FiSHMED
Fishes in Mediterranean Environments

sibic
Sociedad Ibérica de Ictiología
Sociedade Ibérica de Ictiologia

Updating *Anaecypris hispanica* distribution and conservation status in Portugal

Ana Cristina Cardoso

Instituto da Conservação da Natureza e das Florestas, Mértola, Portugal

ana.cardoso@icnf.pt

SUMMARY

Anaecypris hispanica is a small and endangered cyprinid endemic restricted to the south-western Iberian Peninsula. Despite its conservation status, the last assessment of its distribution in Portugal was carried on in the late 1990s. Here, I use data collected through a standardized methodology between 2015 and 2021 to update the information on the main threats, distribution and abundance patterns of *A. hispanica* in Portugal. Results confirm the progressive decrease in the geographic range of the species, involving its disappearance from six sub-basins. The main threats for conservation of *A. hispanica* and other fish in the Guadiana basin are the same identified twenty years ago (i.e., over-abstraction of water, over-grazing and proliferation of exotic species). Conservation actions should be mainly focused on improving water management, and should become priorities for the Guadiana Hydrographic Management Plan.

Keywords: endemic fish, fish conservation, summer pools, water extraction, invasive species, Guadiana River basin

Citation: Cardoso AC (2022) Update on *Anaecypris hispanica* distribution and its conservation status in Portugal. Fishes in Mediterranean Environments 2022.001: 12p. https://doi.org/10.29094/FiSHMED.2022.001

INTRODUCTION

Freshwater fish are one of the most endangered groups of species in the Mediterranean region, where 56% of endemic freshwater fish species are threatened (Smith & William, 2006; Cuttelod *et al.*, 2008). The Guadiana basin hosts a rich and highly endemic fish fauna, which is also severely imperilled, since 8 out of 11 native freshwater species are threatened after IUCN criteria. In fact, thirty years ago SNPRCN (1991) already called for the protection of the Guadiana fish fauna due to the high number of Iberian endemic species found in it.

Anaecypris hispanica is a small Iberian cyprinid (Figure 1), which is classified as Critically Endangered in the Portuguese Red List of Vertebrates (Cabral *et al.*, 2005) and as Endangered in the Spanish Red Data

DOI: 10.29094/FiSHMED.2022.001

Book (Doadrio, 2002) and in the IUCN Red List (IUCN, 2021). Its geographical range is restricted to some tributaries of south-western Iberian Guadiana and Guadalquivir river basins (Doadrio, 2002; Cabral *et al*., 2005; De Miguel *et al*., 2010), all of which are Mediterranean-type streams, with a typical intermittent regime, being reduced to pools during the summer. The fish assemblages depend on these pools to survive and it is during drought months that species are more vulnerable to several pressures. In these pools, large numbers of fish have an increased predation risk, intense competition for space and food, frequent hypoxia conditions, high temperatures, and a higher probability of being affected by infectious diseases (Magoulick & Kobza, 2003; Matthews & Marsh-Mathews, 2003; Dekar & Magoulick, 2007). Additionally, pools face increasing pressures as a result of human activities and climate change (Collares-Pereira *et al.* 2000b; Matono *et al*., 2014). Water pollution and extraction and the proliferation of exotic species are perceived as the main threats for *A. hispanica* (Collares-Pereira *et al*., 2000b; Smith & William, 2006; Blanco-Garrido *et al*., 2009).

Fifty years ago, *A. hispanica* inhabited rivers across the entire upper and lower Portuguese sector of Guadiana basin (Collares-Pereira & Almaça, 1979), but its abundance and geographic range have been progressively diminishing since then (Collares-Pereira *et al*., 1999). This small cyprinid has been the focus of several research, monitoring and conservation projects in Portugal. Saramugo LIFE project (1997-2000) was the lever that triggered several lines of research on the ecology and biology of the species (Collares-Pereira *et al*., 1999; Ribeiro *et al*., 2000; Carrapato & Ribeiro, 2012), which culminated in the presentation of a management plan for its conservation (Collares-Pereira *et al.* 2000a). Phylogenetic analyses were also carried out (Salgueiro *et al*., 2003; Levy *et al*., 2009; Sousa-Santos *et al*., 2014), and six management units were defined according to the genetic proximity (Alves *et al*., 2001). During the 2004/2005 drought, actions were carried out to rescue individuals from threatened nuclei and monitor the situation throughout its distribution area (Rogado *et al*., 2005), revealing that the species could have disappeared from the upper Portuguese Guadiana basin. More recently, work has been carried out on *A. hispanica* habitat preferences and its relationships with exotic species (Blanco-Garrido *et al*., 2009; Muñoz, 2017; Ilhéu *et al*., 2017; Sousa-Santos, 2018; Da Silva *et al*., 2019).

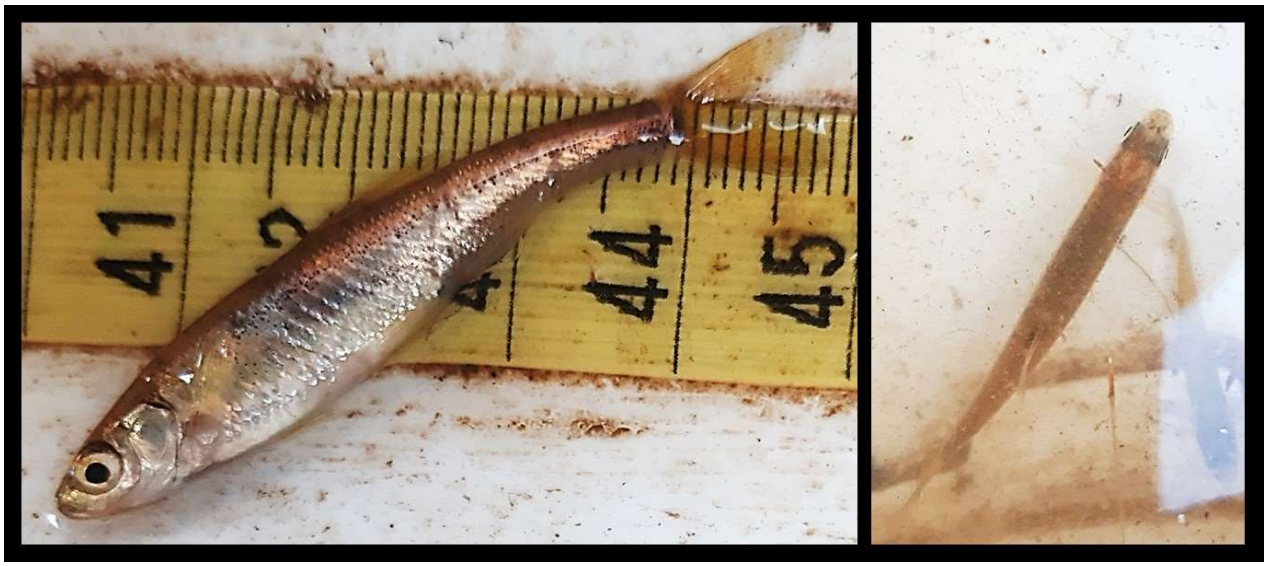

Figure 1. *Anaecypris hispanica* specimens from Murtigão stream.

DOI: 10.29094/FiSHMED.2022.001

All this valuable background information made it possible to update the management plan and produce an action plan for the conservation of *A. hispanica* (Cardoso *et al*., 2014). The purpose of the action plan was to define proactive measures that would likely be effective in preventing further declines of the species and minimising threats. The plan included detailed actions to address specific threats, mainly regarding the recovery and protection of the habitat and the decreasing of the pressure from exotic species. The implementation of the action plan has been achieved through four conservation projects: LIFE (2014-2019); SOMINCOR (2014-2019); VALAGUA (2015 - 2019); POSEUR (2018-2022) (Fragoso *et al*., 2018; Cardoso, 2018a; Cardoso, 2018b).

Monitoring programs allow collecting long-term datasets that are crucial to understand and manage freshwater systems (Dodds, 2012; Cid, 2017). However, the last comprehensive assessment of *A. hispanica* distribution and abundance in Portugal was published by Collares-Pereira *et al.* in 1999. After that, Cardoso & Carrapato (2008, 2010) evaluated the presence/absence of the species in summer pools across its Portuguese range, using different types of nets and variable sampling efforts. Taking into account the lapse of time that has passed, it was urgent to assess the situation of the species in the basin using a standardized methodology. Such an approach was implemented in the framework of a LIFE project (2014-2019), applying a sampling methodology that allows appropriate interannual comparisons and the design of long-term monitoring schemes (INAG, 2008).

Here, I describe patterns and trends in the distribution and abundance *A. hispanica* in the Portuguese sector of the Guadiana basin, with the aim of setting the bases for future long-term monitoring. The work also characterizes the main threats affecting remaining populations, and identifies relevant conservation measures, particularly addressing the problems of collaboration with landowners.

METHODS

The field work was carried out during the springs of 2015, 2018 and 2021. Sampling sites were chosen based on the known presence of *A. hispanica* before 1999 (Collares-Pereira *et al.* 1999; Collares-Pereira, 1990). A total of 45 sites were sampled in 2015, of which only 25 were assessed in 2018 and 2021 (Figure 2). An effort was done in the early autumn of 2017 to confirm *A. hispanica* absences obtained in the 2015 survey and earlier ones (Rogado *et al*, 2004; Cardoso & Carrapato, 2008). To that aim, trawl-net surveys were carried out in ponds between sampled sections where no *A. hispanica* had been caught in previous surveys. Confirmed the absence, sampling was kept only in 25 sites on the streams with *A. hispanica* presence (Figure 2).

Fish populations were sampled by electrofishing, following the protocol developed by the Portuguese Water Agency under the implementation of the Water Framework Directive (INAG, 2008). Usually, river sections of 100 to 120 m were sampled in a single pass with portable electrofishing device (Hans Grassl model EL62) by wading upstream in a zigzag path. After capture and identification, fishes were returned alive to the water at the collection site. Abundance was quantified as the number of individuals collected per $100m^2$.

Since 2005 information related with anthropogenic threats in the *A. hispanica* distribution area has been collected through surveillance, satellite images and non-systematic monitoring. This information was complemented with contributions from the data base of Guadiana Hydrographic Management Plan (APA, 2014).

The data on the presence and abundance of *A. hispanica* and on the incidence of threats were included in a geographic information system that was treated with Quantum Gis 3.4.5.

DOI: 10.29094/FiSHMED.2022.001

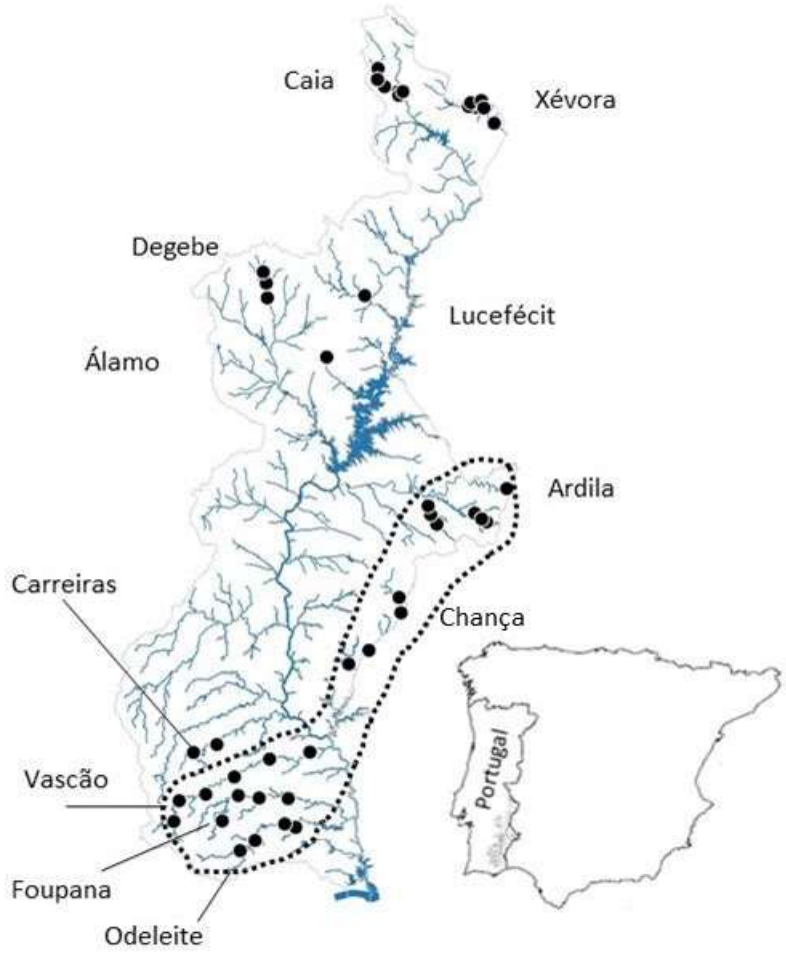

Figure 2. Map of the Portuguese sector Guadiana River basin (see position within the Iberian Peninsula in the lower map), showing the sampling locations in the 2015 survey. In 2018 and 2021 surveys were developed only in the 25 sites inside the dashed polygon.

RESULTS AND DISCUSSION

Distribution and abundance trends

The distribution area of *A. hispanica* has decreased from nine to five sub-basins of the Guadiana basin since the work carried out by Collares-Pereira *et al.* (1999). Presently, the species occurs in the Ardila, Chança, Vascão, Foupana and Odeleite sub-basins.

It is apparently extinct in the upper part of the Portuguese Guadiana basin. Since the work done by Ribeiro *et al.* (2000) *A. hispanica* has not been reported in the sub-basins of Xévora, Caia, Degebe and Carreiras despite the great amount of field work developed in them (Rogado *et al.*, 2004; Cardoso & Carrapato, 2008; EDIA, 2008; EDIA, 2009; Matono *et al.*, 2012a; Godinho *et al.*, 2014; Matono *et al.*, 2014). Distribution patterns seem, however, to have remained rather stable between 2015 and 2021, a period during which no further sub-basin extinctions have been recorded (Figure 3). Occurrence of *A. hispanica* at individual sampling sites was irregular, with the species being consistently detected in only three sampling sites (two in the Chança and one in the Odeleite sub-basin), a pattern that might be related with an unstable occupancy pattern due to the gregarious behaviour, to the generally low densities of the species or to a combination of both effects. In this sense, it

DOI: 10.29094/FiSHMED.2022.001

would be desirable to confirm the extinction of *A. hispanica* from the northern part of the Portuguese Guadiana basin through techniques sensitive enough to detect rare fish, such as environmental DNA approaches (Evans & Lamberti, 2018; Shu, 2020).

The abundance of *A. hispanica* was in general low, since annual values higher than one individual per 100m² were only recorded in Chança and Foupana sub-basins, and only occasionally (Table 1). The temporal and spatial irregularity of *A. hispanica* presence and the very low densities detected may indicate a fragmentation of the populations and an overall very poor conservation status of the species (Fagan *et al.*, 2002).

Table 1. Number of individuals caught and average abundance of *A. hispanica* across the sub-basins surveyed during 2015, 2018 and 2021

	Sampled sites (*A. hispanica* present)	Captured individuals (N)			Abundance (N/100m²)		
	2015-2021	2015	2018	2021	2015	2018	2021
Xévora	6	0	-	-	0	-	-
Caia	6	0	-	-	0	-	-
Degebe	3	0	-	-	0	-	-
Ardila	6 (2)	11	10	9	0.37	0.55	0.64
Chança	4 (4)	36	13	55	**1.76**	0.43	**1.31**
Vascão	6 (3)	4	1	6	0.09	0.13	0.23
Carreiras	2	0	-	-	0	-	-
Foupana	4 (2)	17	1	14	0.56	0.07	**1.82**
Odeleite	4 (4)	4	2	4	0.07	0.13	0.19

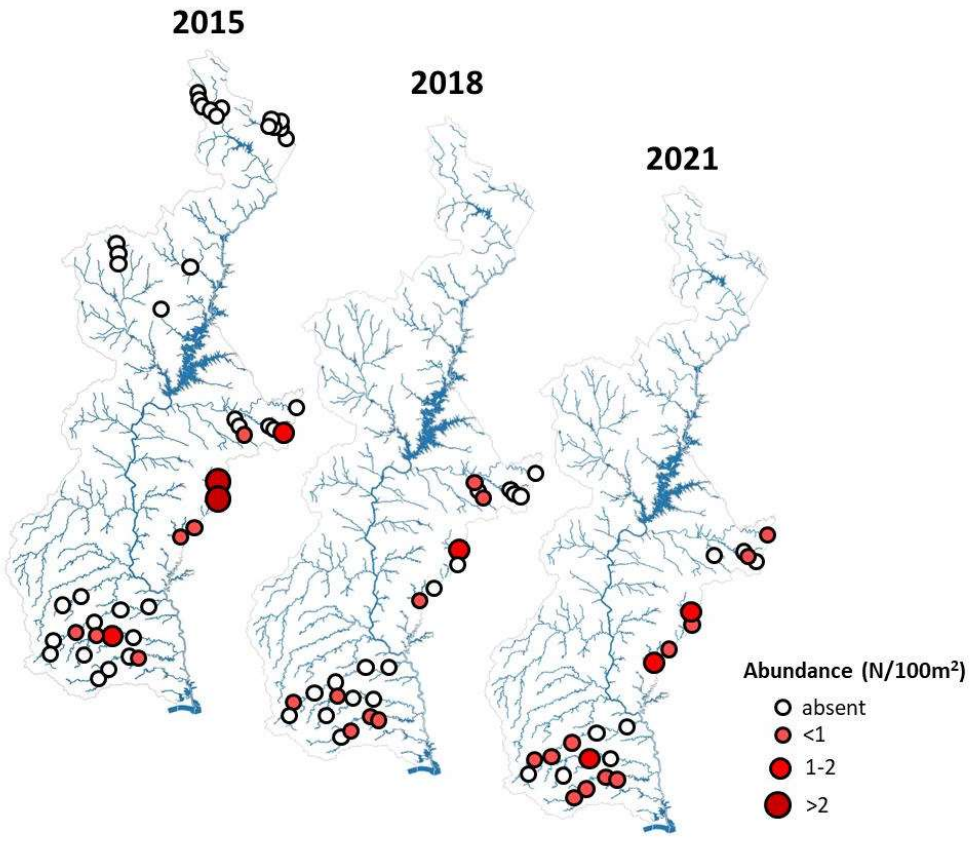

Figure 3. Abundance of *Anaecypris hispanica* (N / 100m²) in the sampling network in 2015, 2018 and 2021.

DOI: 10.29094/FiSHMED.2022.001

Table 2. Threats updated to 2021, for each sub-basin where the species occurred in the past and for the sub-basins where it still occurs: X – Important threat; XX – Predominant threat (sub-basins are listed from north to south).

		***Anaecypris hispanica* absent**						***Anaecypris hispanica* present**				
	THREATS	**Xévora**	**Caia**	**Lucefécit**	**Degebe**	**Álamo**	**Carreiras**	**Ardila**	**Chança**	**Vascão**	**Foupana**	**Odeleite**
Riparian habitats	Cattle grazing		X		XX	X		XX	X	X		
	Ploughing up to limit of stream bed				X							
	Expansion of cane, *Arundo donax*									X		
Water quality	Intensive agriculture	X	X	X	X	X						
	Cattle grazing				XX	X		X	X			
	Ineffective effluents treatment				X	X		X		X		
Hydro-morphology	Water abstraction	X			X				X	X	X	XX
	Flow regime disruption	XX										
	Fragmentation by weirs		X							X		X
Non-native species	> 10% of the captures are exotic species	XX	X	XX	XX	X	XX	XX	X	X		
	Proliferation from dams	XX		X				X	X	X		X

DOI: 10.29094/FiSHMED.2022.001

Threats

Comparing the information presented in Table 2, with the pressures identified by Collares-Pereira *et al.* (2000b), the main threats to the fish conservation in the Guadiana basin remain the same, after twenty years. Habitat loss or degradation caused by various anthropic actions, such as the construction of dams and weirs, pollution, water abstraction, and proliferation of exotic species continue to exist.

Based on data from APA (2014), anthropogenic pressures in the Guadiana basin clearly differ between the northern and southern parts of the basin. In the northern part of the basin - Xévora, Caia and Degebe - economic activities are much more intensive than in the south, including irrigated crops, intensive cattle rising. In the southern area - Ardila, Chança, Vascão, Foupana and Odeleite - agriculture and livestock are mostly extensive and situations of point source pollution are sparser.

Despite the extensive farming, during summer drought periods cattle concentrate along the streams. For example, cattle from different farms wade along 5 km of the Murtigão stream (Figure 4a) to find the only place with green vegetation during summer and autumn, associated with the pools that act as fish refugia. Grazing reduces water quality directly through organic inputs (Hubbard *et al.*, 2004) and indirectly by reducing or even eliminating the riverine vegetation, which is an important element of the *A. hispanica* habitat (Ribeiro *et al.*, 2000; Ilhéu K., 2017). Outside the dry summer season, aquatic and riparian vegetation can also provide shelter to different cyprinid species, particularly under flooding conditions (Baladrón *et al.*, 2021).

The proportion of exotic species was higher in the Ardila sub-basin and in the lower sections of Vascão and Chança. In spite of the proliferation of exotic fish in the Guadiana River, no new species was observed, the four species captured in the south being the same since Collares-Pereira (2000b): *Australoheros facetum*, *Carassius* sp., *Gambusia holbrooki*, and *Micropterus salmoides*.

Water abstractions exhaust all the water from the pools along several kilometres in sections of Vascão, Foupana and Odeleite. In particular in Odeleite, the water abstractions observed are for agricultural purposes and take place in several locations. The size of the agricultural plots is reduced, generally less than 1 ha, but abstraction can lead to the disappearance of all the pools within a radius of around 2 km (Figure 4b). In Foupana and Vascão, water abstractions are for livestock watering and hunting management. For instance, in Vascão a tank could be filled up with 5000 m^3 every week on the same pool, drastically decreasing the water level during the drought months.

The combined effects of exotic species and water abstraction will arguably have effects on fish assemblages and on *A. hispanica* in the near future (Clavero *et al.*, 2010), calling for urgent management action, as discussed below.

In addition, climate changes will arguably increase stressing conditions in aquatic ecosystems (Magalhães *et al.*, 2007; Filipe *et al.*, 2013), not only because of the more frequent drought events but also due to a higher demand of water, and grazing during the drought months. As droughts become longer and more intense, the probability of local extinctions will increase, as happened to the population of *A. hispanica* inhabiting the Caia River, which seemed to have disappeared after the 2004/2005 drought event (Rogado *et al.*, 2005; Cardoso & Carrapato, 2008).

Conservation measures

The four projects concerning *A. hispanica* conservation were designed to address specific problems in a particular pool or river section. The targets included improving riverine vegetation and recovering of riparian habitats, controlling access of cattle to pools, building alternative solutions to water abstraction during drought periods, removing obstacles, or introducing control devices for exotic species in big dams to prevent their proliferation from the dams located in the tributaries to the main course of sub-basins

DOI: 10.29094/FiSHMED.2022.001

where *A. hispanica* occurs (Figure 5). However, the spatial and temporal scope of these actions might be too reduced to have a meaningful positive impact in the overall conservation of Portuguese A. *hispanica* populations. For that reason, and as Collares-Pereira et al. (2000b) advanced in the first place, it is necessary that the next Guadiana Hydrographic Management Plan (GHMP), which is under development, incorporates several actions proposed under those conservation projects in order to expand the effect of those actions and broaden their positive outcomes, in the entire area of distribution of *A. hispanica*.

Increased efforts are required to integrate conservation and management actions across economic sectors and an integrated river basin management approach should be considered for the Guadiana Basin (Maiz et al., 2017; Nikolaos et al., 2017). Since 2000, there have been three GHMP, but they neither effectively integrated measures directly associated with the conservation of endangered freshwater fish, nor achieved the objectives regarding stream water quality indicators (APA, 2014). The future plan must comply with its goals and fulfil the requirements of the the European Habitats and Water Framework Directive.

On the other hand, water abstractions and livestock rising have a local but relevant socio-economic weight, so any measures to control it must be accompanied by alternative solutions and possible financial support through Rural Development Policies, linked with the Common Agricultural Policy (Nikolaos et al., 2017). However, previous conservation projects have failed in preventing damaging uses to *A. hispanica* populations, including water abstraction and cattle use of refuge pools. Even with available funding stakeholders were not willing to change their behaviour. For the conservation of the *A. hispanica*, these uses must be particularly avoided in those streams identified as critical for the conservation of the species in all subbasins where it is still present.

It is thus essential to continue monitoring *A. hispanica* populations, in order to detect its temporal and spatial trends and identify critical threats for their conservation.

Figure 4. Left: sampling site Murtigão stream without *A. hispanica*, where cattle wade along the stream. Right: one of several water abstraction sites in Odeleite, this particular one being just one kilometre downstream from a sampling site hosting *A. hispanica*. The water pump is installed on top of a small trailer that moves from one pool to another.

DOI: 10.29094/FiSHMED.2022.001

Figure 5. Examples of measures developed in the field to enhance the habitat of *A. hispanica*: a) Cane, *Arundo donax*, removed and riverside vegetation recovered along 500 m of Vascão river; b) a section of 10 km of Vascão river reconnected; c) ground water abstraction in order to prevent livestock watering in the pools of Alcaides (Chança tributary); d) Exotic species control device, designed by Aqualogus and co-financed by LPN & LIFE Saramugo, installed in a dam located on a tributary of Vascão river.

ACKNOWLEDGEMENTS

Data collection in 2015 and 2018 was co-funded by the project LIFE Saramugo (LIFE13 NAT/PT/000786). Field work was carried out by three teams: P. Pinheiro, J. Oliveira and F. Reis from Aqualogus; D. Sousa, P. Matono and P. Rodrigues from Évora University; a mixed team with H. Lousa and N. Silva from Liga para a Proteção da Natureza (LPN) and A. Martins, J. Santos, J. Pepe, and C. Carrapato from Instituto da Conservação da Natureza e das Florestas (ICNF). Data collection in 2021 was supported exclusively by ICNF. I would like to thank C. Carrapato, A. Martins, B. Machado, I. Santos, J. Santos, A. Trindade and A. Mota for their help during field work and sample collection. My thanks also to S. Marques for the final English revision.

CITED REFERENCES

APA - Agência Portuguesa do Ambiente (2014). Relatório de caracterização (Art.º 5º da DQA) – Região Hidrográfica (RH7). Plano de Gestão da Região Hidrográfica 2016/2021. Relatório disponível no âmbito da consulta pública.

Alves, M.J., H. Coelho, M.J. Collares-Pereira & M.M. Coelho (2001). Mitochondrial DNA variation in the highly endangered cyprinid fish *Anaecypris hispanica*: importance for conservation. Heredity 87: 463-473.

Baladrón, A., M.J. Costa, M.D. Bejarano, A. Pinheiro, I. Boavida (2021). Can vegetation provide shelter to cyprinid species under hydropeaking? Sci. Total Environ. 769: 145339.

Blanco-Garrido, F., Clavero, M., Prenda, J., 2009. Jarabugo (*Anaecypris hispanica*) and freshwater blenny (*Salaria fluviatilis*): habitat preferences and relationship with exotic fish species in the middle Guadiana basin. Limnetica 28: 139–148.

Cabral, M.J., Almeida, J., Almeida, P.R., Dellinger, T.R., Ferrand de Almeida, N, Oliveira, M.E., Palmeirim, J.M., Queiroz, A.I., Rogado, L., Santos-Reis, M. (2005). Livro Vermelho dos Vertebrados de Portugal. Instituto da Conservação da Natureza, Lisboa, 659pp.

Cardoso, A.C., Carrapato, C. (2008). Intervenção saramugo –2008. Relatório interno do Instituto da Conservação da Natureza e da Biodiversidade, Mértola.

Cardoso, A.C., Carrapato, C. (2010). Intervenção saramugo –2010. Relatório interno do Instituto da Conservação da Natureza e da Biodiversidade, Mértola.

Cardoso, A.C., Carrapato, C., Farinha, J.C., Rito, P., Silva, E. (2014). Plano de Ação do Saramugo – Action Plan for Conservation of the Saramugo (*Anaecypris hispanica*) – *2012-2016*. Editado por Instituto da Conservação da Natureza e das Florestas & SOMINCOR, Mértola, 79pp.

Cardoso, A.C. (2018a). How to deal with the increased demand for water at ponds? Livro de Resumos das apresentações orais no VII Iberian Congress of Icthyology. SIBIC 2018. Where Rivers Meet The Oceans, 12 – 15 de junho no CCMAR, Universidade do Algarve.

Cardoso, A.C. (2018b). An Action Plan and four projects for the conservation of *Anaecypris hispanica*. Abstract book of the Symposium for the conservation of freshwater fish and habitat rehabilitation. 27 to 29 September 2018. University of Évora.

Carrapato, C., Ribeiro, F. (2012). Larval development of the Iberian cyprinid *Anaecypris hispanica*. Limnetica 31: 119–128.

Cid, N., N. Bonada, S. M. Carlson, T. E. Grantham, A. Gasith, V. H. Resh (2017). High variability is a defining component of Mediterranean-Climate Rivers and their biota. Water 9:2-54

Clavero, M., V. Hermoso, N. Levin, S. Kark (2010). Geographical linkages between threats and imperilment in freshwater fish in the Mediterranean Basin. Diversity and Distributions 16:744-754.

Collares-Pereira, M.J., Almaça C. (1979). *Pseudophoxinus hispanicus* (Steind., 1866), Cyprinidae nouveau pour le Portugal. Bull. Mus. Natl. Hist. Nat. *A* 9951: 285–287.

Collares-Pereira, M.J., Cowx, I.G., Rodrigues, J.A., Rogado, L., da Costa, L.M. (1999). The status of *Anaecypris hispanica* in Portugal: problems of conserving a highly endangered Iberian species. Biol. Conserv. 88: 207–212.

Collares-Pereira, M.J., Rodrigues, J.A., Rogado, L., Cowx, I.G., (2000a). Plano de Gestão para a Conservação do Saramugo. Programa Life-Natureza – contrato B4–3200/97/280, CBA-FCUL, Lisboa.

Collares-Pereira, M.J., Cowx, I.G., Ribeiro, F., Rodrigues, J.A., Rogado, L. (2000b). Threats imposed by water development schemes on the conservation of endangered species in the Guadiana River, Portugal. Fish. Manage. Ecol. 7: 167– 178.

Cuttelod, A., García, N., Abdul Malak, D., Temple, H., Katariya, V. (2008). The Mediterranean: a biodiversity hotspot under threat. *In*: J.-C. Vié, C. Hilton-Taylor and S.N. Stuart (eds). The 2008 Review of The IUCN Red List of Threatened Species. IUCN Gland, Switzerland.

Da Silva, J., P. Matono, E. Barata, J.M. Bernardo, A. M. Costa, M. Ilhéu (2019). Behavioural interactions between the endangered native fish Saramugo, *Anaecypris hispanica*, and the invasive bleak, *Alburnus alburnus*. Limnetica 38: 517-533.

De Miguel, R., Pino, E., Ramiro, A., Aranda, F., Pena, J.P., Doadrio, I., Fernández-Delgado, C. (2010). On the occurrence of *Anaecypris hispanica*, an extremely endangered Iberian endemism, in the Guadalquivir River basin. J. Fish Biol. 76: 1454–1465.

Dekar, M.P., Magoulick, D.D. (2007). Factors affecting fish assemblage structure during seasonal stream drying. Ecol. Freshw. Fish 16: 335–342.

Doadrio, I. (2002). *Atlas y Libro Rojo de los Peces Continentales de España*. Dirección General de Conservación de la Naturaleza. Museo Nacional de Ciencias Naturales, Madrid.

Dodds, W.K., E. Gaiser, G.J. Hansen, J.M. Smith (2012). Suprises and Insights from Long-Term Aquatic Data Sets and Experiments. BioScience 62: 709-721.

EDIA (2008). Monitorização da Ictiofauna na Área do Bloco de Rega do Monte Novo: 2006-2008. Relatório final, Universidade de Évora.

EDIA (2009).Monitorização da Qualidade da Água e do Estado Ecológico da Rede Primária do EFMA. Relatório final, Universidade de Évora.

Evans, N. T., & Lamberti, G. A. (2018). Freshwater fisheries assessment using environmental DNA: A primer on the method, its potential, and shortcomings as a conservation tool. Fish. Res. 197: 60– 66.

Filipe, A.F., J.E. Lawrence, N. Bonada (2013). Vulnerability of stream biota to climate change in mediterranean climate regions: a synthesis of ecological responses and conservation challenges. Hydrobiologia 719, 331–351.

Fagan, W.F., Unmack, P.J., Burgess, C., Minckley, W.L. (2002). Rarity, fragmentation, and extinction risk in desert fishes. Ecology 83, 3250–3256.

Fragoso, S., Lousa, H., Silva, N., Alcazar, R., Cardoso, A., Carrapato, C., Ilhéu, M., Matono, P., Bernardo, J., Costa, A.M., Almeida, J., Pinheiro, P., Oliveira B. (2018). Concrete Conservation Actions for *Anaecypris hispanica* in the Guadiana Basin – LIFE Saramugo Project. Livro de Resumos das apresentações orais no VII Iberian Congress of Icthyology. SIBIC 2018. Where Rivers Meet The Oceans, 12 – 15 de junho no CCMAR, Universidade do Algarve

Godinho, F.N., Pinheiro, P.J., Oliveira, J.M., Azedo, R. (2014). Responses of intermittent stream fish assemblages to irrigation development. River Res. Appl. 30: 1248-1256.

Hubbard, R.K., G.L. Newton, G.M. Hill (2004). Water quality and the Grazing Animal. Publications
from USDA-ARS / UNL Faculty. 27.

Ilhéu, M., P. Matono, J.M. Bernardo, A.M. Costa, J. Da Silva, D. Sousa, P. Rodrigues, A.C. Cardoso, C. Carrapato, P. Pinheiro, J. Almeida, H. Lousa, N. Silva, R. Silva, R. Alcazar (2017). Patterns of habitat use of the endangered fish species Saramugo, *Anaecypris hispanica*, and the invasive Bleak, *Alburnus alburnus*: implications for native fish fauna conservation. Proceedings of the 10th Symposium for European Freshwater Sciences, 2-7 July, Olomuc, Czech Republic.

INAG (2008). Manual para a avaliação biológica da qualidade da água em sistemas fluviais segundo a Diretiva Quadro da Água: Protocolo de amostragem e análise para a fauna piscícola. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional, Lisboa. 15pp.

INAG, I.P. & AFN (2012). Desenvolvimento de um Índice de Qualidade para a Fauna Piscícola. Ministério da Agricultura, Mar, Ambiente e Ordenamento do Território.

IUCN (2021). The IUCN Red List of Threatened Species. Version 2021-3. https://www.iucnredlist.org. Accessed on 13/01/2022.

Levy, A., I. Doadrio, V. Almada (2009). Historical biogeography of European leuciscins (Cyprinidae): evaluating the Lago Mare dispersal hypothesis. J. Biogeog. 36: 55-65.

Magalhães, M.F., P. Beja, I.J. Schlosser, M.J. Collares-Pereira (2007). Effects of multi-year droughts on the fish assemblages of seasonally drying Mediterranean streams. Freshw. Biol. 52: 1494–1510.

Magoulick, D.D., Kobza, R.M. (2003). The role of refugee for fishes during drought: a review and synthesis. Freshw. Biol. 48: 1186–1198.

Maiz-Tome, L., W. Darwall, C. Numa, V. Barrios and K.G. Smith (2017). Freshwater Key Biodiversity Areas in the north-western Mediterranean sub-region. IUCN, Gland, Switzerland, Cambridge, UK and Malaga, Spain: viii + 48pp

Matthews, W.J., Marsh-Matthews, E., 2003. Effects of drought on fish across axes of space, time and ecological complexity. Freshw. Biol. 48: 1232–1253.

Matono, P., Bernardo, J.M., Ferreira, M.T., Formigo, N., Almeida, P.R., Cortes, R., Ilhéu, M. (2012). Fish-based groups for ecological assessment in rivers: the importance of environmental drivers on taxonomic and functional traits of fish assemblages. Knowledge and Management of Aquatic Ecosystems 405: 04

Matono, P., Bernardo, J.M., Costa, A.M., Ilhéu, M. (2014). Fish response to anthropogenic pressures in temporary streams: the

importance of environmental drivers. River Res. Appl. 30: 1281-1295

Muñoz, C. (2017). Patrones de uso del hábitat para la espécie em peligro crítico saramugo (*Anaecypris hispanica*) y la espécie invasiva alburno (*Alburnus alburnos*): interacciones negativas potenciales. Mestrado em Biologia da Conservação da Universidade de Évora, Portugal.

Nikolaos, T.S., S. Sabater, T. Datry, M.M. Norais, A. Buffagni, G. Dörflinger, S. Zogaris, M.M. Sánchez-Montoya, N. Bonada, E. Kalogianni, J. Rosado, L. Vardakas, A. M. De Girolamo, K. Tockner (2017). Non-perennial Mediterranean rivers in Europe: Status, pressures, and challenges for research and management. Sci. Total Environ. 577: 1–18.

Ribeiro, F., Cowx, I.G., Collares-Pereira, M.J. (2000). Life history traits of the endangered Iberian cyprinid *Anaecypris hispanica* and their implications for conservation. Archiv Hydrobiologie 149 (4): 569-586.

Rogado, L., Cardoso, A.C., Carrapato, C. (2005). Resumo dos trabalhos efectuados pelo Instituto da Conservação da Natureza (ICN), para a monitorização da ictiofauna na Bacia do Guadiana para avaliação e minimização dos efeitos da seca, de 15 de Junho a 30 de Setembro de 2005. Relatório interno do Instituto da Conservação da Natureza.

Salgueiro, P., Carvalho, G., Collares-Pereira, M.J., Coelho, M.M. (2003). Microsatellite analysis of genetic population structure of the endangered cyprinid *Anaecypris hispanica* in Portugal: implications for conservation. Biol. Conserv. 109, 47–56.

Shu, L., A. Ludwig, Z. Peng (2020). Standards for methods utilizing environmental DNA for detection of fish species. Genes, 11, 296.

Smith, Kevin G. and Darwall, William, R.T. (Compilers), (2006). The Status and Distribution of Freshwater Fish Endemic to the Mediterranean Basin. IUCN, Gland, Switzerland and Cambridge, UK. v + 34 pp.

SNPRCN (Ed.), (1991). Livro Vermelho dos Vertebrados - Vol. 2, Peixes Dulçaquícolas e Migradores. Secretaria de Estado dos Recursos Naturais, Lisboa, 55.

Sousa-Santos, C., Robalo, J.I., Francisco, S.M., Carrapato, C., Cardoso, A.C., & Doadrio, I. (2014). Metapopulations in temporary streams - the role of drought-flood cycles in promoting high genetic diversity in a critically endangered freshwater fish and its consequences for the future. Molecular Phylogenetics and Evolution, 80, 281-96.

Sousa-Santos, C., P. Matono, J. Da Silva, M. Ilhéu (2018). Evaluation of potential hybridization between native fishes and the invasive bleak, *Alburnus alburnos* (Actinopterygii: Cypriniformes: Cyprinidae). Acta Ichthyologica et Piscatoria; Szczecin Vol. 48, Ediç. 2: 109-122.