

RESEARCH ARTICLE



Seasonal patterns of microhabitat selection in the Southern Iberian spined-loach *Cobitis paludica*

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Abstract

The Southern Iberian spined-loach *Cobitis paludica* is an Iberian endemism threatened by human activities, including habitat destruction. For this reason, the development of conservation and the recovery plans for the species calls for a precise knowledge of its habitat requirements. Here, microhabitat use and selection patterns were investigated to determine the limiting factors for the species in different seasons, corresponding to a gradient in flow conditions. The microhabitat of the loach was analysed in the River Jarama (Tagus River basin, central Spain) in the period of maximum activity between March and September 2013. No significant differences in microhabitat use were found between males and females, and only very weak ontogenetic changes were detected. The microhabitat used by the loach varied significantly throughout the study period, generally adapting to the flow-mediated dynamics of available habitat. The most stable pattern throughout the year was the use of very low water velocities. Additionally, the loach made selective use of certain microhabitat features, with slight adjustments to the seasonally changing habitat conditions. The loach significantly selected positions with abundant silt substrate and aquatic vegetation, and avoided coarser substrates. The availability of fine substrates and abundant vegetation is therefore a critical habitat requirement for the loach, which needs instream structures that provide foraging substrate, refuge and safe positions for spawning. Selection of refuge elements (deep habitats, aquatic vegetation) was especially apparent under low-flow conditions. Human activities leading to the loss of these critical microhabitats may threaten the survival of already scarce loach populations.

Keywords Cobitidae · Freshwater fish · Habitat management · Restoration · River · Stream

Introduction

The Southern Iberian spined-loach *Cobitis paludica*, henceforth loach, is an endemic species distributed throughout the centre and south of the Iberian Peninsula, from the Ulla and Ebro River basins towards the south (Doadrio et al. 1988; Perea et al. 2011; Sánchez-Hernández et al. 2018). Their populations are declining throughout its range due to water and gravel extraction that result in habitat destruction, and to the introduction of exotic fish species (Elvira 1990, 1995, 1996; Crivelli 2006). As a result, the species is considered vulnerable by the IUCN (Crivelli 2006; Freyhof and Brooks 2011), and it is listed in the Appendix III (Protected fauna species) of the Bern Convention.

Most of the still scarce studies on loach have mainly addressed aspects, such as its geographical distribution, genetic characterization, taxonomy and phylogeny (Doadrio et al. 1988; Madeira et al. 1992; Vasil'eva et al. 1992; Perdices et al. 1995, 2016; Doadrio and Perdices 1997, 2005; Perdices and Doadrio 2001; Ludwig et al. 2001), or some aspects of its biology, such as growth, feeding and reproduction (Lobón-Cerviá and Zabala 1984; Rodríguez-Jiménez 1987; Valladolid and Przybylski 1996, 2003; Przybylski and Valladolid 2000; Soriguer et al. 2000; Oliva-Paterna et al. 2002; Sánchez-Carmona et al. 2008). Unfortunately, while habitat degradation and destruction are considered within the main factors causing the decline of loach populations, very few studies have investigated the habitat requirements and the ecology of the species. The only existing work describing the microhabitat used by the loach was based on captures per unit of effort using minnow traps in a Mediterranean coastal stream, in an area where euryhaline and freshwater species coexist (Clavero et al. 2005).

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Habitat dynamics are strongly driven by the flow regime, which is markedly variable both within and between years in Mediterranean-type streams, being characterised by a sequence of regular and often extreme winter–spring flooding and summer drying (Gasith and Resh 1999). Although winter–spring high flows lead to increased wet area, use of large areas of stream habitat might be restricted by high water currents, and availability of velocity shelters might become limiting; meanwhile, strong habitat contraction occurs under summer low flows, so biotic controls (e.g., predation, competition) become more important, and refuge availability turns into a limiting factor (Ayllón et al. 2014). In consequence, stream-dwelling fish have developed behavioural adaptations to endure seasonal and yearly variations in flow conditions, including adjustments in their habitat selection patterns (Ayllón et al. 2014; Elvira et al. 2021). Flow regimes can also shift over time as a consequence of changes in climate, land use or flow management, producing alterations in the instream environment that can have strong impacts on fish populations, communities and entire ecosystems (Palmer and Ruhi 2019). Therefore, it is critical to establish how habitat selection patterns may vary during the ontogeny and across seasons to know if flow modifications can create habitat bottlenecks at different moments of the year that may affect the survival of specific age classes. Quantitative insights into the effects of variable flow on habitat selection are basic to design environmental flow regimes according to the Water Framework Directive, which is still an open issue in water management.

As the loach continues to decline, it is imperative to develop priority measures for the conservation and recovery of the species. However, a lack of information on the biology and ecology of threatened species may constrain the design and implementation of appropriate management programmes (Wootton et al. 2000). In particular, research on habitat requirements is essential to inform management and restoration activities when stream habitat is extensively degraded (Rosenfeld 2003). Nonetheless, basic information on loach habitat requirements is missing. To fill this knowledge gap, the main objectives of the study were to: (1) characterise the habitat use and selection patterns of the loach at a microhabitat scale to determine the proximate habitat features that are necessary for its persistence; (2) explore the link between flow-mediated habitat dynamics and variations in habitat use and selection patterns to understand the consequences of flow disturbances on the local persistence of the species.

Materials and methods

Study site

The study was conducted in the River Jarama at Valdepeñas de la Sierra, Guadalajara, Tagus River basin, central

Spain, 40°53'30" N 3°21'22" W, at 755 m a.s.l. (Fig. 1). The study area is embedded within a forest of *Quercus ilex* with some cereal crops, while the gallery forest is mainly composed of *Alnus glutinosa* and *Populus* spp. The River Jarama is the main right-bank tributary of the River Tagus, runs for 190 km and drains an 11,596 km² catchment, with the maximum flow in February–March and the minimum in August–September. Its source is in the Peña Cebollera at 2119 m a.s.l. and it flows into the River Tagus at 482 m a.s.l. The river is regulated by the El Vado dam (862 m a.s.l.). In its upper section, the main environmental impacts are those derived from water withdrawal for irrigation.

Native fish fauna of the study site consists of Brown trout *Salmo trutta*, Iberian barbel *Luciobarbus bocagei*, Iberian straight-mouth nase *Pseudochondrostoma polylepis*, Calandino *Squalius alburnoides*, Southern Iberian chub *Squalius pyrenaicus*, Northern Iberian spined-loach *Cobitis calderoni* and Southern Iberian spined-loach *Cobitis paludica*. Barbel, nase and chub were the dominant species in the past (Lobón-Cerviá and Penczak 1984), but the current fish assemblage is dominated by trout, Southern Iberian spined-loach and an exotic species, the Pyrenean gudgeon *Gobio lozanoi* (the only alien fish present in the study site).

Habitat data collection and fish sampling procedures

From March through September 2013, microhabitat use and availability were characterised in a reach of about 100 m long and 4.25 ± 1.56 m width. The study reach was sampled from one to four times per month, the number needed to capture at least 50 individuals each month;

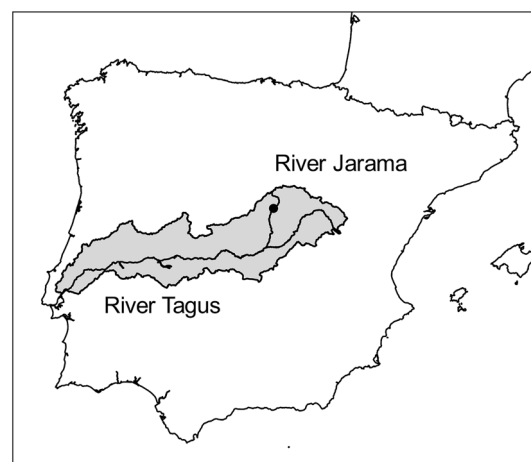


Fig. 1 Map of the study area showing the sampling site (black circle) used to characterise the microhabitat use and selection of the Southern Iberian spined-loach in the River Jarama. The location of the River Tagus basin (light grey colour) in the Iberian Peninsula is also shown (color figure online)

despite such sampling effort, it was not possible to reach that figure in all sampled months (see below). Due to the high winter discharge, the reach was sampled just once in March. In the sampling period, mean daily discharge was markedly higher in March and April (10.8 and $5.0 \text{ m}^3 \text{ s}^{-1}$, respectively) than from May to September (3.5 , 0.25 , 0.15 , 0.1 and $0.01 \text{ m}^3 \text{ s}^{-1}$, respectively). Fish were sampled by electrofishing using a 2200-W DC generator and captured loaches were placed into holding boxes, anaesthetised with tricaine metasulphonate (MS222), sexed, weighed (wet weight, g) and measured (total length, mm), and after recovery, were returned alive to the river. Sex was determined as males bear a *lamina circularis* on the second pectoral ray (organ of Canestrini) that is missing in females. Fish handling was minimised during the beginning of the spawning season (March–May) to avoid reproduction disturbance, so captured fish were not sexed in those samplings. Further explanation of the methods used can be found in Ayllón et al. (2014) and Elvira et al. (2021). All the work complied with the current Spanish and European conservation legislation and strictly adhered to the regulations on the handling of wild animals (Directive 2010/63/EU).

In the microhabitat assessments, the whole study reach was sampled, covering the entire channel width by slowly moving laterally from the starting point until the other bank was reached and then moving slowly upstream and repeating this procedure. Wherever a loach was captured, water depth (cm), current velocity (m s^{-1}) and distance to the nearest bank (m) were measured in the exact point where the fish was captured, while the proportion of aquatic vegetation (%) and substrate composition (%) were visually estimated in a $20 \times 20 \text{ cm}^2$ quadrat placed on the river bed. Particle size composition of the substrate was described following the classification criteria established in Platts et al. (1983) according to particle diameter: silt $< 0.01 \text{ cm}$, sand $0.01\text{--}0.5 \text{ cm}$, gravel $0.5\text{--}7.6 \text{ cm}$, cobble $7.6\text{--}30.5 \text{ cm}$ and boulder $> 30.5 \text{ cm}$. Based on these percentages, the substratum coarseness index (Nicola et al. 2010) was calculated as follows: $(\text{silt \%} \bullet 1) + (\text{sand \%} \bullet 2) + (\text{gravel \%} \bullet 3) + (\text{cobble \%} \bullet 4) + (\text{boulder \%} \bullet 5)/5$. This index measures the particle size and varies from 20 (100% silt) to 100 (100% block). A total of 13 (March), 17 (April), 153 (May), 52 (June), 104 (July) and 29 (September) individuals were captured during the fish surveys. To determine microhabitat availability, the same variables were concurrently measured with the fish samplings at randomly selected points throughout the study site. Five to seven (depending on the channel width) random positions per 10 m of bank were measured. Selected random positions varied across sampling surveys.

Data analyses

We verified whether parametric statistics could be applied in our analyses. To do so, we tested whether the availability and use data satisfied the criteria of normality and homoscedasticity. The assumption of normality of distributions (for both availability and use data) was verified for each habitat variable using the Shapiro–Wilk test. Since most of the variables did not meet normality requirements, they were transformed, using the natural logarithm in the non-percentage variables and the arcsine of the square root of the variable in the percentage variables. Still, not all variables met normality requirements. Consequently, for the subsequent analyses, the original, non-transformed variables were used, and non-parametric tests were applied.

Seasonal dynamics of habitat availability were analysed using both univariate and multivariate tests. The Kruskal–Wallis test by ranks (henceforth K–W) was used to check for significant differences across months in individual habitat variables. The Dunn’s test was used to compute post-hoc comparisons of pairs of groups. A principal component analysis (PCA) on habitat variables was used to summarise spatial (across microhabitat positions) and temporal (across months) changes in microhabitat availability, pooling all microhabitat availability data. Variables were standardised to a mean of 0 and a standard deviation of 1. The principal components with an eigenvalue over 1 were extracted, and analyses of variance (ANOVA) with subsequent post-hoc Tukey tests were performed to compare PC values across months.

Monthly patterns (from March to September) of habitat used by the loach were analysed to characterise the adjustments of habitat use to the seasonal dynamics of the microhabitat: (1) sexual and size-related differences in the use of each habitat variable were tested by means of linear mixed-effects models including sex and total length of captured individuals as fixed effects (as well as their interaction) and month as a random factor; (2) K–W tests were conducted to compare use of each habitat variable across months.

Two different approaches were used to analyse habitat selection (i.e., the use of a habitat in greater/lower proportion of its availability in the environment; Rosenfeld, 2003): (1) K–W tests were used to compare availability and use by loach of each habitat variable; (2) a multivariable logistic regression model was fit to describe the relationship between habitat availability and the relative probability of habitat use, and thus provide the probability that a position was used by the loach as a function of its microhabitat characteristics.

In the logistic regression model, the binary dependent variable indicated whether a position was used by the loach or was measured to characterise habitat availability, while measured habitat variables (except substratum coarseness) were used as continuous predictors. To avoid collinearity

and summarise the substrate characteristics, a PCA was performed only on substrate variables and the two resulting PCs were used as continuous predictors in the logistic regression model. The next steps were followed to develop the optimal model.

Firstly, the global model with all variables was fitted and regression predictors were standardised by centring and dividing by two standard deviations using the *arm* package for R (Gelman et al. 2018). Month was included as a random factor to induce a temporal autocorrelation structure in the data, so a generalised linear mixed-effects model was fitted using the *lme4* package version 1.1–23 (Bates et al. 2020) to estimate the global model. We compared a random intercept model with all possible random intercept and slope models following methods in Zuur et al. (2009) to choose the optimal random structure; the model with a random intercept and a random slope for habitat variables velocity and distance to the nearest bank was the best fit—i.e., it provided the lowest value of the Akaike's Information Criterion adjusted for small samples (AICc).

Secondly, all possible sub-models from the global model (model set) were built by means of the *MuMIn* package version 1.43.15 (Barton 2019). The AICc was then used to evaluate competing models. The top model set (i.e., that encompassing the model having the lowest AICc value and all models with a $\Delta\text{AICc} < 2$; Burnham and Anderson 2002) was obtained from the model set and averaged. Full averaging was used, so the coefficients (and their variance) of predictor variables not included in a model were set to zero.

Finally, the relative importance (defined as the sum of Akaike weights over all models including the predictor) of each predictor included in the final averaged model was calculated.

All statistical analyses were performed with Statistica 13.5 (<https://docs.tibco.com/products/tibco-statistica-13-5-0>), but for the logistic regression model, which was fitted in R version 4.0.4 (R Core Team 2021). We applied Holm's sequential Bonferroni adjustment (Holm 1979) to determine significance for all K–W tests. The significance level was set to $\alpha = 0.05$ for all statistical analyses.

Results

Seasonal variation in microhabitat availability

Depth availability in the River Jarama varied between 1 and 89 cm (mean depth of sampled quadrats = 27.49 cm, standard error = 0.54 cm), water velocity availability between 0 and 2.6 m s⁻¹ (0.27 ± 0.01 m s⁻¹) and distance to the nearest bank between 0.1 to 10.5 m (2.08 ± 0.05 m) (Table 1; Supp. Material, Figure S1). Availability of aquatic vegetation in sampled quadrats was relatively low and rather

variable (mean = 28.25% of quadrat's surface, standard error = 1.18%), while substrate composition in sampled quadrats was dominated by cobble ($32.34 \pm 1.10\%$), gravel ($30.46 \pm 1.18\%$) and silt ($27.73 \pm 1.04\%$) (Table 1; Supp. Material, Figures S1 and S2). The availability of the other substrate types was low, sand ($6.98\% \pm 0.59\%$) and boulder ($6.49\% \pm 0.60\%$).

All microhabitat variables analysed showed significant monthly differences (Table 1). As the river flow decreased steadily from March to September, so did mean water depth and current velocity, and distance to the nearest bank. Due to the channel architecture of the study site, velocity changed more strongly with flow than water depth. Availability of aquatic vegetation in the sampled quadrats significantly increased in summer (June through September) compared to late winter and spring (March through May), because most patches with aquatic vegetation were concentrated on the centre of the channel. As for the substrate composition, sampled quadrats had a higher proportion of silt, cobble and boulder, and lower of sand and gravel, in summer than in winter/spring months.

The PCA revealed four main factors accounting for 67.39% of the spatio-temporal variance in available microhabitat (Table 2). The first factor (PC1) was positively correlated with the proportion of silt substrate and aquatic vegetation and negatively with proportion of gravel substrate and current velocity. The second factor (PC2) discriminated those positions with a high proportion of cobble substrate. The third factor (PC3) characterised deep positions away from the riverbank. Finally, the fourth factor (PC4) was negatively correlated with a high proportion of boulder substrate.

PC1 values differed across months ($F_{5,975} = 66.88$, $p < 0.001$); PC1 values were significantly higher in June, July and September than in March, April and May (Tukey test, $p < 0.001$). There were also significant differences in PC2 values across months ($F_{5,975} = 18.60$, $p < 0.001$), so that PC2 values were significantly higher in June and significantly lower in March and May compared to the other months (Tukey test, $p < 0.001$). Likewise, PC3 values differed across months too ($F_{5,975} = 25.42$, $p < 0.001$), with PC3 values significantly higher in March and April. Finally, there were also significant differences in PC4 across months ($F_{5,975} = 29.71$, $p < 0.001$); PC4 values were highest in March and lowest in September (Tukey test, $p < 0.001$).

Sex-related, size-related, and seasonal variation in microhabitat use

Habitat use data were recorded for a total of 368 loach individuals (range 30–105 mm in total length; mean 68.62 ± 0.75 mm) (Supp. Material, Fig. S3). Neither sex nor individual body size did significantly influence habitat use

Table 1 Characteristics of available habitat (mean $\pm$ standard error, range in brackets) from March through September 2013 in the River Jarama study site

	March (<i>n</i> = 70)	April (<i>n</i> = 180)	May (<i>n</i> = 221)	June (<i>n</i> = 80)	July (<i>n</i> = 160)	September (<i>n</i> = 270)	Kruskal–Wallis test
Depth (cm)	34.86 $\pm$ 2.37 (6–89)	31.35 $\pm$ 1.20 (6–76)	25.52 $\pm$ 0.86 (5–66)	23.01 $\pm$ 1.41 (4–52)	26.46 $\pm$ 1.37 (3–84)	26.55 $\pm$ 1.20 (1–86)	H = 34.41*
Velocity (m s ⁻¹)	0.75 $\pm$ 0.08 (0–2.6)	0.44 $\pm$ 0.04 (0–2.0)	0.39 $\pm$ 0.03 (0–2.3)	0.08 $\pm$ 0.01 (0–0.5)	0.07 $\pm$ 0.01 (0–0.8)	0.10 $\pm$ 0.01 (0–1.2)	H = 198.72*
Distance to the nearest bank (m)	4.01 $\pm$ 0.31 (0.3–10.5)	2.92 $\pm$ 0.16 (0.3–10.5)	2.08 $\pm$ 0.09 (0.1–8.1)	1.66 $\pm$ 0.08 (0.3–3.0)	1.61 $\pm$ 0.07 (0.1–4.0)	1.42 $\pm$ 0.05 (0.1–4.2)	H = 145.68*
Aquatic vegetation (%)	22.00 $\pm$ 4.03 (0–100)	15.17 $\pm$ 2.04 (0–100)	23.33 $\pm$ 2.36 (0–100)	51.00 $\pm$ 4.49 (0–100)	34.09 $\pm$ 3.34 (0–100)	32.41 $\pm$ 2.19 (0–100)	H = 73.93*
Silt (%)	19.14 $\pm$ 3.85 (0–100)	5.97 $\pm$ 1.17 (0–100)	18.35 $\pm$ 2.31 (0–100)	23.56 $\pm$ 2.05 (0–80)	33.12 $\pm$ 2.60 (0–100)	35.63 $\pm$ 2.19 (0–100)	H = 227.97*
Sand (%)	10.57 $\pm$ 2.57 (0–90)	14.17 $\pm$ 2.09 (0–100)	8.78 $\pm$ 1.21 (0–90)	5.94 $\pm$ 1.58 (0–80)	3.50 $\pm$ 1.16 (0–100)	2.15 $\pm$ 0.51 (0–50)	H = 75.55*
Gravel (%)	49.57 $\pm$ 4.89 (0–100)	37.67 $\pm$ 2.65 (0–100)	51.81 $\pm$ 2.46 (0–100)	17.00 $\pm$ 2.98 (0–100)	18.50 $\pm$ 2.59 (0–100)	14.30 $\pm$ 1.78 (0–100)	H = 200.15*
Cobble (%)	18.29 $\pm$ 3.83 (0–100)	38.42 $\pm$ 2.78 (0–100)	20.38 $\pm$ 2.03 (0–100)	53.50 $\pm$ 3.13 (0–100)	37.00 $\pm$ 2.61 (0–100)	32.70 $\pm$ 2.11 (0–100)	H = 85.72*
Boulder (%)	2.43 $\pm$ 1.73 (0–100)	3.78 $\pm$ 1.14 (0–90)	0.68 $\pm$ 0.37 (0–60)	0	7.87 $\pm$ 1.64 (0–90)	15.22 $\pm$ 1.59 (0–100)	H = 138.93*
Substratum coarseness	54.86 $\pm$ 2.14 (20–100)	63.97 $\pm$ 1.14 (20–98)	55.25 $\pm$ 1.14 (20–84)	60.09 $\pm$ 1.30 (30–80)	56.60 $\pm$ 1.57 (20–96)	57.96 $\pm$ 1.39 (20–100)	H = 28.54*

Significant differences in monthly microhabitat availability (Kruskal–Wallis test) are reported in the last column, after *p* values were adjusted using Holm's sequential Bonferroni correction (*ns* not significant, **p* $\leq$ 0.05)

Table 2 Factor loadings for the first four principal components from the PCA on all monthly microhabitat availability samples in the River Jarama study site

Variables	PC1	PC2	PC3	PC4
Depth	0.28	– 0.30	0.70	0.04
Velocity	– 0.63	0.14	0.13	0.08
Distance to the nearest bank	– 0.25	– 0.01	0.63	0.41
Aquatic vegetation	0.55	0.07	– 0.20	0.37
Silt	0.82	– 0.23	– 0.16	0.12
Sand	– 0.05	– 0.49	0.41	0.14
Gravel	– 0.71	– 0.46	– 0.39	0.08
Cobble	– 0.05	0.92	0.19	0.19
Boulder	0.11	0.08	0.30	– 0.86
Explained variance (%)	22.58	16.38	15.89	12.54

Loadings in bold indicate correlations greater than 0.5

from June to September (Table 3). Only a weak relationship between body size and distance to the nearest bank was detected (Table 3), with larger fish using positions further away from the bank.

The loach occupied positions with very low current velocity (mean value for all months: 0.07 $\pm$ 0.01 m s⁻¹), medium depth (30.52 $\pm$ 0.69 cm), dominated by silt substrate (51.18 $\pm$ 2.03%) and with a high proportion of aquatic vegetation (48.98 $\pm$ 2.11%) (Figs. 2 and 3, Supp. Material,

Table 3 Summary of the linear mixed-effects models relating the use of each microhabitat variable with sex and total length of observed loaches from June through September 2013 in the River Jarama study site

Variables	Sex	Length	Sex $\times$ Length
Depth	0.628	0.778	0.460
Velocity	0.158	2.253	0.053
Distance to the nearest bank	0.077	6.420*	0.072
Aquatic vegetation	0.983	0.005	0.988
Silt	0.071	0.292	1.130
Sand	0.000	0.088	0.423
Gravel	2.665	0.004	0.505
Cobble	2.436	0.952	1.033
Boulder	0.044	0.265	0.073
Substratum coarseness	0.397	0.451	1.192

Month was included as a random factor. The F statistic values are shown (all terms were not significant except for: **p* $\leq$ 0.05)

Table S1). However, microhabitat used by the loach differed significantly across months, existing significant differences in all habitat variables (Supp. Material, Table S1 and Fig. S4–S13). The most apparent changes were the use of positions with a significantly higher proportion of aquatic vegetation in late spring/summer, coarser substrates in summer and higher depth in late summer (Figs. 2 and 3, Supp. Material, Table S1).

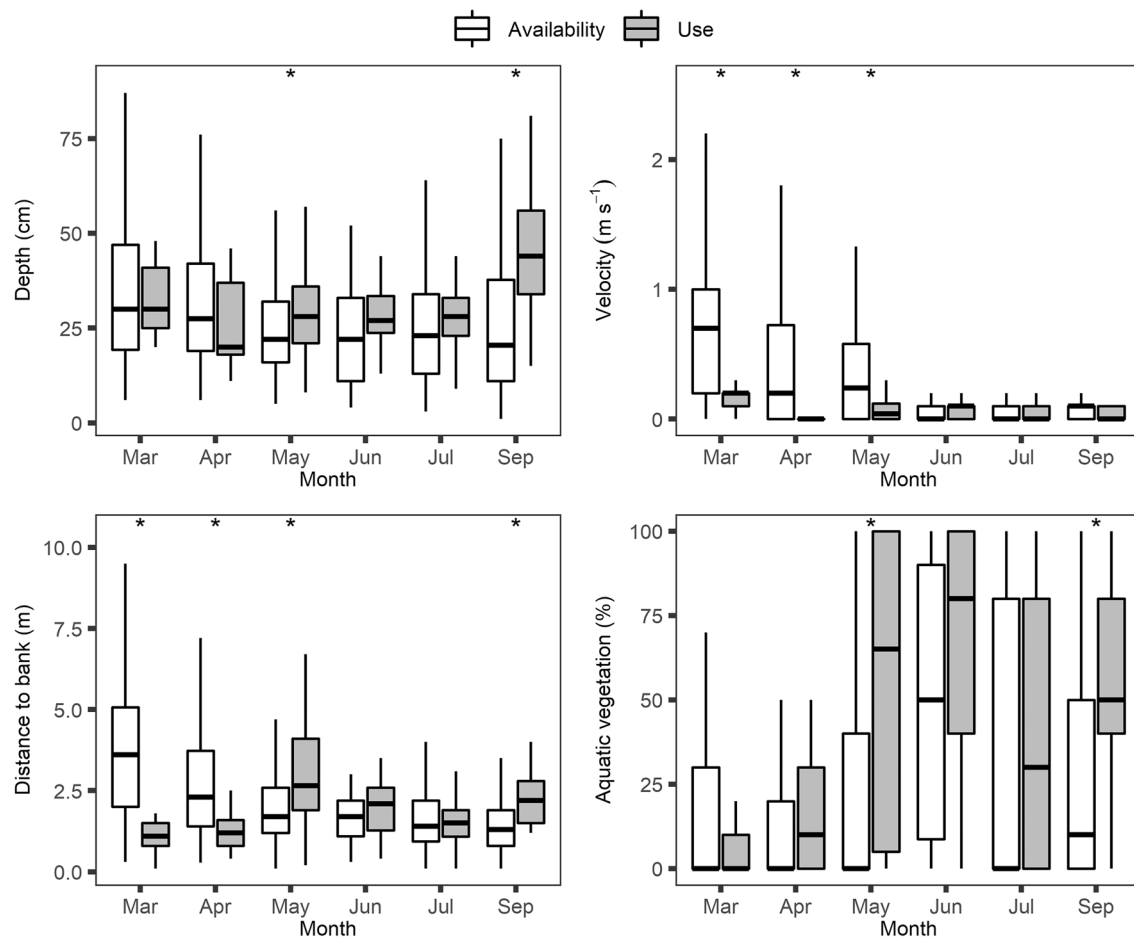


Fig. 2 Monthly availability and Southern Iberian spined-loach use of depth, current velocity, distance to the nearest bank, and proportion of aquatic vegetation in the River Jarama study site from March to September 2013. Graphics show the median value and the first and third quartiles, which correspond with the lower and upper hinges of

the box; the whiskers extend from the hinges 1.5 times the inter-quartile range. Significant differences estimated through the Kruskal–Wallis test are also indicated after the p value was adjusted using Holm's sequential Bonferroni correction ($*p \leq 0.05$). Outliers can be checked in Supp. Material, Fig. S14

Microhabitat selection

Univariate analyses indicated that habitat used by the loach differed significantly from habitat availability in the reach only with regard to certain habitat features and in certain months. In general, the dynamics of habitat use followed the dynamics of habitat availability. In June and July, the loach hardly exhibited a non-random use of available habitat. The most consistent patterns throughout sampled months were a marked preference for positions dominated by silt substrate with low current velocity (Table 4, Figs. 2 and 3). Loaches only showed a differential use of microhabitats regarding depth in May and September, when they positively selected deeper positions, while changed their preference for the location of used habitats relative to the banks throughout the year (Table 4, Fig. 2). Loaches occupied positions with abundant aquatic vegetation from May onwards, but they were significantly overrepresented in those habitats only in

May and September (Table 4, Fig. 2). Regarding the substrate composition, the loach showed the aforementioned marked preference for positions with a high proportion of silt, exhibiting avoidance or just random use of the rest of substrate types (Table 4, Fig. 3).

The two factors of the PCA performed on substrate variables accounted for 65.8% of the variance. The first factor (34.8%) was positively correlated with the proportion of cobble (factor loading: 0.784) and boulder (0.345) substrates and negatively with the proportion of gravel (-0.48) and silt (-0.48) substrates, and thus reflected increasing substrate coarseness (hereafter $PC_{\text{coarseness}}$). The second factor (31.1%) discriminated between positions presenting a high proportion of silt substrate (0.864) from those featuring mainly gravel (-0.864) (hereafter $PC_{\text{silt/gravel}}$).

The optimal multivariable logistic regression model indicated that loaches used selectively throughout the year positions with very slow current velocity linked to silts

Fig. 3 Monthly availability and Southern Iberian spined-loach use of substrate variables in the River Jarama study site from March to September 2013. Methods and formats are the same as for Fig. 2. Outliers can be checked in Supp. Material, Fig. S15

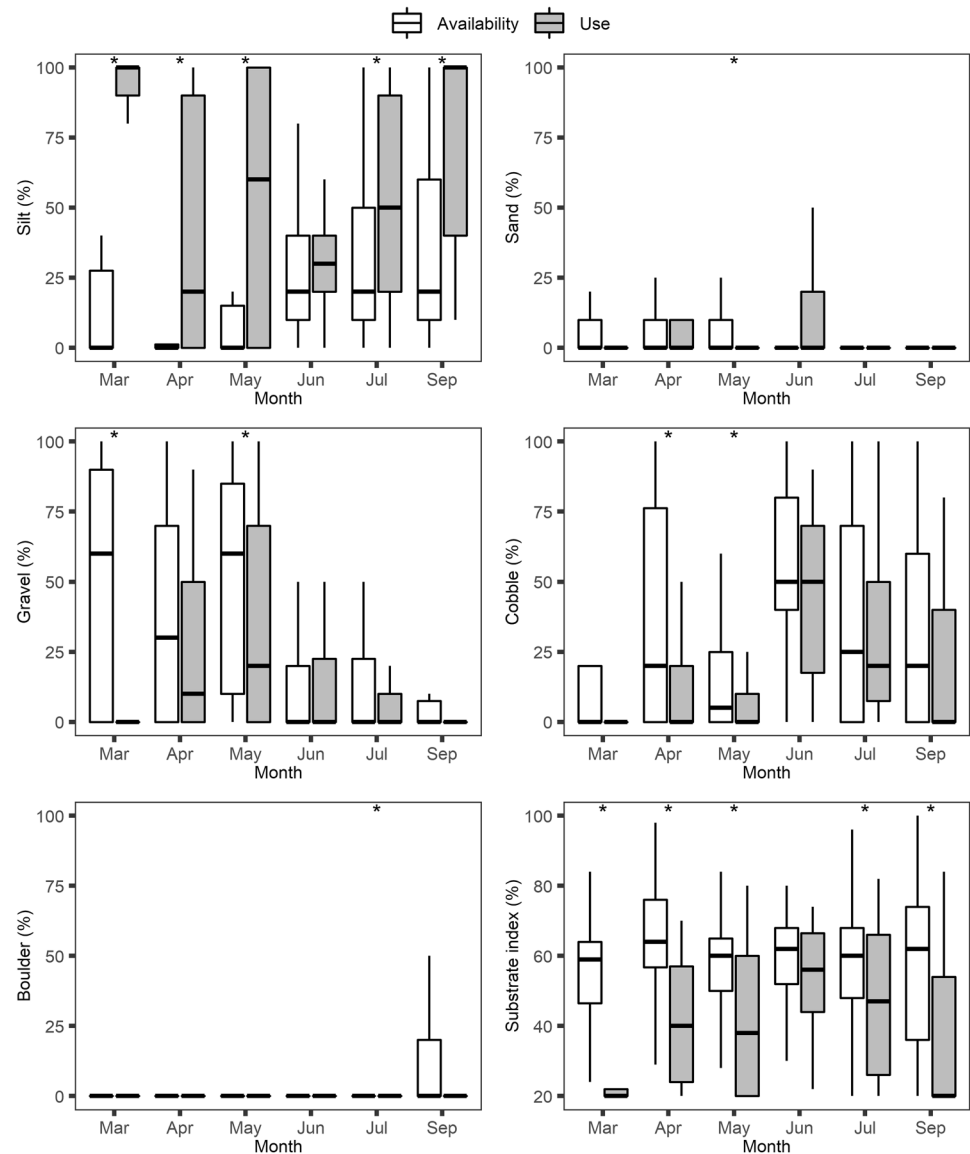


Table 4 Habitat selection by the Southern Iberian spined-loach from March through September in the River Jarama study site

	March (<i>n</i> = 83)	April (<i>n</i> = 197)	May (<i>n</i> = 374)	June (<i>n</i> = 132)	July (<i>n</i> = 264)	September (<i>n</i> = 299)
Depth (cm)	0.09 ns	1.11 ns	15.64* (+)	7.14 ns	5.34 ns	23.59* (+)
Velocity (m s ⁻¹)	11.41* (-)	12.58* (-)	50.87* (-)	0.001 ns	2.62 ns	1.95 ns
Distance to the nearest bank (m)	19.19* (-)	10.75* (-)	41.42* (+)	4.83 ns	0.26 ns	20.83* (+)
Aquatic vegetation (%)	1.08 ns	1.64 ns	49.70* (+)	4.25 ns	3.31 ns	11.94* (+)
Silt (%)	22.71* (+)	22.89* (+)	60.99* (+)	6.32 ns	20.02* (+)	24.36* (+)
Sand (%)	1.67 ns	0.19 ns	22.00* (-)	2.48 ns	4.09 ns	0.11 ns
Gravel (%)	9.97* (-)	1.89 ns	18.44* (-)	0.03 ns	1.25 ns	3.73 ns
Cobble (%)	6.30 ns	8.26* (-)	10.74* (-)	3.03 ns	1.12 ns	6.25 ns
Boulder (%)	0.38 ns	1.41 ns	3.50 ns	—	10.83* (-)	4.77 ns
Substratum coarseness	21.40* (-)	19.11* (-)	42.19* (-)	6.26 ns	16.74* (-)	19.98* (-)

Significant differences between monthly microhabitat use and availability are indicated through the H statistic of the Kruskal–Wallis test and its *p* value adjusted using Holm's sequential Bonferroni correction (ns not significant, **p* ≤ 0.05). When significant (marked in bold), (+) indicates preference, while (−) indicates avoidance

substrates (high $PC_{\text{silt/gravel}}$ values) with abundant aquatic vegetation, and avoided coarse substrates (positions with high $PC_{\text{coarseness}}$ values) (Tables 5 and 6). The model also indicated that the effect of current velocity and distance to the nearest bank on the probability of habitat use varied significantly across months (Table 5), following the patterns observed in the univariate analyses.

Discussion

Sexual and size-related variation in microhabitat use

Despite the existing sexual dimorphism regarding body size in the Southern Iberian spined-loach (Przybylski and Valladolid 2000), no significant differences were found in microhabitat use between males and females in the River

Jarama over the sampled months. Since no previous studies have analysed sex differences in loach microhabitat use, we are unable to determine whether this is a general pattern across its range. This pattern differs, however, from what has been observed in the Northern spined-loach *Cobitis taenia*, which displays sexual variation in substrate preferences, with females choosing coarser (gravels) substrates than males (fines) under experimental conditions in a multisite approach (Culling et al. 2003).

Overall, we did not detect a strong relationship between body size and habitat used by the loach. Smaller individuals tended to use more frequently positions dominated by cobble substrate—which offers higher protection against excessive current velocity—and located closer to the banks than larger fish, but the pattern was weak as the explained variance was really low. This result contrasts with findings by Clavero et al. (2005), who observed a clear size-based spatial segregation along depth gradients, with larger individuals consistently using deeper microhabitats. However, the predominant size classes studied by Clavero et al. (2005) were much smaller (and likely younger) (range 14–95 mm in total length; mean 38.6 ± 0.3 mm) than loaches observed in our study site (range 30–105 mm in total length; mean 68.62 ± 0.75 mm; Supp. Material, Figure S3). Likewise, previous studies have described size-related variations in microhabitat use in other species of *Cobitis* in central Europe, smaller fish being found in positions with slower velocities, finer substrates, and more abundant vegetation (Copp and Vilizzi 2004), although Slavík et al. (2000) reported that the spatial distribution of different loach sizes were driven only by substrate preferences. In the observations of Copp and Vilizzi (2004), the most apparent shifts in loach habitat use took place between age—0 fish and older individuals. Thus, all in all, our and previous studies suggest that main changes in habitat use over ontogeny might take place early in life in loaches.

Table 5 Summary of the multivariable logistic regression model that predicts the probability of loach habitat use

Variables	Coefficients	Importance
Random effects		
Month	0.80	1.00
Month:Velocity	0.34	1.00
Month:Distance	1.75	1.00
Fixed effects		
Intercept	-1.42 ± 0.31	1.00
$PC_{\text{silt/gravel}}$	0.87 ± 0.16	1.00
Aquatic vegetation	0.66 ± 0.14	1.00
$PC_{\text{coarseness}}$	-0.77 ± 0.17	1.00
Velocity	-1.65 ± 0.49	1.00
Depth	0.01 ± 0.08	0.21
Distance to the nearest bank	0.04 ± 0.37	0.21

Model-averaged coefficient estimates ($\pm$ standard error) and relative importance of variables are shown. Relative importance is the sum of Akaike weights over all models within the top model set including the predictor variable. Estimate coefficients for the random structure are the standard deviation of random intercept and slopes in the models. $PC_{\text{silt/gravel}}$ and $PC_{\text{coarseness}}$ are the two factors of the principal component analysis performed on substrate variables

Seasonal microhabitat use and selection patterns

The microhabitat features used by the loach in the River Jarama significantly changed over the sampling period, from March to September, generally adapting to the flow-driven

Table 6 Structure of multivariable logistic regression models that predict the probability of loach habitat use included in the top model set ($\Delta AIC_c < 2$) and their model weights

Model	ΔAIC_c	w_i
Aquatic vegetation—Velocity + $PC_{\text{silt/gravel}}$—$PC_{\text{coarseness}}$	0.00	0.574
Aquatic vegetation—Velocity + $PC_{\text{silt/gravel}}$—$PC_{\text{coarseness}}$ + Depth	1.97	0.213
Aquatic vegetation—Velocity + $PC_{\text{silt/gravel}}$—$PC_{\text{coarseness}}$ + Distance to bank	1.98	0.212

It is indicated whether predictor variables have a positive (+) or negative effect (−) on the probability of detection of the loach. The random intercept (Month) and random slopes (Month:Velocity and Month:Distance) are included in all models. Variables with $p < 0.05$ are marked in bold

dynamics of available habitat. The most stable pattern throughout the year was the use of very low water velocities, which is consistent with the known general ecology of the species (Elvira 1995; Perdices and Doadrio 1997). During high flows (March–April), those positions were located near the banks, where gravels were more abundant. As the flow diminished in late spring and summer, the availability of microhabitats with low velocities was significantly higher and no longer limiting, but loaches tended to move to the centre of the channel, where aquatic vegetation was more abundant.

There was also significant evidence that the study population made selective use of certain microhabitat features during the period analysed, with some adjustments to the seasonally changing habitat conditions. Apart from the observed strong preference for very low water velocities, the loach consistently selected positions in habitats with abundant silt substrate and aquatic vegetation, and avoided the coarsest substrates, which coincides with patterns observed in other species of *Cobitis* (Przybylski et al. 2003; Copp and Vilizzi 2004). Availability of fines is thus a critical habitat requirement for the Southern Iberian spined-loach. The preference of loaches for silty bottoms probably corresponds to a physical structure that provides shelter and foraging substrate (for small invertebrates and detritus) (Valladolid and Przybylski 1996, 2003; Copp and Vilizzi 2004). In fact, Valladolid and Przybylski (1996) reported that in the nearby River Lozoya the loach lives buried in fine substrates, from where it emerges only occasionally. This is in agreement with species distribution analyses conducted at large spatial scales: presence/absence models by Ferreira et al. (2007) indicated that the loach was mainly confined to river reaches dominated by fine substrates in western Iberia. Valero et al. (2017) showed that density of loach increased with fine sediment load in rivers of the River Guadalquivir basin (south-western Iberian Peninsula); indeed, while excess of fine sediment deposition produces a detrimental effect on freshwater fish habitat and food availability, the loach displays a higher tolerance threshold than other native fish species (Valero et al. 2017). While loaches made use of positions with gravels and cobbles, they did not actively select such microhabitats and were even underrepresented in spring therein.

Presence of aquatic vegetation in the riverbed was the third habitat feature critically required by the loach, particularly in late spring and summer. Aquatic vegetation provides visual cover against terrestrial and aquatic predators, and those refuges are thus particularly selected by freshwater fish under low-flow conditions (Ayllón et al. 2014). Clavero et al. (2005) showed that loaches were distributed in the reach as a function of depth and presence of refuge, showing the highest preference for deep, covered habitats. In the River Jarama, the loach did not show a non-random use of water depth, as suitable depths were highly available, except in late

summer, when loaches were significantly overrepresented in pool habitats. Increasing depth reduces the risk of predation (Power 1987), so pool habitats provide extra protection during the period of lowest flows, when fish are more vulnerable to predation.

Implications for loach conservation and management

The present study highlights that the Southern Iberian spined-loach displays an aggregate spatial distribution throughout the year in highly specific microhabitats resulting from the interaction of hydraulic and structural characteristics. Thus, human activities leading to the loss of such critical microhabitats can put the persistence of its populations at risk. The presence of dams can be especially negative for loach populations: (1) Long-term storage and non-seasonal release of water, or hydropeaking operations, can severely alter the downstream river channel and habitat availability (Poff and Hart 2002), causing the lack of the microhabitats with very slow current velocity used by the loach. (2) Capture of sediments by dams produces changes in the natural patterns of fine sediment transport, leading to coarsening of the riverbed downstream (Poff and Hart 2002) and thus to the loss of silt-dominated microhabitats critically required by the loach. Strong changes in sediment granulometry and habitat composition produced by dams can thus alter the structure of benthic fish assemblages (Granzotti et al. 2018). However, an excess of fine sediment load can be also harmful, as it can both clog the riverbed—thus decreasing benthic invertebrate density and diversity—and bury the aquatic vegetation (Ryan 1991) upon which the loach relies to take refuge. Therefore, loach population densities are higher at intermediate than at very high levels of fine sediment load (Valero et al. 2017). Consequently, land use changes leading to increased soil erosion and excessive sediment yield might be detrimental for loach populations, for example, in basins dedicated to intense olive grove, where soil loss produced by the run-off is amplified by sloping tillage (Valero et al. 2017).

Gravel extraction is considered one of the main threats to the loach (Perdices and Doadrio 1997). In general, both species richness and diversity of benthic fishes decline in dredged sites, where assemblages are taken over by eurytopic generalists (Freedman et al. 2013). Gravel dredging increases sedimentation rates, which might benefit silt-tolerant species in the short-term (Freedman et al. 2013), but can have negative consequences for the loach on the long-term, for reasons commented above. In addition, while gravels and cobbles were not actively selected by the loach in the River Jarama, they were used in both spring and summer; this suggests that substrate heterogeneity can be important for the species to a certain extent. Therefore,

human activities leading to riverbed homogenization like gravel dredging or reach channelization—which additionally affects the benthic invertebrate assemblage composition (Blake and Rhanor 2020)—could limit the viability of loach populations.

Direct (e.g., through gravel dredging) or indirect (e.g., through sedimentation processes) removal of aquatic vegetation is likely to produce strong negative impacts on loach populations because the presence of aquatic vegetation as a refuge element was a main determinant of its habitat use and selection patterns. The lack of cover against predators is especially harmful in river reaches presenting high densities of piscivorous fish species, like native eels (Clavero et al. 2005) or exotic largemouth bass (Almeida et al. 2012). The lack of abundant aquatic vegetation can also disrupt loach reproduction. Loach spawning is typically very long and fractional (i.e., they are multi-spawners; Lobón-Cerviá and Zabala 1984; Oliva-Paterna et al. 2002), lasting from mid-May through July in the River Jarama (Lobón-Cerviá and Zabala 1984), during the low-flow period. The Southern Iberian spined-loach—like the Northern spined-loach *C. taenia*—lays its eggs in positions with very low current velocity and over very dense aquatic vegetation (Bohlen 2000a, b, 2003), which provides cover against egg predation and prevents flushing of the eggs. Thus, lack of abundant aquatic vegetation increases loach mortality rates during early development and may even lead to a complete failure in recruitment.

The species is locally protected as it was considered to be “of special interest” in the 1998 Regional Catalogue of Threatened Species of Castilla-La Mancha. However, there is still no management plan for the species, even though it is required by law. Likewise, the study site was included in the Sierra Norte de Guadalajara Natural Park in 2011, a protected natural area that had its own Natural Resources Management Plan since 2010. Despite all these conservation initiatives, the local loach populations are still in serious decline, as the necessary tools for their recovery have not been put in force. The accurate knowledge of the loach microhabitat requirements should be an essential starting point when drawing up the necessary management plans for the species. Since our results may entail critical implications for the conservation and management of the species in other river basins, further research should involve a wider range of river typologies and/or longer time frames to ensure the transferability of the habitat selection models developed in our study to other threatened populations.

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Declarations

Conflict of interest (include appropriate disclosures) The authors declare that they have no conflict of interest.

Ethics approval (include appropriate approvals or waivers) All the work complied with the current Spanish and European conservation legislation and strictly adhered to the regulations on the handling of wild animals (Directive 2010/63/EU).

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication Not applicable.

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