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Determinants of large-scale spatial distribution and seasonal microhabitat selection patterns of the endangered freshwater blenny *Salaria fluviatilis* in the Ebro River basin, Spain

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Abstract

1. The freshwater blenny *Salaria fluviatilis* is an endangered fish species with populations that are in rapid decline, largely owing to habitat degradation caused by human activity. This situation highlights the urgent need to develop measures for the conservation and recovery of the species based on a deep understanding of its specific habitat requirements.
2. In this study, spatial distribution and habitat selection patterns were investigated to determine the limiting factors for the species at different times of the year and at different spatial scales, from macro to microhabitats.
3. The presence of the freshwater blenny was assessed at 127 sites in the Ebro River basin, Spain, between 2002 and 2012. It was only detected at 25 sites, corresponding to the intermediate and lower reaches of medium-sized tributaries and in the main river, in accordance with the ecology of the species. Whether the species was present depended on the physicochemical, habitat and biological conditions of the study sites. Freshwater blenny was very sensitive to organic pollution and eutrophication, the deterioration of substrate composition and channel structure, and the degradation of aquatic and riparian vegetation.
4. Freshwater blenny showed a selective use of microhabitat locations with high current velocity, linked to gravel or cobble substrate. It was also observed that the species is capable of adapting its selection behaviour to the flow-mediated seasonal changes in its physical environment.
5. Although the results presented indicate that the species is not a microhabitat specialist, individual survival is likely to be dependent on the availability of key microhabitats, which must be protected against detrimental human activity.

KEYWORDS

Blenniidae, freshwater fish, habitat management, restoration, river, stream

1 | INTRODUCTION

The freshwater blenny *Salaria fluviatilis* is found in river basins around the Mediterranean Sea, from Portugal to Israel (Bath, 2003). It is a benthic species living in lakes and streams with a moderate current, on gravel or stony bottoms. The female spawns under a large stone and the male defends the clutch until the eggs hatch a week later. The larvae are planktonic, remaining in calm water near the river banks until they are about 13–14 mm in total length, when they become benthic (Kottelat & Freyhof, 2007; Gil, Faria & Almada, 2010).

Several aspects of the biology of the freshwater blenny are already well known (Bath, 2003), in particular its trophic ecology (Prenda & Mellado, 1993; Psarras, Barbieri-Tseliki & Economou, 1997), egg cannibalism (Vinyoles, Côté & de Sostoa, 1999), sexual dimorphism (Alp & Kara, 2007; Fabre et al., 2014; Laporte et al., 2018), growth (Vinyoles & de Sostoa, 2007), reproduction (Psarras, Barbieri-Tseliki & Economou, 1997; Vila-Gispert & Moreno-Amich, 1998; Vinyoles, Côté & de Sostoa, 1999; Vinyoles, Côté & de Sostoa, 2002; Vinyoles & de Sostoa, 2007), reproductive behaviour (Neat et al., 2003; Lengkeek & Didderen, 2006; Gasith & Goren, 2009; Fabre, García-Galea & Vinyoles, 2014a; Quirós & Vinyoles, 2016), and early development (Psarras, Barbieri-Tseliki & Economou, 1997; Gil, Faria & Almada, 2010; Fabre, García-Galea & Vinyoles, 2014b; Çoker, 2019).

From a conservation perspective, the species is considered to be of Least Concern by the International Union for Conservation of Nature (IUCN), but local populations can be threatened (Crivelli, 2006) and it is endangered in several countries, including in Spain, where it is protected by the national catalogue of threatened species. It is also listed (as *Blennius fluviatilis*) in Appendix III (protected species of wild fauna) of the Bern Convention (Council of Europe, 1979). The freshwater blenny has been identified as a potential host for the giant freshwater pearl mussel *Margaritifera auricularia*, a critically endangered species, and could play a key role in its survival in one of its last refuges in Spain (Soler et al., 2019). However, current plans to implement protected river areas are still insufficient to ensure the conservation of the freshwater blenny (Cañedo-Argüelles et al., 2019).

Iberian freshwater blenny populations are in sharp decline as a result of human activity, including:

- Habitat destruction in breeding areas resulting from instream gravel extraction (Côté et al., 1999), as well as habitat degradation and loss owing to excessive water diversion for agricultural use, the construction of dams, and river regulation in lower reaches (Elvira, 1995; Elvira, 1996). Streamflow reduction, in particular, has had a markedly adverse effect on the reproduction of the freshwater blenny, causing a significant decrease in the number of nests and eggs per nest (Quirós & Vinyoles, 2016)
- Water pollution, especially from urban wastewater (Elvira, 1995; Elvira, 1996; Hernández et al., 2000)
- The introduction of non-native piscivorous species that prey on adults and nests (Elvira, 1995; Elvira, 1996; Elvira, Nicola & Almodóvar, 1996; Nicola, Almodóvar & Elvira, 1996)

Although habitat degradation and destruction is regarded as the main driver of freshwater blenny decline, very few studies have researched the habitat and ecological requirements of the freshwater blenny, either for lake- (Vila-Gispert & Moreno-Amich, 1998; Gasith & Goren, 2009) or stream-dwelling populations (Freeman et al., 1990; Hernández et al., 2000; Blanco-Garrido, Clavero & Prenda, 2009; Laporte et al., 2014). With regards to stream-dwelling populations, only Freeman et al. (1990) described the microhabitat use of the species, with the other studies conducted at the mesohabitat scale.

The quality and quantity of the available physical habitat is a main determinant of the distribution and abundance of stream-dwelling fish, and habitat dynamics are strongly dictated by the flow regime (Bunn & Arthington, 2002). Stream-dwelling fish have developed adaptations in various traits, including habitat selection behaviour, to respond to both short- and long-term patterns in flow-mediated habitat variability (Ayllón et al., 2014). In particular, stream-dwelling freshwater blenny show a high degree of morphological plasticity as a response to water velocity (Laporte et al., 2016). Likewise, Laporte et al. (2018) found larger individuals in stream-dwelling populations than in lake-dwelling populations, associated with greater sexual dimorphism in size resulting from differences in the environmental conditions experienced.

The present decline of Iberian freshwater blenny populations indicates the need to develop priority measures for the conservation and recovery of the species. For this, it is essential to obtain basic information on its habitat requirements, which are still relatively unknown. For example, there is contrasting evidence on whether changes in blenny habitat requirements take place alongside ontogeny, with some studies describing size-related habitat segregation (Gasith & Goren, 2009), whereas other studies do not report this (Freeman et al., 1990). In addition, the species displays strong sexual dimorphism in size and behaviour (Laporte et al., 2018), which might also result in habitat segregation between sexes. The main objectives of the study were: (i) to determine the factors influencing the large-scale distribution of the freshwater blenny; (ii) to characterize its habitat use and selection patterns at the microhabitat scale, accounting for sexual differences and ontogenetic changes, to determine the proximate key factors that enable its survival; and (iii) to explore the relationship between these factors and flow-mediated habitat dynamics, in order to understand the possible consequences of flow alterations on the survival of the species.

2 | METHODS

2.1 | Large-scale distribution

2.1.1 | Study area

To characterize the large-scale distribution of the freshwater blenny, 127 sites located on 16 rivers belonging to the Ebro River basin (Figure 1) were sampled. The study area was situated between latitudes 41°58′57″N and 42°41′30″N, and longitudes 1°21′31″W and 2°55′53″W. Sites were chosen to cover the existing variability of environmental and geomorphological conditions within the area. To do this, existing Geographic Information Systems (GIS) data were analysed and complemented with field exploratory surveys to characterize reaches at the mesohabitat scale, following common, standard methods (Bain & Stevenson, 1999). Sites corresponded to third- to seventh-order rivers and were located at altitudes ranging from 221 to 664 m a.s.l.

2.1.2 | Fish sampling procedures

Quantitative assessments of freshwater blenny populations were conducted in 2002 (winter–spring), 2003 (summer–autumn), 2004 (summer–autumn), and 2012 (spring–autumn) by electrofishing using a 2,200-W DC generator. After capture, fish were returned alive to the same sites. Every site was sampled once per season, on a river section varying between 100 and 150 m in length, and the sampling efforts ranged from 30 to 60 min (Blanco-Garrido, Clavero & Prenda, 2009). The winter and spring sampling campaigns were aimed at characterizing populations during the pre-reproductive period (the period of maximum activity); the summer–autumn campaigns characterized populations once the new cohort of alevins had been incorporated. A qualitative assessment of the abundance of fish species other than blenny was simultaneously performed at each site.

2.1.3 | Determinants of the large-scale distribution of freshwater blenny

Twenty-six variables were quantified to characterize the geomorphology, fish community, and ecological status of the study sites (Table 1). Geomorphological attributes (Strahler stream order, altitude, and reach slope) were calculated by means of ARCGIS 9.2 (ESRI Inc., Redlands, CA, USA) from digital data provided by the Hydrographic Confederation of the Ebro River basin (CHEBRO). Characteristics of the fish communities (number and proportion of native and non-native species) were calculated from data collected in field sampling. Twenty physicochemical, hydromorphological, and biological parameters and indices monitored by CHEBRO, in compliance with the European Water Framework Directive (Council of the European Communities, 2000), were used to characterize the ecological status of the study sites. The indices used included: river habitat index (IHF) (Pardo et al., 2002), which assesses the heterogeneity of in-channel physical elements (embeddedness and sedimentation, riffle frequency, substrate composition, velocity and depth regime, shading, heterogeneity of structural elements, and aquatic vegetation cover); the riparian forest quality index (QBR) (Munné et al., 2003), which evaluates riparian conditions (total vegetation cover, cover structure and quality, and channel alterations); and four biotic indices that assess the diversity of aquatic communities of macrophytes (macroscopic aquatic vegetation index, IVAM; Moreno, Navarro & de las Heras, 2006), diatoms (pollution sensitivity index, IPS, and biological diatom index, IBD; Coste, 1982, Coste et al., 2009), and macroinvertebrates (Iberian Biological Monitoring Working Party, IBMWP; Alba-Tercedor et al., 2002). Data

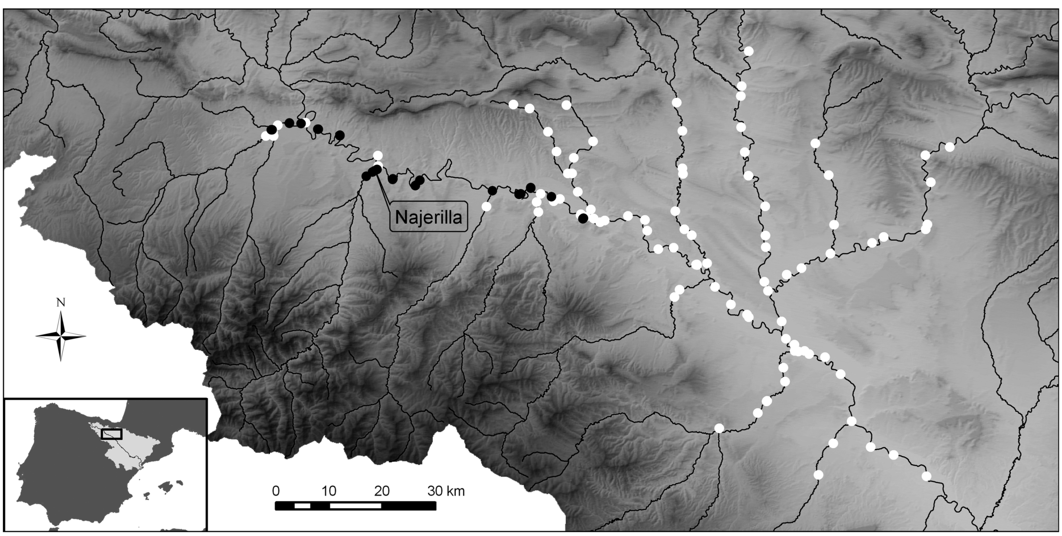

FIGURE 1 Map of the study area showing the sampling sites used to characterize the large-scale spatial distribution of the freshwater blenny. Black/white points indicate the sites where the blenny was detected/not detected, respectively. The study of microhabitat use and selection took place in the River Najerilla sampling site indicated by the label. The location of the study area within the River Ebro basin (light-grey colour) in the Iberian Peninsula is also shown

TABLE 1 Geomorphological, physicochemical, habitat, and biological variables used to characterize the study sites

Type of indicator	Variable	Description (units)
Descriptors of geomorphology		
	Altitude	Altitude (m a.s.l.)
	Stream order	Strahler stream order
	Slope	Reach slope (%)
Descriptors of the fish community		
	n total fish species	Total number of fish species in the community (#)
	n native fish species	Total number of native fish species in the community (#)
	n non-native fish species	Total number of non-native fish species in the community (#)
	% non-native fish species	Proportion of non-native fish species in the community (%)
Indicators of ecological status monitored by the Hydrographic Confederation of the Ebro River basin		
Turbidity	Suspended solids	Mass of suspended solids over water volume (mg L^{-1})
Organic pollution	Dissolved oxygen	Concentration (O_2, mg L^{-1})
	Oxygen saturation	Quantity of oxygen in the water (%)
	Chemical oxygen Demand	Mass of oxygen consumed over water volume (mg L^{-1})
	Nitrite	Concentration (NO_2^-, mg L^{-1})
	Ammonia	Concentration (NH_4^+, mg L^{-1})
Acidification	pH	pH value
Salinity	Conductivity	Conductivity at 20 °C (μS cm^{-1})
	Sulphate	Concentration (SO_4^{2-}, mg L^{-1})
	Chloride	Concentration (Cl^-, mg L^{-1})
Nutrients	Nitrate	Concentration (NO_3^-, mg L^{-1})
	Phosphate	Concentration (PO_4^{3-}, mg L^{-1})
	Total phosphorus	Concentration (P, mg L^{-1})
Habitat diversity	IHF[a]	River Habitat Index (unitless)
Riparian status	QBR[b]	Riparian Forest Quality Index (unitless)
Macrophytes	IVAM[c]	Macroscopic Aquatic Vegetation Index (unitless)
Diatoms	IPS[d]	Polluo-Sensitivity Index (unitless)
	IBD[e]	Biological Diatom Index (unitless)
Macroinvertebrates	IBMWP[f]	Iberian Biomonitoring Working Party Index (unitless)

[a]Pardo et al. (2002).
[b]Munné et al. (2003).
[c]Moreno, Navarro & de las Heras (2006).
[d]Coste (1982).
[e]Coste et al. (2009).
[f]Alba-Tercedor et al. (2002).

from the closest monitoring station to each study site were used for each year sampled (mean ± SD: distance = 3,137.5 ± 2,805.5 m; range 42–12,227 m). If the value of a given parameter was not available for the sampled year, then the closest previous value in the time series was used.

2.1.4 | Data analyses

A multivariate logistic regression model was fitted to the detected/not-detected data using the variables describing the geomorphology, fish community, and ecological status of study sites as continuous predictors. To reduce the high number of continuous predictors, a univariate analysis of each continuous variable was first performed to test for significant correlation with blenny detection at a site (and also to assess nonlinear effects). Eight variables were not significant and so were removed from subsequent analyses. The concentration of nitrates was observed to follow a nonlinear relationship, and thus a second-order term (nitrates^2) was included in the final analysis. Second, correlation analyses were performed and highly correlated variables (Pearson's $r > 0.7$) were removed to avoid multicollinearity (Dormann et al., 2013). Six predictors were removed for this reason, resulting in 12 predictors being used to fit the logistic regression model (11 predictors that were included in the best model, see Section 3, together with the IPS index).

The final model selection was performed using an information theoretic approach (IT; Burnham & Anderson, 2002). Whereas the stepwise approach leads to a final single 'best' model, the IT approach simultaneously compares a set of competing models to identify the subset of models that are equally likely to be the best model. To do this, the methods described by Ayllón et al. (2014) were followed. First, the global model including all variables was fitted and regression predictors were standardized by centring and dividing by 2 SDs using the ARM package in R (Gelman et al., 2018). Second, all possible sub-models from the global model (model set) were generated by means of the MUMIN 1.43.15 package (Barton, 2019). Akaike's information criterion adjusted for small samples (AICc) was then used to assess competing models. The top model set, encompassing the model having the lowest AICc value and all models with a ΔAICc < 2 (Burnham & Anderson, 2002), was obtained from the model set and averaged, i.e. a weighted average of parameter estimates was calculated. Model averaging means that parameter estimates from models that contribute little information about the variance in the response variable are given little weight (Grueber et al., 2011). 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 explanatory variable) of each variable included in the final averaged model was calculated. All statistical analyses were performed with R 3.6.1 (R Core Team, 2019).

2.2 | Microhabitat use and selection

2.2.1 | Study site

The study of microhabitat use and selection was conducted in the River Najerilla at Torremontalbo, La Rioja, Ebro River basin, northern Spain, 42°29′55″N 2°40′58″W (Figure 1). The River Najerilla is a medium-sized stream, a tributary of the River Ebro, that runs for 99.7 km and drains a 1,105-km^2 catchment area with a mean annual flow of 9.81 m^3 s^{-1}, with the maximum flow in March–April and the minimum flow in August–September. Its source is in the Sierra de la Demanda at 1,181 m a.s.l. and it flows into the River Ebro at 400 m a.s.l., with an average gradient of 0.78%. The river is regulated by the Mansilla dam (860 m a.s.l.). In its lower section, the main environmental impacts are those derived from water withdrawal for irrigation, water pollution from urban and agricultural uses, the dredging and channelling of the river bed, and the replacement of the natural riparian vegetation by poplar production crops (*Populus* spp.).

2.2.2 | Habitat data collection

Sampling to investigate microhabitat selection was conducted from January to May 2012. Fish were sampled by electrofishing and captured blennies were anaesthetized with tricaine metasulphonate (MS222), sexed, weighed (wet weight, g), and measured (total length, mm), and, after their recovery, returned alive to the waters where they came from. All of the work complied with current Spanish and European conservation legislation and strictly adhered to the regulations on the handling of wild animals (Directive 2010/63/EU). A qualitative assessment of the relative abundance of fish species other than blenny was performed simultaneously.

The same reach length was electrofished each month (100 m); the mean width was 18.1 ± 2.8 m (the mean sampled area was 1,800 ± 30 m^2). In the sampling period, the mean daily flow was markedly higher in March and May (5.29 and 6.52 m^3 s^{-1}, respectively) than in January, February, and April (2.92, 3.31 and 3.72 m^3 s^{-1}, respectively). In the habitat assessments, wherever a blenny was captured, the water depth (cm), current velocity (m s^{-1}), and distance to the nearest bank (m) were measured at the exact point where the fish was captured, and the proportion of aquatic vegetation (%) and the substrate composition (%) were estimated visually in a 20 × 20 cm^2 quadrat placed on the river bed. The particle size composition of the substrate was described following the classification criteria established by Platts, Megahan & Minshall (1983), according to particle diameter: silt, <0.01 cm; sand, 0.01–0.5 cm; gravel, 0.5–7.6 cm; cobble, 7.6–30.5 cm; and boulder, >30.5 cm. Based on these percentages, the substrate coarseness = (silt % • 1) + (sand % • 2) + (gravel % • 3) + (cobble % • 4) + (boulder % • 5)/5 was then calculated. This index measures the particle size and varies from 20 (100% silt) to 100 (100% boulder). To determine microhabitat availability, the same variables were measured concurrently with fish sampling at randomly selected points in the study site.

2.2.3 | Data analyses

The assumption of the normality of distributions (for both availability and use data) was verified for each habitat variable using the Shapiro–Wilk test. As most of the variables did not meet normality requirements, they were transformed, using the natural logarithm for the non-percentage variables and the arcsine of the square root of the variable for the percentage variables. However, even then not all variables met the normality requirements. Consequently, for the subsequent analyses, the original 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. Dunn's test was used to compute post-hoc comparisons of pairs of groups. The significance level was set to $\alpha = 0.05$ for all statistical analyses. A principal component analysis (PCA) on habitat variables was used to summarize spatial (across microhabitat locations) and temporal (across months) changes in microhabitat availability, pooling all availability data. For this analysis, proportions of silt and sand were aggregated in a new variable called 'fines'. Variables were standardized to a mean of 0 and a standard deviation of 1. The principal components with an eigenvalue greater than 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 January to May) of habitat used by the freshwater blenny were analysed to characterize the adjustments of habitat use to the seasonal dynamics of the microhabitat: (i) sexual differences in monthly habitat use were tested using K–W analyses; (ii) ontogenetic changes in habitat use were tested by means of Spearman's rank correlation analyses between the values of microhabitat variables and the total length of captured individuals throughout the monthly samples; and (iii) K–W tests were conducted to compare the use of each habitat variable across months. Holm's sequential Bonferroni adjustment (Holm, 1979) was applied to determine significance for the K–W tests.

Two different approaches were used to analyse habitat selection (i.e. the use of a habitat in greater or lower proportion of its availability in the environment; Rosenfeld, 2003): (i) K–W tests were used to compare the availability and use of each habitat variable by freshwater blenny; (ii) a multivariate logistic regression model was fitted to describe the relationship between habitat availability and the relative probability of habitat use, following the procedures described in Section 2.1.4. Here, the binary dependent variable indicated whether a location was used by the freshwater blenny or was measured to characterize habitat availability. Thus, the logistic model provides the probability that a location was used by the blenny as a function of its microhabitat characteristics. In this case, month was included as a random factor to induce a temporal autocorrelation structure in the data, so a generalized linear mixed-effects model was fitted using the lme4 1.1–23 package (Bates et al., 2020) to estimate the global model. Statistical analyses were performed with R 3.6.1 (R Core Team, 2019) and STATISTICA 13.5 (https://docs.tibco.com/products/tibco-statistica-13-5-0).

3 | RESULTS

3.1 | Large-scale distribution

The freshwater blenny was detected in 25 out of the 127 study sites (19.7%). The probability that the freshwater blenny was detected in a reach within the study area increased with river order, oxygen saturation, and number of native species in the fish community, and with the IHF, QBR and IVAM habitat and biological indices, and it decreased with the concentration of chlorides, nitrites, and phosphates. Meanwhile, it had a nonlinear relationship with the concentration of nitrates (over a certain threshold, increasing the concentration of nitrates had a negative effect) (Tables 2 and S1). The relative importance metric and the value of the standardized coefficients indicated that the effect of biological variables (native fish species, QBR, and IVAM) was less influential than geomorphological

TABLE 2 Summary of the multivariate logistic regression model that predicts the probability of detection of freshwater blenny

Variables	Coefficients	Importance	Detected	Not detected
(Random intercept)	−7.71 ± 2.68	1.00	–	–
Chloride	−15.43 ± 6.94	1.00	34.03 ± 17.36	130.21 ± 90.23
IHF index	3.07 ± 1.54	1.00	78.50 ± 1.70	63.93 ± 7.69
Nitrate	5.95 ± 4.91	1.00	8.69 ± 5.12	12.54 ± 8.79
Nitrate^2	−2.31 ± 3.67	0.80	–	–
Stream order	5.14 ± 1.98	1.00	5.86 ± 0.58	5.05 ± 1.11
Oxygen saturation	2.53 ± 1.4	1.00	97.39 ± 9.74	82.05 ± 7.67
Nitrite	−2.18 ± 2.05	0.84	0.04 ± 0.02	0.08 ± 0.05
QBR index	1.1 ± 1.09	0.68	59.60 ± 34.27	36.30 ± 25.58
Phosphates	−2.37 ± 3.06	0.55	0.12 ± 0.06	0.20 ± 0.13
IVAM index	1.14 ± 1.85	0.44	4.64 ± 0.38	3.62 ± 0.88
Native fish species	0.73 ± 1.09	0.44	4.44 ± 1.36	3.03 ± 1.59
IBMWP index	−0.28 ± 0.88	0.16	108.22 ± 26.73	91.15 ± 30.58

Note: Model-averaged coefficient estimates (± SDs) and the 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. Mean values (± SDs) of each predictor variable for sites where freshwater blenny was detected versus not detected are also shown. Nitrates^2 is a second-order term included to represent the non-linear relationship between nitrates and the probability of blenny detection.

and physicochemical variables (Table 2). The IBMWP and IPS biological indices had no or very little influence on the probability of blenny detection (Tables 2 and S1).

3.2 | Microhabitat use and selection

3.2.1 | Fish community composition

The freshwater blenny *S. fluviatilis*, the Ebro barbel *Luciobarbus graellsii*, the Pyrenean minnow *Phoxinus bigerri*, and the Pyrenean stone loach *Barbatula quignardi* were the dominant species throughout the microhabitat study site in the River Najerilla at Torremontalbo. Other species that occurred in low numbers include the brown trout *Salmo trutta*, the Ebro nase *Parachondrostoma miegii*, and the Northern Iberian spined-loach *Cobitis calderoni*, together with two non-native species: the Pyrenean gudgeon *Gobio lozanoi* and pumpkinseed *Lepomis gibbosus*.

3.2.2 | Seasonal variation in microhabitat availability

The depth availability in the River Najerilla varied between 12 and 87 cm (mean depth of sampled quadrats = 36.46 cm, standard deviation = 13.70 cm), the water velocity availability varied between 0 and 2.8 m s^{-1} (mean velocity of sampled quadrats = 1.24 m s^{-1}, standard deviation = 0.58 m s^{-1}), and the distance to the nearest bank varied between 0.3 and 11.2 m (mean distance of sampled quadrats = 4.75 m, standard deviation = 2.69 m) (Figure S1; Table 3). The availability of aquatic vegetation in the sampled quadrats was low (mean = 3.24% of surface area, standard deviation = 12.79%), and the substrate composition in the sampled quadrats was dominated by gravel (mean = 48.84% of surface area, standard deviation = 31.20%) and cobble (mean = 37.12% of surface area, standard deviation = 28.42%) (Figure S2; Table 3). The availability of the other substrate types was low, with boulders (mean = 8.38%) predominating over fines (mean below 3.5%).

Most microhabitat variables analysed showed significant seasonal differences (Table 3). The mean water depth increased in the months with higher flow, i.e. March and May. The average current velocity was significantly lower in January and April (Dunn's test, $P < 0.05$). The availability of aquatic vegetation significantly increased in February and to a lesser extent in March. As for the substrate composition, all variables except the availability of boulders showed significant monthly variations. In general, substrate composition was similar across months, with a similar mean daily flow, but differed across flow groups (March and May versus January, February and April).

The PCA revealed three main factors accounting for 63.5% of the spatiotemporal variance in available microhabitat (Table 4). The first factor (PC1) characterized locations close to the river bank, with shallow depth and fine substrate. The second factor (PC2) was

TABLE 3 Characteristics of available habitat (mean ± standard error, range in brackets) from January to May 2012 in the River Najerilla study site

	January (n = 50)	February (n = 40)	March (n = 50)	April (n = 60)	May (n = 50)	Kruskal–Wallis test
Depth (cm)	35.3 ± 1.80 (19–72)	35.1 ± 2.63 (12–87)	39.5 ± 1.86 (17–76)	33.6 ± 1.93 (12–79)	39.1 ± 1.40 (18–65)	H = 10.68 ns
Velocity (m s^{-1})	0.9 ± 0.06 (0–1.7)	1.6 ± 0.09 (0.4–2.5)	1.3 ± 0.06 (0.5–2.3)	1.1 ± 0.08 (0–2.6)	1.5 ± 0.08 (0.3–2.8)	H = 44.46*
Distance to the nearest bank (m)	4.4 ± 0.41 (0.4–10.1)	4.2 ± 0.27 (1.7–7.1)	5.4 ± 0.37 (0.6–10)	4.6 ± 0.38 (0.3–11.2)	5.0 ± 0.39 (0.4–10.8)	H = 5.28 ns
Aquatic vegetation (%)	0	17.2 ± 4.30 (0–80)	2.4 ± 0.98 (0–40)	0	0	H = 72.39*
Silt (%)	10.0 ± 3.97 (0–100)	0	0	5.8 ± 2.22 (0–80)	0	H = 19.13*
Sand (%)	4.2 ± 1.89 (0–70)	5.4 ± 1.80 (0–60)	0	0	2.8 ± 1.74 (0–85)	H = 29.49*
Gravel (%)	46.6 ± 5.11 (0–100)	65.8 ± 4.17 (20–100)	39.9 ± 3.41 (0–90)	57.2 ± 4.08 (0–100)	36.5 ± 3.85 (0–90)	H = 28.80*
Cobble (%)	30.6 ± 3.98 (0–85)	27.4 ± 4.21 (0–80)	49.8 ± 3.73 (0–100)	31.0 ± 2.95 (0–80)	46.1 ± 4.50 (0–95)	H = 24.50*
Boulder (%)	8.6 ± 3.28 (0–90)	1.5 ± 1.50 (0–60)	10.3 ± 3.47 (0–90)	6.0 ± 1.68 (0–50)	14.6 ± 4.33 (0–90)	H = 6.77 ns
Substrate coarseness	64.7 ± 2.38 (20–98)	65.0 ± 1.18 (48–88)	74.1 ± 1.16 (62–96)	66.3 ± 1.22 (32–90)	74.5 ± 1.55 (43–98)	H = 46.31*

Note: 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 4 Factor loadings for the first three principal components from the PCA on all monthly microhabitat availability samples in the River Najerilla study site

Variables	PC1	PC2	PC3
Depth	−0.47	**0.65**	0.29
Velocity	0.28	**0.84**	0.01
Distance to the nearest bank	**0.73**	−0.34	0.12
Aquatic vegetation	0.03	**0.56**	−0.10
Fines	**−0.72**	−0.35	−0.08
Gravel	0.44	0.17	**−0.84**
Cobble	0.25	0.09	**0.91**
Boulder	−0.37	−0.07	0.09
Variance explained (%)	21.6	21.4	20.5

Note: Loadings in bold indicate correlations greater than 0.5.

positively correlated with water depth and velocity, and with aquatic vegetation. The third factor (PC3) discriminated between locations with substrate dominated by cobble (positively correlated) or by gravel (negatively correlated). PC1 values marginally differed across months ($F_{4,245} = 2.3$, $P = 0.06$): PC1 values were highest in March, intermediate in February, April, and May, and lowest in January (Tukey's test, $P < 0.05$). There were significant differences in PC2 across months ($F_{4,245} = 12.8$, $P < 0.001$), so that PC2 values were significantly higher in March and May than in the other months (Tukey's test, $P < 0.05$). Finally, there were also significant differences in PC3 across months ($F_{4,245} = 12.1$, $P < 0.001$): PC3 values were highest in February, intermediate in March and May, and lowest in January and April (Tukey's test, $P < 0.05$).

3.2.3 | Sexual-, size-, and seasonal-related variation in microhabitat use

Habitat use data were recorded for 242 freshwater blenny individuals (145 females, 84 males, and 13 undetermined juveniles; range 40–120 mm in total length) (Figure S3). The average total length of individuals did not show significant monthly differences (mean 8.5 ± 1.5 cm; K–W, $H = 0.73$, $P = 0.95$).

Habitat use did not differ between males and females in any sampled month (Table S2). No ontogenetic changes in habitat use were detected either. The Spearman's rank correlation coefficients between the values of the microhabitat variables and the total length of the individuals were not significant ($P > 0.05$) throughout the monthly samples.

Microhabitat used by the freshwater blenny differed significantly across months. There were significant monthly differences in all habitat variables except for the proportion of silt and boulder substrate types (Figures S4–S9; Table 5). The average depth of the locations used by the freshwater blenny was significantly lower in January and significantly higher in March and April (Dunn's test, $P < 0.001$), compared with the average value of 40.4 cm (Figure 2). The freshwater blenny occupied locations with very high current velocity (1.48 ± 0.50 m s^{-1}). The average current velocity of the locations used in April was significantly lower than those used in the other months (Dunn's test, $P < 0.01$), which showed no significant differences among them, with an average value of 1.6 m s^{-1} (Figure 2).

The average distance to the nearest bank where the freshwater blenny were captured differed across months (Table 5), with the distances observed in February and May being significantly shorter and longer, respectively, than those observed in the other months (Dunn's test, $P < 0.05$) (Figure 2). The aquatic vegetation was used by the freshwater blenny whenever it was available in the reach, i.e. in February and March (Table 5).

Regarding substrate composition, blennies occupied locations comprised mostly of gravel and cobble (Table 5). There was a significant decrease in the use of gravel areas as the sampling period progressed, which matched a gradual and significant increase in the use of areas dominated by cobble substrate (Figure 2). Locations with silt, sand, and boulders were rarely used by blennies, and this pattern did not change over the sampling period (Table 5). In accordance with these patterns, the substrate coarseness index of the occupied locations showed intermediate values that increased over the sampling period. Within the range of the very high current velocities used by the blenny, the locations with the lowest velocities were in general linked to the coarsest substrates, and this was consistent over time (with Spearman's rho ranging between −0.4 and −0.7, $P < 0.05$), except for May, when velocity was independent of the substrate coarseness index in the occupied locations.

3.2.4 | Microhabitat selection

Univariate analyses indicated that the freshwater blenny showed differential use, relative to availability in the reach, of only certain habitat features and only in certain months. In general, the dynamics of habitat use followed the dynamics of habitat availability. Freshwater blennies only showed a differential use of microhabitats regarding depth in April, when they positively selected deeper

TABLE 5 Characteristics of habitat used by the freshwater blenny (mean ± standard error, range in brackets) from January to May 2012 in the River Najerilla study site

	January (n = 47)	February (n = 38)	March (n = 50)	April (n = 58)	May (n = 49)	Kruskal–Wallis test
Depth (cm)	33.6 ± 0.94 (19–49)	38.1 ± 1.53 (20–55)	43.3 ± 1.43 (20–59)	41.4 ± 1.24 (26–66)	38.1 ± 1.12 (23–61)	H = 31.25*
Velocity (m s^{-1})	1.5 ± 0.04 (0.9–2.4)	1.8 ± 0.09 (0.8–3.6)	1.5 ± 0.07 (0.2–2.4)	1.2 ± 0.06 (0.1–2.5)	1.6 ± 0.07 (0.5–2.6)	H = 34.88*
Distance to the nearest bank (m)	4.6 ± 0.24 (2.0–8.4)	3.1 ± 0.24 (0.5–7.9)	4.8 ± 0.35 (0.5–10.5)	4.8 ± 0.34 (0.7–11.1)	6.5 ± 0.32 (1.1–11.2)	H = 43.65*
Aquatic vegetation (%)	0	23.6 ± 4.58 (0–80)	5.9 ± 1.57 (0–40)	0	0	H = 81.91*
Silt (%)	0	0	0	0.5 ± 0.52 (0–30)	0	H = 3.17 ns
Sand (%)	1.4 ± 0.47 (0–15)	1.1 ± 0.50 (0–10)	0	0	1.8 ± 0.63 (0–20)	H = 21.40*
Gravel (%)	66.0 ± 4.18 (0–100)	55.9 ± 4.19 (0–90)	50.1 ± 4.24 (0–100)	45.0 ± 3.68 (0–100)	28.0 ± 2.57 (0–85)	H = 44.18*
Cobble (%)	28.4 ± 3.95 (0–90)	37.2 ± 3.76 (0–80)	36.5 ± 3.45 (0–85)	47.8 ± 3.52 (0–90)	56.1 ± 3.64 (0–100)	H = 31.01*
Boulder (%)	4.3 ± 2.33 (0–80)	5.8 ± 3.01 (0–80)	13.4 ± 3.51 (0–70)	6.7 ± 2.39 (0–80)	14.3 ± 3.57 (0–80)	H = 8.68 ns
Substrate coarseness	67.1 ± 1.09 (60–96)	69.6 ± 1.29 (58–92)	72.7 ± 1.39 (60–94)	72.0 ± 0.98 (60–94)	76.7 ± 1.06 (62–94)	H = 41.58*

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

locations (Figure 2; Table 6). There was a positive selection of locations with higher velocities than average available locations in January and March (Figure 2; Table 6). Blennies significantly selected locations near the bank in February but selected locations closer to the centre of the channel in May (Figure 2; Table 6). Whenever aquatic vegetation was available in the reach (in February and March) it was used by blennies, but in a way that was proportional to its availability (Table 6). Blennies used the substrates most available in the reach and only showed a positive differential use of gravel in January and of cobble in April (Table 6).

The multivariate logistic regression model indicated that freshwater blennies selected positions with high current velocity linked to cobble, preferably in deep habitats far from the bank, and avoided fine substrates (Tables 7 and S3).

4 | DISCUSSION

4.1 | Determinants of large-scale distribution patterns

This is the first study describing the factors driving the distribution of the endangered freshwater blenny at such a large spatial scale. Ten geomorphological, physicochemical, and biological characteristics were identified as the most relevant predictors of freshwater blenny presence or absence in the study area within the Ebro River basin. First, the stream order operates as a landscape filter, as it summarizes a wide range of large-scale features and upstream–downstream gradients (e.g. altitude, catchment size, stream size, water volume, etc.) affecting the structure and functioning of aquatic ecosystems. The freshwater blenny was not present in low-order upstream reaches and typically inhabited only the intermediate and lower reaches of medium-sized rivers, where the fish community was also more diverse. This pattern is in accordance with the known ecology of the species in running water (Elvira, 1995). In Iberian basins, the freshwater blenny has been typically found in downstream reaches of tributaries or in the main river channel, in reaches characterized by high water flow, fast currents, and wide channels (Godinho, Ferreira & Cortes, 1997; Hernández et al., 2000; Blanco-Garrido, Clavero & Prenda, 2009). However, blenny populations living in flowing waters near the sea may have different behaviour and different habitat preferences (Laporte et al., 2014).

Second, the logistic regression model indicated that water quality is a critical factor for the presence of the freshwater blenny. The species was only present in sites with very low levels of organic pollution (with low values of nitrites and high values of oxygen saturation), without eutrophication (as indicated by its absence in sites with an excessive concentration of phosphates and nitrates), and low salinity. This pattern agrees with the studies of Hernández et al. (2000), Laporte et al. (2011) and Laporte et al. (2014), who reported that water pollution (faecal and organic contamination) limited the distribution of the blenny in the Júcar River basin, eastern Spain, and in 10 Corsican rivers, respectively. Similar to the present

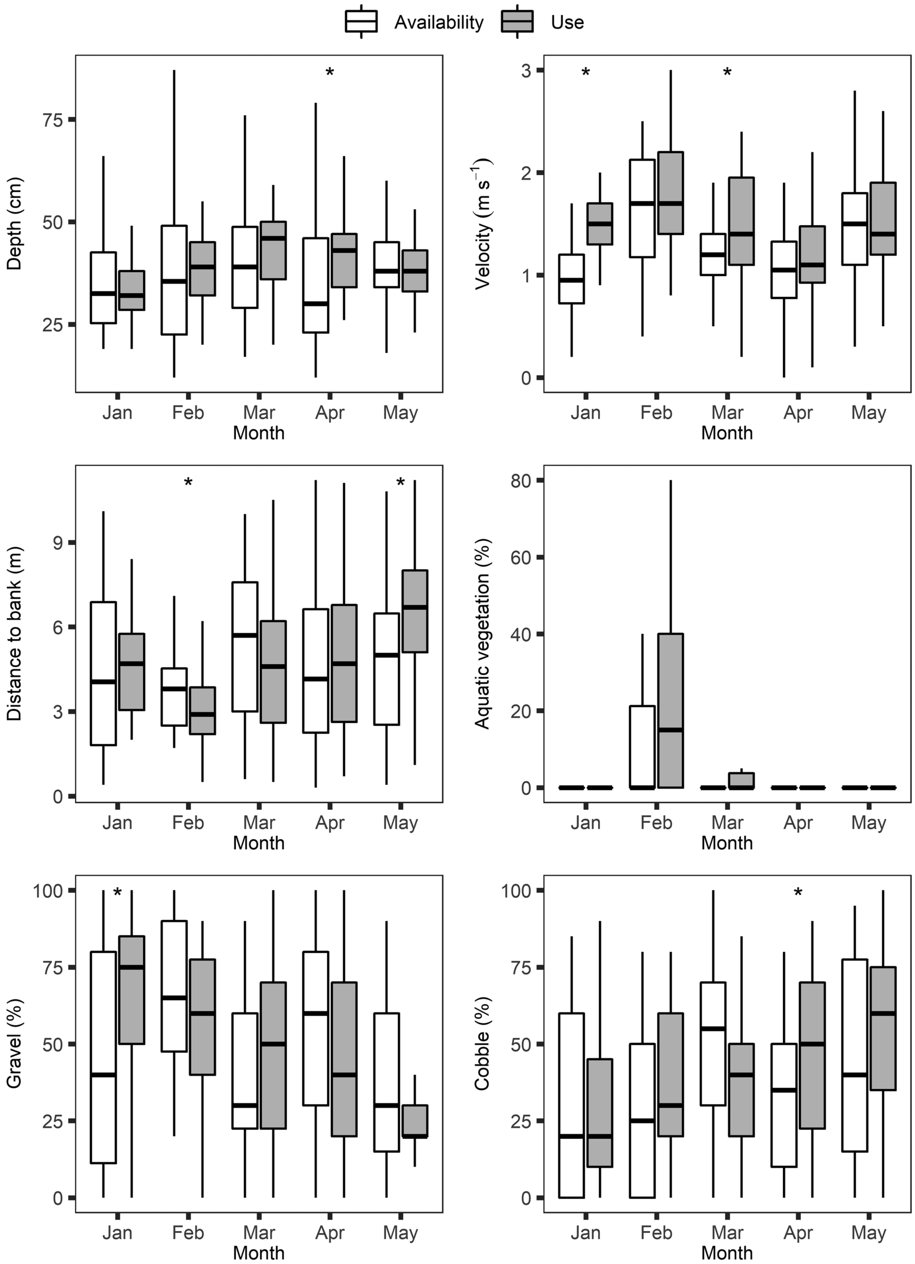

FIGURE 2 Monthly availability and freshwater blenny use of depth, current velocity, distance to the nearest bank, and proportion of aquatic vegetation, gravel, and cobble in the River Najerilla study site from January to May 2012. Graphics show the median value and the first and third quartiles, which correspond to the lower and upper hinges of the box; the whiskers extend from the hinges 1.5 times the interquartile range. Significant differences estimated through the Kruskal–Wallis test are also indicated after the *P* value was adjusted using Holm's sequential Bonferroni correction (*$P \le 0.05$)

findings, the freshwater blenny occurred more frequently in reaches with high levels of dissolved oxygen and with high pH levels in the Guadiana River basin (Blanco-Garrido, Clavero & Prenda, 2009).

Third, the presence of the species was linked to high values of the IHF index, which evaluates instream habitat heterogeneity based on several physical variables of the stream channel. This includes variables such as the frequency of riffles, the diversity of the water velocity and depth regimes, substrate heterogeneity, sedimentation, the presence and diversity of structural elements (e.g. woody material or tree roots in the banks) and aquatic vegetation cover, and shading. Godinho, Ferreira & Cortes (1997) showed that the presence of the freshwater blenny in the lower Guadiana River basin, southern Iberian Peninsula, was highly linked to reaches with fast-flowing water and high substrate heterogeneity. The probability of finding the species in a river reach of the study system also increased with the value of the QBR index, which assesses the total riparian cover, its structure and quality, and the naturalness of the channel morphology. Therefore, the freshwater blenny was absent in sites with highly degraded instream or riparian habitats. Previous studies, however, reported only a weak positive relationship (Blanco-Garrido, Clavero & Prenda, 2009) or no relationship at all (Godinho, Ferreira & Cortes, 1997) between the presence of freshwater blenny and riparian cover.

The presence of the freshwater blenny was also linked to high values of biological indicators, such as the richness of native fish species or the IVAM index, which measures the richness and diversity of the aquatic macrophyte community. High values of the IVAM index are typically correlated with high invertebrate richness and low levels of organic pollution and eutrophication. The presence of non-native species did not seem to exert an evident influence on the distribution of blenny, as suggested by previous studies (García-Berthou &

TABLE 6 Habitat selection by the freshwater blenny from January to May 2012 in the River Najerilla study site

	January ($n = 97$)	February ($n = 78$)	March ($n = 100$)	April ($n = 118$)	May ($n = 99$)
Depth	0.02 ns	0.88 ns	4.02 ns	**12.92*** (+)	0.25 ns
Velocity	**41.69*** (+)	1.26 ns	**6.84*** (+)	1.89 ns	0.69 ns
Distance to the nearest bank	0.31 ns	**9.08*** (+)	1.37 ns	0.36 ns	**8.50*** (+)
Aquatic vegetation	–	1.20 ns.	1.59 ns	–	–
Silt	**7.01*** (−)	–	–	4.73 ns	–
Sand	0.08 ns	4.33 ns	–	–	0.59 ns
Gravel	**6.81*** (+)	2.59 ns	2.85 ns	5.26 ns	0.48 ns
Cobble	0.03 ns	3.57 ns	5.46 ns	**12.06*** (+)	2.05 ns
Boulder	1.78 ns	2.06 ns	0.69 ns	0.24 ns	0.16 ns
Substrate coarseness	0.02 ns	5.83 ns	1.61 ns	**13.63*** (+)	1.83 ns

Note: 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, $^*P \leq 0.05$). When significant (marked in bold), (+) indicates preference, whereas (−) indicates avoidance.

TABLE 7 Summary of the multivariate logistic regression model that predicts the probability of freshwater blenny habitat use

Variables	Coefficients	Importance
(Random intercept)	0.09 ± 0.04	1.00
Velocity	0.89 ± 0.21	1.00
Sand	−0.48 ± 0.32	0.93
Cobble	0.22 ± 0.19	0.59
Depth	0.19 ± 0.10	0.37
Distance to the nearest bank	0.18 ± 0.09	0.35
Gravel	−0.02 ± 0.09	0.08
Aquatic vegetation	−0.01 ± 0.07	0.07

Note: Model-averaged coefficient estimates (± SDs) 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.

Moreno-Amich, 2000; Hernández et al., 2000; Blanco-Garrido, Clavero & Prenda, 2009; Laporte et al., 2014).

4.2 | Sexual- and size-related variation in microhabitat use

No significant differences were found in microhabitat use between males and females in the River Najerilla throughout the months studied. Freeman et al. (1990) also found that males and females occupied almost indistinguishable habitats in a population of freshwater blenny from the River Matarraña (Ebro River basin), even when males were defending their territories during the breeding season. Sexual differences in habitat occupation were also not detected in lake populations (Gasith & Goren, 2009). The year-round territoriality is typical of many species of Blenniidae, probably to ensure that shelter and food resources are used efficiently (Gonçalves & Faria, 2009), but differences in habitat use between sexes are unknown (Gonçalves & Faria, 2009).

No ontogenetic changes in habitat use were detected for the size range analysed (40–120 mm in total length) in the River Najerilla. However, only 41 of the 242 individuals in the sample were smaller than 70 mm in total length. The apparent lack of ontogenetic changes in microhabitat use was also documented by Freeman et al. (1990), but the size range analysed was not reported in that study. The authors suggested that this result could be linked to the structural characteristics of the reaches analysed. They argued that if individuals of different sizes occupy similar microhabitats, the available habitat should not be a limiting resource, whereas if habitat conditions were limiting, smaller individuals would occupy less favourable areas because they are less competitive. Alternatively, it might be possible that unoccupied habitats offered too few opportunities for growth and survival. This may also explain the vulnerability of the species to alteration of the substrate. In addition, Freeman et al. (1990) suggested that individual variability in habitat preferences may also contribute to the absence of significant variation in microhabitat use between different sizes of freshwater blenny, as has been observed in other fish species.

In contrast, Gasith & Goren (2009) found that the freshwater blenny shows size-related habitat segregation in Lake Kinneret, Israel. In their study, smaller individuals were more abundant in river beds dominated by small cobbles, whereas larger individuals were mostly found in habitats with large boulders. This habitat segregation could reduce cannibalism (Gasith & Goren, 2009). However, blennies from lake populations demonstrate different ecology, shape, and size to stream-dwelling individuals, as well as a distinct level of sex differentiation (Laporte et al., 2018). Furthermore, the size range analysed in Lake Kinneret (28–96 mm in total length) was slightly lower than in the River Najerilla, but the main difference was that most individuals (174 of the 197 analysed individuals) were smaller than 70 mm in total length. This suggests that the main ontogenetic shifts in microhabitat use may occur early in life, during the first year of the benthic stage of the freshwater blenny.

4.3 | Seasonal microhabitat use and selection patterns

The characteristics of the physical habitat used by the freshwater blenny in the River Najerilla from January to May are like those reported in winter and spring by Freeman et al. (1990) in the River Matarraña using underwater observation techniques. This was to be expected, as both sites are in the Ebro River basin in north-eastern Spain, in rivers with relatively similar hydrological conditions. However, the microhabitat use of the freshwater blenny from the River Najerilla changed over the sampling period, adapting to the flow-driven dynamics of the available habitat. These observations indicate a close relationship between microhabitat availability and blenny use, as documented in previous studies (Freeman et al., 1990). Thus, habitat availability dynamics seemed to drive the temporal pattern of habitat use. Blenny showed a seasonal tendency (from January to May) to use locations further away from the bank, as well as to occupy locations with coarser substrates, with less gravel, and with more cobble. In addition, blennies used habitats with aquatic vegetation whenever they were available in the river.

There was also significant evidence that the study population made selective use of certain microhabitat features during the period analysed. In the study system, blennies selected deep habitats with very high current velocities linked to cobble, while avoiding fine substrates. However, this general pattern changed over the sampling period. Whereas the preferential use of habitats dominated by gravels and close to the river bank was observed in the winter months (January–March), the selection of habitats with cobble substrate further away from the river bank occurred during the spring months (April and May).

The instream distribution of the freshwater blenny seems to be related to the physical characteristics of the river systems that they occupy. In the River Najerilla, the species selected areas of very high velocity in all months sampled, as captured individuals always occupied locations with a current velocity greater than 1.2 m s^{-1}. Laporte et al. (2014) also found a consistent relationship between high current velocity and abundance of freshwater blenny in the streams of Corsica, France; however, the current velocities selected ranged between 0.5 and 1.0 m s^{-1}.

In the River Najerilla, the species occupied refuges determined by an interaction of structural (cobble/gravel ratio) and hydraulic (water velocity) parameters. The importance of these areas was highlighted by Freeman et al. (1990) and Côté et al. (1999), who observed that the freshwater blenny was abundant in microhabitats with high water velocity and substrate composed mainly of cobble and gravel. However, Freeman et al. (1990) observed that water depth and velocity were the most important variables influencing microhabitat use, whereas substrate composition affected microhabitat use less consistently. These authors related their results to the intra-annual flow dynamics of the River Matarraña, which is characterized by very sharp fluctuations throughout the year. Therefore, blennies select areas with sufficient depth and suitable substrates for shelter and nesting to endure the periods of low flow. However, in the River Najerilla the flow rate, and therefore the water depth, does not diminish to levels that are limiting for the freshwater blenny; nevertheless, the freshwater blenny in the River Najerilla also showed a preference for deep areas. In contrast, Alp & Kara (2007) reported that species abundance was greater in shallow waters with stony bottoms in the Ceyhan River basin, Turkey. Hence, the relevance of water depth for the survival of the species seems to be more context dependent than the availability of habitats with high velocities and medium-sized substrates, especially cobble.

The observations on the higher use of coarser substrate in May matched the start of the breeding season, which lasts from the end of May to the end of July (Vinyoles, Côté & de Sostoa, 2002). During this period, the availability of large stones in the river bed is a key habitat requirement for the freshwater blenny, as they are used for nest construction by males (Freeman et al., 1990; Côté et al., 1999). Females preferentially spawn under larger stones (Freeman et al., 1990), so large stones tend to have larger clutches (Côté et al., 1999).

4.4 | Implications for river restoration and management

4.4.1 | Threats to freshwater blenny habitat

The conservation problems of the freshwater blenny in the Ebro River basin are common to other small benthic fish species living in streams. Furthermore, the occurrence of this fish is closely linked to reaches with highly heterogeneous hydraulic and structural conditions (as shown by its relationship with the IHF index) that generate microhabitats with very specific characteristics, as has been shown in this and other previous studies (Freeman et al., 1990; Côté et al., 1999). Strong physical alterations of the benthic aquatic environment resulting in the loss of critical microhabitats for the blenny can limit the viability of their populations to the point of extirpation, as shown by this study of the large-scale spatial distribution of the species.

The main threat to blenny populations is the destruction of nesting sites by the extraction of gravel from rivers. Côté et al. (1999) observed that freshwater blenny populations from different Spanish basins show similar habitat preferences and that habitat alteration by gravel extraction substantially reduces their survival. Thus, the reduction in the availability of intermediate-sized substrates resulting from the extraction of gravel from the river bed leads to a significant reduction in the density of nests (Côté et al., 1999). The extraction of cobble and gravel from watercourses is therefore a serious threat, as this type of substrate is a critical requirement for blenny reproduction. Activities that involve the compression or clogging of the substrate, or the sedimentation of suspended solids that can saturate the nooks and crannies that serve as a refuge for the freshwater blenny, are potentially a threat to the survival of this species. This was demonstrated by the absence of the species in the study sites with poor habitat heterogeneity, unsuitable substrate composition, high levels of sedimentation, and lack of structural elements.

The aggregate distribution of freshwater blenny observed in this study is an additional risk factor, as it limits the resilience of populations after a mortality event, especially if fragmentation is associated with a low population density. The strict requirements of the species, especially regarding substrate composition and heterogeneity (with both gravel and cobble being important at different times of the year) together with water velocity, lead to population nuclei distributed far from each other, sometimes with additional intercommunication problems caused by the human alteration or fragmentation of rivers. Although the freshwater blenny shows high plasticity in the degree of sexual dimorphism, reproductive tactics, or body shape (Fabre et al., 2014; Laporte et al., 2016; Laporte et al., 2018), which facilitates acclimation to new environmental conditions, the species appears to be very sensitive to the alteration and destruction of its most preferred habitats.

The excessive withdrawal of water for irrigation is another factor that leads to the loss of habitat for the freshwater blenny (Vinyoles & de Sostoa, 2007). In addition, this study has shown that good water quality status is a critical requirement for blenny populations to persist. Water quality degradation because of point- or diffuse-source contaminants can threaten the persistence of populations and thus the conservation of the species (Hernández et al., 2000; Laporte et al., 2011; Laporte et al., 2014).

4.4.2 | Freshwater blenny conservation in practice

The results for habitat use and selection by the freshwater blenny as well as the large-scale analysis of human impacts on populations presented here were reported to the local competent authority: the Regional Ministry for the Environment of La Rioja, Spain. The recovery plan for the freshwater blenny, which was adopted in 2014 and has been implemented since 2015, accounted for most of our proposed guidelines for population management and the recovery of river habitat for the conservation of the species. In particular, the recovery plan for freshwater blenny in La Rioja legally warrants habitat conservation in watercourses and river banks, as well as the preservation of good water quality and the establishment of minimum flows to safeguard required habitats. For example, the Regional Ministry for the Environment must report on any actions, plans, or projects that would result in the modification of the habitat used by blenny for reproduction or refuge.

The recovery plan also states that future actions must be compatible with the conservation of blenny populations and, if necessary, must implement the required measures to minimize disturbance and ensure that habitat conditions are maintained or restored to conditions favourable to the species. Furthermore, particular attention must be paid to actions causing disturbances to which the blenny is particularly sensitive, including: (i) fragmentation of the habitat by weirs, dams, and other barriers that limit the movement of this species, which, owing to its small size and epibenthic habits, displays a low capacity for dispersal; (ii) modifications of the river bed by dredging, channelling, the construction of breakwaters, or any other action that simplifies the river bed or its banks and causes the loss of the substrates used for nesting; (iii) alteration of the structure of the natural vegetation of the bank and the river bed by cutting; (iv) degradation of water quality caused by organic pollution from wastewater discharge, and alteration of the natural flow regime by water withdrawal for irrigation, hydroelectric exploitation, and other uses; and (v) gravel extraction, as this is considered incompatible with the conservation of the freshwater blenny insofar as it implies a significant alteration of the species' habitat.

The implementation of the recovery plan has made it possible to maintain the few extant stream-dwelling populations of the freshwater blenny in La Rioja, as well as enabling the recovery of a previously extinct population in one tributary of the River Ebro by means of habitat improvement and restocking. This is a successful example of the application of scientific research to the effective conservation and management of a threatened species (Boon & Baxter, 2020). However, additional conservation management strategies are likely to be needed to ensure population stability and the persistence of this fish, which is still endangered. In addition, we recommend that recovery programmes, regardless of species or location, use long-term monitoring to assess their efficacy and to ensure the full recovery of threatened fish populations.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest associated with this work.

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