Alarming decline of freshwater trigger species in western Mediterranean key biodiversity areas

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Article impact statement: The delineation of some KBAs and their focal areas has shortcomings related to flawed data or lack of distribution data for trigger species.

Abstract

Theidentification of key biodiversity areas (KBA) was initiated by the International Union for Conservation of Nature in 2004 to overcome taxonomic biases in the selection of important areas for conservation, including freshwater ecosystems. Since then, several KBAs have been identified mainly based on the presence of trigger species (i.e., species that trigger either the vulnerability and or the irreplaceability criterion and thus identify a site as a KBA). However, to our knowledge, many of these KBAs have not been validated. Therefore, classical surveys of the taxa used to identify freshwater KBAs (fishes, molluscs, odonates, and aquatic plants) were conducted in Douro (Iberian Peninsula) and Sebou (Morocco) River basins in the Mediterranean Biodiversity Hotspot. Environmental DNA analyses were undertaken in the Moroccan KBAs. There was a mismatch between the supposed and actual presence of trigger species. None of the trigger species were found in 43% and 50% of all KBAs surveyed in the Douro and Sebou basins, respectively. Shortcomings of freshwater KBA identification relate to flawed or lack of distribution data for trigger species. This situation results from a misleading initial identification of KBAs based on poor (or even inaccurate) ecological information or due to increased human disturbance between initial KBA identification and the present. To improve identification of future freshwater KBAs, we suggest selecting trigger species with a more conservative approach; use of local expert knowledge and digital data (to assess habitat quality, species distribution, and potential threats); consideration of the subcatchment when delineating KBAs boundaries; thoughtful consideration of terrestrial special areas for conservation limits; and periodic field validation.

KEYWORDS
focal areas, Iberia, Morocco, protected areas, trigger species

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Resumen: La identificación de las áreas clave de biodiversidad (ACB) fue iniciada por la Unión Internacional para la Conservación de la Naturaleza en 2004 con el objetivo de sobreponerse a los sesgos taxonómicos en la selección de áreas importantes para la conservación, incluyendo los ecosistemas de agua dulce. Desde entonces, varias ACB han sido identificadas principalmente con base en la presencia de especies desencadenantes (es decir, especies que desencadenan el criterio de vulnerabilidad o de carácter irremplazable y por lo tanto identifican a un sitio como una ACB). Sin embargo, a nuestro conocimiento, muchas de estas ACB no han sido validadas. Por lo tanto, los censos clásicos de taxones utilizados para identificar las ACB de agua dulce (peces, moluscos, odonatos y plantas acuáticas) fueron realizados en las cuencas de los ríos Duero (Península Ibérica) y Sebou (Marruecos) en el Punto Caliente de Biodiversidad del Mediterráneo. Realizamos análisis de ADN ambiental en las ACB de Marruecos. Hubo una discrepancia entre la supuesta presencia y la actual presencia de especies desencadenantes. Ninguna de las especies desencadenantes se encontró en 43% y 50% de las ACB censadas en las cuencas del Duero y del Sebou, respectivamente. Las deficiencias en la identificación de las ACB de agua dulce están relacionadas con la carencia de datos o datos erróneos sobre la distribución de las especies desencadenantes. Esta situación resulta en una identificación inicial engañosa de las ACB con base en información ecológica deficiente (o incluso incorrecta) o también puede deberse al incremento en las perturbaciones humanas ocurridas entre la identificación de la ACB y el presente. Para mejorar la identificación de ACB de agua dulce en el futuro, sugerimos que la selección de especies desencadenantes se realice con un enfoque más conservador; que se usen el conocimiento local de los expertos y los datos digitales (para evaluar la calidad del hábitat, la distribución de las especies y las amenazas potenciales); que se consideren las subcuencas cuando se delimiten las fronteras de las ACB; que se consideren cuidadosamente las áreas de especies terrestres para los límites de conservación; y que se realicen validaciones periódicas de campo.

Palabras Clave:
área focal, áreas protegidas, especie desencadenante, Iberia, Marruecos

INTRODUCTION

The implementation of protected areas (PAs) is one of the most important conservation tools available to protect biodiversity (Pringle, 2017). However, it is still strongly biased toward the protection of terrestrial charismatic species, such as birds and mammals (Darwall et al., 2011; Mammola et al., 2020). Additionally, many PAs were established because they had high aesthetic values and low agriculture value and human density. Therefore, many of them may consistently fail to conserve substantial fractions of biodiversity (Joppa & Pfaff, 2009).

The concept of key biodiversity areas (KBA), developed at the beginning of the present century, aims to overcome these biases. The process of KBA identification was built on previous site-selection approaches (e.g., important bird and biodiversity areas, important plant areas, Alliance for Zero Extinction sites) and highlights areas that make a significant contribution to the global persistence of biodiversity across taxonomic groups and ecosystems. Many KBAs overlap previously established PAs (Eken et al., 2004). In 2004 the International Union for Conservation of Nature (IUCN) initiated a worldwide consultative process to establish an overarching method to identify

KBAs that culminated in the publication of the Global Standard for the Identification of Key Biodiversity Areas in 2016 (International Union for Conservation of Nature and Natural Resources, 2016). Subsequent guidelines were published in 2019 and 2020 (KBA Standards & Appeals Committee, 2019, 2020). The IUCN KBA approach uses a set of standardized criteria and thresholds that are based on data on threatened or geographically restricted species or both, ecological integrity, important biological processes, and irreplaceability. These criteria are mainly based on the presence of so-called trigger species (i.e., species that trigger either the vulnerability and or the irreplaceability criterion and thus identify a site as a KBA [Langhammer et al., 2007]). One method of identifying the trigger species for each KBA has been the use of experts, participating in workshops, who confirm the likelihood of occurrence and persistence of these trigger species for proposed KBAs. Some concerns have been raised regarding KBAs usefulness because they do not have the same legislative status as PAs and hence, may not have ongoing site management aimed at protecting biodiversity and ecosystems. Also, unlike systematic conservation planning, the KBA selection approach uses mostly biodiversity data, not accounting for ecosystem services, threats, and costs. Thus, these 2 methods should be combined to better achieve conservation goals (Smith et al., 2019). Other authors claim that the use of global-scale data without local experts' input to identify local-scale KBAs can lead to omission and commission errors (Knight et al., 2007).

Nevertheless, efforts have been made to overcome these major drawbacks (see KBA Standards & Appeals Committee, 2020), and the KBA identification approach has a high potential to characterize biodiversity patterns, identify biodiversity hotspots, and potentially help define important areas for conservation, especially for noncharismatic taxa and underrepresented ecosystems. This may be especially true for developing countries with fewer designated PAs (Waldron et al., 2013), as well as for freshwater taxa and ecosystems that, despite being among the most threatened worldwide, have a lower conservation investment (Darwall et al., 2011; Di Marco et al., 2017). Furthermore, current data show that the terrestrial taxa that generally inspire the creation of PAs are poor surrogates for freshwater biodiversity (Darwall et al., 2011; Leal et al., 2020; Nogueira et al., 2021). Given the evident discrepancy in spatial prioritization between freshwater and terrestrial or marine ecosystems, the creation of KBAs for freshwater taxa (primarily based on fishes, molluscs, odonates, decapods, and aquatic plants) was a critical and logical step that IUCN has initiated with vigor. Also, the creation of these KBAs could be a step forward to help achieve Target 11 (increase of inland waters protection) of the Convention on Biological Diversity and also its successor target of the post-2020 Global Biodiversity Framework (Donald et al., 2019). Since the inception of this program, 3894 potential KBAs for freshwater were delineated in the Mediterranean Biodiversity Hotspot (Darwall et al., 2014). However, most of these KBAs were desk-based exercises based on available data on species status refined by expert knowledge and have yet to be confirmed in the field. Given the pace that freshwater biodiversity and ecosystems are disturbed by human activities (Reid et al., 2019), it is opportune to confirm the conservation status of proposed or validated KBAs.

We assessed the representativeness of the trigger species' distributions and the conservation status of the freshwater KBAs identified in 2 large river basins of the Mediterranean Biodiversity Hotspot: Douro River Basin in the Iberian Peninsula (Maíz-Tomé et al., 2017) and the Sebou River Basin in Morocco (Darwall et al., 2014). Both regions provide excellent case studies because of the distinct availability of biodiversity data, human economic revenue and investment in scientific research, their spatial and climatic heterogeneity, and presence of habitats that encompass many endemic and evolutionarily unique freshwater species (Froufe et al., 2014; Sousa et al., 2016; Kalkman et al., 2018; Gomes-dos-Santos et al., 2019; Sousa-Santos et al., 2019). This contrasts with the high level of disturbance and large number of threatened species present in both areas (Cuttelod et al., 2008). Currently, the Iberian and Moroccan KBAs we assessed have not been validated through the Global Standard for the Identification of Key Biodiversity Areas because they are considered legacy KBAs and therefore are not included in the World Database of KBAs (Darwall et al., 2014; BirdLife International, 2020). Their legacy status was validated based on stakeholder consultation (Darwall et al., 2014). Thus, the shortfalls we identified here and our proposed guidance can help further validation and improve delineation of these KBAs and guideline efficacy, especially in freshwater ecosystems.

METHODS

Of all freshwater KBAs in the Mediterranean region (Darwall et al., 2014; Maíz-Tomé et al., 2017), we focused on the 14 in the Douro River basin, Iberian Peninsula (Figure 1a), and on the 4 in Sebou River basin, Morocco (Figure 1b). Special attention was given to headwaters, lakes, and springs (i.e., focal areas) that were previously defined as regions inside the KBAs of critical importance for the survival and reproduction of freshwater biodiversity (Abell et al., 2007) but are now considered boundaries of the KBAs. Apart from El Rebollar (Douro basin) and Oued Bouhlou (Sebou basin), all the KBAs we assessed have a designated focal area.

The effectiveness of the KBAs was assessed by determining whether the trigger species that were used to define them (fishes, molluscs, odonates, and plants) were present. For this, 43 and 37 sites were selected and surveyed for fishes, molluscs, odonates, and aquatic plants in Douro and Sebou basins, respectively. The research team already knew the study areas where some of the chosen survey sites represented the few permanent freshwater habitats available for aquatic species during summer. These sites were sampled with help from local experts and were carried out in 2018 and 2019.

Fishes were surveyed by electrofishing in river stretches of 100 m. We used a portable Hans Grassl (Schönau am Königssee, Germany) ELT60II with a pulsed DC-300-600 V generator. The fish were identified, counted, and returned to the river.

Macroinvertebrates were sampled with a hand net (mesh 0.05 cm) in river stretches of 50 m. Six replicate surface sweeps

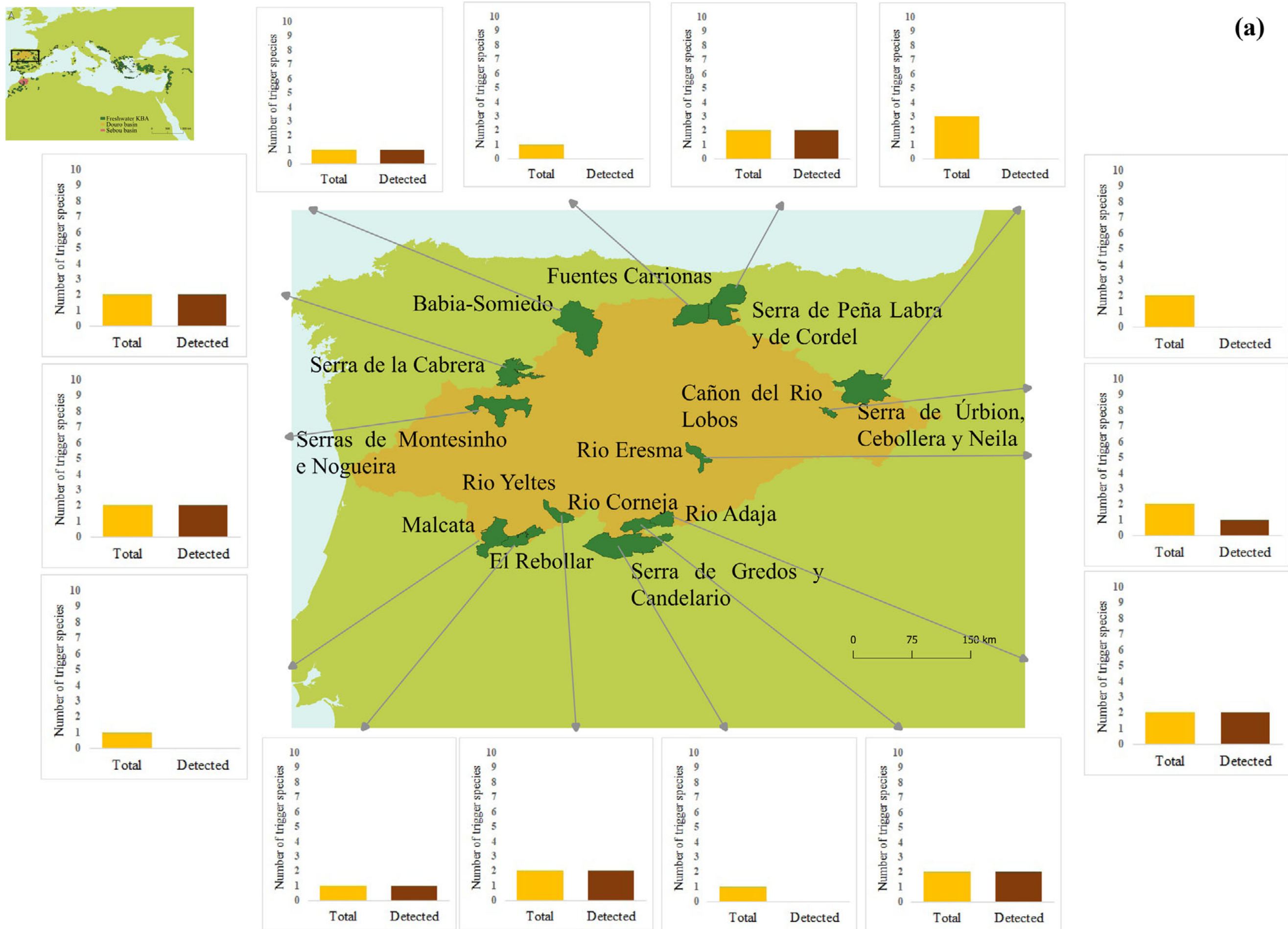

FIGURE 1 Legacy freshwater key biodiversity areas (KBAs) comparison of total number of trigger species and number of trigger species found in each KBA in the (a) Douro River (orange) and (b) Sebou River (blue) basins

(1 m long and 0.25 m wide) were performed. Kick sampling was also used: the sampling net was placed downstream, whereas the substratum was kicked upstream to guide the macroinvertebrates into the net. Sampling covered all types of microhabitats (e.g., lentic and lotic, banks, and center of the channel), sediments (e.g., pebbles, cobbles, sand, silt, clay) as well as macrophytes. Organisms were stored in alcohol for later sorting and identification. Adult Odonata assessment involved timed counts (1 h) for species and individuals along and above the water, as well as in the margin vegetation and on stones. These surveys assessed the most suitable areas along stretches of several hundred meters along the river or around ponds and were complemented with larval data from macroinvertebrate sampling. Freshwater mussels were surveyed along river stretches of 50 m by snorkeling and hand searching (detailed methods in Cummings et al. [2016]). All the live specimens were identified and counted and returned to their original locations.

Aquatic plants were surveyed by walking selected river reaches and parts of the margins and water column of standing water bodies. Abundance was expressed as percent cover.

In the Moroccan KBAs, water samples were collected for environmental DNA (eDNA) analysis following the protocols described by Valentini et al. (2016) for fishes and by Prié et al. (2021) for freshwater bivalves. This step provided a check for species that may not have been observed in the field when classical methods (described above) were used or that were present nearby. In addition to number of trigger species, we also assessed the number of species of conservation importance based on their IUCN Red List status: vulnerable (VU), endangered (EN), and critically endangered (CR).

Our research design was approved by and permission to conduct fieldwork was granted by Moroccan (Université Cadi Ayyad, Marrakech), Portuguese (Instituto da Conservação da Natureza e das Florestas), and Spanish (Junta de Castilla y León) authorities.

RESULTS

In 43% (Iberia) and 50% (Morocco) of the KBAs assessed, no trigger species were found (Figure 2a). At least some trigger species were detected in 7% (Iberia) and 50% (Morocco) of all assessed KBAs (Figure 2a). Although we detected all trigger species in 50% of the Iberian KBAs, all trigger species were not detected in any of the Moroccan KBAs (Figure 2a). In Iberia we only found all designated trigger species in 7 KBAs

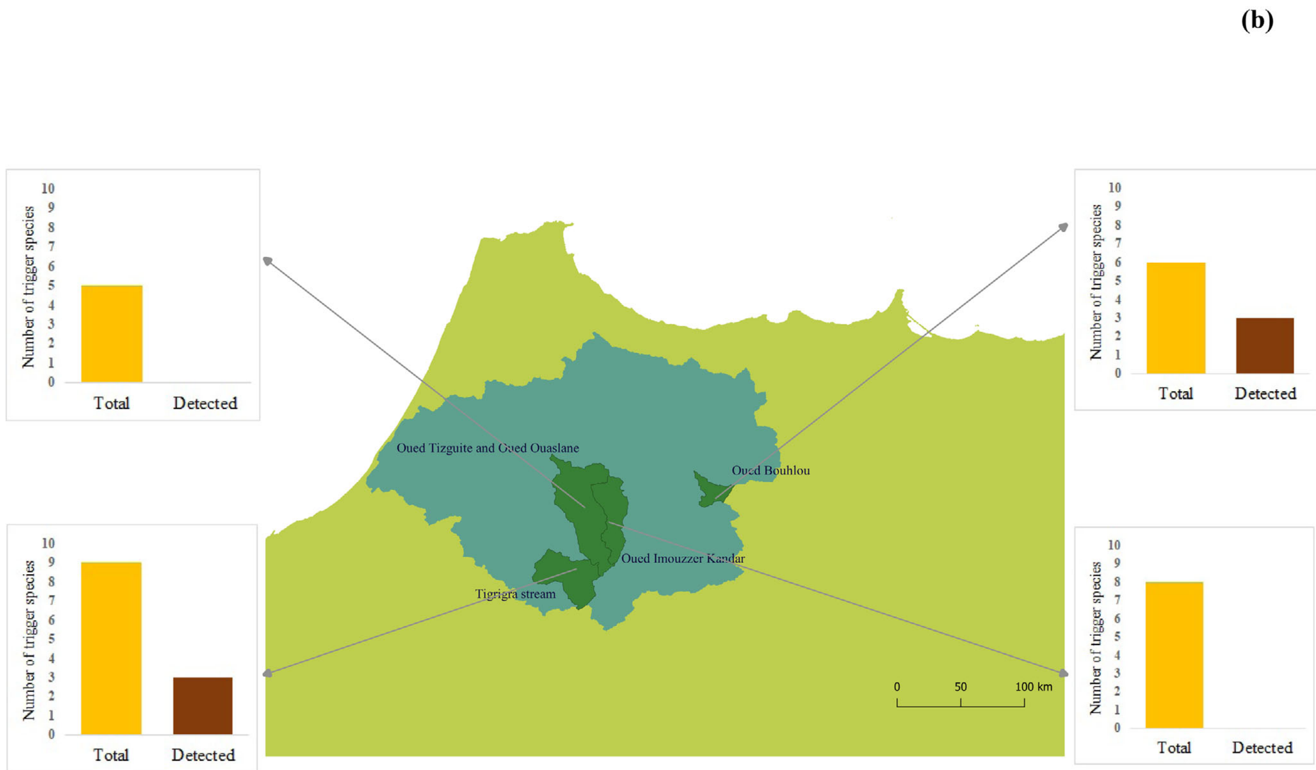

FIGURE 1 Continued

(Babia-Somiedo, El Rebollar, Serras de Montesinho e Nogueira, Rio Adaja, Rio Corneja, Rio Yeltes, and Serra de la Cabrera). One KBA had half of them (Rio Eresma), and the remaining 6 had none of the trigger species (Cañon del Rio Lobos, Fuentes Carrionas, Serra da Malcata, Serra de Gredos y Candelario, Serra de Peña Labra y de Cordel and Serra de Úrbion, Cebollera y Neila) (Figure 1a & Table 1). In Morocco we failed to detect any trigger species in Imouzzer Kandar and Oued Tizguite and Oued Ouaslane KBAs. Only half of trigger species were detected in the Oued Bouhlou, and only 2 were found in Oued Tigrigra from a total of 9 trigger species described previously (Figure 1b & Table 1).

Threatened species were detected in almost all KBAs, except for Cañon del Rio Lobos, Rio Adaja, Serra de Úrbion, Cebollera y Neila, and Imouzzer Kandar (Table 1). Almost all threatened species found in Iberian freshwater KBAs were fishes, except in Serras de Montesinho e Nogueira, where the pearl mussel (*Margaritifera margaritifera*) (EN) was found (Figure 2b). Forty-four percent of threatened species were VU, 44% were EN, and the remaining 12% were CR (Figure 2c). In Moroccan KBAs, 47% of threatened species were molluscs, 33% were plants, 13% were fishes, and 7% were Odonata (Figure 2b), of which 56% were VU, 31% were EN, and 13% were CR (Figure 2c).

The eDNA analyses allowed detection of all the fish and bivalve species found using traditional methods. It also allowed detection of 1–3 more species (including 1 trigger species) in each site that had been overlooked during field surveys conducted with classical methods (Table 2). In some cases (e.g., *Potomida littoralis*), the number of DNA reads was very low, suggesting that the species was rare or may live upstream of the surveyed sites.

DISCUSSION

To our knowledge, this study represents the first field validation of the freshwater KBAs identified before the Global KBA Standard was approved. Overall results suggest there are some shortcomings in the definition of the studied KBAs and their respective focal areas, mostly related to flawed or outdated data or lack of distribution data for trigger species. Some of the assessed KBAs were poorly identified due to selection of incorrect trigger species and poor definition of their ranges. Others possibly failed due to the rapid extirpation of species caused by ongoing and accelerating threats (e.g., introduction of invasive species, habitat loss and fragmentation, and water abstraction) inside the KBAs. The results observed inside Douro's KBAs are better explained by the latter, but for Morocco, due to the general lack of historic data, it is not possible to accurately pinpoint a major cause for these results, but it is most likely a combination of the 2.

Our results are consistent with some of the known shortfalls of the KBA approach identified by Knight et al. (2007). In some cases, the trigger species were wrongly identified during the IUCN Red List assessment, and the species never occurred inside a specific KBA. For instance, in Oued Tigrigra, the EN bivalve *Unio durieui* was included as a trigger species but this species is now known to occur only in Tunisia and eastern

TABLE 1 Presence (+) or absence (–) of trigger species and other threatened species in key biodiversity areas (KBA) assessed, main threats to species in KBAs, and KBA ecological condition and suggested action

	KBA	Trigger species	Other threatened species	Main threats	KBA ecological condition and suggested action
Iberia	Babia-Somiedo	*Cobitis calderoni* (EN) +	*Achondrostoma arcasii* (VU)	Lack of connectivity to the watershed	Good condition, identify additional taxa
	Cañon del Río Lobos	*Achondrostoma arcasii* (VU) –	–	Lack of connectivity to the watershed	Poor condition, change target taxa andreview KBA's limits
		Pseudochondrostoma duriense (VU) –		Water abstraction	
	El Rebollar	*Cobitis vettonica* (EN) +	*Pseudochondrostoma duriense* (VU)	Lack of connectivity to the watershed	Good condition, identify additional taxa
			Cobitis paludica (VU)		
			Squalius alburnoides (VU)		
	Fuentes Carrionas	*Pseudochondrostoma duriense* (VU) –	*Achondrostoma arcasii* (VU)	Lack of connectivity to the watershed	Moderate condition, change target taxa
	Malcata	*Eryngium viviparum* (EN) –	*Pseudochondrostoma duriense* (VU)		Moderate condition, change target taxa
			Cobitis paludica (VU)		
			Squalius alburnoides (VU)		
	Serras de Montesinho e Nogueira	*Cobitis calderoni* (EN) +	*Pseudochondrostoma duriense* (VU)	Invasive species	Good condition, identify additional taxa
		Margaritifera margaritifera (EN) +		Lack of connectivity to the watershed	
	Río Adaja	*Cobitis paludica* (VU) +	–	Habitat fragmentation	Good condition
		Achondrostoma arcasii (VU) +			
	Río Corneja	*Achondrostoma arcasii* (VU) +	*Pseudochondrostoma duriense* (VU)	Water abstraction	Good condition, identify additional taxa
			Squalius alburnoides (VU)	Siltation	
	Río Eresma	*Cobitis calderoni* (EN) –	*Pseudochondrostoma duriense* (VU)	Water abstraction	Moderate condition, change target taxa
		Achondrostoma arcasii (VU) +		Siltation	
	Río Yeltes	*Achondrostoma salmatinum* (EN) +	*Pseudochondrostoma duriense* (VU)	Invasive species	Good condition, identify additional taxa
		Cobitis paludica (VU) +	*Squalius alburnoides* (VU)	Habitat fragmentation	
	Sierra de Gredos y Candelario	*Iberhoratia aurorae* (DD) –	*Pseudochondrostoma duriense* (VU)	Lack of connectivity to the watershed	Moderate condition, change target taxa
	Sierra de la Cabrera	*Cobitis calderoni* (EN) +	*Anguilla anguilla* (CR)	Lack of connectivity to the watershed	Good condition, identify additional taxa
		Achondrostoma arcasii (VU) +	*Pseudochondrostoma duriense* (VU)		
	Sierras de Peña Labra y Cordel	*Pseudochondrostoma duriense* (VU) –	*Achondrostoma arcasii* (VU)	Lack of connectivity to the watershed	Moderate condition, change target taxa
	Sierras de Úrbion, Cebollera y Neila	*Cobitis calderoni* (EN) –	–	Lack of connectivity to the watershed	Poor condition, no trigger or threatenedspecies found
		Achondrostoma arcasii (VU) –			
		Pseudochondrostoma duriense (VU) –			

(Continues)

TABLE 1 (Continued)

	KBA	Trigger species	Other threatened species	Main threats	KBA ecological condition and suggested action
Morocco	Imouzzer Kandar	*Cobitis maroccana* (VU) –	–	Water abstraction	Poor condition, no trigger or threatened species found; review KBA's limits
		Horatia sp. nov. *haasei* (EN) –		Invasive species	
		Melanopsis scalaris (EN) –		Climate change	
		Theodoxus marteli (VU) –			
		Theodoxus numidicus (VU) –			
		Calopteryx exul (EN) –			
		Cordulegaster princeps (NA) –			
		Plantago lacustris (VU) –			
	Oued Tigriga	*Cobitis maroccana* (VU) –			Moderate condition, change target taxa
		Horatia aghbalensis (EN) +			
		Melanopsis arbalensis (NA) –	*Unio foucauldianus* (CR)	Water abstraction	
		Theodoxus numidicus (VU) –	*Scrophularia eriocalyx* (EN)	Pollution	
		Melanopsis scalaris (NA) +	*Apium repens* (VU)	Recreational activities	
		Unio durieui (EN) –	*Damasonium polyspermum* (VU)	Habitat degradation	
		Calopteryx exul (EN) +	*Rorippa hayanica* (VU)	Habitat fragmentation	
		Cordulegaster princeps (NA) –			
		Lepidium violaceum (VU) –			
	Oued Tizguite & Oued Ouaslane	*Cobitis maroccana* (VU) –			Poor condition, change target taxa
		Giustia midarensis (EN) –	*Astacus astacus* (VU)*	Pollution	
		Heideella knidirii (EN) –	*Scrophularia eriocalyx* (EN)	urbanization	
		Horatia 'aghbalensis' (EN) –	*Damasonium polyspermum* (VU)	Water abstraction	
		Calopteryx exul (EN) –			
	Oued Bouhlou	*Cobitis maroccana* (VU) +	*Salaria atlantica* (VU)		Good condition, change target taxa andreview KBA's limits
		Heideella knidirii (EN) –	*Pseudunio marocanus* (CR)	Water abstraction	
		Horatia haasei (EN) –	*Unio foucauldianus* (CR)	dams	
		Calopteryx exul (EN) +	*Potomida littoralis* (EN)		
		Cordulegaster princeps (NA) –	Horatia aghbalensis (EN)		
		Plantago lacustris (VU) +	*Theodoxus numidicus* (VU)		

*Non-native invasive species.

TABLE 2 Presence (+) or absence (–) of species in key biodiversity areas based on environmental DNA analysis versus classical surveys

Species	Oued Tizguite & Oued Ouaslane		Oued Bouhlou		Oued Tigrigra		Imouzzer Kandar 1		Imouzzer Kandar 2	
	eDNA	classical	eDNA	classical	eDNA	classical	eDNA	classical	eDNA	classical
Margaritifera marocana	–	–	+	+	–	–	–	–	–	–
Potomida littoralis	+	–	+	+	+	–	–	–	–	–
Unio foucauldianus	–	–	+	+	+	+	–	–	–	–
Salaria atlantica	–	–	+	–	–	–	–	–	–	–
Cobitis maroccana	–	–	+	–	–	–	–	–	–	–
*Gambusia holbrooki**	–	–	–	–	–	–	+	–	+	+
Gobio gobio	+	+	–	–	–	–	–	–	–	–
*Lepomis gibbosus**	+	–	+	+	+	–	–	–	–	–
Luciobarbus labiosa	+	–	+	+	+	+	–	–	+	+
*Oncorhynchus mykiss**	–	–	–	–	–	–	+	–	+	–
*Scardinius erythrophthalmus**	+	+	–	–	–	–	–	–	–	–
Carasobarbus fritschii	–	–	+	+	+	+	–	–	–	–

*Non-native invasive species.

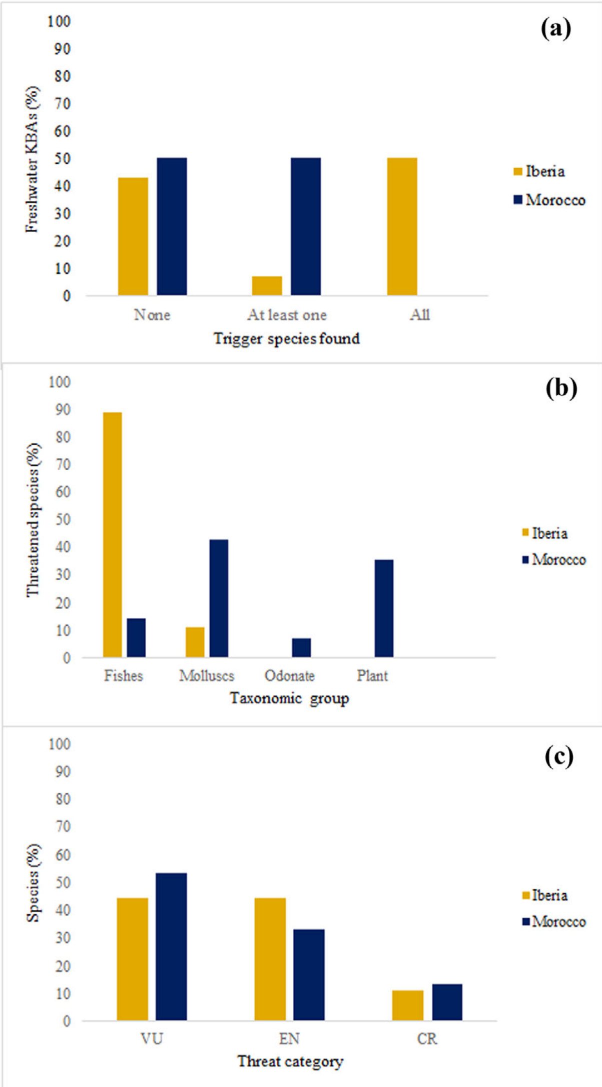

FIGURE 2 (a) Percentage of freshwater key biodiversity areas (KBAs) where none, some, and all of the trigger species occur, (b) percentage of threatened species inside KBAs by taxonomic group, and (c) percentage of threatened species inside KBAs by International Union for Conservation of Nature threatened category (VU, vulnerable; EN, endangered; and CR, critically endangered)

Algeria. The original assessor likely mistook it for the congeneric *Unio foucauldianus* (CR) found in this KBA. Because of inaccurate distribution data, some trigger species occurred in different sites from the ones expected. For instance, in sites surveyed in Imouzzer Kandar none of the 8 trigger species were found. However, in areas adjacent to this KBA, 2 trigger species, *Cobitis maroccana* (VU) and *Melanopsis scalaris* (EN), were present as were 2 other threatened species that were not considered trigger species, *Potomida littoralis* (EN) and *Unio foucauldianus* (CR). These results could justify an extension of Imouzzer Kandar limits to include the known area of occurrence of these species. In other KBAs, although the trigger species were properly identified, given the rapid changes caused by intense human activities (discussed below), they may have been extirpated from these areas. Therefore, the trigger species and status of the KBA need to be reviewed urgently. This was the case for the odonate trigger species *Calopteryx exul* (EN). We found *C. exul* in some streams in Oued Tigrigra during the 1990s (G.D.K., personal observation), but when revisiting these sites for this study, the streams had dried up or just a few disconnected pools remained, not enough for the survival of a rheophilic species (Ferreira et al., 2015).

Some KBAs contain a large number of species with conservation concern; however, there was a mismatch between the originally designated trigger species and the species found in our assessment. For instance, in Oued Bouhlou, although we detected only half the trigger species, there were many other species of conservation importance, including the freshwater mussels *Pseudunio marocanus* (CR), *Unio foucauldianus* (CR), and *Potomida littoralis* (EN), which were not considered originally as trigger species during the KBA selection process. The fact that these are threatened species does not automatically grant them the status of trigger species. However, given that *P. marocanus* is an endemic species restricted to 2 basins in Morocco (Sousa et al., 2016, 2018), there is a large chance that it meets the KBA criteria.

Although the poor knowledge about freshwater biodiversity, especially in Morocco, may have driven these results, the rapid changes in species composition caused by human activities are also a highly plausible explanation for the discrepancies found. The intensification of several human impacts, such as water shortage (drought and overexploitation), organic pollution, presence of dams, and introduction of invasive species, has also promoted a large decline in freshwater diversity, especially in the driest regions of central Iberia and Morocco (Sousa et al., 2018; Sousa, Ferreira, et al., 2020; Gomes-dos-Santos et al., 2019). For example, the increase in water extraction for agriculture purposes at Aoua and Hachlaf Lakes, the designated focal areas of the Imouzzer Kandar KBA, left them dry (Figure 3a), such that a temporary wetland habitat has replaced them. Consequently, most freshwater species, except for the plants, have rapidly disappeared from these lakes, invalidating its status as a KBA for freshwater taxa. The Aghbal spring, focal area of Oued Tigrigra, has been gradually transformed into a small reservoir used for recreational activities. In addition, an increasing number of invasive species is being reported in the Moroccan KBAs, such as the crayfish *Astacus astacus*, the fishes *Gambusia holbrooki*, *Lepomis gibbosus*, *Gobio gobio*, *Scardinius erythrophthalmus*, *Oncorhynchus mykiss*, and the Asian clam *Corbicula fluminea*, but their impacts are still largely unknown (Clavero et al., 2012).

In the same vein, many native fish species in Iberia have declined dramatically over the last few years, possibly due to water shortage, eutrophication, habitat fragmentation, and the spread of invasive species (Figure 3b) (Hermoso et al., 2011). The growing number of invasive species in Iberian KBAs is alarming when combined with the lack of connectivity due to the presence of hundreds of dams and weirs (Terêncio et al., 2021). For instance, we found a substantial number of specimens of the invasive minnow *Phoxinus bigerri*, which can potentially replace endemic species, such as *Achondrostoma arcasii*. The presence of non-native piscivorous fish, such as *Lepomis gibbosus* and *Exox lucius* (Figure 3b), and crayfish, such as *Pacifas-*

FIGURE 3 (a) Completely dry Lake Aoua, the focal area of the Imouzzer Kandar key biodiversity area (KBA), and (b) an *Esox lucius*, one of the many invasive non-native species found in Douro River KBAs

tacus leniusculus and *Procambarus clarkii*, may be responsible for the decrease or even extirpation of native cyprinid species and freshwater mussels (Almeida et al., 2014; Sousa et al., 2019).

Given that most of the Iberian KBAs were selected based on the presence of trigger fish species that are highly susceptible to habitat fragmentation and introduction of invasive species, it is not surprising that we did not find the trigger species inside the KBAs. However, one could argue that because we were dealing with rare species and a limited number of surveyed sites, we may have overlooked them. Therefore, future field surveys with more sites complemented by other methods (e.g., environmental DNA) should be performed. Our eDNA analysis for the Moroccan KBAs was particularly efficient in the detection of rare species that would otherwise be missed with classical sampling. For instance, *Cobitis maroccana*, a trigger species from Oued Bouhlou, was only detected through this method. Nevertheless, eDNA analysis did not detect the majority of the trigger species mentioned for each site, which corroborates the hypothesis that they are absent in these sites and supports the efficiency of classical sampling methods used to detect the trigger species.

Although this work highlights some of the main problems associated with the previous KBA approach in freshwater ecosystems, we fully recognize its importance for the conservation of aquatic biodiversity. KBAs represent a low-cost standardized approach to identify important conservation areas, filling some gaps related to the lack of representativeness of freshwater ecosystems and less charismatic species in PAs, and the lower PA coverage in countries with fewer resources (Butchart et al., 2014). Given its importance and considering the Guidelines for the Identification of KBAs and our results, we offer the following steps for the improvement of freshwater KBA designation. First, select trigger species with a more conservative approach by delineating their distributions, using only recent data, and conducting a more discerning evaluation of the IUCN Red List data (i.e., use expert opinion and field validation). Trigger species are identified with IUCN Red List information, so it is necessary to reassess the conservation status of data-deficient species, such as *Iberhoratia aurorae* (Serra de Gredos y Candelario) and *Melanopsis arbalensis* (Oued Tigriga). The Global Standard for the Identification of KBAs acknowledges this flaw, and more restrictive evidence is recommended, based on the same suggestions we make here, to confirm the presence and conservation status of a trigger species inside a proposed KBA.

Second, increase contributions from local experts in assessment of habitat quality (special attention to focal areas) and species distribution either by bringing local experts and stakeholders to workshops or by using questionnaires or face-to-face interviews (e.g., Sousa, Nogueira, et al. 2020). The exploration

FIGURE 3 Continued

of available digital data (e.g., iEcology [Jarić et al., 2020], text, images, videos, online activity, etc.) should be also pursued. Special attention should be given to the identification (and if possible mitigation) of the most important disturbances threatening biodiversity and ecosystems.

Third, KBAs boundaries should be planned at the subcatchment level to ensure long-term persistence of trigger species, given that spatial (longitudinal, vertical, lateral) and temporal connectivity play a major role in the dynamics of freshwater ecosystems (Hermoso et al., 2012). Focal areas identified for freshwater KBAs will likely become the boundaries of the validated KBAs, instead of the wider subcatchment, which, as demonstrated here, can lead to the omission of important trigger species.

Fourth, provide a more thoughtful consideration of the use and the limits of previously established special areas for conservation (SACs) (Habitats Directive 92/43/EEC) for terrestrial taxa applied to the definition of freshwater KBAs. Some of the KBAs we assessed (e.g., Serras de Nogueira e Montesinho and Cañon del Rio Lobos) were delineated using terrestrial SACs boundaries. As shown previously (Leal et al., 2020), spatial prioritization based on terrestrial species does not necessarily benefit freshwater taxa.

Fifth, establish baseline surveys of the trigger taxa for KBA validation, periodically monitor, and consider a systemic validation based on classical monitoring tools (as described here). If possible, complement these with eDNA analyses (Thomsen & Willerslev, 2015). Indeed, eDNA analysis is efficient, is easily standardized for long-term monitoring, and does not require special skills or taxonomic expertise. The most recent Guidelines for the Identification of KBAs state that confirmed KBAs should be reassessed at least every 8–12 years, but more frequently if possible. Given the rapid pace of ecosystem changes and species extirpation in freshwaters, we believe this reassessment should ideally be conducted every 4 years (following the important bird area monitoring framework [BirdLife International, 2006]). These KBAs should be considered for long-term ecological research sites (Reinke et al., 2019) that emphasize the need to establish an effective protocol for KBA monitoring based on freshwater experts' knowledge worldwide. To ensure that there is sufficient and reliable biodiversity data available to identify freshwater KBAs, it is necessary to devote more resources to field surveys and to improve biodiversity databases and facilitate their use. It is undoubtedly true that this sort of improvement requires investment (economic and human resources); thus, it is necessary to encourage long-term support of such initiatives. This should be an ongoing process in which cooperation among researchers, stakeholders, local citizens, and politicians pursues the best (and less expensive) methods and finds the best solutions to protect freshwater ecosystems.

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