2017

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Knowl. Manag. Aquat. Ecosyst. 2017, 418, 56
© F. Banha et al., Published by EDP Sciences 2017
https://doi.org/10.1051/kmae/2017045

www.kmae-journal.org

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Management of
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Journal fully supported by Onema

RESEARCH PAPER OPEN ACCESS

Forensic reconstruction of *Ictalurus punctatus* invasion routes using on-line fishermen records

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Abstract – In this work, the presence of the channel catfish *Ictalurus punctatus* in the Portuguese section of the Guadiana drainage (Iberian Peninsula) is confirmed based on morphological and molecular species identification. The spatial and temporal dispersal of this non-native catfish was also reconstructed for the Guadiana drainage, based mostly on online fishermen records with minor contributions from the few scientific reports available. The obtained records (mainly from angling fora) span the period since the species' first reported presence in Iberia (1980s) up to the present, and support a westward invasion pattern of non-native fish (NNF) reported for the Iberian fish invasion hotspot. The invasion pathway is driven mainly by natural dispersal downstream at a rate between 8 and 42 km per year. Yet, at least four introduction events within the Guadiana drainage can unambiguously be assigned to human translocations after the initial human-mediated introduction. The present study reinforces the usefulness and relevance of using validated on-line fishermen records, provides a more complete and updated distribution range of NNF species and enables assessment of their dispersal patterns. This is of particular importance because it allows near real-time monitoring of NNF dispersal, including first occurrences of NNF, at minimal cost.

Keywords: dispersal / channel catfish / portugal / non-native fish / distribution

Résumé – Reconstruction scientifique des voies d'invasion d'*Ictalurus punctatus* à l'aide d'articles en ligne de pêcheurs. Dans ce travail, la présence du poisson-chat *Ictalurus punctatus* dans la partie portugaise du bassin hydrographique du Guadiana (Péninsule Ibérique) est confirmée sur la base de l'identification morphologique et moléculaire de l'espèce. La dispersion spatiale et temporelle de ce poisson-chat non indigène a également été reconstituée pour le bassin du Guadiana, en se basant principalement sur des articles en ligne de pêcheurs, avec des contributions mineures provenant des quelques rapports scientifiques disponibles. Les enregistrements obtenus (principalement à partir de forums de pêche à la ligne) couvrent la période depuis la première présence signalée de l'espèce en Péninsule Ibérique (1980) jusqu' à aujourd'hui, et confirment un schéma d'invasion vers l'ouest des poissons non indigènes (NNF) signalé par le hotspot d'invasion des poissons ibériques. La voie d'invasion est faite principalement par la dispersion naturelle en aval, à un rythme de 8 à 42 km par année. Cependant, au moins quatre événements d'introduction dans le bassin hydrographique du Guadiana peuvent être attribués sans ambiguïté à des translocations humaines après l'introduction initiale par l'homme. La présente étude renforce l'utilité et la pertinence d'utiliser des informations en ligne validées de pêcheurs, afin de fournir une carte de distribution plus complète et mise à jour des espèces de poissons non indigènes et d'évaluer leurs modèles d'invasions. Ceci est d'une importance particulière, car il permet une surveillance quasi en temps réel de la dispersion des NNF, y compris les premières occurrences de NNF, et ce, presque gratuitement.

Mots-clés : dispersion / poisson-chat / portugal / poisson non indigène / distribution

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1 Introduction

Non-native species are a leading threat to aquatic biodiversity worldwide (Sala *et al.*, 2000), and to freshwater fishes in particular (Moyle and Light, 1996). The main vectors of non-native fish (NNF) introductions are aquaculture, recreational fisheries and ornamental fish trade (Welcomme, 1988; Gozlan *et al.*, 2010), although the importance of these vectors varies geographically. For instance, while aquaculture and waterways are the main reasons for NNF introduction and establishment in central Europe, recreational fisheries are the predominant vector in the Iberian Peninsula (Elvira and Almodóvar, 2001; Ribeiro *et al.*, 2009a; Rabitsch *et al.*, 2013). The Iberian Peninsula has been referenced as a fish invasion hotspot (Leprieur *et al.*, 2008), which is of major concern given its large number of endemic species with restricted distributions (Filipe *et al.*, 2009). Indeed, Iberian freshwater ecosystems exhibit a high rate of NNF arrival, with one new species being recorded every two years (Elvira and Almodóvar, 2001; Ribeiro *et al.*, 2009a). In fact, more than five new NNF were identified in this region in the last decade (*e.g.* Ribeiro and Veríssimo, 2014; Banha *et al.*, 2015), of which many are listed as high impact NNF (*e.g.* Ribeiro *et al.*, 2015). Despite the high rate of recent NNF introductions in the Iberian Peninsula, little is known about how and where illegal recreational fish introductions occur.

Early detection of non-native species is crucial to minimize the negative impacts of a potential invasion to the recipient ecosystems and to reduce the associated costs of subsequent control/remediation actions (Mehta *et al.*, 2007). Indeed, it has been shown that eradication of invasive species is more difficult following species' establishment and involves increased costs (Simberloff, 2001).

Early detection of NNF can be made using online information about recreational fishermen catches. The use of online fishermen fora is growing at an increasing rate, providing an unparalleled opportunity for improved and "near-real time" knowledge on recent NNF arrivals (Ribeiro and Veríssimo, 2014; Banha *et al.*, 2015). These records can be posteriorly verified *in situ* by scientists and/or managers allowing for unambiguous species identification, *e.g. via* morphological and/or molecular studies, while helping to direct sampling efforts in the field and subsequent monitoring of the species' establishment and control actions. These non-scientific data also have great potential in helping the reconstruction of the spatial invasion patterns of NNF. A recent study in the Iberian Peninsula used records of the non-native *Silurus glanis* from online fishermen fora to unveil the progression of this species in the Tagus drainage and provided information about the species' habitat and invasion through time (Gago *et al.*, 2016).

The general spatial invasion pattern of NNF in Iberian Peninsula (*i.e.* westward invasion) provides a unique opportunity to distinguish human-mediated introductions from natural dispersal (Ribeiro *et al.*, 2009b; Gago *et al.*, 2016). In fact, most NNF Iberian introductions initially occur in Catalonian watersheds (northeastern Iberia), followed by secondary introductions in central Iberia (upper reaches of international rivers), that drain westwards and eventually arrive to the Portuguese section in the lowland areas of international rivers (Ribeiro *et al.*, 2009b). The channel catfish, *Ictalurus punctatus* (Rafinesque, 1818) is one such species apparently following this spatial pattern (Hermoso *et al.*, 2008). The species was supposedly firstly introduced in the lower Ebro (northeastern Iberia) in the late 1980s (Doadrio, 2001; Elvira and Almodóvar, 2001; López *et al.*, 2012), but subsequent records were on the mid part of the Guadiana drainage, an international river (Hermoso *et al.*, 2008). Despite this frequent Peninsula-wide spatial invasion pattern, there is little information about NNF dispersal within watersheds, although such knowledge is particularly relevant for the management of international watersheds.

The channel catfish is originally from the eastern United States and northeastern Mexico but has been introduced worldwide, including at least five European countries, mostly for aquaculture and for recreational fisheries (Elvira and Almodóvar, 2001; Uzunova and Zlatanova, 2007; Ligas, 2008; Zogaris *et al.*, 2012). This species is an omnivorous opportunistic feeder but large individuals become piscivorous (Perry Jr., 1969; Cannamela *et al.*, 1978). The channel catfish exhibits parental care (nest guarding), relatively high fecundity, and is tolerant of a wide range of environmental conditions (Tucker and Hargreaves, 2004). It also exhibits high longevity (up to 40 years old) and attains large sizes (over 10 kg and 1 m long) (Moyle, 2002). Although the channel catfish may exert a major negative effect on native fishes populations, through competition for food and habitat, or through predation (Marsh and Brooks, 1989; Hawkins and Nesler, 1991; Olden and Poff, 2005), there are no studies that address the ecological impact of the species (Savini *et al.*, 2010).

In this work, the presence of the non-native channel catfish *I. punctatus* is confirmed for Portugal, using both morphological and molecular species identification. Correct taxonomic identification of NNF is mandatory for a species to be included in the national inventory of non-native species. It is also extremely important in delineating adequate management plans (*e.g.* Goolsby *et al.*, 2006) and in future monitoring of species' establishment and impact. Additionally, the spatial and temporal invasion of the channel catfish within the Guadiana watershed was reconstructed based on anglers' online records, and the usefulness of this type of approach for invasion pathway reconstruction was demonstrated.

2 Materials and methods

2.1 Confirmation of *Ictalurus punctatus* presence in Portugal

On 12 October 2016, a professional fisherman captured ten *I. punctatus* individuals using a 80 mm mesh gillnet in the Alqueva reservoir (38°11′57.89″ N; 7°29′47.93″ W). The fish were brought to the laboratory for morphological identification following Hubbs *et al.* (2008) and Paruch (1986), based on meristic features. Total length (TL), fork length (1 mm) and total weight of all specimens were measured to the nearest 1 mm and 0.01 g. Fishes were dissected to determine the sex and the maturity stage. A fin clip was also collected from each fish and preserved in absolute ethanol for molecular species identification using a fragment of the cytochrome oxidase subunit I (COI) gene. Briefly, genomic DNA (gDNA) was extracted using the EasySpin Genomic DNA Tissue Kit (Citomed, Lisbon, Portugal), following the manufacturer's protocol. A 619 bp fragment of the COI was amplified using the Polymerase chain reaction (PCR) and the following primers: FishF2 5′ TCGACTAATCATAAAGATATCGGCAC 3′, and FishR1 5′ TAGACTTCTGGGTGGCCAAAGAATCA 3′ (Ward *et al.*, 2005). PCR reactions were performed in a total volume of 10 μl including 5 μl of MyTaqTM HS Mix 2X (Bioline, London, U.K.), 0.4 μl of each primer (10 μM), 3.2 μl of ultrapure autoclaved water, and 1 μl of gDNA. After optimization, the PCR temperature profile included an initial denaturation of 5 min at 95 °C, followed by 35 cycles of 1 min at 95 °C, 30 s at 57.2 °C, 1 min at 72 °C and a final extension step of 10 min at 72 °C. The amplicons were sequenced at Macrogen Europe (Amsterdam, The Netherlands) and quality control of the resulting sequences was performed in Geneious (Biomatters, 2013). Sequences were aligned using the Geneious algorithm under default conditions and compared to those available in GenBank for other *Ictalurus* species.

The specimens and respective tissue samples were deposited in the zoological collection " Museu Bocage" of the Museu Nacional de História Natural e da Ciência (MUHNAC; University of Lisbon, Portugal): whole voucher specimen MB05-003527 in the Fish Collection; Tissue and DNA Collection samples MB85-016900 to MB85-016909.

2.2 *Ictalurus punctatus* invasion

Extensive searches on different information sources such as literature, social media websites (*e.g.* YouTube), recreational fisheries fora and blogs, and news-media channels were consulted to obtain channel catfish *I. punctatus* records aiming at tracking the invasion pathway and timing within the Guadiana drainage. Following Gago *et al.* (2016), a Boolean search was performed during October and November 2016 using different combinations of keywords, including common names in Portuguese and Spanish (*i.e.* peixe-gato; pez gato; pez gato del canal), and the river name (Guadiana). As in previous works (*e.g.* Gago *et al.*, 2016), confirmed species' records were accepted only when including locality, year and accompanying media (picture or video that allows confirmation of morphometric features of channel catfish such as four pairs of barbels, adipose fin clearly separated from the caudal fin, deeply forked caudal fin). Additionally, only the earliest record was retained when multiple records were reported for the same location in different information sources. The geographical coordinates were extracted from Google Maps, and when a reservoir name was given as locality, the coordinates from the reservoir mid-point were used. The river distance (in km) between each pair of records was measured based on satellite photos available in Google Earth® 7.1.7.2606 version using the measure tool. Annual expansion rates within the Guadiana catchment were calculated based on the two most distant records per year.

3 Results

3.1 Confirmation of *Ictalurus punctatus* presence in Portugal

The specimens captured in the Portuguese section of the Guadiana watershed had four pairs of barbels, dorsal fin with one spine and six soft rays, pectoral fins with a spine, and naked skin, characteristics of the family Ictaluridae. The specimens also had eyes present, adipose fin clearly separated from the caudal fin, deeply forked caudal fin, assigning them to the genus *Ictalurus*. The deep caudal fork, the silvery body with dark spots along the dorsal area, anal fin ray counts under 30 (median = 27, range between 24 and 29), and serrated barbs on the posterior side of the pectoral spine supports their identification as *I. punctatus*. The individuals captured presented a mean TL of 416 ± 57 mm SD and mean total weight of 646 ± 286 g SD. All individuals had undeveloped gonads and thus it was not possible to determine their sex macroscopically.

Molecular analyses provided concordant identification of the specimens as *I. punctatus*. Of the ten sampled specimens, eight produced good quality COI sequences and the final alignment showed all sequences to be identical (GenBank Accession no. KY471388). BLAST searches showed the above haplotype exhibited 98–99% similarity (99% query cover; E-value: 0.0) to available *I. punctatus* sequences from the North American native range (*e.g.* GenBank Accession nos. JF292392–JF292380; Wong *et al.*, 2011). Other *Ictalurus* species' entries showed a lower sequence similarity (<94%) for the same level of query coverage (*i.e.* 99%).

3.2 *Ictalurus punctatus* invasion

A total of 42 records were obtained for channel catfish on the Guadiana river catchment, with the angling fora representing the main source of information with 59% of all records. A mean of three records per year were registered from 2006 to 2016, with a maximum of 9 records in 2012. Although the number of records grew regularly over the years, the number of locations was stable until 2010, but doubled thereafter (Tab. 1). Almost 50% of all new records were within a 50 km distance of previous ones, with the number of records generally decreasing with increasing distance. Records at distances of 50–100 km represent less than 20% and from 100 km to 150 km only 12% of total records. However, approximately 25% of the occurrences were registered at distances over 200 km. Records corresponding to large expansions in the invaded area occurred in two periods: 2010–2011 and 2015–2016, with the addition of nearly 100 km of river stretch in both cases (Fig. 1).

Reconstruction of the spatial invasion pattern of the channel catfish along the Guadiana River showed that dispersal was mainly downstream, at a rate ranging between 8 and 42 km per year (Fig. 2). At least four introduction events within the Guadiana drainage can unambiguously be assigned to human assisted introductions because these locations are isolated (by downstream dams) from previously invaded areas. The first record in the Guadiana drainage is from 1983 at Castilseras dam located in a tributary of the Zújar river, based on a catfish anglers' video on Youtube. Subsequent records are from the Zújar and La Serena dams in the 1990s, both located in the Zújar River downstream from the Castilseras dam. These two locations represented 62% of the records gathered in this work (12 records in Zújar dam and 14 in La Serena dam). The second introduction event occurred during the 1990s at Orellana dam, located in the mainstream of the Guadiana river and with no direct connection to the Zújar river. Thus, the most probable source might be a translocation from the Zújar river (11 km by

Table 1. Number of *Ictalurus punctatus* records per time period in the River Guadiana drainage between the 1980s and 2016, collected from various information sources: literature (papers and books); angling forums; youtube.com; blogs and other web sources (*e.g.* tourist promoter's sites, environmental groups, popular science sites, instagram.com, flickr.com, facebook.com).

Year	Angler forums	Youtube	Literature	Blogs and others web sources	Total	Number of new locations
80s	0	1	0	0	1	1
90s	0	0	2	0	2	2
2006	1	0	1	0	2	1
2007	2	0	0	0	2	0
2008	3	0	0	0	3	1
2009	1	0	0	0	1	0
2010	6	0	0	0	6	1
2011	0	1	0	1	2	1
2012	5	2	0	2	9	1
2013	2	0	0	1	3	0
2014	2	1	0	0	3	1
2015	2	0	0	1	3	1
2016	1	2	1	1	5	2
Total	25	7	4	6	42	12

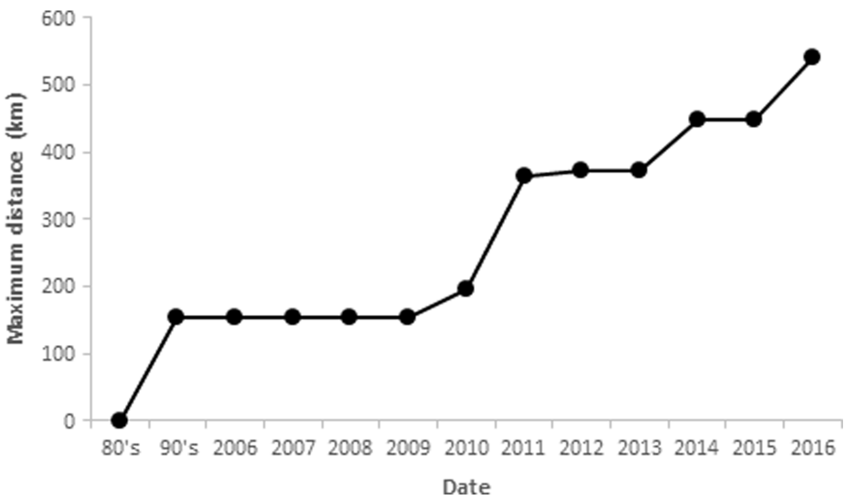

Fig. 1. Maximum river stretch distance (km) invaded by *Ictalurus punctatus* in the Guadiana river, as a function of time. The invaded river stretch corresponds to the sum of distances between the furthest up- and downstream locations for a given year.

road) or from Castilseras dam (79 km by road). The third introduction event occurred around 2010 at the Garcia-Sola dam, located upstream from the Orellana dam but without any fish passage. Finally, the fourth introduction event occurred around 2014 at La Colada dam, a smaller reservoir located upstream from La Serena dam (46 km by road). Downstream natural dispersal from Orellana and Zújar dams occurred along the past decade reaching the upper part of the Alqueva dam in 2011 (*e.g.* Ajuda bridge) and reaching the lower reaches of the Guadiana (Mértola) in 2016. Currently, the total invaded area covers 541 km of river stretch in the Guadiana drainage (337 km in the main drainage; 41% of total river length) (Tab. 2).

4 Discussion

The current work confirms the presence of the non-native channel catfish *I. punctatus* in Portugal and documents in detail its spatial invasion pattern within the Guadiana drainage (one of the largest rivers in the Iberian Peninsula). As seen in other freshwater NNF within Iberian waters, the channel catfish showed a progression from a north-eastern entryway *via* the Ebro drainage, into the central part of the peninsula and the upper reaches of international rivers that flow into the Atlantic (Ribeiro *et al.*, 2009b). Additionally, the present study reinforces the relevance of using online fishermen's records to unveil the invasion patterns of NNF and to provide a more complete and updated distribution range for these species (Ribeiro and Veríssimo, 2014; Banha *et al.*, 2015; Gago *et al.*, 2016). Indeed, online records by anglers suggest that *I. punctatus* original introduction in Spain occurred in 1983 at Castilseras dam (Almadén), probably before the first record (1987) in the Lower Ebro (López *et al.*, 2012). For the Guadiana drainage, this record at Castilseras dam occurs more than one decade before the first scientific record from the late 1990s (Pérez-Bote, 2006). The

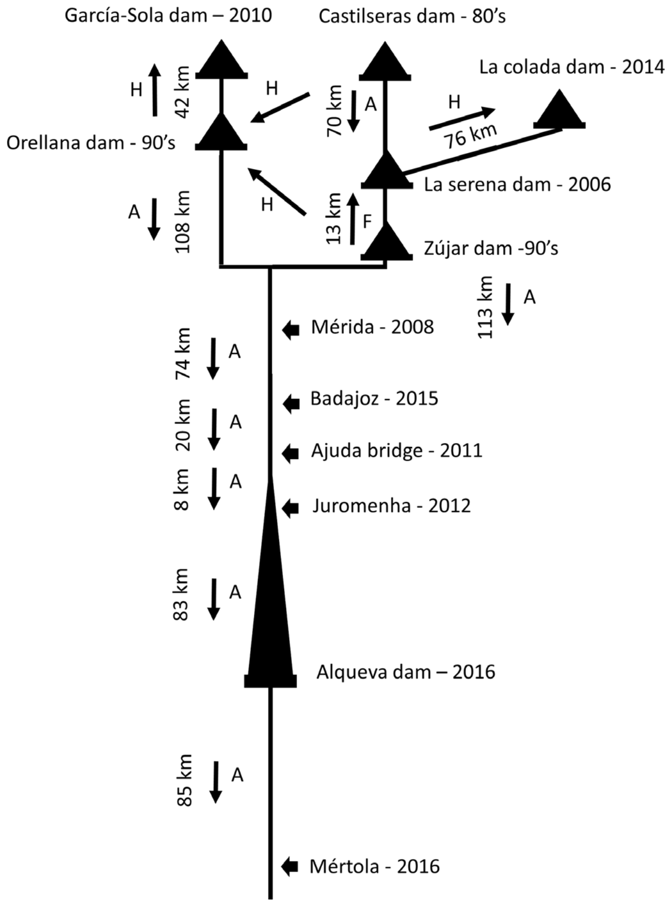

Fig. 2. Forensic reconstruction of *Ictalurus punctatus* invasion in the Guadiana river drainage, based on online and literature records. Arrows indicate dispersal direction and the associated letter describes the probable means of dispersal (A – active dispersal; H – human translocation; F – dam constructed in previously flooded area by a downstream dam). River distance in kilometers between successive locations and year of first detection for each location were given in corresponding stretch of river and location.

usefulness of fishermen records is further augmented by the relative easiness to access both past, and near real-time information on NNF occurrence and dispersal, at minimal cost.

Previous work involving online angler fora records identified three potential sources of bias for this data type. First, bias due lag time between detection by fishermen and online availability (Banha *et al.*, 2015). Second, bias on angler demographics, with higher incidence of information from younger fishermen (*e.g.* Sax *et al.*, 2003). Third, bias due to omissions for sake of secrecy, prestige, or fear that records may influence the actions of fishery management agencies (Venturelli *et al.*, 2017).

Our forensic reconstruction of *I. punctatus* invasion in the Guadiana river basin also showed that natural active dispersal downstream is probably the major mechanism of range expansion by this species. The estimated range expansion rate for the channel catfish was between 8 and 42 km per year according to the time and distance between records. Several studies show that channel catfish have a great dispersal ability: its movement ranges from 0.3 to 1.4 km/day in ponds (Ziebell, 1973), while reported downstream and upstream movements in the Minnesota river were 219 km/year and 124 km/year, respectively (Hubley Jr., 1963). The maximum distance moved by specimens of *I. punctatus* in rivers were 74 km downstream in 45 days (McCammon, 1956), and 469 km upstream in 72 days (Dames *et al.*, 1989). Data gathered in this study indicate that reservoirs may provide particularly suitable habitats for catfish populations, since most of the channel catfish records available online were from reservoirs (88%). This is not surprising given that these are the areas preferred by recreational fishermen (Marta *et al.*, 2001). Indeed, the large majority of NNF in the Iberian Peninsula occur mainly in lentic habitats (Ribeiro *et al.*, 2009b), although the channel catfish is also well adapted to rivers (Moyle, 2002). Significant habitat alterations that occurred in the Guadiana drainage, *e.g.* the construction of the largest reservoir in Western Europe (Alqueva reservoir, established in 2002), might have aided the expansion and establishment of the channel catfish in Portugal as suggested for the black bullhead *Ameiurus melas* (Rafinesque, 1820) by Ribeiro *et al.* (2006). The specimens collected in Alqueva were 4 to 5 years old (Appelget and Smith Jr., 1951; Sneed, 1951; Tucker and Hargreaves, 2004) and thus were born approximately in 2011 after dam construction, suggesting the establishment of a breeding population locally.

The ecological consequences of the arrival of the channel catfish are unpredictable, but there is potential for a very negative impact on the Guadiana river fish community, given its richness in endemic species (Filipe *et al.*, 2004). Currently, the number of NNF in the lower Guadiana equals the number of native freshwater fish, with eleven species each (Ribeiro *et al.*, 2009a; Filipe *et al.*, 2010). In mainland Portugal, there are 20 NNF including six new species since the last inventory conducted in the late 2000s (Ribeiro *et al.*, 2009a), representing an incredibly fast rate of species introduction and establishment. Such a high establishment rate also raises concerns related to the introduction of other non-native species, particularly species that can accidentally be carried by recreational fishermen (*e.g. Procambarus clarkii*, *Crangonyx pseudogracilis* (Banha and Anastácio, 2015) and *Dreissena polymorpha* larvae (Banha *et al.*, 2016)).

To conclude, we confirm the presence of *I. punctatus* in Portugal and report the invasion pathway of this non-native freshwater fish, within an international drainage. We demonstrate that the species current distribution within the Guadiana drainage results from multiple independent introductions/translocations coupled to natural downstream dispersal, eventually extending beyond national boundaries. Our approach was based mostly on available "non-scientific" data collected by recreational fishermen, one of the main stakeholder groups involved in NNF introduction and spread. This approach can easily be implemented for other NNF and may eventually allow the identification of areas of recurring first occurrences within a river drainage. These areas may be primary targets for close monitoring and environmental awareness campaigns directed at recreational fishermen, aiming at the prevention/reduction of future introductions. Additionally, the obtained results highlight the need for urgent coordination at the international level due to the transnational nature of the

Table 2. Detailed sources consulted for construction of *I. punctatus* invasion route on Guadiana river basin.

Source type	Year capture	Local name	Location coordinates		Source
Youtube	1983	Castilseras dam	38°44′14.70″ N	4°47′48.47″ W	https://www.youtube.com/watch?v=2Lyt8oL8jeQ
Literature	1999	Orellana dam	39°0′2.84″ N	5°28′3.01″ W	Pérez-Bote (2006)
Literature	1999	Zújar dam	38°55′45.07″ N	5°26′44.24″ W	Pérez-Bote (2006)
Forums	2006	La Serena dam	38°55′38.83″ N	5°19′52.16″ W	http://foro.carpamania.com
Literature	2006	La Serena dam	38°55′38.83″ N	5°19′52.16″ W	Hermoso *et al.* (2008)
Forums	2007	La Serena dam	38°55′38.83″ N	5°19′52.16″ W	http://foro.carpamania.com
Forums	2008	Orellana dam	39°0′2.84″ N	5°28′3.01″ W	http://foro.carpamania.com
Forums	2008	Zújar dam	38°55′45.07″ N	5°26′44.24″ W	http://www.passioncarp.es
Forums	2008	La Serena dam	38°55′38.83″ N	5°19′52.16″ W	http://foro.carpamania.com
Forums	2008	Orellana dam	39°0′2.84″ N	5°28′3.01″ W	http://www.passioncarp.es
Forums	2009	La Serena dam	38°55′38.83″ N	5°19′52.16″ W	http://foro.carpamania.com
Forums	2009	La Serena dam	38°55′38.83″ N	5°19′52.16″ W	http://www.passioncarp.es
Forums	2010	García-Sola dam	39°11′43.95″ N	5°10′31.75″ W	http://foro.carpamania.com
Forums	2010	Zújar dam	38°55′45.07″ N	5°26′44.24″ W	http://www.passioncarp.es
Forums	2010	Zújar dam	38°55′45.07″ N	5°26′44.24″ W	http://www.passioncarp.es
Forums	2010	La Serena dam	38°55′38.83″ N	5°19′52.16″ W	http://foro.carpamania.com
Forums	2011	Zújar dam	38°55′45.07″ N	5°26′44.24″ W	http://foro.carpamania.com
Blogs and others web sources	2011	Ajuda bridge	38°46′34.07″ N	7°10′18.24″ W	http://alandroalandia.blogspot.pt
Youtube	2011	Zújar dam	38°55′45.07″ N	5°26′44.24″ W	https://www.youtube.com/watch?v=nWANI5yKQeY
Forums	2012	Zújar dam	38°55′45.07″ N	5°26′44.24″ W	http://foro.carpamania.com
Forums	2012	Juromenha	38°44′12.24″ N	7°14′15.58″ W	http://www.portugalpesca.com
Forums	2012	La Serena dam	38°55′38.83″ N	5°19′52.16″ W	http://www.passioncarp.es
Forums	2012	La Serena dam	38°55′38.83″ N	5°19′52.16″ W	http://foro.carpamania.com
Forums	2012	La Serena dam	38°55′38.83″ N	5°19′52.16″ W	http://foro.carpamania.com
Blogs and others web sources	2012	Zújar dam	38°55′45.07″ N	5°26′44.24″ W	http://www.todoslospecestodaslastecnicas.com
Blogs and others web sources	2012	Juromenha	38°44′12.24″ N	7°14′15.58″ W	http://alandroalandia.blogspot.pt
Youtube	2012	Castilseras dam	38°44′14.70″ N	4°47′48.47″ W	https://www.youtube.com/watch?v=2Lyt8oL8jeQ
Youtube	2012	La Serena dam	38°55′38.83″ N	5°19′52.16″ W	https://www.youtube.com/watch?v=FNL5UwbMI34
Forums	2013	La Serena dam	38°55′38.83″ N	5°19′52.16″ W	http://foro.carpamania.com
Forums	2013	La Serena dam	38°55′38.83″ N	5°19′52.16″ W	http://foro.carpamania.com
Blogs and others web sources	2013	Zújar dam	38°55′45.07″ N	5°26′44.24″ W	http://lapescaenbadajoz.blogspot.pt
Forums	2014	La Serena dam	38°55′38.83″ N	5°19′52.16″ W	http://www.passioncarp.es
Blogs and others web sources	2014	La Colada dam	38°31′34.04″ N	5°0′17.13″ W	http://luciobass.blogspot.pt
Youtube	2014	Castilseras dam	38°44′14.70″ N	4°47′48.47″ W	https://www.youtube.com/watch?v=LjNg4BYo-BE
Forums	2015	Zújar dam	38°55′45.07″ N	5°26′44.24″ W	http://www.passioncarp.es
Forums	2015	Mérida	38°55′8.06″ N	6°21′21.37″ W	http://foro.carpamania.com
Blogs and others web sources	2015	Badajoz	38°51′49.29″ N	7°0′51.61″ W	http://amoscaporextremadura.blogspot.pt
Forums	2016	Zújar dam	38°55′45.07″ N	5°26′44.24″ W	http://www.passioncarp.es
Literature	2016	Alqueva dam	38°11′57.89″ N	7°29′47.93″ W	This work
Literature	2016	Mértola	37°38′36.40″ N	7°39′11.20″ W	This work
Youtube	2016	Orellana dam	39°0′2.84″ N	5°28′3.01″ W	https://www.youtube.com/watch?v=DGkipnhTg5s
Youtube	2016	Zújar dam	38°55′45.07″ N	5°26′44.24″ W	https://www.youtube.com/watch?v=hTsvxsqcH7o

Iberian Peninsula river's watersheds, aiming particularly at the new European Union Alien Invasives legislation (EU regulation 2016/1141) and the recently published list of invasive species of concern.

Acknowledgements. We thank Mr. Paulo Mata Pinto for his help in *Ictalurus punctatus* capture in the Alqueva reservoir. This work was supported by the FRISK Project (Ref. PTDC/AAG-MAA/0350/2014); strategic plan of MARE – Marine and Environmental Sciences Centre (UID/MAR/04292/2013) and by the MARE mini-grants project "Análise das rotas de dispersão de peixes invasores predadores na Península Ibérica". F. Banha and F. Ribeiro were supported by post-doctoral grants from MARE (MAR-04292). A. Veríssimo was funded by Fundação para a Ciência e Tecnologia (SFRH/BPD/77487/2011).

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Cite this article as: Banha F, Veríssimo A, Ribeiro F, Anastácio PA. 2017. Forensic reconstruction of *Ictalurus punctatus* invasion routes using on-line fishermen records. *Knowl. Manag. Aquat. Ecosyst.*, 418, 56.