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# Jarabugo (*Anaecypris hispanica*) and freshwater blenny (*Salaria fluviatilis*): habitat preferences and relationship with exotic fish species in the middle Guadiana basin

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**ABSTRACT**

**Jarabugo (*Anaecypris hispanica*) and freshwater blenny (*Salaria fluviatilis*): habitat preferences and relationship with exotic fish species in the middle stretch of the Guadiana basin**

In this work the habitat preferences of two endangered freshwater fish species, jarabugo (*Anaecypris hispanica*) and freshwater blenny (*Salaria fluviatilis*) are analysed, and the distribution of both species in relation to the presence of exotic fish species in the middle stretch of the Guadiana basin is assessed (28 sampling sites). The jarabugo as well as the blenny showed a very restricted distribution, mainly jarabugo (only present in 14.3 % of the sites) which in comparison with previous studies suffered a strong reduction on its distribution area (86 %). This species preferred small streams (generally less than 10 m channel width) and with submerged aquatic vegetation, while the blenny appeared mostly in large downstream stretches (> 10 m channel width), a higher water flow, and water availability (permanent water flow) . Based on a randomisation test (Monte Carlo simulation), blenny distribution was random with respect to exotic species. However, the jarabugo and the centrarchidae (*Lepomis gibbosus* and *Micropterus salmoides*) never coexisted in the same locations, despite a high probability of co-occurrence expected by chance (Monte Carlo simulation $P = 0.965$). The spatial segregation between the jarabugo and centrarchids, together with the decline of the former in areas which experienced an increase in centrarchid abundance, suggest these exotics are a threat for the conservation of jarabugo.

**Key words:** Iberian endemism, biotic interactions, large dams, Mediterranean streams, conservation.

***RESUMEN***

***El jarabugo (*Anaecypris hispanica*) y el blenio de río (*Salaria fluviailis*): preferencias de hábitat y relación con los peces exóticos en el tramo medio de la cuenca del Guadiana***

*En este trabajo se analizan las preferencias de hábitat de dos peces continentales amenazados, el jarabugo (*Anaecypris hispanica*) y el blenio de río (*Salaria fluviatilis*), así como la distribución de ambas especies en relación a la presencia de especies exóticas en el tramo medio de la cuenca del Guadiana (28 localidades de muestreo). Tanto el jarabugo como el blenio mostraron una distribución muy restringida, especialmente el primero (sólo presente en el 14.3 % de los sitios muestreados) que, en comparación con estudios previos, vio reducida enormemente su área de distribución (86 %). Esta especie prefirió arroyos pequeños (generalmente de menos de 10 m de anchura de cauce) y con vegetación acuática sumergida, mientras que el blenio ocupó principalmente los tramos bajos, con cauces amplios (> 10 m) y un mayor caudal y disponibilidad de agua (caudal permanente). Según la aplicación de un test de aleatorización (simulación de Monte Carlo) la distribución del blenio fue aleatoria respecto a la presencia de especies exóticas. Sin embargo, el jarabugo y los centrárquidos (*Lepomis gibbosus *y*

Micropterus salmoides*) nunca coexistieron en las mismas localidades, a pesar de existir una elevada probabilidad de coexistencia esperable por azar (*P = *0.965, test de Monte Carlo). La segregación espacial entre el jarabugo y los centrárquidos, junto con el declive del primero en zonas que han experimentado un aumento en la abundancia de centrárquidos, apunta a estas especies exóticas como una amenaza para su conservación.*

***Palabras clave:*** *Endemismos ibéricos, interacciones bióticas, infraestructuras hidráulicas, ríos mediterraáneos, conservación.*

## INTRODUCTION

The jarabugo (*Anaecypris hispanica*), an Iberian fish species endemic to the Guadiana basin, and the freshwater blenny (*Salaria fluviatilis*, simply blenny hereafter) are both among the most threatened fish in Iberian freshwaters (Collares-Pereira *et al*., 1999; Doadrio, 2002). The jarabugo is considered "Endangered" in Spain and "Critically Endangered" in Portugal, while the blenny is considered "Endangered" both in Spain and Portugal (Doadrio, 2002; ICN, 2005).

Both species exist in highly fragmented and localized populations (Changeux & Pont, 1995;

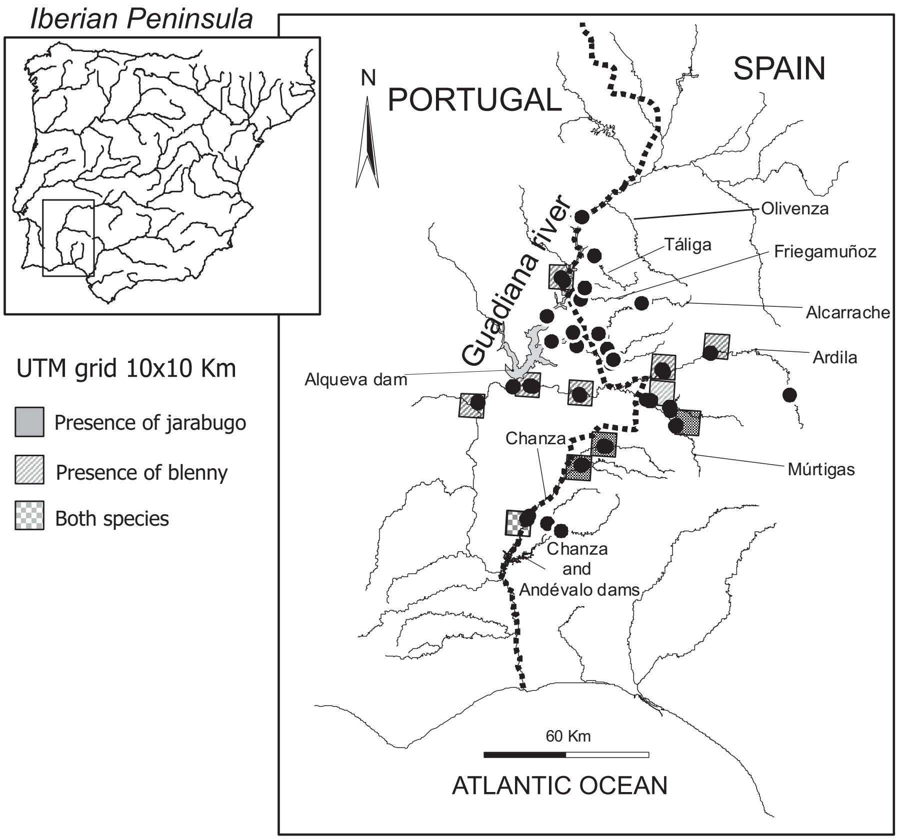


**Figure 1.** Map of the middle-lower stretch of the Guadiana river basin showing the location of the sampling points. Field work was carried out before the construction of Alqueva and Andévalo dams. *Mapa del tramo medio de la Cuenca del Guadiana y localización de los puntos de muestreo. El trabajo de campo se realizó antes de la construcción de las presas de Alqueva y Chanza-Andévalo.*

Corbacho & Sánchez, 2001; Salgueiro *et al.*, 2003), and little is known about their specific ecological requirements, such as habitat preferences (see Ribeiro *et al.*, 2000; Vinyoles, 1993). The expansion of exotic fish species and habitat disturbance are supposed to be important threats for both endangered species (Collares-Pereira *et al.*, 2000; Collares-Pereira & Cowx, 2001; Doadrio, 2002); however, there has been insufficient analytical effort to clarify factors that threaten them. Understanding the factors that influence the distribution and conservation of these endangered species is essential in order to identify the problems and to propose strategies for their conservation.

This work addresses three main objectives: (i) to determine the distribution range of jarabugo and blenny in the middle stretch of the Guadiana basin, (ii) to describe the habitat preferences of both species, and (iii) to examine the spatial relationships between these endangered species and exotic fish.

## METHODS

The study area is located in the middle stretch of the Guadiana basin (Fig. 1). The climate is typically Mediterranean, with rainfall being concentrated between autumn and spring and featuring a severe summer drought (Gasith & Resh, 1999). These climatic characteristics generate a high intra- and inter-annual variability in flow regime. In summer, most tributaries become a succession of pools of variable dimensions with little or no water flow. More permanent and stable hydrological conditions exist in downstream stretches and in the main river.

The fieldwork was carried out from April to June 2001, before the construction of the Alqueva and Chanza-Andévalo dams, two large reservoirs located in the study area (Alqueva: 3150 $Hm^3$; Chanza-Andévalo: 973 $Hm^3$). A total of 28 locations (Fig. 1) were sampled by electrofishing (230 V, 1-5 A). Sampling was conducted once in

**Table 1.** Environmental variables used to characterise the 28 sampling locations. *Variables ambientales utilizadas para caracterizar las 28 localidades de muestreo.*

| Macrohabitat variables | Method |
|---|---|
| Drainage area in each sampling site ($km^2$) | Extracted from maps (1:100 000) |
| Stream order (Strahler, 1964) | Extracted from maps (1:100 000) |
| Distance to the main river (km) | Extracted from maps (1:100 000) |
| Altitude (m) | Extracted from maps (1:100 000) |
| **Mesohabitat variables** | **Method** |
| *Physico-chemical parameters* | |
| Temperature (°C) | Portable probes |
| Conductivity (μS/cm) | Portable probes |
| Turbidity (FTU) | Portable probes |
| Dissolved oxygen (mg/l and %) | Portable probes |
| pH | Portable probes |
| *Stream structure parameters* | |
| Current velocity | Floating object, 3 replicates |
| Depth (cm) | Rigid meter |
| Channel width (m) | Flexible meter |
| Substrate coarseness | Wentworth scale |
| Submerged vegetation cover ( %) | Visual estimate, 2-3 observers |
| Emergent vegetation cover ( %) | Visual estimate, 2-3 observers |
| Herbaceous cover ( %) | Visual estimate, 2-3 observers |
| Shrub cover ( %) | Visual estimate, 2-3 observers |
| Tree cover | Visual estimate, 2-3 observers |
| Fish shelter (submerged stones and branches, cavities between rocks...) | Visual estimate, 2-3 observers |

each site, over a length of between 100 and 250 m and during a period of 1-1.5 hours.

Fifteen habitat variables at a mesohabitat scale were measured or estimated in each of the sampled locations, including both physico-chemical and stream structure parameters (Table 1). In addition, a set of macrohabitat variables was extracted from topographical and hydrological maps (1:100 000) (Table 1). Mesohabitat and macrohabitat variables were considered separately for statistical analyses.

Principal Component Analysis (PCA) techniques were applied separately to mesohabitat and macrohabitat variables to describe the main habitat gradients. Principal components extracted from both PCA are hereafter referred to as $PC_{mes}$ (mesohabitat variables) and $PC_{mac}$ (macrohabitat variables). Presence/absence data for both jarabugo and blenny were used in a habitat use-availability analysis according to Prenda *et al*. (1997).

A probability analysis (Monte Carlo simulation test) was performed to assess the relationship between the presence of exotic species and the observed distribution of jarabugo and blenny, testing the null hypothesis of random fish distribution in reference to exotic species. This analysis generates curves that represent the probability of coincidence between jarabugo/blenny and the exotic species expected by chance. This probability was calculated as follows:

$$P(i) = C(i)/\text{total number of randomized coincidences},$$

where $P(i)$ is the probability of coincidence in $i$ sites, $C(i)$ is the number of coincidences observed in the field in $i$ sites ($i = 0, 1, 2, \ldots,$ 28 sites). The significance level was assigned to $P < 0.05$ (Manly, 1997).

A specific monitoring for jarabugo was carried out at one stream stretch within the Chanza sub-basin (Fig. 1), the best preserved spe-

**Table 2.** Loading factors habitat variables included in the PCA. The percentage of the original variance explained by each component is included. Before PCA, percentages were arcsine transformed, distance to the main channel was square-root transformed and the remaining variables, except temperature, conductivity and pH, were log transformed [log($x$ + 1)]. *Factores de carga de las variables de hábitat incluidas en el Análisis de Componentes Principales (ACP). Se incluye el porcentaje de varianza explicado por cada componente. Antes de realizar el ACP, los porcentajes se transformaron con la función arcoseno, la distancia al canal principal se transformó con la función raíz cuadrada. El resto de variables, excepto la temperatura, conductividad y el pH, se transformaron con logaritmos decimales [log(*x + 1)].*

| **Mesohabitat variables** | **$PC1_{mes}$** | **$PC2_{mes}$** |
|---|---|---|
| | 25.37 % | 17.63 % |
| Temperature | −0.42 | 0.28 |
| Conductivity | 0.56 | 0.62 |
| Turbidity | 0.34 | 0.42 |
| Dissolved $O_2$ | 0.07 | 0.78 |
| pH | 0.13 | 0.64 |
| Current velocity | 0.74 | 0.25 |
| Flow | 0.90 | 0.03 |
| Channel width | 0.85 | −0.005 |
| Substrate coarseness | 0.02 | 0.20 |
| Submerged vegetation cover | −0.75 | 0.24 |
| Emergent vegetation cover | −0.47 | 0.46 |
| Herbaceous cover | −0.33 | 0.38 |
| Shrub cover | −0.24 | 0.56 |
| Tree cover | 0.28 | 0.12 |
| Fish shelter | −0.24 | 0.36 |
| **Macrohabitat variables** | **$PC1_{mac}$** | |
| | 73.57 % | |
| Stream order | 0.91 | |
| Drainage area | 0.88 | |
| Distance to the main river | −0.84 | |
| Altitude | −0.80 | |

cies population in the study area. Given the extremely restricted distribution of jarabugo (see Results below), this monitoring was carried out in three different periods (springs 2001, 2005 and 2006) to detect possible changes in its population associated to changes in environmental conditions (alterations of habitat features, introduction of exotic species, ...).

## RESULTS

### Distribution range of jarabugo and blenny

Jarabugo showed a reduced distribution, occurring only in four sampling stations (14.3 %), and having suffered a strong reduction on its occupancy area, compared with previous works (Doadrio, 2002). Of the seven UTM (Universal Transverse Mercator) 10 × 10 km squares prospected in this study where the species had been previously reported by Doadrio (2002), it was not detected in six (86 % reduction of the distribution area).

Blenny was recorded at nine sampling stations (32.1 %). This species had been previously reported in just one UTM 10 × 10 km square (Doadrio, 2002), in which it was not found during the present work. However, it was found in seven new UTM 10 × 10 km squares.

### Habitat preferences

The mesohabitat variables were summarized in two main gradients (Table 2), related to (1) stream size ($PC1_{mes}$), ranging from narrow stretches and low flow to larger streams and high flow, and to (2) water physico-chemical parameters ($PC2_{mes}$). At the macrohabitat scale, an upstream-downstream gradient could be defined ($PC1_{mac}$) (Table 2).

Jarabugo occurred preferentially in small streams (generally < 10 m channel width) with high submerged vegetation coverage ($PC1_{mes}$, partitioned $\chi^2$-analysis, $p = 0.032$) and was absent from the largest river stretches, with high flow, channel width (> 10 m channel width) and current velocity ($PC1_{mes}$, partitioned $\chi^2$-analysis, $p < 0.001$) (Fig. 2). The species was distributed randomly in relation to the water physico-chemical gradient defined by $PC2_{mes}$ ($\chi^2$-analysis, $p = 0.127$) and to the upstream-downstream gradient ($PC1_{mac}$; $\chi^2$-analysis, $p = 0.127$).

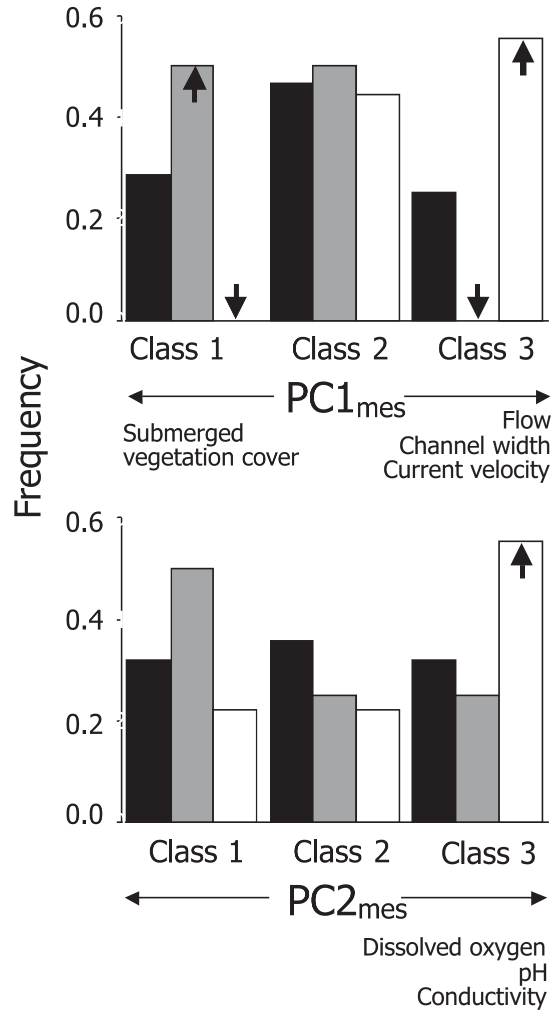


**Figure 2.** Habitat preferences of jarabugo (*Anaecypris hispanica*) and freshwater blenny (*Salaria fluviatilis*). Black bars are habitat availability, grey bars are habitat use by jarabugo and white bars are habitat use by blenny. $PC1_{mes}$ and $PC2_{mes}$ represent habitat gradients. The meaning of each gradient is under the arrows. Habitat types in which the species was over-represented or under-represented are indicated by vertical arrows (partitioned $\chi^2$-analysis, $p < 0.05$). Jarabugo $n = 4$; freshwater blenny $n = 9$. *Preferencias de habitat del jarabugo (*Anaecypris hispanica*) y el blenio de río (*Salaria fluviatilis*). Las barras negras son el hábitat disponible, las grises son el hábitat usado por el jarabugo y las blancas el hábitat usado por el blenio.* $PC1_{mes}$ *y* $PC2_{mes}$ *representan los gradientes de hábitat. El significado de los gradientes se indica bajo flechas. Los tipos de hábitat en los que cada especie está sobre- o infra-representada se indican con flechas verticales (*$\chi^2$*-subdividido,* $p < 0.05$*). Jarabugo* $n = 4$*; blenio* $n = 9$*.*

Blenny used sites with opposite habitat features to those of jarabugo (Fig. 2). It was overrepresented in the largest rivers (> 10 m channel width and > 3 stream order) ($PC1_{mes}$, partitioned $\chi^2$-analysis, $p < 0.001$), and was mainly located in the lowermost stream stretches, close to the main river ($PC1_{mac}$, partitioned $\chi^2$-analysis, $p = 0.004$). Freshwater blenny occurred more frequently in stretches with high dissolved oxygen, pH and conductivity ($PC2_{mes}$, partitioned $\chi^2$-analysis, $p = 0.035$) (Fig. 2).

## Relationships between jarabugo, blenny, and exotic fish species

During the field work, until six exotic fish species were collected in the area (*Gambusia holbrooki*, *Australoheros facetus*, *Cyprinus carpio*, *Carassius auratus*, *Lepomis gibbosus*, and *Micropterus salmoides*), Centrarchids (*L. gibbosus* and *M. salmoides*) were the most abundant and widespread exotic species (see Blanco-Garrido *et al*., 2008). Blenny was distributed randomly in reference to exotic species (Fig. 3), in contrast, jarabugo and centrarchids were not found together, although the probability of co-occurrence expected by chance was very high (Monte Carlo test $P = 0.965$, prob. no co-occurrence $P = 0.035$, Fig. 3). This result revealed a strong spatial segregation between jarabugo and these exotic species.

The monitoring of jarabugo in the Chanza sub-basin showed that its population suffered a strong decline between 2001 and 2006. In the first sampling (spring 2001) the population of jarabugo presented a high density of individuals (Fig. 4). However, its abundance decreased during the following sampling (spring 2005), until its apparent disappearance in spring 2006. This

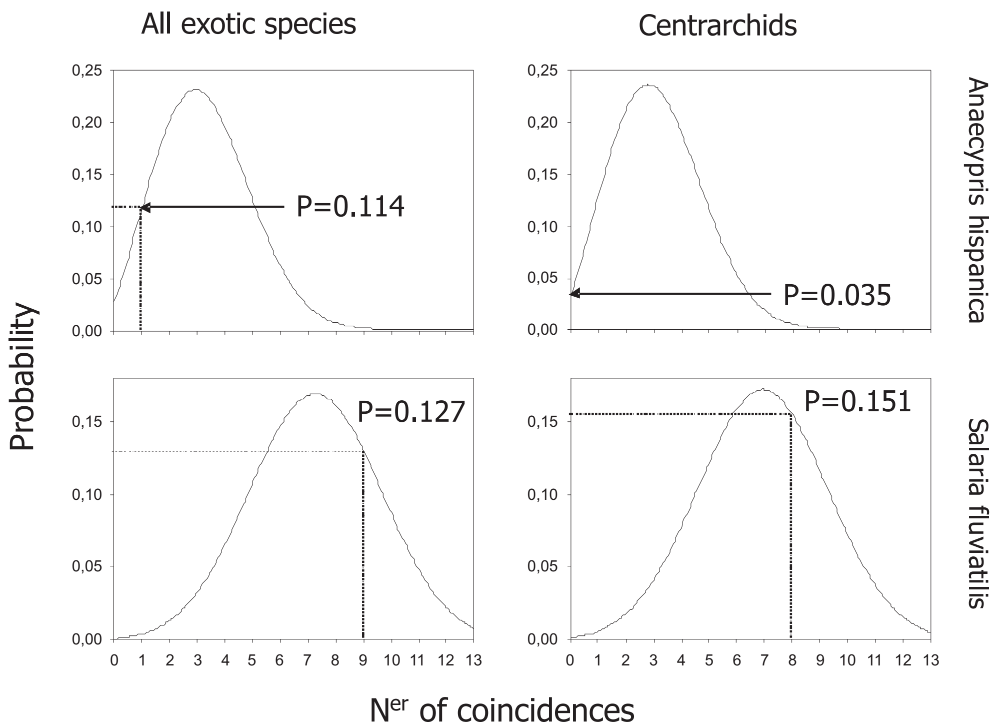


**Figure 3.** Probability coincidence models between jarabugo/blenny and the exotic species derived from a Monte Carlo simulation test. P values are the probability of coincidence expected by chance for the number of locations where each species pair coexists in the field. See methods for a more detailed description of the analysis. *Modelos de probabilidad de coincidencias entre jarabugo/ blenio y las especies exóticas a partir de un test de simulación de Monte Carlo. P es la probabilidad de coincidencia esperable por azar entre cada par de comparaciones para el número de localidades observadas en el campo en que realmente coinciden. Ver métodos para una descripción más detallada del análisis.*

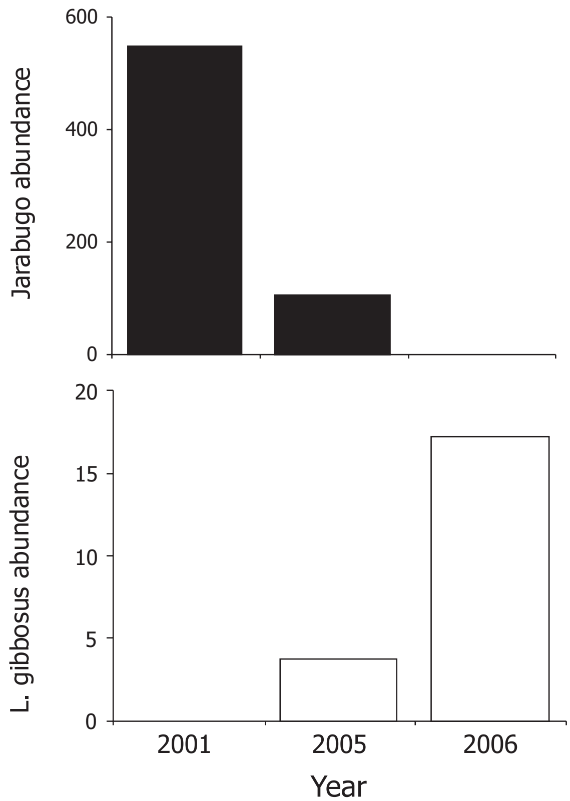


**Figure 4.** Decline of jarabugo abundance in Chanza subbasin (Guadiana basin, SW Iberian Peninsula) during the period 2001-2006 and the parallel increase of *L. gibbosus* abundance. Abundance data are given in CPUE (number of individuals/100 m*1 hour fishing). Note that Y-axis are given in different scales. *Disminución del jarabugo en la subcuenca del Chanza (cuenca del Guadiana, suroeste ibérico) en el periodo 2001-2006 y el aumento paralelo de la abundancia de centrárquidos. Los datos de abundancia se expresan en CPUE (número de individuos/100 m*1 hora de pesca). El eje Y de ambas figuras están en escalas diferentes.*

was accompanied by a parallel increase of *L. gibbosus* abundance (Fig. 4). Changes in habitat features were not detected during the study period.

## DISCUSSION

### Habitat preferences

As shown in the results, jarabugo and blenny showed different habitat preferences. Jarabugo inhabited small streams with low current and water flow and with abundant submerged vegetation. These results are in agreement with previous observations on habitat use in the Portuguese sector of the Guadiana basin (e.g. Collares-Pereira et al., 1999; Ribeiro *et al*., 2000). Blenny was generally found in downstream stretches or in the main river channel, areas characterized by high water flow, high current velocity, and wide channels. This species has specific requirements for heterogeneous substrates made of large stones (more than 180 $cm^2$ on average), which are used for nesting (Côté *et al*., 1999). This type of substrate is abundant in downstream stretches and in the main river, which had the highest current velocity in the study area. Conversely, the breeding period of the blenny, which in other Iberian basins extends from the end of May to the beginning of August (Vinyoles, 1993; Vila-Gispert & Moreno-Amich, 1998), coincides with the driest period in the Iberian Peninsula. Although data on blenny breeding periods in the study area are not available, it can be assumed to be similar to those observed in other Iberian basins. This may force this species to select those zones with more permanent water availability, usually found downstream (Filipe *et al*., 2002). In fact, Freeman *et al*. (1990) observed that prolonged drought conditions apparently prevented successful reproduction and also caused high juvenile and adult mortality in this species.

### Relationship with exotic fish species

The presence of exotic species did not seem to exert an evident influence on the distribution of blenny. This species seems to be tolerant to the presence of exotic fish (e.g. Prenda & Mellado, 1993). Blenny was the only common native species remaining after the introduction of more than 12 exotic fish species to Lake Banyoles (García-Berthou & Moreno-Amich, 2000a). These authors suggested that the persistence of blenny is related to particular ecological features such as its cryptic morphology, small size and benthic preferences.

In contrast to blenny, this work shows a strong spatial segregation between jarabugo and centrarchids. They were never found together, despite the high probability of coexistence expected by chance. Jarabugo was not detected in most of the

Guadiana's tributaries studied here, where it had been previously reported (Doadrio, 2002). Five of the six UTM squares where jarabugo seems to have disappeared are currently invaded by centrarchids (the exotics were not cited by Doadrio (2002) in these UTMs). Bernardo *et al*. (2003) reported an increase of the proportion of centrarchids (mainly *L. gibbosus*) in the Guadiana, in the period of 1980–1995, coinciding with a strong reduction of jarabugo populations, both in abundance and distribution range (Collares-Pereira *et al*., 1999). Moreover, the decline of the jarabugo population in the Chanza sub-basin was followed by a parallel increase in *L. gibbosus* abundance, without any other detected changes in the remaining environmental features. A similar process could be occurring in other areas invaded by centrarchids, given the capacity of exotic species to extirpate local populations (e.g. Ross, 1991), resulting in the spatial segregation observed here.

Adult and juvenile jarabugo could be seen as potential preys for *M. salmoides* or *L. gibbosus* because of the small size of this cyprinid (maximum total length 7 cm). Also, jarabugo's eggs consumption by sunfish may be significant, given the ability of sunfish to consume fish eggs (Garcia-Berthou & Moreno-Amich, 2000b). Fish eggs predation might strongly affect species recruitment. Finally, aggressive behaviour has been cited as an important cause of exclusion between fishes (Ortubay *et al*., 2002). Agonistic behaviour between centrarchids and Iberian native fish fauna has not been described yet. Nevertheless, this type of aggressive behaviour is likely to occur, since centrarchids display territoriality, active parental care and nest defence (Colgan *et al*., 1981; Popiel *et al*., 1996; Cooke *et al*., 2002). Agonistic behaviour might also account for the absence of jarabugo in the presence of centrarchids. All the evidence suggest that centrarchids are one of the factors responsible for the decline of jarabugo in the Guadiana basin.

### Jarabugo and blenny conservation status

In the past, jarabugo was well distributed across the Guadiana basin, at least in the Portuguese sector (Collares-Pereira *et al*., 1999, 2002), but also presumably in the Spanish one (Lozano Rey, 1935). However, its distribution range and abundance has drastically reduced in the last 30 years. If no efficient conservation measures are put into practice, their disappearance seems unavoidable (Collares-Pereira *et al*., 1999, 2000). The situation of jarabugo is particularly concerning because centrarchids, both *L. gibbosus* and *M. salmoides*, are expanding their distribution range in the Guadiana river basin, while new exotic fish species are being introduced into this basin (Hermoso *et al*., 2008). Concerning blenny, its specific requirements for permanent water areas and elevated flows make this species vulnerable to water management policy. Proposed hydraulic policies into the Guadiana basin, such as impoundment of rivers or interbasin transfers of water (Collares-Pereira *et al*., 2000), are altering and diminishing flow regimes. This represents a potential threat to the species conservation in the Guadiana, a basin in which the most diverse freshwater fish fauna of the Iberian Peninsula still persists (Filipe *et al*., 2002).

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