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Habitat use of an endangered endemic freshwater fish from Southern Iberia: The Malaga chub *Squalius malacitanus* in Genal River (Guadiaro basin)

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SUMMARY

The Malaga chub *Squalius malacitanus* is an endangered endemic freshwater fish inhabiting a few small basins around the Strait of Gibraltar, Southern Iberia. This work describes the habitat use displayed by the species in Genal River (Guadiaro basin), as well as its population structure along the river and seasons. The fish community was exclusively formed by native species, although this characteristic could change due to the spreading of invasive species previously established in the basin and/ or the arrival of new invaders. Our results suggest that the Malaga chub is a habitat generalist, since it occupied the different types of the habitats within the Genal River in proportion to their availability. However, chub abundance tended to decrease in reaches with higher current velocities. Also, the species' abundance increased in deeper zones, usually with fine substrate, and in the middle reaches of the stream, where the habitat stability was maximum. The abundance decreased in the lower reaches, following the reduction of habitat stability. This pattern was consistent with the size structure displayed along the river and seasons. The scarcity or even the absence of many size classes out of the zones with higher habitat stability was frequent, while these reaches were characterized by a better representation of all size classes. The Genal River seems to be an ideal stream to study the ecology of this unique fish community composed exclusively by native species, a rare fact in the Iberian Mediterranean basins

Keywords: *Squalius malacitanus*, habitat use, habitat stability, size structure

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INTRODUCTION

The Malaga chub *Squalius malacitanus* (Doadrio & Carmona, 2006), Fam. Cyprinidae, is an Iberian endemic freshwater fish inhabiting a few small basins around the Strait of Gibraltar, Southern Iberia (Perea et al., 2016; Figure 1). The species occurs at both sides of the Strait, occupying drainages flowing to the Atlantic Ocean (Barbate, Valle, Jara, Vega and Miel) and the Mediterranean Sea (Guadiaro, Guadalmina and Guadaiza). Chub populations from these basins have traditionally been identified as Iberian chub, *Squalius pyrenaicus* (Günther, 1868) (e. g. Clavero et al., 2005), but Doadrio & Carmona (2006) described them as a new species based on genetic and morphological characters. Furthermore, phylogenetic, geographic and population structure analyses revealed two lineages within the species, the Atlantic and the Mediterranean lineages (Perea et al., 2016).

The separation of *S. pyrenaicus* and *S. malacitanus* is estimated to be very old, dating from the Upper Miocene (around 11.6 Ma), and related to the rising of the Betic Mountains (Perea et al., 2016). The separation of the Atlantic and Mediterranean lineages within Malaga chub is also old (around 4.9 Ma), originating from the formation of the present-day river basins after the opening of the Strait of Gibraltar (Perea et al., 2016).

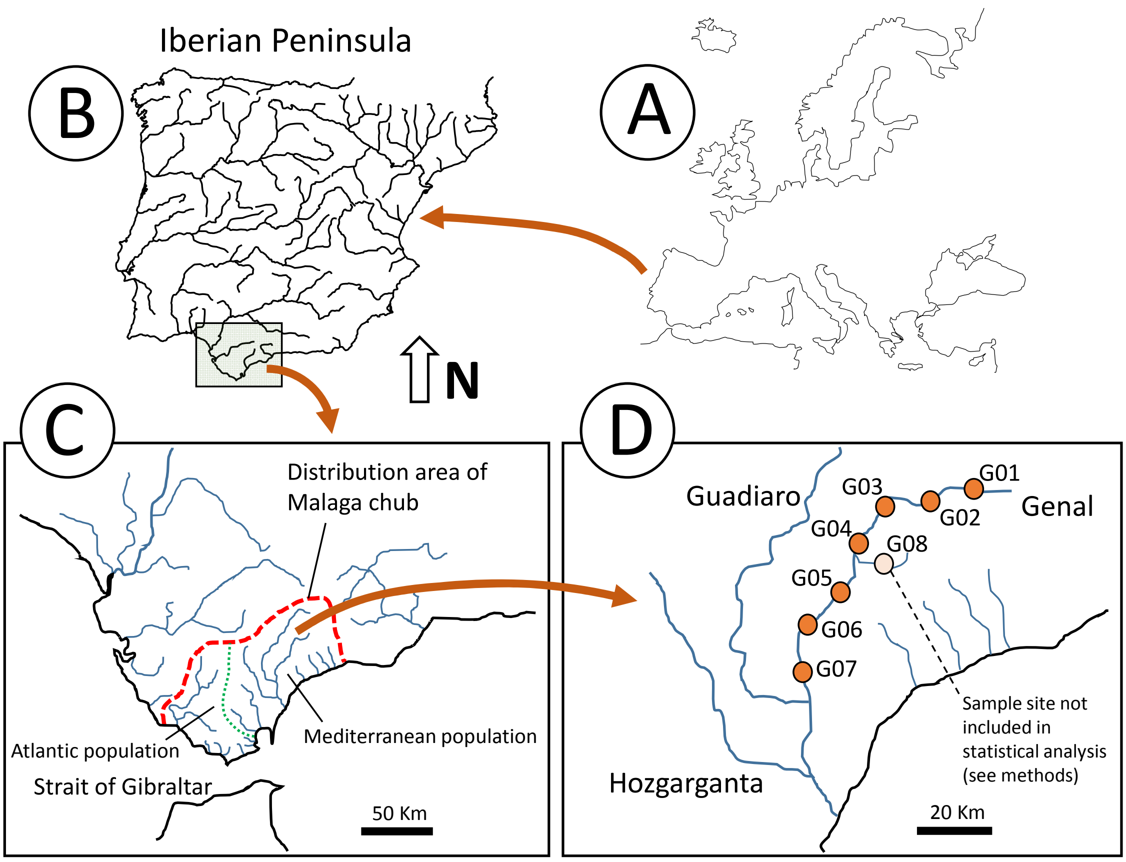

Figure 1. A: Map of Europe. B: Map of the Iberian Peninsula highlighting the area of the Strait of Gibraltar. C: The surroundings of the Strait of Gibraltar showing the distribution area of Malaga chub. The Atlantic and Mediterranean populations of the species are also indicated. D: Map of the study area in Genal river (Guadiaro basin) with the sampling sites.

Extinction risk is high for freshwater fishes in small Mediterranean basins, such as those occupied by Malaga chub, due to the extreme seasonal fluctuations of the typical Mediterranean flood-drought cycle (Clavero et al., 2005). Moreover, the loss of species due to local extirpation processes in the isolated systems cannot be compensated by natural recolonization (Martin-Smith & Laird, 1998), resulting in permanent extinctions events (Clavero et al., 2002; 2005).

Given the restricted distribution range of Malaga chub and the potential risk of loss of populations, the species has been classified as "Endangered" (Freyhof & Kottelat, 2008; Doadrio et al., 2011). Indeed, the distribution range of Malaga chub may be drastically reduced as a result of the aridification in the Mediterranean region from global climate change (Seager et al., 2014). Therefore, understanding the factors regulating the ecological aspects of this endangered species, such as habitat use, is important not only to identify preferential stream zones for the species, but also for detecting threats and designing strategies for its conservation (Filipe et al., 2002; 2004).

This paper analyses the distribution patterns and habitat use of Malaga chub in Genal River, one of the three main tributaries of the Guadiaro River, the larger basin occupied by the species across its distribution range. Specifically, we address the three following objectives: (i) to determine the habitat occupancy by the species in relation to the stream habitat gradients; (ii) to describe the species abundance patterns in relation to these gradients and the habitat stability profile described by the river form the headwaters to downstream; and (iii) to analyse the species' size structure variation along the river and between seasons.

STUDY AREA

The Genal River is one of the main tributaries in the Guadiaro River basin (1484 km^2) (Figure 1). The Genal sub-basin drains an area of 337 km^2 and the main river channel is about 50 km long. To the north and northeast, the basin is bounded by the western face of Sierra de las Nieves, Sierra del Oreganal (maximum elevation at Jarastepar peak, 1427 masl) and Sierra de los Castillejos (maximum elevation at Conio peak, 1269 masl). Three main lithological domains (limestone rocks, metamorphic rocks and peridotites) can be found in the Genal sub-basin (Castillo, 2002), in addition to flysch sedimentary formations.

The temperature and rainfall regimes are typical of the Mediterranean climates. The average annual temperature is around 14-18ºC, fluctuating between 3 and 13ºC during the coldest months (December-February) and between 18 and 32ºC in the summer months. Average rainfall is high, exceeding 1000 mm throughout the sub-basin, being higher in upper areas. The "horizontal rain", a meteorological phenomenon due to the precipitation contributed by the mists that accumulates in the summits and canyons, is also important in certain areas within the basin, where it contributes between 500 and 600 mm precipitation (Castillo, 2002). The snowfall is very sporadic and restricted to elevations above 900-1000 masl.

The study area presents an extraordinary floristic richness due to the mild climate and the lithological diversity of the basin. A total of 1155 floristic taxa have been described within the area (Gavira-Romero & Pérez-Latorre, 2003; 2005). In the middle zone of the basin there are extensive plantations of chestnut trees (*Castanea sativa*).

Generally, the Genal River is perennial, except in the highest reaches and lowest reaches near the confluence with the Guadiaro River, where the flow ceases during the summer. The upper course runs through permeable limestone beds with a limited capacity to retain water, especially during the dry season. The lower reaches run through an alluvial riverbed with a high infiltration capability that, in conjunction with water extraction for irrigation of crops, cause the flow to cease in summer.

METHODS

The field work was carried out between November 2008 and July 2009, covering a complete hydrological cycle. A total of seven sites (numbered G01 to G07, with numbers increasing downstream) were sampled along the main river channel by electrofishing with an Electracatch International Limited WFC4 pulse box (230 V, 2-5 A) in four occasions (one sample per season, Figure 1). An additional site on a tributary (Almárchal stream, G08; Figure 1) was sampled on a single occasion (spring), but was not included in the statistical analysis. Sampling was conducted over a reach of 80 m and for an average of 40 min (range: 68.5-100 m, 18-83 min), through a single-pass without block-nets. Previous works in neighbouring Mediterranean streams indicate that a sampling effort of 50 m and 20 minutes fishing is effective at capturing most fish species (Magalhães et al., 2002b; Filipe et al., 2004) and provide good estimates of fish abundance (Mesquita et al., 2006). Catches per unit of effort (CPUE) values were calculated as the number of fish captured divided by the sampling distance (m). Fish captured were identified to species level, measured for total length (to the nearest mm), weighted (in g) and returned to the water.

Nineteen habitat variables were measured or estimated in each sample occasion (sites and seasons), including both physico-chemical and stream structure descriptors (Table 1). Variables related to current velocity, water depth, river width, substrate coarseness and shelter availability were recorded from perpendicular transects to the river channel located every 20 m within the surveyed river stretch (3–6 transects per reach, depending on the length of the surveyed stretch). Following Hermoso et al., (2009), each variable was measured at three points along each transect (one at the beginning of the transect, one in the middle and the last one at the end), generating 9-18 measures for each variable per reach. Then, mean values were used for the analysis, as described in Hermoso et al. (2009).

A Principal Component Analysis (PCA) was applied to a matrix of habitat variables x sampling occasions (sites x seasons) to describe the main habitat gradients. Principal Components (PCs) with eigenvalues ≥ 3 were retained for further analysis. Each extracted PC (interpreted as habitat gradients) was split into four equal-sized portions in terms of factor scores to evaluate whether the Malaga chub distributed randomly or non-randomly along it. Presence/absence data of the species were used in the habitat use-availability analysis. The intensity of use displayed by the species in each portion was measured as the number of sites-sampling occasions where the species was present. Habitat availability was estimated as the total number of sites-sampling occasions within each portion along the gradients. The null hypothesis of random association between the amount of habitat available and used for the species was tested through a chi-squared test (Prenda et al., 1997; Blanco-Garrido et al., 2009; Hermoso et al., 2009). Habitat use by Malaga chub was also assessed by accounting for the correlation (Pearson) between the abundance of the species [$Log_{10}(x+1)$-transformed] and the habitat gradients represented by PCs. Moreover, one-way ANOVA was employed to assess possible seasonal patterns in Malaga chub abundance and habitat gradients.

As habitat instability, knowing as the amplitude of the seasonal fluctuation of habitat attributes, is a typical feature of Mediterranean streams following the characteristic Mediterranean flow cycle (Gasith & Resh, 1999; Magalhães et al., 2002a), we explored the effect of this factor on fish abundance pattern. For this purpose, an index of habitat stability (IHS) that compiles the multidimensional fluctuation of habitat variables in each sample sites along the hydrological cycle was used. IHS was calculated for each site (i) as follows:

$$IHS\ (i)=1/\{[e_1*SD_{(i)(PC1)}]+[e_2*SD_{(i)(PC2)}]+[e_3*SD_{(i)(PC3)}]\}$$

where $e_{1,2,3}$ is the eigenvalue of the first 3 PCs (PC1, PC2 and PC3) extracted from the

PCA and $SD_{(i)(PC1,2,3)}$ is the Standard Deviation of the factor scores of the four values of the site (i) (autumn, winter, spring and summer) along PCs (PC1, PC2 and PC3).

IHS values were calculated for each site (G01-G07) describing the profile of habitat stability from upstream to downstream reaches. This habitat stability profile was compared to the abundance pattern of the Malaga chub and other fish species along the longitudinal profile. Finally, the species size structure was analysed accounting for the variation of the frequency of each size class along the longitudinal profile and across seasons.

Table 1. Habitat variables used to characterize fish habitat in Genal River (Southern Iberia). All variables, except substrate coarseness, are continuous variables. The three main Principal Components (PC) extracted from the Principal Component Analysis applied to habitat variables are presented. Those variables with factor loadings ≥ |0.65| appears in bold (red-negative, green positive loadings) in order to simplify the ecological meaning of each PC. Also, eigenvalues, explained variance of each PC and the range of values of the original variables are given.

	PC 1	PC 2	PC 3	Range
Altitude (m)	0.20	-0.57	**0.70**	19-643
Current velocity (m/s)	**-0.83**	0.00	0.17	0.00-1.14
Mean depth (cm)	-0.37	**0.76**	0.15	14.7-69.1
Maximum depth (m)	-0.12	**0.74**	0.07	0.3-2.0
Channel width (m)	-0.56	0.60	-0.36	1.3-23.6
Substrate coarseness*	-0.22	**-0.65**	0.38	1.0-9.0
Shelter (m^2/4m)**	0.08	0.50	-0.37	0-30
pH	0.07	0.16	0.62	7.6-8.5
Conductivity (µS/cm)	**0.76**	0.38	0.22	475-1513
Dissolved oxygen (mg/L)	-0.56	0.52	0.39	5.84-12.76
Temperature (ºC)	**0.68**	-0.17	-0.62	8.6-24.3
Turbidity (NTU)	-0.21	-0.11	**-0.72**	0.00-2.92
Chlorides (mg/l)	-0.14	0.39	-0.30	4-12
Alkalinity (mmol/l)	-0.12	-0.59	-0.42	2.1-6.6
Hardness (mmol/l)	**0.84**	0.31	0.11	2.7-8.8
Nitrates (mg/l)	-0.58	0.00	0.41	0.0-6.3
Nitrites (mg/l)	0.42	0.26	0.10	0.00-0.02
Amonia (mg/l)	-0.28	0.02	0.04	0.00-0.37
Phosphates (mg/l)	-0.41	-0.14	-0.25	0.24-12.20
Eigenvalue	4.75	3.78	3.00	
% explained variance	23.7%	18.9%	15.0%	

* Wentworth scale, values : 1.0 finest-9.0 thickest
** m^2 of shelter in a band of 4m-width perpendicular to the stream channel

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RESULTS

The fish community was exclusively formed by native species. Malaga chub was the most abundant species, comprising 80% of total captured fish, and representing more than 60% of total fish biomass (Table 2). Andalusian barbel (*Luciobarbus sclateri* Gunther, 1868) was the second most abundant species, comprising 10.2% of total catches. Nase (*Pseudochondrostoma willkommii* Steindachner, 1866) and the European eel (*Anguilla anguilla* Linnaeus, 1758) constituted 4.9% and 3.9% of total captured fish, respectively. Eels were the second species in terms of biomass (22% of total biomass). Sandsmelt (*Atherina boyeri* Risso, 1810) was rare, representing only 1% of total catches (Table 2). Chub was the most widely distributed fish species, being found in seven out of eight sites (Table 2) while barbel and eel were present in six out of the 8 sampling sites. Two putative hybrids of chub and nase were captured in G04 during spring sample (Figure 2) but further work is required to confirm hybridisation between these two species.

The three first PCs accounted for 57.6% of total variance. PC1 was negatively related to current velocity and positively related to water characteristics (hardness and conductivity) (Table 1, Figure 3). This gradient was also related to water temperature, nitrates, channel width and dissolved oxygen (Table 1). PC2 was positively correlated with depth (maximum and mean) and negatively correlated to the substrate coarseness (Table 1, Figure 3). PC3 was defined by altitude and turbidity (Table 1, Figure 3).

Chub was randomly distributed with respect to the aforementioned habitat gradients ($\chi 2$-test $p>0.05$ in all cases), occupying the different portions of the habitats in proportion to their availability in terms of presence-absence data (Figure 3). However, the species abundance was correlated to PC1 ($r=0.42$ $p=0.033$; Figure 4) and that means that the species tended to be more abundant in areas with higher hardness and conductivity, decreasing in reaches with higher current velocities. Also, the abundance was correlated to PC2, so the species tended to be more abundant in deeper zones, usually with fine substrate ($r=0.49$ $p=0.012$; Figure 4 and 5).

Table 2. Fish species captured in Genal River. Mean CPUE, number of captures, mean BPUE, total biomass and frequency of occurrence are given. Numbers in brackets (captures and biomass) are percentages; SE: standard error. *The hybrid taxon was not included in further analysis.

Species	Mean CPUE±SE	N	Mean BPUE±SE	Biomass	Frequency (%)
Malaga chub *Squalius malacitanus*	91.9±23.6	1909 (80.0)	266.3±58.0	5346.2 (60.7)	87.5
Barbel *Luciobarbus sclateri*	10.9±4.3	243 (10.2)	54.4±15.3	1125.4 (12.8)	75.0
Nase *Pseudochondrostoma willkommii*	3.9±1.8	116 (4.9)	18.0±6.1	373.5 (4.2)	50.0
Eel *Anguilla anguilla*	4.8±1.5	92 (3.9)	104.4±34.5	1939.5 (22.0)	75.0
Sandsmelt *Atherina boyeri*	0.5±0.3	24 (1.0)	1.2±1.2	24.8 (0.3)	25.0
* *S. malacitanus x P. willkommii*	0.1±0.1	2 (0.1)	0.3±0.3	5.4 (0.1)	12.5

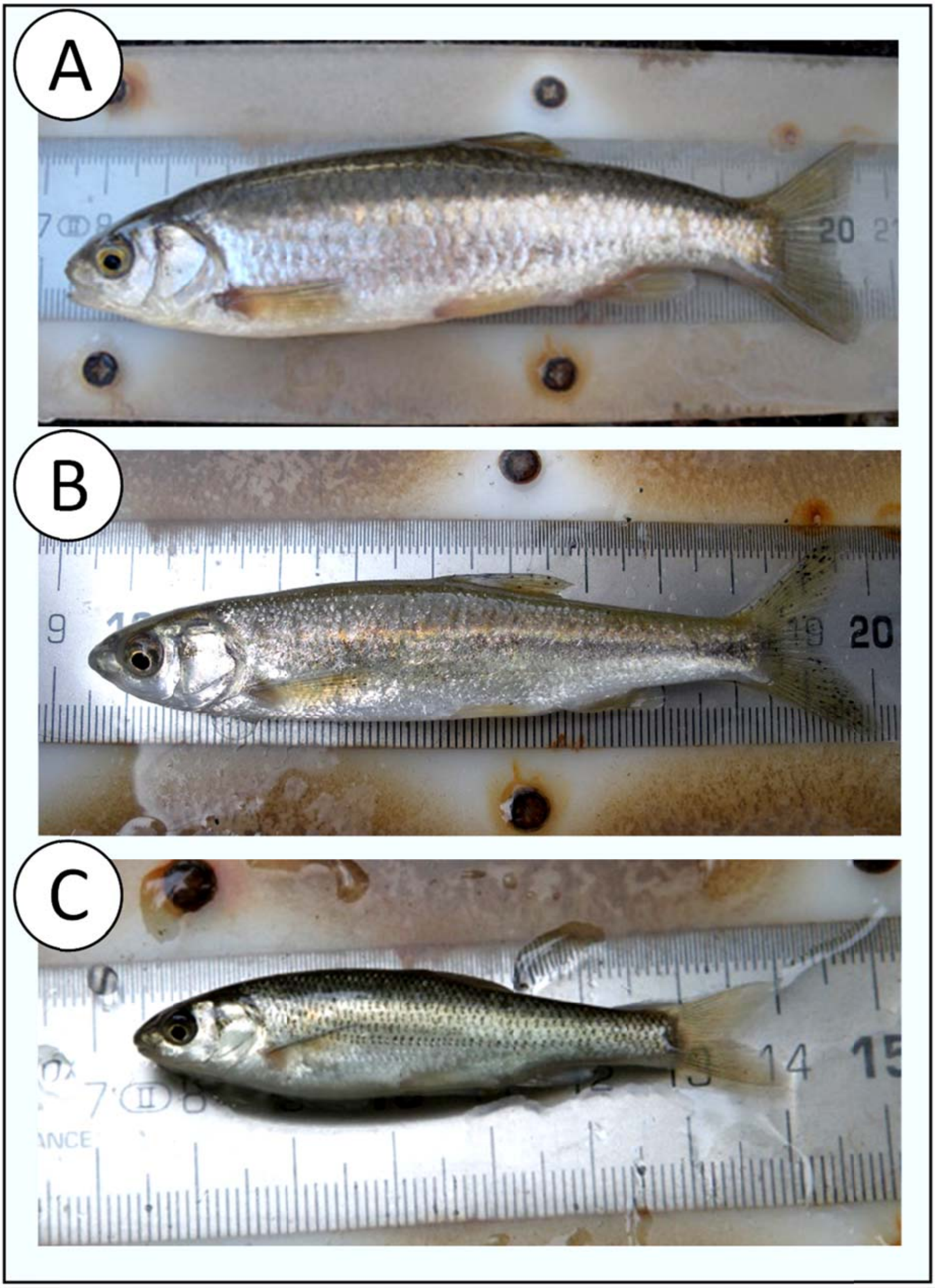

Figure 2. . A: Malaga chub (*Squalius malacitanus*). B: nase (*Pseudochondrostoma willkommii*). C: possible hybrid between these two species.

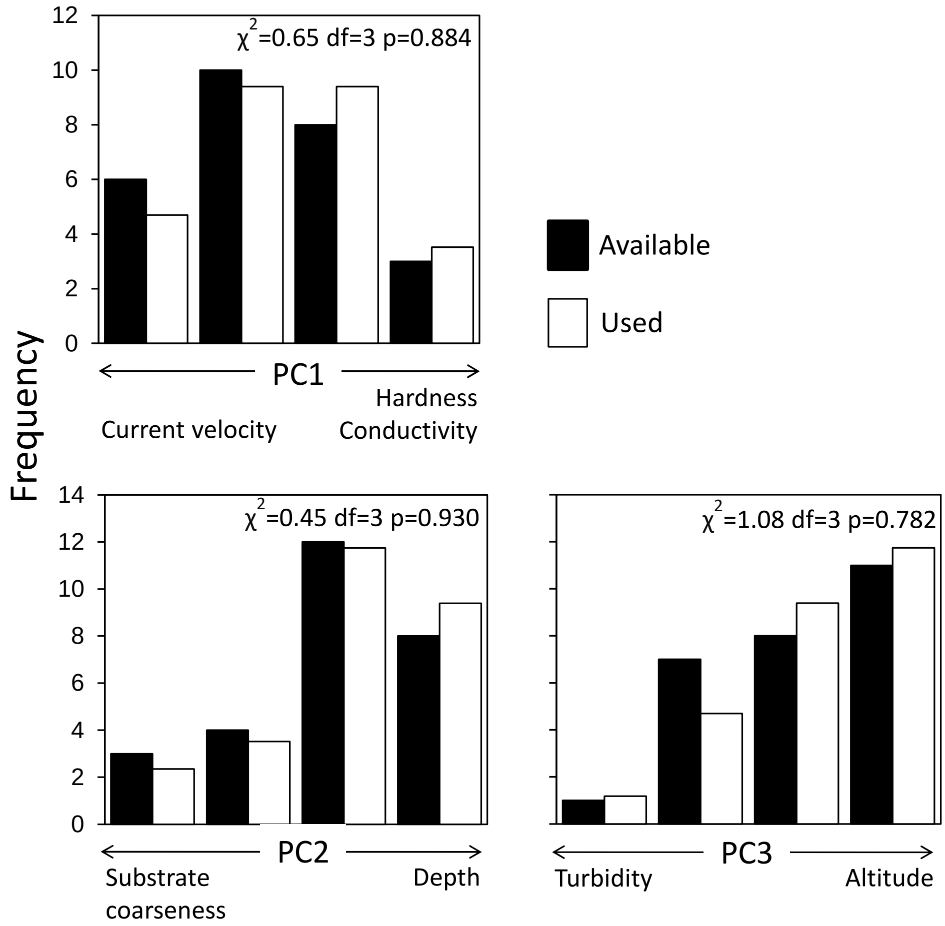

Figure 3. Analysis of chub preferences for the four equivalent portions in which habitat gradients (PC1, PC2 and PC3) were split. The meaning of the habitat gradients are given under arrows (see Table 1). The chi-squared statistic and its associated p-value are also given.

The habitat gradient represented by PC1 showed some changes across the seasons in the overall stream profile, with an increase of hardness and conductivity in spring and summer, followed by a parallel decrease of current velocity in the same seasons (ANOVA: F(3, 22)=36.09 p<0.001; Figure 4). In contrast, the habitat gradient represented by PC2 and the abundance of Malaga chub seem quite constant across the seasons (F=0.06 p=0.976, F=0.94 p=0.440, respectively; Figure 4).

The values of the IHS increased from the upper reaches to the middle reaches, where the index reached its maximum values, decreasing towards the lower stretches (Figure 6). The abundance of chub along the river closely mirrored the habitat stability (r=0.91, p=0.005; n=7). The species' abundance clearly increased from the headwaters towards the middle reaches, where the habitat stability was maximum and then decreased in the lower reaches (Figure 6). Other species, such as nase and sandsmelt, also reached their maximum abundances in the middle reaches, although their abundance patterns were apparently more independent of the habitat stability profile than that for chub (r=0.47 and 0.23 respectively, p>0.05 in both cases; Figure 6). In contrast, barbel and eels reached their maximum of abundance outside the zone with the highest habitat stability, showing greater independence of the habitat stability profile (r=-0.06 and r=0.55 respectively, p>0.05 in both cases; Figure 6).

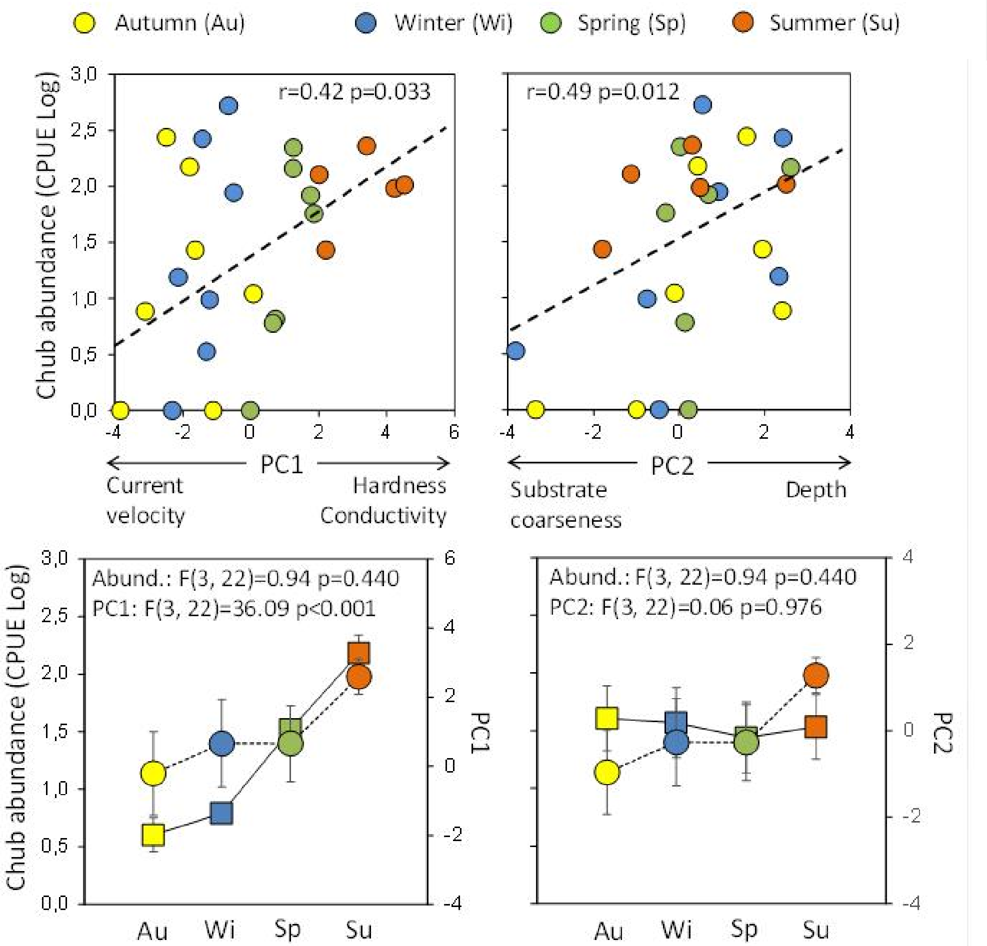

Figure 4. Above: Relationship (Pearson correlations) between the abundance of chub (CPUE $Log_{10}(x+1)$-transformed) and the habitat gradients represented by PC1 and PC2. The meaning of the habitat gradients are given under arrows (see Table 1). Below: Changes of abundance (circles) and habitat gradients (squares) (ANOVA) across the seasons (mean ± SE). The r (correlation) and **F** (ANOVA) statistics and their associated p-values are given.

Figure 5. Typical habitat of Malaga chub in the Genal River. The area inside the dashed line is a pool with fine substrate and slow current where chubs can reach high densities

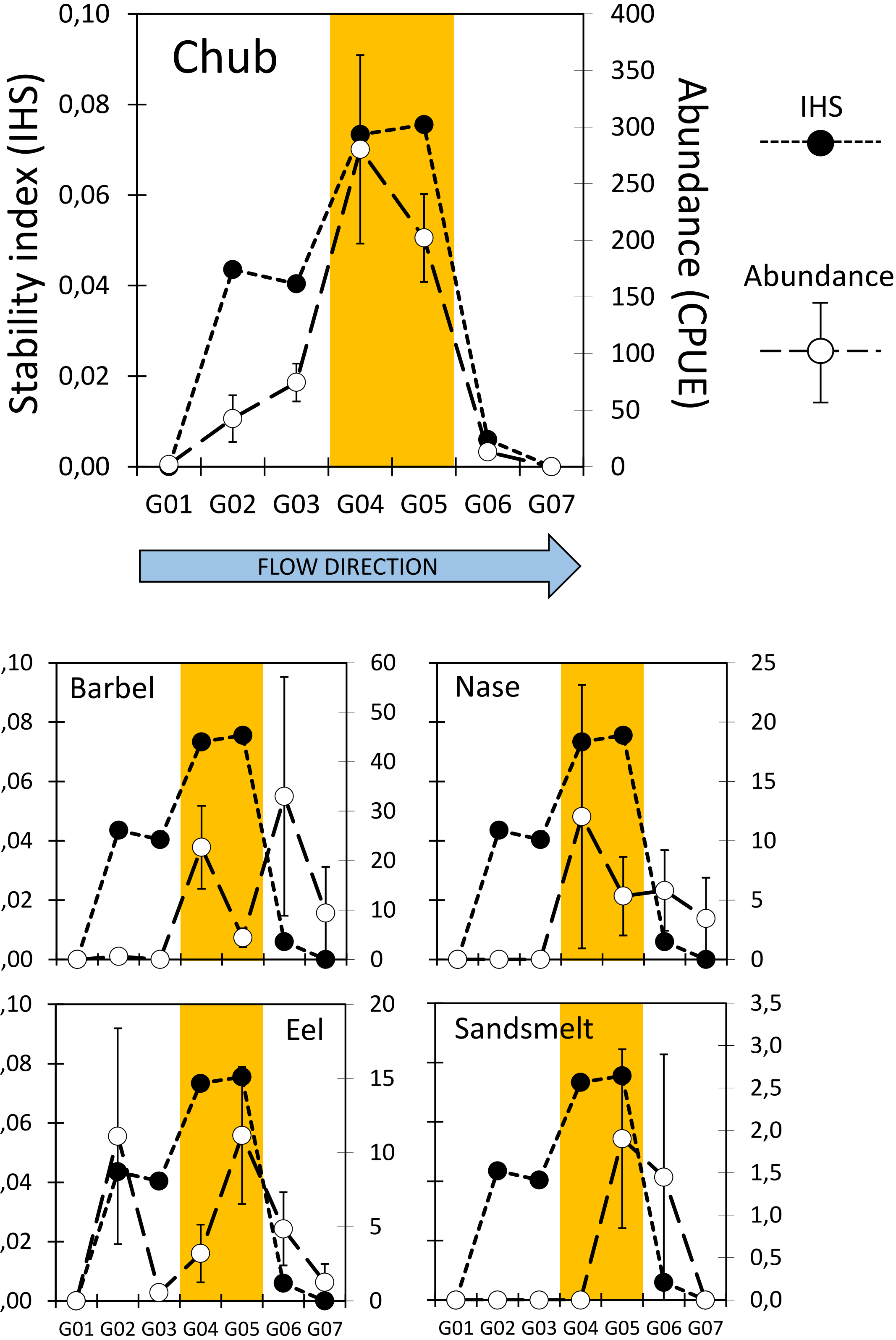

Figure 6. Relationship between the profile of habitat stability (IHS) and the species abundance patterns (CPUE±SE) along the Genal River. The sites where IHS reached the highest values (highest habitat stability) are indicated by a yellow band.

The relationship between the abundance of the Malaga chub and habitat stability was consistent with the size structure displayed by this species along the river and among seasons. The scarcity or even the absence of many size classes out of the zones with higher habitat stability was frequent, while these reaches were characterized by a better representation of all size classes (Figure 7). This result showed that, apparently, size distribution was better structured in the zones with a higher habitat stability.

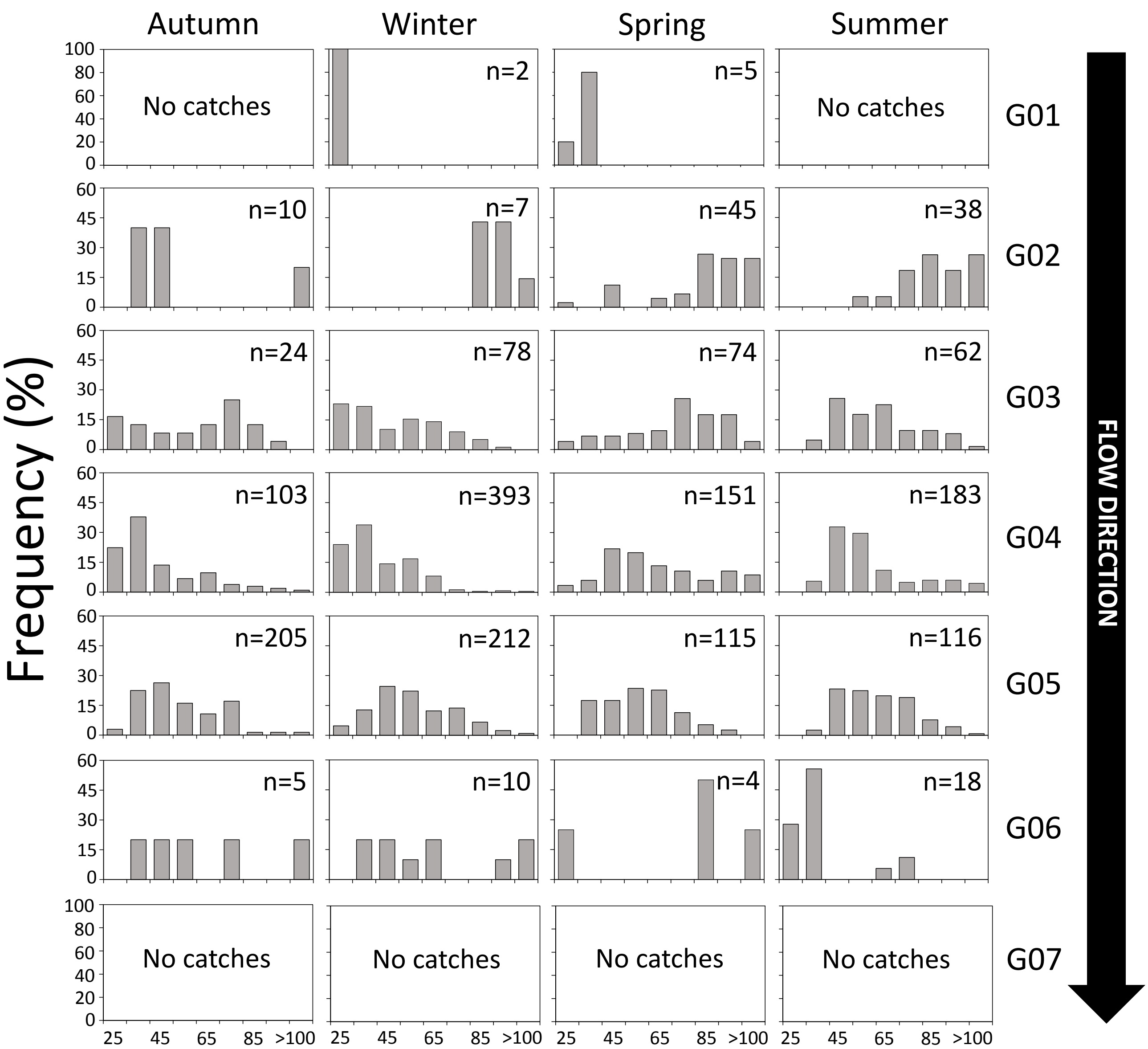

Figure 7. Size distribution of chub in each sample sites and seasons. Note that size classes are better represented in those sites with the highest habitat stability (G04 and G05, but also G03: see Figure 5).

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DISCUSSION

As far as we know, this is the first study explicitly focused on the habitat use of the endangered Malaga chub. Previous works citing punctual habitat preferences of the species are mere descriptions, without a specific analytical background (f. ex. Doadrio & Carmona, 2006; Freyhof & Kottelat, 2008; Doadrio et al., 2011; Sousa-Santos et al., 2014; Salvador, 2016). In the article that described *S. malacitanus* as a new species, Doadrio & Carmona (2006) wrote that it "typically inhabits streams with clear waters and gravel bottoms and prefers moderate flowing stretches", a statement that has been directly reproduced in subsequent works.

Our results suggest that the Malaga chub is a habitat generalist, since it occupied the different configurations of the habitats within the Genal River basin in proportion to their availability. This finding fits well with previous observations made on *S. pyrenaicus*, an ecological equivalent species traditionally considered as ubiquitous (Magalhães, 1993). In any case, the species seems to become more abundant under certain habitat configurations, such as river zones with moderate-low current, relative high water hardness, conductivity, and in stream sections characterized by relative depth and fine substrates, usually represented by pools.

On the other hand, the abundance pattern of chub varied in parallel to habitat stability along the Genal River. Both parameters were minimum at upstream zones, increasing their values towards the middle reaches where they reached the maximum, and clearly decreased in lower stream sections (Figure 6). Animal populations are more likely to reach abundances close to their carrying capacities in more stable environments (Townsend et al. 2003). A strong and continuous flow stability gradient and, hence, an habitat stability gradient can be defined in Mediterranean basins from the relatively stable river mouths to upper stream stretches, which experience huge flow variations following the characteristic Mediterranean flow cycle (Gasith & Resh, 1999; Magalhães et al. 2002a). In Genal River, the maximum stability level is reached towards the middle stretches, showing a clear unimodal pattern of habitat stability, as observed in other Mediterranean basins (Clavero et al., 2010; 2017). Downstream zones of Genal River are affected by substrate with a high capability of infiltration and the extractions of water for irrigation, which cause the cease of superficial flow during the summer drought period, leading to a decrease in the stability of fluvial habitat from the middle reaches to the river mouth. These longitudinal differences in stability in small Mediterranean catchments are related to fish abundance, which decreases in higher positions within catchments (Magalhães et al. 2002b) or those stretches with higher instability (Clavero et al., 2008). Habitat stability seems to be a key factor in the configuration of freshwater fish communities to the point that piscivorous predators, such as otters, can change the amount of fish consumed depending on the stability of the habitat, due to the influence of this factor on structuring fish populations (Clavero et al., 2008).

Nonetheless, our results show that not all fish species in the Genal River responded equally to the variation in habitat stability. The abundance patterns of barbel and eel seemed to be independent from the habitat stability profile of Genal River, while nase and sandsmelt reached their maximum abundances in the most stable stretches. In any case, the pattern of abundance of the Malaga chub was the one most closely linked to the habitat stability variation. Our results are somehow contradictory with the observations regarding the habitat use of the Malaga chub made by Doadrio et al. (2011), since these authors stated that the species prefers upstream stretches.

The relationship between the Malaga chub and habitat stability is also supported by the more diverse and better structured size spectra in more stable areas. On the contrary, the most fluctuating zones, usually the ones located to the extremes of the stability profile (headwaters and low reaches), presented an evident scarcity or even absence of many size classes. It is known that the abundance of all size classes of fish tend to increase in stable Mediterranean stream stretches (Magalhães et al., 2002b). The intensity of changes in channel morphology, flow characteristics and resource availability along time, or, in other words, the stability-instability habitat gradient (or profile), exert a strong influence on fish community organization (Schlosser, 1982). On the other hand, size structure has been frequently employed as a measure to assess the 'health status' of fish populations (Oberdoff & Hughes, 1992; Oberdoff & Porcher, 1994; Belpaire et al., 2000). Therefore, it is not surprising that the size spectra appears as better structured in areas that exhibit a higher habitat stability. All these results suggest that the middle reaches of Genal River zone act as a key core area from where the less suitable areas (less stable areas) are colonized when habitat conditions allow it. This seems to be especially true in the case of chub, a species that shows a clear dependence on habitat stability in Genal River.

Comments on species conservation

The fish community of the Genal River was exclusively comprised of native species, a rare occurrence in Iberian basins (Clavero & García-Berthou, 2006). Moreover, a hybrid taxon between Malaga chub and nase also inhabit the Genal River, although we cannot confirm the hybridization between these species cause the lack of genetic analysis. These results highlight the high conservation value of the fish community inhabiting in the Genal River. However, this pristine image could change due to the spreading of invasive fish species previously established in the Guadiaro basin and/or the arrival of new invaders. In fact, the topmouth gudgeon (*Pseudoraspora parva* Temminck & Schlegel, 1846) was detected in Guadiaro and Hozgarganta Rivers in 2013 (Dana et al., 2015), being a potential threat for the native species of the basin.

The results obtained in this work can help to design some measures for the conservation of the Malaga chub in the Genal River. The maintenance of the natural structure of the stream channel, mainly in the middle reaches, seems to be an essential issue. In this line, the application of certain measures, such as avoiding the removal of sediments from pools, a widespread practice that is carried out in the middle stretch of the river to generate swimming areas in summer, could be of great interest. Furthermore, the maintenance of the connectivity upstream and downstream from the middle reaches seems to be fundamental for the conservation of the species, since this zone may act as a key core area for the species, as shown in this work. Finally, monitoring the fish community is essential to early detect the arrival of invaders and control or erradicate them in the initial invasion stages. The intentional fragmentation, i. e. the construction of certain barriers to prevent the colonization of invasive species (Rahel, 2013; Beatty et al., 2017), could be an interesting point to be considered in the zone. These barriers must be compatible with the migration of species such as the eel and the in-stream movement of native species.

The data on habitat use by the Malaga chub presented here could be a starting point for future studies. The Genal River could be an ideal stream to assess the possible reproductive relationships between chub and nase, as well as to study the ecology of this unique fish community.

AUTHORS CONTRIBUTIONS

FBG lead the writing and the analytical procedures aided by THG and OGR. The three authors contributed to the field data collection.

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