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CHALLENGES IN ANOSTRACAN RESEARCH

How threatened are large branchiopods (Crustacea, Branchiopoda) in the Iberian Peninsula?

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Abstract The Iberian Peninsula harbours 24 taxa of native large branchiopods (LBs). Most of them inhabit Mediterranean temporary ponds, which are priority habitats under the EU Habitats Directive. In this work, Iberian LBs were evaluated using IUCN Red List criteria based on geographic range (extent of occurrence, area of occupancy, number of locations, habitat fragmentation and expected decline). Our results show that 46% of the Iberian LBs are threatened: four taxa should be considered as Critically Endangered (*Linderiella baetica*, *Triops emeritensis*, *Cyzicus tetracerus* and *Leptestheria mayeti*), three taxa fall under the category Endangered (*Artemia salina*, *Tanymastigites lusitanica* and *Triops vicentinus*) and four species (*Artemia* sp. parthenogenetic strains, *Branchinecta orientalis, Lepidurus apus* and *Triops gadensis*) are Vulnerable. Two species (*Phallocryptus spinosus,* and *Maghrebestheria maroccana*) are considered Near Threatened. Our results highlight the worrying risk of extinction of Iberian LBs at the regional level, mainly related to the disappearance and degradation of their habitats and the relatively low degree of habitat protection. For Iberian endemic species, this evaluation is also valid at the global level and gives strong support for their inclusion in the IUCN Red List.

Keywords Temporary ponds · Distribution · Conservation · Endangered · IUCN · Red List

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Introduction

In the Iberian Peninsula, large branchiopods (hereinafter LBs) are represented by the orders Notostraca, Anostraca and Spinicaudata (Brendonck et al., 2008). The primary scientific knowledge on this group of animals was provided by Vianna-Fernandes (1951), Margalef (1953) and Alonso (1985, 1996). In the last two decades, however, notable improvements of the knowledge of LB distribution in the Iberian Peninsula were achieved (e.g. Prunier et al., 2011; Rodríguez-Flores et al., 2016; Machado et al., in press) and six new species were described (Alonso & García-de-Lomas, 2009; Korn et al., 2010; Machado & Sala, 2013). On the whole, the native fauna of LBs from the Iberian Peninsula sums up to 24 taxa: 13 Anostraca, seven Notostraca and four Spinicaudata, eight of these species (33%) being Iberian endemics (García-de-Lomas et al., 2015a, b, c). The particular richness of LB species in the Iberian Peninsula may be the result of small-scale geographic and geological diversity and comparatively low impact of the glaciations (Miracle, 1982; Sahuquillo & Miracle, 2013). LBs are predominantly associated with seasonal wetlands such as temporary (rain and snow-melt) pools, salt flats, and alkali pans, but certain species occur in permanent playas, fishless alkali lakes, and salt lakes (Brendonck et al. 2008). In the Iberian Peninsula, due to its Mediterranean climate, wetlands are predominantly temporary and with very diverse degrees of salinity (Alonso, 1998).

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Temporary ponds are suffering a worrying decline in many countries (e.g. Zacharias & Zamparas, 2010; van den Broeck et al., 2015). Land use changes and the water demand associated with intensive farming have a strong impact on the conservation of inland water bodies and their associated biological communities (e.g. Suso & Llamas, 1993; Alvarez-Cobelas et al., 2001; Reques, 2005). In spite of the fact that Mediterranean temporary ponds are an endangered priority Habitat of Community Interest under the EU Habitats Directive (Camacho et al., 2009), these changes in land use have led to the disappearance of thousands of small ponds and floodplains, which are essential for the life of LB crustaceans and many other organisms.

With regard to conservation status, the IUCN Red List of Threatened Species includes assessments for 29 species of Anostraca, 12 of Spinicaudata (of which four taxa could be considered LBs) and one of Notostraca (IUCN, 2016). Some taxa are only preserved in captivity (C. Rogers, pers. comm.). Despite the fact that the number of records for some taxa is particularly low (Alonso, 1996), currently, none of the species that occur in the Iberian Peninsula is included in the IUCN Red List. To our knowledge, published data on their conservation status are very limited. *Linderiella baetica* Alonso & García-de-Lomas, 2009, an endemic Anostraca from the Iberian Peninsula, is endangered due to the alarming status of a single known population located in Cádiz province (southern Spain). Therefore, its inclusion in the IUCN Red List has recently been proposed (García-de-Lomas et al., 2016).

In this study, the conservation status of the Iberian LBs is assessed at the regional level based on IUCN criteria and considering their geographic range (IUCN, 2012; IUCN Standards and Petitions Subcommittee, 2016). Specifically, we try to answer the following general question: what is the IUCN category of threat for each LB taxon occurring in the Iberian Peninsula? For this purpose, we assessed specific questions such as: (I) How many locations are recorded for each LB species? (II) What is the proportion of habitats of LBs that is included in the Natura 2000 network? (III) Are LBs habitats severely fragmented? (IV) Is a continuing decline for each LB species observed, inferred or projected? This is the first assessment of the category of threat for Iberian LBs.

Materials and methods

Study area

The Iberian Peninsula is located in the south-west of Europe, between 10°07′W and 2°54′E (~1000 km) longitude, and between 43°42′N and 35°49′N (~850 km) latitude. This region comprises the mainland of Spain (84.49% of total surface) and Portugal (15.32%) and the country of Andorra (0.08%). Though mostly belonging to the Mediterranean region, it shows a great climatic variability. The spatial differences of annual mean temperatures surpass 18°C and the average annual accumulated precipitation ranges from barely 200 mm to over 2500 mm. The climate ranges from warm temperate, fully humid with warm summers (Cfb) in the north to warm temperate, steppe with hot summers (Csa) in the southern half. Also, southeastern Spain presents an arid and steppe climate (Kottek et al., 2006; Iberian Climate Atlas, 2011). The Balearic islands were not considered in the frame of this study.

The Iberian Peninsula is a territory traditionally characterized by an important proportion of land dedicated to farming and livestock (54 and 40% in Spain and Portugal, respectively) (The World Bank, 2016). Further land-use changes occurred in the last 50 years (Stoate et al., 2001), giving rise to: (I) the increase in the area of irrigation, with more than 4,220,000 ha (MAGRAMA, 2015), which was especially noticeable (+135% between 1956 and 2003) in regions with scarce rainfall (CMAOT, 2016); (II) an increase of urban surface (+286% between 1956 and 2003); (III) a reduction of 60% of wetland surface, that involved the desiccation of major wetlands such as La Janda (ca. 4000 ha) in Cádiz province, Antela (ca. 3600 ha) in Orense province and La Nava (ca. 4000 ha) in Palencia province (Alario, 1989; Dueñas & Recio, 2000; Fernández-Soto et al., 2011).

Species and IUCN Red List criteria

All the 24 species of LBs from the Iberian Peninsula were evaluated according to IUCN Red List criteria (IUCN, 2012; IUCN Standards and Petitions Subcommittee, 2016). Only georeferenced records of wild populations (N = 1410) were selected from a literature review (see Online Resource 1). Assessments were applied at the species level. In the genus *Artemia* Leach, 1819, parthenogenetic strains and ploidies (diploid and tetraploid) represented in the Iberian Peninsula were not treated as separate species according to Baxevanis et al. (2006) and Rogers (2013), who considered *A. parthenogenetica* Bowen & Sterling, 1978 as "nomen dubium".

The analysis performed in the present study was based on the application of IUCN Red List Criterion B, which evaluates the geographic range in the form of the extent of occurrence, EOO (criterion B1) and the area of occupancy, AOO (criterion B2) (Table 1). EOO is defined as the area contained within the shortest continuous imaginary boundary which can be drawn to encompass all the known, inferred or projected sites of present occurrence of a taxon, excluding cases of vagrancy (IUCN, 2012). AOO is defined as the area within this EOO that is actually occupied by this taxon, excluding cases of vagrancy. AOO estimates were obtained by counting the number of occupied cells in a uniform grid (2 × 2 km) that covers the entire range of a taxon and then tallying the total area of all occupied cells. Both EOO and AOO were calculated using the GEOCAT tool available at http://geocat.kew.org/. Given the isolated nature of habitats colonised by LBs, we gave priority to criterion B2 (based on AOO) over B1 (based on EOO), especially if B1 would have suggested a lower level of threat (see Discussion for further details).

To address the criterion B, the general distributional threshold of a taxon must first be met for one of the categories of threat (Critically Endangered, Endangered or Vulnerable), either in terms of EOO or AOO. The taxon must then also meet at least two of the three subcriteria listed for criterion B. These options are (a1) known to exist in a reduced number of locations or (a2) severely fragmented, (b) continuing decline, or (c) extreme fluctuation (Table 1). Therefore, if a taxon meets, e.g. the distributional requirement for the category Endangered and option a2 (severely fragmented), but none of the other options, it would not meet the requirements to be classified as Endangered under criterion B (IUCN Standards and Petitions Subcommittee, 2016). Data that could reveal the possible occurrence of extreme fluctuations of LB population sizes is not available for most of Iberian temporary habitats. In fact, population sizes of LBs would be particularly difficult to measure on a large scale because high

Table 1 IUCN Red List categories and underlying criteria obtained for Iberian large branchiopods as inferred from distribution records available in the literature

Taxon	B1: EOO (km^2) [EOO < 100 (CR); 100 < EOO < 5000 (EN); 5000 < EOO < 20,000 (VU)]	B2: AOO (km^2) [AOO < 10 (CR) 10 < AOO < 500 (EN) 500 < AOO < 2000: (VU)]	(a1) Number of locations in the Iberian Peninsula [=1 (CR); ≤5 (EN); ≤10 (VU)]	(a2) Severely fragmented? ["yes" if >50% EOO or AOO is separated by a large distance; or most localities (>50%) are at ≤50 km to the closest highway, railway, waterway and urban settlement (see Table 2)]	(b) Continuing decline expected? [based on: [(b1) protected localities ≤50% (see the next column); (b2) Habitat loss or unsustainable uses; (b3) Climate change; (b4) Invasive alien species)]	(b1) Number of protected localities/total number of localities (% of protected localities)	IUCN Red List Category
Anostraca							
Artemia Leach, 1819 sp. (parthenogenetic strains)	300,745 (LC)	44 (EN)	9 (VU)	Yes	Yes (b4) i, ii, iv	9/11 (81.8%)	Regional assessment: Vulnerable B2ab(i, ii, iv)
Artemia salina (Linneaus, 1758)	78,526 (LC)	28 (EN)	4 (EN)	Yes	Yes (b4) i, ii, iv	6/7 (85.7%)	Regional assessment: Endangered B2ab(i, ii, iv)
Branchinecta ferox (Milne-Edwards, 1840)	27,535 (NT)	32 (EN)	5 (EN)	No	No serious decline expected	9/9 (100%)	Regional assessment: Least concern
Branchinecta orientalis Sars, 1901	4,136 (EN)	40 (EN)	8 (VU)	Yes	Yes (b2) i, ii, iii, iv	7/10 (70%)	Regional assessment: Vulnerable B1ab(i, ii, iii, iv), +B2ab(i, ii, iii, iv)
Branchinectella media	(Schmankewitsch, 1873)	45,764 (LC)	124 (EN)	7 (VU)	Yes	No serious decline expected	31/35 (88.6%)
Regional assessment:							
Least concern							
*Branchipus cortesi** Alonso & Jaume, 1991	153,989 (LC)	284 (EN)	>20 (LC)	Yes	No serious decline expected	83/114 (72.8%)	Global assessment: Least concern
Branchipus schaefferi Fischer, 1834	398,392 (LC)	748 (VU)	>20 (LC)	Yes	Yes (b1, b2) ii, iii, iv	136/273 (49.8%)	Regional assessment: Least Concern

Table 1 continued

Taxon	B1: EOO (km^2) [EOO < 100 (CR); 100 < EOO < 5000 (EN); 5000 < EOO < 20,000 (VU)]	B2: AOO (km^2) [AOO < 10 (CR) 10 < AOO < 500 (EN) 500 < AOO < 2000: (VU)]	(a1) Number of locations in the Iberian Peninsula [=1 (CR); ≤5 (EN); ≤10 (VU)]	(a2) Severely fragmented? ["yes" if >50% EOO or AOO is separated by a large distance; or most localities (>50%) are at ≤50 km to the closest highway, railway, waterway and urban settlement (see Table 2)]	(b) Continuing decline expected? [based on: [(b1) protected localities ≤50% (see the next column); (b2) Habitat loss or unsustainable uses; (b3) Climate change; (b4) Invasive alien species)]	(b1) Number of protected localities/total number of localities (% of protected localities)	IUCN Red List Category
Chirocephalus diaphanus Prévost, 1803	525,669 (LC)	1296 (VU)	>20 (LC)	Yes	Yes (b1, b2) ii, iii, iv	193/402 (48.0%)	Regional assessment: Least Concern
*Linderiella baetica** Alonso & García-de-Lomas (2009)	4 (CR)	4 (CR)	1 (CR)	Yes	Yes (b1, b2) i, ii, iii, iv	0/1 (0%)	Global assessment: Critically Endangered B1ab(i, ii, iii, iv) + B2ab (i, ii, iii, iv)
Phallocryptus spinosus (Milne-Edwards, 1840)	70,273 (LC)	56 (EN)	7 (VU)	Yes	Unknown (b4)	14/15 (93.3%)	Regional assessment: Near Threatened B2ab?(i, ii, iv)
Streptocephalus torvicornis (Waga, 1842)	334,560 (LC)	260 (EN)	>20 (LC)	Yes	Yes (b1, b2) i, ii, iv	23/68 (33.8%)	Regional assessment: Least Concern
*Tanymastigites lusitanica** Machado & Sala (2013)	664 (EN)	36 (EN)	3 (EN)	Yes	Yes (b2) i, ii, iv	5/9 (55.6%)	Global assessment: Endangered B1ab(i, ii, iv) + B2ab(i, ii, iv)
Tanymastix stagnalis (Linnaeus, 1758)	499,488 (LC)	404 (EN)	>20 (LC)	Yes	No serious decline expected	126/173 (72.8%)	Regional assessment: Least Concern
Notostraca							
Lepidurus apus (Linnaeus, 1758)	68,329 (LC)	48 (EN)	6 (VU)	Yes	Yes (b2) i, ii, iv	6/11 (54.5%)	Regional assessment: Vulnerable B2ab(i, ii, iv)
*Triops baeticus** Korn, 2010	54,467 (LC)	272 (EN)	20 (LC)	Yes	Yes (b1, b2) i, ii, iv	32/81 (39.5%)	Global assessment: Least Concern

Table 1 continued

Taxon	B1: EOO (km^2) [EOO < 100 (CR); 100 < EOO < 5000 (EN); 5000 < EOO < 20,000 (VU)]	B2: AOO (km^2) [AOO < 10 (CR) 10 < AOO < 500 (EN) 500 < AOO < 2000: (VU)]	(a1) Number of locations in the Iberian Peninsula [=1 (CR); ≤5 (EN); ≤10 (VU)]	(a2) Severely fragmented? ["yes" if >50% EOO or AOO is separated by a large distance; or most localities (>50%) are at ≤50 km to the closest highway, railway, waterway and urban settlement (see Table 2)]	(b) Continuing decline expected? [based on: [(b1) protected localities ≤50% (see the next column); (b2) Habitat loss or unsustainable uses; (b3) Climate change; (b4) Invasive alien species)]	(b1) Number of protected localities/total number of localities (% of protected localities)	IUCN Red List Category
Triops cancriformis (Bosc, 1801)	309,226 (LC)	188 (EN)	25 (LC)	Yes	Unknown	25/57 (43.9%)	Regional assessment: Data deficient
*Triops emeritensis** Korn & Pérez-Bote, 2010	8 (CR)	8 (CR)	1 (CR)	Yes	Yes (b2) i, ii, iv	2/2 (100%)	Global assessment: Critically Endangered B1ab(i, ii, iv) + B2ab(i, ii, iv)
*Triops gadensis** Korn & Garcia-de-Lomas 2010	1,171 (EN)	44 (EN)	9 (VU)	Yes	Yes (b1, b2) i, ii, iv	2/13 (15.4%)	Global assessment: Vulnerable B1ab(i, ii, iv) + B2ab(i, ii, iv)
Triops simplex Ghigi, 1921	4 (CR)	4 (CR)	1 (CR)	Yes	Unknown	1/1 (100%)	Regional assessment: Data deficient
*Triops vicentinus** Korn, Machado, Cristo & Cancela da Fonseca, 2010	1,031 (EN)	52 (EN)	3 (EN)	Yes	Yes (b2) i, ii, iv	19/23 (82.6%)	Global assessment: Endangered B1ab(i, ii, iv) + B2ab(i, ii, iv)
Spinicaudata							
*Cyzicus grubei** (Simon, 1886)	113,722 (LC)	228 (EN)	>20 (LC)	Yes	Yes (b1, b2) i, ii, iv	25/67 (37.3%)	Global assessment: Least Concern
Cyzicus tetracerus (Krynicki, 1830)	4 (CR)	4 (CR)	1 (CR)	Yes	Yes (b2) i, ii, iv	1/1 (100%)	Regional assessment: Critically Endangered B1ab(i, ii, iv) + B2ab(i, ii, iv)

Table 1 continued

Taxon	B1: EOO (km^2) [EOO < 100 (CR); 100 < EOO < 5000 (EN); 5000 < EOO < 20,000 (VU)]	B2: AOO (km^2) [AOO < 10 (CR) 10 < AOO < 500 (EN) 500 < AOO < 2000: (VU)]	(a1) Number of locations in the Iberian Peninsula [=1 (CR); ≤5 (EN); ≤10 (VU)]	(a2) Severely fragmented? ["yes" if >50% EOO or AOO is separated by a large distance; or most localities (>50%) are at ≤50 km to the closest highway, railway, waterway and urban settlement (see Table 2)]	(b) Continuing decline expected? [based on: [(b1) protected localities ≤50% (see the next column); (b2) Habitat loss or unsustainable uses; (b3) Climate change; (b4) Invasive alien species)]	(b1) Number of protected localities/total number of localities (% of protected localities)	IUCN Red List Category
Leptestheria mayeti (Simon, 1885)	4 (CR)	4 (CR)	1 (CR)	Yes	Yes (b1, b2) i, ii, iv	0/1 (0%)	Regional assessment: Critically Endangered B1ab(i, ii, iv) + B2ab(i, ii, iv)
Maghrebestheria maroccana Thiéry, 1988	269,242 (LC)	64 (EN)	12 (NT)	Yes	Yes (b1, b2) i, ii, iv	12/24 (50%)	Regional assessment: Near Threatened B2ab(i, ii, iv)

Iberian endemic species are marked with an asterisk (*). When there is only one or two localities, EOO = AOO. Abbreviations for each IUCN Red List category are: Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near threatened (NT), Least Concern (LC) and Data Deficient (DD). Letters i–v indicate the subcriteria: i: extent of occurrence; ii: area of occupancy; iii: area extent and/or quality of habitat; iv: number of locations or subpopulations; v: number of mature individuals. Percentage of protected localities refers to the proportion of sites included in Natura 2000 network. Note that the term "location" in (a1) defines a geographically or ecologically distinct area in which a single threatening event can rapidly affect all individuals of the taxon present, whereas the term "locality" in (b1) refers to the number of ponds in which the taxon was found fluctuations are typical of LBs (e.g. related to different water levels and associated salinity contents among different flooding events). Inference of population sizes from the resting egg bank would also pose problems because this would necessitate the identification of the amount of viable eggs. However, LBs may not be expected to hatch all upon first inundation, and suitable hatching conditions often vary among species. Furthermore, the determination of species at early larval stages may cause problems. Therefore, in this study we assessed the parameters "number of locations" (a1), "severely fragmented" (a2) and "continuing decline" (b) (Table 1).

The "number of locations" refers to geographically or ecologically distinct areas in which a single threatening event can rapidly affect all individuals of the taxon present.

"Severely fragmented" refers to a situation in which increased extinction risk to a taxon results from the fact that most of its individuals are found in small and relatively isolated subpopulations (IUCN, 2012). A taxon can be considered to be severely fragmented if most (> 50%) of its total AOO is in habitat patches that are (1) smaller than would be required to support a viable population, and (2) separated from other habitat patches by a large distance (IUCN Standards and Petitions Subcommittee, 2016). However, it remains unknown which is the habitat size for each species to support a viable population. Therefore, we inferred this parameter by calculating the percentage of AOO that is separated from other habitat patches by a large distance. Intrinsically, a "large distance" can be assumed as one that precludes LB dispersal from neighbouring locations. Dispersal distances may be highly variable depending on the dispersal vector (both physical and biological vectors), local environmental conditions or the availability of suitable habitats (Incagnone et al., 2015 and references therein). For the purpose of this study, we regarded distances greater than, or equal to 50 km as representing a large distance. To calculate distances among the different localities for each LB species, we used the "Point distance" proximity tool in Arctoolbox (ArcGIS® 10.2.2). Also, the presence of fenced infrastructures (highways, railways), waterways (artificial channels and rivers) and urban settlements (cities, towns and villages; hamlets were not considered) between neighbouring ponds were assumed to represent barriers for dispersal that may have similar effects to a large distance. Thus, we considered that a LB species was under a severe fragmentation when > 50% of localities were at ≤50 km for all the four parameters (distance to the closest highway, railway, waterway and urban area) (Table 2). To calculate distances to the nearest highway, railway, waterway and urban areas, we used the "Near" proximity tool in Arctoolbox (ArcGIS® 10.2.2). Based on European standards, we used the projected coordinate system UTM and datum ETRS89, and worked with the different zones included in the Iberian Peninsula (29, 30 and 31) in separate projects. Projected polyline (for highways, railways and waterways) and point (for urban settlements) shapefiles from Spain and Portugal were obtained at http://www.eurogeographics.org/ and http://www.mapcruzin.com. In the particular case of urban settlements, points represented the centroid. Thus, distance calculations of Iberian LB localities to the closest urban settlement may be slightly (ca. hundreds of metres) overestimated. In the particular case of coastal localities of *Artemia* spp. (i.e. saltmarshes and saltpans) that lie within migration routes of waterbirds (Figuerola & Green, 2002; Green et al., 2005), we did not consider the presence of such barriers in the assessment of severe fragmentation.

The parameter "continuing decline" is defined as a recent, current or projected future decline (which may be smooth, irregular or sporadic) which is liable to continue unless remedial measures are taken (IUCN Standards and Petitions Subcommittee, 2016). We have inferred the continuing decline of either EOO, AOO, the quality of habitat or the number of locations from four different sources of information. First, for each LB species, we calculated the proportion of localities (i.e. the number of ponds in which the respective taxon was found) that belong to the Natura 2000 Network. This Network includes Special Protection Areas (SPA) and Sites of Community Importance (SCI). We assumed that protected habitats are much less likely to undergo direct loss or deep transformation than unprotected habitats. We assumed that for a certain species there is a "continuing decline" in EOO or AOO when the percentage of localities included in the Natura 2000 network is ≤50%. Shapefiles of SPA and SCI from Spain and Portugal were obtained from the official websites of the Spanish Ministry of Agriculture, Food and Environment (http://www.mapama.gob.es/es/biodiversidad/

Table 2 Parameters related to fragmentation of geographical range for Iberian LB species

Species	N	Distance to the closest highway		Distance to the closest railway		Distance to the closest waterway		Distance to the closest urban settlement	
		Mean ± SD (km)	% of localities at ≤50 km	Mean ± SD (km)	% of localities at ≤50 km	Mean ± SD (Km)	% of localities at ≤50 km	Mean ± SD (Km)	% of localities at ≤50 km
Artemia sp. (partenogenetic strains)	11	13.1 ± 12.4	100	17.8 ± 9.1	100	14.7 ± 15.4	100	8.0 ± 6.3	100
Artemia salina	7	11.2 ± 9.7	100	22.0 ± 10.5	100	6.4 ± 7.1	100	8.9 ± 9.8	100
Branchinecta ferox	9	10.2 ± 6.8	100	34.5 ± 26.7	*44.4*	6.4 ± 7.1	100	10.9 ± 6.9	100
Branchinecta orientalis	10	11.7 ± 7.2	100	9.6 ± 8.2	100	19.0 ± 12.2	100	8.5 ± 6.5	100
Branchinectella media	35	8.4 ± 5.0	100	12.5 ± 9.8	100	24.1 ± 14.8	88.6	7.4 ± 4.1	100
Branchipus cortesi	114	18.2 ± 11.9	100	28.3 ± 14.9	97.4	25.8 ± 17.6	82.5	7.3 ± 6.9	100
Branchipus schaefferi	273	4.9 ± 6.2	100	14.2 ± 11.8	98.2	11.8 ± 12.1	97.4	5.4 ± 5.0	100
Cyzicus grubei	67	15.3 ± 15.2	97.0	28.6 ± 17.3	92.5	19.5 ± 12.1	98.5	8.1 ± 5.6	52.2
Cyzicus tetracerus	1	1.3	100	34.5	100	26.5	100	60.6 ± 49.0	100
Chirocephalus diaphanus	402	17.4 ± 20.1	88.3	26.4 ± 19.7	85.3	24.6 ± 18.8	88.1	18.5	100
Lepidurus apus	13	8.6 ± 9.1	100	33.3 ± 19.5	69.2	20.9 ± 14.4	100	5.2 ± 4.6	100
Leptestheria mayeti	1	11.6	100	12.3	100	7,4	100	10.9	100
Linderiella baetica	1	0.4	100	0.0	100	8,6	100	0.6	100
Maghrebestheria maroccana	24	16.5 ± 15.1	100	19.5 ± 13.1	100	23.5 ± 20.0	79.2	11.4 ± 9.4	100
Phallocryptus spinosus	15	11.9 ± 5.5	100	14.8 ± 12.5	100	31.5 ± 11.3	93.3	7.2 ± 5.0	100
Streptocephalus torvicornis	68	8.5 ± 12.6	97.1	23.6 ± 16.7	92.6	19.9 ± 13.6	98.5	7.6 ± 4.3	100
Tanymastigites lusitanica	9	7.2 ± 5.5	100	31.4 ± 14.3	100	30.2 ± 17.6	77.8	14.5 ± 5.0	100
Tanymastix stagnalis	173	16.8 ± 15.9	96.0	24.5 ± 15.8	96.5	22.6 ± 17.6	88.4	6.2 ± 5.8	100
Triops baeticus	81	16.3 ± 12.4	100	25.1 ± 13.3	98.8	22.0 ± 12.3	97.5	12.0 ± 7.6	100
Triops cancriformis	57	6.1 ± 5.5	100	16.0 ± 10.3	100	15.8 ± 18.4	93	6.3 ± 3.9	100
Triops emeritensis	2	20.2 ± 0.9	100	45.0 ± 0.4	100	22.3 ± 1.0	100	8.6 ± 0.4	100
Triops gadensis	13	10.0 ± 8.8	100	16.6 ± 14.3	100	15.5 ± 12.0	100	3.9 ± 2.6	100
Triops simplex	1	4.2	100	28.3	100	38,1	100	7.3	100
Triops vicentinus	23	11.2 ± 8.1	100	15.8 ± 8.9	100	18.3 ± 5.0	100	3.7 ± 1.3	100

Italics indicate percentages of localities located at <50 km to the closest infrastructure below 50%. In such cases, the LB species was assumed not to be under severe fragmentation servicios/banco-datos-naturaleza/informacion-disponible/rednatura2000_descargas.aspx) and the Portuguese Institute of Nature and Forest Conservation (http://www.icnf.pt/portal/naturaclas/cart). For analysis, we calculated the proportion of habitats of LBs that are included in Natura 2000 habitats using the "join" tool in ArcGIS® v.10.2.2. Secondly, we evaluated land uses in the 50 km surrounding each pond. Land use data were obtained from shapefiles available at http://www.siose.es/and http://epic-webgis-portugal.isa.ulisboa.pt/. Unsuitable uses and habitats for LBs include urban areas, roads, railways, harbours, airports, treatment plants (water or wastewater), active mining, dumps, intensive croplands, fish farms without outdoor hatcheries, waterways (artificial channels, rivers, ramblas and estuaries) and permanent lakes and wetlands (with fish) (see Online Resource 2). Third, we revised climatic change models for particular areas and their potential impact on the hydrology of temporary ponds (in the study region this concerns Doñana and Daimiel, see Discussion for details). And fourth, we evaluated the occurrence of invasive alien species. This applies to *Artemia franciscana* (Amat et al., 2007). Finally, it was also assessed whether the causes of threat have ceased or not and if they would be reversible (see Online Resource 2).

For the regional evaluations, the Guidelines for Application of IUCN Red List Criteria at Regional and National Levels (IUCN, 2012) were applied. Following this methodology, a preliminary category for each species was obtained based on its EOO and AOO as inferred from Iberian localities. Afterwards, the distribution of the species outside the Iberian Peninsula was analysed, in order to evaluate the probability of natural immigration from populations outside the study region ("rescue effect") (Hanski & Gyllenberg, 1993). Apparently, long-distance dispersal of a sufficiently high number of LB resting cysts to allow a successful establishment in the new habitat may be regarded as a very scarce event for most types of temporary bodies of water (Incagnone et al., 2015). In practice, the Iberian Peninsula is surrounded by the Mediterranean Sea and the Atlantic Ocean; its only connection with France and central Europe is through the Pyrenees, a mountain range of more than 400 km in length with more than 14 peaks that exceed 3000 m above the sea level. Several source of evidence support that the Pyrenees act as an extremely effective barrier. For instance, Reniers et al. (2013) found genetic divergence between Iberian and French haplotypes of *Chirocephalus diaphanous* Prévost, 1803. Nevertheless, this species is the most widespread and common European anostracan (Brtek & Thiéry, 1995). Also, *C. diaphanus* is a cold-tolerant species which can colonise high mountain habitats and may survive under ice sheets (Zarattini & Mura, 2007; Reniers et al., 2013). The fact that Iberian and French specimens belong to different clades suggests that the Pyrenees are an effective barrier for dispersal. Regarding diaptomid copepods, beta diversity patterns showed higher affinities of the Iberian Peninsula with northern African regions of the western Mediterranean than to western central Europe (Marrone et al., 2017). This result may be explained by glaciations (when glacial refugia, such as those southern regions, have retained diaptomid copepod assemblages during longer periods of time, allowing speciation processes to accumulate) and geographical particularities (the east–west arrangement of the Pyrenees, that has prevented southern species to migrate northward during post-glaciation periods). Surrounded by natural barriers, the possibilities of a rescue effect for Iberian LBs are generally remote and were thus treated in this study as being unlikely.

Results

Among the Iberian endemic LBs, *Linderiella baetica* and *Triops emeritensis* Korn & Pérez-Bote, 2010 should be considered as "Critically Endangered" globally (Table 1). Two species (*Tanymastigites lusitanica* Machado & Sala, 2013 and *Triops vicentinus* Korn, Machado, Cristo & Cancela da Fonseca, 2010) should be considered as "Endangered" globally, whereas *Triops gadensis* Korn & García-de-Lomas 2010 should be considered as "Vulnerable" globally. The remaining endemic species (*Cyzicus grubei* (Simon, 1886), *Triops baeticus* Korn, 2010 and *Branchipus cortesi* Alonso & Jaume, 1991) are considered to be of "Least Concern".

Among the non-endemic LBs evaluated, *Cyzicus tetracerus* (Krynicki, 1830) and *Leptestheria mayeti* (Simon, 1885) should be considered as "Critically Endangered" at the regional scale; *Artemia salina* (Linneaus, 1758) should be considered "Endangered" and three species (*Artemia* Leach, 1819 sp.-parthenogenetic strains, *Branchinecta orientalis* Sars, 1901 and *Lepidurus apus* (Linnaeus, 1758) should be considered "Vulnerable" (Table 1). *Maghrebestheria maroccana* Thiéry, 1988 should be regarded "Near Threatened". For *Phallocryptus spinosus* (Milne-Edwards, 1840) (Anostraca), more than 90% of its known localities are protected, and mainly associated with inland saline wetlands. A decline of EOO or AOO caused by a land use change is not expected. However, this species inhabits hypersaline ponds (García & Niell, 1993) that are being invaded by *Artemia franciscana* (Amat et al., 2007). This species has a restricted distribution and their habitats are severely fragmented. Therefore, *P. spinosus* was also considered as "Near Threatened".

The remaining species (*Branchinecta ferox* (Milne-Edwards, 1840), *Branchinectella media* (Schmankewitsch, 1873), *Branchipus schaefferi* Fischer, 1834, *Chirocephalus diaphanus*, *Streptocephalus torvicornis* (Waga, 1842) and *Tanymastix stagnalis* (Linnaeus, 1758)) are categorized as being of "Least Concern" at the regional scale (Table 1). Presently, the single population reported as representing gonochoric *Triops cancriformis* (Bosc, 1801) cannot be differentiated from Northern African *Triops simplex* Ghigi, 1921 based on available morphological characters. Furthermore, the single record of *Triops simplex* in the Iberian Peninsula is entirely based on molecular data so that to date no morphological data are available for Iberian *Triops simplex* (Zierold et al. 2007; Korn et al. 2010). Therefore, the *Triops cancriformis—Triops simplex* morphogroup is considered as "Data deficient".

Overall, currently 46% of the LB fauna represented in the Iberian Peninsula is threatened (i.e. fall into the categories Critically Endangered, Endangered or Vulnerable) according to IUCN Red List criteria (Table 1).

The confluence of fenced infrastructures such as highways and railways and other barriers to walking animals such as waterways and urban settlements (see Online Resource 3) evidenced a severe fragmentation throughout the Iberian Peninsula for almost all Iberian LB species. *Branchinecta ferox* was the only species that did not meet the four parameters related to fragmentation (Table 2).

Discussion

LBs are often considered to be the flagship group of temporary aquatic habitats (Belk, 1998). LBs are often stenoic species that allow distinguishing different typologies of temporary ponds (Alonso, 2010). Despite Mediterranean temporary ponds are priority habitats of Community Interest under the EU Habitats Directive and are suffering a deep decline in many countries, the assessment of LBs according to the IUCN Red List criteria has received no attention in the Mediterranean basin. The assessment of an entire group at the regional level is an inclusive approach and can be useful for defining conservation priorities and decision making.

Problems related to the application of IUCN Red List criteria for LBs

The assessment of LBs here developed highlights some considerations for further discussion. Available data did not allow to rigorously assess the criteria A (population size reduction measured over a period of 10 years or three generations), C (small population size and decline), and E (quantitative analysis), since most published works include mere occurrence records of the different LB species in the territory. In addition, the number of mature individuals of LBs in each habitat is unknown as it is highly dependent on the time at which the sampling is performed in relation to the flood cycle (e.g. days after inundation and water characteristics at the sampling date) (e.g. Lahr et al., 1999; Alonso & García-de-Lomas, 2009). Therefore, the only reliable IUCN criterion to be used was the geographical range.

Temporary ponds are 'islands' in a 'sea' of habitats not suitable for LBs. Therefore, it is difficult to conceive EOO as a realistic area through which there is an effective dispersal and ongoing gene flow (Korn et al., 2010; Reniers et al., 2013). LBs are skin-breathing filter feeders that will readily absorb many harmful substances very fast and show a fast reaction (in fact, many branchiopods are regularly used for toxicity tests). If a toxic substance enters a habitat in sufficient concentration (which may be readily reached in small ponds) then it may easily become meaningless if the inhabiting LBs showed a population decline or had a stable population before. This is a relevant peculiarity if compared to an endangered island population of a terrestrial organism, for which a pollution event may usually not affect the whole population at once.

Regarding potential dispersal, due to their ability to produce resting eggs, LBs are generally assumed to have a high potential for passive dispersal (via wind, birds, or other animals) in environments that show natural conditions (Incagnone et al., 2015). For LB species living in coastal salterns, natural vectors (birds) apparently persist (despite they also spread invasive species such as *Artemia franciscana*). However, it remains uncertain if LB species that have a short life span (as may be expected for *Tanymastigites lusitanica*) and/or typically colonise very small and shallow ponds (e.g. *Branchipus schaefferi* or *Tanymastix stagnalis*) (e.g. Pino et al., 2004; Machado & Sala, 2013) may be dispersed by small birds (e.g. passerines) or flying insects (Beladjal & Mertens, 2009). In such a case, neighbouring ponds that are separated by fenced infrastructures would not be considered as severely fragmented. Additional studies are therefore recommended to investigate the potential role of small birds in LB dispersal.

Due to the stochastic nature of passive dispersal and the predicted risk of a fast extinction in disturbed localities (see above), we expect that EOO may show fast fluctuations that often could be misleading. For example, long-distance dispersal to a new, isolated locality (possibly too small for long-term survival) might result in a large increase of inferred EOO, possibly underestimating the risk of extinction of the species. Likewise, disappearance from such a site could easily lead to an overestimation of the risk of extinction in a species that otherwise has a rather small range. We thus assume that in taxa-like LBs that are exclusively passively dispersed, the EOO values are generally subjected to high levels of fluctuations. Therefore, AOO appears a more reliable parameter than EOO when assessing the category of threat for large branchiopods. Even if molecular studies report some evidence of gene flow among LB populations, it is impossible to infer if such gene flow would have occurred at a time pre-dating the land transformation, or if the remaining density of inhabited ponds is still sufficient to support gene flow at sufficient frequencies. It is also possible that extensive use for farming and livestock, using traditional methods, may have facilitated passive dispersal by aquatic birds (which avoid ponds with densely forested shorelines).

Regarding the "rescue effect", the frequency of occurrence of long-distance dispersal in LBs may often be too low to allow for regular gene flow among populations, so that successful dispersal is likely to result in new, but isolated populations. For example, evidence of gene flow among SW Iberian populations of *Triops* was only reported for two geographically restricted areas (within the Guadalquivir delta, and between Tahivilla and Benalup in southern Cádiz province, involving populations of *T. baeticus* and *T. gadensis*, respectively; see Korn et al., 2010). The presently existing populations may often be the result of a single dispersal event that occurred hundreds, or thousands of years ago (depending on the longevity of involved habitats). The probability of repeated dispersal to the same site may usually be so low that it may be regarded negligible. This means that for taxa that need peculiar habitat characteristics that are present only in very few locations, a rescue effect may be regarded highly unlikely. This also implies that the more habitats are lost, the less likely are potentially new habitats (like "cattle ponds") to be populated by large branchiopods.

Assessment of single LB species

Regarding the conservation status of Iberian LBs, the endemic *Linderiella baetica* (Anostraca) is probably the most worrying case. One locality in El Cuervo (southern Spain) disappeared decades ago due to urbanization, mining and agriculture. The remaining, single known locality is not protected and has undergone a major alteration and its urbanization is imminent (García-de-Lomas et al., 2016). The disappearance from this site would lead to the global extinction of *L. baetica*.

Also the cases of *Cyzicus tetracerus* and *Leptestheria mayeti* (Spinicaudata) are alarming, since at the regional level each of these species is represented by a single population. *Cyzicus tetracerus* is a species with Palearctic distribution whose only record in the Iberian Peninsula dates from 1983 (Alonso, 1996). M. Korn (unpubl. results) confirmed the presence of this species in 2006, but its current status is unknown. Although its habitat ("La Zaida" pond) is included in a SPA, agricultural use in the area surrounding the pond offers few long-term conservation guarantees (Table 1). The closest known occurrence locality for the species lies in mainland France (Defaye et al., 1998); therefore, a rescue effect appears unlikely, due to the barrier effect of Pyrenees. *Leptestheria mayeti* is distributed in the Arabian Peninsula, North Africa, southern Italy and the Balearic Islands (Thiéry, 1986, 1996; Alonso, 1996; Alfonso, 2017). The only known population in the Iberian Peninsula is located in Alcublas (East of Spain). The closest occurrence locality for the species in the island of Majorca (Balearic islands archipelago) (Pretus, 1991; Boix et al., 2009) is suggestive of the existence of actual successful long-range dispersal by birds using the Mediterranean migratory flyway (e.g. Barriocanal & Robson, 2007)). However, considering the geological history of the Balearic islands (Dewey et al., 1989; Bidegaray-Batista & Arnedo, 2011) and later connections during glaciations (Bover et al., 2008), genetic analysis among *L. mayeti* populations from the Iberian Peninsula and the Balearic islands would shed some light on the origin of these populations. However, the number of localities make a rescue effect unlikely and we kept the category of "Critically Endangered" for this species. Although this site is not included in the Natura 2000 Network, it was proposed to be included among the "important areas for ponds" (Ewald et al., 2010). Currently, this site is locally protected as a Wildlife Reserve for LBs and amphibians (Order 1/2014; at http://www.dogv.gva.es/portal/ficha_disposicion.jsp?L=1&sig=001311/2014). However, it may be affected by land use changes or habitat alteration (Sahuquillo & Miracle, 2010) and hitherto remains insufficiently protected.

Regarding Iberian species of *Triops* (Notostraca), a particular problem arises from the difficulties of morphological determinations. Based on integrative taxonomic investigations, the Iberian populations formerly attributed to *Triops cancriformis mauritanicus* or *Triops mauritanicus* have been described as four separate species that are endemic to the Iberian Peninsula (Korn et al., 2010). For *Triops emeritensis*, a single location (represented by two ponds) is known in the Iberian Peninsula. Both ponds occur within a SPA, but one of them can be affected by agriculture (ploughing and sowing) (Pérez-Bote et al., 2006). Nevertheless, in order to verify the actual number of localities in the Iberian Peninsula, it would be necessary to test further records that were initially reported as *Triops cancriformis mauritanicus* in Pérez-Bote et al. (2006) but were not included in Korn et al. (2010). It is presently not possible to infer if all these records belong to *T. baeticus* (the predominant species in the region based on available molecular data), or if some of these records might actually refer to *T. emeritensis*. In case all the undetermined additional records reported in Pérez-Bote et al. (2006) (i.e. three additional ponds south of the Guadiana river) would turn out to belong to *T. emeritensis* (although this appears unlikely), the resulting category for the species would change to "Endangered".

Triops vicentinus has 23 known localities in southern Portugal (Korn et al., 2010; Machado et al., in press). EOO and AOO of this species are small (1031 and 52 km^2, respectively) and meet IUCN Red List criteria of Endangered species (Table 1). Although ca. 83% of the known localities are protected, the habitats of this species are severely fragmented and a reduction in the number of its localities is expected, especially in the vicinity of Faro (Algarve, Portugal). Faro is the main administrative city of the Algarve region and some *T. vicentinus* localities are very close to the urban area of Faro suburbs, although inside the Ria Formosa Natural Park. Some unprotected sites, located about 30 km from Faro, are within agricultural areas.

For *Triops simplex*, the available data (one single location) would suggest that this species is "Critically endangered" at the regional scale. However, additional records of the genus *Triops* have been reported in the eastern Iberian Peninsula (e.g. Forner i Valls & Brewster, 2013; Verdiell-Cubedo & Boix, 2014) which are close to the only known site in the Castellón province (the determination of this population is based on molecular data). Due to an overlap in key morphological characters, it presently cannot be inferred whether these records belong to *T. cancriformis* or *T. simplex*. Therefore, *Triops cancriformis* and *Triops simplex* should conservatively receive the category "Data deficient". Other recent records from southern Spain also do not allow attribution to species (e.g. Prunier & Saldaña, 2010; Prunier et al., 2011). Further studies are needed in order to provide molecular based determinations of populations from the known sites, or in order to detect morphological characters that might be used for reliable determinations.

Lepidurus apus (Notostraca) shows an AOO = 48 km^2 and ≤10 locations (Table 1). The habitats are severely fragmented and 54% of them are protected. The closest localities outside the Iberian Peninsula are located in southern France (e.g. departments of Landes and Haute Garonne) (Brtek & Thiéry, 1995) and Morocco (a single pond confirmed in the Middle Atlas) (van den Broeck et al., 2015). In theory, western Maghreb might be a potential area for a rescue effect; however, the single locality known in Morocco is in the Middle Atlas, at 1878 m.a.s.l. and between 880 and 1120 km away from Iberian localities and separated by the Strait of Gibraltar. In addition, the taxonomic classification of Moroccan *Lepidurus* (referred to either as *L. couesii* or *L. apus*) (Thiéry, 1986) remains unclear so that it is presently not clear if these populations represent potential sources for a rescue effect. In contrast, localities in southern France are between 350 and 600 km away from Iberian localities (which is within the distance range found in the Iberian Peninsula) (see Online Resource 3). This fact suggests that *L. apus* might be eventually dispersed through the passage of Irún (Basque Country) more easily than through the Pyrenees. However, the passage of Irún (with altitudes below 1000 m.a.s.l.) represents a narrow strip (ca. 7%) of the total border between France and Spain and no biological and\or physical dispersal vectors for *L. apus´* resting stages able to cross the Pyrenees are known. Therefore, the probability of a rescue effect from southern France was considered unlikely.

Tanymastigites lusitanica (Anostraca) shows EOO = 664 km^2 and AOO = 36 km^2 (Table 1). With its habitats being severely fragmented and merely 56% of them being protected, a continuing decline is expected in the AOO of this species due to a change in land use. Although some of the populations are located in a natural park, the known populations inhabit puddles on dirt roads (Machado & Sala, 2013; J. Sala, pers. comm.). Therefore, if these roads are asphalted or if the puddles are frequently filled, all known populations will be lost. According to IUCN Red List criteria, we propose the category "Endangered" (Table 1).

Branchinecta orientalis (Anostraca) is a species widely distributed in Asia Minor and Central Asia (e.g. Manca & Mura, 1997; Alonso 2010; Marrone et al., 2015). It is also present in Austria, Hungary and Serbia (Horváth et al., 2013). Although it has 70% of its Iberian habitats included in SCI or SPA, all its populations are severely fragmented. The intense agricultural use that surrounds its habitats infers a continuous decline in the quality of its habitats. Therefore, this species should be considered "Vulnerable".

Branchinecta ferox (Anostraca) is a Palearctic and circum-Mediterranean species (Brtek & Thiéry, 1995; Hovarth et al., 2013; Marrone et al., 2016). It shows a restricted AOO in the Iberian Peninsula (Table 1), with its populations being fragmented by highways, waterways and urban settlements but not for railways. Since all of its currently known localities are protected, a reduction in its AOO or habitat quality cannot be inferred. Likewise, *Branchinectella media* (Anostraca) has a small AOO (124 km^2), but most of its known localities are included in the Natura 2000 network. Therefore, a continuous decline in EOO or AOO is not expected. Thus, *Branchinecta ferox* and *Branchinectella media* are interpreted to be of "Least Concern" at the regional scale. It is important to emphasize that "least concern" simply means that, in terms of extinction risk, these species are of lesser concern than species in other threat categories. It does not imply that these species are of no conservation concern.

The case of *Artemia* (Anostraca) is particularly worrying due to the decline of a large part of the coastal populations of the native species caused by the invasion of *Artemia franciscana* (Amat et al., 2007). In the case of *Artemia salina*, all known localities are coastal and therefore prone to invasion of *A. franciscana* due to a high abundance of aquatic birds, which act as vectors of *A. franciscana* (Green et al., 2005). Birds can also spread the native species but the number of remaining populations is so low that birds carry mostly *A. franciscana* resting eggs (Green et al., 2005). Even if *Artemia salina* and *Artemia* sp. (parthenogenetic strains) colonize new locations, it may be quickly displaced by the invasive species (Sánchez et al., 2017). No effective control methods for the invasive *A. franciscana* are known. In addition, the average loss rate of habitats (mainly saltmarshes and saltpans) reached 71% in mainland Spain and 50% in mainland Portugal in 20 years (Amat et al., 2007). Therefore, the causes of threat have not ceased and are irreversible. Although the EOO for *A. salina* is relatively large and most of its localities are included in the Natura 2000 network (85.7%) (Table 1), the small AOO (28 km^2) suggests that it deserves consideration as an "Endangered" species. Half of the *Artemia* sp. (parthenogenetic strains) localities are coastal saltpans, which may easily be connected by the aquatic bird flyways. Therefore, also this species is prone to be outcompeted by *A. franciscana* and should be considered a "Vulnerable" species. In fact, some locations have been invaded recently (Sánchez et al., 2017). The case of the native *Artemia* illustrates that protected areas are not free from biological invasions and, therefore, the protection of the territory does not guarantee by itself the conservation of its fauna in the long term if an adequate management of other threats is not additionally implemented.

For *Phallocryptus spinosus* (Anostraca) more than 90% of its known localities are protected, and mainly associated with inland saline wetlands. A decline of EOO or AOO caused by a land use change is not expected but locations are severely fragmented. However, this species inhabits permanent wells besides temporary hypersaline ponds (García & Niell, 1993; García et al., 1997) that are being invaded by *Artemia franciscana* (Amat et al., 2007). In fact, *A. franciscana* has already reached one of the main locations of *P. spinosus* in Fuente de Piedra lagoon in the province of Malaga (Amat et al., 2007). It presently remains unknown whether the invasion by *A. franciscana* is provoking a negative impact on *P. spinosus*, similar to that observed for native *Artemia* populations. Therefore, further research should be conducted to determine the actual effect of *A. franciscana* on this species.

In sum, for almost half (46%) of Iberian LBs, our results highlight the urgent need to ensure the legal protection of their habitats in accordance with Directive 92/43/EC. In this Directive (and its transposition in Spain and Portugal as member countries of the European Union), "Mediterranean temporary ponds" are included as priority areas of Community interest (Habitat 3170*). Effective protection involves not only land protection but also sustainable uses surrounding these habitats (e.g. to avoid aquifer overexploitation, see below). The use of LBs as a flagship group would help to promote a better protection of this habitat category, which comprises a diverse group of distinct aquatic habitats with faunal richness adapted to particular environmental conditions.

The current categories of threat of Iberian LBs are the consequence of a worrying reality: First, despite we found a high proportion ($>50\%$) of temporary ponds included in the Natura 2000 network (Table 1), this fact does not ensure their long-term viability. Emblematic National parks like Doñana in the south of Spain or Las Tablas de Daimiel in central Spain are not affected by a severe fragmentation but suffer from the overexploitation of aquifers by the surrounding croplands. Both temporary ponds and crops share the same aquifer in these protected areas and their surroundings (Suso & Llamas, 1993; Alvarez-Cobelas et al., 2001). In practice, crop development that surrounds these National parks is far from being at a stop, so a continuing decline in temporary ponds was inferred even in some protected areas. Secondly, the current landscape fragmentation in the Iberian Peninsula may compromise LB dispersal by walking animals. Fenced infrastructures such as highways and railways sum up to 18,000 and 18,600 km, respectively, whereas forests in hands of private owners (very often fenced) reach 66 and 93% in Spain and Portugal, respectively (Feliciano et al., 2015; Quiroga et al., 2015). As a consequence, the flow of animal vectors for LBs (e.g. wild mammals, cattle or amphibians) may often have been disrupted even between neighbouring ponds (even if only tens of metres apart) (Hobbs et al., 2008; Harris et al., 2009). Finally, climate change is projected to provoke a reduction in groundwater recharges between 14 and 57% (Guardiola-Albert & Jackson, 2011). Among these threats, the only one that could be reversed is an insufficient low proportion of currently protected habitats. It is therefore expected that even common species with a low proportion of occurrence in protected habitats (e.g. *Branchipus schaefferi*, *Chirocephalus diaphanus* or *Streptocephalus torvicornis*) that have been considered to be of Least Concern in the Iberian Peninsula will experience a significant decline in the future unless measures are taken to increase their proportion of protected habitats in order to partially compensate for the expected loss caused by those threats that cannot be controlled.

Considering the importance of the Mediterranean temporary ponds in the conservation of LBs and the advancing loss of these habitats in many countries (e.g. Wood et al., 2003; Zacharias & Zamparas, 2010), the present work also aims to serve as an example of what could be done in other regions or countries to evaluate the current status of the temporary aquatic environments and their associated fauna.

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