

Eurasian otter *Lutra lutra* distribution and habitat use in a Mediterranean catchment managed for the control of invasive giant reed *Arundo donax*

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Received: 24 July 2022 / Accepted: 14 November 2022

Abstract – We assessed the distribution and habitat use of Eurasian otter *Lutra lutra* on the middle course of the River Segura and its tributaries (Murcia, SE Spain) within the framework of the project LIFE13BIO/ES/001407 RIPISILVANATURA (2014–2019). Between April 2016 and June 2018, we surveyed for otter spraints 42 sampling stretches (mean length $\pm$ SD = 0.5 ± 0.2 km), covering a 109.8 km long section of the river. The study time frame coincided with the main restoration activities of the LIFE project, consisting in the eradication of widespread, invasive alien giant reed *Arundo donax* and plantation of autochthonous riparian vegetation. Otter habitat preferences were assessed by measuring ten environmental parameters potentially affecting its occurrence. The relationship between the presence/absence of the otter and habitat variables was evaluated by both linear multiple regression and non-parametric random forest regression model. Otters preferred river stretches crossing forested areas, avoided polluted waters and their occurrence was inversely related to the width of the emerged aquatic vegetation, mostly consisting of *Arundo* reed beds. Moreover, in managed stretches, otter marking intensity increased after the removal of alien reeds and was higher in RIPISILVANATURA restored transects (1.18 spraints/100 m) than in the downstream, unmanaged half of the watercourse (0.76 spraints/100 m). The combined effects of water pollution and giant reed spread may alter fish distribution and abundance, reducing prey availability to otters. The improvement of chemical and physical habitat quality of streams through alien reeds removal seems thus pivotal to enhance the recovery of otter's populations in the south-eastern Iberian Peninsula.

Keywords: Invasive alien species / freshwater habitat / habitat restoration / riparian vegetation / environmental management

1 Introduction

The current scale and intensity of human-induced environmental change is unprecedented, and the rate of species extinctions is still accelerating, representing a major threat to biodiversity (Brooks *et al.*, 2002; Hanski, 2011), and humankind (United Nations, 2019). Economic activities affect species abundance and distribution through landscape modifications which alter ecosystem properties and functions (Foley *et al.*, 2005), including productivity (Haberl *et al.*, 2007) and food availability (Muhly *et al.*, 2013). The main causes of the degradation of natural ecosystems are landscape transformation, direct exploitation of organisms, pollution, climate change, and invasive alien species (United Nations, 2019).

Environmental degradation is especially worrying in inland aquatic ecosystems, which have been recognized among the most threatened in the world (Saunders *et al.*, 2002). Rivers have experienced intense and long-standing human pressures (*e.g.*, agriculture, water storage and diversion, urban and industrial sewage discharge), which have caused the impairment of aquatic and riparian ecosystems worldwide (Dudgeon *et al.*, 2006; Pletterbauer *et al.*, 2018; Schmutz and Sendzimir, 2018; Oberdorff, 2022).

Mediterranean landscapes have suffered intense human influence for centuries, which has led to the progressive loss of native riverine species and habitats and spread of opportunistic and non-native species (Tierno de Figueroa *et al.*, 2012). In particular, the giant reed (*Arundo donax*), one the 100 most problematic Invasive Alien species (IAs) worldwide (Lansdown, 2013), has progressively colonized the Mediterranean

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region. *Arundo donax* is a perennial, rhizomatous grass which has been introduced from Asia for bioconstruction (e.g., roofs) and erosion control (Elorza *et al.*, 2004). More recently, European projects have enhanced its cultivation for biomass production (El Bassam and Dalianis, 1998). *Arundo donax* large rhizomes enhance its tolerance towards environmental stress and enable rapid re-growth after mowing, mechanical removal and fire (Quinn and Holt, 2008; Lambert *et al.*, 2010). For these reasons, *Arundo donax* flourishes in the riparian habitats of urban and agricultural areas, where it outcompetes native reeds and trees, increasing flooding, siltation (Lambert *et al.*, 2010), and the frequency and intensity of fire (Giessow *et al.*, 2011). *Arundo donax* also affects soil arthropods (Maceda-Veiga *et al.*, 2016) and can have a dramatic impact on fish, amphibian and riparian bird populations (Giessow *et al.*, 2011; see also Bruno *et al.*, 2019, and references therein).

Flowing in the semi-arid southeastern Iberian Peninsula, the River Segura (Murcia, Spain) is one of the most regulated European catchments. Reservoirs, water withdrawal and inter-basin water transfer deeply alter natural flow regimes (Vidal-Abarca *et al.*, 2002; Belmar *et al.*, 2010) and the outstanding spread of irrigated agriculture and urban areas have deeply transformed the landscape (Martinez-Fernandez *et al.*, 2000; Grindlay *et al.*, 2011). Favoured by these man-driven environmental alterations, the giant reed has widely spread in the midlands and lowlands of the catchment, displacing the native riparian vegetation (Bruno *et al.*, 2019).

With the aim of controlling this invasive reed and enhancing the recovery of native riparian habitats, soft-engineering techniques were applied between winter 2015-16 and 2019 in 37 selected river stretches of the middle segments of the River Segura, within the framework of the LIFE+ project RIPISILVANATURA (LIFE13BIO/ES/001407–<https://www.chsegura.es/es/cuenca/restauracion-de-rios/segura-ripisilva/index.html>). The removal of above-ground stems was carried out either mechanically (using compact excavators) or manually (by portable electric lawnmowers), using the latter method wherever native species were still occurring, and was followed by monthly or quarterly maintenance to enhance the effects of competition (Curt *et al.*, 2017). In addition, since winter 2016 site-specific combinations of native riparian species have been planted in mowed patches, depending on local environmental conditions and preexisting native vegetation.

Till now, the effects of restoration actions have been tested using ecological indicators such as riparian vegetation, aquatic macroinvertebrates and birds. A significant increase in the richness of riparian plant species and, for extensive treatments, bird richness and abundance were observed (Bruno *et al.*, 2019). However, the assessment of restoration success at the ecosystem scale should also consider other levels of biological organization (Pedersen *et al.*, 2007; Golet *et al.*, 2011; Pander and Geist, 2013), including top predators, which may play a major role in restored ecosystem functioning (Ritchie *et al.*, 2012).

The Eurasian otter (*Lutra lutra*) is a semi-aquatic mustelid which, being at the top of the trophic chain, is particularly sensitive to the environmental variations affecting freshwater ecosystems, particularly in Mediterranean climates (Gasith and Resh, 1999; Magalhães *et al.*, 2002). In Murcia, the otter suffered a large reduction in its distribution between the 1960s and 1990 s, (Palazón and Carmona, 1998; Pastor *et al.*, 2008),

mainly due to water pollution, habitat fragmentation and hunting (Cortés *et al.*, 1998). As in other Mediterranean areas (Prigioni *et al.*, 2007), this species has progressively recovered during the last 30 years and is currently reported on a ca. 244 km long stretch of the river (Dirección General de Medio Natural, 2019). As otter recovery can be affected by freshwater habitat quality (Prenda *et al.*, 2001; Ruiz-Olmo *et al.*, 2004; Remonti *et al.*, 2008), we aimed to assess the effects of environmental factors on otter distribution and habitat use on the River Segura. Concomitant restoration activities within RIPISILVANATURA allowed us to include the effects of giant reed removal, which were assessed by comparing otter marking intensity in restored vs. unrestored river stretches. We expected habitat quality to be positively related to otter occurrence and hypothesized restored stretches to be used by otters in a greater extent than those invaded by giant reed.

1.1 Study area

Flowing in one of the driest European regions (SE Spain), the River Segura (Fig. 1) is a semi-arid (mean annual rainfall: 382 mm; mean annual temperature: 17 °C) catchment (14,432 km²). Water availability (on average 318×10^6 m³/year) is insufficient to meet water demand (totalling 1483×10^6 m³/year), of which 75% is used for irrigation-dependent agriculture (Uche *et al.*, 2015). To solve this large deficit, water is mainly supplied from saltwater desalination plants and through the Tagus-Segura inter-basin water transfer (IBT, 286 km) connecting, since 1978, the Entrepeñas and Buendía dams in the upper River Tagus, to the Talave Dam in the River Segura catchment (Pittock *et al.*, 2009).

Despite the remarkable cover of forested or semi-natural areas (45.2%), the expansion of agricultural land (52.1%) and increasing flow regulation have caused the progressive reduction of natural areas and the spread of invasive alien species (Bruno *et al.*, 2014a). Agriculture is the main source of diffuse water pollution, mainly through the increase in phosphate and nitrate concentrations (Pellicer-Martínez and Martínez-Paz, 2018).

The study area coincided with the middle sector of the river (Fig. 1), showing a mixture of Eurasian and Ibero-African flora (*Salix* spp., *Fraxinus* spp., *Populus* spp., *Tamarix* spp., *Nerium oleander*; Bruno *et al.*, 2014b). Notwithstanding native habitats have been progressively invaded by the giant reed, which can cover up to 90% of riverbanks (mean $\pm$ SD = $54 \pm 27\%$; authors' unpublished data). To control the spread of this invasive species, restoration activities were carried out on a 57 km long stretch of the river (Fig. 1).

2 Methods

From April 2016 to July 2018, 42 sampling sites (mean length $\pm$ SD = 0.5 ± 0.2 km) were monitored on the River Segura and its tributaries, covering a 109.8 km long stretch of the river (Fig. 1). Each site was surveyed 1–10 times (mean $\pm$ SD = 6.1 ± 2.5) using the widely used “standard method” (Reuther *et al.*, 2000). Typical otter marking sites (e.g., large stones, bridges, pool borders, confluences; Macdonald and Mason, 1983; Prigioni, 1997) were searched for otter spraints (*i.e.*, feces and anal secretions) on both

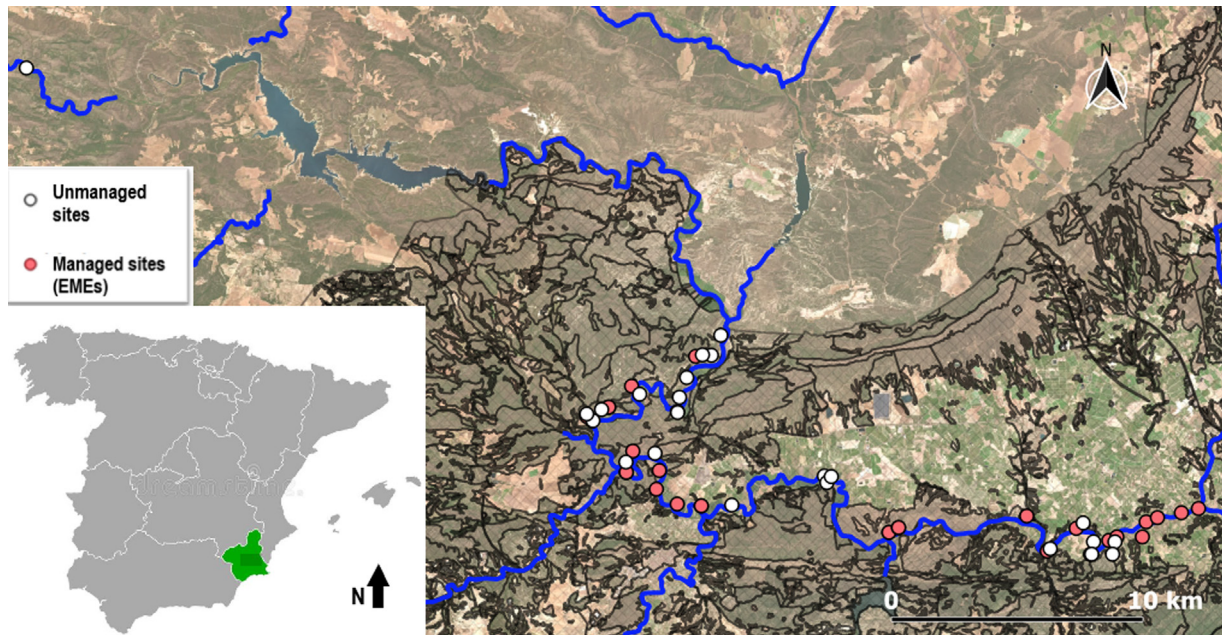


Fig. 1. Study area; surveys for otter were carried out in both the upstream half of the River Segura (Calasparra), included in the Life project RIPISILVANATURA (giant reed removal) and in the downstream, unmanaged river stretch (Cieza, Ulea and Murcia).

Table 1. Habitat variables potentially affecting otter distribution recorded for each transect (*: excluded from subsequent analysis after testing for collinearity).

N	Acronym	Variable	Measure
1*	Dis	Discharge	Classes by eye (0–3)
2	Vel	Water speed	m/s
3	Tur	Water turbidity	Classes by eye (0–4)
4*	WW	Width of wet riverbed	(m)
5	RW	Width of the whole riverbed	(m)
6	RD	Water depth	(m)
7	Pol	Pollution	Classes by eye (0–3)
8	VeW	Width of emerged aquatic vegetation	(m, on both banks)
9	RiV	Area occupied by riparian vegetation	% cover in a 30 m belt on both banks
10*	WaV	Aquatic vegetation cover	% cover

riverbanks and around small islands. To gather additional information to the standard method, surveys were not halted as soon as signs of the species occurrence were found (Balestrieri *et al.*, 2011). Otter marking activity was expressed as the percentage of surveys positive for otters [$P\% = (\text{number of positive surveys} / \text{total number of surveys}) \times 100$], and as the mean number of spraints per 100 m (MI).

Variation in yearly mean MI was compared by Mann–Whitney *U* test between the 52 km domain of the RIPISILVANATURA project ($N=27$ ecological monitoring sites, EMEs) and the unmanaged, downstream river stretch (58 km, $N=15$ sampling sites). Between 2016 and 2018, the relationship between MI and variation in *A. donax* cover was tested, using Spearman non-parametric correlation (ρ), for the nine EMEs positive to otter presence throughout the study period. Giant reed cover was assessed by counting the number of stems in five, 1×1 m plots (see Bruno *et al.*, 2019 for further details).

For each monitored stretch, ten habitat variables potentially affecting otter distribution were recorded during each visit (Tab. 1): 1. Discharge (0: dried, 1: low, 2: medium, 3: high); 2. Water velocity (m/s); 3. Water turbidity (0: none, 1: very low, 2: low, 3: high, 4: very high); 4. Mean width of the wet riverbed (m); 5. Mean width of the riverbed (m); 6. Mean river depth (m); 7. Pollution (0: none, 1: low, 2: medium, 3: high); 8. Mean width of emerged aquatic vegetation on both river banks (m); 9. Mean percent cover of (semi-)natural vegetation in a 30 m large belt on both banks; 10. Mean percent cover of aquatic vegetation.

Discharge was assessed visually, based on the distribution and cover of hydromorphologic units (HMU; Parasiewicz, 2007), which broadly reflect the progressive increase in water velocity and surface turbulence (1: runs and large isolated pools caused by summer drought; 2: ruffles and slow ruffles; 3: riffles and rapids). Water turbidity and vegetation cover were

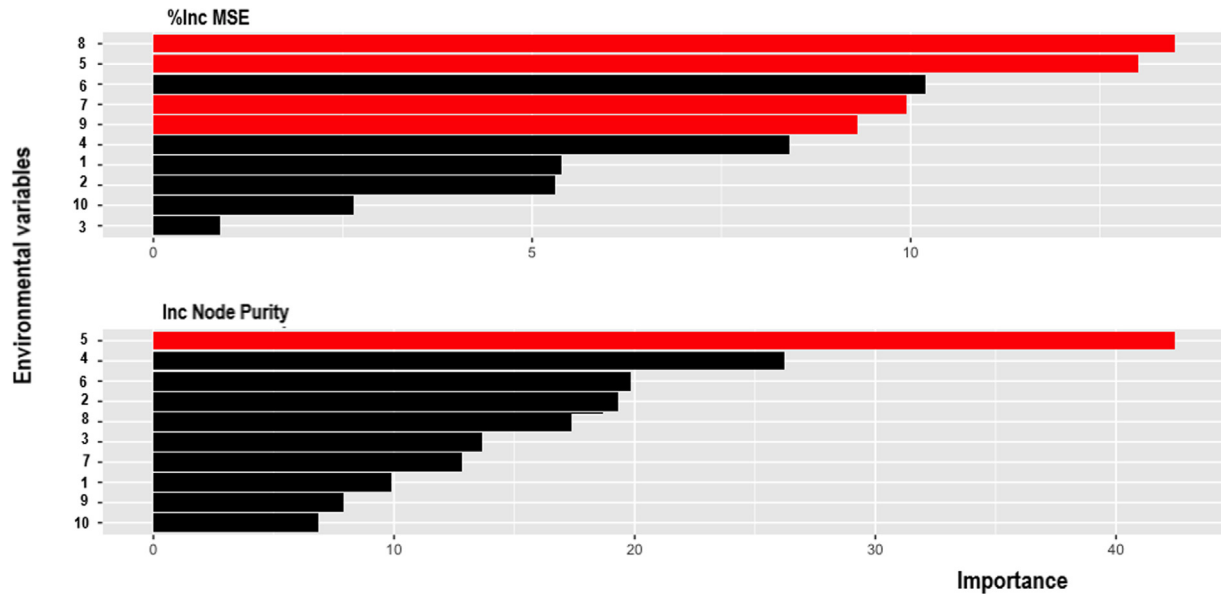


Fig. 2. Variables importance, as assessed by both the percent Increase of Mean Square Error and Increase in Node Purity, in the Random Forest Regression model; significant variables (permutation test) are shown by red bars (1. Discharge; 2. Water speed; 3. Water turbidity; 4. Mean width of the wet riverbed; 5. Mean width of the riverbed; 6. Mean river depth; 7. Pollution; 8. Mean riparian vegetation width; 9. Mean % cover of (semi-)natural vegetation; 10. Mean % cover of aquatic vegetation).

Table 2. Parameters of the multiple regression, with otter marking intensity as dependent variable.

Variables	<i>B</i>	SE	<i>t</i>	<i>P</i>
Constant	−1.107	0.274	−4.041	<0.001
Pollution	−0.257	0.083	−3.114	0.002
Aquatic vegetation width	−0.202	0.052	−3.896	<0.001
% cover of riparian vegetation	0.216	0.051	4.212	<0.001

assessed by eye, while pollution was scored based on the recording of a total of six signs of presence (foam, oil, wastewater, garbage, algal blooms, anoxic sediment). The remaining variables were measured in 7–10 sections per site and averaged. Considering that in Mediterranean rivers otters tend to mark suitable feeding sites, such as pools (Remonti *et al.*, 2011), the first six variables aimed to assess the role played by discharge variation and river morphology on the distribution of marking sites. Variables 7–10 were measured to assess the environmental quality of aquatic (7, 10) and riparian (8–9) habitats. Emerged aquatic vegetation (8) mostly consisted of giant reed, although sometimes mixed with minor patches of native *Phragmites australis*. To account for seasonal variation in MI, only the sites sampled at least once per season ($N=21$) were included in the analysis.

The relationship between each environmental variable and MI was first tested by non-parametric correlation (Spearman's test). To avoid collinearity, those variables clearly representing redundant information ($p < 0.01$; 1, 4, 10 in Tab. 1) were omitted, choosing the one to be rejected according to the strength of its correlation with the dependent variable. The influence of the measured variables on MI was then tested by a backward stepwise linear multiple regression, using Fisher's *F* test to check the level of significance of the model and to enter

or remove the variables (SPSS 12.0.1; SPSS, Chicago, IL, USA). Before the analysis, whenever habitat variables were not normally distributed, the best transformation to improve the distribution of data was identified using Box-Cox's method (Box and Cox, 1964).

As the use of variable transformation may result in some misinterpretations (Robert and Casella, 2004; Tang *et al.*, 2012), data were also analysed using Random Forest (RF; Breiman, 2001). This nonparametric, machine learning method is highly suitable for analysing both compositional data and longitudinal settings with the aim of identifying non-linear relationships amongst both untransformed continuous and categorical variables (Vincenzi *et al.*, 2011; Brückner and Heethoff, 2017). A random forest model is made up of hundreds of unpruned classification and regression trees, each trained by selecting a random bootstrap subset (X_i) and a random set of predictor variables. Predictor variables are evaluated by how much they decrease node impurity or how often they make successful predictions in the forest of classification and regression trees (Breiman, 2001). We assessed variable importance by both, the percent Gini increase of mean square error in nodes (%Inc MSE) and the increase in node purity (Inc Node Purity). The RF regression model was applied 1000 times, assessing the significance level

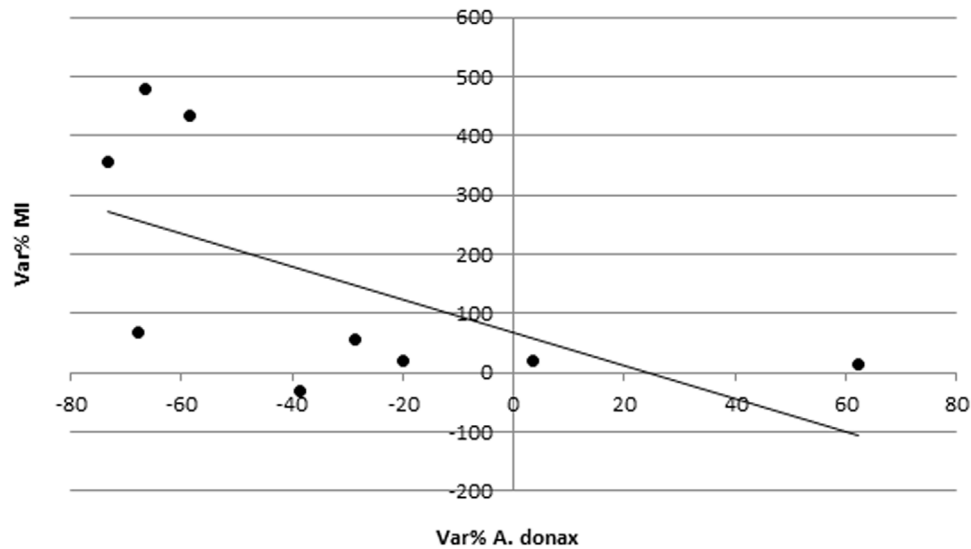


Fig. 3. Relationship between the percent variation in *Arundo donax* cover and otter marking intensity (MI), as assessed between 2016 and 2018 for nine sampling stretches where the removal of giant reed was carried out.

Table 3. Differences in mean Marking Intensity (MI) between unmanaged sites ($N=15$) and Ecological Monitoring Stations (EMEs, $N=27$).

	Marking Intensity		
	2016	2017	2018
Unmanaged sites	0.07	2.34	0.89
EMEs	0.87	2.18	1.19
Mann–Whitney U	128.5	264	318.5
P	0.036	0.96	0.62

of each individual variable by the 95 percentiles of the ordered distribution of node impurity values (Balestrieri *et al.*, 2013).

3 Results

The otter was detected throughout the whole study area. The percentage of positive surveys remained constant (ca 80%) throughout the study period (2016–2018), while mean marking intensity increased, on average, from 0.81 spraints/100 m in 2016 to 0.88 and 1.25 in 2017 and 2018, respectively, for a total of 585 spraints recorded in the study period.

Mean marking intensity was higher in RIPISILVANATURA transects (Calasparra and Cieza municipalities, 1.18 spraints/100 m) than in the downstream half of the watercourse (Cieza, Ulea and Murcia, 0.76 spraints/100 m). We found no significant difference in yearly MI between unmanaged areas and EMEs, except for 2016 (Tab. 3), when MI was markedly higher in the latter (0.07 vs. 0.87 spraints/100 m; $U=128.5$, $P=0.036$). Percent variation in *A. donax* cover between 2016 and 2018 was negatively correlated with MI ($\rho=-0.7$, $P=0.036$; Fig. 3).

Results of the multiple linear regression indicated that otters preferred stretches surrounded by natural vegetation ($P < 0.001$) and tended to avoid polluted waters ($P=0.002$, Tab. 2). In contrast, marking intensity decreased with increasing width of reedbeds ($P < 0.001$, Tab. 2). The Random Forest model displayed similar results and trends by

including as most important variables the width of aquatic vegetation and riverbed, followed by pollution and percent cover of (semi-) natural vegetation (Fig. 2).

4 Discussion

Our results provide an overview of the variables explaining otter distribution in Murcia Region, a relevant contribution to the framework of its Regional Recovery Plan (Directive n° 59/2016). Although our main objectives were to assess the current distribution of the Eurasian otter in the catchment of the River Segura and highlight the environmental variables that may affect habitat use by otters, the coincidence with a restoration initiative provided a unique opportunity to incorporate giant reed management as a factor potentially affecting habitat quality to otters.

In general, the effects of invading giant reed on riparian habitats of semiarid Mediterranean river corridors have been poorly assessed. Usually, these effects are inferred through the examination of invertebrate based-food chains (e.g., Herrera and Dudley, 2003; Maceda-Veiga *et al.*, 2016). The effects on bird communities have also been dealt with in a few studies (Bruno *et al.*, 2018, 2019, and references therein), but rarely on other vertebrates.

Otters being quite elusive and mainly nocturnal, indirect signs of presence are a major tool for assessing their relative

abundance and habitat preferences. Although the use of spraints was long debated in the 1980s (Kruuk *et al.*, 1986; Jefferies, 1986; Mason and Macdonald, 1987; Kruuk and Conroy, 1987), more recently, sprainting activity has been demonstrated to reflect changes in the distribution of otters (Chanin, 2003), MI increasing with both, habitat use (Clavero *et al.*, 2006) and otter numbers (Mason and Macdonald, 1993; Strachan and Jefferies, 1996; Lanszki *et al.*, 2008).

If we assume that MI is an index of habitat use, our results suggest that giant reed spread may affect the suitability of freshwater habitats to otters and that habitat restoration may enhance their recovery in the Murcia region.

As recorded for birds (Bruno *et al.*, 2019), the otter showed short-term responses to giant reed mowing, marking intensity starting to increase since the first year of management and being higher in managed than unmanaged stretches. As suggested by the 2016 values, the sudden availability of reed-free banks rapidly turned into an increase in otter occurrences.

We acknowledge that dense giant reed may hinder the surveyors from accurately looking for spraints over large sections of riverbanks, making the recorded variation in marking intensity partly depending on sign detectability rather than otter occurrence per se. However, sampling stretches were long enough to include both reed-covered and reed-free banks and, anecdotally, we observed that conspicuous, potential sprainting sites (*e.g.*, boulders) found unmarked inside reedbeds were effectively marked by otters after reed removal.

The apparently counter-intuitive negative relation between marking intensity and the width of emerged aquatic vegetation further supports the hypothesis that otters tended to avoid unmanaged river stretches, where giant reed has mostly displaced native species. Invading the whole riverbed, giant reeds reduce the width of the water channel, affecting the distribution of fish species (Giessow *et al.*, 2011). Moreover, dense reeds may hinder otter movements and offer refuge to fish, lowering the hunting success of the predator. Accordingly, on river stretches covered with giant reeds, otter diet has been reported to consist mostly of alien red swamp crayfish *Procambarus clarkii* (Rubio *et al.*, 2019). As the use by otters of alternative-to-fish prey has been related to fish shortage, (Karamanlidis *et al.*, 2013; Smirollo *et al.*, 2019), our results suggest that giant reed spread may lower fish availability to otters. Our results are consistent with those of previous studies showing that high-density stands lower significantly the use of riparian habitats by large and medium mammals (Hardesty-Moore *et al.*, 2020), due to the difficulty and moving and hunting through *Arundo* patches (Coffman *et al.*, 2004).

The other selected variables confirmed that habitat quality is a major factor determining the recovery of otters on the River Segura. The occurrence of forest cover adjacent to the riparian corridor has been previously demonstrated to enhance the diversity of terrestrial carnivores (Matos *et al.*, 2009; Santos *et al.*, 2011; Grilo *et al.*, 2016). Despite being strictly linked to aquatic habitats, otters may also benefit from well-preserved riparian forests, which may offer suitable resting sites especially in human-disturbed areas (Weinberger *et al.*, 2019). Lack of riparian forest (Karr and Schlosser, 1978; Jones *et al.*, 1999; Gregory *et al.*, 1991), as so as water pollution (Changeux and Pont, 1995; Vila-Gispert *et al.*, 2002),

may also alter the abundance and distribution of fish, otters' main food resource (Dettori *et al.*, 2022), affecting the use by otters of deforested stretches.

While, in the Iberian Peninsula, the presence of *Arundo donax* is frequently assumed as a typical component of the habitat of Eurasian otters (Melero *et al.*, 2008, 2011), our results suggest that the encroachment of freshwater habitats by this invasive species may affect otter distribution. Long-term monitoring is required to confirm the positive effects of giant reed management on both the overall quality of freshwater habitats and otter expansion in the catchment of the River Segura. The ongoing recovery of such a charismatic species as the Eurasian otter may contribute to the positive perception of management actions by both government agencies and public audience, enhancing the application of further river restoration projects.

Acknowledgements. Daniel Bruno was funded by “PTI ECOBIODIV” through the “Vicepresidencia Adjunta de Áreas Científico-Técnicas (VAACT-CSIC)”. Part of the field work was financed through the participation of the University of Murcia in the RIPISILVANATURA Project (LIFE13BIO/ES/001407). The preliminary results of this research were presented at RestauraRios 2019 Conference (Rubio *et al.*, 2019, in Spanish).

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Cite this article as: Dettori EE, Balestrieri A, Zapata-Pérez VM, Bruno D, Rubio-Saura N, Robledano-Aymerich F. 2022. Eurasian otter *Lutra lutra* distribution and habitat use in a Mediterranean catchment managed for the control of invasive giant reed *Arundo donax*. *Knowl. Manag. Aquat. Ecosyst.*, 423, 26.