



EUROPEAN UNION OF AQUARIUM CURATORS

REPORTING FORM

FOR *IN SITU* CONSERVATION PROJECTS FUNDED

1 TITLE OF PROJECT “Conservation of *Achondrostoma occidentale*, a freshwater fish endemic to the westernmost tip of Europe”

2 NAME OF APPLICANT Maria de Fátima Santos Gil

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WHAT YEAR WAS YOUR PROJECT FUNDED BY EUAC?

2017

3 LOCATION OF PROJECT (REGION & COUNTRY)

River Safarujo – region: Extremadura, country: Portugal

4 PROJECT START AND END DATES:

01.09.2017 to 31.08.2018

5 PROJECT CO-ORDINATOR, ADDRESS AND INSTITUTIONAL AFFILIATION

(IF DIFFERENT FROM APPLICANT)

(same as applicant)

6 PROJECT TYPE

(TICK ANY COMPONENTS THAT APPLY)

- BIOLOGICAL/ECOLOGICAL RESEARCH
- VETERINARY/CONSERVATION MEDICINE
- ANIMAL WELFARE
- CAPTIVE BREEDING
- RE-INTRODUCTION/RE-STOCKING/TRANSLOCATION
- HUMAN-WILDLIFE CONFLICT

- EDUCATION/PUBLIC AWARENESS
- TRAINING/WORKSHOPS
- COMMUNITY-BASED/SOCIAL POLICY
- ECOTOURISM/SUSTAINABLE DEVELOPMENT
- SUSTAINABLE USE
- WARDENING/LAW ENFORCEMENT
- PROTECTED AREAS MANAGEMENT
- OTHER: _____

7 FOCAL SPECIES (COMMON AND SCIENTIFIC NAME)

Western Ruivaco (*Achondrostoma occidentale*)

8 IUCN RED LIST STATUS (OR OTHER THREAT LISTING) OF FOCAL SPECIES

Endangered

CITES -- NO

APPENDIX

9 PROJECT BACKGROUND

A. occidentale is a small cyprinid endemic to three small (<40km) river basins located at the westernmost tip of Europe, isolated from its geographically closest congeneric species for more than 7My. It inhabits typical Mediterranean-type intermittent rivers, with winter floods and summer droughts of variable intensity. The dry season is particularly threatening for fish, due to major habitat contraction/population fragmentation (and consequent genetic bottlenecks) and exposure to higher competition for the limited space/food and poorer water quality. Other threats include habitat destruction, pollution and spread of the exotic crayfish *Procambarus clarkii*. The eminent risk of extinction of *A. occidentale* after a severe drought prompted the launching of an Ex-situ Conservation program in 2007. Captive stocks founded with wild caught adults are maintained at the Aquário Vasco da Gama (AVG) and reproduction occurs under a naturalist approach to prevent natural selection relaxation. Over 8.000 captive bred fish were already released in their natural populations for population reinforcement and, consequently, to minimize the risk of extinction *in situ*. Since this program was pioneer in Portugal, it promptly gathered a high level of attention by the media and local communities, which have been invited to participate in restocking and environmental education actions.

10 WAS THE OVERALL PROJECT PURPOSE FULFILLED?

The overall project purpose was:

1. Assess *in situ* benefits from the recently conducted restocking actions (monitoring the current density of individuals and compare it with previously gathered baseline data). **This purpose was fulfilled.**
2. To support the logistics of a third restocking action (in 2018). **This purpose was fulfilled.**
3. To implement *in situ* conservation measures. These tasks will be conducted with local students, in order to consolidate the need to have locals as allies in the conservation of the fluvial ecosystem. Without this funding restocking actions would continue to be mere releases of fish without a formal involvement of the society. **This purpose was fulfilled.**
4. Funding allocated to genetic analyses for the prosecution of the captive breeding program targeting this species, since the quantification of an eventual loss of genetic diversity is essential to maintain or to change the ongoing procedures in the future. **This purpose was fulfilled to the extent that it was possible with the existing knowledge and technology, but we are still working to obtain our intended goal.**

11 WHAT OBJECTIVES WERE MET?

1. Monitor the benefits of past restocking actions, by conducting two field surveys aiming at characterize fish distribution and densities along the river; **Objective met.**
2. Raise and release another generation of captive bred fish; **Objective met.**
3. Assess the eventual loss of genetic diversity during captive breeding, due to inbreeding and lineage sorting effects; **Objective partially met.**
4. Invest in the environmental education of local students through hands-on conservation activities in the wild and in the Aquário Vasco da Gama (AVG) facilities. **Objective met.**

Objectives 1, 2, and 4 were met and objective 3 was partially met, but work is still going so that it will be met in the near future.

WHAT OBJECTIVES WERE NOT MET?

None.

12 WHAT PROJECT ACTIVITIES WERE UNDERTAKEN?

1. MONITORING

Activity proposed: Fish will be sampled by electrofishing after the dry and wet seasons (September 2017 and April 2018). We will characterize habitat conditions and population parameters (fish density, proportion of size classes, condition factor). Since the dorsal fin tip of previously released fish was cut, recaptures will be quantified and fish condition factor will be evaluated. Exotic crayfishes will ideally be removed and sent to feed birds at recovering centres.

Activity undertaken: Monitoring of the target population of the Safarujó River has been made in an annual basis since 2017, in the end of the summer (September). We selected this time of the year since it is important to evaluate the recruitment of the species (which typically spawns between March and June) and to assess habitat conditions in the most threatening season for its survival. Indeed, in the end of the summer, this temporary river is fragmented into a series of disconnected pools (Fig. 1), where fish congregate in extremely narrow water volumes and are more vulnerable due to poorer water quality, lower available oxygen levels, increased risk of predation and increased risk of having contagious diseases.

We selected 2 sampling points located in the middle section of the river, known to be the most representative summer refugia for *A. occidentale* regarding water availability and fish density (SAF1 and SAF6 sampling points).



Fig. 1 – Sampling point (SAF6) in September 2017 (left) and in September 2018 (right).

2. RESTOCKING

Activity proposed: In 2018, two brood stocks of the captive population of *A. occidentale* (founded in 2016), which is being raised according to the naturalistic approach implemented by the *Ex-situ* Conservation Program, will be sufficiently grown to be released, contributing to the reinforcement of the wild population and, therefore, to the *in situ* conservation of the species. Fish will be transported from AVG to the river and released after being individually marked.

Activity undertaken: 17 April 2018, 100 captive bred *A. occidentale* born in 2016 and 2017 in Aquário Vasco da Gama, from a parent wild population of 30 captured on river Safarujó, were released at SAF6.

3. GENETIC SURVEY

Activity proposed: Fin clips taken during activity#2 will be used to extract DNA and amplify microsatellites. The genetic pattern of the offspring will be compared to that of the initial captive stock (fin clips already collected in 2016) and the eventual loss of genetic diversity will be quantified. This result is extremely important to validate the *ex-situ* conservation as an effective tool to minimize the extinction risk.

Activity undertaken: Conventional genetic sequencing of 20 individuals using a mitochondrial and a nuclear marker (cytochrome b and beta-actin, respectively) showed that the *A. occidentale* population from the Sarafujo River had an extremely low genetic diversity (Fig. 2), with only two cytochrome b haplotypes and three beta-actin haplotypes. However, since these markers are slow-paced (cytochrome b is the fastest and has a mutation rate of 1% per million years) and do not allow us to address properly the eventual loss of genetic diversity due to inbreeding and genetic drift effects, as a consequence of captive breeding from a limited breeding stock (N=30 adult individuals), we started searching for other methodologies. Initially, we intended to use microsatellites but realize that it was expensive to develop a library for the species and find a number of polymorphic loci that was high enough to address our goal. At the same time, the prices to conduct genomic analysis were rapidly becoming lower. Thus, we started evaluating the available techniques and requesting quotes from different companies to obtain data at a genome-wide level which will allow us to obtain millions of single nucleotide polymorphisms (SNP's) instead of the dozens of loci that will be analysed if we had opted by the microsatellite analyses approach. Funds were allocated to the extraction of high quality DNA (with integrity, concentration and mass that meet the minimum standards of the outsourcing genomic company) from 3 wild breeders, 3 F1 individuals and 3 F2 individuals born at the Aquário Vasco da Gama. These DNA samples will be shipped to LGC Genomics GmbH in July 2020 for genotyping-by-sequencing/ddRAD to obtain approximately 1.5M read pairs per sample. This delay in sending the samples for genomic sequencing was due to several constraints: first, we spend much time searching for the best techniques, asking advices and comparing quotations; second, laboratorial work to obtain high quality DNA was much more laborious than that of conventional genetics and required some adaptations of our procedures; third, as these companied work in batches of 96-well plates, we had to wait for the processing of other samples (and for the respective funding) until the whole plate was completed and ready to be send; and, finally, in March 2020 we had to stop working due to the covid-19 pandemic and started gradually to return to lab work in the beginning of June. Results from this analysis will be sent to us in 6-8 weeks and afterwards we will be analysing data from at least the following three months. Despite of the considerable delay regarding the original chronogram of the Project, we strongly believe that these results are worth the time because they will allow us to estimate the level of diversity loss by captive breeding, a crucial aspect of this conservation tool that was never addressed for threatened fish to date.

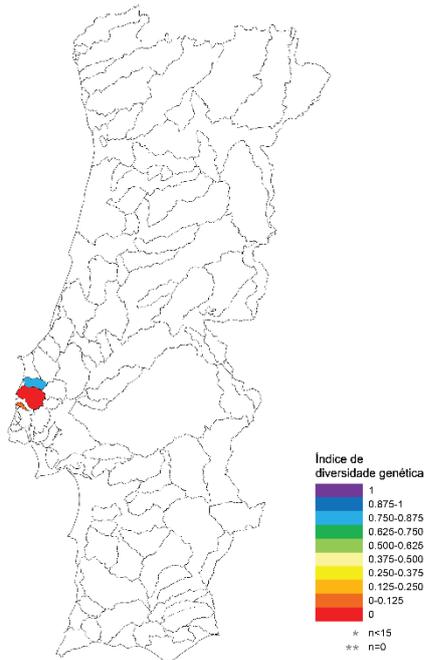


Fig. 2 – Genetic diversity values for *Achondrostoma occidentale* populations (Safarujó River is orange coloured), based on the cytochrome b gene.

4. ENVIRONMENTAL EDUCATION

Activities proposed: Two “clean-up the river” days with the participation of the local community (removal of plastic debris); hands-on activities for students in the field during monitoring and restocking actions.

Activities undertaken:

26 october 2017 visit to the river with the students from the primary school nearest to point in river Safarujó where the restocking actions were to be taken, presentation of the conservation project, cleaning up the river activity with students and teachers. The students and teachers participated in a ceremony for the establishment of a local Safarujó tribe committed to the river protection.

14 april 2018 visit to the river with the same local students who had the opportunity to participate on the restocking action and were oriented to conduct a survey of the river sides biodiversity and to detect potential environmental threats to the river.

Activity proposed: Visit of local students to the captive breeding facility at the AVG (preceded by a talk explaining the project);

Activity undertaken: 16 january 2018. This activity occurred with the presence of 48 students and 5 teachers and technicians from the primary school nearest to point in river Safarujó where the restocking actions were to be taken. The students were divided in two groups, both visited the reproduction tanks, observed the target species on the exhibition tank and participated in a workshop. The workshop focused on ecological aspects of the target species using models produced with the project funding and the students prepared field guides and booklets to be used on the next visit to the river.

Activity proposed: Distribution of project flyers in the postal boxes of local communities and of pedagogic materials in local primary schools. Public awareness will also be raised by disseminating project details in social media.

Activity undertaken: Local school students were oriented to produce banners and painted rocks with messages on the importance of the local river and the uniqueness of the fish species targeted on this conservation project. These materials were scattered, by the students, on the village public spaces on 12 january 2018.

June to August 2018, two posters were produced: one with a schematic of the river and river margins stating the importance of trees, shrubs, aquatic vegetation, rock falls and summer refuges, for water quality and fish survival; the other telling the story of young local students, the river Safarujo's tribe, and how they are responsible for the river's surveillance and conservation.

All the actions were published on facebook and site by Aquário Vasco da Gama and the participating schools.

All project activities were undertaken, although the one stated on nº3 (GENETIC SURVEY) is still ongoing.

13 WHAT OUTCOMES WERE ACHIEVED DURING THE COURSE OF THE PROJECT?

1. MONITORING

Results obtained during the 2017 and 2018 field campaigns were compared with the reference situation (2014), however, due to the significant fluctuation of hydrological conditions (related mostly due to the rainfall registered during the winter) and absence of a long time series of data it is premature to draw conclusions. Despite of that, we can see from the results (Table 1) that SAF1 sampling point showed a lower water volume but a significantly higher fish density in 2017 than in 2014 (almost 12 times more individuals per m³ than the reference situation), likely as a result of the restocking action conducted in 2016. In the following field campaign (2018), after a drier hydrological year, the water volume was even lower in this sampling point and the number of fish captured was reduced from 56 to only 10 individuals (Table 1). This depletion was also observed in the sampling point SAF6, where only one adult individual was captured although dozens of larvae were observed (Table 1). This was the sampling point where captive bred fish were released in April but, due to the lowering water levels in this section of the river and to its complete drought upstream from this point, we believe that fish moved downstream where there was higher water availability. These typically Mediterranean streams are extremely dynamic but, in the long term, the most important is that fish are able to survive in adequate summer refugia from where they can colonize the whole water course once the hydrological connectivity is restored by the first strong rainfalls.

These data reinforce the need to proceed with the *ex-situ* conservation program, to provide fish that will mitigate the species trend to depletion and restock populations that otherwise would be extirpated during a severe drought. These restocking actions need to be conducted in parallel with the implementation of habitat restoration measures (especially by improving shadowing with the rehabilitation of the riparian vegetation, removing of the invasive *Arundo donax* and deepening summer pools) and better water management policies. Both these aspects are being promoted in coordination with local and governmental authorities and are one of the major in situ benefits of the conducted restocking actions.

sampling point	year	Nb Fish	length (m)	average width (m)	average depth (m)	volume (m ³)	Fish density (ind/m ³)	water temperature (°C)	dissolved oxygen (mg/l)	pH
SAF1	2014	31	10,80	4,10	0,55	24,35	1,27	20,7	3,0	8,0
SAF1	2017	56	5,70	2,75	0,29	4,52	12,39	17,8	0,49	7,47
SAF6	2017	102	26,3	2,71	0,51	36,05	2,79	20,10	1,75	7,31
SAF1	2018	10	4,34	2,20	0,30	2,89	3,46	16,2	10,63	7,50
SAF6	2018	1	almost dry							

Table 1 – Habitat conditions and number of fish captured in September 2014 (reference situation), 2017, 2018 and 2019.

2. RESTOCKING

Note the presence of larvae on the releasing point SAF6. Adult fish were released in the spring just at the beginning of the spawning season and probably contributed to the larvae population observed.

For the restocking action we invited the local authorities and the local land owners and we used this activity as an opportunity for communicate our work and to address local conservation problems. One of the positive outcomes was the local government request to become a partner on an existing environmental education and monitoring project dedicated to

endemic freshwater fish and freshwater habitats.

4. ENVIRONMENTAL EDUCATION

The two posters produced for the project communication were offered to the school in an event that took place at the school.

A set of papier maché models of fish eggs and aquatic vegetation was also offered to the school.

A second set of poster prints was offered to the local authorities.

ARE ANY ONGOING?

As explained in nº12 the 3.GENETIC SURVEY objective is still ongoing.

DID ANY EXPECTED OUTCOMES FAIL?

None.

14 DID LOCAL PEOPLE/COMMUNITIES PARTICIPATE IN THE PROJECT? IF SO, HOW MANY AND WILL CONTINUED CONTACT BE MADE?

The local primary school: 48 students plus 5 teachers and school technicians.

Local authorities: the local environmental policy deputy, the local government president and her secretary, the owners of the river bank's property where the restocking actions occurred and people who live nearby.

After the end of this project we did another restocking action in spring 2019, with the presence of local government authorities, with the help of students from two more local schools and with the presence of the owners of river bank's properties. We visited the school who participated in the 2017/18 actions and offered a preserved fish specimen for the school Natural History cabinet.

In the autumn 2019, [MARE-ISPA \(Research Group of the ISPA-University Institute\)](#), a partner in the project, has conducted a monitoring action.

This year we will visit the same local school. We are preparing an event for the presentation of a children's book on rivers conservation, written by one of our colleagues and illustrated by the artist who illustrated the posters produced for the project. This book has a special dedication for this school (it will be a surprise!).

We have renewed the parenting captive population in 2019 and continue the captive reproduction of this species. New restocking is scheduled for 2022 and monitoring actions will continue once a year. Mare-ISPA also continues to advise the local authorities and local land owners on the riparian zone restoration.

15 DID THE GOVERNMENT OF THE HOST COUNTRY RECEIVE INFORMATION ON THE PROJECT'S RESULTS?

The National Authority on Conservation (Instituto Conservação da Natureza e Florestas – ICNF) received a report on the project actions.

16 HOW DID THE RELATIONSHIP WITH OTHER NGOS WORK? WERE THERE ANY ISSUES?

The major Portuguese conservationist NGO is a partner with Aquário Vasco da Gama on a wider project for the conservation of endemic freshwater species, which includes the species population targeted in this in situ project. Their representative has been present for the restocking and educational action and has been very supportive.

17 TOTAL PROJECT BUDGET AND EXPENDITURE (IN EUROS)

11584,86

18 AMOUNT OF MATCHING FUNDS

SPENT:

Circa 2800€ (as expected)

19 AMOUNT SPENT FROM EUAC FUNDS:

8784,86

20 EXPENDITURE BREAKDOWN (IN EUROS)

TRAVEL	911,83
SALARIES	
ACCOMMODATION	695,49
EQUIPMENT	859,16
COMMUNICATION	850,0
MISCELLANEOUS	5468,38
TOTAL	8784,86

21 PUBLICATIONS PRODUCED AS A RESULT OF THE PROJECT

. Two posters for project communication on the local communities.

. A scientific poster presented at the IMMR'18 | International Meeting on Marine Research 2018, Peniche, Portugal, 5 Jul - 6 Jul, 2018: "GUIDELINES FOR CONSERVATION OF CRITICALLY ENDANGERED CYPRINIDS - REVISED PROTOCOL FOR EX-SITU REPRODUCTION." Rui P. Calado, Fátima Gil, Carla S. Santos and Teresa M. Baptista.

. A communication was presented at EUAC meeting Hull 2018: "Conservation of *Achondrostoma occidentale*, a freshwater fish endemic to the westernmost tip of Europe" – Report of an *in situ* conservation project funded by EUAC, Fátima Gil, Carla Sousa-Santos.
