

Two potential disadvantages of underwater observation (Heggenes *et al.* 1990) are the lower probability of observing fish in very shallow water (compared with electric fishing), and difficulties in small fish identification. The classification of the small fish up to 7 cm allowed species identification in most cases, but some young of the year were excluded from the analysis because the species were unknown, which was considered to be a limitation derived from the methods of the study. Another potential shortcoming was the impracticability of surveying similar study areas or habitat proportions across sites because of the habitat conditions. Under such circumstances, the combination of different habitat types in the whole study was considered the best option to address the general objectives.

The relationship between habitat availability and microhabitat use was treated in two steps. First, all the data were filtered to ensure that fish groups showing random use of habitat at a site were discarded. Second, the lack of correlation between flow rate and microhabitat variables (summarised in PCs) indicated that flow, season or inter-site differences were not the main factors determining microhabitat use. The water velocities selected by barbel and the focal heights by chub were correlated with flow, suggesting that changes in flow between seasons could affect the three species differently, which has been observed in larger-scale studies in Iberia (Filipe, Cowx & Collares-Pereira 2002); however, because all the variables interact in microhabitat selection, these two correlations out of nine were not considered very important to the general results.

Regarding the variables involved, depth was the most relevant factor in microhabitat selection, and the segregation by species and size classes are shown for the first time for these three species. Underwater observation was restricted to clear water and so are the results presented here; but studies in different types of rivers support the idea that depth could be the key variable to understand the microhabitat selection by European cyprinids (Copp & Jurajda 1993; Grossman & De Sostoa 1994a, b).

In conclusion, this study highlights the importance of habitat diversity in spring and summer. As found in previous studies, not only the pools but also shallower habitats with intermediate or high velocities are necessary for the conservation of these endemic Iberian species (Ilhéu, Costa & Bernardo 1999; Magalhães et al. 2002), especially for the survival of small fish and *Chondrostoma* (which selects larger velocities than the other species). The availability of habitat suitability criteria is essential for the implementation of environmental flow regimes in regulated rivers, in the actual framework of European water management. The information regarding fish height and velocity in the water column also allows the application of 2- and 3-dimensional habitat models. This information can also be useful to managers, because they can apply conservation measures for critical habitats to maintain and enhance the native fish populations in Mediterranean rivers.

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