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Distribution and demography of the critically endangered Lisbon arched-mouth nase, *Iberochondrostoma olisiponense*

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SUMMARY

The Lisbon arched-mouth nase, *Iberochondrostoma olisiponense*, is a cyprinid fish endemic to the lowlands of the Tagus drainage (Portugal, Iberian Peninsula). Originally described as *Chondrostoma olisiponensis* about one decade ago, the species lacks national conservation status despite its classification as critically endangered (CR) by the IUCN Red List of Threatened Species. Threat level was based on evidence of decreasing population size and a contraction of the species' distribution area. In an effort to provide a robust assessment of the current distribution of *I. olisiponense*, we performed a comprehensive survey of 19 tributaries in the lower Tagus and its main channel between late 2015 and late 2016, totaling 87 unique sites. Species presence was confirmed only at two of the tributaries with previous records (Muge and Trancão), and two new population nuclei were detected at Cabanas and at the Tagus main stem. Evidence of successful recruitment was found at Muge and Trancão only. On the other hand, extensive sampling at tributaries with previous species' occurrence (Rio Maior, Magos and Ulme) proved unsuccessful in detecting *I. olisiponense*, suggesting that the species has been extirpated there.

These results extend the species' previously known distribution area but also suggest contemporary local extinctions. Further research and conservation efforts are thus urgently needed to monitor the extant populations and unveil the species' habitat requirements, biology, and genetic parameters of diversity and structure.

Keywords: Boga-de-boca-arqueada de Lisboa, conservation, distribution, demography, lowland, Portugal, *Chondrostoma olisiponensis*

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INTRODUCTION

Monitoring the presence and status of persisting populations of highly endangered species with fragmented distributions is essential to direct and adjust conservation actions. Such efforts are particularly important for critically endangered freshwater fish occurring in areas with marked seasonality, where ongoing climate change may pose increased challenges due to reduced water and habitat availability and quality. This is the case of Mediterranean-type streams in the Iberian Peninsula, which are also home to a large proportion of endemic species within a biodiversity hotspot (Smith & Darwall 2006; de Figueroa *et al*. 2013).

The Lisbon arched-mouth nase, *Iberochondrostoma olisiponense* (Gante, Santos & Alves 2007), hereon referred to as the Lisbon nase, is a recently described small cyprinid fish (< 18 cm total length; Figure 1) endemic to the lower Tagus drainage [Rio Tejo (Pt)] in Portugal. This species was detected in a few small tributaries in the late 1970s (Figure 2), but currently it is reported in three tributaries covering a total area of ca. 10 km² (Gante *et al*. 2007, 2012). Each tributary harbors small, localized population nuclei apparently isolated from each other. Indeed, preliminary genetic analyses revealed strong differentiation among tributaries indicative of low connectivity between populations, as well as low levels of molecular diversity and high inbreeding, suggestive of small effective population sizes (Sousa-Santos *et al*. 2014).

Worrying signs of decreasing population trends in the Lisbon nase came from surveys done in 2006, 2008 and 2011 that detected this species at only three small tributaries (Rio Maior, Ribeira de Muge and Rio Trancão), i.e., a smaller current distribution than its known area of occurrence based on museum records (Gante *et al*. 2007). These surveys failed to detect the species in previous occurrence sites (e.g. Ribeira de Ulme), while evidence of extensive hybridization with the congeneric *Iberochondrostoma lusitanicum* was found at Rio Maior and, to a much lesser extent, at Rio Trancão (Gante *et al*. 2010; Sousa-Santos *et al*. 2013, 2014, 2016). Low local abundances of the Lisbon nase can compromise persistence of this species by increasing the likelihood of hybridization with *I. lusitanicum*, which is often more abundant in areas of co-occurrence (Sousa-Santos *et al*. 2014). Based on the restricted and fragmented distribution, and on the evidence of decreasing population trends, the Lisbon nase was classified as Critically Endangered by the International Union for Conservation of Nature (Gante *et al*. 2012).

Despite the current scenario of range contraction in the Lisbon nase, no comprehensive assessment of its distribution has been conducted to date. It is assumed that the original distribution area of the species was likely more continuous throughout the lower Tagus than at present, when consider-

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ing all species' records to date including recent, as well as museum and historical records (Gante *et al*. 2007, 2010, Figure 2). On the other hand, detection may have been hindered by species misidentification in previous fieldwork conducted in the area (see Oliveira 2005). To improve our knowledge on the current distribution range of this rare species, we conducted the first comprehensive field survey in the lower Tagus drainage targeting specifically the Lisbon nase (Veríssimo *et al*. 2016). Here we provide the most updated and exhaustive information on distribution, abundance and demographics of the persisting Lisbon nase populations, aiming at improving and informing urgently needed conservation actions.

Figure 1. Female (upper photo, approx. 9 cm FL) and male (lower photo, approx. 7 cm FL) Lisbon nases (*Iberochondrostoma olisiponense*) collected in Rio Trancão. Note the sexual dimorphism in fin length, with males displaying longer fins.

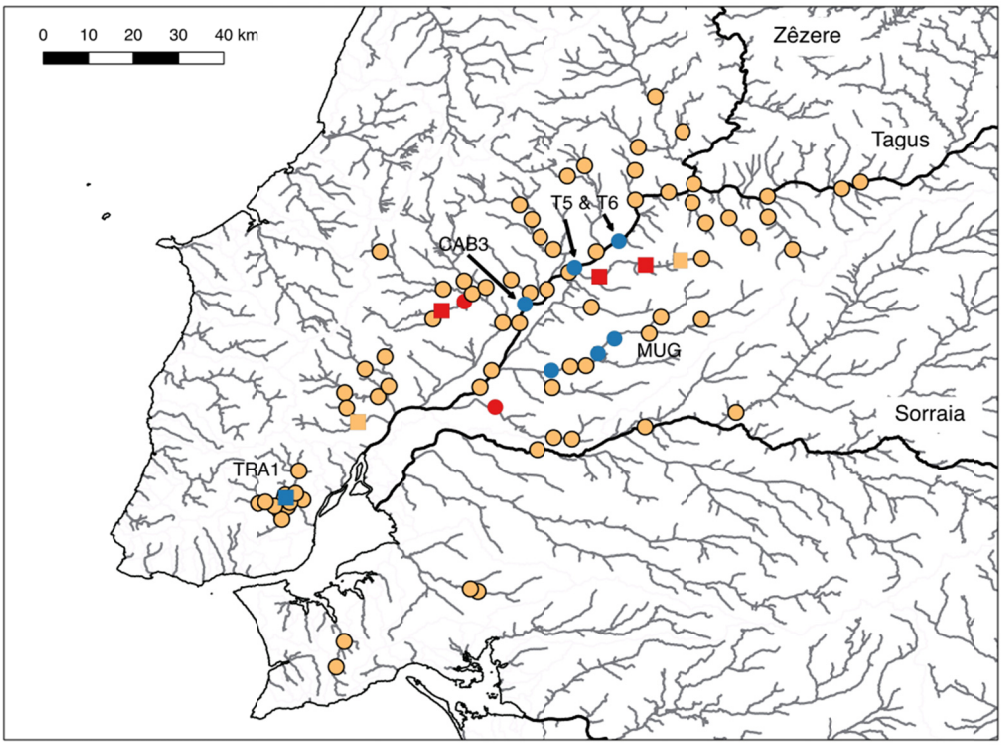

Figure 2. Location of the sampling sites and occurrence of the Lisbon nase within the lower Tagus drainage. Sites sampled once in 2016 are marked with circles, and sites sampled in both years (2015 & 2016) are marked with squares. Sites with confirmed species presence are filled in blue, those with previous detections are filled in red, and those with no detections are filled in orange.

METHODS

A total of 87 sites were sampled across 19 tributaries and main stem of the lower Tagus drainage (Figure 2). The number of sampled sites per tributary ranged from 2 to 14 and was set in relation to its length, considering either one sampling site per 10 km or a minimum of 2 sites per tributary. The exception to these criteria was the Trancão tributary, where 14 sites were surveyed within its 30 km extension, aiming at obtaining more fine-scale detail on the distribution of the longest known Lisbon nase population (C. D. Santos, personal observation). All sites were sampled between April and October of 2016, of which 11 sites were sampled also in the previous year between late July and early November in 2015. Sites with temporal replicates represent four tributaries with previous positive detections of the Lisbon nase, namely Trancão (TRA1), Maior (RMA1-4), Muge (MUG1-3) and Ulme (ULM1-3) (Gante *et al*. 2007, 2010; Sousa-Santos *et al*. 2014), and allowed assessments of the temporal persistence of these population nuclei.

At each site, the fish community was surveyed using electrofishing (300 –500 V, 2–3 A, DC) for about 30 minutes, and covering a length of 365±195 m (average ± SD), including all available habitats (e.g. riffle, run and pool areas). All sampled fish were identified *in situ* to species (or genus in the case of *Lampetra*, *Liza*, *Carassius*, *Pomatoschistus*), and up to 30 Lisbon nase specimens per site were measured to the nearest mm (fork length; FL). Age was estimated based on the length distribution

of the modal size classes, following previous age studies on Iberian cyprinids (Ribeiro *et al.* 2000, 2003). Specifically, fish smaller than 40 mm were considered as 0+, while fish ranging from 40-60 mm were considered as 1+, and longer than 60 mm, > 1+. Native fish were returned alive to the water, or preserved in 10% formalin and deposited in the ichthyological collection Museu Bocage – Museum of Natural History and Science (MUHNAC) in the rare event fish died during sampling. Non-native fishes were given a lethal dose of anesthetic in accordance with Portuguese legislation.

RESULTS

Distribution area

The Lisbon nase was found at five sites within three different tributaries: Trancão (TRA1), Muge (MUG2, MUG5 and MUG7) and Cabanas (CAB3), and at two sites in the main stem of the Tagus River (T5 and T6) (Figure 2). The species was detected for the first time in Cabanas and Tagus main stem. The number of Lisbon nase caught per site varied between 1 and 341 individuals (both at TRA1), totalling 504 specimens (Table 1). Conversely, the species was not detected in tributaries with previously reported populations, namely Rio Maior (RMA1-9), Magos (MAG1-3) and Ulme (ULM1-5), despite the high sampling effort including temporal replicates in Ulme and Rio Maior (Figure 2).

Within tributaries, the Lisbon nase was detected only at a single site in Trancão and in Cabanas, despite 14 and 3 sites being surveyed, respectively. In contrast, the species was detected at three of the nine sites surveyed in Muge, along nearly 20 km of this tributary. Likewise, the Lisbon nase was detected in two adjacent sites in the Tagus main stem out of the ten surveyed, both of which were less than 20 km from Cabanas and Muge outflows (Figure 2). Abundance of the Lisbon nase at Trancão displayed marked variation between years, although the area surveyed in 2015 was ~20% of the one surveyed in 2016 (Table 1). At Muge, the abundance of Lisbon nase and the area surveyed were more consistent between years, especially when comparing MUG2 (2015) and MUG7 (2016), although the lower abundance values at MUG5 in 2016 suggest a patchy distribution along the tributary (Table 1).

Population size-structure

The length frequency distribution of the Lisbon nase showed evidence of recent recruitment in Trancão and Muge, given the presence of young-of-the-year (< 40 mm; Figure 3), in contrast to Cabanas and the Tagus main stem. Muge and Cabanas exhibited the widest length range of all populations detected, while Trancão was dominated by small fish (<60 mm, probably ages 0+ to 1+) and Tagus by larger fish (>60 mm, probably ages > 1+, Figure 3).

Community composition

The relative contribution of Lisbon nase to the local fish community at sites with positive detection was highest at Trancão (TRA1: 33% in 2016) and Cabanas (CAB3: 21%), and lowest at the Tagus main stem sites (T5: 2%; Table 1). The co-occurring fish community included a total of fourteen native species (Table 1) but varied widely among sites (from 3 to 8, average: 5.8). The number of native species was highest at Muge (n = 7-8) and Tagus (7), and was lowest at Trancão and Cabanas (4). *Anguilla anguilla*, *Pseudochondrostoma polylepis*, *Luciobarbus bocagei* and *Cobitis paludica* were the most commonly found native species in association with the Lisbon nase. In contrast, the native *I. lusitanicum*, known to hybridize with the Lisbon nase, co-occurred at only two sites in Muge in low numbers (n = 1-2 individuals). A total of eight co-occurring non-native fish were detected, with most (n = 6-7) being present at Muge, Cabanas and Tagus, while only two were found in Trancão. Among the non-native species, *Carassius* spp. and *Gambusia holbrooki* were generally found at sites where the Lisbon nase was detected (Table 1).

Table 1. Species composition, date of collection and sampled area at sites with detection of the Lisbon nase (*Iberochondrostoma olisiponense*) in the lower Tagus catchment.

	Muge			Trancão		Cabanas	Tejo main stem		
Site	MUG2	MUG5	MUG7	TRA1		CAB3	T5	T6	**TOTAL**
Date	Nov 2015	Jul 2016		Jul 2015	Jul 2016	Aug 2016	Oct 2016		
Area surveyed (m²)	400	500	450	400	2150	280	1000	600	5780
Lisbon nase, *Iberochondrostoma olisiponense*									
Number of fish	30	2	21	1	341	88	11	10	504
Density (fish per 100 m²)	7.5	0.4	4.7	0.25	15.9	31.4	1.1	1.6	
Native species									
Pseudochondrostoma polylepis	78	5	38	0	0	0	1	0	122
Anguilla anguilla	4	15	1	2	3	9	10	2	46
Luciobarbus bocagei	6	12	2	0	0	8	74	44	146
Cobitis paludica	0	0	6	5	10	0	199	23	243
Squalius alburnoides	8	1	13	0	0	0	0	0	22
Lampetra sp.	6	8	0	0	0	0	0	0	14
Squalius pyrenaicus	4	2	5	0	0	0	0	0	11
Liza sp.	0	0	0	0	0	3	0	0	3
Iberochondrostoma lusitanicum	1	0	2	0	0	0	0	0	3

Table 1. Continued.

	Muge			Trancão		Cabanas	Tejo main stem		
Station	MUG2	MUG5	MUG7	TRA1		CAB3	T5	T6	**TOTAL**
Gasterosteus aculeatus	0	0	0	0	1	0	0	0	1
Petromyzon marinus	0	0	0	0	0	0	6	0	6
Atherina boyeri	0	0	0	0	0	0	1	0	1
Platichthys flesus	0	0	0	0	0	0	3	0	3
Non-native species									
Carassius sp.	0	2	0	160	458	63	65	0	748
Gambusia holbrooki	35	15	1	1500	206	114	17	37	1925
Gobio lozanoi	34	135	53	0	0	82	6	2	312
Alburnus alburnus	21	42	12	0	0	41	0	0	116
Cyprinus carpio	0	17	0	0	0	3	1	6	27
Lepomis gibbosus	2	5	0	0	0	4	104	0	115
Micropterus salmoides	0	0	0	0	0	0	24	0	24
Sander lucioperca	0	0	0	0	0	0	4	0	4
No. native species	**8**	**7**	**8**	**3**	**4**	**4**	**8**	**4**	**14**
No. of non-native species	**4**	**6**	**3**	**2**	**2**	**6**	**7**	**3**	**8**
Total fish sampled per site	**229**	**261**	**154**	**1668**	**1019**	**415**	**526**	**124**	**4396**
Total species richness	**12**	**13**	**11**	**5**	**6**	**10**	**15**	**7**	**22**

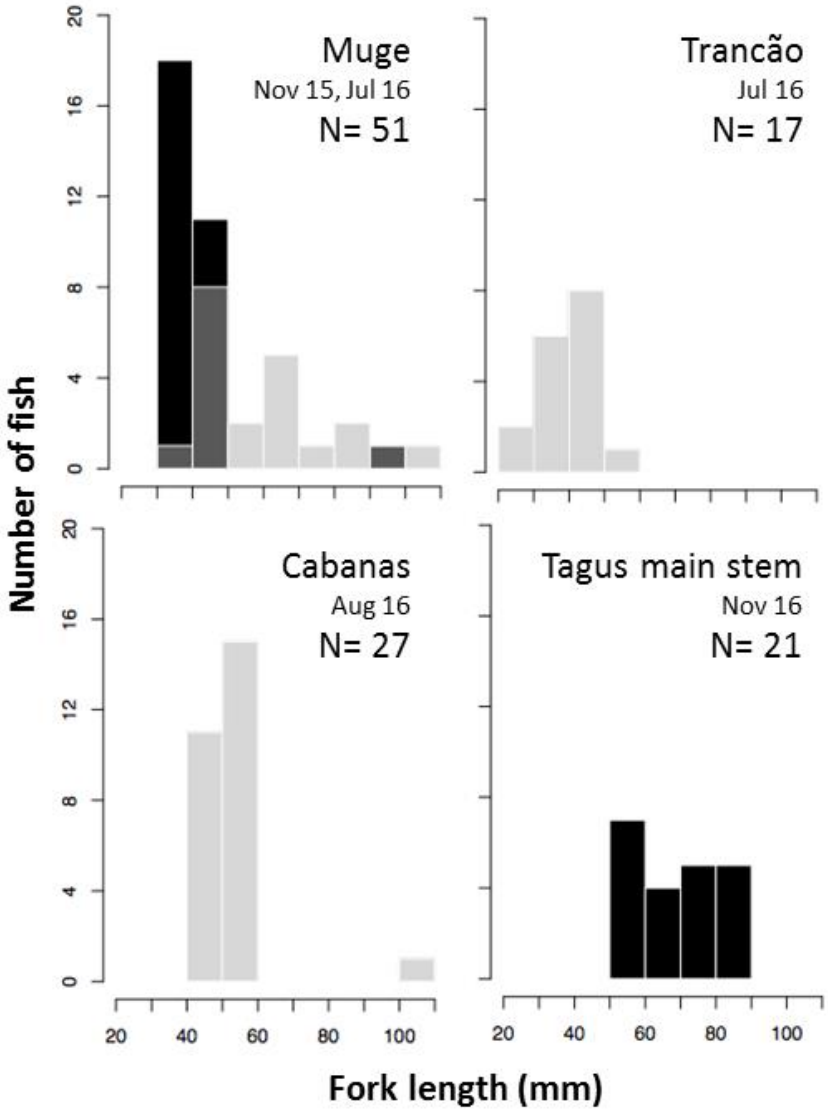

Figure 3. Fork length-frequency distributions at the three tributaries (Trancão, Muge, Cabanas) and Tagus main stem, where the Lisbon nase was detected. Light grey bars indicate data from summer (July and August), black bars indicate data from fall (November), dark grey bars indicate overlapping data from both seasons (in Muge).

DISCUSSION

Updated species distribution

The results shown here provide an updated perspective of the distribution of the Lisbon nase, with population nuclei detected in Cabanas, Muge and Trancão in addition to the Tagus main stem. The population recently known from Rio Maior was not detected in our survey, and those from Magos and Ulme have not been detected in any recent field surveys and are solely known from specimens sampled in 1979-1980 and deposited in museum collections (Gante *et al.* 2007; this work). The current work thus confirms the concerning signals of loss or reduction of population nuclei in

just a few decades (Gante *et al*. 2007, 2012; Figure 2).

Failure to detect the Lisbon nase in Rio Maior is most concerning given the high sampling effort implemented there (i.e. 9 sites). Previous records of the species in this catchment refer to Ribeira de Almoster (Gante *et al*. 2007, 2010; Sousa-Santos *et al*. 2014), which was sampled in two consecutive years in our study (2015 and 2016) in both up- and downstream reaches. Previous studies found low genetic diversity in the Rio Maior population compared to Muge and Trancão, as well as high levels of hybridization and introgression from *I. lusitanicum* (Sousa-Santos *et al*. 2014), suggesting lower adaptive potential and possible low density in this tributary. In addition, Rio Maior has been affected by recurrent pollution events, either from local pig farms or deficient water treatment plants (as observed during fieldwork), which may ultimately lead to local extirpation of fish communities. Taken together, the data collected thus far indicate that this population may be near extinction or already extinct. However, it is possible that the species occurs at very low densities and is patchily distributed in this area, thus precluding detection using electrofishing.

The detection of the Lisbon nase in Cabanas and in the Tagus main stem is very positive as it expands its previously known distribution. While the occurrence of the Lisbon nase in Cabanas is not completely unexpected, its occurrence in the Tagus main stem is particularly striking as the species was thought to occur only in peripheral flooded habitats (Gante *et al*. 2007). In spite of the terminal main stem being the highest order stretch of the Tagus, the Lisbon nase was collected in shallow, low current, side channels with abundant riparian vegetation and not in fast flowing, main channel areas (Figure 4), in agreement with a hypothetical preference for more lentic habitats (Gante *et al*. 2007). Overall capture data suggests that the Lisbon nase is restricted to lowland riverine habitats, with sandy/silty substrate, and high prevalence of aquatic and riparian vegetation, including seasonally flooded areas (Figure 4).

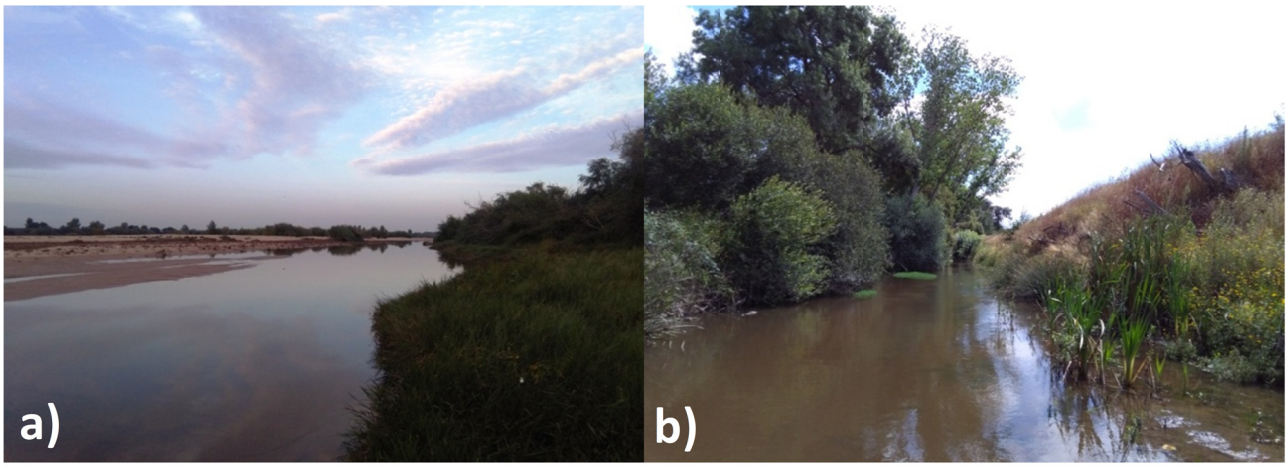

Figure 4. Freshwater habitats with Lisbon nase occurrence: a) side channel of Tagus main stem, and b) Muge tributary

Population demographics

Among all presently detected populations of the Lisbon nase, those from Trancão and Muge appear to be the most temporally stable, possibly reflecting larger population sizes sustained through time. Despite this, the two tributaries have remarkably different habitat stability and availability, which may have contributed to the observed patterns of abundance and age structure. The Trancão population is spatially restricted to a small wetland (~13 ha), subject to marked seasonal fluctuations in water level and habitat availability (Figure 5). The area is flooded in almost all its extension until early summer, but the available habitat becomes reduced to a few (about 10) man-made wells in late summer-early fall. Consequently, the maintenance of these wells and adequate water levels are essential for species survival, particularly given the high pressure imposed by local water extraction for agriculture during the dry season. The increasing frequency and severity of observed and projected heat waves and dry spells in the Iberian Peninsula (e.g. Jerez *et al.* 2013; Soares *et al.* 2016; Fonseca *et al.* 2016), may lead to a progressive decrease in water and habitat availability and quality. Consequently, the Trancão population may become dependent on the few surviving individuals retained in the wells, which are the source of new recruits in the following breeding season(s).

In Ribeira de Muge, the Lisbon nase occupies a larger area (Figure 2), extending for at least ~20 km of river length. Water levels here are less variable between seasons and likely sustain more stable habitats throughout the year and among years. The wider size range and more consistent temporal abundances observed at Muge support the existence of a relatively stable population with a more balanced demographic structure (Figure 3). Indirect support for this hypothesis is provided by the higher genetic diversity levels found in Muge compared to Trancão and Rio Maior (Sousa-Santos *et al.* 2014). Temporal stability and connectedness of habitats in Muge are further supported by the co-occurrence of the Lisbon nase with larger-sized potamodromous *Luciobarbus bocagei* and *Pseudochondrostoma polylepis*, and with the native *Squalius pyrenaicus* (Table 1, Oliveira *et al.* 2007).

The lack of recruits in Cabanas and Tagus in our survey raises concerns over the long-term persistence of the Lisbon nase at these sites. However, the reasons behind the absence of recruits may differ among sites and have differing conservation implications. Specifically, the area of species' occurrence at Cabanas is limited to ~600 m^2 of riverbed immediately adjacent to the Tagus main stem. In this short river section there is direct discharge of untreated urban effluents, which tend to accumulate given the low to absent water flow (A. Veríssimo, F. Ribeiro, personal observation). These conditions are particularly severe in the dry season and may limit successful recruitment in this population (Figure 3). Alternatively, Cabanas may be only temporarily inhabited by the Lisbon nase whereby individuals may move in and out of this section and stay only for some time without reproducing. The lack of young-of-the-year in the Tagus main stem may be due to recruitment probably occurring in adjacent flooded areas of low current. Further sampling effort is needed throughout the year to test alternative hypotheses.

Community composition

The Lisbon nase often co-occurs with the native *Cobitis paludica* and the introduced *Carassius* sp. and *Gambusia holbrooki*, which are generally found in habitats with reduced water current. Conversely, the close-relative *I. lusitanicum* was not frequently found to co-occur with the Lisbon nase, a pattern that suggests different ecological preferences between these species, which are known to hybridize (Gante *et al.* 2010; Sousa-Santos *et al.* 2014). In fact, *I. lusitanicum* occurs in low order and small streams, being restricted to the upper reaches of the main tributaries (Oliveira 2005; Robalo *et al.* 2009). The two most common non-native species co-occurring with the Lisbon nase, *Carassius* sp. and

Gambusia holbrooki, may compete for food, space or oxygen with the threatened native species, particularly in receding lotic environments (Caiola & De Sostoa 2005; Fagernes *et al*. 2017). However, the ecological impacts of non-native fish on the Lisbon nase have not been specifically examined. Directed studies should be conducted on this topic, particularly because the Lower Tagus river has been recently invaded by highly predatory fish such as *Sander lucioperca* and *Silurus glanis* (Ribeiro *et al*. 2009; Gago *et al*. 2016).

Figure 5. Water wells in Paul das Caniceiras (Trancão drainage) during wet (Top: January of 2016) and dry seasons (Low: October of 2016). The wells function as summer refugia for the Lisbon nase after the waters recede from the floodplains. Please note white arrow indicating the tree base line for comparison between photos.

Conservation recommendations

Effective habitat recovery and legal protection of the Lisbon nase by governmental agencies and local communities are urgently needed. Improvements in habitat and water quality and availability for this species should be prioritized, including control of pollution and water extraction during dry summer periods. These measures are particularly urgent at Cabanas and Trancão, but also at the Tagus main stem, which has been subject to increased pressure on water quality and quantity. In severe drought years, survival could be improved by collecting fish prior to dry season bottlenecks, maintained safely in captivity and released back to their original habitat in the autumn, after the return of prosperous conditions such as increased water/habitat availability. Specific measures to control or eradicate non-native species should be implemented to minimize their potential impact, particularly at geographically isolated populations (e.g. Trancão). Population monitoring and more detailed research on connectivity, abundance and genetic diversity of persisting population nuclei are also essential to inform adequate conservation actions. Creation of natural local preserves (e.g. sanctuaries) in areas where the Lisbon nase is known to persist could have a positive impact on the short-term survival of the species, in particular if these areas could be buffered against recurrent effects of climate change, pollution and non-native species introduction and spread. These measures would also have positive impacts on the long-term survival of other native species.

AUTHORS CONTRIBUTIONS

This work was planned and executed by AV, FR, HFG and CDS, with significant contributions from GC, JMO and RC.

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