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## Habitat use of an endangered cyprinodontid fish in a saline wetland of the Iberian Peninsula (SW Mediterranean Sea)

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### Abstract

*Aphanius iberus* is an endemic fish restricted to a few populations along the Spanish Mediterranean coastline and included on international red lists. Information on its ecological requirements is needed to implement effective recovery and conservation measures. This two-year study aimed to analyse the effect of habitat changes, mainly in water salinity and refuge availability, on the life-history traits and microhabitat use of an *A. iberus* population inhabiting a littoral wetland managed for salt exploitation. The species was more abundant at the intake pond, which was characterised by lower water conductivity values and higher cover of the submerged macrophyte *Ruppia cirrhosa*. The pond with the highest values of water conductivity showed no presence of newborn individuals (< 10 mm), which probably indicates the reproduction failure of *A. iberus* or high mortality rates for younger individuals. Overall, the species' selection of microhabitats was related to refuge presence (submerged vegetation and pond dykes). Juvenile individuals showed a strong dependence on sheltered microhabitats through the studied ponds, with *R. cirrhosa* meadows as important refuge areas for this age group. Results highlighted the importance of developing traditional maintenance and management measures for the conservation of such endangered fish species.

**Keywords:** Littoral wetlands, salt pond systems, Cyprinodontidae, *Aphanius iberus*, habitat selection, management.

### Introduction

Cyprinodontidae is a family of fishes naturally located in fresh and brackish waters comprising species adapted to extreme and fluctuating environmental conditions. These fish species have a high level of tolerance to extreme environmental conditions such as hypersalinity, elevated water temperatures and pH values that are typical of saline aquatic systems (Leonardos *et al.*, 1996; Plaut, 2000; Martin & Saiki, 2005). They represent an important group of endemisms in Mediterranean shallow coastal wetlands, being often considered key species in these ecosystems (Oliva-Paterna *et al.*, 2006; Economou *et al.*, 2007; Leonardos, 2008). Due to the high level of endemism of the cyprinodontids and their level of imperilment, the family is considered the most endangered among freshwater fish in Europe (83% of the species are highly threatened) (Freyhof & Brooks, 2011) and urgent conservation measures are required for the species of this family. The causes of their endangered status vary throughout the Mediterranean basin, depending on regions and countries, but are mostly related to human activities such as the transformation of littoral wetlands

that are one of its common habitat type; water abstraction related to agricultural purposes and the effects of competition from invasive species (Smith & Darwall, 2006; Clavero *et al.*, 2010). In the context of conservation of imperilled fish species, effective management measures should rely on basic information on their habitat requirements at different spatial scales and during different life-span stages (Cooke *et al.*, 2012).

The Iberian toothcarp *Aphanius iberus* (Valenciennes, 1846) is an endemic cyprinodontid of the Iberian Peninsula restricted to isolated populations along the Spanish Mediterranean coastline (Oliva-Paterna *et al.*, 2006). The species is catalogued as Endangered A2ce (IUCN 2012) and is one of the few Iberian fish species protected by national and international laws. As with other native Mediterranean cyprinodontids (Maltagliati, 1999; Clavero *et al.*, 2007; Leonardos, 2008), the current distribution of Iberian toothcarp has mostly been restricted to isolated saline aquatic systems (Oliva-Paterna *et al.*, 2006; Araguas *et al.*, 2007; Alcaraz *et al.*, 2008), mainly those with eusaline and hypersaline habitats.

When dealing with endangered cyprinodontids, small man-made structures related to water management

(e.g. ponds and irrigation channels) can provide alternative or complementary habitats for these threatened species (Casas *et al.*, 2011). In fact, *Aphanius* species are able to become locally abundant in salt exploitation wetlands (Gutiérrez-Estrada *et al.*, 1998, Oliva-Paterna *et al.*, 2009) and the importance of these aquatic systems as a typical habitat for *Aphanius* species in some areas along the Mediterranean coastline has been noted (Leonardos, 2008; Oliva-Paterna *et al.*, 2006; Zammit-Mangion & Deidun, 2010). However, for the last decades these habitats have been subjected to severe anthropogenic disturbances, mainly related to agriculture and the development of tourism. Thus, many of them have been abandoned or their use has changed with deleterious effects on biodiversity (Ortega *et al.*, 2004; López *et al.*, 2010) and, in particular, on the conservation of Mediterranean cyprinodontids.

Due to species conservation concerns, several studies have recently increased our knowledge of the biological characteristics of the Iberian toothcarp, including feeding ecology (Alcaraz & García-Berthou, 2007), eco-physiology (Oliva-Paterna *et al.*, 2007; García-Alonso *et al.*, 2009), interactions with exotic species (Rincón *et al.*, 2002; Caiola & De Sostoa, 2005) and ecological interactions (Casas *et al.*, 2011; Compte *et al.*, 2012). There are, however, few studies analysing the habitat preferences of Iberian toothcarp at the meso-, or microhabitat scale and no studies have been done in artificial systems such as salt exploitation wetlands. Alcaraz *et al.* (2008) examined its habitat use in a natural saltmarsh showing the importance of glasswort and algal mats for increasing species density.

Information on foraging and refuge microhabitat preferences of the species is required for a more complete approach to its conservation needs. This research focuses on the Marchamalo salt pond system, a wetland located in the south-eastern Iberian Peninsula, which presents high spatial and temporal variation in water salinity. Previous studies on the Iberian toothcarp population in the study area showed temporal changes in several life-history traits of the species in relation to water level and salinity changes (Oliva-Paterna *et al.*, 2009). The aim of this two-year study was to analyse the effect of changes in water salinity on several biological traits and habitat use of Iberian toothcarp at two different scales: ponds and microhabitats within ponds. Our specific objectives were (i) to analyse the variation in species abundance and population structure among differentiated ponds according to water salinity and (ii) to characterise microhabitat use by analysing possible temporal and spatial variations. It was hypothesized that changes in water salinity might have indirect effects on the population dynamics and microhabitat use of the Iberian toothcarp. As mentioned above, an understanding of species habitat use has important management implications for its conservation in similar wetlands, as well as for other endangered cyprinodontids

inhabiting similar aquatic systems. Moreover, knowledge of the Iberian toothcarp microhabitat use might be important for wetland ecologists, in order to gain a better understanding of the effects of its activity on the structure and composition of the invertebrate community in shallow wetlands (Compte *et al.*, 2012).

## Methods

### Study area

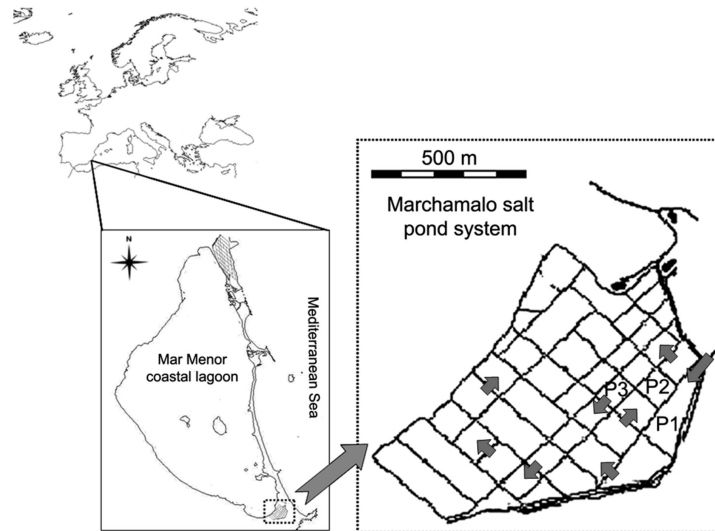
The study was carried out in the Marchamalo salt pond system (SE Spain; UTM 30SYG06) (Fig. 1), an isolated wetland of about 100 ha managed for salt exploitation, although it is currently in a process of abandonment, which is adjacent to the Mar Menor coastal lagoon. The climate is semiarid Mediterranean, with low annual rainfall (< 350 mm) and warm temperature (18° C mean annual temperature).

The Marchamalo wetland consists of several salt ponds (about 50 ponds of different sizes) separated by artificial dykes or levees made of compacted clay covered with boulders (Fig. 1). These levees are naturally vegetated with plant species adapted to salinity such as *Arthrocnemum macrostachyum* (Moris.) Moris, and *Sarcocornia fruticosa* (L.) A. J. Scott. Salt ponds are characterized by low depth (50 cm maximum), soft substrate (mainly muddy bottoms) and isolated patches of submerged vegetation consisting of the phanerogam *Ruppia cirrhosa* (Petagna) Grande, and the filamentous algae *Cladophora* spp.

The Iberian toothcarp is largely the dominant fish in the Marchamalo wetland, while other species of marine origin, i.e. grey mullets (Mugilidae) and the peacock blenny *Salaria pavo* (Risso, 1810), have been captured occasionally but they showed very low densities (Oliva-Paterna *et al.*, 2009). The studied population has inhabited the wetland for more than 20 years and the immigration effect is minimal because the artificial connection with the coastal lagoon is only occasional, and outmigration is absent because the wetland has no natural communication with the Mar Menor lagoon.

### Sampling methods

A spatial sampling design in three ponds representing the salinity gradient of the studied wetland was applied (Fig. 1). Sampling ponds (P1, P2 and P3) range in size from 1.0 to 2.0 ha and vary in mean salinity from 45 to 90. Pond 1 is the largest one and constitutes the intake pond receiving the pumped water from the Mar Menor lagoon. The higher water level in P1 supplies water to the rest of the salt ponds through manually operated sluice gates. The environmental characteristics of each pond slightly differ because of the salinity gradient. The study period spanned from March 2006 to May 2008 and sampling was performed regularly twice a season (except in summer with



**Fig. 1:** Location of Marchamalo salt pond system and distribution of sampling sites: P1, Pond 1; P2, Pond 2; P3, Pond 3. Grey arrows indicate the direction of waterflow through the system.

three samplings), resulting in 20 sampling events. For each one, water temperature ( $^{\circ}\text{C}$ ) and water conductivity ( $\text{mS}/\text{cm}$ ) were measured three times at each sampling pond by a multiparameter WTW-400<sup>®</sup>. Fish sampling consisted of setting 12 minnow-traps (30 mm entrance diameter; 1 mm mesh size) (Harrison *et al.*, 1986), uniformly distributed in each sampling pond for roughly 24 hours.

Microhabitat measurements were recorded at trap level (12 samplings). Using the minnow-trap position as the centre of a 1-m-diameter circle (microhabitat unit), six variables related to habitat structure and presence of surrounding fish refuge were considered: water depth (cm), distance to the nearest shore (cm), submerged vegetation cover (%), submerged vegetation density, substrate size and substrate heterogeneity. The assessment of submerged vegetation cover and density was made visually by two observers, the former recorded as the percentage of the area covered by vegetation and the latter as an ordinate categorical variable from 0 (low meadow density) to 5 (high meadow density). Substrate was classified according to Bain (1999) [mud (1), sand (2), gravel (3), pebble (4) and boulder (5)] and estimated as substrate size (average) and substrate heterogeneity (standard deviation).

In order to obtain additional information about the importance of *R. cirrhosa* meadows as a refuge for the target species, a specific survey throughout the intake pond (P1) was made in summer 2007. The fish sampling method consisted of a  $0.64 \text{ m}^2$  enclosure trap ( $0.8 \times 0.8 \text{ m}$  surface;  $0.5 \text{ m}$  height;  $2 \text{ mm}$  mesh size), which was quickly thrown on the sampling site and then dug into the substrate (Jordan *et al.*, 1997). After water depth (cm) and vegetation cover (%) in the enclosure trap were measured as mentioned above, a quadrangular hand net ( $0.4 \times 0.4 \text{ m}$ ;  $2 \text{ mm}$  mesh size) was used to remove fish; the hand net was swept through the enclosure trap until

five consecutive empty sweeps were obtained. Fish density (number of individuals per litre) in the enclosed area was obtained for three classes of *R. cirrhosa* cover: class 1 ( $< 20\%$ ), class 2 ( $40\text{-}60\%$ ), and class 3 ( $> 80\%$ ). Six replicates were made for each class on the same day.

All captured fish were sexed (male, female, juvenile), counted and measured for total length ( $\text{TL} \pm 1 \text{ mm}$ ), and then released alive to their habitat.

#### Data analysis

The relative abundance of the Iberian toothcarp in minnow-traps was expressed as the number of individuals captured per trap per 24 hours (catches per unit of effort, CPUE). The fish abundance variation among the sampling ponds (total, males, females and juveniles) was tested by Kruskal-Wallis's and Mann-Whitney's tests using pond as factor. Sex ratio was calculated as the ratio of females to males in each pond. Data on total length (TL) of individuals was separately analysed for males, females and juveniles using two-way ANOVA and Tukey's test to assess possible differences among ponds. Bivariate relationships between water temperature, water conductivity and abundance and total length of the species were analysed using Spearman's correlation coefficients.

The analysis of microhabitat use of the Iberian toothcarp was made using data from P1 and P2 sampling ponds only since species abundance in P3 was too low. Sampling events were classified in two differentiated periods of the fish population temporal dynamics according to Oliva-Paterna *et al.* (2009): Period 1 characterised by low species densities and dominated by large individuals (during late autumn, winter and early spring), and Period 2, just in the recruitment period, when fish density is high and there was an abundant presence of juveniles (late spring, summer and early autumn).

To explore patterns in microhabitat use by the species, Principal Component Analysis (PCA) was applied to the environmental variables matrix of each pond in order to extract independent components (henceforth PCs), which could be interpreted as gradients describing patterns of habitat variation for each pond. Only microhabitat gradients with eigenvalues larger than 1 were selected for further analyses. The scores of the selected microhabitat gradients were divided into three segments of equal amplitude (PC segments), the number of available microhabitat units in each one and the number of juveniles, males and females was counted.

Selection of microhabitat variables was analysed by applying Ivlev's electivity index ( $D$ ) with Jacobs' modification (Jacobs, 1974) to each of the three segments defined in the different PCs. This index ranges from -1 (total avoidance) to 1 (absolute positive selection) and was calculated as:  $D = (r-p) / (r+p-2rp)$ , where  $D$  is the electivity measure,  $r$  is the percentage use of the resource (i.e. proportion of the Iberian toothcarp individuals included in a PC segment), and  $p$  is the percentage of the resource in the environment (i.e. proportion of available microhabitat units included in the PC segment).  $D$  values between 0.25 and 0.5 were considered to be a moderate positive selection and those higher than 0.5 were a strong positive selection (negative selection in the case of negative  $D$  values). In order to reinforce the analysis of microhabitat selection, the factors extracted by the PCAs were correlated (Spearman's correlation coefficients) with the

Iberian toothcarp abundance (Quinn & Keough, 2002).

The comparison of species density in enclosure traps among the three classes of vegetation cover was performed using the Kruskal-Wallis and Mann-Whitney tests.

Statistical analyses were performed using the SPSS® statistical package (Version 15.0) and significant differences were recorded at  $p < 0.05$ .

## Results

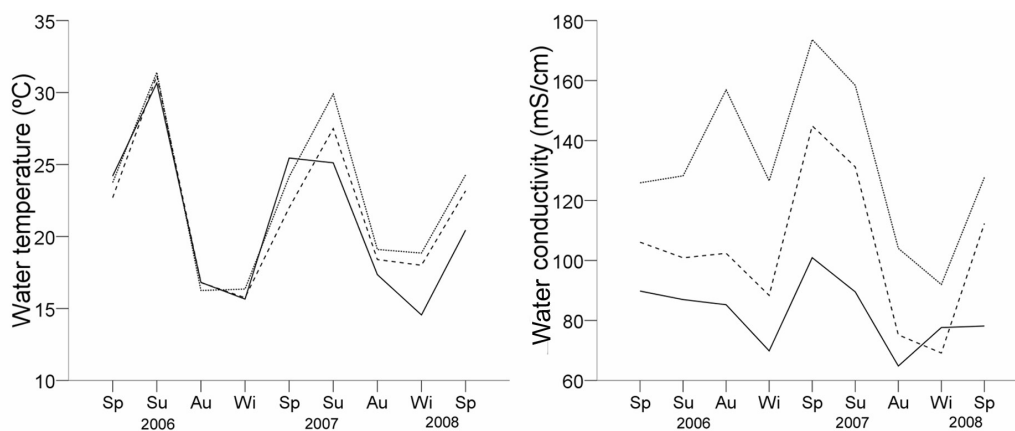
### Environmental characteristics of ponds

The mean values of environmental parameters during the study period at each pond revealed that water temperature, conductivity and depth were higher in P3 than in the other two ponds (Table 1). Submerged vegetation cover and density were higher in P1, with intermediate values in P2, whereas in P3 there was no presence of submerged vegetation (Table 1). Thus, the lower conductivity values facilitated submerged vegetation growth, which was composed mainly of the phanerogam *R. cirrhosa* and filamentous algae of the genus *Cladophora*. The maximum coverage of submerged vegetation occurred during warm seasons; P2 presented only isolated patches of filamentous algae during that period.

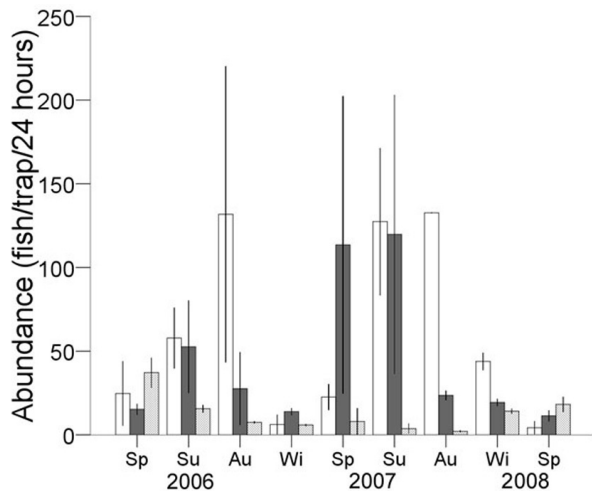
Water temperature and conductivity showed a high degree of temporal variation (Fig. 2). In general, water temperature reached highest values during summer across all ponds, with higher values during summer 2006

**Table 1.** Average values of environmental variables ( $\pm$  SD) in each study pond of the Marchamalo salt pond system.

Environmental variables	Pond 1	Pond 2	Pond 3
Water temperature (°C)	21.5 $\pm$ 0.6	22.5 $\pm$ 0.5	23.1 $\pm$ 0.6
Water conductivity (mS/cm)	81.9 $\pm$ 1.4	101.8 $\pm$ 2.9	132.9 $\pm$ 2.1
Water depth (cm)	26.3 $\pm$ 1.0	28.6 $\pm$ 1.1	29.8 $\pm$ 0.9
Submerged vegetation cover (%)	31.7 $\pm$ 5.1	10.8 $\pm$ 3.2	0
Submerged vegetation density (0-5)	2.4 $\pm$ 0.7	1.3 $\pm$ 0.6	0



**Fig. 2:** Mean seasonal values of water temperature (°C) and water conductivity (mS/cm) at each study pond of the Marchamalo salt pond system. Pond 1: black lines; Pond 2: dashed lines; Pond 3: grey lines.



**Fig. 3:** Mean seasonal abundance of *A. iberus* ( $\pm$  SE) in each study pond of the Marchamalo salt pond system. Pond 1: white bars; Pond 2: black bars; Pond 3: grey bars.

(> 30°C) in relation to summer 2007. The lowest water temperatures were recorded in winter (< 20°C), with minimum values during winter 2008 (< 15°C at P1). Spatial patterns showed that from summer 2007 to spring 2008 water temperature was lower in P1 compared to the other two ponds, with maximum values in P3 (Fig. 2). With regard to water conductivity, there was no regular seasonal pattern (Fig. 2). P1 showed the lowest values compared to the other two ponds across all seasons (except during winter 2008 when it was higher than in P2), with mean values lower than 100 mS/cm. In P3, water conductivity was the highest, reaching maximum values during spring 2007 (> 170 mS/cm) (Fig. 2).

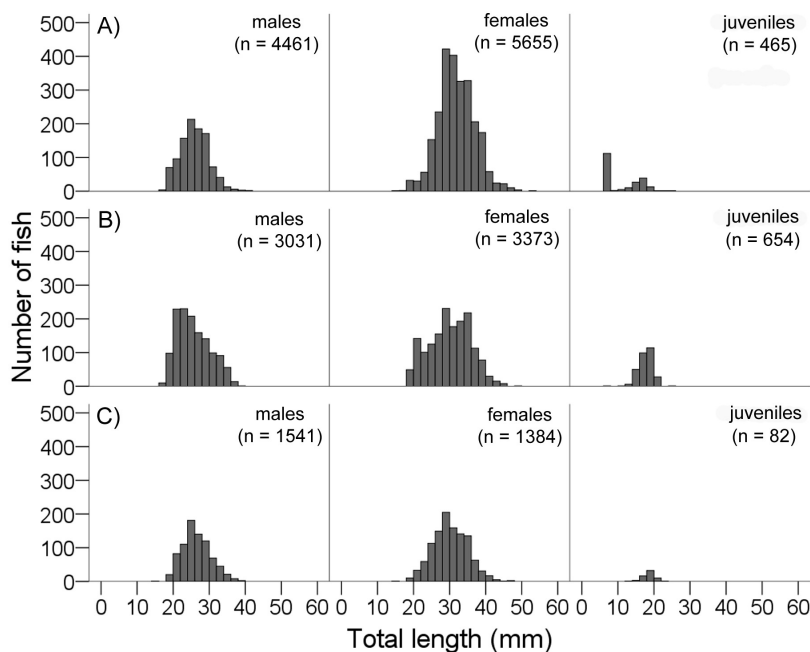
### Abundance and population structure

Total abundance of the Iberian toothcarp showed a high degree of seasonal and spatial variation (Fig. 3). The mean abundance of the species was highest during summer 2007: 83.6 individuals ( $\pm$  33.8 SE), and lowest during winter 2007: 8.7 individuals ( $\pm$  2.3 SE). There were significant differences in species abundance among sampling ponds (Kruskal-Wallis:  $H = 13.70$ ,  $p < 0.001$ ) (Fig. 3). Pair-wise comparisons revealed that captures in P3: 12.2 individuals ( $\pm$  2.5 SE), were lower compared to P1: 64.4 individuals ( $\pm$  14.7 SE), and P2: 48.3 individuals ( $\pm$  16.1 SE) (Mann-Whitney:  $U = 84.0$ ,  $p < 0.01$ ;  $U = 85.0$ ,  $p < 0.01$ , respectively). There was no significant difference in species abundance between P1 and P2 (Mann-Whitney;  $U = 158.5$ ,  $p > 0.05$ ) (Fig. 3).

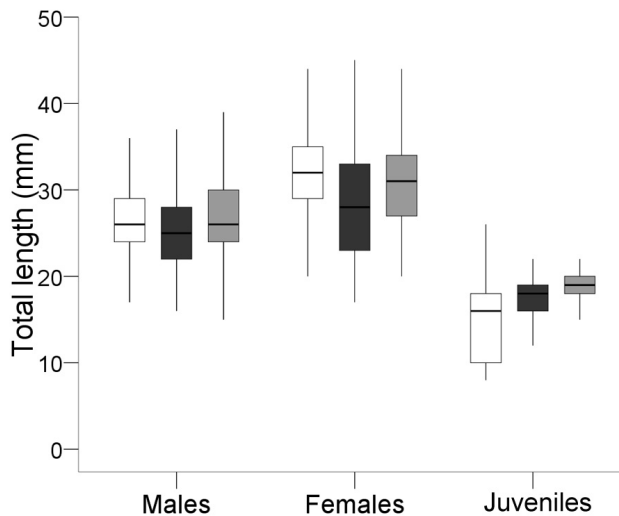
During the study period, a significant negative relationship was detected between water conductivity and total abundance (Spearman's correlation;  $\rho = -0.33$ ,  $p < 0.01$ ), but not with water temperature ( $\rho = 0.18$ ,  $p > 0.05$ ). Furthermore, there was a negative relationship between male and female abundances and water conductivity ( $\rho = -0.32$ ,  $p < 0.05$ ;  $\rho = -0.30$ ,  $p < 0.05$ , respectively). No significant correlations were detected, however, between abundance of juveniles and either water conductivity ( $\rho = -0.21$ ,  $p > 0.05$ ), or water temperature ( $\rho = 0.05$ ,  $p > 0.05$ ).

The sex ratio varied among ponds, with the proportion of females being higher in P1 (4.04:1) in relation to P2 (1.35:1) and P3 (1.44:1).

With regard to population size structure, length-frequency distributions showed that newborn individuals (TL < 10 mm) were much more abundant in P1 than in the other two ponds (Fig. 4). In fact, there were no fish



**Fig. 4:** Length-frequency distributions of *A. iberus* males, females and juveniles in each study pond of the Marchamalo salt pond system. A) Pond 1; B) Pond 2; C) Pond 3. n = number of individuals measured for total length.



**Fig. 5:** Box-plots for total length of *A. iberus* males, females and juveniles in each study pond of the Marchamalo salt pond system. Pond 1: white bars; Pond 2: black bars; Pond 3: grey bars

smaller than 10 mm (TL) in P3 (Fig. 4). In relation to this, there were significant differences in the TL of females and juveniles among ponds (ANOVA:  $F = 4.74$ ,  $p < 0.05$ ;  $F = 4.90$ ,  $p < 0.05$ , respectively), but not in the TL of males (ANOVA:  $F = 0.76$ ,  $p > 0.05$ ) (Fig. 5). Tukey's test showed that the length of females was higher ( $p < 0.01$ ) in P1 ( $31.7 \text{ mm} \pm 0.1 \text{ SE}$ ) compared to P2 ( $28.4 \text{ mm} \pm 0.1 \text{ SE}$ ) and the length of juveniles was also higher ( $p < 0.01$ ) in P3 ( $19.1 \text{ mm} \pm 0.2 \text{ SE}$ ) compared to P1 ( $14.4 \text{ mm} \pm 0.2 \text{ SE}$ ) (Fig. 5). Among the correlations between fish size and the environmental variables, there was only a significant negative relationship between the TL of males and water temperature (Spearman:  $\rho = -0.58$ ,  $p < 0.001$ ).

#### Microhabitat use

The PCA on the habitat characteristics of available microhabitat units produced two gradients (PC1 and PC2) that, together, explained 73.5% and 77.1% of the original variation within the data set of P1 and P2, respectively (Table 2). In both cases, PC1 described a gradient

**Table 2.** Factor loadings, eigenvalues and explained variances resulting from the PCAs applied to the matrix of microhabitat variables recorded in each of the study ponds (Pond 1 and Pond 2) of the Marchamalo salt pond system.

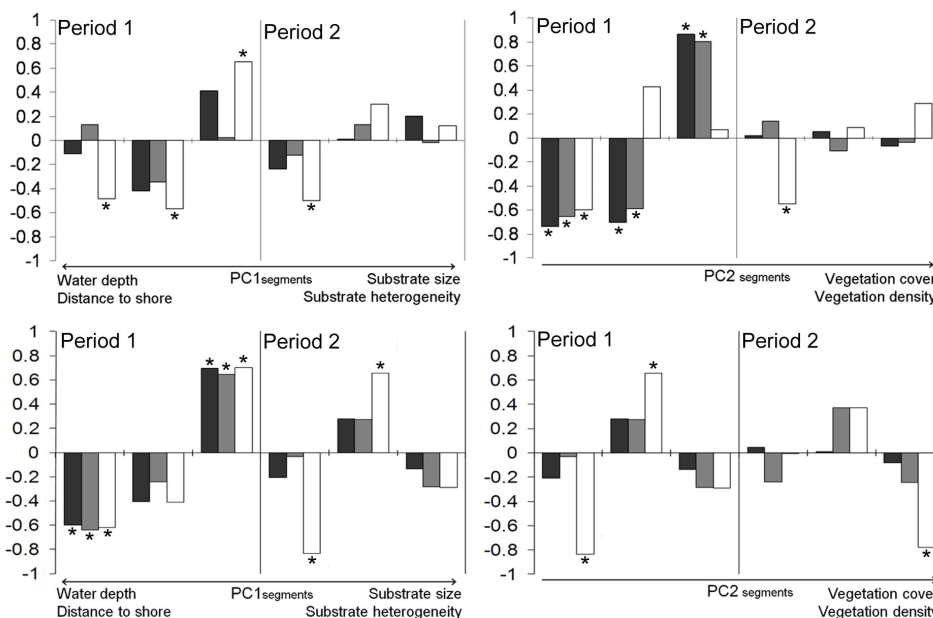
Environmental variables	Pond 1		Pond 2	
	PC1	PC2	PC1	PC2
Water depth	-0.41	0.21	-0.55	0.04
Distance to the nearest shore	-0.87	-0.11	-0.84	-0.10
Submerged vegetation cover	-0.04	0.92	0.12	0.96
Submerged vegetation density	0.10	0.90	0.07	0.96
Substrate size	0.92	0.09	0.90	0.21
Substrate heterogeneity	0.95	0.08	0.93	0.14
Eigen value	2.73	1.68	2.99	1.64
Explained variance	45.5%	28.0%	49.8%	27.3%

running from microhabitat units located farthest from the shore to microhabitat units dominated by more heterogeneous and rougher substrate, close to pond dykes. PC2 was positively associated with submerged vegetation cover and density.

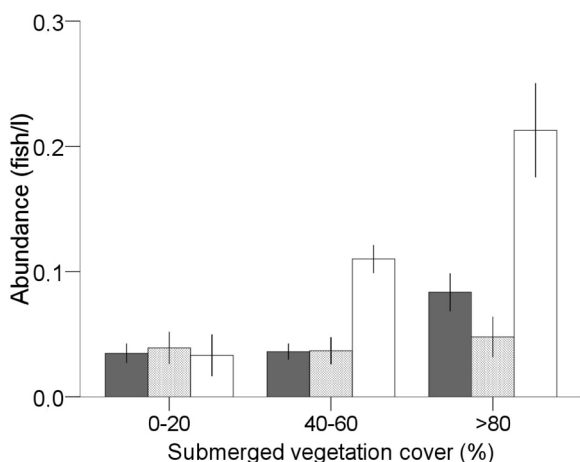
The Iberian toothcarp showed significant spatial and temporal differences in microhabitat use (Fig. 6). In sampling pond P1, during the low density period (Period 1), males and females positively selected microhabitats with a high cover and density of submerged vegetation, whereas juveniles demonstrated a strong avoidance of areas without submerged vegetation. Moreover, juveniles positively selected microhabitats with a rougher and more heterogeneous substrate (Fig. 6). Furthermore, species abundance (males, females and juveniles) was significantly positively correlated with PC2 (Table 3). Thus, the Iberian toothcarp showed maximum abundance values in microhabitats with well-developed meadows of submerged vegetation during the period of low density and large fish dominance. During Period 2, during re-

cruitment and with higher population densities, juveniles showed a negative selection of microhabitats located farthest from the shore and with scarce presence of submerged vegetation (Fig. 6). Moreover, their abundance was positively correlated with PC2 (Table 3), indicating higher abundance in locations with high submerged vegetation cover and density.

In sampling pond P2, during Period 1 there was a positive selection of microhabitats close to pond levees with high substrate size and heterogeneity, and a negative selection of locations farthest from the shore by each group of individuals (Fig. 6). Additionally, juveniles avoided microhabitats with lower submerged vegetation density and cover, and positively selected those with intermediate values (Fig. 6). Correlation analysis showed that the relative abundance of all groups (males, females and juveniles) was positively correlated with PC1 (Table 3). These results indicate that Iberian toothcarp abundance was higher in microhabitats close to levees with more available refuge areas (rough and heterogeneous



**Fig. 6:** *A. iberus* electivity (Ivlev's index,  $D$ ) for each of the three segments (1, 2, 3) in which the gradients of variation of microhabitat features were divided (PC1 and PC2) (Pond 1 in top graphics and Pond 2 in down graphics). Data are shown separately for males (black bars), females (grey bars) and juveniles (white bars) captured in each of the population dynamic periods: Period 1 (low densities of the species) and Period 2 (high densities of the species). Asterisks denote strong positive or negative selection ( $D > 0.5$  or  $D < -0.5$ , respectively).



**Fig. 7:** Mean abundance of *A. iberus* ( $\pm$  SE) according to the three classes of *R. cirrhosa* cover in Pond 1. 0-20: vegetation cover < 20% of the enclosed area; 40-60: vegetation cover > 40% and < 60% of the enclosed area; > 80: vegetation cover > 80% of the enclosed area. Data are shown separately for males (black bars), females (grey bars) and juveniles (white bars).

substrate). During Period 2, juveniles avoided microhabitats located farthest from the shore and those with high cover and density of submerged vegetation, whereas they positively selected intermediate values of the PC1 gradient (Fig. 6). However, no significant correlations between abundance and microhabitat gradients were encountered during this period (Table 3).

As regards enclosure traps, there were significant differences in the abundance of males among submerged vegetation classes (Kruskal-Wallis:  $H = 6.90$ ,  $p < 0.05$ ) (Fig. 7). Pair-wise comparisons revealed that significantly higher values were registered in traps with submerged vegetation cover higher than 80% (Mann-Whitney:  $U = 4.0$ ,  $p < 0.05$ , for both comparisons, class 3 vs. class 1 and class 3 vs. class 2). The abundance of juveniles was also significantly different between vegetation cover classes (Kruskal-Wallis:  $H = 12.23$ ,  $p < 0.01$ ), with higher values for class 3 compared to class 1 and class 2 (Mann-Whitney:  $U = 0.1$ ,  $p < 0.01$ ;  $U = 5.0$ ,  $p < 0.05$ , respectively). Moreover, abundance was higher for class 2 compared to

**Table 3.** Spearman's correlation coefficients between the Iberian toothcarp abundance and each of the components extracted by the PCA analysis in each of the study ponds (Pond 1 and Pond 2) of the Marchamalo salt pond system. \*  $P < 0.05$ ; \*\*  $P < 0.01$ .

	Pond 1				Pond 2			
	PC1		PC2		PC1		PC2	
	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2
Males	-0.10	0.08	0.35**	0.03	0.43**	-0.06	-0.05	-0.04
Females	-0.07	0.02	0.37**	0.02	0.48**	0.03	-0.20	-0.16
Juveniles	0.15	0.14	0.22*	0.22*	0.32**	-0.03	-0.19	-0.20



class 1 (Mann-Whitney:  $U = 2.5$ ,  $p < 0.01$ ). There were no significant differences in the abundance of females among classes of submerged vegetation (Kruskal-Wallis:  $H = 0.17$ ,  $p < 0.05$ ).

## Discussion

In the Marchamalo wetland, the Iberian toothcarp showed a non-random pattern of habitat use with spatial differences (environmental-effect) in the selection of microhabitat patches, according to the salinity level of the studied ponds. The observed spatial differences in abundance and population structure are probably related to the different environmental conditions in the studied ponds. The species showed higher abundances at the intake pond (P1), which was characterised by lower water conductivity values and high cover of submerged vegetation, consisting mainly of the phanerogam *R. chirrosa*. These habitat conditions may favour the species, since well-developed meadows of submerged vegetation could provide refuge against predators and abundant food resources for this species (Alcaraz *et al.*, 2008; Oliva-Paterna *et al.*, 2009). Furthermore, it has been demonstrated that lower values of salinity in these systems promote high diversity and biomass of macroinvertebrates (Takekawa *et al.*, 2006; López *et al.*, 2010), which are potential prey for *A. iberus*. In a study on *Aphanius fasciatus* (Nardo, 1827), Leonardos *et al.* (1996) reported that populations of this species inhabiting lower salinity habitats had higher growth rates and lower values of mortality due mainly to the abundant presence of aquatic macrophytes, which offered abundant food resources and refuge against piscivorous birds. Marchamalo wetland and the surrounding area of the Mar Menor lagoon maintain an important assemblage of these bird species mainly during summer months, which probably exert high predation pressure on *A. iberus*. In fact, together with its extreme physicochemical conditions, the absence of refuge created by submerged vegetation in the sampling pond with high water salinity (P3) may account for the lowest species density. Therefore, the observed spatial and temporal variability in population abundance at Marchamalo wetland is determined by the particular environmental conditions of each pond, as well as by the life-history of the species (Oliva-Paterna *et al.*, 2009).

As regards population size structure, it is noted the presence of a large proportion of newborn individuals (TL < 10 mm) in Pond 1 and their total absence in the most saline sampling pond, which is reflected by the lower mean size of juveniles in Pond 1 compared to Pond 3. This indicates reproduction failure or the presence of high mortality rates among younger individuals in Pond 3, which could be related primarily to the extreme water conductivity values recorded in this pond. In fact, previous data from the studied population showed a significant

decline in newborn individuals as a consequence of the drastic increases in salinity (Oliva-Paterna *et al.*, 2009). In general, the first life stages of fish are the most sensitive to salinity stress (Holliday, 1969) and particularly in cyprinodontid fish. A substantial increase in water salinity has been related to negative effects on several biological parameters such as growth, condition, oxygen consumption, activity and survival (Leonardos *et al.*, 1996; Plaut, 2000; Yildirim & Karacuha, 2008), thus affecting reproductive success. Moreover, it was noted that high salinity *per se* does not provide any reproductive advantage for our target species (Oltra & Todolí, 2000).

There were spatial differences, among the sampling ponds, as regards mean total length for females and juveniles, but not for males. This was probably due to differences in behavioural attributes between sexes, such as the territorial breeding behaviour of males, which has been observed for cyprinodontid fish species (Kodric-Brown, 1977; Clavero *et al.*, 2005). The Iberian toothcarp males also develop territorial behaviour (Rincón *et al.*, 2002), mainly during the breeding season, from May to September, defending small territories that function as sites for courtship, mating and ovoposition. This behaviour may reduce their dispersal through ponds or contribute to the dispersion of all male size-classes and consequently, there were no spatial differences in male sizes. On the contrary, females were significantly smaller in Pond 2 compared to Pond 1, probably as a result of a movement process involving small females from Pond 1. The higher proportion of females observed in this pond could force this demographic group to move away due to limited resource availability (McMahon & Matter, 2006). Results obtained with enclosure traps could reflect this pattern, since in summer the Iberian toothcarp males were more abundant in areas with well-developed meadows of *R. cirrhosa* (cover higher than 80%), which may be indicative of the territorial behaviour of males during the breeding season (spawning is made on aquatic macrophytes). In contrast, the abundance of females was not dependent on submerged vegetation cover, probably because they perform more displacements related to mating behaviour.

In Marchamalo wetland, *A. iberus* selected microhabitat as a function of refuge presence (submerged vegetation patches and zones close to levees), mainly during the period of low density and dominance of larger fish in its population dynamics. This period comprises the unfavourable time from November-December to March-April, when water temperature was normally below 20°C. During this period the metabolism and the somatic condition of Iberian toothcarp decrease (Oliva-Paterna *et al.*, 2009) and their activity and interactions could be reduced probably because of the search for refuge to overwinter (Valdimarsson & Metcalfe, 1998; Millidine *et al.*, 2006). The different use of sheltered microhabitats between ponds indicates a clear preference for vegetated microhabitats in

the sampling pond with lower salinity, whereas in the more saline pond, which was characterised by scarce submerged vegetation, the species selected microhabitats close to the levees with high substrate heterogeneity. The zones next to pond levees also provide protection against piscivorous birds because they are made of natural materials, such as boulders that offer shelter in the form of crevices and, unlike in industrial ponds, are covered with natural vegetation, which adds particular relevance to these structures as toothcarp refuge.

During the two periods juveniles showed high dependence on sheltered microhabitats throughout the sampling ponds. In this sense, results obtained using the enclosure traps also highlighted the valuable role of submerged meadows of *R. cirrhosa* as refuge areas for *A. iberus* juveniles. The trend to occupy sheltered microhabitats (mainly aquatic macrophytes) has been demonstrated previously for smaller individuals of *Aphanius baeticus* populations (Clavero *et al.*, 2005, 2007). The risk of predation could probably play a role in the use of sheltered microhabitats, although the high biomass and density of invertebrates occurring in submerged vegetation mats might also be an important factor (Alcaraz & García-Berthou, 2007).

The high adaptability of some cyprinodontids to extreme saline wetlands makes knowledge of their population dynamics and ecological traits in such habitats imperative for conservation management. Identification of the most relevant habitat characteristics necessary for the success of target populations inhabiting these aquatic systems is fundamental in designing effective recovery programmes. Salinity is an important stress factor for cyprinodontids, since osmotic pressure negatively affects their metabolism, growth performance and survival (Leonardos *et al.*, 1996; Plaut, 2000; Yildirim & Karacuha, 2008; Oliva-Paterna *et al.*, 2009). However, these effects do not contradict the fact that hypersaline systems are essential habitats for the recovery and conservation of Mediterranean toothcarps (Oliva-Paterna *et al.*, 2006; Freyhof & Brooks, 2011). The increase in abundance of native toothcarps and the improvement of their population status could be good indicators of the well-being of both natural and managed coastal wetlands.

Our results suggest the importance of management programmes that consider biological criteria in salt exploitation type wetlands. The conservation of *R. cirrhosa* meadows by controlling water salinity, since high salinity values prevent *R. cirrhosa* establishment (Sim *et al.*, 2006), is of special importance. Thus, the maintenance of meso-, or euhaline ponds should be used as a key tool in designing these programmes for the Iberian toothcarp. It would also be necessary to develop restoration and maintenance measures for the conservation of pond dykes according to the traditional techniques and materials, in order to increase toothcarp refuge.

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