



# Ichthyofauna of Doñana, southern Spain: checklist and historical variation

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#### SUMMARY

The Doñana Natural Area is one of the most important protected areas in the Iberian Peninsula and has a wide variety of aquatic environments hosting a diverse biota. Despite the ecological and conservation relevance of fish, there is a scarce knowledge of the fish fauna in the Doñana Natural Area. In this study, we present an updated checklist of the ichthyofauna of Doñana and describe its long-term (1975-2014) temporal variation, based on fish records from scientific and grey literature and our own field surveys. The Doñana Natural Area hosts 28 fish species (19 native and 9 non-native). Native species have tended to decline in parallel with an increase in the richness of non-native species. The number of UTM squares 1×1 km hosting native species has not varied significantly along time, while squares with non-native species have increased, especially since 2002. Three threatened species, three-spined stickleback (Gasterosteus aculeatus), Iberian arched-mouth nase (Iberochondrostoma lemmingii) and the southern Iberian chub (Squalius pyrenaicus), have not been detected in the last years, and should be considered as locally extinct. Fish assemblages of the Doñana Natural Area feature a poor conservation status, probably related with the increasing ecological pressure from the non-native biota, among other causes.

Keywords: Iberian Peninsula, fish assemblages, species richness, occurrence

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#### INTRODUCTION

The Donana Natural Area encompasses a series of terrestrial and aquatic ecosystems around the estuary of the Guadalquivir River, in southwestern Iberian Peninsula. It has an extraordinary diversity of fauna and flora, related to its high environmental heterogeneity and its geographic location between the European and African continents (García-Novo & Marín-Cabrera 2006). The aquatic environments of the Doñana Natural Area (henceforth, simply Doñana) are considered one of the biodiversity hotspots in the Iberian Peninsula and Europe (Drake et al. 2002, Fernández-Delgado 2006, 2017). The high diversity of aquatic environments favours a high species richness of several animal groups (Díaz-Paniagua et al. 2005, Fahd et al. 2009, Ministerio de Medio Ambiente 2009).

Fish are one of the least studied vertebrate groups in Doñana. Studies on fish communities have been developed in the area for many years, but most of them were limited to the estuary of the Guadalquivir River (Hernando 1978, Fernández-Delgado 1987, 2005 and 2006, Fernández-Delgado et al. 1994, Prieto et al. 1998, Fernández-Delgado et al. 2000, Drake et al. 2002, Sobrino et al. 2005, among others). However, an increase in the interest for the ichthyology in Doñana has been developed in recent years (Bravo-Utrera 2010). Here, we review all the information on the Doñana fish assemblages excluding those of the estuary, in order to summarize their main characteristics and describe their main trends between 1975 and 2014.

# STUDY AREA

Doñana covers approximately 1225 km² and includes two protected areas, the Doñana National and Natural Park, with different levels of protection (more restrictive in the National Park) (Fig. 1). It features a sub-humid Mediterranean climate (Siljeström et al. 2002) and has two main geomorphological units: the silt-clay

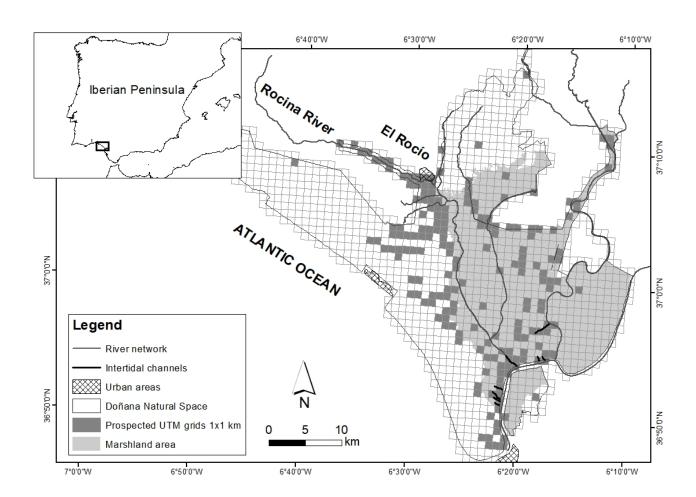
marshland and the aeolian sands (Fig. 1) (Gómez-Rodríguez et al. 2011). The contact zone between the aeolian system and the marshland, locally termed la Vera, is a humid ecotone (Clemente et al. 1997). The Guadalquivir River estuary originally had about 2500 km<sup>2</sup> of marshland area, but during the first half of the 20th century much of this area was transformed for agricultural uses and the original marshland area was reduced by about 80%. Most remnants of those shallow water areas are included in the protected Doñana marshland. The hydrological functioning of most of the present-day marshland is strongly affected by the artificial isolation from the Guadalquivir River estuary due to the construction of dykes and the management of the sluices, having lost all tidal influence (Fernández-Delgado 2005). The flooding of the marsh follows the Mediterranean-climate precipitation regime. In an average hydrological cycle the flooding starts in autumn and the highest water levels are reached in winter or spring. With the temperature increases and the lack of summer precipitations the progressive drying phase of the marshland is usually completed by mid-summer. The marshes remain dry until the next wet period with the exception of a few permanent water spots (Serrano et al. 2006; Moreno-Valcárcel et al. 2013).

The anthropogenic degradation of the aquatic ecosystems of Doñana has a long history, and is mainly caused by the many modifications of the main course of the Guadalquivir River (which has been rectified and simplified, losing several branches) (Bayán-Jardín 2006) and, especially since the second half of the 20th century, by the proliferation of irrigated croplands and their associated hydraulic infrastructures (Rivera 2002; Fernández-Delgado 2017). Most of the small rivers draining into the marshland are nowadays altered in their hydromorphology, especially those of the left margin of the Guadalquivir River, in order to adapt them for irrigation purposes. Consequently, the marshland area has suffered several negative impacts derived from a reduction in quality and quantity of the water inputs (Bayán-Jardín 2006, Paredes et al. 2020).

For the aims of this work, we have defined four different aquatic habitat types: 1) intertidal creeks and ponds; 2) permanent ponds in the marshland; 3) small streams; and 4) aeolian sand ponds (Fig. 2).

We have collected information on fish occurrences from 336 sampling events throughout the study area from 1975 to 2014, based on different sources (Table 1).

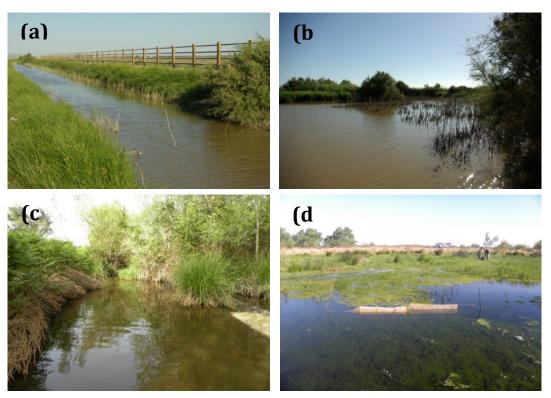
We summarized this information in 1×1 km UTM cells, obtaining fish records for 185 of them (Fig. 1). We have classified as "historical situation" the information before 2002, when the Doñana Biological Station – CSIC started the implementation of a monitoring scheme that included fish (see Table 1) and as "current situation" the subsequent information (2002-2014).



**Figure 1**. Map of the Doñana Natural Area, including the Doñana National Park and the Doñana Natural Park. The 1×1 km UTM grid used in the present study is presented, with the 188 prospected cells being shown in dark grey. River network, intertidal channels, urban areas and marshland area (soft grey) are shown.

 $\textbf{Table 1}. \ \ \textbf{Documentary sources used in the study of the fish assemblage status in Do\~nana}.$ 

Analyzed period	Documentary source	Reference
1975-1976	PhD thesis "Structure of the fish community in the Guadalquivir marshlands"	Hernando 1978
1991-1992	"Inventory of the fish species in Doñana National Park. Biology, ecology and conservation status"	Research project
1996	"Inventory of the fish species in Doñana National Park" (Supplementary sampling)	Research project
1998-2006	"Monitoring of the effects of the toxic spill from the Aznalcóllar mines on the fish community of the Guadiamar River"	Research project
2002-2013	Monitoring network of the Doñana Biological Station	EBD-CSIC
2003-2005	"Macroinvertebrates and fish assemblages affected by the Project <i>Doñana 2005</i> "	Research project
2003-2005	Scientific article "Fish composition and assemblage in the anthropogenic-modified tidally-restricted Doñana (Spain) marshlands"	Moreno-Valcárcel et al. 2013
2006-2009	"Conservation status and conservational issues of the autochthonous continental fish in the Guadalquivir River basin. Inventory of the most important fluvial stretch to fish protection"	Research project
2009-2011	"Fishes as indicator of the elimination and permeabilization of the Mountain of the River"	Research project
2011	Scientific article "Long-term effects of tidal restriction on fish assemblages in east Atlantic coastal marshlands"	Moreno-Valcárcel et al. 2016
2010-2014	PhD "Fish assemblage in the marshland of Doñana Protected Area: composition, dynamic and influence of the tidal restriction"	Moreno-Valcárcel 2015



**Figure 2**. Examples of different aquatic habitats found in Doñana: (a) an intertidal creek in the external marsh area; (b) permanent freshwater pond in the marshland; (c) *La Rocina* stream; (d) pond in the aeolian sands.

#### FISH CHECKLIST

Thirty-four fish species (25 native and 9 non-native) belonging to 18 families have been cited in Doñana since 1975 (Table 2). The families with a higher number of species were Cyprinidae (n= 8), Mugilidae (n= 5) and Gobiidae (n= 3). This inventory includes freshwater, estuarine, catadromous and marine migrant species (sensu Franco et al. 2008). Some marine straggler species have been excluded from this checklist due to their rare occurrence in Doñana (e.g. Pomatomus, Lichia, Trachinotus).

Six native species have not been recorded in the area since 2002 (Table 3). Two of them, the Iberian arched-mouth nase [Iberochondrostoma lemmingii (Steindachner, 1866)] and the Southern Iberian chub [Squalius pyrenaicus (Günther, 1868)] are cyprinids catalogued as vulnerable (VU) in the Spanish Red List, following IUCN criteria (Doadrio 2002). The other

four species are the European anchovy [Engraulis encrasicholus (Linnaeus, 1758)], the three-spined stickleback (Gasterosteus aculeatus Cuvier, 1829), the peacock blenny [Salaria pavo (Risso, 1810)] and the black-stripped pipefish (Syngnathus abaster Risso, 1827).

Four non-native species were present in the Doñana before 1975 and are still found there: the common carp [Cyprinus carpio (L., 1758)], the largemouth bass [Micropterus salmoides (Lacepède, 1802)], the Eastern mosquitofish (Gambusia holbrooki Girard, 1859), and the mummichog [Fundulus heteroclitus (Linnaeus, 1766)] (Table 3). Five new non-native have been introduced since 1975, these being the bleak, [Alburnus alburnus (Linnaeus, 1758)], the black bullhead [Ameiurus melas (Rafinesque, 1820)], the goldfish [Carassius auratus (Bloch, 1782), the Prussian carp [Carassius gibelio (Bloch, 1782)] and the pumpkinshed [Lepomis gibbosus (L., 1758)] (Table 3).

**Table 2.** Checklist of fish species in Doñana, excluding the Guadalquivir River estuary.

Family	Species	Status	Recent records
Anguillidae	Anguilla anguilla (Linnaeus, 1758)	Native	YES
Atherinidae	Atherina boyeri Risso, 1810	Native	YES
Engraulidae	Engraulis encrasicholus (Linnaeus, 1758)	Native	NO
Cobitidae	Cobitis paludica (de Buen, 1930)	Native	YES
Cyprinidae	Alburnus alburnus (Linnaeus, 1758)	Non-native	YES
	Carassius auratus (Bloch, 1782)	Non.native	YES
	Carassius gibelio (Bloch, 1782)	Non-native	YES
	Cyprinus carpio Linnaeus, 1758	Non-native	YES
	Iberochondrostoma lemmingii (Steindachner, 1866)	Native	NO
	Luciobarbus sclateri (Günther, 1868)	Native	YES
	Squalius alburnoides (Steindachner, 1866)	Native	YES
	Squalius pyrenaicus (Günther, 1868)	Native	NO
Cyprinodontidae	Aphanius baeticus Doadrio, Carmona & Fernández-Delgado, 2002	Native	YES
Fundulidae	Fundulus heteroclitus (Linnaeus, 1766)	Non-native	YES
Poeciliidae	Gambusia holbrooki Girard, 1859	Non-native	YES
Gasterosteidae	Gasterosteus aculeatus Cuvier, 1829	Native	NO
Bleniidae	Salaria pavo (Risso, 1810)	Native	NO
Centrarchidae	Lepomis gibbosus (Linnaeus, 1758)	Non-native	YES
	Micropterus salmoides (Lacepède, 1802)	Non-native	YES
Moronidae	Dicentrarchus labrax (Linnaeus, 1758)	Native	YES
	Dicentrarchus punctatus (Bloch, 1792)	Native	YES
Sparidae	Diplodus bellottii (Steindachner, 1882)	Native	YES
Mugilidae	Chelon labrosus (Risso, 1827)	Native	YES
	Liza aurata (Risso, 1810)	Native	YES
	Liza ramada (Risso, 1827)	Native	YES
	Liza saliens (Risso, 1810)	Native	YES
	Mugil cephalus Linnaeus, 1758	Native	YES
Gobiidae	Pomatoschistus microps (Krøyer, 1838)	Native	YES
	Pomatoschistus minutus (Pallas, 1770)	Native	YES
	Gobius niger Linnaeus, 1758	Native	YES
Soleidae	Solea senegalensis Kaup, 1858	Native	YES
	Solea vulgaris Quensel, 1806	Native	YES
Ictaluridae	Ameiurus melas (Rafinesque, 1820)	Non-native	YES
Syngnathidae Syngnathus abaster Risso, 1827		Native	NO

Table 3. Temporal changes in the presence of fish species in Doñana.

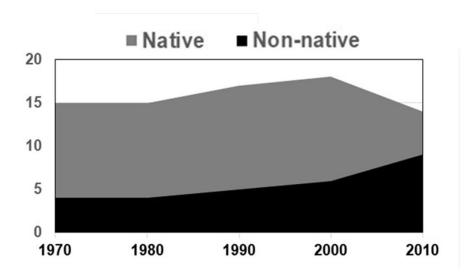
		1975-89	1990-99	2000-09	2010-14
	Anguilla anguilla		_	_	
	Atherina boyeri				
	Engraulis encrasicholus				
	Cobitis paludica				
ľ	Iberochondrostoma lemmingii				
	Luciobarbus sclateri				
	Squalius alburnoides				
	Squalius pyrenaicus				
	Aphanius baeticus				
es	Gasterosteus aculeatus				
eci	Salaria pavo			•	
$\mathbf{ds}$	Dicentrarchus labrax				
ve	$Dicentrarchus\ punctatus$				
Native species	Diplodus bellottii				
Z	Chelon labrosus				
	Liza aurata				
	Liza ramada				
	Liza saliens				
	Mugil cephalus				
	Pomatoschistus microps				
	Pomatoschistus minutus				
	Gobius niger				
	Solea senegalensis				
	Solea vulgaris				
	Syngnathus abaster				
	Alburnus alburnus				
ies	Ameiurus melas				
Exotic speci	Cyprinus carpio				
	Carassius auratus				
	Carassius gibelio				
	Fundulus heteroclitus				
	Gambusia holbrooki				
	Lepomis gibbosus				
	Micropterus salmoides				

#### FISH BIODIVERSITY TRENDS

The richness of native fish species slightly increased from 1975 to the 2000s decade (Fig. 3), a pattern that could be linked to an increment in the sampling effort during this period, since colonisations by new native species seem less plausible. In fact several native species have not been regularly recorded over time, and only 14 of them have been detected during the period 2010-14 (Table 3). Contrastingly, the richness of non-native species has clearly increased over the time, notably since the year 2000.

The globally threatened European eel (Anguilla anguilla L., 1758) has a stable occurrence in Doñana (Table 3), although data on abundances would be necessary to accurately evaluate the temporal evolution of its status. The lower Guadalquivir River is thought to be a critical area for the conservation of the eel (Kettle et al. 2011, Arribas et al., 2012), and Doñana might be an important component of this system for the species. Other globally threated fish species found in Doñana is the Baetican toothcarp (Aphanius baeticus Doadrio, Carmona Fernández-Delgado, which is present in a low number of locations, being scarce in all of them. This

species has been at risk of local extinction in Doñana for decades, arguably due to negative interactions with non-native fish such Eastern mosquitofish mummichog (Oliva-Paterna et al. 2006). The three-spined stickleback, a threatened species in Spain, was cited in the Rocina stream (see Fig.1c) in the 1970s (Hernando 1978), but has not been recorded again Predation by (Table 3). non-native largemouth bass may have driven this local extinction (Fernández-Delgado & Elvira 2004), as has been reported in other Iberian aguatic systems (Moreno-Amich et al. 2006). Predation by the large-mouth bass, as well as pollution episodes, may have also driven the local extinction of the Iberian archedmouth nase and the Southern Iberian chub in the area (Hermoso & Clavero 2011, Fernández-Delgado et al. 2014). southern Iberian spined-loach, paludica (de Buen, 1930), occurs regularly in streams and ponds, although always at very low densities. Different population nuclei of this species in the Doñana have disappeared during the period of our review (Fernández-Delgado et al. 1994). southern Iberian barbel, Luciobarbus sclateri (Günther, 1868), is the only cyprinid with permanent presence and ample distribution in the area over time (Table 3).



**Figure 3**. Temporal variation in the number of native and non-native fish species recorded in Doñana.

Some estuarine and marine migrant species, such as the peacock blenny, the black goby (Gobius niger Linnaeus, 1758), the European anchovy and the Senegal seabream [Diplodus bellottii (Steindachner, 1882)], have been captured occasionally inside the marshland (Table 3). Probably, the actual state of the Doñana marshland does not provide suitable habitats for these species, due to the progressive reduction of natural tidal flooding (Moreno-Valcárcel et al. 2013). However, other estuarine species, probably less sensitive to reduction of connectivity, such as the big-scale sand smelt (Atherina boyeri Risso, 1810), the common [Pomatoschistus] micropsgoby (Krøyer, 1838)] and the sand goby [Pomatoschistus minutus (Pallas, 1770)], have permanent presence inside the marshland (Fernández-Delgado et al. 2000, Moreno-Valcárcel et al. 2013, 2016).

The marine and estuarine Moronidae, Soleidae and Mugilidae fish have been frequently captured in the saline external marshland. We think that their density there is lower than what would be expected in a naturally functioning marshland area, arguably due to the several modifications of the Guadalquivir marshland, involving a severe loss of connectivity (Fernández-Delgado et al. 2000, Moreno-Valcárcel et al. 2013, 2016).

The number of non-native fish has notably increased in recent times in Doñana (Fig. 3), with new three invasive species detected since 2009: the bleak, the Prussian carp and the black bullhead (Table 3). The bleak has only been recorded twice, in both cases related flood episodes in the Guadalquivir River, so the species does not seem to be established in the study area. Although the presence of the Prussian carp has only been recently confirmed, it is possible that the species had been introduced several years ago, passing undetected due to its similarity with the goldfish, Carassius auratus (Bloch, 1782) (Ribeiro et al. 2015). The black bullhead was detected in the Guadalquivir basin in 2007 (García de Lomas et al. 2009) and in Doñana in 2009.

The common carp was arguably the first non-native species established in Doñana, although the introduction date is unknown. It has been suggested that the common carp would have been present in Doñana at least since the 19<sup>th</sup> century (Fernández-Delgado 1990, Fernández-Delgado & Elvira 2004). The high environmental tolerance of the common carp, specifically to low oxygen levels and high salinities, has allowed its successful spread in the stagnant waters of the marshland (Fernández-Delgado et al. 2000).

Another abundant and widely distributed non-native species mummichog, which is present in the estuary of the Guadalquivir River at least since the 1970s (Fernández-Delgado et al. 1986, Gutiérrez-Estrada et al. 1998). The species was already very abundant in the Doñana marshes during the first 1980s (Fernández-Delgado orbs. pers.). It has been proposed that the mummichog would cause ecological impacts mainly through its competition with several other species (Arias and Drake 1989, Fernández-Delgado 1989a, Gutiérrez-Estrada et al. 1998). Probably the most affected species is the Baetican toothcarp, which could be negatively affected by both mummichog and the mosquitofish (Rincón et al. 2002, Clavero et al. 2007). The eastern mosquitofish was introduced in Spain in 1921 by the health authorities to reduce the larvae of the mosquito that transmits malaria, and is currently widespread through most of the lentic water bodies in the Iberian Peninsula (Doadrio 2002).

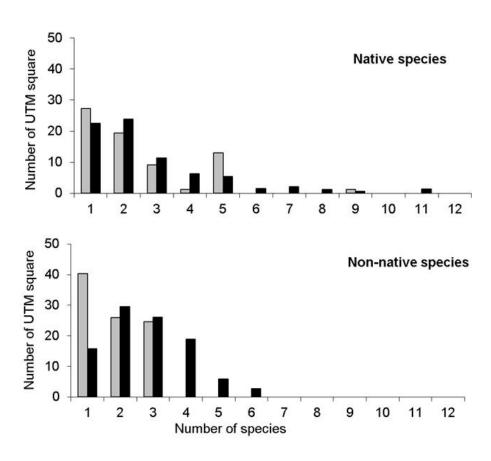
The pumpkinshed has been present in Doñana since the 1990s, while the other centrarchid fish, the largemouth bass, was introduced in the study area in the 1970s (Hernando & Pareja 1974) (Table 3). Both species are mostly present in the streams flowing into the Doñana marshland.

In spite of the decline on several native species, the number of 1×1 km UTM squares occupied by these native species has not shown drastic changes between the historical and current situation (Fig. 4). The small increment in number of the native

species since 2003 may be related to a higher sampling effort. Non-native species have increased not only in number, but also in geographical spread. Before 2002 most (40.3%) 1×1 km squares had only one nonnative species and there were no squares with four or more. After 2002 more than 25% of the squares had four or more nonnative species (Fig. 4). Results from other studies show how the number of invasive species tend to be higher than the number of natives in lentic Mediterranean heavily modified systems (Clavero & Hermoso, 2011), and how the abundance of the aguatic organism varies in response to invasions, ending in local extinctions after a time lag (Gallardo et al. 2016).

In summary, the composition in fish communities in Doñana has been very dy-

namic in recent decades. While the richness of native species has tended to decline, with six species being lost up to 2014, that nonnative species has increased, from four species in 1975 to nine species in 2014, and their ranges have notably expanded. The whole impoverishment of the fish assemblage in Doñana increases with the loss of 3 threatened species that can be considered as locally extinct. These patterns arguably indicate a generalized degradation of aquatic systems in Doñana. The information provided here can be used to set comparison baselines for future studies and to set conservation targets in habitat restoration programs.



**Figure 4**. Citations of native and non-native species per 1×1 km UTM square among 188 prospected squares. Grey bars represent the 1975-2002 period and black bars represent the 2003-2014 period.

#### **AUTHOR CONTRIBUTIONS**

RMV lead the review, analysis and writing the manuscript. CFD and FJOP contributed to the conceptualization, writing and interpretation.

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