Limnetica, 37 (1): 105-115(2018). DOI: 10.23818/limn.37.09

Growth and age structure in captive and wild stocks of the endangered western ruivaco *Achondrostoma occidentale* (Cyprinidae)

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Received: 11/04/17 Accepted: 14/11/17

ABSTRACT

Growth and age structure in captive and wild stocks of the endangered western ruivaco *Achondrostoma occidentale* (Cyprinidae)

Declines in freshwater fish populations are occurring at a fast rate, increasing the importance of ex-situ conservation programs supported by sound knowledge of population dynamics and life-history traits of the target species. We analysed the growth and age structure of wild and captive stocks of the western ruivaco *Achondrostoma occidentale*, a Portuguese endangered cyprinid, targeted for captive breeding and restocking since 2007. Specifically, we compared maximum size, longevity, and length-at-age among captive and wild populations, restocked and non-restocked. We found considerable variation in length-at-age and longevity between captive-bred and wild fish, with the former generally growing faster and living longer. Analysis of length-age distributions among wild populations suggested a positive effect of restocking actions in fish abundance. Results highlight the value of captive breeding for the conservation of endangered cyprinids, and reinforce the need for detailed data on life-history trait variation between captive and wild stocks to assess the efficiency of ex-situ conservation programs.

Key words: captive breeding, endangered cyprinids, Mediterranean rivers, recruitment, life-history traits

RESUMO

Crescimento e estrutura etária de stocks selvagens e de cativeiro de um ciprinídeo ameaçado, o ruivaco-do-Oeste* Achondrostoma occidentale *(Cyprinidae)

A ictiofauna presente em sistemas de água doce encontra-se em declínio acentuado sendo, por isso, crucial a implementação de medidas preventivas para a protecção destas espécies. Uma das soluções passa pela implementação de programas de conservação ex-situ, fundamentados com conhecimentos sólidos acerca da dinâmica populacional e características da história vital das espécies-alvo. Neste estudo são apresentados dados acerca do crescimento e estrutura etária de stocks selvagens e de cativeiro de um endemismo lusitano, o ruivaco-do-Oeste Achondrostoma occidentale*, alvo de programas de reprodução em cativeiro e de acções de repovoamento desde 2007. Foram comparados os comprimentos máximos, longevidades e comprimentos à idade entre stocks de cativeiro e selvagens. De um modo geral, os resultados revelaram que indivíduos de cativeiro têm taxas de crescimento e longevidades superiores aos indivíduos selvagens. As distribuições bimodais de comprimento–idade encontradas em duas das populações selvagens sugerem um efeito positivo das acções de repovoamento nas abundâncias de ruivaco-do-Oeste. Os resultados apresentados neste estudo destacam a importância de programas de reprodução em cativeiro para a conservação de ciprinídeos ameaçados, e revelam de que forma as comparações de características da história vital entre stocks selvagens e de cativeiro podem contribuir para avaliar a eficácia destes programas.*

Palavras chave: *reprodução em cativeiro, ciprinídeos ameaçados, rios Mediterrânicos, recrutamento, caracteres de história de vida*

INTRODUCTION

Freshwater ecosystems are among the most endangered ecosystems in the world and declines in their biodiversity are expected to be more intense than in terrestrial or marine environments, affecting invertebrates, amphibians and fishes (Sala *et al.*, 2000; Cooke *et al.*, 2012). Cyprinids, the most diverse family of freshwater fishes, currently face a wide range of threats, including habitat degradation, damming and water extraction, invasive species and climate change (Clavero *et al.*,2010), and are thus considered among the most threatened biota worldwide (Hermoso & Clavero, 2011).

Captive breeding is increasingly valuable in the conservation of Mediterranean endemic freshwater fishes (Carrizo *et al.*, 2013; Maceda-Veiga, 2013; Sousa-Santos *et al.*, 2014). In particular, Iberian cyprinids, with one of the highest proportions of threatened species recorded to date (Darwall *et al.*, 2008), are good candidates for captive breeding and stock enhancement programs (Gil *et al.*, 2010).

In Portugal, there is an ongoing captive breeding program targeting endangered and critically endangered Iberian cyprinids, since 2007 (Sousa-Santos *et al.*, 2014). This program involves the capture of breeders in wild populations, the maintenance of brood stocks for three consecutive generations, the restocking of wild populations with captive-reared fish (ideally after river management actions) and, afterwards, the foundation of new stocks of wild breeders to proceed with the program, maximizing the preservation of the original genetic pool of target populations (see Sousa-Santos *et al.* 2014 for details).

Among other species, this captive breeding program has been focused on the western ruivaco *Achondrostoma occidentale* (Robalo, Almada, Sousa Santos, Moreira & Doadrio 2005), a cyprinid listed as "Endangered" in the IUCN Red List

Table 1. Captive stocks and restocking actions targeting the western ruivaco (based on Sousa-Santos *et al.* 2014). For each stock, we present the year of foundation at the Vasco da Gama Aquarium (VGA, coordinates: 38°41'57.4"N 9°14'21.2"W) and at the Campelo Station (CS, coordinates: 39°59'56.3"N 8°15'57.5"W), the number of breeders used, and the year and number of fish release in the wild. Stocks sampled in the present study are highlighted in grey. * Initial stocks including only wild fish; ** Restocking action conducted after the acquisition of data presented in this paper. *Stocks de cativeiro e acções de repovoamento de ruivaco-do-Oeste (baseado em Sousa-Santos* et al. *2014). Para cada stock, são apresentados o ano de fundação do stock em cativeiro em cada estação de cativeiro (Aquário Vasco da Gama – VGA, coordenadas: 38°41'57.4"N 9°14'21.2"W; Estação Aquícola de Campelo – CS, coordenadas: 39°59'56.3"N 8°15'57.5"W), o total de fundadores do stock, e o número de indivíduos libertados na natureza. Os stocks amostrados neste estudo encontram-se destacados a cinzento. * Os stocks iniciais incluíam apenas peixes capturados na natureza. ** As acções de repovoamento ocorreram após a recolha dos dados apresentados neste estudo.*

River of origin	Breeding facilities	Year of foundation	Number of breeders	Year of release	Number of fish released
Alcabrichel	VGA	2006	16*	2011	400
Alcabrichel	VGA	2011	60	2013	446
Alcabrichel	CS	2009	45*	2011	400
Alcabrichel	CS	2011	60	2013	1.190
Alcabrichel	CS	2013	60	2015	2.482
Alcabrichel	CS	2015	60	-	-
Sizandro	CS	2009	19*	2013	1.309
Sizandro	CS	2013	60	2016	1.259
Sizandro	CS	2016	60	-	-
Safarujo	VGA	2012	12*	2015	350
Safarujo	VGA	2015	60	2016	448**

(IUCN 2016) that was only described in 2005 (Robalo *et al.*, 2005). This small-sized species (<100 mm total length) is endemic to three small basins (<40 km^2) in western Portugal (Fig. 1), where it is found in low numbers and faces severe pollution and water scarcity (Robalo *et al.*, 2008).

The captive breeding of the western ruivaco has been very successful, and 8284 captive-reared fish were released in the wild between 2011 and 2016 (Sousa-Santos *et al.*, 2014; Sousa-Santos *unpublished data*). However, the life-history traits of wild and captive populations have yet to be assessed, although this was considered critical to evaluate the efficiency of conservation measures designed for freshwater fishes (e.g. Ribeiro *et al.*, 2000; Vinyoles & De Sostoa, 2007). In order to fill this gap, this study quantified the variation in growth and age structure of western ruivaco in captive and restocked and non-restocked wild populations. Assuming that fish generally have a shorter life span and smaller body size in fluctuating versus stable environments (e.g. Schlosser, 1990; Winemiller & Rose, 1992), we predicted that fish bred in captivity would live longer and grow faster than wild fish. As this would likely result in longer reproductive span, and production of more and larger eggs that favour larval survival (Matthews, 1998), we also predicted that captive-reared fish will have positive effects in the recruitment and structure of restocked populations, contingent on local environmental conditions. Results were used to discuss the value of captive breeding programs of threatened cyprinids.

MATERIAL AND METHODS

Study populations

Wild populations (henceforth designated by wild stocks) of the western ruivaco occur in the Alcabrichel (25 km, 39º1'N, 9º1'W), Sizandro (37 km, 39º0'N, 9º1'W) and Safarujo rivers (18 km, 39º0'N, 9º2'W), in the Torres Vedras and Mafra municipalities, north of Lisbon. All these rivers are typically Mediterranean, drying to isolated pools during summer-early fall (Gasith & Resh, 1999). There is a high human settlement in the catchments, resulting in severe habitat destruction and water pollution (Robalo *et al.*, 2005; Robalo *et al.*, 2008). Along the margins, the high abundance of exotic giant cane *Arundo-donax* prevents native vegetation from growing and stabilizing the river banks (Mameri, 2015).

Other native fish in the rivers are the loach *Cobitis paludica* (deBuen, 1930) and the European eel *Anguilla anguilla* (Linnaeus, 1758). Non-native fish are restricted to a few *Gambusia holbrooki* in the Sizandro River, but non-native American crayfish *Procambarus clarkii* (Girard, 1852) has been increasing in density in the last few years, especially in the Alcabrichel and Sizandro rivers (Sousa-Santos and Mameri *unpublished data*).

The first captive populations (henceforth designated by captive stocks) of western ruivaco from the Alcabrichel, Sizandro and Safarujo rivers were founded in 2006, 2009 and 2012, at the Vasco da Gama Aquarium and the Campelo Station (Table 1). Although an effort was made to maximize the number of breeders and guarantee the representation of the genetic pool of each target population, the initial stocks of breeders, between 12 and 45 fish, were largely constrained by the low abundance of fish in the wild (Table 1). As the program proceeded and all captive-bred individuals were released in the wild, a second round of captive breeding was initiated using slightly larger stocks of breeders, including 30 wild and 30 captive bred fish (Sousa-Santos *et al.*, 2014). Captive stocks were kept in outdoor tanks, under natural conditions of light and temperature (but see Sousa-Santos *et al.*, 2014, for details).

In order to reduce vulnerability to genetic diversity loss, interbreeding depression and deleterious mutations associated with small population size (e.g. Madeira *et al.*, 2005; Hoffman & Williams, 2009), fish were released in the wild generally after three consecutive generations in captivity, and new stocks of breeders were founded as described above (Sousa-Santos *et al.*, 2014). To maximize the chances of survival and persistence of captive-bred fish in the wild, fish were only released in upper river stretches with adequate habitats and no pollution (Sousa-Santos *et al.*, 2014). When this study started, in September 2014, restocking actions had already been conducted twice in the Alcabrichel, in 2011 and

2013, and once in the Sizandro, in 2013, but not in the Safarujo River (Table 1).

Fish sampling

Wild stocks of western ruivaco from the Alcabrichel, Sizandro and Safarujo rivers were sampled between September and October 2014, in isolated dry-season pools. Fish were sampled in 6 pools in the Alcabrichel, 3 in the Sizandro, and 5 in the Safarujo. Each pool was electro-fished in an upstream zigzag pattern, using a SAMUS725G portable device fitted with a single anode, and discharging 1000 V electric impulses (frequency: 80 Hz; duration: 1.40 ms). Fish were counted, measured for fork-length (to the nearest mm), and returned to the stream.

Captive stocksfoundedat the Vasco da Gama Aquarium (VGA) and Campelo Station (CS) between 2012 and 2013 (Table 1) were sampled in March 2015, shortly before the spawning season, using hand nets (2 mm mesh). Prior to sampling, the water depth in each tank was lowered to approximately 50 cm to increase netting efficiency. Fork-length (FL) was measured before release, except individuals below 60 mm from the Safarujo, which were merely counted due to logistical constraints.

Age and growth analyses

Age distribution of each stock was estimated using the stratified Ketchen's procedure (Ricker, 1975). Individuals were divided into FL groups with 10 mm intervals. Scales were removed from individuals above 40 mm (Table 2). Smaller fish were not considered for analysis because handling could result in high mortality risk. Scales from the third row below the dorsal fin were mounted on microscope slides and the annuli were interpreted using the criteria of Bagenal & Tesh (1978). All scales were read twice and a third reading was made if the first two differed; when two of the three measures did not agree, the scale was discarded. Age determinations were used to construct separate age-length keys for each stock. This allowed the conversion of length distributions into age distributions, assuming age 0 for fish below 60 mm from the captive stock of the Safarujo and below 40 mm from the remaining stocks. Comparisons of length distributions were restricted to wild stocks only, to account for changes in size classes caused by different sampling seasons in wild and captive stocks.

Growth of each individual fish was described in terms of length increments between annuli. The total radius and distance from the focus to each annulus was measured for each scale. Length at annulus formation was back-calculated

Table 2. Wild and captive stocks of western ruivaco surveyed for age and growth analyses. For each stock, we present the number of individuals measured for fork-length (N) and sampled for scales (n). Note that wild and captive stocks were sampled September-October 2014 and March 2015, respectively. *Stocks selvagens e de cativeiro de ruivaco-do-Oeste amostrados para determinação da idade e taxas de crescimento. Para cada stock é apresentado o número de indivíduos para os quais foi medido o comprimento à furca (N) e retiradas escamas (n). Nota: a amostragem dos stocks selvagens e de cativeiro decorreu em Setembro-Outubro de 2014 e Março de 2015, respectivamente.*

River	Stock	Code	N	n	Fork length (mm)	
					Mean ± SD	Range
Alcabrichel	Wild	ALC_wild	376	98	46 ± 21	18–110
Alcabrichel	Captive	ALC_cap	148	39	73 ± 29	27–155
Sizandro	Wild	SIZ_wild	494	27	54 ± 20	19–130
Sizandro	Captive	SIZ_cap	102	28	58 ± 13	25–125
Safarujo	Wild	SAF_wild	135	37	56 ± 21	23–107
Safarujo	Captive	SAF_cap	385	51	81 ± 24	61–141

from linear regression equations ($R^2 \geq 0.87$, $p<0.001$) following the body proportional hypothesis (Bagenal & Tesh, 1978). Prior to back calculations, scale increments from the last annuli were analysed to account for variation in the timing of annulus formation associated with differences in sampling dates between wild and captive stocks. There was no significant variation in scale increments, with mean distances from the last annulus to the scale margin ranging between 181.42 and 182.67 µm in captive and wild stocks, respectively. Therefore, we considered that measurable differences in length increments between wild and captive stocks could indicate variation in fish growth patterns.

Data analyses

Prior to analysis, data were assessed for normality and homogeneity of variances, using the

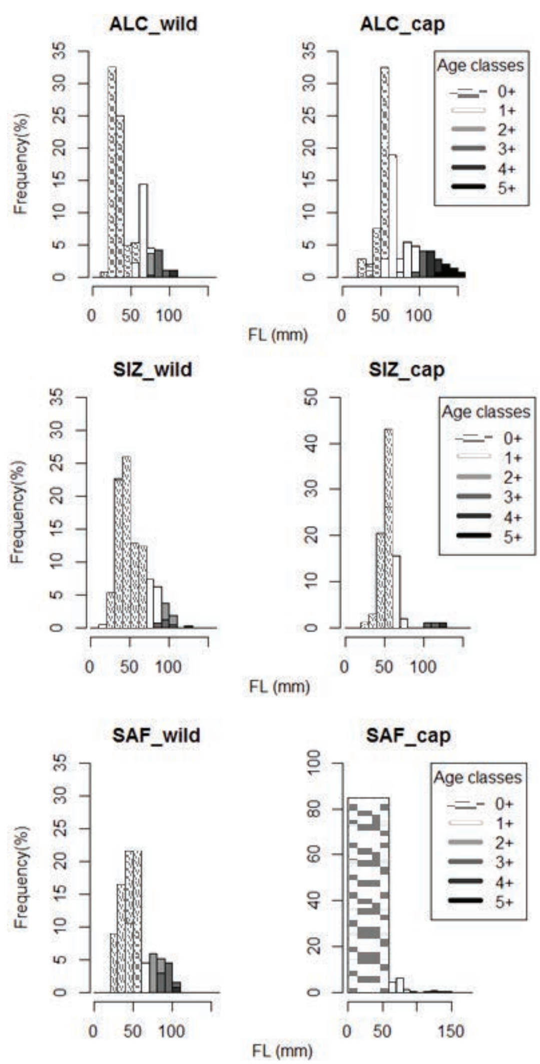

Figure 1. Age-length distributions of western ruivaco in wild and captive stocks from the Alcabrichel (ALC_wild and ALC_cap), Sizandro (SIZ_wild and SIZ_cap) and Safarujo rivers (SAF_wild and SAF_cap). Note that YY axes are different and that fish less than 60 mm in the captive stock of the Safarujo were assumed to be 0+. *Distribuições de comprimento-idade em stocks selvagens e de cativeiro do ruivaco-do-Oeste dosrios Alcabrichel (ALC_wild e ALC_cap), Sizandro (SIZ_wild e SIZ_cap) e Safarujo (SAF_wild e SAF_cap).Notas: para melhor leitura dos gráficos, os eixos dos YY foram ajustados para cada stock; no stock SAF_cap, os indivíduos com menos de 60 mm foram considerados 0+.*

Shapiro-Wilk and the Levene tests, respectively. Variation in fork-length was assessed among wild stocks only, using the Kruskal-Wallis and the Dunn's post- hoc tests. Back-calculated length-at-age was compared among wild and among captive stocks using one-way ANOVA and Tukey HSD post-hoc tests, and between pairs of wild and captive stocks using t-tests for the Alcabrichel and Safarujo rivers, and the Mann-Whitney test for the Sizandro River. Analyses were conducted using the R software, version 3.3.1 (R Development Core Team 2016), and statistical significance testing was assessed at $p<0.05$.

RESULTS

Fish in wild stocks ranged from 18 mm to 130 mm (Table 2). There was significant variation in fork-length among stocks (H=70.209, $p<0.001$),

Table 3. Variation in length (FL, mm) at each age and annual increment in length (mm) in wild and captive stocks of western ruivaco from the Alcabrichel, Sizandro and Safarujo rivers. Codes for stocks are given in Table 2; n is the sample size. *Variação dos comprimentos à furca (FL, mm) a cada classe de idade e respectivo incremento anual (em mm) para cada stock selvagem de cativeiro do ruivaco-do-Oeste, nas ribeiras do Alcabrichel, Sizandro e Safarujo. As siglas para cada stock são apresentadas na Tabela 2; n refere-se à dimensão da amostra.*

Population	Age (years)				
	1	2	3	4	5
ALC_wild					
Mean FL (SD)	56.2 (3.4)	68.7 (1.8)	81.1 (2.2)	94.0 (2.9)	-
Annual increment	39	13	12	13	-
n	80	28	13	4	-
ALC_cap					
Mean FL (SD)	60.2 (3.0)	77.7 (3.2)	102.2 (4.2)	116.5 (5.3)	130.9 (2.9)
Annual increment	40	18	23	15	14
n	39	29	16	13	5
SIZ_wild					
Mean FL (SD)	72.5 (3.8)	86.6 (3.8)	102.4	128.3	-
Annual increment	52	16	15	26	-
n	16	6	1	1	-
SIZ_cap					
Mean FL (SD)	65.5 (2.0)	86.0 (0.6)	101.5 (3.2)	119.5	-
Annual increment	42	21	15	18	-
n	11	3	3	1	-
SAF_wild					
Mean FL (SD)	59.7 (3.7)	73.0 (3.2)	85.1 (2.8)	96.4	-
Annual increment	45	13	14	13	-
n	20	13	5	1	-
SAF_cap					
Mean FL (SD)	63.8 (2.8)	80.9 (2.7)	101.8 (2.6)	122.5 (4.3)	133.5 (0.9)
Annual increment	50	17	21	20	11
n	30	8	6	5	2

with fish from the Safarujo (mean ± SD: 56±21 mm; range: 23–107 mm) and Sizandro (54±20 mm; 19–130 mm) being larger than those from the Alcabrichel (46±21mm; 18–110 mm). Among captive stocks, maximum length was found in individuals from the Alcabrichel (155 mm, mean ± SD: 73±29 mm), followed by those from the Safarujo (141 mm, 81±24 mm) and Sizandro (125 mm, 58±13 mm).

Wild stocks only included fish aged 0 to 4, while fish reached age 5 in the captive stocks of the Alcabrichel and Safarujo rivers (Fig. 1). Both wild and captive stocks were dominated by age 0 fish (73.1 % and 53.7 %, respectively) and, among wild stocks, a considerable percentage of age 1 fish was found in the Alcabrichel (16.8 %). Fish age 2 were rare in the captive stock of the Safarujo (8.0 %) and fish age 3 were absent in both the wild and captive stocks of the Sizandro.

Back-calculated length-at-age 1 varied significantly among wild stocks (F=143.75, d.f.=2, *p*<0.001), with the highest and lowest growth rates in the Sizandro and Alcabrichel rivers, respectively (Table 3). There were also significant differences in growth among captive stocks (F=13.99, *p*<0.001), with individuals from the Alcabrichel attaining larger sizes than the remainder (Table 3). When comparing wild and captive stocks, significant differences in length-at-age 1were found for all rivers. Individuals were smaller in wild than in captive stocks of the Alcabrichel (T=-6.273, *p*<0.001) and the Safarujo (T=-4.262, *p*<0.001), but the reverse pattern was found for the Sizandro (Z=-4.805, *p*<0.001).

DISCUSSION

Iberian freshwater fishes face important conservation threats, with 25 of the 31 cyprinids classified as vulnerable, endangered or critically endangered in the IUCN Red List (IUCN, 2016). In this situation, ecological research should increasingly support conservation efforts, particularly for species with restricted distributions, suffering progressive and generalized declines, such as the western ruivaco *A. occidentale*.

Overall, patterns in growth, age and longevity of the wild and captive stocks of western ruivaco highlighted in this study were consistent with those described for other small Iberian cyprinids, with individuals growing quickly in the first year and having a short life-span (e.g. Fernandez-Delgado & Herrera, 1995a; Fernandez-Delgado & Herrera, 1995b; Pires *et al.*, 2000; Magalhães *et al.*, 2003; Ribeiro *et al.*, 2003). There was, however, considerable variation in size and growth of the western ruivaco, both among wild and captive stocks. Among wild stocks, individuals in the Safarujo and Sizandro were larger than in the Alcabrichel, and tended to grow faster in the Sizandro and slower in the Alcabrichel. These patterns may at least partially reflect variation in habitat conditions among rivers, with previous habitat surveys indicating that dry-season pools in the Sizandro were larger and had higher instream and macrophyte cover than in the Alcabrichel and Safarujo (Mameri *et al.*, 2016). Most likely this reflects higher refugia availability and habitat stability (Maddock, 1999; Petry *et al.*, 2003), which may favour growth, as suggested for other Iberian cyprinids (Aparicio & Sostoa, 1998; Ferreira *et al.*, 2007).

Both wild and captive stocks were dominated by age 0 fish, but there was an increase in relative abundance of age 1 fish in the Alcabrichel and Sizandro, where restocking actions had been carried out. The high abundance of age 0 fish in all rivers reflects successful recruitment in 2014. The high proportion of age 1 fish in the Alcabrichel likely reflects some positive effects of restocking with captive-reared individuals conducted in 2013. Although it is not possible to validate this hypothesis based in this study, few age 1 fish were found in the Safarujo, consistent with the lack of previous restocking actions and population enhancement effects.

Length-at-age 1 was higher in captive fish versus Alcabrichel and Safarujowild stocks, suggesting that growth has been favoured by mild conditions prevailing in captivity, including high water quality and availability, stable food intake, no extreme flow changes and consequently low energy expenditures (King *et al.*, 2003; Piffady *et al.*, 2010; Grossman *et al.*, 2016). Indeed, conditions may be harsh for fish in the Alcabrichel, which has been classified as "Very polluted" (Teixeira *et al.*, 2008), and in the Safarujo, which tends to be severely affected by summer droughts, lacking surface flow for most of its extension. However, the growth pattern between captive and wild stocks was not general, with fish from the Sizandro tending to grow faster in the wild, probably reflecting particularly favourable local conditions, as discussed above

Final remarks

This study highlights that fish raised in captivity may tend to attain larger size, grow faster and live longer than in the wild. This improved performance, which is also associated with better body condition (Mameri, 2015), can potentially result in higher fitness and reproductive potential for captive than for wild individuals (e.g. De Miguel *et al.*, 2013). Future studies on the survival of captive fish released in the wild and the abundance of ages 0 and 1 are thus needed to clarify the effects of restocking actions on recruitment and population recovery.

Under normal circumstances and in the absence of mass mortality inducing factors, the recruitment of restocked populations can be enhanced throughout generations, contributing to a higher representation of all age classes and to minimizing extinction risk due to demographic factors. Because small populations of endangered species tend to be disturbed sooner by demographic than by genetic factors (Lande, 1988; Caro & Laurenson, 1994; Caughley, 1994; Lacy, 2000), population reinforcements may constitute an effective conservation tool, when combined with in-situ measures targeting river habitat restoration and water quality improvement (Sousa-Santos *et al.*, 2014). Nevertheless, more baseline information on the species targeted for captive breeding and restocking is critical to increase the effectiveness of these conservation measures in terms of population size, structure and persistence in the wild.

ACKNOWLEDGEMENTS

This study was conducted as part of the ex-situ conservation project targeting endangered cyprinid fish species endemic to Portugal (partners: ISPA, Quercus, Vasco da Gama Aquarium, Figueiró dos Vinhos Municipality and Veterinary Medicine Faculty of the University of Lisbon). We thank the staff from the Vasco da Gama Aquarium and the Campelo Station, for allowing and supporting the sampling of captive stocks of the western ruivaco. Thanks are also due to André Levy for the English revision and the two anonymous referees for their insightful comments on earlier versions of the manuscript. Permits for field work were given by ICNF. This study was financed by the European Fund for Economic and Regional Development (FEDER) through the Program Operational Factors of Competitiveness (COMPETE) and National Funds through the FCT - Portuguese Foundation of Science and Technology, under the Pluriannual Program UI&D 331/94, the strategic projects UID/MAR/04292/2013 and UID/BIA/00329/2013 granted to MARE and to CE3C, respectively, and the grants awarded to C. Sousa-Santos (SFRH/BPD/29774/2006 and MARE-ISPA/BPD/001/2015).

REFERENCES

APARICIO, E. & A. SOSTOA. 1998. Reproduction and growth of Barbus haasi in a small stream in the N.E . of the Iberian Peninsula. *Archiv Fur Hydrobiologie*, 142(1): 95–110. DOI: 10.1127/archiv-hydrobiol/142/1998/95

BAGENAL, T.B., F. W. TESCH. 1978. Age and growth. In: *Methods for Assessment of Fish Production in Fresh Waters* (Bagenal, T. B., ed.): pp. 101–136. Blackwell Scientific Publications, Oxford, UK.

CARRIZO, S. F., K. G. SMITH & W. R. T. DARWALL. 2013. Progress towards a global assessment of the status of freshwater fishes (Pisces) for the IUCN Red List: Application to conservation programmes in zoos and aquariums. *International Zoo Yearbook*, 47: 46–64. DOI: 10.1111/izy.12019

CARO, T. M. & M. K. LAURENSON. 1994. Ecological and Genetic Factors in Conservation: A Cautionary Tale. *Science*, 263: 485 –486.

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. DOI: 10.1111/j.1472-4642.2010.00680.x

COOKE, S. J., C. PAUKERT & Z. HOGAN. 2012. Endangered river fish: factors hindering conservation and restoration. *Endangered Species Research*, 17: 179–191. DOI: 10.3354/esr00426

DANNEWITZ, J. 2003. Genetic and Ecological Consequences of Fish Releases. Ph.D. thesis. Uppsala University. Sweden.

DARWALL, W., K. SMITH, D. ALLEN, M. SEDDON, G. M. REID, V. CLAUSNITZER& V. J. KALKMAN. 2008. Freshwater biodiversity: a hidden resource under threat. In: *The 2008 Review of The IUCN Red List of Threatened Species* (edited by Vié J.-C., Hilton-Taylor C., Stuart S.N.): pp. 1–11. Gland, Switzerland.

DE MIGUEL, R. J., F. J. OLIVA-PATERNA, L. GÁLVEZ-BRAVO, C. FERNÁNDEZ-DELGADO. 2013. Habitat quality affects the condition of *Luciobarbus sclateri* in the Guadiamar River (SW Iberian Peninsula): effects of disturbances by the toxic spill of the Aznalcóllar mine. *Hydrobiologia*, 700: 85–97. DOI: 10.1007/s10750-012-1211-0

FERNÁNDEZ–DELGADO, C. & M. HERRERA. 1995a. Age structure, growth and reproduction of *Leuciscus pyrenaicus* in an intermittent stream in the Guadalquivir river basin, southern Spain. *Journal of Fish Biology*, 46: 371–380. DOI: 10.1111/j.1095-8649.1995.tb05977.x

FERNÁNDEZ–DELGADO, C. & M. HERRERA. 1995b. Age, growth and reproduction of *Rutilus lemmingii* in an intermittent stream of the Guadalquivir River basin, southern Spain. *Hydrobiologia*, 299: 207–213. DOI: 10.1007/BF00767327

FERREIRA, M. T., L. SOUSA, J. M. SANTOS, L.REINO, J. OLIVEIRA, P. R. ALMEIDA & R. V. CORTES. 2007. Regional and local environmental correlates of native Iberian fish fauna. *Ecology of Freshwater Fish*, 16: 504–514. DOI: 10.1111/j.1600-0633.2007.00241.x

FLEMING, I. A.1994. Captive Breeding of Wild Conservation Salmon. *Conservation Biology*, 8(3): 886–888.

FRASER, D. J. 2008. How well can captive breeding programs conserve biodiversity? A review of salmonids. *Evolutionary Applications*, 1: 535–86. DOI: 10.1111/j.1752-4571.2008.00036.x

GASITH, A. & V. H. RESH. 2009. Streams in Mediterranean Climate Regions: Abiotic Influences and Biotic Responses to Predictable Seasonal Events. *Annual Review of Ecology and Systematics*, 30: 51–81. DOI: doi.org/10.1146/annurev.ecolsys.30.1.51

GIL, F., C. SOUSA-SANTOS & V. ALMADA. 2010. A Simple and Inexpensive Technique for the Ex Situ Reproduction of Critically Endangered Cyprinids – *Achondrostoma occidentale* as a Case Study, 41(4): 661–664.

HERMOSO, V. & M. CLAVERO. 2011. Threatening processes and conservation management of endemic freshwater fish in the Mediterranean basin: a review. *Marine and Freshwater Research*, 62: 244–254. DOI: 10.1111/j.1749-7345.2010.00408.x

IUCN 2016. The IUCN Red List of Threatened Species. Electronic version, updated 7 December 2016. http://www.iucnredlist.org. Accessed 5 February 2017.

KING, J., C. BROWN & H. SABET. 2003. A scenario-based holistic approach to environmental flow assessments for rivers. *River Research and Applications*,19: 619–639. DOI: 10.1002/rra.709

LACY, R. C. 2000. Should We Select Genetic Alleles in Our Conservation Breeding Programs? *Zoo Biology*, 19, 279–282. DOI: 10.1002/1098-2361(2000)19:4<279::AID-ZOO5>3.0.CO;2-V

LEBERG, P. L., & B. D. FIRMIN. 2008. Role of inbreeding depression and purging in captive breeding and restoration programmes. *Molecular Ecology*, 17: 334–343. DOI: 10.1111/j.1365-294X.2007.03433.x

MACEDA-VEIGA, A. 2013. Towards the conservation of freshwater fish: Iberian Rivers as an example of threats and management practices. *Reviews in Fish Biology and Fisheries*, 23(1): 1–22. DOI: 10.1007/s11160-012-9275-5

MADDOCK, I., & H. GROVE. 1999. The importance of physical habitat assessment for evaluating river health. *Freshwater Biology*, 41: 373–391. DOI: 10.1046/j.1365-2427.1999.00437.x

MAGALHÃES, M. F., I. J. SCHLOSSER & M. J. COLLARES-PEREIRA. 2003. The role of life history in the relationship between population dynamics and environmental variability in two Mediterranean stream fishes. *Journal of Fish Biology*, 62: 1–18. DOI: 10.1046/j.1095-8649.2003.00148.x

MAMERI, D. 2015. Habitat use, growth and reproductive behaviour of the western ruivaco (Cyprinidae): contributes to the restocking of wild populations. Master's thesis. Universidade de Lisboa, Portugal.

MAMERI, D., C. SOUSA-SANTOS, M. F. MAGALHÃES, J. I. ROBALO. *In press*. Conservation of the critically endangered western ruivaco *Achondrostoma occidentale* (Pisces: Cyprinidae): integrating habitat data in restocking programs to improve population recovery. *Gaia Scientia*. DOI: 10.21707/gs.v10.n02a01

MATTHEWS, W. J. 1998. In: *Patterns in Freshwater Fish Ecology*. 1st edition, Chapman and Hall, New York, USA, 756 pp.

PETRY, P.,P. B. BAYLEY & D. F. MARKLE. 2003. Relationships between fish assemblages, macrophytes and environmental gradients in the Amazon River floodplain. *Journal of Fish Biology*, 63: 547–579. DOI: 10.1046/j.1095-8649.2003.00169.x

PIFFADY, J.,Y. SOUCHON, H. CAPRA & E. PARENT. 2010. Quantifying the effects of temperature and flow regime on the abundance of 0+ cyprinids in the upper River Rhone using Bayesian hierarchical modelling. *Freshwater Biology*, 55: 2359–2374. DOI: 10.1111/j.1365-2427.2010.02453.x

PIRES, A. M., I. G. COWX & M. M. COELHO. 2000. Life history strategy of *Leuciscus pyrenaicus* (Cyprinidae) in intermittent streams of the Guadiana basin (Portugal). *Cybium*, 24(3): 287–297.

RIBEIRO, F., I. COWX & M. COLLARES-PEREIRA. 2000. Life history traits of the endangered Iberian cyprinid *Anacypris hispanica* and their implications for conservation. *Archiv für Hydrobiologie*, 149(4): 569–586. DOI: 10.1127/archiv-hydrobiol/149/2000/569

RIBEIRO, F., I. G. COWX, P. TIAGO, A. F. FILIPE, L. MOREIRADA COSTA & M. J. COLLARES-PEREIRA. 2003. Growth and reproductive traits of diploid and triploid forms of the *Squalius alburnoidescyprinid* complex in a tributary of the Guadiana River, Portugal. *Archiv Fur Hydrobiologie*, 156(4): 471–484. DOI: 10.1127/0003-9136/2003/0156-0471

RICKER, W. E.1975. Computation and Interpretation of Biological Statistics of Fish Populations. *Bulletin of the fisheries research board of Canada*,191: 1–382.

ROBALO, J. I., V. C. ALMADA, C. SOUSA SANTOS, M. I. MOREIRA & I. DOADRIO. 2005. New species of the genus *Chondrostoma* Agassiz, 1832 (Actynopterigii, Cyprinidae) from western Portugal. *Graellsia*, 61(1): 19–29. DOI: 10.3989/graellsia.2005.v61.i1.3

ROBALO, J. I., C. SOUSA-SANTOS, I. DOADRIO & V. C. ALMADA. 2008. Threatened fishes of the world: *Achondrostoma occidentale* Robalo, Almada, Sousa-Santos, Moreira & Doadrio 2005 (Cyprinidae). *Environmental Biology of Fishes*, 83: 347–347. DOI: 10.1007/s10641-008-9348-4

SALA, O. E., F. S. CHAPIN III, J. J. ARMESTO, E. BERLOW, J. BLOOMFIELD, R. DIRZO, E. HUBER-SANWALD, L. F. HUENNEKE, R. B. JACKSON, A. KINZIG, R. LEEMANS, D. M. LODGE, H. A. MOONEY, M. OESTERHELD, N. L. POFF, M. T. SYKES, B. H. WALKER, M. WALKER, & D. H. WALL. 2009. Global Biodiversity Scenarios for the Year 2100. *Science*, 287: 1770–1774. DOI: 10.1126/science.287.5459.1770

SCHLOSSER, I. J. 1990. Environmental Variation, Life History Attributes, and Community Structure in Stream Fishes: Implications for Environmental Management and Assessment. *Environmental Management*, 14(5): 621–628. DOI: 10.1007/BF02394713

SOUSA-SANTOS, C., F. GIL & V. C. ALMADA, 2014. Ex situ reproduction of Portuguese endangered cyprinids in the context of their conservation. *Ichthyologi-*

cal Research, 61(2): 193–198. DOI: 10.1007/s10228-013-0383-6

TEIXEIRA, A., A. M. GERALDES, J .M. OLIVEIRA, J. BOCHECHAS & M. T. FERREIRA. 2008. Avaliação da Qualidade Ecológica de Rios Portugueses (Projecto AQUARIPORT): Síntese dos resultados referentes à análise das comunidades de macroinvertebrados bentónicos. *Proceedings of 9o Congresso da Água*. April 2–4, Cascais, Portugal.

WILLIAMS, S. E. & E. A. HOFFMAN. 2009. Minimizing genetic adaptation in captive breeding programs: A review. *Biological Conservation*, 142: 2388–2400. DOI: 10.1016/j.biocon.2009.05.034

WINEMILLER, K. O. & K. A. ROSE. 1992. Patterns of life-history diversification in North American fishes: implications for population. *Canadian Journal of Fisheries and Aquatic*, 49(10): 2196–2218. DOI: 10.1139/f92-242