
The occurrence, distribution and biology of invasive fish species in fresh and brackish water bodies of NE Morocco

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Abstract

The occurrence, distribution and biology of invasive fish species in fresh and brackish water bodies of NE Morocco. Monitoring the presence and expansion of alien species and upgrading their biological and ecological knowledge seems crucial to mitigate their possible impact on native communities. Within inland superficial waters, alien fish represent an important threat to the biodiversity and studies on their impact on native communities have increased around the world in the last years. However, little is known about their occurrence, biology and influences in North Africa in general, and more specifically in Morocco. In the present work we aimed to: 1) investigate the presence of any native *Aphanius* species, especially the Mediterranean killifish *Aphanius fasciatus* recorded from the lower basin of the Moulouya River (NE Morocco); 2) monitor the presence and expansion of two invasive species, the eastern mosquitofish *Gambusia holbrooki* and the mummichog *Fundulus heteroclitus*; and 3) contribute to the understanding of the ecological and abiotic affinities that govern the distribution of these alien fishes in North Africa. To achieve these goals, several field sampling campaigns were carried out between 2014 and 2018 across eastern Morocco, comprising the administrative Oriental Region and the Moulouya River Basin and covering an area of 119,268 km². No native *Aphanius* species were found. The eastern mosquitofish has invaded the freshwater hydrosystems of the northern part of Morocco, including the study area, while the mummichog is currently limited to the brackish and salty wetlands of Lower Moulouya. Our results show that the known ranges of these two alien species have expanded.

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Key words: Alien fishes, Mediterranean killifish, Eastern mosquitofish, Mummichog, Competition, Range expansion

Resumen

Incidenia, distribución y biología de las especies invasivas de peces en masas de agua dulce y salobre del NE de Marruecos. Monitorizar la presencia y expansión de especies exóticas y mejorar el conocimiento biológico y ecológico de las mismas parece clave para mitigar su posible impacto en las comunidades nativas. Las especies de peces exóticas constituyen una importante amenaza para la diversidad en las aguas superficiales continentales, por lo que los estudios sobre su impacto en las comunidades nativas se han incrementado en todo el mundo en los últimos años. No obstante, el conocimiento sobre la incidencia, biología e influencias

de este fenómeno en el norte de África en general, y más específicamente en Marruecos, es escaso. En este trabajo nos propusimos: 1) investigar la presencia de alguna especie nativa de *Aphanius*, especialmente del fartet mediterráneo *Aphanius fasciatus* anteriormente registrado en la cuenca inferior del río Moulouya (NE de Marruecos); 2) monitorizar la presencia y expansión de dos especies invasoras, el pez mosquito *Gambusia holbrooki* y *Fundulus heteroclitus*; y 3) ayudar a comprender las afinidades ecológicas y abióticas que rigen la distribución de estos peces exóticos en el norte de África. Para alcanzar estos objetivos realizamos varias campañas de muestreo sobre el terreno en el este de Marruecos entre 2014 y 2018, incluidas la Región Oriental y la cuenca del río Moulouya, cubriendo una extensión de 119.268 km². No encontramos especies nativas de *Aphanius*. El pez mosquito oriental ha invadido los sistemas hídricos de agua dulce de la parte norte de Marruecos, incluida el área estudiada, mientras que *Fundulus heteroclitus* está limitado en la actualidad a las aguas salobres y saladas de los humedales del curso bajo del Moulouya. Como resultado de este estudio, las áreas de distribución conocidas de ambas especies exóticas se han ampliado.

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Palabras clave: Peces exóticos, Fartet mediterráneo, Pez mosquito oriental, *Fundulus heteroclitus*, Competencia, Expansión potencial

Resum

Incidència, distribució i biologia de les espècies invasives de peixos en masses d'aigua dolça i salabrosa del NE del Marroc. Monitorar la presència i l'expansió d'espècies exòtiques i millorar-ne el coneixement biològic i ecològic sembla clau per mitigar l'impacte que poden exercir en les comunitats natives. Les espècies de peixos exòtiques constitueixen una amenaça important per a la diversitat a les aigües superficials continentals, per la qual cosa els estudis sobre el seu impacte en les comunitats natives s'han incrementat arreu del món els darrers anys. Tanmateix, el coneixement sobre la incidència, la biologia i les influències d'aquest fenomen al nord d'Àfrica en general, i més específicament al Marroc, és escàs. En aquest treball ens vam proposar: 1) investigar la presència d'alguna espècie nativa d'*Aphanius*, especialment del fartet mediterrani *Aphanius fasciatus* registrat anteriorment a la conca inferior del riu Moulouya (NE del Marroc); 2) monitorar la presència i l'expansió de dues espècies invasores, el peix mosquit *Gambusia holbrooki* i *Fundulus heteroclitus*; i 3) ajudar a entendre les afinitats ecològiques i abiótiques que regeixen la distribució d'aquests peixos exòtics al nord d'Àfrica. Per assolir aquests objectius vam portar a terme diverses campanyes de mostreig sobre el terreny a l'est del Marroc entre 2014 i 2018, incloent-hi la Regió Oriental i la conca del riu Moulouya, cobrint una extensió de 119.268 km². No hi vam trobar espècies natives d'*Aphanius*. El peix mosquit oriental ha envaït els sistemes hídrics d'aigua dolça de la part nord del Marroc, incloent-hi l'àrea estudiada, mentre que *Fundulus heteroclitus* està limitat actualment a les aigües salabreses i salades dels aiguamolls del curs baix del Moulouya. Com a resultat d'aquest estudi, les àrees de distribució conegudes de les dues espècies exòtiques s'han ampliat.

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Paraules clau: Peixos exòtics, Fartet mediterrani, Peix mosquit oriental, *Fundulus heteroclitus*, Competència, Expansió potencial

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Introduction

Biological invasions are one of the most important human impacts on a wide range of ecosystems and a major cause of global change (Ricciardi, 2006). The introduction and invasion of alien species are one of the main threats to biodiversity and ecosystem functioning worldwide, and represent the cause of huge ecological and economic costs around the world (Fausch and García–Berthou, 2013). Many invasive species have been implicated in species extinction, habitat degradation and ecosystem alteration (Cox, 1999; Pimentel et al., 2005; Coccia, 2015). Freshwater invasions have been less studied than terrestrial invasions, and the ecological and evolutionary consequences of most invasions remain unknown (Ricciardi and MacIsaac, 2011). Freshwaters are the most invaded and threatened ecosystem worldwide, with proportionally more invaders than terrestrial systems (Vitousek et al., 1997; Strayer, 2010).

Many members of the genus *Aphanius* are “victims” of biological invasion, such as the Mediterranean killifish, *A. fasciatus* (Valenciennes, 1821), a cyprinodontid fish whose native range includes coastal brackish–waters mainly in the coastal zone of the central and eastern Mediterranean (Bianco, 1995). Nevertheless, it can also be found in various inland water bodies, such as lakes, inland streams and even in some North African oases (Hrbek and Meyer, 2003; Güçlü et al., 2013). *Aphanius fasciatus* is also present in various Mediterranean islands, such as Sardinia, Corsica and Cyprus (Bianco et al., 1996; Kottelat and Freyhof, 2007; Englezou et al., 2018). The Mediterranean killifish was reported in Morocco for the first time at the wetland of the Moulouya River mouth by Melhaoui (1994), but the species has not been reported since this record.

Aphanius fasciatus has disappeared from many sites where it once existed, and its populations have declined dramatically. In many cases it has even reached local extinction due to problems such as pollution of continental and coastal waters, brackish–water habitat degradation, destruction and reduction of salt–works and, most importantly, introduction of exotic fishes (Bianco, 1995). Competition with the alien species *Gambusia holbrooki* Girard, 1859 has strongly reduced the presence of *A. fasciatus* in many parts of its range (Kessabi et al., 2009; Valdesalici et al., 2015).

Commonly known as 'Eastern mosquitofish', *Gambusia holbrooki* is a freshwater poeciliid native of America (Lloyd and Tomasov, 1985). It has been considered one of the world's one hundred worst invasive alien species by the GISP (Global Invasive Species Programme, <http://www.issg.org/database/>). It was introduced extensively for biological control against mosquito larvae and distributed in all continents except Antarctica, becoming a notorious pest worldwide (Cote et al., 2011; Pyke, 2005; Srean, 2015; Arnett, 2016) and causing serious problems for native fish such as *A. fasciatus*, *Aphanius iberus* (Valenciennes, 1846) or *Valencia hispanica* (Valenciennes, 1846) (Rincón et al., 2002; Caiola and de Sostoa, 2005; Valdesalici et al., 2015).

Another alien fish which has been reported to cause range reductions in native toothcarps is the mummichog *Fundulus heteroclitus* (Linnaeus, 1766), a small teleost fish, naturally occurring in saltmarshes of the Atlantic coast of North America from the Gulf of St Lawrence, southwards to north-eastern Florida (Shute, 1980). It was found in Iberia for the first time between 1970 and 1973 (Bernardi et al., 1995). Since then, *F. heteroclitus* has been found in many parts of Spain and Portugal (Morim et al., 2019). We found it for the first time in Morocco and Northern Africa at the Moulouya River mouth

This study aimed to evaluate the presence of *A. fasciatus* or any native *Aphanius* species in NE Morocco, in parallel with the two invasive species *G. holbrooki* and *F. heteroclitus*. Furthermore, our goal was to study the interaction between the invasive fish, and their occurrence, distribution and biology in the fresh and brackish water bodies of the study area.

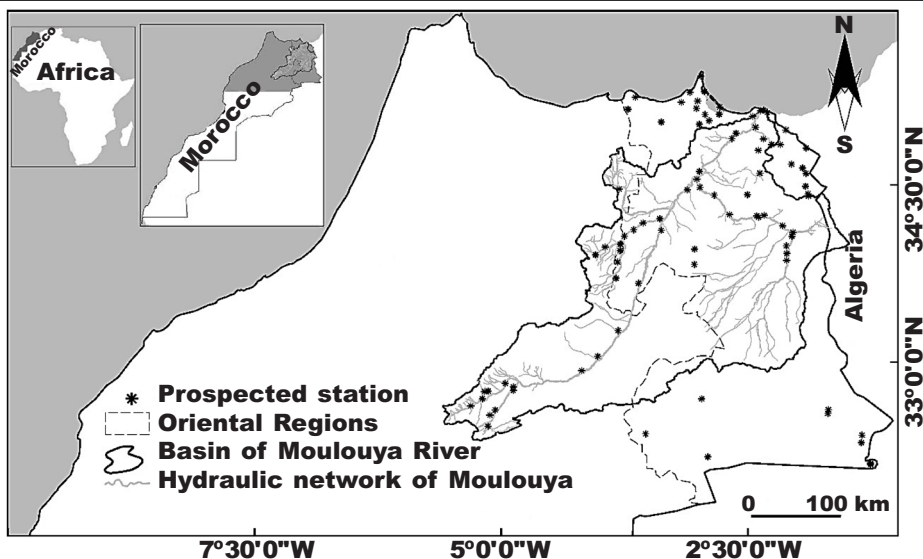


Fig. 1. Location of study area in Morocco and the prospected stations.

Fig. 1. Localización del área de estudio en Marruecos y puntos de muestreo.

Material and methods

Study area

Morocco is currently divided into 12 administrative regions, including the Oriental Region (fig. 1), which occupies almost all the eastern side of the country and covers an area of 88,681 km² (see Mabrouki et al., 2018 for details). The Oriental Region includes the wilaya of Oujda (Oujda–Angad prefecture) and the provinces of Berkane, Driouch, Figuig, Guercif, Jerada, Nador and Taourirt. The watershed of the Moulouya (fig. 1), which includes nearly 43,412 km² of eastern Morocco, covers much of the Oriental Region. With a length of 600 km, the Moulouya is the longest Moroccan River flowing into the Mediterranean. Its main tributaries are the Oueds Anzegmir, Melloulou, Za and Msoun, all permanent. Other tributaries are presently intermittent (3–5 flash floods on average per year) (Bensaad et al., 2017; Mabrouki et al., 2017).

Surveys

Field investigations (often in the framework of various hydrobiological studies) have been carried out since 2014 at 45 stations throughout the Moulouya River basin, including its main affluents: Oued Anzegmir (side of the High Atlas), Oued Melloulou (Middle Atlas slope) and Oued Za (High Plateau), and at about 60 stations spread throughout eastern Morocco from the northern regions of Nador and Saïdia, to Figuig in the southeast and Talessint and Bouanane in the southwest (fig. 1). The stations that showed the occurrence of the mentioned fish species were re-examined between 2017 and 2018. Quantitative sampling of fish fauna was carried out using nets, searching in the most suitable places for the studied species. Sampling lasted an average of one hour over an area of 10 m² at each station, sufficient time

to trap and catch virtually all the fish in each area. Invasive species caught were preserved in formalin solution, while native fish (especially fry) were returned to the water. Permission to perform these studies was granted by the authorities.

Abiotic factors

For this study, ten environmental parameters were selected: sulfate (SO_4^{2-}), biological oxygen demand after 5 days (BOD_5), phosphate (PO_4^{3-}) and nitrate (N-NO_3) were measured in the laboratory. Conductivity, salinity, pH, dissolved oxygen, temperature and the mean depth were measured *in situ* using a multiparametric measuring device (WTW, Multi-Line P4). Two replicas of water samples from each station were taken in 500 ml polyethylene bottles. The water samples were preserved with 2 ml of concentrated hydrochloric acid ($\text{pH} = 2$). According to standards ISO 5667–6 (1990), ISO 5667–2 (1991) and ISO 5667–3 (1994), water samples were transported in a cooler at a low temperature ($\pm 4^\circ\text{C}$) to stop the metabolic activities of organisms in the water.

The current velocity, well known for its selective action on habitat and species distribution (Mabrouki et al., 2019a), was quantified by its mean value at three different locations of the same station. In the absence of a hydrometric reel to measure current velocity it was estimated using a stopwatch at various points of the watercourse by measuring the time it takes for a floating object to cross a given path. The average speed (converted to cm^{-1}) was semi-quantitatively estimated, followed by a transformation into three modalities: 1, very low to no current $< 5 \text{ cm}^{-1}$; 2, low current $5 < 2 < 25 \text{ cm}^{-1}$; and 3, average current $25 < 3 < 50 \text{ cm}^{-1}$.

Statistical processing

Statistical analyses were carried out using software R package version 3.3.1. (R Core Team, 2019). In the modelling of counting processes, here the abundance of a species, two kinds of models are commonly implemented, namely the Poisson model and the negative binomial model (Hilbe, 2011). Frequently, counting data is characterized by overdispersion, whereby the sample variance is greater than its average. In the case where the anomalous dispersion is proved, the Poisson regression is no longer suitable for modelling this distribution, and the negative binomial regression model, allowing more flexibility in the dispersion, should be used. The relevance of Poisson regression or negative binomial models was evaluated by the Pearson Residue Test (Plackett, 1983).

Results

Occurrence, distribution and habitat types of the studied species

Despite our repeated sampling and the large number of prospected stations, we were unable to find *A. fasciatus* (or any *Aphanius* species). In contrast, its probable range was totally occupied by two exotic fish species *F. heteroclitus* and *G. holbrooki*. The species *F. heteroclitus* was found in six of 45 sampling sites while *G. holbrooki* appeared in 35.

In Morocco, the distribution of *F. heteroclitus* seems to be limited to the Oriental region so far, from the wetlands of Ain Chabbak to the mouth of the Moulouya River (fig. 2). Its habitat consists of coastal swamps and large sections of the Moulouya to its confluence with the Mediterranean, at an altitudinal ranging between 0 and 307 m above sea level. In shallow habitats, where predators are less abundant, such as in the Ain Chebbak wetland, *F. heteroclitus* can be found swimming freely in pelagic and superficial parts along with *G. holbrooki*. On the other hand, in the main watercourse of the lower Moulouya, where the depth is greater and predatory fishes are more abundant, *F. heteroclitus* was only caught among the aquatic vegetation, always together with *G. holbrooki*, except near the river mouth in extreme lower Moulouya (stations S37, S78 and S39), where it was captured alone.

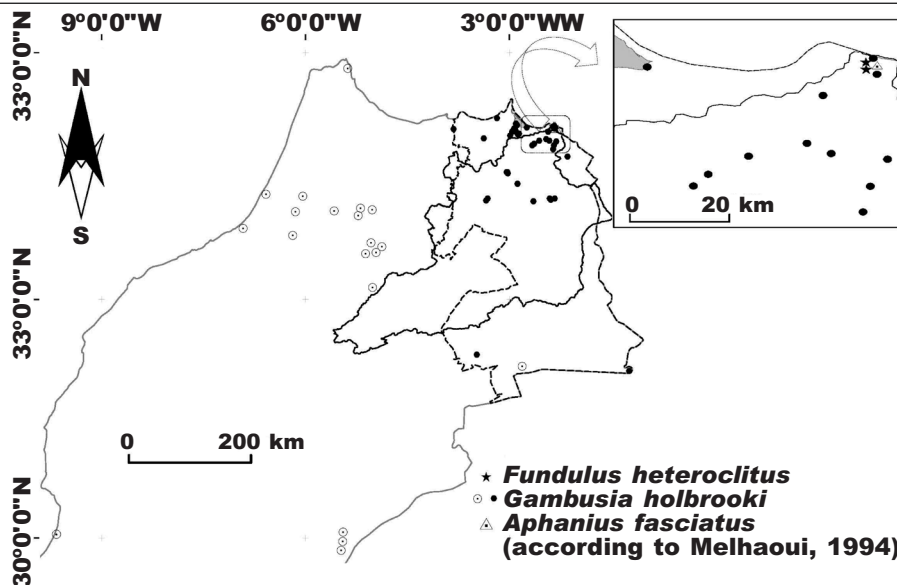


Fig. 2. Distribution of the studied species in Morocco (black dots represent new records, white circles represent bibliographic).

Fig. 2. Distribución de las especies estudiadas en Marruecos (nuevos registros en negro, registros bibliográficos en blanco).

With regard to *G. holbrooki*, the species appears to be widely distributed through Morocco, including the Moulouya River Basin (fig. 2). It is highly confined to brackish waters near the coast (as in Nador lagoon and the Moulouya SIBE), and it is omnipresent along Za watercourses, Sebra and the complex Zeghzel–Cherraa rivers. It can go up to Melloulou and the Middle Moulouya during the summer period with the weakening current. The species also appears to be well established in other areas of the oriental region of Morocco, from Nador and Al Hoceima north and to Figuig south–east and the Talessint region south–west. The eastern mosquitofish seems able to cope remarkably well in a wide range of habitats, even those that are highly degraded as long as there is water. Its habitats range from large permanent streams of average and low altitudes, to dams, natural and managed springs, and small temporary tributaries (provided that they are connected to a permanent source or underground water). It can be also found in human constructions such as irrigation canals and cement tanks for water retention.

The physicochemical variables of water

Both *G. holbrooki* and *F. heteroclitus* species are well known for their tolerance to large variations in abiotic parameters (Kneib, 1986; Lipcius and Subrahmanyam, 1986; Pyke, 2008). However, in the study area, *G. holbrooki* appears to excel *F. heteroclitus* in term of oscillation intervals of most of the studied parameters (see appendix). *G. holbrooki* showed great plasticity in terms of salinity; was recorded in low mineralized waters with a minimum of 0.587 g/l (587.67 ppm), and also in the brackish waters of the coastal system with a maximum of 1,837 g/l (1,839.09 ppm). The greater plasticity of the species was demonstrated mainly by

the large intervals of the organic pollution indicators, showing that it can tolerate BOD₅ concentrations between 1.49 and 20.3 mg/l, nitrate between 1.25 and 67 mg/l, sulfate between 33 and 403 mg/l phosphate between 0.016 and 2.82 mg/l and finally pH between 6.65 and 8.3. This same observation was noted for dissolved oxygen; the species seems to withstand great fluctuations of this vital element and it can be found not only in very well oxygenated waters 11.3 mg/l but also in weakly oxygenated waters with a minimum of 2.01 mg/l.

Fundulus heteroclitus is instead more demanding in terms of the physicochemical quality of the water and showed weak ranges of variation for most of the studied parameters (see appendix). However, it can survive in a hypersaline waters compared to *G. holbrooki*, with a maximum of 2,757 g/l (2,760.15 ppm) recorded at the Moulouya mouth (S39).

Concerning temperature, it is difficult to accurately evaluate variation for these two species in the study area because we did not perform a monthly follow-up in all the stations because of the violent floods recorded during the cold period. However, water temperature is linked to local conditions (climate, duration of sunshine, flow and altitude) and to seasonality in the Moulouya River Basin (Mabrouki et al., 2016; Taybi, 2016). During the study period, the maximum temperature did not exceed 26°C for either species. Given the lack of sampling during winter, our study does not report the lowest temperature at which the fish remain alive or active.

Modelling results

Poisson regression and negative binomial regression showed the same explanatory variables of the abundance frequency, with similar effects. However, the Poisson regression model showed an abnormal over-dispersion, and its statistical relevance was rejected by the Pearson Residue Test. The negative binomial regression model was better adapted to these data, and its statistical relevance was verified.

Discussion

Growth in international trade and concurrent increases in transport capacity have accelerated the rate of introduction of alien species worldwide, with freshwater ecosystems, and their native fish communities, being particularly susceptible (Sala et al., 2000; Kolar and Lodge, 2002; Macdonald and Tonkin, 2008). The most important pathways of freshwater invasions are aquaculture, pet/aquarium trade and stocking activities, shipping, and inland canals, without forgetting that some species can be introduced intentionally (Coccia, 2015; Nunes et al., 2015). Invasive species are a major threat to global biodiversity, causing the decline and extinction of native freshwater fish species throughout the world (Strauss et al., 2006; García-Berthou et al., 2005; Srean, 2015). The piscivorous 'Nile perch' *Lates niloticus* (Linnaeus, 1758) is a classical example, as its introduction in the 1950s in Lake Victoria in East Africa led to the extinction of over 200 endemic fishes (Kitchell et al., 1997).

During our survey, we did not find *A. fasciatus* in Morocco in spite of our exhaustive sampling. This could be due to several reasons, such as pollution and destruction of the habitat on one hand, and the impact of alien fish on the other, as has occurred in many areas of its range (IUCN, 2006). We should take into account that the record of *A. fasciatus* from Morocco could be erroneous due to misidentification and the fact that scientific collections from Morocco contain no samples. Although healthy populations still exist in some parts of the Maghreb, occupying the same type of habitats in Algeria and Tunisia (Kraiem, 1983; Boumendjel et al., 2015), the palaeogeographic history of Morocco differs. Until 5.3 million years ago (Krijgsman et al., 1999a, 1999b), together with Spain, the north of Morocco formed the Betico-Rifian massif. As Spain and Morocco shared fauna not present in the other countries, in Morocco populations it would be more likely to find species related to Spanish aphanidiids, such as *A. baeticus* Doadrio, Carmona and Fernández-Delgado, 2002 or *A. iberus* than *A. fasciatus*. On the other hand, the present distribution of *A. iberus*, *A.*

baeticus, and even *A. saourensis* Blanco, Hrbek and Doadrio, 2006 occurs along the coastline that existed in the Miocene before the separation of the Betico–Rifian Massif at the Miocene–Pliocene boundary 5.3 million years ago (Doadrio, 1994; Krijgsman et al., 1999a, 1999b). It is therefore more likely to find *Aphanius* in inland Morocco than in coastal rivers.

In consensus with these observations, the previous record of *A. fasciatus* from Morocco would be the misidentification of a different species of the genus. Nevertheless, the idea that alien fishes have driven the Moroccan population of a native *Aphanius* species to extinction is also possible, especially in eastern Morocco, where *G. holbrooki* and *F. heteroclitus* have invaded all suitable hydrosystems, from the fresh continental waters of rivers and springs, to brackish and salty waters of coastal lagoons and wetlands.

The nature of *G. holbrooki* with its high reproductive potential, fast maturation rate, flexible behavior and broad environmental tolerances have contributed to its success as an invader, and the species is considered to pose a serious threat not only to native fishes worldwide but also to amphibians, macro–invertebrates, zooplankton and phytoplankton communities (Courtney and Meffe 1989; Howe et al., 1997; Caiola and de Sostoa, 2005; Macdonald and Tonkin, 2008; Rowe et al., 2008; Mabrouki et al., 2019b). Usually, the species of the genus *Aphanius* can take refuge in estuarine habitats to escape competition with *G. holbrooki* due to its strong salt–tolerant character like all cyprinodontids, as has previously been reported (Nordlie and Mirandi, 1996; Doadrio, 2002; Alcaraz et al., 2008; Valdesalici et al., 2015). However, in our case these refuges are occupied by another alien fish, which is also a cyprinodontiform specialized in mesohaline and hyperhaline waters and which is possibly responsible for the population regression of *A. baeticus* in the Iberian Peninsula (Oliva–Paterna et al., 2006).

Regarding the ecological affinities of the two invasive species, the results of the negative binomial regression of mosquitofish abundance as a function of the environmental variables selected for this study (table 1) showed the existence of a highly significant static relationship between *Gambusia* abundance and salinity, and water velocity (p -value < 0.001), and a very significant relation with depth (p -value between $[0.001, 0.01]$). This correlation is negative for the three variables, i.e., if the salinity of the environment decreases by 1.4, the abundance of *G. holbrooki* will increase by one. *G. holbrooki* has extensive tolerance for several environmental factors as reported in other works (Pyke, 2008), but according to our results it seems to prefer shallow waters with average to low salinity and slow current velocity, in agreement with findings in previous studies (Stearns and Sage, 1980; Brown–Peterson and Peterson, 1990). Its peak of colonization will thus be carried out, most probably, in shallow lentic water bodies with low or moderate salinity. Brown–Peterson and Peterson (1990) reported that females from freshwater were in better somatic condition and matured later, since ovarian development was significantly more advanced in oligohaline waters. The same model was used for *F. heteroclitus*; a positive correlation was detected between this species and salinity (table 2), and the relevance of this regression model was verified by the Pearson residue test; the model is well adapted to the data.

To conclude, both invasive species generally occur in water that is shallow, stagnant or slow moving, with dense aquatic and riparian vegetation. In water bodies that are moderately deep, they are generally found along the shallow edges, especially those that are well vegetated. However, *F. heteroclitus* seems to be specialized in brackish saline waters of coastal hydrosystems, while *G. holbrooki* seems specialized in freshwater and slightly brackish water, showing scarce selectivity in terms of habitat, as was observed in its native area (Moyle and Nichols, 1973). Indeed, this species can occur in environments that range from almost completely undisturbed swamps, lakes, and streams to highly disturbed water bodies including urban drains with various water quality problems such as pollution and dense growth of aquatic and surface weeds. *G. holbrooki* is often extremely abundant in disturbed habitats near urban areas (Arthington and Lloyd, 1989; Pyke, 2008). This could be explained by its high tolerance to fluctuations in abiotic factors (Odum and Caldwell, 1955; Keup and Bayliss, 1964; Cherry et al., 1976; Fastelli et al., 2012), this trait being one of the main causes behind its expansion success and outcompeting *F. heteroclitus* in the

Table 1. Estimates of the parameters of the negative binomial regression model (for *Gambusia holbrooki*): SE, standard error; IC, interval of confidence.

Tabla 1. Estimaciones de los parámetros del modelo de regresión binomial negativa (para *Gambusia holbrooki*): SE, error estándar; IC, intervalo de confianza.

Coefficients					
	Estimate	SE	z-value	Pr(> z)	Sign.
(Intercept)	6.247685	0.750739	8.322	< 2.10 ⁻¹⁶	***
Depth	-0.017242	0.005463	-3.156	0.001600	**
Salinity	-1.430826	0.399830	-3.579	0.000345	***
Velocity	-0.708805	0.184655	-3.839	0.000124	***

IC (95%)			Quality of fit of the model	
	Inf.	Sup.		
(Intercept)	4.776263	7.719107	Residual deviance	38.825
Depth	-0.027949	-0.006533	Degrees of freedom	32
Salinity	-2.214478	-0.647172	AIC	262.1
Velocity	-1.070722	-0.346887	Pearson residue test	0.24688

Table 2. Estimates of the parameters of the negative binomial regression model (for *Fundulus heteroclitus*): SE, standard error; IC, interval of confidence.

Tabla 2. Estimaciones de los parámetros del modelo de regresión binomial negativa (para *Fundulus heteroclitus*): SE, error estándar; IC, intervalo de confianza.

Coefficients					
	Estimate	SE	z-value	Pr(> z)	Sign.
(Intercept)	1.3836	0.4029	3.434	0.000595	***
Salinity	0.4141	0.2045	2.025	0.042867	*

IC (95%)			Quality of fit of the model	
	Inf.	Sup.		
(Intercept)	0.59391502	2.1733113	Residual deviance	7.2169
Salinity	0.01330016	0.8148827	Degrees of freedom	4
			AIC	37.282
			Pearson residue test	0.1661

study area. Nevertheless, the apparently limited tolerance towards different environments observed in *F. heteroclitus* might also be due to its recent introduction in the study area, or to the presence of *G. holbrooki*, that could outcompete it in more internal water bodies.

Research on invasive species is an urgent research topic for the conservation of biodiversity (Dukes and Mooney, 2004; Rice and Sax, 2005; Taybi et al., 2020a). Studies on the impact of invasive fish on native fauna have flourished around the world in recent years. However, little is known about their occurrence, biology and influence in North Africa in general or in Morocco in particular. Since *G. holbrooki* was first introduced in Morocco in 1929 to fight against waterborne diseases, it has invaded all the northern Moroccan hydrosystems (Azeroual, 2003; Clavero et al., 2014). Our surveys in eastern Morocco have significantly increased its known range. Future assessments in other parts of the country could expand the species' distribution area, especially in the Western Rif and the High and the Anti-Atlas valley, and even in the Sahara. On the other hand, *F. heteroclitus* seems to be limited to the wetland of Moulouya mouth for the time being, but thanks to its 'salt-tolerant' character, this newcomer could soon invade habitats of the same type. Indeed, considering the habitat destruction and deterioration of the quality of surface waters currently occurring in Morocco (Mabrouki et al., 2016; Taybi et al., 2016; Bensaad et al., 2017; Mabrouki et al., 2017; Taybi et al., 2020b), in addition to the climate change effects and intensification of saline stress (Moss et al., 2009), both alien fishes would be in a better position to invade North African ecosystems. It is also of note that freshwater ichthyofauna of Morocco is still little known and new species are being recorded (Ford et al., 2020). Several native fish species could co-occur with the two invaders in eastern Morocco and in the Moulouya River basin, such as the North African shad *Alosa algeriensis* Regan, 1916 and many endemic barbels (i.e. *Luciobarbus quercifensis* Doadrio, Casal-López and Yahyaoui, 2016; *L. lanigarensis* Brahim, Libois, Henard and Freyhof, 2018 and *L. yahyaoui* Doadrio, Casal-López and Perea, 2016). Knowledge of the distribution of both native and non-native freshwater fishes in Morocco therefore merits urgent research.

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Appendix. Abundance and value of abiotic parameters measured at the sampling sites where *G. holbrooki* and *F. heteroclitus* were found: Gam, abundance of *Gambusia*; Fun, abundance of *Fundulus*; Depth, in cm; Temp, water temperature in °C; Sali, salinity in g/l; Dis_O, dissolved oxygen in mg/l; NO₃, nitrate in mg/l; SO, sulfate in mg/l; PO, phosphate in mg/l; BOD₅ in mg/l; Vel, current velocity in cm/s.

Apéndice. Abundancia y valor de los parámetros abióticos medidos en los puntos de muestreo donde se encontraron *G. holbrooki* y *F. heteroclitus*. (Para las abreviaturas, véase arriba).

Code	GPS	Dates	Gam	Fun	Dept	pH	Temp	Sali	Dis_O	NO ₃	SO	PO	BOD ₅	Vel	
S01	34.886388	-2.662500	15/10/2017	23	0	50	7.35	20	0.778	8.87	5.35	279	0.495	4.93	2
S02	34.907647	-2.635794	22/10/2017	49	0	40	7.5	23	0.796	7.87	2.90	227	0.023	1.49	2
S03	35.051583	-2.428444	22/10/2017	4	0	100	7.55	23	0.988	7.97	9.95	49	0.805	5.65	2
S04	35.097611	-2.388611	22/10/2017	5	0	100	7.8	25	1.337	8.3	9.93	33	0.160	3.30	2
S05	34.838972	-2.356000	15/10/2017	9	0	60	7.1	19	0.421	7.15	1.35	51	0.015	3.93	2
S06	35.101091	-2.350840	15/10/2017	13	8	80	7.1	20	0.43	7.2	2.27	55	0.010	5.89	2
S07	34.945444	-2.413472	15/10/2017	35	0	54	6.75	22	1.011	5.01	5.95	129	1.105	5.75	1
S08	34.964310	-2.457187	15/10/2017	29	0	60	6.65	23	1.048	2.01	100	209	2.095	20.30	1
S09	35.119388	-2.337583	30/07/2018	0	8	40	7	27	1.456	8	19.6	320	1.5	9.2	1
S10	35.105194	-2.345833	30/07/2018	6	0	50	7.1	25	1.466	7.25	1.25	52	0.017	5.93	1
S11	35.099722	-2.328361	22/02/2016	17	0	20	7.6	25	1.837	9	9.83	90	0.4	5.7	1
S12	34.935222	-2.311416	30/07/2018	26	0	46	8.2	25	0.912	9	21.4	70	0.3	9	1
S13	34.216986	-3.344555	08/07/2018	5	0	43	7.6	26	0.587	5.6	7.37	65	0.05	6.80	3
S14	34.242113	-2.336661	01/07/2018	19	0	60	7.9	26	0.455	6.82	3	79	0.035	6	2
S15	34.226888	-2.392916	01/07/2018	9	0	65	7.5	26	0.511	7	1.25	51	0.016	5.49	3
S16	34.239333	-2.409666	01/07/2018	5	0	40	7.9	26	0.616	5.8	1.3	53	0.023	5	3
S17	34.206416	-2.647861	22/07/2018	5	0	100	7.3	25	1.58	8	3	69	0.02	5.2	1
S18	34.421000	-2.881361	22/07/2018	12	0	50	7.3	25	0.874	7.4	2.2	51	0.019	4.2	3
S19	34.546252	-3.025305	29/07/2018	6	0	50	6.9	24	0.909	5.15	2.79	52	0.029	10.16	3
S20	34.241627	-3.320388	08/07/2018	5	0	60	7.6	24.6	0.707	4.23	49.2	362	1.34	10	2
S21	34.560083	-3.042611	29/07/2018	6	0	40	7.42	24.3	0.745	5.36	14	298	0.9	6	3
S22	34.561413	-3.030491	29/07/2018	6	0	45	7.35	25	0.781	6.24	16.64	342	1.05	8	3
S23	35.059201	-2.948783	17/08/2018	24	0	43	7.6	25	0.975	6	32.1	261	2.82	11.21	2
S24	35.129972	-2.883833	19/08/2018	16	0	35	8.3	25	1.178	5	53.62	179	1.37	11	1
S25	35.051722	-2.962944	17/08/2018	12	0	43	7.6	25	0.91	8	8.22	96	0.4	10	2
S26	35.053055	-2.905250	12/08/2018	29	0	56	8.1	24	0.534	12	2.4	53	0.2	6	2
S27	35.063500	-2.906388	12/08/2018	65	0	53	8.1	25	0.644	11.3	2.32	78	0.32	10	2
S28	35.087305	-2.934388	12/08/2018	23	0	60	7.42	25	0.946	9	10.23	65	0.04	8	2
S29	35.017750	-2.876666	13/08/2018	6	0	130	8.1	25	0.944	10	3.32	62	0.32	9.3	1
S30	35.103469	-2.745860	22/05/2018	13	0	80	8	24	1.127	10	40	403	2.04	12.5	1
S31	35.147185	-2.902922	22/05/2018	9	0	60	8.3	24	1.078	8	25	82	1.1	10.4	1
S32	35.151729	-2.904918	22/05/2018	14	0	64	8.2	24	1.097	7	31	70	1.3	17.5	1
S33	35.213583	-3.183722	30/08/2018	13	0	48	7.42	24.5	0.634	7	15	98	0.9	7	2
S34	34.972793	-3.374910	30/08/2018	10	0	50	7.42	25	0.591	8	13.53	120	0.01	6	2
S35	35.085162	-3.818661	31/08/2018	45	0	53	7.5	24.5	0.636	11	3.7	73	0.42	8	1
S36	32.313449	-3.479673	13/05/2018	12	0	64	7.6	24	0.912	10	2	47	0.027	6	2
S37	35.104580	-2.344717	30/07/2018	0	2	25	7.8	25	0.864	9	12.83	98.4	0.5	5.6	1
S38	35.109127	-2.362519	10/06/2018	0	5	100	7.8	26	2.055	8	9.50	46	0.35	4.78	1
S39	35.114235	-2.345846	10/06/2018	0	10	100	8	26	2.211	7.6	8.90	42	0.6	5.75	1
S40	35.122983	-2.34121	23/10/2017	0	16	120	7.5	25	2.757	8.5	9.41	35	0.46	4.40	1