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 out 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

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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 endangered 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
Anaecypris hispanica distribution, status and threats

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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.
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 100m².

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.
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
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

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

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

<table>
<thead>
<tr>
<th>THREATS</th>
<th>Anaecypris hispanica absent</th>
<th>Anaecypris hispanica present</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Riparian habitats</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle grazing</td>
<td>X</td>
<td>XX X</td>
</tr>
<tr>
<td>Ploughing up to limit of stream bed</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Expansion of cane, Arundo donax</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td><strong>Water quality</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensive agriculture</td>
<td>X X X X X X</td>
<td></td>
</tr>
<tr>
<td>Cattle grazing</td>
<td>XX X</td>
<td>X X</td>
</tr>
<tr>
<td>Ineffective effluents treatment</td>
<td>X X</td>
<td>X X</td>
</tr>
<tr>
<td><strong>Hydro-morphology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water abstraction</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Flow regime disruption</td>
<td>XX</td>
<td></td>
</tr>
<tr>
<td>Fragmentation by weirs</td>
<td>X</td>
<td>X X</td>
</tr>
<tr>
<td><strong>Non-native species</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 10% of the captures are exotic species</td>
<td>XX X XX XX X XX X XX X XX X X</td>
<td></td>
</tr>
<tr>
<td>Proliferation from dams</td>
<td>XX X</td>
<td></td>
</tr>
</tbody>
</table>
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.
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 sub-basins 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 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.

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