Disruption of salinity regimes in Mediterranean coastal wetlands and its impact on the coexistence of an endangered and an invasive fish

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SUMMARY

Mediterranean coastal wetlands have been extensively eliminated and degraded by human activities. Salinity regimes are one of the main abiotic factors structuring biological communities in coastal wetlands and can be disrupted outflows from irrigated fields, especially through dilution. Here, we analyse patterns in salinity regimes as well as their temporal trends in coastal lagoons of the Ebro Delta, focusing on the impacts of rice farming. We then analyse the relationships between salinity regimes and the populations of two fish species, the Iberian toothcarp (*Aphanius iberus*) and the Eastern mosquitofish (*Gambusia holbrooki*). The former is a globally threatened cyprinodontid endemic to Spain and its decline has been linked to the impacts of mosquitofish.

The influence of rice farming in the Ebro Delta has disrupted the salinity regimes of its coastal lagoons, most of which have winter salinity peaks and low values during summer due to irrigation drainage from inundated rice paddies. Active management of these outflows has favoured increases of summer salinity in some lagoons between years 2001 and 2009, but during the same period summer salinity values have decreased in other lagoons. Between years 2005 and 2009 we sampled fish 55 times in the autumn, within 16 sites, capturing more than 10,000 fish. We related fish captures to the seasonal variation in water salinity. Toothcarp were more abundant in sites with higher summer salinities, i.e. with little influence of drainage effluent from rice fields. Mosquitofish tended to be less abundant when winter conductivities were high, suggesting that the maximum salinity levels during winter act as a filter for the species.

The agriculture-induced drop in salinity during summer seems to allow mosquitofish to occupy coastal lagoons. We discuss the interactions of rice culture with natural wetlands as well as the management implications for the conservation of the Iberian toothcarp.

Keywords: *Aphanius*, *Gambusia*, salinity, rice fields, Ebro Delta, wetland functioning, fish conservation, coastal lagoons

INTRODUCTION

Coastal wetlands are amongst the most threatened and most intensively degraded ecosystems in the world (Gibbs, 2000). Large amounts of coastal wetland areas have been drained due to land reclamation for agricultural uses and urban expansion. Moreover, the ecological functioning of many of the coastal wetland systems that remain have been strongly altered through human activities, such as hydrological infrastructures that change physicochemical cycles or hydroperiods (Moser *et al.*, 1996; Brinson & Málvarez, 2002). The situation is of special concern in the Mediterranean Basin, where most of the population is concentrated along the coastal strip (EEA, 1999) and there is a long history of wetland drainage and degradation (e.g. Brinson & Málavarez, 2002; Levin *et al.*, 2009). The massive loss of wetland areas during the 20th century in large Mediterranean deltas, such as those of the Po (Cencini, 1998) and the Rhône (Tamisier & Grillas, 1994) rivers, illustrate the threats posed to coastal wetlands.

Water salinity is one of the key abiotic factors structuring biological communities in coastal wetlands (Nordlie & Haney, 1998; Cardona *et al.*, 2008). Salinity is highly variable both temporally and spatially in Mediterranean coastal wetlands with naturally functioning regimes, due to the influences of sea water, river floods and rainfall as well as the effects of summer droughts (Pearce & Crivelli, 1994). Due to this natural variability, coupled with other sources of variation (such as periodic desiccation episodes), biological communities occupying coastal wetlands tend to be highly dynamic in time and space (e.g. Quintana *et al.*, 1998; Pérez-Ruzafa *et al.*, 2007). However, the influence of agricultural activities on many Mediterranean wetlands has often produced disruptions of their functioning. For example, many temporary systems have been converted in permanent ones and salinity has decreased due to agricultural outflows, which often dominate salinity regimes (e.g. Tamisier & Grillas, 1994).

The Iberian toothcarp (*Aphanius iberus*; Plate 1) is a Mediterranean fish species that has been driven to imperilment due in part to the loss of natural wetland habitats. This species, henceforth simply toothcarp, is a small (<60mm in total length) euryhaline and eurythermic cyprinodontid fish endemic to the Spanish Mediterranean coastal strip, which is categorized as Endangered (EN) in the IUCN Red List (Oliva-Paterna *et al.*, 2006). Coupled with wetland habitat loss and degradation, toothcarp decline is thought to have been caused by the impacts of Eastern mosquitofish (*Gambusia holbrooki*, henceforth simply mosquitofish) (Oliva-Paterna *et al.*, 2006), an invasive North American species introduced in the

early 1920s to the Iberian Peninsula for mosquito (and malaria-associated) control (Moreno-Valcárcel & Ruiz-Navarro, 2009). Aided by its ability to live in a wide variety of habitats, mosquitofish is presently one of the most widespread freshwater fish in the Iberian Peninsula, as it is worldwide

(Doadrio, 2002; Pyke, 2005). The negative ecological impacts of mosquitofish, as well as those of the congeneric Western mosquitofish (*G. affinis*), have been reported in many areas and affect different biological groups, such as native amphibians, fish and invertebrates (see Pyke 2008).

PLATE 1. Female (above) and male Iberian toothcarp (*Aphanius iberus*) from the Ebro Delta. Photograph by Mariano Cebolla

The impacts of mosquitofish on toothcarp populations have been shown to be modulated by water salinity. Toothcarp has disappeared from most of the freshwater areas that it had once inhabited, which are usually occupied by mosquitofish (Alcaraz & García-Berthou, 2007). Extant toothcarp populations are mainly restricted to saline coastal habitats, which have acted as effective refuge areas due to toothcarp's higher salinity tolerance (e.g. Moreno-Amich *et al.*, 1999). However, mosquitofish is also able to inhabit saline habitats (Pyke, 2005), even though salinity modifies life-

history features, such as reproductive strategy (Brown-Peterson & Peterson, 1990) and individual behavior (Alcaraz *et al.*, 2008). In fact, the closely-related Western mosquitofish has been shown to experience rapid, genetically-fixed adaptations in saline systems that increase its salinity tolerance (Purcell *et al.*, 2008) and similar processes are also plausible in mosquitofish.

We studied toothcarp and mosquitofish populations coexisting in saline environments in a large Mediterranean coastal wetland, the Ebro River Delta, which is one of the main toothcarp strongholds across the

whole range of the species. Most of the original wetland area in the Delta is currently devoted to intensive rice culture that involves the seasonal inundation of rice fields. Agriculture outflows from rice fields have been shown to have large influence on the salinity regimes of coastal lagoons within the Ebro Delta, which can become the opposite to what one would expect in similar naturally-functioning systems (Comín *et al.*, 1987; Rodríguez-Climent *et al.*, 2013). The main aims of our work are: i) to evaluate the level of disruption of natural salinity regimes in toothcarp habitats in the Ebro Delta; ii) to analyse whether there is any temporal trend in the influence of freshwater inputs on the salinity regimes of coastal lagoons; and iii) to analyse whether the disruption of salinity regimes is hindering the long-term conservation of toothcarp populations that occupy the Ebro Delta by favouring the occupation of brackish and saline habitats by mosquitofish.

STUDY AREA

The Ebro Delta is a large alluvial plain, of ca. 330 km^2, formed by the deposition of sediments as the river enters the Mediterranean Sea and divided by the river in two sectors, the northern and southern hemideltas (e.g. Valdemoro *et al.*, 2007) (Figure 1). The Delta has two sand spits that form two semi-closed bays. Originally, the alluvial plain was mainly covered by a mosaic of saline and freshwater wetlands, including lagoons, marshes and subterranean water outflows that maintained dynamic contact with the river and the Mediterranean Sea (Plate 2). Since the second half of the 19th and the beginnings of the 20th centuries the natural mosaic of habitats in the Delta have been largely changed to irrigated agricultural lands. Today, lagoons and marshes occupy only 5% of the original Delta surface. These remaining natural wetlands are included in the Ebro Delta Natural Park, which protects an area of around 78 km^2

(Curcó, 2006) (Figure 1). Extant toothcarp populations in the Ebro Delta are mainly included within the Natural Park, occupying lagoons, marshes and saltpans, although the species can also be occasionally found in bays and drainage canals of the irrigated agricultural lands. We studied fish communities in 8 coastal lagoons (Table 1) that have been traditional toothcarp habitat within the Ebro Delta (de Sostoa, 1983).

Almost two thirds of the Delta surface is currently devoted to rice culture and the hydrological characteristics of aquatic ecosystems in the Delta, including that of natural wetlands and bays, are strongly influenced by rice culturing activities (Comín *et al.*, 1987; Palacín *et al.*, 1992). Due to the saline nature of soils, rice fields are inundated between April and September with a high water renovation rate (3 to 5 days). Two irrigation canals begin at the Xerta dam, some 60 km upstream from the river mouth, diverting a water flow of some 40 m^3/s. Once the canals enter the Delta, they are subdivided to form a complex network of canals, moving low-conductivity water from the river to rice fields. Drainage outflows from fields are guided back to the river or the sea through an equally complex network of drainage canals. Inflow and outflow canals sum to more than 1000 km in length (March & Cabrera, 1997).

METHODS

Physicochemical parameters and fish sampling

We measured water temperature (ºC) and conductivity (mS×cm^{-1}) between 2001 and 2009 in 16 sites belonging to 8 different coastal lagoons within the Ebro Delta Natural Park (Table 1). We intended to conduct these water quality surveys on a monthly basis, although the final dataset has numerous gaps. Overall, we collected 1371 conductivity and temperature measurements.
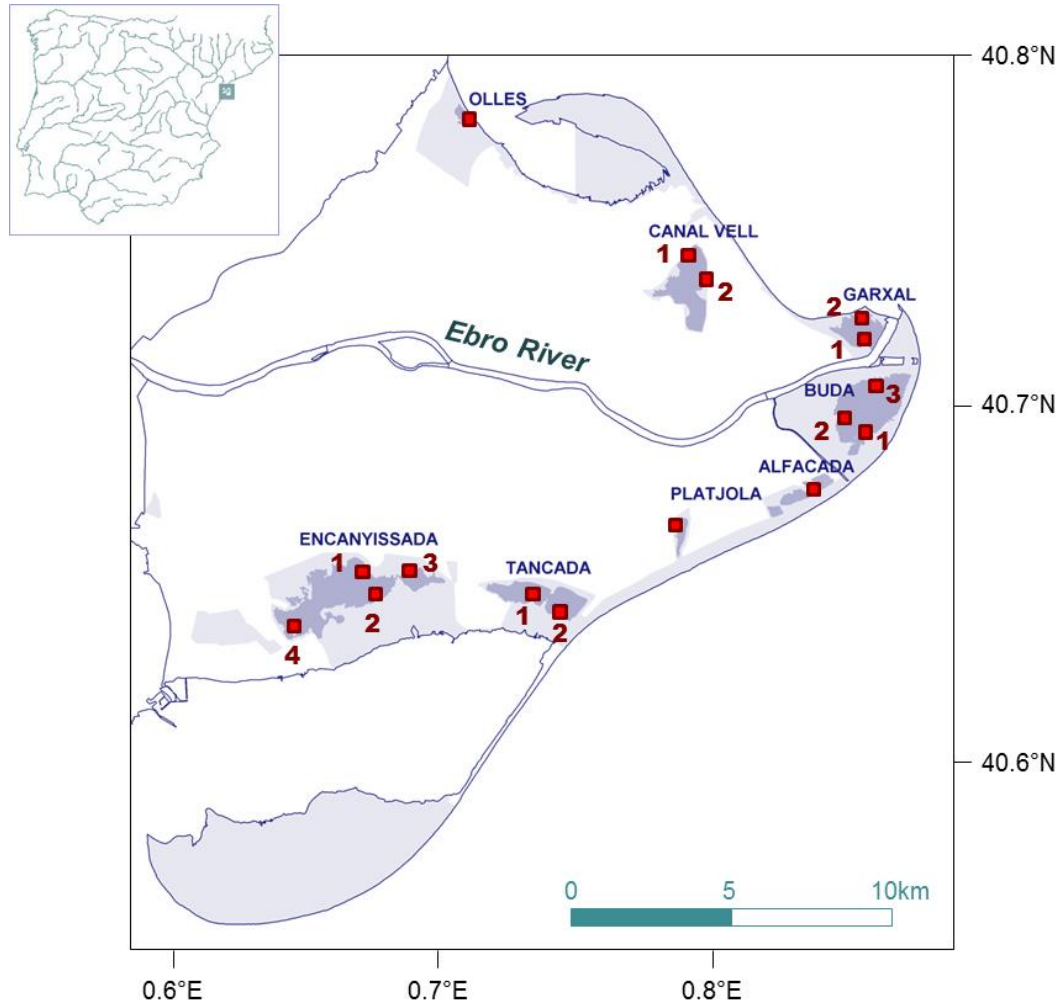
FIGURE 1. Map of the Ebro Delta showing the location of the 8 main coastal lagoons and the 16 sampling sites (red squares). In lagoons with more than one sampling site shown numbers are the same as those presented in Table 1. Light shading marks the extent of the Ebro Delta Natural Park. The position of the Ebro Delta within the Iberian Peninsula is shown in the upper left map, which also marks the main Iberian watercourses.

We monitored fish populations between 2005 and 2009 in the 16 sites mentioned above. Overall, we conducted 55 fish samplings (i.e. not all sites were sampled all years), all of them taking place between August 27th and October 18th, a period that coincides with the maximum abundance of toothcarp populations (e.g. Oliva-Paterna *et al.*, 2009). We used two alternative methods to sample fish: i) a hand drag net, locally known as *gamber*; and ii) fyke nets. The *gamber* is a semicircular net, with a radius of 100 cm and a 3 mm mesh-size, with which fish are captured by pulling it in a backward direction (i.e. the operator is in front of the net's mouth), preferably in vege-

tated microhabitats. Time was measured during fish sampling with the *gamber* and Catch-per-unit-effort (CPUE) was expressed as captures of individuals per minute of fishing. Fyke nets used here were about 100 cm in length, with two funnels and a 3.5 mm mesh size. We set between 1 and 8 fyke nets (mean, 3.8) for whole-day periods and CPUE was expressed as individuals captured per fyke net per 24 hours (e.g. Clavero *et al.*, 2006). Since CPUE had different units for each survey method, we used different statistical techniques (e.g. using "method" as random effect or as covariate) to control for the possible effects of survey method in the variability of catch data (see *Data anal-*

yses). The CPUEs of toothcarp and mosquitofish resulting from the two sampling techniques at the same sites showed a moderate (sensu Taylor, 1990) and positive correlation (Pearson's *r* values 0.42 and 0.44 for toothcarp and mosquitofish, respectively for log-transformed values in Table 1). These relationships were not statistically significant (*P*≤ 0.13) due to the low sample sizes (N= 14, since two of the sites were not sampled with *gamber*, see Table 1), although they can still be interpreted in terms of effect sizes (*r*). More importantly, both sampling techniques produced similar relationships between relative abundances and environmental data (e.g. Figure 5). Data obtained through the two fishing method are always identified in the graphics. Captured fish were identified to species level, with the exception of grey mullets that were pooled at the family level (Mugilidae), and released.

In each fish sampling event we recorded water conductivity and temperature, henceforth referred to as autumn conductivity and autumn temperature. Data from the water quality monitoring described above were used to compile the following variables: i) winter conductivity, as the average conductivity recorded in a given site in the winter preceding the fish survey (in fact winter-early spring, February to April); ii) summer conductivity, as the average conductivity recorded in the summer preceding the fish survey (July-August); and iii) summer temperature, as the temperature recorded at that site in June preceding the fish survey.

TABLE 1. Mean conductivity (mS cm^{-1}) and CPUEs, per sampling technique, of the two target species in the 16 sites surveyed for this study. The situation of the eight coastal lagoons is shown in Figure 1. Relative abundance values do not have units, since capture per unit of effort (CPUEs) values were calculated using two different capture techniques (see Methods); Fyke nets (Fyk): ind/(trap×day); *Gamber (Gbr)*: ind/min.

Lagoon	Code	Conductivity (mS cm^{-1})			Toothcarp		Mosquitofish	
		Winter	Summer	Autumn	Fyk	Gbr	Fyk	*Gbr*
Alfacada	AL	13.0	13.5	14.6	102.3	17.3	14.0	0.3
Buda	BU1	17.2	16.4	17.8	3.3	8.7	0.3	0.1
	BU2	14.4	8.9	12.4	3.0	2.2	0.8	28.0
	BU3	29.0	14.1	16.5	1.7	-	0.2	-
Canal Vell	CV1	20.5	11.1	11.7	12.5	10.1	1.4	7.7
	CV2	23.9	10.0	11.8	7.0	57.3	0	21.2
Encanyissada	EN1	36.2	6.9	21.2	0.7	4.8	0	5.0
	EN2	41.9	9.9	21.1	4.3	3.8	0	0.2
	EN3	18.4	3.0	6.5	0	-	0	-
	EN4	43.1	4.8	25.7	6.9	27.4	0.3	0.2
Garxal	GA1	8.5	15.5	13.5	8.4	28.6	331.3	100.3
	GA2	10.8	13.0	10.2	23.5	0.8	61.0	28.6
Olles	OL	7.7	1.7	5.3	0	0	116.5	4.8
Platjola	PL	15.8	2.4	3.3	3.3	0	2.7	53.8
Tancada	TA1	49.9	24.6	24.3	2.8	0.4	0	2.3
	TA2	48.4	27.6	27.9	4.5	13.7	2.0	0

Data analyses

We first analysed the seasonal variation in water conductivity in the Ebro Delta lagoons. We transformed conductivity values by using their square roots, since this transformation improved the normality of the data. We averaged monthly conductivity values for each sampling site and then standardized monthly data for each site by subtracting the site's mean from each value and dividing by the site's standard deviation. Through this standardization procedure we were able to compare in a direct manner the conductivity values among different lagoons in the Ebro Delta and data from other sites. We used standardized monthly salinity values as dependent variable in a one-way ANOVA analysis, using month as factor (i.e. there were 16 replicates for each month, one for each study site). To compare seasonal conductivity patterns observed in the Ebro Delta with those from a Mediterranean coastal lagoon system without agriculture-related freshwater inflows we compiled monthly data from La Pletera lagoons, located some 300 km to the north from the Ebro Delta (see Badosa *et al.*, 2006 for site description). Data from La Pletera were analyzed in the same way as those from the Ebro Delta.

We then analysed inter-annual trends in water conductivity. To do so, we selected summer (July-August) and winter (February-April) square root-transformed conductivity values and analyzed their variation between 2001 and 2009. These data were used as dependent variable in analyses of covariance (ANCOVAs), in which site was used as factor (16 levels) and calendar year was used as covariate. Our main goal was to test: i) whether there was a general temporal trend in conductivity values among Ebro Delta lagoons (tested through the calendar year significance); and ii) whether temporal trends were homogeneous across the sixteen study sites (tested through the site × year interaction). The effect sizes were assessed through the partial Eta squared (η_p^2), a statistic that (as R^2) is independent of the degrees of freedom used in the analyses (Tabachnick & Fidell, 2001). Different ANCOVAs were run for summer and winter conductivity data.

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To summarize the variation in the composition of fish communities in relation to physicochemical parameters, we performed a partial canonical correspondence analysis (CCA). CCA is a direct gradient multivariate analysis in which main gradients in species composition are directly related to a set of environmental variables, through a combination of ordination and multiple regression techniques (ter Braak & Smilauer, 1998). The species matrix used in the CCA included the CPUE of 7 species [transformed as $\log_{10}(\text{CPUE}+1)$] recorded in at least 3 of the 55 fish samplings. The environmental matrix included two temperature variables (summer and autumn) and three conductivity variables (winter, summer and autumn). Since we used two fish sampling methods (*gamber* and fyke nets), and this could influence ability to describe fish communities, we included the survey method as a covariable in order to control for the effects of sampling methodologies in the variability of fish data.

PLATE 2. Above, Tancada lagoon, surrounded by flooded rice fields. Bellow, the southern bay of the Ebro Delta (Badia dels Alfacs), with the Tancada lagoon in the foreground, followed by Encanyassda lagoon. Photographs by Mariano Cebolla

Preliminary detrended correspondence analysis produced a first axis of 2.9 SD units in length, and thus we used the biplot scaling in the CCA, which is recommended for gradient lengths up to 4 SD (ter Braak & Smilauer, 1998).

Finally, we analyzed the relationships between the abundances

[log$_{10}$(CPUE+1)] of toothcarp and mosquitofish with water conductivity and its temporal variability. For each species we performed a partial least squares regression (PLSR) analyses, using winter, summer and autumn conductivities as predictors. In the toothcarp PLSR, we also included the abundance of mosquitofish as predictor variable, to account for possible biotic interactions. PLSR is a statistical technique that combines features of multiple regression and principal components analyses (PCA) (Abdi, 2003) that is especially useful when predictors are highly correlated, a frequent feature of ecological datasets. PLSR searches for a set of components (called latent vectors) that maximize the covariation between the predictor dataset and the dependent variable and that, as for PCA components, are interpreted through the weights of the original variables (Abdi, 2003). The strength of the relationships between each original predictor and the dependent variable can also be evaluated through standardized coefficients (ß). PLSR is useful in many ecological analyses, which often include large amounts of predictors, and it has been shown to provide more reliable results than multiple regression or principal component regression (i.e. running PCA with environmental variables followed by multiple regression) (Carrascal *et al.*, 2009).

In spite of this, PLSR doest not account for possible unimodal responses or for the effect of possible confounding factors (e.g. survey methodology) that should be considered in the analyses as random effects. Therefore, we complemented PLSR results with linear mixed models (LMM) testing the effects of conductivity-related variables on log-transformed toothcarp or mosquitofish relative abundances. Survey method (2 levels) and site (16 levels) were used as random factors. For each species, we selected minimum adequate models through a stratified backward selection procedure. We fitted a LMM including all predictors and their quadratic terms. In a first step, deleted non-significant quadratic terms and then deleted non-significant predictor provided that its quadratic term had

been previously deleted (i.e. quadratic terms could only be included in the final models accompanied by the original variable).

RESULTS

Conductivity values ranged between 1.0 and 69.1 mS×cm^{-1} (i.e. salinity from ca. 0.5 to ca. 45), and averaged 16.2 mS×cm^{-1}. There were clear monthly variations in water conductivity (standardized values; $F_{11,180}$= 4.9; $P<$ 0.001). Maximum conductivity values were recorded in February and March, while minimum values were measured between July and September. From April to August there was a continuous decrease in water conductivity, coinciding with the period during which rice fields are flooded (Figure 2). These patterns are in clear contrast with those recorded in La Pletera, a pool of Mediterranean coastal lagoons that do not receive freshwater input from agricultural lands. In La Pletera maximum conductivity coincides with the summer drought period (July to September) during which minimum conductivity values were recorded in the Ebro Delta lagoons (Figure 2). The disrupted conductivity regime was a feature common to most surveyed lagoons, with the exception of el Garxal and, to some extent, Buda (Figure 3). There was not any clear interannual trend in conductivity common to all lagoons, although summer values showed a significant, though very weak temporal increase (Table 2). In contrast with that, the interaction term (Site × Year) had strong effects both in summer and winter ANCOVAs, showing that the conductivity of different sites followed different interannual trends. For example, summer conductivity increased in some sites within Encanyissada (EN1, EN2), Canal Vell (CV1) and Alfacada lagoons, while it decreased in Olles and Garxal (GA1) (Figure 3). Temporal trends of winter conductivity seem even more variable, with approximately half of the sites showing interannual decreases and the other half, increases.
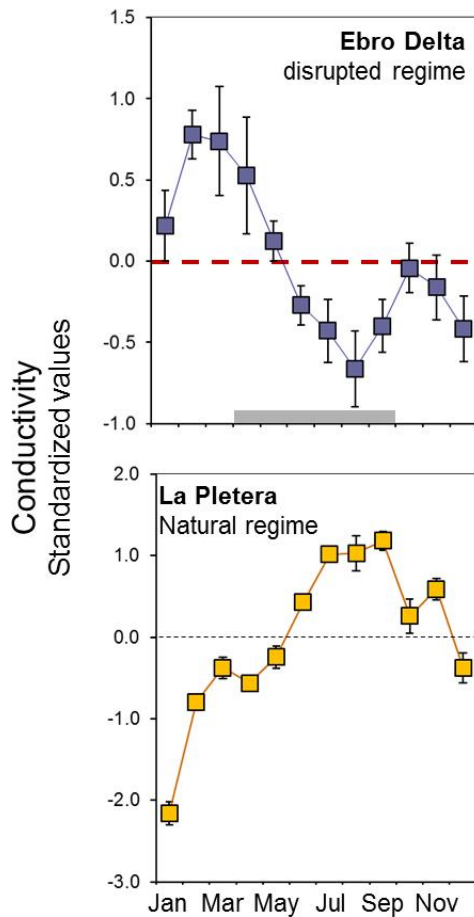
FIGURE 2. Monthly variation of conductivity values in two groups of coastal lagoons: the Ebro Delta and la Pletera. La Pletera lagoons are used as an example of salinity regimes in naturally functioning Mediterranean coastal wetlands, since they are not influenced by agricultural-related freshwater outflows. The thick grey line at the bottom of the upper panel marks the period during which rice paddies are inundated by water with a high renovation rate. Conductivity values were standardized prior to analyses to allow comparisons among different lagoons. Squares are average values (for 16 sites in the Ebro Delta and 4 lagoons in La Pletera) and whiskers are standard errors of the mean.

The main axis of variation in the composition of fish communities was positively related to winter conductivity and negatively to summer temperature (Figure 4). Mosquitofish were the dominant species in sites where low conductivity predominated during winter, while sites with high winter conductivity were dominated by species that are characteristic of estuarine and coastal wetland environments, such as pipefish (*Syngnathus abaster*), common goby (*Pomatoschistus microps*) and sand smelt (*Atherina boyeri*). The second axis of variation mainly discriminated communities characterized by the high abundance of grey mullets from those dominated by toothcarp, the latter having high values of summer conductivity (Figure 4).

In concordance with CCA results, the PLRS reported a positive relationship between toothcarp relative abundance and summer conductivity, although it also showed a negative relationship with winter conductivity. We did not detect any negative relationship between mosquitofish abundance and that of toothcarp. In fact this relationship was positive, although rather weak (Table 3). The final LMM analyzing the variation in toothcarp abundance retained only summer conductivity ($P= 0.015$) and its quadratic term ($P= 0.051$), denoting a unimodal response of toothcarp populations to salinity conditions experienced during summer (Figure 5). The PLRS using mosquitofish abundance as dependent variable showed a clear negative influence of winter conductivity and a positive, rather weak relationship with summer conductivity. Accordingly, the final LMM retained only the linear effects of winter conductivity ($P= 0.002$) (Figure 5).

DISCUSSION

Salinity regimes

Our results show that salinity regimes in the Ebro Delta lagoons are the opposite of what could be expected from naturally functioning Mediterranean coastal wetlands: conductivity is lowest during summer, when it should be highest. This pattern is due to the dilution effect of water from the Ebro River that is used to inundate rice fields and then is diverted to peripheral areas of the Delta, including the river itself as well as lagoons and the two bays. The disruption of the salinity regimes in lagoons of the Ebro
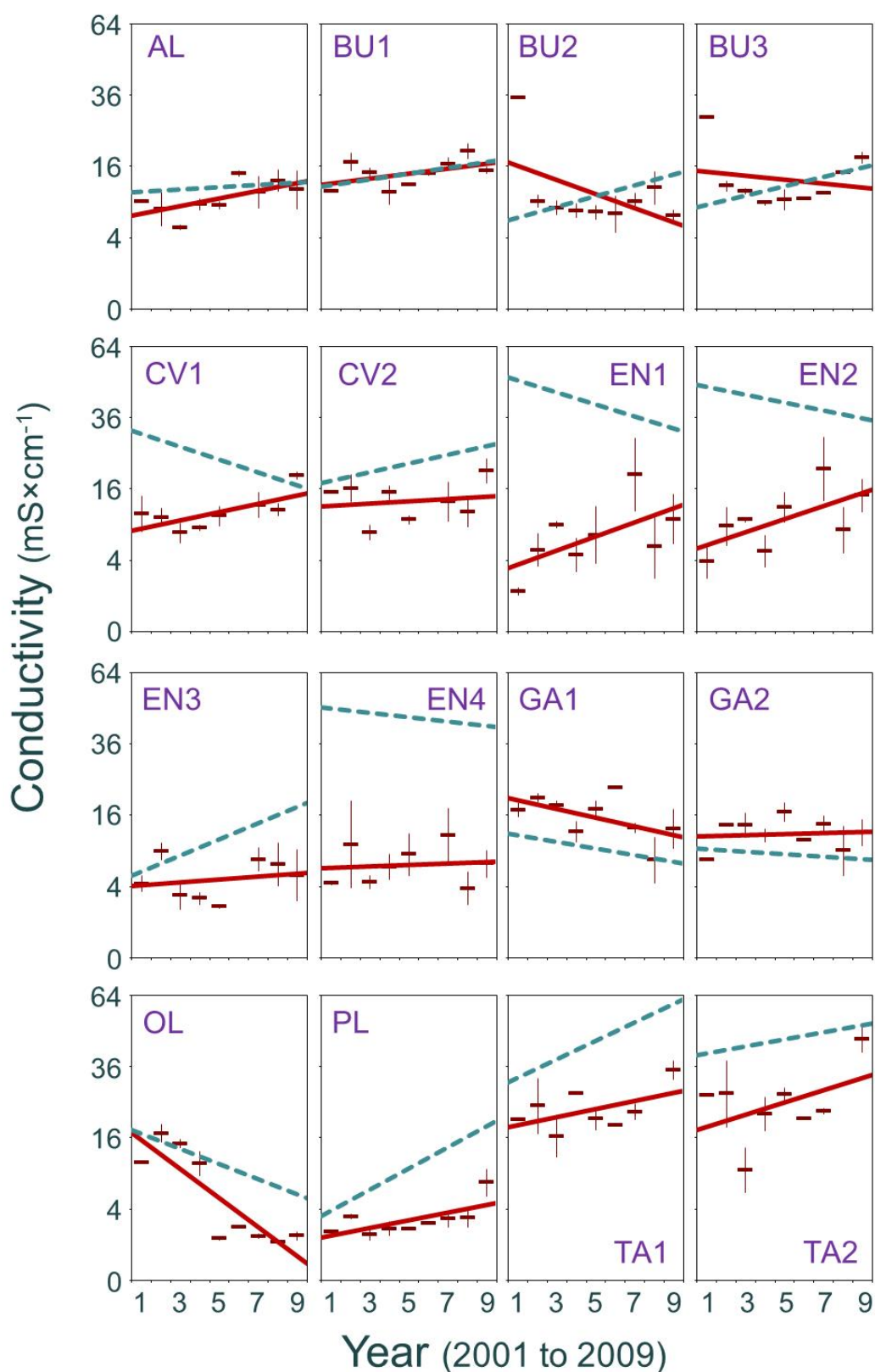
FIGURE 3 Temporal evolution of summer (brown lines and symbols; whiskers denoting standard errors) and winter (blue broken line) conductivity values in the 16 sites surveyed in the Ebro Delta. Average winter values are not shown. Codes for sites as in Table 1. Note that Y-axes follow quadratic scale because conductivity values had been square root-transformed.

TABLE 2. Results of the ANCOVAs testing the effect of site (the 16 sites listed in Table 1), year (calendar year between 2001 and 2009) and their interaction on the variation of summer and winter conductivity values. Significant interactions denote that the temporal evolution of salinity values have differed among sites (see Figure 3)

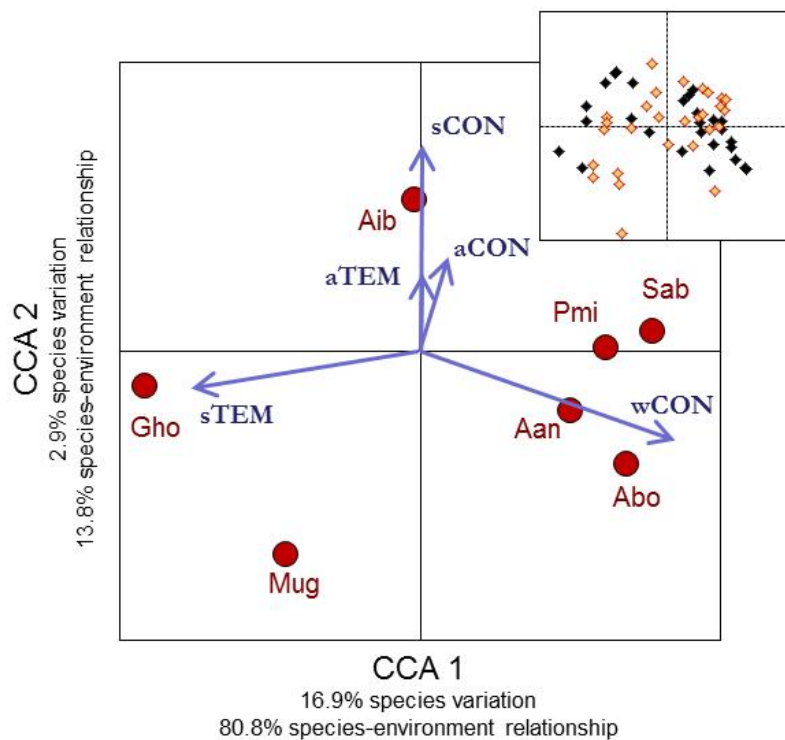|		d.f.	F	P	η_p^2
Summer	Site	14	4.5	<0.001	0.16
(July-August)	Year	1	4.8	0.03	0.01
R^2= 0.59	Site × Year	15	5.8	<0.001	0.21
Winter	Site	14	2.4	0.004	0.10
(February-April)	Year	1	0.1	0.75	< 0.01
R^2= 0.68	Site × Year	15	2.5	0.002	0.11

FIGURE 4 Graphic summary of the results from the partial canonical correspondence analysis (CCA) relating freshwater fish communities to conductivity and temperature variables in 55 fish sampling performed in the 16 study sites listed in Table 1. Species scores are marked by red points while the relationships between environmental variables and CCA axes 1 and 2 are represented by arrows. Species codes: Gho- *Gambusia holbrooki*; Mug- Mugilidae (pooled species); Aib- *Aphanius iberus*; Aan- *Anguilla anguilla*; Pmi- *Pomatoschistus microps*; Abo- *Atherina boyeri*; Sab- *Syngnathus abaster*. Environmental variables codes: sTEM- summer temperature; aTEM- autumn temperature; sCON- summer conductivity; aCON- autumn conductivity; wCON- winter conductivity. The upper-right panel shows the position of the 55 sampling events. Black diamonds represent surveys using *gamber* and yellow diamonds mark surveys using fyke nets. Sampling method was included as a covariate in the CCA in order to control for variations in fish communities derived from changes in sampling technique.

Delta was first reported by Comín *et al.* (1987), who measured seasonal variations in water conductivity in all lagoons analyzed here, with the exception of Garxal, in 1983 and 1984. Freshwater runoff from rice paddies has also been also shown to alter the salinity patterns in the Ebro Delta bays (e.g. Palacín *et al.*, 1992). Comín *et al.* (1987) further showed that the summer minimum in water conductivity coincided with peaks in phosphorus concentration, found to be a strong driver of eutrophication processes. Uncontrolled diversion of nutrient-rich freshwater into lagoons during the 1970s and 1980s produced radical changes in the characteristics of those systems, such as the disappearance of submerged macrophyte beds (e.g. Menéndez & Comín, 2000; Prado *et al.*, 2013).

Changes in Management actions and changes in agricultural practices were implemented after the establishment of the Ebro Delta Natural Park (in 1983), and especially since the 1990s, in order to reduce the environmental degradation of Ebro Delta lagoons. For example, a circumvallation channel was constructed around the Encanyssada lagoon to collect freshwater run-offs from rice fields, leading to a clear improvement of parameters such as turbidity, or cover of submerged macrophytes (Menéndez *et al.*, 1995). Circumvallation canals have been also built around the Tancada and Cana Vell lagoons and the connections of some lagoons with the sea are periodically dredged to increase the entry of marine water. However, as is clearly represented in Figures 2 and 3, current salinity regimes still departed strongly from expected natural patterns during our study period, and there was not a generalized trend towards increasing summer salinity among Ebro Delta lagoons. Some of the lagoons that have been intensively managed, such as the Encanyissada lagoon, seem to be recovering a more natural salinity regime, at least in some parts. However, the influence of freshwater drainage outflows seems to be increasing in other areas, such as Olles lagoon, which during our sampling period had

much lower conductivity values than those reported by Comín *et al.* (1987).

We also detected a trend towards decreasing salinity in Garxal lagoon (Figure 3), which was the only one among those sampled by us that maintained a natural salinity regime (i.e. with summer salinities being higher than winter ones). However, the Garxal, which is the youngest lagoon in the Delta, does not receive direct drainage outflows from rice paddies. Therefore the recorded trend towards decreasing salinity in GA1 was probably related to the direct influence of the Ebro River, to which the lagoon is connected. In recent years the water management authority has implemented flushing floods in the lower Ebro in order to reduce the density of aquatic macrophytes (e.g. Batalla & Vericat, 2009), which could arguably be increasing the penetration of freshwater into the Garxal lagoon.

Toothcarp and mosquitofish populations

Several authors have shown that water salinity has a strong effect on the distribution and abundance of mosquitofish (e.g. Nordlie & Mirandi, 1996) and hence modulates its impacts on toothcarp populations (e.g. Alcaraz & García-Berthou, 2007). Complementing previous observations, our work highlights the role of seasonal variability in salinity in the regulation of the populations of both species (see also Prado *et al.*, 2014). Each species responded to the salinity recorded in lagoons in different periods, mosquitofish being more abundant in sites with low salinity during winter and toothcarp thriving in sites with high salinity during summer (although this relationship was not linear, as discussed below). Strikingly, conductivity values recorded simultaneously to fish sampling (i.e. autumn values) were inferior predictors of toothcarp and mosquitofish abundance than the conductivity that their populations had experienced in the months preceding fish surveys.

The responses of mosquitofish and toothcarp populations to salinity fluctua-

tions fit well what could be expected given the differences in the salinity tolerance of both species (e.g. Alcaraz & García-Berthou, 2007). The least tolerant species, mosquitofish, was more intensively affected by maximum salinity episodes during winter, while in summer, when salinity is lowered by agricultural dilution, salinity values do not seem relevant for mosquitofish. The response of toothcarp, which is better adapted to saline environments, was more related to water salinity recorded during summer, the period of most intense dilution due to rice field outflows. Toothcarp were more abun-

dant in those lagoons that maintained a more natural salinity regime, with relatively high salinities during summer.

As said above, the abundance of toothcarp had a unimodal relationship with summer conductivity (Figure 5). Moreover, once the effects of summer salinity had been accounted for, the abundance of toothcarp had a negative relationship with winter salinity (see Table 3). These results might seem counter intuitive, given capacity of the species to inhabit even hyperhaline aquatic systems (e.g. Sanz-Brau, 1985). In the Ebro
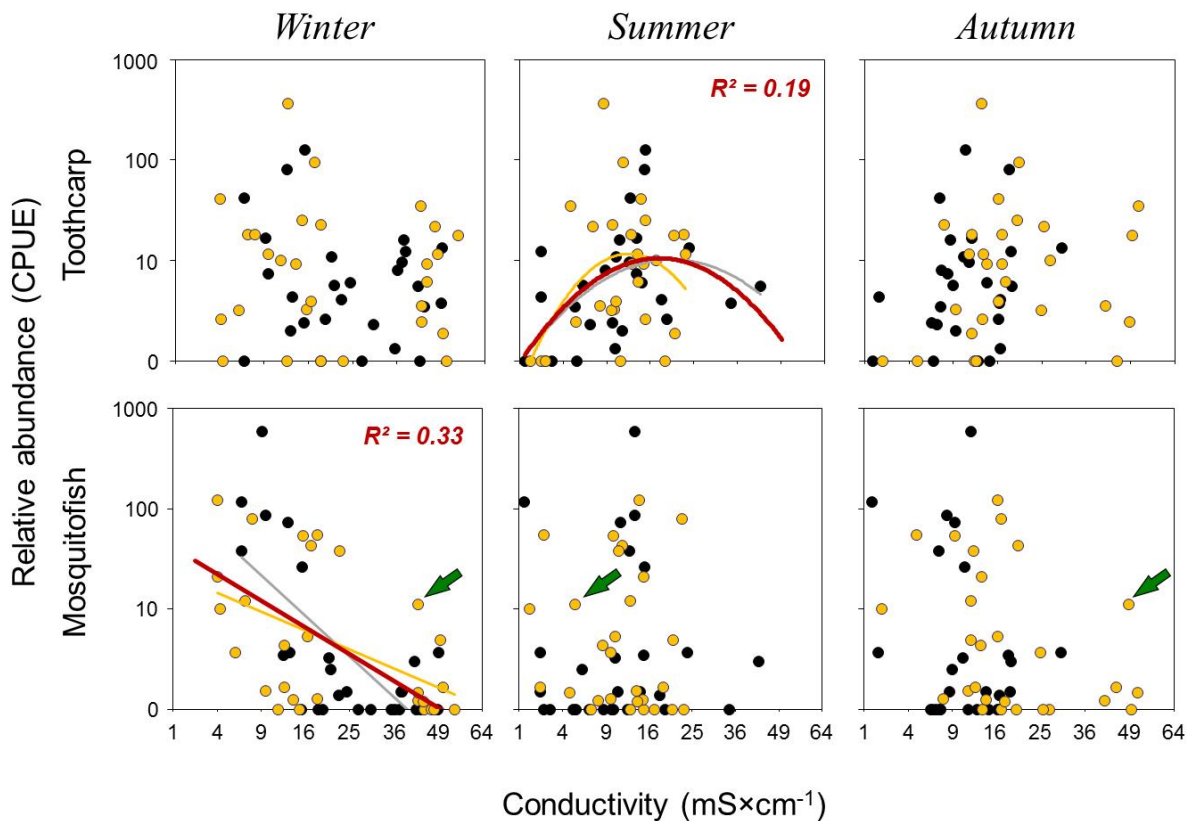

FIGURE 5. Relationships between the relative abundance of Iberian toothcarp and Eastern mosquitofish and the water conductivity recorded at different seasons. All fish data derive from autumn surveys, while conductivity values represent that recorded the previous winter, the previous summer and in the time of the fish survey. The arrows in the lower panels mark the position of a single survey in one locality (i.e. relative abundance does not change). Black circles (and grey lines) represent surveys using *gamber* and yellow circles (and yellow lines) mark surveys using fyke nets. The fitting brown lines represent the significant relationships found in the linear mixed models (see results section) and R^2 values are presented as an approximation to effect sizes. Capture per unit of effort (CPUE) are represented in a base-10 logarithmic scale and their units are not given since they were calculated using two different capture techniques. Conductivity is represented using a quadratic scale.

TABLE 3   Results of the partial least squares regressions (PLSR) analyses run for the two fish species under analysis. The table shows the weights of the original variables for the two first latent vectors extracted by the PLSR as well as the standardized regression coefficients (ß). Bottom rows show the proportion of the variance in fish relative abundance (R^2 of Y) and the proportion of the variance in the original predictor dataset (R^2 of X) accounted for by the extracted vectors

	Toothcarp			Mosquitofish		
	v1	v2	ß	v1	v2	ß
Winter conductivity	-0.31	-0.99	-0.21	-0.91	-0.59	-0.57
Summer conductivity	0.76	-0.10	0.28	-0.05	0.62	0.13
Autumn conductivity	0.50	0.02	0.19	-0.40	0.51	-0.06
Mosquitofish	0.27	-0.01	0.10			
R^2 of Y	0.15	0.01		0.29	0.05	
R^2 of X	0.27	0.41		0.51	0.30	

Delta the species is often abundant in salt-pans, with higher salinities than those recorded in lagoons. Notwithstanding, adverse effects of high salinity periods on toothcarp physiology and condition have been previously reported from other areas (Ruiz-Navarro *et al.*, 2008, Oliva-Paterna *et al.*, 2007). It is feasible that the energetic cost of osmoregulation may be reflected at the population level, leading to lower toothcarp abundances at very high salinities, and this may be the plausible explanation of what seems to occur in the Ebro Delta lagoons. On the other hand, the present use of saline habitats by toothcarp may not be reflecting actual habitat preference, since the species has disappeared from almost all previously occupied freshwater systems, arguably due to displacement by mosquitofish (e.g. Rincón *et al.*, 2002). For example, the species is currently absent from Les Olles lagoon, which has very low salinity values, while it had been recorded there in the early 1980s (de Sostoa, 1983) and up to the early 2000s (authors personal observations). The range of salinities currently used by toothcarp seems to be constrained by the presence of mosquitofish within the freshwater end of the salinity regime and by the physiological tolerance of the species at the saline extreme, hence producing a unimodal response to water salinity.

In spite of this, we did not detect any negative effect of mosquitofish on toothcarp. This contradicts widely reported deleterious effects of mosquitofish on toothcarp populations (Alcaraz & García-Berthou, 2007), which have been demonstrated in controlled experimental conditions (Rincón *et al.*, 2002; Caiola & de Sostoa, 2005). We believe that the expected negative relationships did not show up in our dataset because interspecific relationships are influenced by past unrecorded changes in the distribution of both species. At present, toothcarp has been completely eradicated from freshwater systems in the Delta, while its coexistence with mosquitofish in lagoons of the Ebro Delta may be possible due to the higher salinity of these systems. This was noted by Vargas (1993), who studied populations of toothcarp and mosquitofish between 1983 and 1984 in Canal Vell lagoon, where both species still coexist 25 years later. In agreement with Vargas (1993) our results suggest that this long-lasting coexistence is made possible by winter increases in salinity that limits mosquitofish populations. In addition, Alcaraz *et al* (2008) found that the level of mosquitofish aggressive interactions with another Mediterranean toothcarp species (*Aphanius fasciatus*) is negatively related to salinity. Therefore, salinity could possibly control mosquitofish abundance and at the same time reduce its *per capita* agonistic impacts

on toothcarp, which may facilitate the coexistence of both species. However, there may be other factors related to habitat structure that may facilitate coexistence between toothcarp and mosquitofish. For example, it has been suggested that the presence of abundant macrophyte cover (such as *Ruppia* beds) could allow the persistence of toothcarp in sympatry with mosquitofish, possibly by reducing the intensity of interspecific interactions (Fernández *et al.*, 2009)

Based on mosquitofish relationships with salinity in the Ebro Delta (Figure 5) and elsewhere (e.g. Bachman & Rand, 2008), it can be argued that its presence in coastal lagoons of the Ebro Delta is made possible by the dilution effects of rice field outflows. Mosquitofish would probably be unable to occupy these coastal lagoons if salinity regimes were less influenced by agricultural outflows and conductivity increased during spring and summer from winter minima (e.g. see La Pletera trend, Figure 2). Presently, mosquitofish populations probably pass through a high-salinity bottleneck during the winter after which they experience a low-conductivity period that coincides with the peak of its reproductive season in the Iberian Peninsula (Moreno-Varcárcel & Ruiz-Navarro, 2009). Mosquitofish in the Ebro Delta may have adapted genetically to higher salinities similarly to its congener Western mosquitofish (Purcell *et al.*, 2008). This adaptation may have been facilitated by selection pressure that is experienced by overwintering individuals and might explain the presence of relatively abundant populations in sites where for several months conductivity is over 40 mS×cm^{-1} (salinity over 25), as shown by the arrows in Figure 5 and as previously observed elsewhere (e.g. Ruiz-Navarro *et al.*, 2011, 2013). This possible high adaptability of mosquitofish casts doubt on the long-term sustainability of its coexistence with toothcarp, since more tolerant selected forms may displace toothcarp to increasingly saline habitats. These issues are currently speculative in nature and deserve further research, for which the Ebro Delta offers an ideal area for hypotheses testing.

Management implications and conflicts of interest

Our results show that water management related to rice culturing developed in the Ebro Delta modifies the physicochemical regimes of natural wetland areas and that these modifications affect fish populations, by facilitating the thriving of an invasive species. This is a clear example of how the interaction of habitat degradation and invasive species may drive the loss of biodiversity (Didham *et al.*, 2007). The conservation of native fish communities in the Ebro Delta lagoons, as well as that of a globally-threatened species such as toothcarp, largely relies on the maintenance of natural salinity regimes that limit the populations of invasive fish species such as the mosquitofish. Different management actions have been implemented in order to minimize the influences of anthropogenic activities on natural systems within the Delta (e.g. circumvallation canals, see above), but further attempts to restore the naturalness of coastal lagoons in the Ebro Delta are needed.

In recent times there has been a debate on the role of rice fields in the conservation of biodiversity, resulting in different, even contrasting points of view. For example, Tourenq *et al.* (2001) argued that rice fields are poor substitutes of natural wetland areas in the Rhône Delta, while Toral & Figuerola (2010) highlighted the importance of rice fields for the conservation of aquatic birds. However, most of this debate has been focused in the importance of rice fields for populations of aquatic birds, while not considering their influence on other components of biodiversity. This would seem illogical if we consider that the European populations of most aquatic bird species that benefit from rice fields are increasing (see Galewski *et al.*, 2011), while coastal ecosystems themselves and many components of their biota are highly threatened and declining. Most rice culture in Europe is currently viable due to economic subsidies that are tailored to fulfil agro-environmental measures designed to provide habitat for migrating and wintering

birds. In the case of the Ebro Delta, such measures ensure that the inundation of rice fields is maintained until January. We believe that agro-environmental cultivated wetland areas should expand their targets beyond maintaining abundant bird stocks and should focus on the natural functioning of coastal wetland ecosystems.

However, it may be difficult to regulate water circulation across the Ebro Delta only in terms of the natural functioning of wetland areas. The Delta achieved a stable human population only after the establishment of the irrigation system, in the second half of the 19th century, and the local population today is still tightly linked to rice cultivation. Moreover, there are other social groups with different interests in relation to the management of salinity regimes, most notably hunters and fishermen. Hunters prefer to maintain inundation periods in rice fields that promote abundant populations of their target game bird species. Artisanal lagoon fisheries managed by the local fishermen receive the most benefit from moderate to high salinities. Current management of the freshwater circulation across the Ebro Delta searches equilibrium between the pressures exerted by the local population and conservation objectives, but management aimed at biodiversity conservation should move in the direction of recovering the natural functioning of the Ebro Delta wetlands. Our results suggest that this would favour the populations of the globally threatened Iberian toothcarp. This could be directly tested by the experimental isolation of specific lagoons from irrigation canals accompanied by the monitoring of fish communities. This research would be valuable in evaluating the effectiveness of potential conservation measures as well as providing an opportunity to involve the local population in the recovery of the Ebro Delta wetlands and their biodiversity.

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AUTHOR CONTRIBUTIONS

All authors discussed the original idea; NF and JMQ collected the field data; MC and QPR analysed the data; MC lead the writing, which had contributions for all other authors.

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