

Rice, fish and crayfish in the Ebro Delta

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2 **Use of seasonally flooded rice fields by fish and**
3 **crayfish in a Mediterranean wetland**

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1 **ABSTRACT**

2 Rice fields constitute a significant proportion of the existing wetlands in the
3 Mediterranean basin and are important areas for the conservation of different vertebrate
4 species, especially birds. However, little is known on how fish and crayfish use rice
5 fields in Mediterranean areas. In this work we analyze fish communities and crayfish
6 populations occupying rice fields and their associated irrigation network (inflow and
7 outflow channels) in the Ebro Delta (NE Spain). We set fyke nets in 104 sites and
8 captured almost 23,000 fish belonging to 19 species, 9 of which were found to occupy
9 rice fields, as well as over 3,000 red swamp crayfish (*Procambarus clarkii*). Stone
10 moroko (*Pseudorasbora parva*), common carp (*Cyprinus carpio*), dojo loach
11 (*Misgurnus anguillicaudatus*) and Eastern mosquitofish (*Gambusia holbrooki*) were the
12 most common fish found in rice fields. More than 95% of the fish individuals captured
13 belonged to non-native species. Dojo loach, a recently introduced species well adapted
14 to rice cultivation cycles in its native range, used rice fields as reproduction ground.
15 Outflow channels seemed to be a more important source of fish colonizing rice fields
16 than inflow channels. Colonization was the main limitation for the establishment of fish
17 populations in rice fields and fish tended to be more abundant in rice fields than in
18 channels for any given frequency of occurrence. The importance of fish as trophic
19 resource for natural predators and the possible interactions between fish occupying rice
20 fields and rice yield, largely unexplored in the Mediterranean areas, could be managed
21 by modulating connectivity between rice fields and irrigation channels. Rice fields,
22 however, are not important areas for the conservation of native fish biodiversity, being
23 largely occupied by non-native fishes. Moreover, the influence of low-conductivity
24 water diverted for rice cultivation on natural wetlands favors the establishment and
25 expansion of different non-native fish species.

26

27 **KEYWORDS**

28 Rice, fish, crayfish, Invasive species, Ebro Delta, Agro-environmental measures

1 **1. INTRODUCTION**

2 Rice is one of the most important crops worldwide, being the staple food for almost
3 half the whole human population (Tsuruta et al., 2011). In 2009 rice fields occupied
4 over 158 million hectares around the world, almost 90% of them in Asia (FAOSTAT,
5 2011). The majority of rice fields are placed in former natural wetlands and
6 approximately 15% of the world's wetland area corresponds to rice paddies (Lawler,
7 2001). Thus, apart from their socio-economic importance, rice fields are a prominent
8 component of the planet's wetlands. It is therefore important to understand how wetland
9 biota uses rice fields and the role of these human-created wetlands in the conservation
10 or decline of biodiversity. This is especially relevant in the current context of
11 degradation and loss of natural wetlands, which have resulted in the disappearance of
12 over 50% of the original wetland areas in regions such as Europe or the USA (e.g.
13 Keddy et al., 2009; Strum et al., 2013).

14 From a global perspective, the area devoted to rice cultivation in south western
15 European countries is relatively small (less than 0.3% of global area), but these figures
16 grow in importance when put in the context of the available wetland area. For example,
17 the total area designated by Portugal, Spain and Italy as Wetlands of International
18 Importance in the framework of the Ramsar convention is around 432,000 hectares
19 (data from www.ramsar.org; accessed September 2011), while 386,000 hectares were
20 devoted to rice fields in 2009 in those three countries. Moreover, the crop has expanded
21 in Western Europe in the last decades, increasing by 53% between 1961-65 and 2005-09
22 (5-year averages) (Figure 1). These increases have been especially important in Italy
23 (+90%) and Spain (+75%), while the extension of rice fields decreased in the same
24 period in France and Portugal. Rice yields have also increased in these four countries
25 (Figure 1), arguably due to the use of agrochemicals (e.g. Suárez-Serrano et al., 2010a).

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1 European rice fields are placed mainly in lowland river plains or deltas under
2 Mediterranean-type climatic conditions. Fields are inundated between spring and
3 summer, thus having an inverted hydroperiod to that of surrounding natural temporal
4 wetlands, which tend to be dry during summer (Pearce and Crivelli, 1994). In fact, rice
5 fields provide the only available surface freshwater during summer droughts in many
6 Mediterranean wetlands. Rice fields are drained and kept dry during autumn and winter
7 to allow the oxidation of organic matter in soils, although some fields may be
8 maintained flooded during autumn to enhance water bird populations, whether for
9 hunting or with a focus on conservation (Forés and Comín, 1992; Elphick, 2004).

10 Rice fields can be occupied by a rich biota, including algae, aquatic plants, many
11 invertebrate taxa and a variety of aquatic and semi-aquatic vertebrates (Lawler, 2001).
12 Fish are often absent from natural temporary wetlands (Batzer and Wissinger, 1996),
13 but they can occupy seasonally flooded rice fields due to their high connectivity to
14 larger aquatic systems through irrigation infrastructures. As happens in natural
15 wetlands, fish can be key elements in the dynamics of rice field biota, since they can
16 structure communities through top-down mechanisms (Batzer and Wissinger, 1996) and
17 be important prey for other organisms that use rice fields, such as reptiles (Santos et al.,
18 2000) or birds (Lane and Fujioka, 1998).

19 Most of the research focused on the use of rice fields and their associated aquatic
20 habitats by fish has been developed in Asian countries (e.g. Bambaradeniya and
21 Amerasinghe, 2003; Katano et al., 2003). Many Asian rice landscapes are managed as
22 different types of rice-fish systems, in which farmers favour the populations of aquatic
23 animals in rice fields (mainly, but not only, fish) to increase their harvest (Amilhat et
24 al., 2009b; Koseki, 2014). There is only sparse information on the occurrence of fish on
25 south western European rice fields, where fish populations are not enhanced by farmers

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1 for fish production. In fact, to our knowledge, no study has specifically analysed fish
2 communities in European rice fields.

3 Here we provide information about the composition of fish communities that use
4 rice fields in the Ebro Delta, a large coastal wetland area in north eastern Iberian
5 Peninsula. We also characterised fish communities in associated irrigation channels,
6 both inflow and outflow ones, attempting to identify the origin and features of fish that
7 colonise rice fields. Specifically, our aims are: i) to characterise the identity and relative
8 abundance of fish occupying rice fields and their associated irrigation network; ii) to
9 assess the importance of the different possible pathways of occupation of rice fields by
10 fish; and iii) to analyse the population structure of the most common fish species in the
11 different aquatic environments linked to rice cultivation. The results are used to discuss
12 the possible interactions between fish communities and rice cultivation as well as the
13 importance of rice fields for the conservation of fish and other biodiversity components.

14 **2. STUDY AREA**

15 The Ebro Delta is a large alluvial plain formed in a West-East direction by the
16 deposition of sediments as the Ebro River enters the Mediterranean Sea. Around 20,000
17 hectares (more than 60% of the delta surface) are nowadays used for rice cultivation.
18 Rice fields are irrigated with water from the river. Some 40 m³/s are diverted at the
19 Xerta dam (some 60 km upstream from the delta) through two main channels, one at
20 each side of the river, built in 1860 (right side) and 1912 (left side) (March and Cabrera,
21 1997). Once entering the Delta, these two main channels are subdivided to form a
22 complex network of smaller channels, taking low-conductivity water from the river to
23 rice fields. Inundated rice fields have relatively shallow waters (in general less than 15
24 cm) and, due to the saline nature of soils, a high water renovation rate (3 to 5 days)

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1 during rice growing season, between April and September. After harvest, inundation is
2 maintained until January following agro-environmental measures, mainly to benefit
3 water birds. Water outflows from fields are conducted either back to the river or the sea
4 through an equally complex network of drainage channels. Inflow and outflow webs of
5 channels are connected exclusively through rice fields. The total channel network sums
6 more than 1000 km in length (March and Cabrera, 1997). Inflow channels are made of
7 concrete and have strong water current, while most outflow channels have a ground
8 (silty) bottom and carry much more calmed waters. Inflow channels are dried once
9 every year (between January and February) for maintenance operations.

10 **3. METHODS**

11 3.1. *Sampling*

12 Fish communities were sampled between June and October in 2007, 2008 and 2009.
13 We sampled fish communities in 104 sites: 40 inflow channels, 29 rice fields and 35
14 outflow channels. We chose outflow channels that were more dependent on water
15 leaving rice fields, avoiding those that were near the sea, the natural lagoons or the Ebro
16 River. Fish were captured with unbaited fyke nets, which had a single wing (of approx.
17 1m), two funnels and a 3.5mm mesh-size (Clavero et al., 2006). We chose this sampling
18 method because of its versatility, since it could be used in all three surveyed
19 environments. We usually set 3 fyke nets per site (mean 2.4; SD 0.6; range 1-3) leaving
20 them for one day (mean 21.8 hours; SD 0.6 hours; range 16-25.5 hours). Overall we set
21 255 fyke nets.

22 Captured fish were identified to species level, measured for total length and
23 released. Figures for three different grey mullet species (*Liza ramada*, *L. aurata* and
24 *Mugil cephalus*) were pooled in a single category (Fam. Mugillidae). We also counted

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1 red swamp crayfish (*Procambarus clarkii*) captured in fyke nets and analyzed these
2 catches as done with fish species (see below). Red swamp crayfish is an invasive
3 species present in the Ebro Delta since the 1980s, where it is currently a keystone
4 species (e.g. Suárez-Serrano et al., 2010b)

5 3.2. *Data analyses*

6 We compared fish species richness and abundances in the different surveyed
7 environments using individual fyke nets as sampling units in generalized linear mixed
8 models (GLMMs), specifying in all cases Poisson data distributions and logit link
9 functions. GLMMs were run using the library lme4 (Bates et al., 2013) as available for
10 R (R Core Development Team, 2011). Different sites could have different number of
11 fyke nets (1 to 3) and results from different fyke nets within any given site were not
12 independent. Therefore we included site (104 levels) as random factor, nesting it within
13 environment (3 levels), because there was no possible replication of the factor “site”
14 among environments (i.e., each site could only belong to one environment).. We tested
15 the effect of environment on the variation of total, native and introduced species
16 richness and on the relative abundances (i.e. total catch) of the most common fish
17 species and red swamp crayfish. Survey was assigned to a numbered 15-day period
18 starting June 1st (date) and this variable was introduced as a fixed-effect covariate in the
19 models, in order to control for possible temporal patterns in the dependent variables
20 along the survey period. Sampling effort (i.e. the number of hours that each fyke net
21 was set) was also introduced in GLMMs as a fixed-effect covariate, in order to control
22 for the influence of effort on the variation of the dependent variables. Graphic
23 representation of catch results were done in terms of catch per unit of effort (CPUE),
24 measured as $\text{individuals} \times \text{trap}^{-1} \times \text{day}^{-1}$ and $\log_{10}(X+1)$ transformed.

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1 Since uncertainty regarding the residual degrees of freedom preclude the use of p-
2 values in mixed models, the effects of fixed terms in the GLMMs were assessed
3 through the variation of the Akaike information criteria (AIC). For each variable, we
4 first fitted the full model, with environment, date and effort as fixed terms. The effect of
5 each fixed term was evaluated by deleting it and comparing the resulting model with the
6 full one. Following Burnham and Anderson (2002), the term was considered to have a
7 moderate effect when the AIC of the full model was at least 2 units smaller than the
8 AIC of the model excluding that term. If this difference was of at least 7 units, the effect
9 was considered to be strong. This strategy for the evaluation of effects was maintained
10 in all the mixed-model analyses explained below.

11 Fish abundance can be influenced not only by the quality of habitats, but also by the
12 capacity of species to reach those habitats, which in temporary habitats such as rice
13 fields is dependant on colonization events. To take into account the influence of the
14 frequency of occurrence on abundance we used the lme4 library to run mixed-effect
15 analysis of covariance (ANCOVA), testing the effects of number of occurrences
16 (covariate; square-root transformed) and environment (factor) on the number of caught
17 individuals [dependent variable; $\log_{10}(X)$ transformed]. The sampling unit of this
18 analysis was species \times environment and, since most species had been recorded in more
19 than one environment, we included species (17 levels) as random factor to ensure the
20 independency of data. We first performed a homogeneity-of-slopes test focusing on the
21 effect of the covariate \times factor interaction. The AIC of the model with the interaction
22 term was almost 3 units larger than that of the model without the interaction, denoting
23 parallel slopes among environments. Therefore we deleted the interaction from the
24 model and run a standard ANCOVA mixed model.

1 Finally, we compared the size of fish captured in rice fields and inflow and outflow
2 channels. To do so, we selected data from species of which at least 20 individuals had
3 been measured in all three environments and modelled their average size by means of
4 linear mixed models (LMMs) using the lme4 . As above, LMMs used site nested within
5 environment as random effect, since individual measures from any given site may not
6 be independent. We also introduced date as fixed-effect covariate, but in this case
7 sampling effort was not included as a predictor. Only female eastern mosquitofish
8 (*Gambusia holbrooki*) were included in the size analysis, due to the strong sexual
9 dimorphism of the species.

10 **4. RESULTS**

11 Overall, we captured almost 23,000 fish belonging to 19 species, 9 native (including
12 3 grey mullet species) and the rest introduced, as well as over 3,000 crayfish and several
13 other organisms such as frogs, snakes, shrimps and insects. Stone moroko
14 (*Pseudorasbora parva*) and eastern mosquitofish were the dominant fish species in
15 terms of abundance. We detected 9 fish species in rice fields, the most frequent of
16 which were common carp (*Cyprinus carpio*), stone moroko and dojo loach (*Misgurnus*
17 *anguillicaudatus*). Stone morko and Ebro barbel (*Luciobarbus graellsii*) were the most
18 frequent species in inflow channels (Table 1).

19 Almost two-thirds (63%) of the fyke nets set on rice fields captured fish, this
20 percentage rising to 86% in inflow channels and to 100% in outflow channels. Crayfish
21 was more often recorded in rice fields (81%) than in inflow or outflow channels (40%
22 and 67%, respectively). Fish species richness was lower in rice fields than in irrigation
23 channels, a pattern that was especially strong for native species. Average native species
24 richness in rice fields was roughly 8 times lower than that of introduced species.

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1 Richness also tended to be higher in outflow channels than in inflow ones, especially in
2 the case of introduced species. Fyke nets set on outflow channels captured on average
3 2.7 introduced species, over two-times the richness recorded by nets on rice fields or
4 inflow channels (Figure 2; Appendix A).

5 The relative abundance of most species clearly varied among the three surveyed
6 environments (Figure 3; Appendix A). The maximum abundance of Ebro barbel was
7 recorded in inflow channels, while stone moroko and eastern mosquitofish were much
8 more abundant in outflow channels than in the other environments. Common carp and
9 dojo loach were also more abundant in outflow than in inflow channels, having
10 intermediate abundances in rice fields. Eel (*Anguilla anguilla*) and wels catfish (*Silurus*
11 *glanis*) had similar abundance patterns, being slightly more abundant in outflow
12 channels than in inflow ones and very scarce (eel) or absent (wels catfish) from rice
13 fields. Crayfish was clearly more abundant in rice fields than in channels, as well as in
14 outflow than in inflow channels.

15 As expected, the number of occurrences of the different fish species had a strong,
16 positive influence on the number of individuals caught in the different environments
17 ($\Delta AIC = 74.7$; Figure 4). But, in apparent contrast with results presented in Figure 3,
18 fish species were more abundant in rice fields than in channels for any given frequency
19 of occurrence ($\Delta AIC = 14.2$).

20 Stone moroko and dojo loach individuals caught in inflow channels were larger than
21 those from rice fields or outflow channels. These differences are arguably caused by the
22 scarcity of young-of-the-year (YOY) individuals of these species in inflow channels.
23 Common carp YOY were also very rare in inflow channels, although size differences
24 among environments were relevant, as shown by small variation of AIC between the
25 model with and without the “environment” term. The sizes of eastern mosquitofish and

1 Ebro barbel did not differ among environments (Figure 5), but were related to the date
2 covariate (Appendix A).

3 **5. DISCUSSION**

4 Our results show that Mediterranean rice fields can host an important number of fish
5 species. Nine species occupied rice fields, while the whole system (including channels)
6 had 19 fish species belonging to 11 families. The scarce existing literature on the biota
7 of rice fields in Mediterranean areas reports less fish species and/or fish occurring in
8 much lower frequency. For example, Fernando (1993) did not detect fish occupying rice
9 fields in the Camargue (southern France), during a visit in the early 1980s. Santos and
10 Llorente (2009) surveyed 49 rice fields in the Ebro Delta and recorded the presence of
11 fish, which were not identified, in 18 (37%) of them. González-Solís et al. (1996) and
12 Marques and Vicente (1999), studying food availability for waterbirds, captured only 2
13 species, mosquitofish and common carp, in rice fields of the Ebro Delta and the Sado
14 estuary (Portugal), respectively. Fish richness in the Ebro Delta rice fields is lower than
15 most values reported for fields in tropical areas, mainly in south-east Asia, where more
16 than 30 species can be recorded (e.g. Fernando, 1993). However, overall figures are
17 similar or larger than those from rice ecosystems studied in India (19 species, Aditya et
18 al., 2010), Japan (19 species, Katano et al., 2003), or Brazil (11 species, Rodrigues et
19 al., 2011).

20 Since fish can only occupy rice fields during the flooded season, colonization of fish
21 must occur yearly from the irrigation channel network. As explained in the description
22 of the study area, inflow channels are dried up each winter for maintenance operations.
23 Arguably as a consequence of this, average fish species richness was almost two-times
24 larger (see Figure 2) and CPUE was more than one order of magnitude higher (258.5 vs.

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1 13.9 individuals \times trap⁻¹ \times day⁻¹) in outflow channels than in inflow ones. These
2 differences may be also promoted by the more natural, silt substrate of outflow channels
3 and their high connectivity to permanent aquatic habitats (marshes, lagoons, the Ebro
4 River or the sea). Outflow channels would thus be a more prominent source of fish
5 colonizing rice fields than inflow channels. This seems to be confirmed by the fact that
6 the most frequent fish species found in rice fields (stone moroko, common carp, dojo
7 loach and mosquitofish) are clearly more abundant in outflow than in inflow channels.
8 But some fish species can also occupy fields from inflow channels, as seems to be the
9 case of Ebro barbel and pumpkinseed sunfish (*Lepomis gibbosus*). The occupation of
10 rice fields by dense fish populations seems to be mainly constrained by the ability of
11 fish to colonize them, with no apparent limitation due to habitat quality. As shown in
12 Figure 3, when colonization barriers are overcome fish can be abundant in rice fields,
13 more so than in channels for any given frequency of occurrence. In contrast, red swamp
14 crayfish, which is able to permanently occupy intermittent aquatic habitats (e.g.,
15 Aquiloni et al., 2005), develop denser populations in rice fields than in any other aquatic
16 environment within the Ebro Delta.

17 Stone moroko and dojo loach are recent non-native additions to the Iberian
18 ichthyofauna that were first cited in the Ebro Delta (Caiola and de Sostoa, 2002; Franch
19 et al., 2008). Both of them are Asian species which are frequently found in rice fields in
20 their native areas, especially the dojo loach, which is often the most common species
21 (e.g., Katano et al., 2003). Dojo loach is known to make reproductive migrations into
22 rice fields from associated channels (Fujimoto et al., 2008) and is able to colonize a
23 wide range of types of rice fields (Katayama et al., 2011). Since flooding regime is
24 similar to that reported in Japanese studies, it is plausible that dojo loach could be using
25 rice fields in the Ebro Delta as nursing grounds. This seems to be supported by the large

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1 proportion of small young-of-the-year individuals found in rice fields (see Figure 5) and
2 could explain the successful establishment and secondary expansion of the species in
3 the Ebro Delta (Franch et al., 2008). Although patterns are not as clear as in the case of
4 dojo loach, the analysis of the size structures of captured individuals suggest that stone
5 moroko and common carp could also be reproducing in rice fields.

6 Fish have been shown to have positive effects on rice yields, mainly through the
7 control of potential animal pest and weeds (Tsuruta et al., 2011; Xie et al., 2011). The
8 presence of fish within rice fields also reduces the inter-annual variability of the yield
9 and minimizes the use of agrochemicals (Xie et al., 2011). But these knowledge comes
10 from Asian countries in which there is a millenary tradition of rice-fish co-culture (Lu
11 and Li, 2006), which often involves the annual stocking of fish into fields (Amilhat et
12 al., 2009b). Nothing is known about the possible interactions between wild fish
13 populations and rice production in Mediterranean areas, but it seems likely there could
14 also be positive effects of the presence of fish. For example, the apple snail (*Pomacea*
15 *caniculata*) is an agricultural pest first cited in the Ebro Delta in 2009 (López-Soriano et
16 al., 2009) that could be in part controlled through predation by fish and other aquatic
17 animals (Yusa et al., 2006). Since obstacles to colonization seem to be an important
18 constraint of fish occupations of rice fields, management of fish populations within
19 fields could be undertaken by modulating the connectivity with the irrigation network,
20 especially with outflow channels. Further improvement of fish habitats could involve
21 the establishment of small ponds, which could offer refuge for fish in the very shallow
22 waters of rice fields. These structures are widely employed in Asia to enhance fish
23 production (e.g., Amilhat et al., 2009a).

24 In contrast with the plausible positive effect of fish, red swamp crayfish has clear
25 negative impacts on rice cultivation, both by seed and seedling consumption (Anastácio

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1 et al., 2005) and by its borrowing behavior that can lead to the collapse of field and
2 channel margins (Barbaresi et al., 2004; Arce and Diéguez-Uribeondo, 2015). However,
3 any hypothetical change made in the rice agroecosystem in the Ebro Delta to favor fish
4 populations (e.g., enhanced connectivity, habitat improvement) is not likely to affect
5 crayfish populations in rice fields, because it is precisely in the fields where the species
6 attains its maximum densities.

7 Fish inhabiting rice fields, together with amphibians, crayfish and insects, are
8 important prey for many waterbirds, including herons, gulls, ducks and waders (Lane
9 and Fujioka, 1998; Elphick, 2000; Czech and Parsons, 2002). In Mediterranean areas
10 rice fields remain flooded during summer, when natural wetlands are dry or much
11 reduced in extent, and thus in that period fields concentrate large numbers of birds
12 (Toral and Figuerola, 2010). Fasola et al. (1996) highlighted the importance of rice
13 fields for heron conservation in large Mediterranean wetlands (including the Ebro
14 Delta), showing that six species obtained more than half of their trophic resources from
15 fields. The high availability of red swamp crayfish in rice fields favored the growth of
16 the Audouin gull (*Larus audouinii*) colony of the Ebro Delta (Navarro et al., 2010),
17 which in the early 2000s included about one half of the global breeding population of
18 the species. The benefits obtained by aquatic predatory birds from the availability of
19 fish, crayfish and other prey could be however thwarted by the increased contact with
20 agrochemical pollutants (Ochoa et al., 2012) or the establishment of trophic dependency
21 with an anthropogenically managed systems, which are disconnected to natural
22 phenomena (Fasola and Ruiz, 1996).

23 In contrast with the positive effects of rice fields and its biota on the conservation
24 status of aquatic birds in Mediterranean areas, rice fields do not seem to be positive for
25 the conservation of the aquatic biodiversity itself. Less than 5% of fish individuals

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1 captured during the work (557 out of 22966) or specifically in rice fields (22 out of 614)
2 belonged to native species. This dominance of non-natives has probably increased in
3 recent times due to the establishment of fish species that are well adapted to occupy rice
4 fields (dojo loach and stone moroko, see above). In addition, the invasive red swamp
5 crayfish was clearly a dominant species within rice fields, where an important number
6 of other non-native invertebrate taxa occur, notably gastropods and ostracods (Oscoz et
7 al., 2010). The high degree of invasion in rice fields could be related to the decline of
8 native taxa, such as the green frog (*Pelophylax perezi*) (Santos and Llorente, 2009).
9 Amphibians are sensitive to predation by fish species present in Ebro Delta rice fields,
10 such as stone moroko (Teplitsky et al., 2003), as well as to the presence of red swamp
11 crayfish (Cruz et al., 2006). Furthermore, the high availability of trophic resources in
12 rice fields, mainly in the form of non-native fish and crayfish, could be triggering
13 hyperpredation processes (e.g. Courchamp et al., 2000; Pope et al., 2008), through
14 which increasing waterbird population, based on non-native resources, would be driving
15 the decline of native taxa, such as amphibians or snakes (Santos and Llorente, 2009).
16 The facilitation effects of rice cultivation on the non-native aquatic biota of the Ebro
17 Delta expand outside rice fields and their associated channel network. Most non-native
18 fish species occupying the Ebro Delta are freshwater dwellers and their presence is
19 often associated with the freshwater inputs transported from the Ebro River to the rice
20 fields (e.g., Franch et al., 2008). The irrigation system has modified the hydrological
21 functioning of natural aquatic systems within the Ebro Delta, such as coastal lagoons,
22 often implying clear reductions in salinity (Comín et al., 1987). This disruption of
23 natural salinity regimes has been shown to favor invasive mosquitofish at the expense of
24 the endangered Spanish toothcarp (*Aphanius iberus*) (Clavero et al., 2015).

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1 In conclusion, rice fields in the Ebro Delta host a much more diverse fish and
2 crayfish community than it had been previously reported for Mediterranean rice fields.
3 This biota has probably positive interactions with rice cultivation (fish) but also
4 important negative effects (crayfish). Aquatic organisms occupying rice fields in the
5 Ebro Delta have been important in the recovery of many waterbird species, but they are
6 mainly non-native species that interact negatively and through diverse pathways with
7 native aquatic fauna. Besides focusing on yield, rice fields should be managed taking
8 into account their importance for the biota that inhabits them (with a focus extending
9 beyond waterbirds), as well as their influence on surrounding natural systems.

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Table 1. List of fish species captured in rice fields (Rice) and their associated irrigation channels (Inflow and Outflow) in the Ebro Delta, showing the total number of individuals of caught for each species, their status (I, introduced; N, native) and their frequency of occurrence in the three environments associated to rice cultivation. Grey mullets (Fam Mugilidae) include at least three species: *Mugil cephalus*, *Chelon labrosus* and *Liza ramada*. Species codes are later used in Figures 3 and 5. Data for red swamp crayfish and other captured taxa are also shown.

					Frequency of occurrence (%)		
					Inflow	Rice	Outflow
					(N=108)	(N=59)	(N= 88)
		Code	Status	Individuals			
<i>Pseudorasbora parva</i>	Stone moroko	PPA	I	12838	39.8	27.1	92.0
<i>Gambusia holbrooki</i>	Eastern mosquitofish	GHO	I	8611	12.0	18.6	77.3
<i>Misgurnus anguillicaudatus</i>	Dojo loach	MAN	I	547	27.8	23.7	38.6
<i>Luciobarbus graellsii</i>	Ebro barbel	LGR	N	293	39.8	10.2	30.7
<i>Anguilla Anguilla</i>	Eel	AAN	N	152	29.6	3.4	44.3
<i>Cyprinus carpio</i>	Common carp	CCA	I	150	11.1	27.1	31.8
<i>Silurus glanis</i>	Wels catfish	SGL	I	65	15.7	-	18.2
Fam. Mugilidae	Grey mullets		N	106	-	-	34.1
<i>Alburnus alburnus</i>	Bleak	AAL	I	159	3.7	5.1	6.8
<i>Lepomis gibbosus</i>	Pumpkinseed sunfish	LGI	I	15	7.4	1.7	1.1
<i>Carassius auratus</i>	Goldfish		I	6	3.7	-	2.3
<i>Scardinius erythrophthalmus</i>	Rudd		I	9	1.9	-	3.4
<i>Salaria fluviatilis</i>	Freshwater blenny		N	4	3.7	-	-
<i>Gobio lozanoi</i>	Iberian gudgeon		N	7	0.9	3.4	-
<i>Sander lucioperca</i>	Pikeperch		N	2	1.9	-	-
<i>Atherina boyeri</i>	Sandsmelt		N	1	-	-	1.1
<i>Aphanius iberus</i>	Spanish toothcarp		N	1	-	-	1.1
Total fish catches (n)				22966	1364	614	20988
<i>Procambarus clarkii</i>	Red swamp crayfish	PCL	I	3238	39.8	81.4	67.0
<i>Pelophylax perezi</i>	Iberian green frog			60	1.9	22.0	5.7
<i>Natrix maura</i>	Viperine snake			5	-	3.4	3.4
	Shrimps			17	4.6	-	4.5
	Insects			31	0.1	8.5	1.1

Figure 1. Evolution along a 49-year period (1961-2009) of: i) the area devoted to rice cultivation (lines); and ii) rice yield (crosses) in south-western Europe, presented also separately for Portugal, Spain, France and Italy. Data obtained from FAOSTAT (2011)

Figure 2. Average species richness (\pm SE) caught per fyke net set during 24 hours in each of the aquatic environments associated to rice cultivation: INFLOW- inflow channels; RICE- rice fields; OUTFLOW- outflow channels. Results are shown for all fish captures (A) and separately for native (B) and introduces (C) fish species. The asterisks indicate the relevance of the “environment” term in generalized linear mixed models analyzing richness while controlling for sampling date and sampling effort, based on changes of AIC (see methods): ** strong effect (Δ AIC > 7); * moderate effect ($7 \geq \Delta$ AIC > 2).

Figure 3. Average relative abundance [$\log_{10}(\text{CPUE}+1)$] (\pm SE) of the seven most widespread fish species in the aquatic systems associated to rice cultivation in the Ebro Delta, plus that of the red swamp crayfish. The asterisks indicate the relevance of the “environment” term based on changes of AIC, as explained in Figure 2. Species codes are the same as those in table 1.

Figure 4. Relationship between the number of occurrences of a species (note the quadratic progression) and the number of individuals of that species caught (note the exponential progression), shown separately for the three environments analyzed. Each dot represents a species in an environment, and thus a single species can be represented by up to three dots.

Figure 5. A) Size-structure of the most abundant fish species in the three surveyed habitats. Numbers in the X-axes of histograms show the range of sizes represented for each species and, in parenthesis, the magnitude of the size classes employed (in mm). The number of individuals measured is indicated by the number within each panel. B) Average size (\pm SE) of the same species in the three environments, with asterisks denoting the relevance of the “environment” term based on changes of AIC, as explained in Figure 2. Species codes as in Table 1.

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FIGURE 1

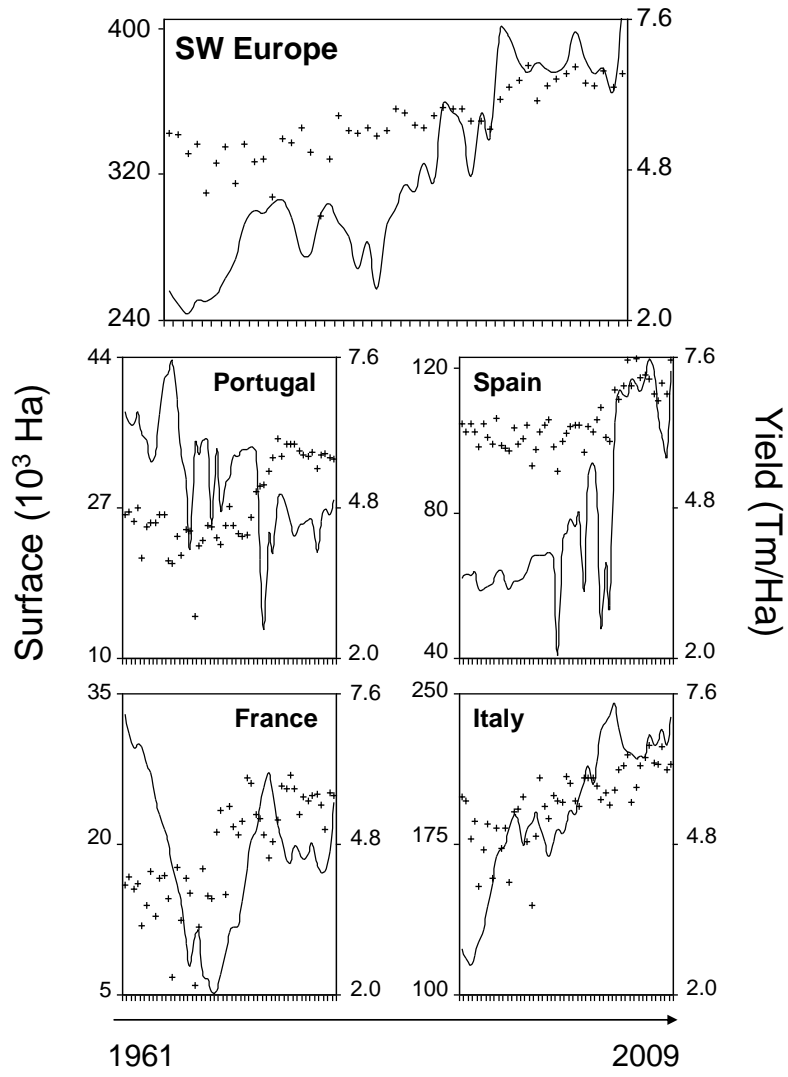
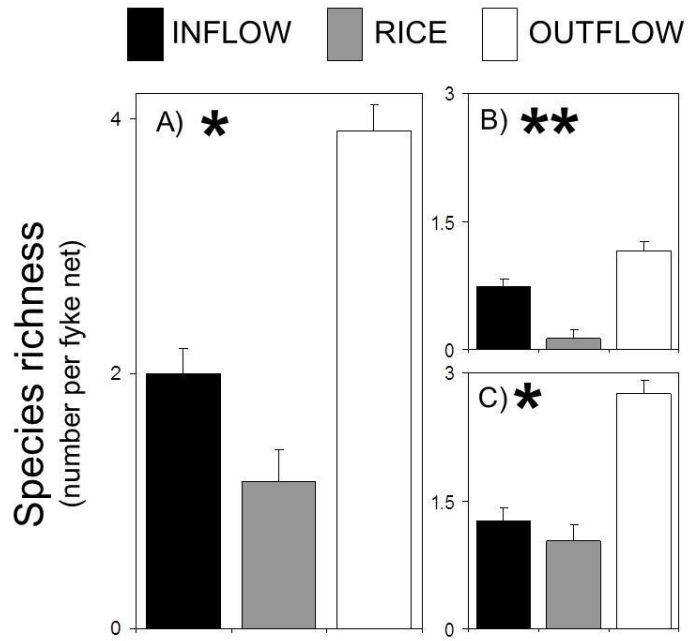


FIGURE 2



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FIGURE 3

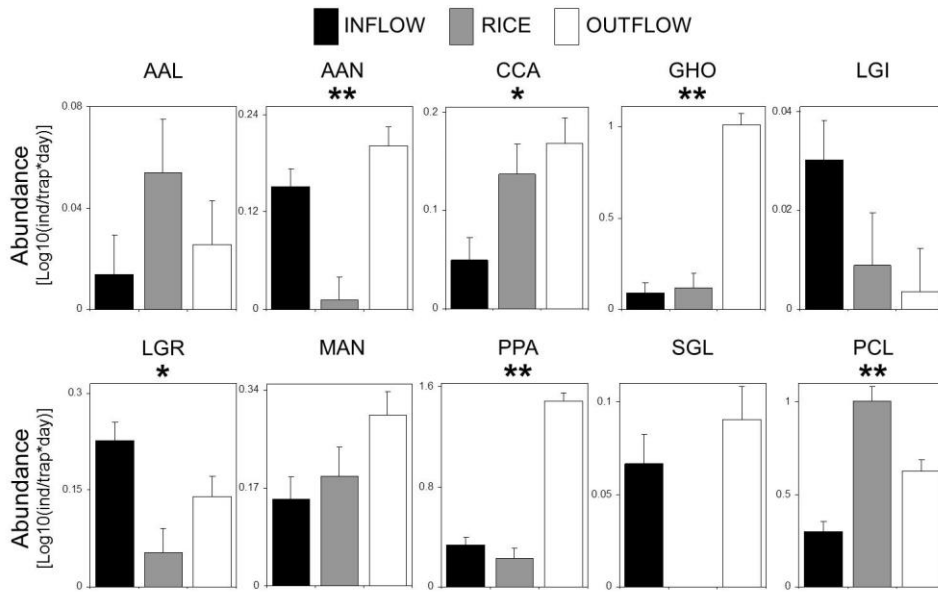


FIGURE 4

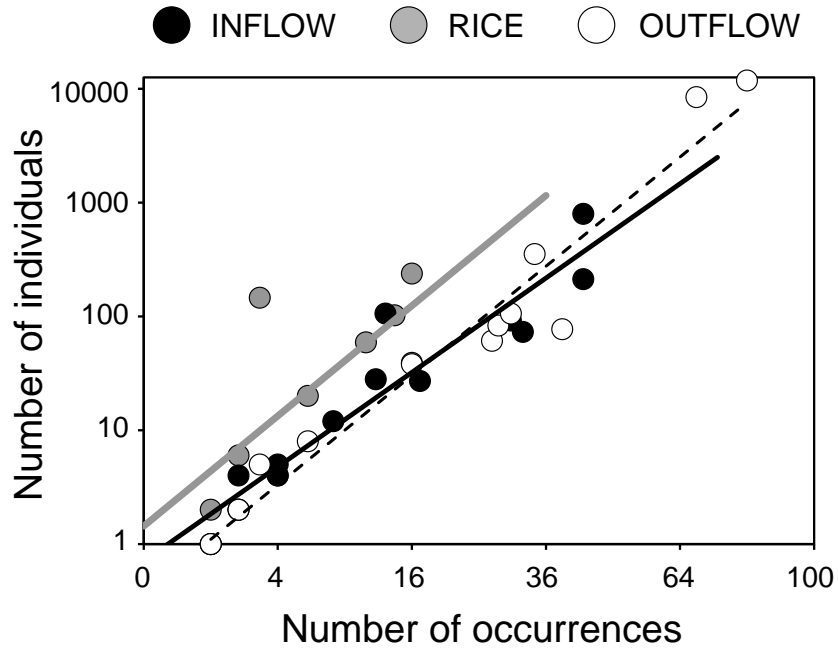
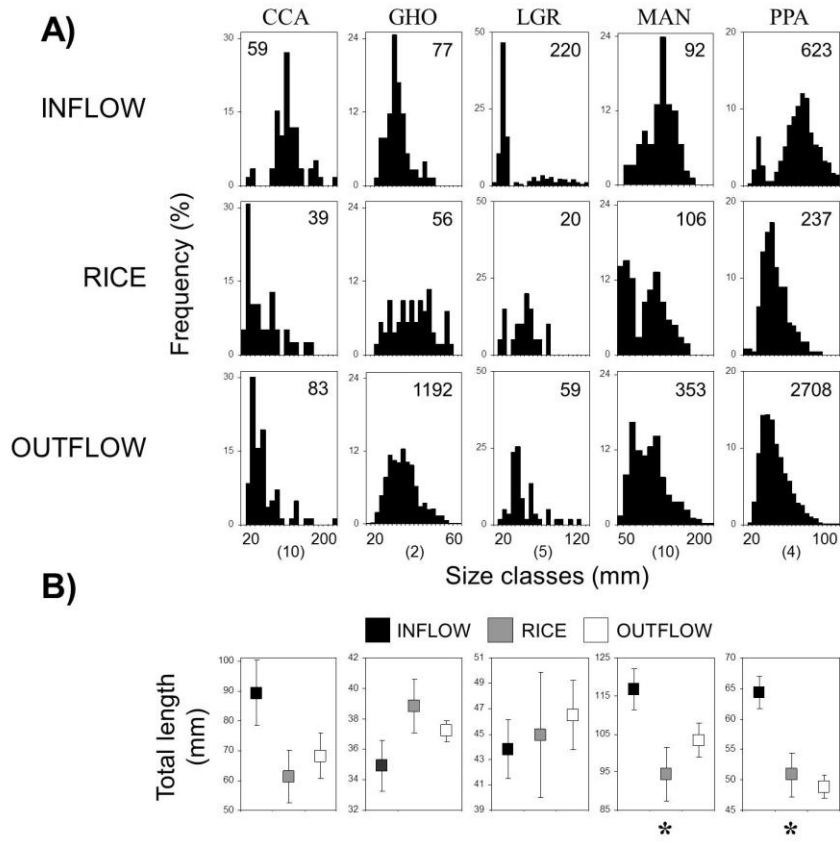


FIGURE 5



Appendix A. Results of the fixed-effect terms of mixed models

Table S1. Results of the generalized linear mixed models (GLMMs) relating species richness and abundances of the most common fish species (as well as that of red swamp crayfish, *Procambarus clarkii*) to the different environments studied (3 levels: inflow channels, rice fields and outflow channels) and two continuous covariates, the sampling date (an ordinal variable coding 15-day periods) and the sampling effort (number of hours that fyke nets were functioning). Sampling unit was the individual fyke net, so site (104 levels, nested within environment) was included as random effect to ensure data independence. Year, with three levels (2007, 2008 and 2009) was included as an additional random term. The asterisks indicate the relevance of the fixed terms based on changes of AIC of GLMMs with and without each particular variable: ** strong effect ($\Delta AIC > 7$); * moderate effect ($7 \geq \Delta AIC > 2$). The positive or negative nature of the relationships with covariates is reported whenever effects are relevant.

	ENVIRONMENT	DATE	slope	EFFORT	slope
SPECIES RICHNESS	39.3*	5.7*	+	5.0*	+
NATIVE SPECIES RICHNESS	19.3**	0.1		8.2*	+
INTRODUCED SPECIES RICHNESS	26.3*	7.9*	+	1.4	
<i>Alburnus alburnus</i> ABUNDANCE	> 0.1	> 0.1		> 0.1	
<i>Anguilla anguilla</i> ABUNDANCE	6.4**	2.1		0.6	
<i>Cyprinus carpio</i> ABUNDANCE	4.5*	5.9*	-	0.7	
<i>Gambusia holbrooki</i> ABUNDANCE	27.6**	1.7		> 0.1	
<i>Lepomis gibbosus</i> ABUNDANCE	0.4	0.6		0.1	
<i>Luciobarbus graellsii</i> ABUNDANCE	7.2*	5.5*	-	4.2	
<i>Misgurnus anguillicaudatus</i> ABUNDANCE	2.9	9.4*	+	1.6	
<i>Pseudorasbora parva</i> ABUNDANCE	44.7**	4.4*	+	0.9	
<i>Silurus glanis</i> ABUNDANCE	1.5	0.2		5.6*	+
<i>Procambarus clarkii</i> ABUNDANCE	11.3**	14.8**	+	2.7	

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Table S2. Results of the linear mixed models (LMMs) relating the sizes of captured fish to the different environments studied (3 levels: inflow channels, rice fields and outflow channels) and the date (an ordinal variable coding 15-day periods, used as a continuous covariate). LMMs were run for fish species with at least 20 individuals measured in each of the three studied environments. Site (104 levels, nested within environment) and Year (three levels, 2007, 2008 and 2009) were included as random terms. The asterisks indicate the relevance of the fixed terms based on changes of AIC of GLMMs with and without each particular variable: ** strong effect ($\Delta AIC > 7$); * moderate effect ($7 \geq \Delta AIC > 2$). The positive or negative nature of the relationship with date is reported whenever effects are relevant.

	ENVIRONMENT	DATE	slope
<i>Cyprinus carpio</i>	10.6	41.5**	+
<i>Gambusia holbrooki</i>	3.4	9.4**	-
<i>Luciobarbus graellsii</i>	4.8	237.7**	+
<i>Misgurnus anguillicaudatus</i>	3.5*	0.5	
<i>Pseudorasbora parva</i>	21.1*	26.1**	+