EXTINCT IN THE WILD PUPFISH

Conservation Action Plan **2024-2029**


Charco Palma pupfish (*Cyprinodon veronicae*)


La Palma pupfish (*Cyprinodon longidorsalis*)


Potosi pupfish (*Cyprinodon alvarezi*)

Charco pupfish 2 males. © Barbara Nicca

Acknowledgements

This proposal is the product of an exciting, optimistic, and highly collaborative series of discussions and individual work from the named partnership. It has involved in-person meetings between the project leaders and other partners in México, site visits, as well as regular online meetings to develop and review the plan. All partners have taken time to respond to anonymous questionnaires to best build out our aspirations and any concerns. We believe the product is a well-considered and bold plan that will achieve wild recovery and IUCN Red List downlisting for each of the three pupfish species. This processes has cemented a partnership that will see successful conservation of these precious species.

Cover images by Barbara Nicca. Images property of ZSL unless otherwise stated. ZSL would like to thank Dante Fenolio, Heiko Kärst, Maria Martinez, Barbara Nicca, Joel Sartore, Daniel Garza Tobón and fishesoftexas.org for sharing their images for inclusion in this guide.

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CONTENTS

Abbreviations

AZA	Association of Zoos and Aquariums
CTSG	Conservation Translocation Specialist Group
EAZA	European Association for Zoos and Aquaria
EEP	EAZA Ex Situ Programme
EW	Extinct in the Wild
IUCN	International Union for the Conservation of Nature
RCP	Regional Collection Plan
SSC	Species Survival Commission
TAG	Taxon Advisory Group
ZAA	Zoological Association of America
ZSL	Zoological Society of London

ORGANIZATION INFORMATION

Organization Descriptions

Co-Lead: Zoological Society of London (ZSL), United Kingdom

ZSL has been building stronger connections to wildlife for 200 years, through our Zoos, our conservation work in the field and through the Institute of Zoology, a center of research excellence. ZSL leads ground-breaking work in in situ and ex situ conservation and has a long history in nurturing Extinct in the Wild species. Beyond our expertise in ex situ care of Extinct in the Wild species, we are involved in a range of reintroductions from scimitar-horned oryx in Chad, to Partula snails in French Polynesia and sihek (Guam kingfisher). **EWPupfish** builds off this experience. ZSL has cared for the three Extinct in the Wild Mexican pupfish since 2000 and currently hosts the **EAZA EEP** (European Association for Zoos and Aquaria Ex situ Programme) coordinator. ZSL is one of only two major holders of all three pupfish species globally. ZSL is highly capable of administering grants, receiving grant income in the region of £12.5m per year, including multiyear six and seven figure grants.

Co-Lead: Universidad Michoacana de San Nicolás de Hidalgo, México

The Laboratory of Aquatic Biology established the Fish Ark México project in 1998, with an aim to conserve highly threatened central Mexican fish species through ex situ and in situ conservation. Since its origin, enthusiastic students and researchers have collaboratively and successfully implemented several conservation projects. Our most recent success was the reintroduction of the Extinct in The Wild Tequila splitfin, *Zoogoneticus tequila*, in its natural habitat. Our collaboration with the IUCN and Mexican Legislation to catalog freshwater fish extinction risk across all of México resulted in a groundbreaking conclusion that more than one third of Mexican freshwater fishes are threatened with extinction. We continue to work closely with our partners, including the IUCN Species Survival Commission (SSC) Freshwater Fish Specialist Group, to identify and implement effective conservation.

Partner: Bristol Zoological Society (BZS), United Kingdom

BZS is a conservation charity founded in 1836. The FishNet Programme was created by the BZS in 2005 with an aim of saving the most threatened species of freshwater fish from extinction. BZS currently hosts the chair of the **EAZA Freshwater Teleost Taxon Advisory Group (TAG)**. This group has published a Regional Collection Plan (RCP) with the aim of focusing on those species that need conservation attention, including the three species of pupfish that this project focuses on. BZS also hosts the co-chair of the **IUCN Freshwater Fish Specialist Group**. This group has the vision of: Thriving populations of native freshwater fishes sustained in their natural environments as part of functioning healthy ecosystems for nature and people. One of its key goals is to lead and support the science upon which freshwater fish conservation can succeed. It also aims to support best practice in situ and ex situ conservation for freshwater fishes.

Partner: Universidad Autónoma del Estado de Morelos (UAEM), México

The Freshwater Biodiversity Conservation Group has ample experience in implementing freshwater species conservation in México, as its members have worked, and are working with local communities, governments, and other stakeholders in the conservation of several fish (*Graodus boucardi, Astyanax salvatoris, Poeciliopsis balsas*, among others) and other species. Our university hosts the chair of the **IUCN/SSC Freshwater Conservation Committee**. This committee has been involved in assessing and communicating issues related to freshwater biodiversity and has been establishing alliances to develop conservation projects in different regions of México, and other parts of the world, seeking to address the global biodiversity crisis occurring in freshwater ecosystems.

Partner: Acuario Inbursa (AI), México

AI is the product of the serious deterioration of marine and freshwater ecosystems and the need to raise awareness among its visitors to help the conservation of these. AI has collaborated with environmental authorities like PROFEPA (Federal Attorney for Environmental Protection) in charge of monitoring and compliance with the good use of natural resources in environmental matters. AI has a highly successful threatened species breeding program and has experience in the movement of fish internationally and domestically.

Partner: Sealand Monterrey, México. Sealand is under construction in Monterrey

Our educational teams will meld entertainment with important environmental messaging, helping reach large numbers of the general public with the aim of inspiring behavior change toward improved care of freshwater ecosystems. Sealand Monterrey is located in the state that encompasses the indigenous ranges of all three Extinct in the Wild pupfish species.

Partner: Berkeley University, California, USA

The Department of Integrative Biology explores the diversity of life to help conserve natural ecosystems. Researchers work worldwide in the field, in our world-class museums, and in pioneering laboratories, to drive natural science breakthroughs. The Fish Speciation lab focusses on the rapid evolution of new fish species, including the Death Valley pupfishes, highly threatened and managed desert fishes where we study the complex relationships between genotype, phenotype, fitness, and environment.

Partner: Guadalajara Zoo, México

Is a decentralized public body of the Guadalajara City Council, located in the Barranca de Huentitán. It opened in 1988, providing a place where families could go and find a space that would allow them to awaken their interest in nature conservation. It is considered one of the few self-financing zoos in the country. In 2008, a new aquarium was built with ample outdoor space and available tanks for holding and breeding large numbers of pupfish. With its environmental education focus it is also well-placed to lead on pupfish educational programs.

Partner: Universidad Autónoma de Nuevo León (UANL), México

The Facultad de Ciencias Biológicas of the UANL is a universally recognized government academic institution in México. It trains both undergraduate and postgraduate students. The Ichthyology laboratory houses the third largest Ichthyological collection in the country. In the laboratory, studies are carried out on freshwater fish, focused on taxonomy and systematics, as well as ecological studies related to ecological vulnerability, trophic ecology, contamination of water bodies, biogeography, and population structure.

Partner: San Antonio Zoo, USA

Is operated by San Antonio Zoological Society, a non-profit organization. Through its expertise in animal care, conservation, and education, the zoo's mission is to inspire its community to love, engage with, act for, and protect animals and the places they live. The zoo welcomes more than a million visitors each year and is accredited by the Association of Zoos and Aquariums (AZA), the Zoological Association of America (ZAA), and Humane Certified by American Humane. It is one of only three major holders of Extinct in the Wild pupfish globally.

Partner: RioVivo, México

RioVivo is a new initiative based in Mazatlán, México, with the mission to conserve, study, and reproduce the threatened freshwater species of México. Based at the recently inaugurated Gran Acuario Mazatlán (GAM), the aim is to adapt GAM facilities to display species and to construct narratives around the importance of conservation of freshwater habitats and create a research group focused on freshwater conservation biology. The participating institutions in the "RioVivo Centre for Freshwater Species Conservation" are Centro de Investigacion en Alimentacion y Desarrollo A.C. (CIAD, CONAHCYT), and Instituto de Ciencias del Mar y Limnologia (ICML, UNAM).

Partner: Vienna Zoo, Austria

Vienna Zoo is the world's oldest zoo and was awarded the best European zoo in the past 6 years. Several in and ex situ conservation and science-oriented breeding programs were implemented in the past decades and their results are reported continuously to more than 2 million yearly visitors. The long-term expertise of the aquarist team at Vienna Zoo supports the conservation and research of freshwater fish and many other species. Vienna Zoo is one of only two major holders of all three pupfish species which the **EWPupfish** plan focuses on.

Charco pupfish close up. © Barbara Nicca

Partner: University of the West of England, United Kingdom

The Centre for Water, Communities and Resilience is an interdisciplinary collective of researchers and practitioners working to address national and international water security issues. Our mission is to promote world-class interdisciplinary research of climate change impacts, extreme weather and water risk, vulnerabilities and what makes communities resilient. We work at the intersection of (i) water science and engineering, (ii) security, policy, and governance, (iii) community/stakeholder development and resilience (iv) representation, perception, and behavior.

Partner: Instituto Mexicano de Tecnología del Agua IMTA, México

The Mexican Institute of Water Technology is a Public Research Center, coordinated by the Ministry of Environment and Natural Resources, whose mission is to produce, implement and disseminate knowledge and technology for the sustainable use of water in México. Its objectives are: Generate, apply and transfer knowledge to increase the capacities of applied research, technological development and innovation in the water sector and strengthen regional capacities in water science and technology.

Partner: IUCN Species Survival Commission Conservation Translocation Specialist Group (IUCN CTSG)

IUCN CTSG's mission is "To empower responsible conservation translocations that save species, strengthen ecosystems, and benefit humanity." IUCN CTSG had developed the global standard for conservation translocations in terms of the "IUCN Guidelines for Reintroductions and Other Conservation Translocations". IUCN CTSG helps to implement these guidelines by working with governments, academics, Indigenous Peoples, local communities, and practitioners through science, training, and facilitation around the world. These activities are aligned with IUCN CTSG's Extinct in the Wild Task Force which has helped to raise profile and action for Extinct in the Wild species globally and in México. In terms of the Mexican pupfish situation, preliminary meetings were already held in México in spring of 2023 which has served a basis for cooperation and collaboration.

Project Leader Biographies

Professor John Ewen, ZSL

John graduated from Massey University, Aotearoa New Zealand, with a Bachelor of Science (Zoology) (1994) and a Master of Science (Ecology) (1st class honors; 1998). During his studies he led the successful reintroduction of hihi (*Notiomystis cincta*) to Tiritiri Matangi Island. This species and reintroduction have become a model system in the field of reintroduction biology and John continues his close involvement as Co-Chair of the Aotearoa New Zealand government's Hihi Recovery Group.

Following his Master of Science, John moved to Australia where he completed his Doctor of Science (Ecology) at La Trobe University (2002). John then spent time as a post-doctoral researcher at the Université Pierre et Marie Curie in Paris and at the Institute of Zoology, ZSL before settling into a permanent role at ZSL and ultimately promotion to Professor in 2023.

John leads a large research group focused on species recovery involving conservation translocations. He has published more than 150 peer reviewed papers, accruing more than 5500 citations, including a recent review of the status and conservation focus of the world's Extinct in the Wild species (published in Science, 2023). He has published two texts on conservation translocations as edited volumes (in 2012, Reintroduction Biology, Wiley Blackwell & in 2023, Conservation Translocations, Cambridge University Press) and contributed to all other non-taxonomically focused books on the subject.

He has been a member of the **IUCN CTSG** since the 1990's and has led on development of the IUCN CTSGs training course for effective application of the 'IUCN Guidelines for Reintroductions and Other Conservation Translocations'. This training has been delivered to senior government officials, executive directors of leading environmental organizations, professors, graduate students, national park managers and other conservation translocation practitioners from more than 33 countries since its first iteration in 2016.

John is an invited member of the **IUCN CTSG Extinct in the Wild Task Force**, set up in 2018, and co-led on drafting a motion for the 2021 IUCN World Conservation Congress in France, 'Improving Process and Action to Identify and Recover Extinct in the Wild Species' which was officially endorsed through 95% of 115 governments and 99% of 572 non-government organizations globally.

John is the Chair of the Extinct in the Wild Sihek Recovery Team for the US Fish and Wildlife Service and is leading efforts for releases of sihek to the wild in 2024. Beyond his work leading ZSL's Extinct in the Wild initiative, John is also an invited member of the UK Government's Reintroduction Task Force and the BIAZA (British and Irish Association of Zoos and Aquariums) Reintroduction Advisory Group.

In his IUCN CTSG role, he advises on species conservation translocations on a diverse taxonomic range of species in many countries ranging from European sturgeon, *Acipenser sturio*, in England to Wild Camel, *Camelus ferus*, in Mongolia.

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John is an invited member of the IUCN CTSG Extinct in the Wild Task Force, set up in 2018, and co-led on a motion for the 2021 IUCN World Conservation Congress in France, 'Improving Process and Action to Identify and Recover Extinct in the Wild Species'.

Project Leader Biographies continued

Alex Cliffe, ZSL

Alex Cliffe graduated with a Bachelor of Science in Freshwater and Marine Ecology at John Moores University, Liverpool, UK (1998). Alex is a member of the **IUCN Freshwater Fish Specialist Group** and a member of the European Union of Aquarium Curators supporting and advising on key strategic conservation, research, and husbandry. He has been the vice chair for the British Association of Zoos and Aquariums for the last 10 years, supporting and driving the aquarium industry in line with government legislation, husbandry practices, staff training and exhibit development. He has co-authored a variety of research publications related to breeding programs of European freshwater fishes, seahorse reproduction and field conservation. Alex is the **EAZA EEP** co-ordinator for the family of pupfish and killifish (*Cyprinodontidae* and *Aphaniidae*) under the **EAZA Freshwater Teleost TAG**.

Within this role, he oversees priority species across this family, developing robust ex situ and in situ conservation plans. The three species of pupfish that are the focus of this proposal fall under this EAZA EEP as priority species and Whipsnade Zoo, a ZSL Conservation Zoo holds the largest of only two substantial ex situ populations of Potosi pupfish (*Cyprinodon alvarezi*), and the largest of only three substantial ex situ populations of La Palma pupfish (*Cyprinodon longidorsalis*) and Charco Palma pupfish (*Cyprinodon veronicae*). He also is a committee member of two other freshwater fish EAZA EEP's; *Poeciliidae* (Poeciliids) and *Bedotidae* (Malagasy rainbowfish).

Alex has worked extensively in the field from monitoring seahorse populations in the Dutch Antilles and UK waters to more recent work leading projects in both Turkey and México. The projects in Turkey and México are focused on supporting Critically Endangered and Extinct in the Wild freshwater fish, working directly with zoos, aquariums, universities, NGOs, and government officials.

His role as Assistant Curator of Fish at ZSL oversees one of the largest ex situ populations of threatened freshwater fish in the world, ensuring that cutting edge husbandry, research, education, and conservation is at the heart of his role.

> Alex is the EAZA EEP co-ordinator for the family of pupfish and killifish (*Cyprinodontidae* and *Aphaniidae*) under the EAZA Freshwater Teleost TAG.

Professor Omar Domínguez Domínguez, Universidad Michoacana de San Nicolás de Hidalgo

Omar Domínguez Domínguez graduated with a Bachelor in Biological Sciences, by the Universidad Michoacana de San Nicolas de Hidalgo (1997), then as a Master of Science in Management and Conservation of Biodiversity in the Tropics, Seville, Spain (2002), then a Master of Marine Sciences and Limnology, honorable mention (2004), by the Universidad Nacional Autónoma de México (UNAM), and finally a Doctor of Marine Sciences and Limnology, graduated with honorable mention and winner of Alfonso Caso Medal (2008) by the UNAM.

Omar has been a member of the National System of Researchers level III since 2004. He is also a long-time member of the **IUCN Freshwater Fish Specialist Group** and scientific advisor for several natural protected areas in México.

He continues to work on different research projects focusing on taxonomic, biological, biogeographic, phylogenetic, genetic and conservation aspects of Mexican freshwater fish, which has allowed him to publish more than 120 articles in journal citation report (JCR) journals, with more than 2400 citations. Omar has helped catalogue Mexican fish species into IUCN Red List threat categories, and they have also been registered in the Official Mexican Standard. This included, in 2018, assessment of the risk status of 536 Mexican fish species and, in 2022, to 65 Galapagos species according to the IUCN Red List.

He has trained a large number of Biology students at various levels, including producing 36 undergraduate theses, 18 master's and 8 doctoral theses, with another 12 in process. He has also been able to participate as a reviewer and synod of another 41 bachelor's theses, 18 master's and five doctoral theses. Omar has also welcomed five postdoctoral researchers to his group to date. All this has resulted in Omar receiving the Vasco de Quiroga, Jose Alvarez del Villar and distinguished Nicolaita medals for his success in the research of fish studies and conservation.

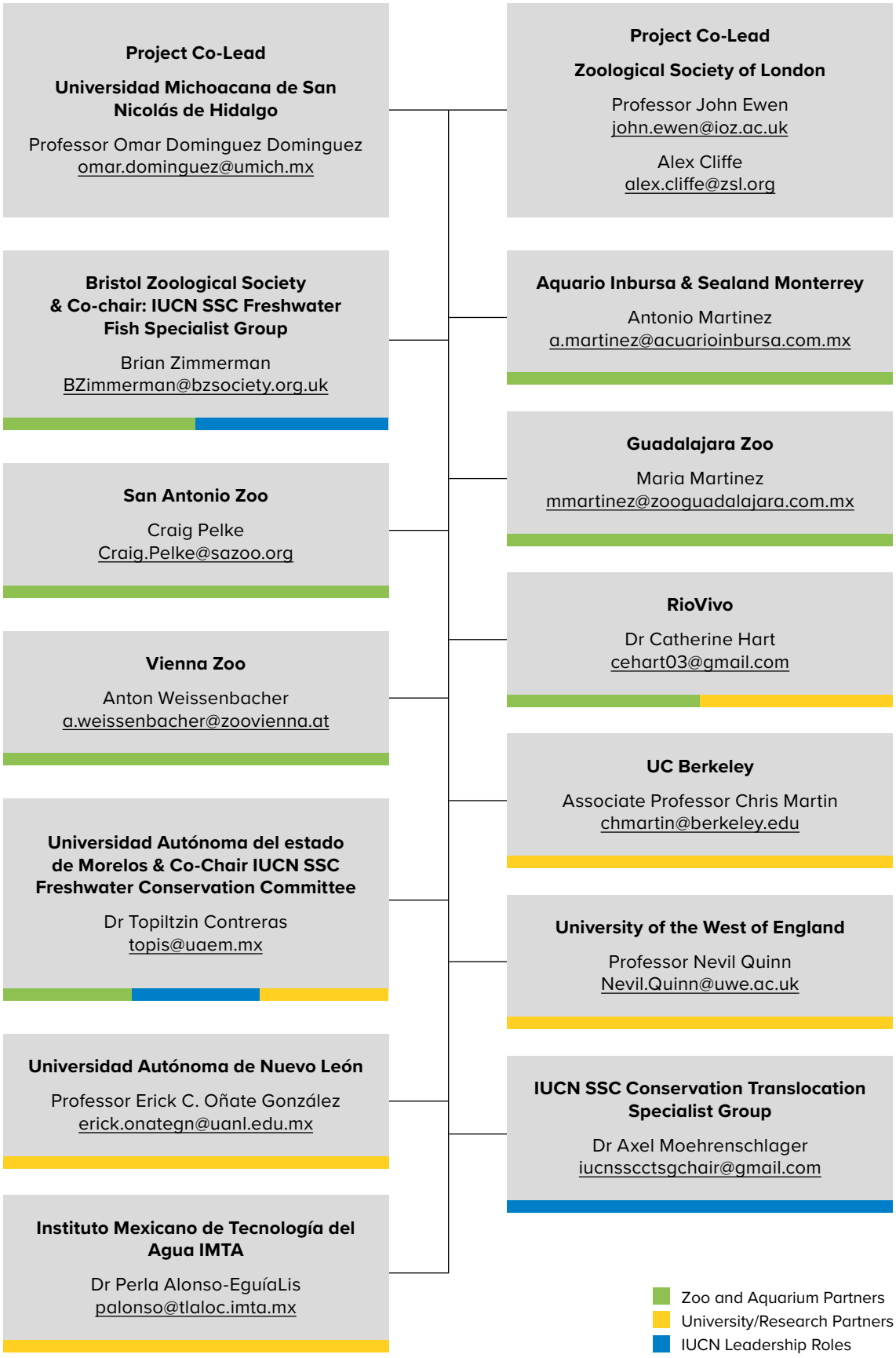
The different projects that he has directed, or in which he has collaborated, have also led to the Aquatic Biology Laboratory, including the Fish Ark Project, to increase in size to now maintain 43 endangered and Extinct in the Wild species. These species make up the Fish Collection of the Universidad Michoacana (Registration with SEMARNAT Mich. PEC-227-07-09).

In addition, Omar has helped create and curate an associated collection of stored fish tissues. This makes the collection of fish and tissues at the Universidad Michoacana an invaluable resource for the study of the world's fish biodiversity, and one of the most important biological collections in México.

> He is the leader of two important conservation projects focused on Extinct in the Wild freshwater fish that have been recognized worldwide, including the successful reintroduction of the Tequila splitfin, *Zoogoneticus tequila*.

He is currently participating in seven research projects with national and foreign collaborators. He has also participated in the preparation of management plans for Natural Protected Areas such as the Zacapu Lagoon and the La Mintzita spring (Michoacán). **He is the leader of two important conservation projects focused on Extinct in the Wild freshwater fish that have been recognized worldwide**, the reintroduction of the Tequila splitfin, *Zoogoneticus tequila*, and the reintroduction of the Golden skiffia, *Skiffia francesae*, to their native ranges.

Organizational Chart for the Implementation Team



SPECIES INFORMATION

La Palma pupfish (male). © Barbara Nicca.

History and Taxonomy

KINGDOM	PHYLUM	CLASS	ORDER	FAMILY
Animalia	Chordata	Actinopterygii	Cyprinodontiformes	Cyprinodontidae

SCIENTIFIC NAME	ENGLISH NAME	SPANISH NAME
Cyprinodon alvarezi	Potosi pupfish	Cachorrito de Potosí
Cyprinodon longidorsalis	La Palma pupfish	Cachorrito de La Palma
Cyprinodon veronicae	Charco Palma pupfish	Cachorrito de Charco Azul / Cachorrito de Charco Palma

México's ichthyofauna is highly diverse and relatively well-studied, with first formal investigations beginning some 250 years ago (Miller et al. 2005). The order Cyprinodontiformes was revised by Parenti (1981) and split into four families: Profundulidae, Fundulidae, Valenciidae, and Cyprinodontidae. This proposal was not widely accepted at the time but there has been very little subsequent debate, and this classification stands (Miller, 2005).

The family Cyprinodontidae is made up of killifishes and pupfish. In spite of their name, cyprinodontids are not closely related to the *Cyprinidae*, the carp family. There are nine genera within the Cyprinodontidae family: *Cualac, Cubanichthys, Cyprinodon, Floridichthys, Garmanella, Jordanella, Megupsilon, Orestias,* and *Pseudorestias* (Freyhof et al. 2017).

The genus *Cyprinodon* (from the Ancient Greek κυπρῖνος (kuprinos), meaning 'carp', and ὀδούς (odous), meaning 'tooth'), the pupfish, represents a unique lineage of fish species with their ancestors being traced as far back as 10-11 million years during the late Miocene. There are more than 40 extant species of pupfish with a global distribution primarily found in México but also the southern United States and the Caribbean islands. Many of these species have very limited ranges and are highly threatened (Martin and Wainwright 2013; Contreras-Balderas and Lozano-Vilano 1996; Martin, Crawford, Turner and Simons 2016). Our proposal focusses on three of these species (Potosi pupfish, La Palma pupfish and Charco Palma pupfish), which were endemic to springs within the Nuevo León State in México and are all currently classified as Extinct in the Wild by the IUCN Red List (IUCN 2019; see **Ex Situ Status**).

The first of these pupfish to be described was the Potosi pupfish (*Cyprinodon alvarezi*) whose scientific name honors Jose Alvarez del Villar (1903-1986) who had originally collected the type specimen in 1952 and intended to describe the species, but instead passed on the type specimen to Robert Miller who took on the task of describing the species about 20 years after collection, in the 1970s. The discovery and description of both the La Palma (*C. longidorsalis*) and Charco Palma pupfish (*C. veronicae*) was much more recent (Lozano-Vilano and Contreras-Balderas 1993) and coincided closely with their rescue into captivity and last records in the wild (for both species the time between discovery and extinction from the wild was about ten years). The Charco Palma pupfish's scientific name honors the niece of María de Lourdes Lozano Vilano and daughter of Salvador Contreras Balderas, Verónica Contreras Arqueita, who assisted on the trip during which the type specimen was collected.

Morphology and Physiology

The specific epithet for *Cyprinodon longidorsalis* (La Palma pupfish) is in reference to the long dorsal fin. La Palma pupfish average around 39mm in length with mature males having a compressed diamond-shaped body, deeper than in females. A depressed dorsal fin at its base is longer than any other species of the group (Figure 1). The head and body are bright blue, with golden reflections. Females average 33.8mm in length and have shallower bodies, dorsal posterior, paler in colour with greyish green appearance and speckled along flanks. In both sexes, the mouth cleft is strongly folded.

The standard length of a male Charco Palma pupfish (*C. veronicae*) is around 39mm long and are distinguished from all congeners by several unique characters, including an anal fin base in length almost double that of maxilla´s length. The body is strongly compressed and diamond shaped, higher in males than in females. Females average 37.6mm in length and have an irregular ocellus on the dorsal fin. Both the head and anterodorsal body is a violaceous blue with each scale with a violet blue centre. The pectoral fins are yellow with a black border. The head and body of the female is a yellowish brown with 6-9 large and darker spots (Figure 2).

Both Charco Palma pupfish and La Palma pupfish apparently form a monophyletic lineage closely related to the Potosi pupfish. The three species have four lachrymal (Potosi pupfish sometimes only has three) and two mandibular pores (Lozano-Vilano and Contreras-Balderas 1993).

The caudal fin of the male Potosi pupfish has a terminal black bar, and the pectoral fins are grey-black. It is the largest of the three species and mature individuals are typically 50-60 mm in length.

All three species have widely overlapping counts of scales, gill rakers, fin rays and cephalic lateral pores, except preopercular ones. Mature males of all three species are metallic blue, although shades and colour distribution may vary with season and species. The

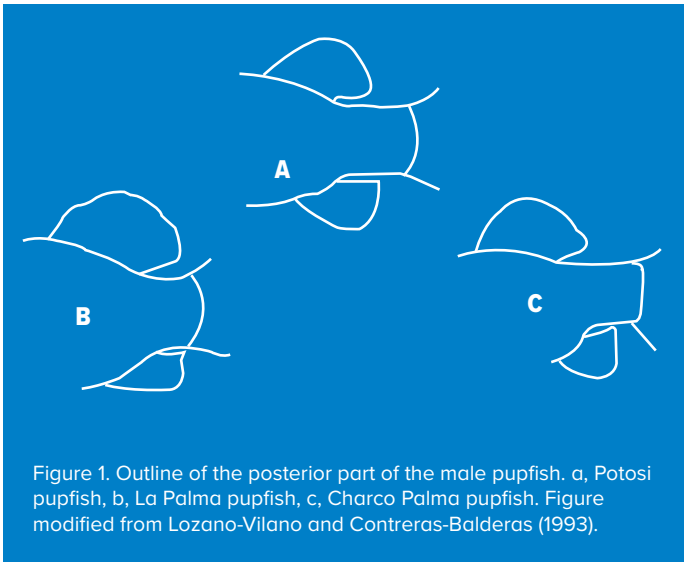


Figure 1. Outline of the posterior part of the male pupfish. a, Potosi pupfish, b, La Palma pupfish, c, Charco Palma pupfish. Figure modified from Lozano-Vilano and Contreras-Balderas (1993).

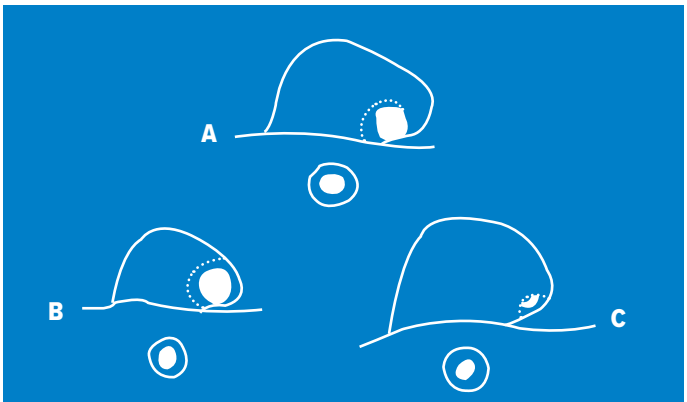


Figure 2. Dorsal fin outlines of female pupfish. a, Potosi pupfish, b, La Palma pupfish, c, Charco Palma pupfish. All scaled in proportion to the eye drawn under each dorsal fin. Figure modified from Lozano-Vilano and Contreras-Balderas (1993).

Potosi pupfish possess distinctively blue rings covering about half of the iris, whereas the Charco Palma pupfish and La Palma pupfish have two coloured rings; the inner golden yellow and the outer pale yellow (Lozano-Vilano and Contreras-Balderas 1993).

All three species possess a long mandible and a prominent jaw which is ideal in grazing filamentous algae which is their predominant food source along with crustaceans and aquatic insect larvae (Moyle et al. 1995).

Pupfishes are capable of persisting in highly mineralized habitats of arid regions (Deacon and Minckley 1974; Miller 1981; Smith 1981). Mineral conditions have proven important for successful ex situ management of all three pupfish, where buffering the water chemistry with minerals such as gypsum, sodium chloride, magnesium and sodium bicarbonate is very effective in improving breeding successes. Cyprinodontids also hold the record for the highest temperature tolerance of any aquatic vertebrate - the Mexican Julimes pupfish, *Cyprinodon julimes*, existing in temperatures of 46/48°C (Miller et al. 2005).

La Palma pupfish male-top, female-bottom. © Dante Fenolio.

Charco Palma pupfish, male-top, female-bottom. © Dante Fenolio.

Potosi pupfish males. © Barbara Nicca.

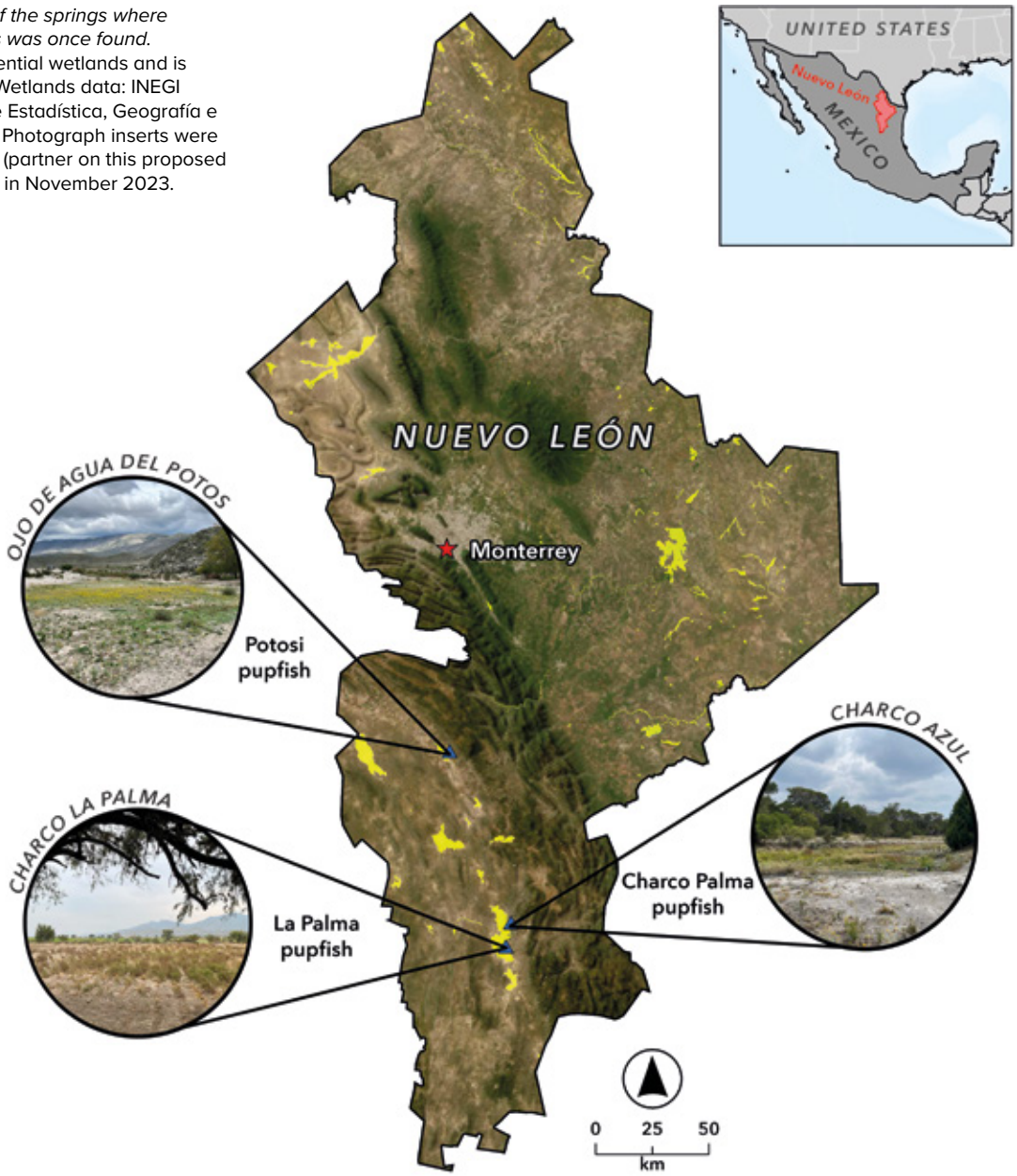


Figure 3. Locations of the springs where each pupfish species was once found. Basemap shows potential wetlands and is from ESRI; Potential Wetlands data: INEGI (Instituto Nacional de Estadística, Geografía e Informática, México). Photograph inserts were taken by Craig Pelke (partner on this proposed project) during a visit in November 2023.

Distribution

The vast majority of pupfish species have extremely limited ranges. We summarize the historic range for each Extinct in the Wild pupfish species here (Figure 3) with current distributions summarized under **Ex Situ Status**.

Potosi pupfish: Potosi pupfish were once restricted to the Potosi Springs northwest of Galeana, Nuevo León, México (Figure 3; Miller et al. 2005).

La Palma pupfish: This species had one of the smallest native range of any vertebrate species, being restricted to a spring pool that covered an 18m^2 shelf (Contreras-Balderas and Lozano-Vilano 1996; Helfman et al. 2009). The Charco la Palma Spring is a small spring within the Bolson de Sandia in Nuevo León, México (Figure 3; Miller et al. 2005; Lozano-Vilano and De la Maza-Benignos 2017).

Charco Palma pupfish: Charco Palma pupfish were restricted to the isolated Ojo de Agua Charco Azul Spring located within the Bolson de Sandia in Nuevo León, México (Figure 3).

Abundance combined with Status and Population Trend

There are no wild populations, with all three species classified as Extinct in the Wild (EW) by the IUCN Red List (Valdes Gonzales 2019a,b,c). No IUCN Green Status assessment – an assessment of the impact of conservation action and recovery potential for a species (IUCN 2021b) has been completed and such an assessment for each species forms part of our proposed project. Details of last wild records and subsequent field searches are summarized from the IUCN Red List accounts (Valdes Gonzales 2019a,b,c) and Smith et al. (2023);

- Potosi pupfish has not been seen in the wild since 1994 (Burkhead 2012) with subsequent surveys of nearby springs also not recording presence of the species.

Potosi pupfish habitat – mineral deposits in spring

- La Palma pupfish has not been seen in the wild since 1994. Given its unique evolutionary and spatial isolation and specific habitat requirements, it is highly unlikely that this species remains extant at other localities.
- Charco Palma pupfish has not been seen in the wild since 1995. There was complete desiccation of the isolated spring system within which it was found by 1997, and there was a subsequent underground fire in 2003. Surveys in nearby springs (in at least 30 km radius) have not recorded the species (A. Valdes pers. comm. 2018 cited in Valdes Gonzales 2019c).

Habitat Use

Pupfish are found in fresh to hypersaline waters (Lyons et al. 2020). Potosi, La Palma and Charco Palma pupfish each inhabited an isolated spring system. The springs that all three species inhabited, ranged from depths of 0.5 m to 2.5 m with males preferring a relatively large surface area to establish their territories. Spawning occurs amongst vegetation, filamentous algae and suitable substrates for the fertilized eggs to adhere to. The recovery of each species will require a stable water table and a good balance of key minerals based on the strata on which each spring is located.

The descriptions of each species' spring were summarized in the IUCN Red List assessments (Valdes Gonzales 2019a,b,c based on Miller et al. 2005) and within an unpublished manuscript from Contreras and Lozano as;

Potosi pupfish: Potosi Springs. High altitude (1,880 m above sea level), clear springs and spring-fed ditches with dense aquatic macrophytes over clay, mud, firm limestone and sandy substrates (Miller et al. 2009). Water temperature fluctuated annually from 16.5-23°C. The pond was also home to another endemic pupfish, Catarina pupfish, *Megupsilon aporus* (now extinct; see **Previous Conservation Actions and Implications**), and an endemic crayfish, *Cambarellus alvarezi*, now also extinct (Alvarez et al. 2010). All species were lost from the spring due to it becoming completely desiccated with the water having been exploited for irrigation and other human uses (Miller et al. 2005). Contreras and Lozano (unpublished) noted that this spring once covered 1 ha from records spanning 1968 to 1983, and that the pools had a maximum depth of about 2.5 m. They noted abundant vegetation of *Ceratophyllum, Najas, Lemna, Nasturtium, Utricularta,* and sparse clumps of *Typha* and *Scirpus*, as described by Smith (1980). They also noted that the original spring must have been smaller and was expanded by means of an earthen and rock dam at some time, one piece of cement had a date inscribed in August 1924.

La Palma pupfish: Charco la Palma Springs. Prior to its desiccation, Charco la Palma was a clear water spring with a temperature of approximately 21°C. It had a muddy substrate with dense algal growth and submerged vegetation (Miller et al. 2009). Contreras and Lozano (unpublished) recorded that in 1984 the spring was about 40 cm deep and slanted on one side to a depth of about 50 cm in other parts; it was fenced around and had a small outlet and little running water that formed a small pool of around 6 m2 and 0.1 m deep. The spring water was used for human consumption and for irrigation of a small orchard, while the outlet pool was used for cattle. They noted that the water was clear and had plenty of aquatic plants. In 1985, Contreras and Lozano (unpublished) noted that the water was 10 cm lower, and the outlet pool was dry, by 1989 they found the water level had dropped another 10 cm and few fishes were visible, a situation that worsened by June 5, 1990.

Charco Palma pupfish: Ojo de Agua Charco Azul Spring. Similarly, to Charco la Palma, the spring was characterized by clear water with a temperature of 19-20 °C. It had muddy and loamy substrates, and vegetation made up of several aquatic macrophytes (Miller et al. 2009). Contreras and Lozano (unpublished) noted that during multiple visits in the 1980s that the site showed signs that it was larger in the recent past. They described an extensive spring complex that had beautifully clear water and a surface of 1.5 ha, with several pools >2 m deep, giving rise to a small creek. This condition appeared stable, except for a drop in water level that stopped flow in the creek by 1985. As with Charco La Palma springs, water extraction led to ever shallower water depth until complete desiccation occurred.

Charco pupfish Whipsnade Zoo.

Local community school child with fish.

Major Threats

Although past conservation interventions prevented certain extinction, continued viability of the three Extinct in the Wild pupfish remains precarious, and each species now faces similar and major threats. We address the major threats here following a progression toward successful wild recovery that will result in downlisting each species' IUCN Red List category.

Threat One: Species Extinction Under Human Care

Being Extinct in the Wild can represent a critical waypoint on the pathway to recovery. Whilst 12 species that were at some point since 1950 restricted to ex situ only populations now have recovered wild populations, there is a contrasting fate where 11 species have gone extinct after only existing in ex situ care, including one pupfish species (Smith et al. 2023). The risk of extinction is increased when a given species has a small number of holders without an integrated management plan and when population sizes remain chronically small such that demographic security is compromised, and populations continue to suffer loss of genetic diversity through genetic drift. The exact population sizes required to achieve demographic security are species- and context-dependent but previous analyses argue for census (total) population sizes in the low thousands of individuals (Brook et al. 2006; Traill et al. 2007; Reed et al. 2003; Frankham et al. 2014). Having several holders for an Extinct in the Wild species helps buffer against any single catastrophe, such as a disease outbreak from causing population failure, or financial failure causing strains on continued population management (e.g. Trask et al. 2020).

Each pupfish species is currently restricted to a small number of holders, all of which are outside of México (see **Ex Situ Status**). Furthermore, the same limited set of holders care for all three species such that any catastrophic event within one holder may well compromise more than one species. The global population sizes for each species only reach into the low hundreds of individuals (see **Ex Situ Status**). Extinction remains a considerable risk and has already occurred for another previously Extinct in the Wild pupfish species, the Catarina pupfish (see **Previous Conservation Actions and Implications**).

Threat Two: Lack of Awareness

That the three pupfish species have been lost from their indigenous range for nearly 30 years and there are no other ex situ holders of the species in México, loss from their homeland risks a loss of connection and awareness that these species were ever an important component of México's freshwater ecosystems. There is a perceived lack of awareness about the crisis facing many of México's freshwater fishes and especially the pupfish (Contreras-MacBeath et al. 2022). An entire generation has grown up using groundwater (see below) without regard to those species that once relied on it. It follows that with no fish there is no competing concern related to water use and no urgent need for balancing environmental concerns alongside other demands for sustainable water management. Successful wild recovery of pupfish will require changes in local behavior toward pupfish and environmental protection (similar to what was found with successful reintroduction of the formerly Extinct in the Wild Tequila Splitfin, *Zoogoneticus tequila* (Domínguez-Domínguez et al. 2018).

Threat Three: Threats to Water

A wide range of ecological threats across México have directly affected many freshwater fish species including urban growth, unsustainable agricultural development, extensive groundwater extraction, and water basin overexploitation. Water basins are further compromised by pollution from agricultural runoff and sewage waste (Lyons et al. 2020).

For all three pupfish species, groundwater overexploitation was the major driver for extinction from the wild with the complete desiccation of their historical habitat (Contreras-Balderas and Lozano-Vilano 1996). Groundwater extraction caused a human induced decline in the water table underneath these springs which in turn caused an underground fire fueled by organic material and heat (Lyons et al. 2020). By 1990 more than 80 wells of >100 m depth had been dug for irrigation of corn and potato fields in the El Potosi and Sandia Valleys, causing the desiccation of springs and creeks in the region. The last pupfish were seen in the wild in 1994 - 1995 (see **Abundance combined with Status and Population Trend**).

Groundwater overexploitation remains a considerable driver of water loss exacerbated by climate change. Our recent site visit (October 2023) shows that the remnant springs for each species, however, are not beyond repair and replenishment. Water remains available and water capture and spring rehabilitation are achievable.

Threat Four: Release and Reinforcement Challenges

The IUCN Motion 119 (IUCN 2021a) calls for the use of reintroductions supported by the IUCN Guidelines for Reintroductions and Other Conservation Translocations (IUCN 2013) for urgent wild recovery of Extinct in the Wild species. Reintroductions and reinforcement (in the sense of both additional releases and other management support such as protection from pollution) can be highly successful actions to recover species. They are essential if the species reintroduced are Extinct in the Wild. However, they are also made challenging due to uncertainties in how the released individuals will settle and survive at release sites (establishment phase; IUCN 2013) and what additional support may be required to allow populations to grow and remain viable (growth and regulation phases; IUCN 2013). These uncertainties are greater when previous releases from which we can learn have not occurred, and as the time (generations) between extinction in the wild and reintroduction increases. Although this combination of factors raises challenges, there are powerful methods for adaptive management (Canessa et al. 2016; Ewen et al. 2023) and a detailed set of guidelines (Guidelines for Reintroductions and Other Conservation Translocation; IUCN 2013) that greatly improve the likelihood of success.

Potosi pupfish habitat.

Charco pupfish habitat evidence of irrigation.

Charco pupfish habitat water container for irrigation.

Use and Trade

Use and trade is not a threat for Extinct in the Wild pupfish. The IUCN Red List accounts for each species conclude no evidence for previous use or trade (Valdes Gonzales 2019a,b,c).

Ex Situ Status

Each species was rescued into captivity during the 1980s and 1990s in response to critical loss of habitat, largely resulting from water extraction for agricultural use. Given the complete loss of wild populations this makes ex situ efforts central to any recovery; each species' fate is literally in our hands. Here we cover three aspects of each species ex situ status, including their rescue, management, and current population size and distribution.

Rescue:

Potosi pupfish - The ex situ population was founded between 1989 and 1994. There were an unknown number of founders.

La Palma pupfish - The ex situ population was founded between 1984 (species discovery) and 1995. There were an unknown number of founders.

Charco Palma pupfish - The ex situ population was founded between 1984 (species discovery) and 1995. There were an unknown number of founders.

Management:

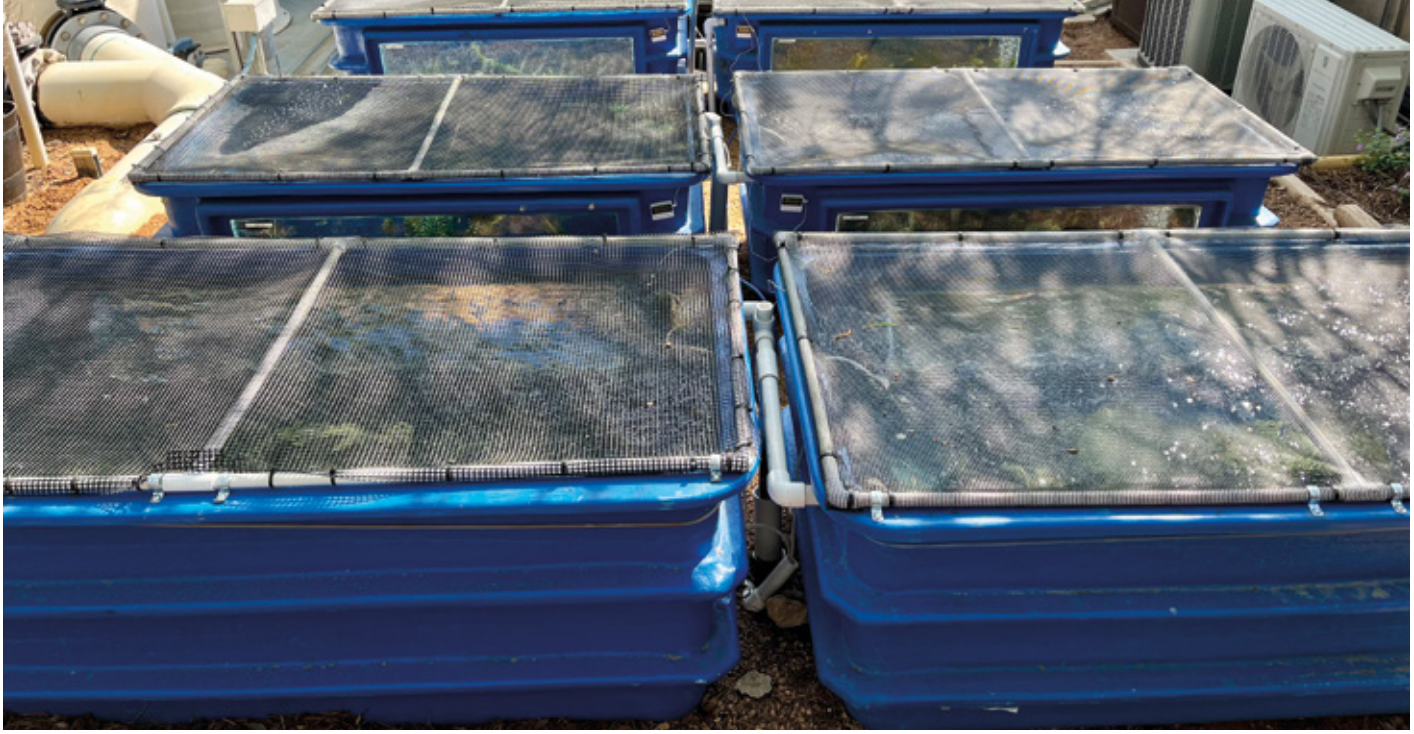
Partly in response to the catastrophic extinction of the then Extinct in the Wild Catarina pupfish (see below), the EAZA approved a new population management structure in April 2017. This established the EAZA Freshwater Teleost Taxon Advisory Group (Freshwater Teleost TAG) currently chaired by Brian Zimmerman (Project partner on this grant and co-chair of the IUCN SSC Freshwater Fish Specialist Group). One of the first tasks of the Freshwater Teleost TAG was the development of a Regional Collection Plan (Weissenbacher et al. 2020) which was approved in November 2019 with the following mission statement:

"To achieve conservation by managing freshwater teleost populations that mainly function as Ark or Rescue populations. A large number of freshwater fishes are threatened with extinction, and several are already Extinct in the Wild. For many of these species ex situ populations can be maintained with relatively few resources and high chances of a successful reintroduction to the wild in the future. The EAZA community has the ability in many cases to rapidly respond to a changing situation in the wild and prevent extinction."

Catching pupfish at Whipsnade Zoo.

An EAZA EEP was then formed under the umbrella of the Freshwater Teleost TAG with a specific focus on Cyprinodontidae including Aphaniidae. Within this EEP, there are 11 priority species of which three are classed as Extinct in the Wild on the IUCN Red List (Valdes Gonzales 2019a,b,c); those being the Potosi pupfish, La Palma pupfish and Charco Palma pupfish covered by our proposal. The Cyprinodontidae including Aphaniidae EEP is hosted by ZSL and currently chaired by Alex Cliffe (Project Co-Leader on this grant).

The global ex situ distribution of each pupfish species is centred in Europe with an additional single holder at San Antonio Zoo in the USA, managed by Craig Pelke (Project partner on this grant). The EEP collaborates directly with San Antonio Zoo and their populations are closely integrated into each species' ex situ management.



San Antonio Zoo pupfish holding.

POTOSI PUPFISH		LA PALMA PUPFISH		CHARCO PALMA PUPFISH	
Holder	**Population**	**Holder**	**Population**	**Holder**	**Population**
Vienna	30	Barneveld	2	Berlin	15
Whipsnade	249	Berlin	2	Münster	12
		Brno	5	San Antonio	101
		Köln	1	Vienna	30
		Münster	12	Whipsnade	108
		Rostock	5		
		Rotterdam	14		
		San Antonio	107		
		Vienna	30		
		Whipsnade	112		
TOTAL	**279**	**TOTAL**	**290**	**TOTAL**	**266**

Table 1. Current populations for Potosi pupfish, La Palma pupfish and Charco Palma pupfish globally. Species 360 Oct 2023.

Current Population:

The same three holders care for the largest and most viable populations of all three species: Whipsnade Zoo, a ZSL Conservation Zoo in the United Kingdom, Vienna Zoo in Austria and San Antonio Zoo in the USA (Table 1). The global populations for each species, however, remain critically small and at risk with 279 individuals of the Potosi pupfish, 290 individuals of the La Palma pupfish, and 266 individuals of the Charco Palma pupfish (Table 1). As a guide, effective populations sizes below 500 do not meet recommendations for maintaining genetic diversity (note that an effective population size of 500 requires an estimated 1900 census population size; Smith et al. 2023; see **Major Threats – Threat One: Species Extinction Under Human Care**). The current ex situ population is in urgent need of revitalisation, substantially increasing global population size for each species and spreading them across more holders. Our first goal targets this by returning each species to México and spreading them safely across multiple holders.

Vienna Zoo off show facilities.

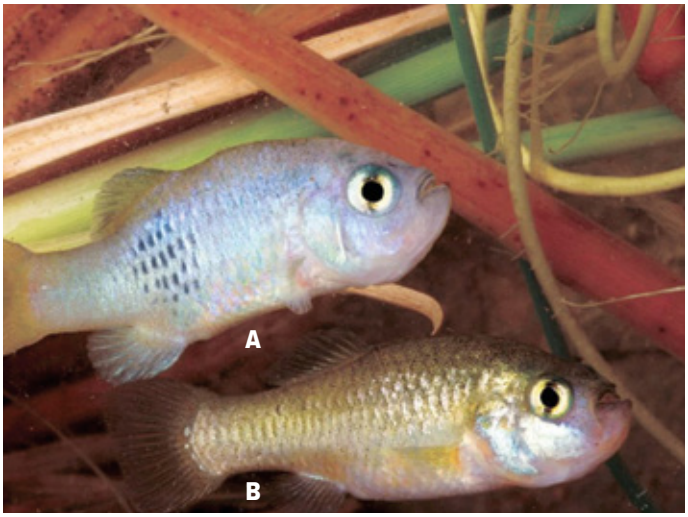
Previous Conservation Actions and Implications

All three pupfish species were successfully rescued to captivity in ex situ facilities over the same decade (1984 to 1995). The establishment of the EAZA Freshwater Teleost TAG and Cyprinodontidae EEP in 2019 set Potosi pupfish, La Palma pupfish and Charco Palma pupfish as priority species, providing them with a more cohesive and effective ex situ management. Through dedicated efforts, the ex situ zoo and aquarium community have established highly successful captive husbandry protocols to keep and breed each species. Whilst each species remains extant, the current distributions and population sizes remain critically small (Smith et al. 2023; see **Ex Situ Status**). To date, no attempts have been made to reintroduce any of these species.

Previous conservation efforts for other pupfish species, however, provide a historical perspective that is highly relevant to our action plan. A combination of extreme failure leading to species extinction contrasts with highly successful release site management and reintroduction outcomes. The lessons learnt have shaped our plan and provided confidence to undertake the first ever actions for wild recovery for three pupfish species. We summarize two key examples here.

Species Extinction from Ex Situ Care:

There are two starkly contrasting options for Extinct in the Wild species to change category on the IUCN Red List; either extinction or recovery of wild populations (see **Major Threats**). One of the 11 species that have gone extinct after only existing in ex situ care was the Catarina pupfish (Valdez-González et al. 2018; Smith et al. 2023). Its extinction in 2014 provides a sobering reminder that rescued pupfish species held in zoos and aquariums as Extinct in the Wild species are not free from risk. The Catarina pupfish is the most recently extinct endemic freshwater fish in México (Valdez-González et al. 2018).



The Catarina pupfish, *Megupsilon aporus*, was a freshwater fish endemic to México; it is now extinct: (a) male, (b) female. Photograph by Daniel Garza Tobón.

The Catarina pupfish was once found alongside the Potosi pupfish in the Potosi Spring. Their habitat was lost primarily due to desiccation (Miller et al. 2009). However, the ultimate failure for this species was insufficient investment in providing coordinated captive care. The species was difficult to maintain, and the already small population was scattered across multiple zoos and aquariums with little integrated infrastructure and monitoring. Catastrophic declines occurred in 2013 without the necessarily alarms being raised and a resulting coordinated response. The last individual tragically died in 2014.

Learning from this catastrophic event has shaped the conservation of the remaining Extinct in the Wild pupfish. There is now a dedicated Cyprinodontidae EEP within the EAZA Freshwater Teleost TAG (see **Ex Situ Status**) that provides much needed coordinated effort to the remaining Extinct in the Wild pupfish species. That said, more needs to be done to revitalize the ex situ population. Our project aims to both secure the ex situ population of each species from extinction, and ready each species for wild recovery, through extending representation within Mexican aquariums, zoos, and universities.

Successful Pupfish Reintroduction:

Threatened fish, particularly those with short breeding cycles like pupfish, are extremely good candidates for reintroduction (Minkley 1995). This is because they can breed readily in captivity, providing individuals for release without jeopardizing the source population, and they have the ability for rapid population establishment and growth if release sites are suitable.

Leon Springs pupfish, *Cyprinodon bovinus*. Female on left and male on right. Images from Thomas, Bonner and Whiteside (2007) (fishesoftexas.org).

For example, the Vulnerable (NatureServ 2013) Leon Springs pupfish, *Cyprinodon bovinus*, was successfully reintroduced to a new site in 2015 (Al-Shaer et al. 2018). Conservation efforts focused on restoring the species' natural habitat, including the removal of bulrush (*Scirpus sp.*) and other vegetation and expanding breeding areas. Extensive post-release monitoring showed the population quickly established over a 14-month period with released males forming territories within 2 weeks and juveniles observed within 14 months (Al-Shaer et al. 2018). The methods used in this study will be highly applicable to other endangered desert pupfish including the Potosi pupfish, La Palma pupfish and Charco Palma pupfish (Al-Shaer et al. 2018). Such rapid population establishment provides confidence in our project's objective for downlisting IUCN Red List category for each species within the required timeframe.

Data Gaps

Reintroducing an Extinct in the Wild species is made more difficult due to uncertainty in which individuals to release and what support they, and their progeny, may require to successfully re-establish a wild population (Canessa et al. 2016). Furthermore, removing individuals from ex situ populations needs to be done such that the ex situ population's viability remains intact, at least until wild populations have fully restored with long-term security (e.g., Trask et al. 2021; Canessa et al. 2016). In most cases, successful biological recovery cannot be achieved in isolation from public enthusiasm and committed support, this has been recognized through work with other species of Mexican freshwater fish (Contreras-MacBeath et al. 2022; Domínguez-Domínguez et al. 2018). Species which are Extinct in the Wild may have two contrasting relationships with local communities; either a strong sense of loss and desire for their successful return, or sadly a loss of connection with little awareness that these species were once an important part of local biodiversity.

Uncertainty, through a lack of knowledge or data gaps, does not prevent conservation action but neither should it be ignored. Responsible and bold action is encouraged but with full account of uncertainty and within a powerful adaptive management framework (IUCN, 2013; Ewen et al. 2023). Critically, this helps to ensure that monitoring is directly tied to management such that decisions about what should be done are clearly stated and data collected during monitoring are fed back into management to adjust actions strategically (Ewen et al. 2023). Formal adaptive management is powerful and efficient. Here we briefly summarize data gaps which our project will learn about through monitoring and analysis. We view resolving these data gaps as providing an extremely high value of information (Canessa et al. 2015) for successful recovery of each pupfish species.

Pupfish biology:

There are three broad aspects of pupfish biology that are important to inform management both pre- and post-reintroduction:

1. **Genetics.** We need to learn about the current distribution of genetic diversity across the ex situ population as well as confirm population provenance. This will allow us to select the right individuals to send to México for establishment of a Mexican ex situ population and select the right individuals for reintroduction.
2. **Disease.** Any movement and release of pupfish also risks movement and releases of fish pathogens which could compromise individuals' health. Knowing what pathogens may be present and how to best manage those risks is crucial for a successful reintroduction.
3. **Demography.** Any movement and release of pupfish needs to balance the continued demographic viability of the ex situ population with the successful establishment and growth of a wild population. Population trends in both the ex situ and wild populations will be driven by survival and reproduction (dispersal is less relevant in isolated springs). Survival and reproduction may be different between ex situ and wild fish and may also be different for released fish and those subsequently born in the wild. Our decisions for ex situ harvest for releases and release site management (see below) will be made using the best current knowledge of each pupfish species and knowledge accrued through strategic monitoring (Ewen et al. 2007) and adaptive management (Canessa et al. 2016). Monitoring establishment and growth of wild populations is an essential component of our project to confirm conditions are met for IUCN Red List downlisting.

Public awareness and support:

Each pupfish species has been Extinct in the Wild since the mid-1990s and remains absent from México completely. This means many Mexicans, and especially local communities, have not likely seen these pupfish for at least 30 years. We don't currently know how this has affected local public awareness about pupfish and attitudes to their conservation. Successful recovery is highly dependent on local public support (Contreras-MacBeath et al. 2022; Domínguez-Domínguez et al. 2018), especially given the shared and limited resource of water for both local communities and fish. We plan to understand the current public awareness and attitude and monitor how effective our educational and awareness goals are for driving long-lasting public pride and commitment to pupfish conservation.

Release site condition:

Although our knowledge of each species' reintroduction site is improving and we are feeling confident that we can achieve successful reintroduction, there are still key data gaps in knowing the exact requirements to ensure suitable habitat in the short and long term. Firstly, we need to understand the current and future hydrological profiles of each site so we can ensure continued water availability. Secondly, linked to this is the structural integrity of each release site and what might be needed to better capture and ensure water security as well as protection from any agricultural pollutants such as herbicides. Thirdly, we need to better understand any other minimal management required to protect establishing populations from any other threats including non-native animal and plant species.

Section 3

CONSERVATION ACTION PLAN

Overview

For the last 30 years México's Extinct in the Wild pupfish have existed in precariously small numbers, cared for in a small number of dedicated ex situ facilities including zoos and aquariums, outside of México. These three species, the Potosi pupfish, La Palma pupfish and the Charco Palma pupfish once occurred in relatively close proximity in the Nuevo León State in México, in similar habitats, and suffered the similar threats that resulted in their extinction from the wild by the early- to mid-1990s.

They were not the only pupfish rescued from these springs, with one other species tragically going extinct whilst under ex situ care (the Catarina pupfish) in 2014. This extinction event prompted an increased focus on careful management of the three remaining pupfish species. At the same time there was an increased effort to understand the wider plight of freshwater fishes in México and efforts to conserve them (Lyons et al. 2020). Whilst ex situ pupfish management is much improved, the critical risks to the remaining populations continue to be significant. This action plan will **revitalize** the ex situ populations of each species to guard against extinction as well as provide numbers of pupfish for upcoming planned **releases** over the next five years, **establishing the first wild populations for each species in nearly three decades**. Following this, populations will be reinforced and supported as required, resulting in the successful downlisting of each of these three species on the IUCN Red List. Our plan is responsible yet bold and will draw on cutting edge conservation science in line with IUCN best practice.

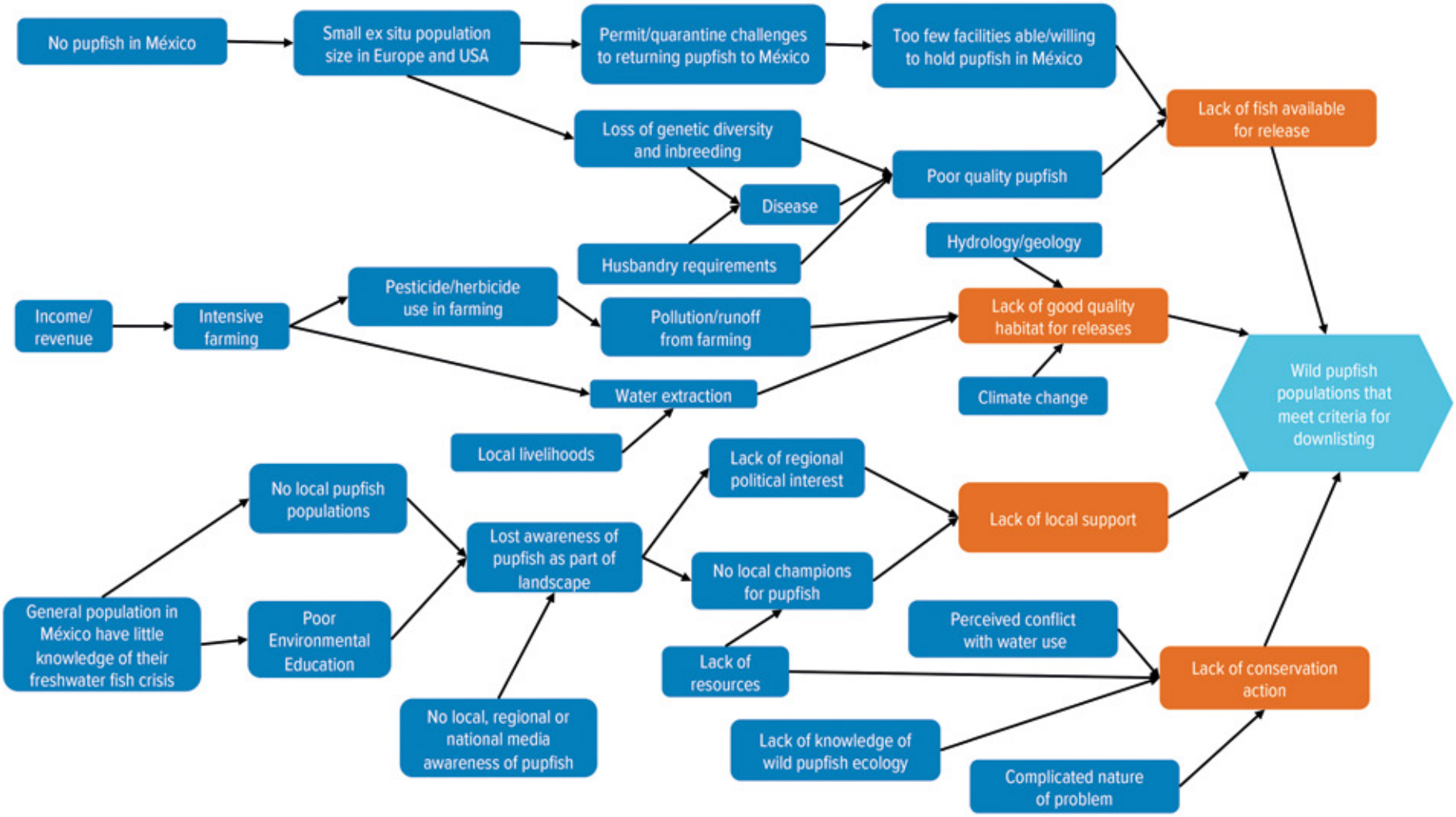
The IUCN has recognized that Extinct in the Wild species sit in an awkward position on the Red List. Species in this category are not assessed in detail under the Red List process given that extinction risk is based on status and trends in wild populations only. As such, the extent of, and variation in, extinction risk is largely overlooked in the set of species for which humans are most responsible and whose fates are far from assured (Smith et al. 2023). In response to this the IUCN SSC Conservation Translocation Specialist Group established an Extinct in the Wild Taskforce in 2018 to champion awareness about, and action to recover, this group of species. At the 2021 IUCN World Conservation Congress in France, the task force's motion for "Improving Process and Action to Identify and Recover Extinct in the Wild Species" was officially endorsed through 95% of 115 governments and 99% of 572 non-government organizations globally (IUCN 2021). Our plan is a direct response to these calls and will simultaneously deliver wild recovery of three currently Extinct in the Wild animal species. Through the example of these pupfish, we will inspire hope that a return to the wild is possible for species ranging from plants to birds and mammals, currently only existing in ex situ populations under human care in zoos, aquaria and botanic gardens.

Our plan brings together a powerful collaborative group of Mexican and international specialists, capable of delivering success across four key conservation goals. Together and with the support of this funding, we will achieve our shared vision of thriving wild populations restored within each species indigenous range, alongside carefully managed and viable ex situ populations. **A fundamental mark of success will be the downlisting of each species on the IUCN's Red List**. Ex situ specialists that currently care for each pupfish species will align and facilitate the transfer of fish and knowledge to Mexican partners at zoos, aquariums and universities. This significant step will bring three species, endemic to México, back home for the first time since their extinction from the wild. Social science and educational specialists will engage with local and national communities to raise critical awareness of the plight of México's pupfish, leading to improved environmental awareness and ultimately local pride and leadership in protecting wild habitats for pupfish. Reintroduction biologists, freshwater fish ecologists and hydrologists will combine with all partners to deliver the monumental return of wild populations of each species.

Vision

All three species of Extinct in the Wild pupfish will be restored to their indigenous lands where they will exist as viable and vibrant in situ and ex situ populations. All three species will meet downlisting criteria to no longer be considered as Extinct in the Wild on the IUCN red list within 10 years. Communities will become active stewards of the fish and take in the restoration of their local environment. Through the example of the pupfish we will inspire hope that a return to the wild is possible for Extinct in the Wild species.

Issues



Goals:

GOAL 1:	GOAL 2:	GOAL 3:	GOAL 4:
Repatriate pupfish to México as thriving ex situ populations	Maximise local project pride, ownership and support of pupfish recovery in the wild	Establish wild populations of each pupfish species built through cutting edge adaptive management	Maximise the education potential of both ex situ and in situ pupfish conservation to inspire sustainable land use and pupfish protection

Goals, Objectives and Tactics Overview

Goal 1: Repatriate pupfish to México as thriving ex situ populations

Objective 1. Implement best practice selection of individuals from each pupfish species for transfer to México.

Tactic 1.1 Undertake genetic analyses of the ex situ population across three primary holders to confirm provenance and develop a management plan to best increase genetic diversity in the founder populations.

Tactic 1.2 Undertake a disease risk analysis for shipping pupfish to identified facilities in México and determine appropriate quarantine procedures.

Tactic 1.3 Increase breeding effort to maximize yield of fish from European and/or USA Zoos to allow sustainable harvest for transfer to México.

Objective 2. Ensure legal shipment and holding of each pupfish species in México.

Tactic 2.1 Ensure all permits for export of pupfish from source countries are obtained.

Tactic 2.2 Obtain all permits for import of pupfish from CONACYT including permissions to disseminate pupfish across participating zoos, aquariums and universities as the ex situ population establishes.

Objective 3. Improve Mexican capacity for holding and breeding each pupfish species.

Tactic 3.1 Improve Mexican aquarist husbandry skills through hosting and training at current pupfish holding zoos in the EU, UK, and the USA as well as visits by experienced staff from current holding zoos to Mexican facilities receiving pupfish.

Tactic 3.2 Provide Mexican zoos, aquariums and universities who will hold pupfish with up-to-date standard operating procedures for pupfish care and aquarium design and maintenance.

Tactic 3.3 Transport of pupfish to México and then distribution between holders in México.

Tactic 3.4 Develop a national network for pupfish breeding management in México as an extension of the current EAZA EEP.

Tactic 3.5 Initiate a national knowledge sharing forum in México to inspire sharing of latest best practice.

Goal 2: Maximise local project pride, ownership and support of pupfish recovery in the wild

Objective 4. Raise local awareness of pupfish recovery and environmental management of water resources.

Tactic 4.1 Survey local public attitudes to environmental management and pupfish recovery.

Tactic 4.2 Recruit local leaders and influencers as local pupfish champions.

Tactic 4.3 Develop an environmental education campaign focused on local villages for each species' release site.

Tactic 4.4 Develop a communications strategy for local and national media and local community forums including campaign materials to help 'spread the word'.

Tactic 4.5 Develop a pupfish film that provides core messages about each species, their conservation and wider environmental awareness.

Objective 5. Involve locals directly in all phases of pupfish recovery.

Tactic 5.1 Actively involve local participation in all phases of the project.

Tactic 5.2 Ultimately hand over long-term management to local communities.

Goal 3: Establish wild populations of each pupfish species built through cutting edge adaptive management

Objective 6. Select the best release site/s for each pupfish species.

Tactic 6.1 Determine best location for release of each pupfish species based on currently available water resources, isolation from pollutants and non-native species, and ability to restore or create persistent water bodies (either or both reintroduction and assisted colonization).

Tactic 6.2 Model the best minimal management required to ensure release sites remain suitable for the short- (10 yrs) and medium-term (25 yrs).

Tactic 6.3 Model hydrological robustness of sites into the future under predicted land use and climate change (>25 yrs).

Objective 7. Restore or enhance water security at selected release sites.

Tactic 7.1 Small scale engineering to prevent water loss and to store excess water at each release site.

La Palma pupfish. © Heiko Kärst.

Tactic 7.2 Small scale engineering to capture pond runoff and recycle for agricultural use.

Tactic 7.3 Create a natural buffer around each release site to prevent pollution from farm runoff.

Objective 8. Initiate releases of each species, testing critical assumptions about how long-term captivity, and changed in situ habitat, influence survival and reproduction.

Tactic 8.1 Select appropriate individuals from each pupfish species for sustainable releases.

Tactic 8.2 Reduce disease risks through the release of each pupfish species.

Tactic 8.3 Establish a robust post-release monitoring methodology that tracks population vital rates (survival and fecundity) in closed release sites for each pupfish species.

Tactic 8.4 Establish a robust monitoring methodology for tracking release site water volume and quality. Adjust if needed.

Tactic 8.5 Establish a robust monitoring methodology for tracking pupfish foraging and anti-predator behavior's post-release. Adjust pre-release conditioning as needed.

Tactic 8.6 Establish a robust monitoring methodology for presence and impact of non-native vegetation and fish competitors/predators. Remove as needed.

Tactic 8.7 Track genetic diversity over time in each natural population by collecting fin-clips and sequencing the genomes of all founder individuals.

Tactic 8.8 Green status assessment for the three species.

Objective 9. Ensure criteria for downlisting is met within life of the project.

Tactic 9.1 Obtain accurate estimates of fish reproduction and recruitment. Track population growth through a bespoke integrated population model. Show survival, breeding and recruitment occurring and sustained across the 'five-year rule' and project the population beyond.

Tactic 9.2 Establish long-term minimum management that is of low intensity such that each pupfish population continues its growth and reaches its regulation phase without need for continual releases of animals.

Goal 4: Maximise the educational role of both ex situ and in situ pupfish conservation to inspire sustainable land use and pupfish protection

Objective 10. Create educational exhibits across México's zoos, aquariums and universities.

Tactic 10.1 Distribute each pupfish species nationally to zoos and aquariums who can create an educational exhibit and contribute to conservation breeding.

Tactic 10.2 Distribute each pupfish species nationally to universities for applied research from graduate and post-graduate students.

Tactic 10.3 Target creation of educational exhibits within the Nuevo León state including in the capital of Monterrey.

Objective 11. Create ongoing opportunities for local interaction and study of pupfish in México.

Tactic 11.1 Build educational ponds or aquariums at schools and/or municipal buildings local to release sites for each pupfish species.

Tactic 11.2 Partner with Mexican universities to provide community research projects for education and applied outcomes (i.e. citizen science).

Goals, Objectives and Tactics

Goal 1: Repatriate pupfish to México as thriving ex situ populations

Ensuring that:

- These three species of Mexican pupfish are finally back in México.
- Each species is well represented in México with a viable ex situ population with suitable numbers and quality for releases and for education.
- Specialist husbandry skills and holding tank parameters are transferred to Mexican partners and these are continually improved and updated to ensure the best possible captive care.

Objective 1. Implement best practice selection of individuals from each pupfish species for transfer to México.

Rationale:

The global population of each pupfish species remains precariously small and only exists outside of México. We desperately need to revitalize the ex situ populations of each species to reduce extinction risk (tactic 1.3). It is important that this is led by repatriation of fish to México and supporting Mexican partners on pupfish conservation both in captivity but also in releases and wild recovery. Each pupfish species has been restricted to human care in captivity since the 1990s and there has been limited movement of animals between the three major holders. There are demographic (tactic 1.3), genetic (tactic 1.1) and health (tactic 1.2) risks with harvesting the current ex situ population for translocation and establishment of an ex situ breeding population in México. Completing the relevant assessments and growing breeding capacity will ensure the correct individuals are selected for translocation, using the best methods available to reduce risk, and therefore establish a thriving ex situ population of each species in México.

NO.	TACTIC	PRIORITY	RESPONSIBILITY	INDICATORS OF PROGRESS	WHAT WE HAVE	WHAT WE NEED
1.1	Undertake genetic analyses of the ex situ population across three primary holders to confirm provenance and develop a management plan to best increase genetic diversity in the founder populations.	Year 1	UC Berkeley; ZSL; Vienna Zoo; San Antonio Zoo;	DNA samples collected from each species and population. Genomic sequencing and analysis completed. Populations for shipping to México identified.	Tissue banks available for DNA extraction. Expertise, methods and a genetics lab.	Funding to pay for genomic sequencing and data analysis.
1.2	Undertake a disease risk analysis for shipping pupfish to identified facilities in México and determine appropriate quarantine procedures.	Year 1	ZSL; Vienna Zoo; San Antonio Zoo; Aquario Inbursa (receiving aquarium)	List of disease hazards. Understanding of quarantine requirements for export and import. Expert report on risk and effective mitigation.	Veterinary specialists who deliver disease risk analyses for animal movements.	Funding for the collation of disease hazards for pupfish transfer, review of country requirements for export and import, and expert report on risk and mitigation.

NO.	TACTIC	PRIORITY	RESPONSIBILITY	INDICATORS OF PROGRESS	WHAT WE HAVE	WHAT WE NEED
1.3	Increase breeding effort to maximize yield of fish from EU, UK and/or USA Zoos to allow sustainable harvest for transfer to México.	Year 1	ZSL; Vienna Zoo; San Antonio Zoo	Increased ex situ population size that allows harvesting for shipping to México without decreasing current population sizes. Successfully increased productivity of EU, UK &/or USA Zoo populations. Harvest model established to confirm sustainable harvest rate.	Current ex situ breeding population size (Potosi pupfish 279; La Palma pupfish 290; Charco Palma pupfish 266). Studbook management through EAZA EEP.	Increase in current population breeding effort. Additional tanks for holding fish. Development of a sustainable harvest model.

Objective 2. Ensure legal shipment and holding of each pupfish species in México.

Rationale:

These three species of endemic Mexican pupfish are currently absent from México and a core vision of our project is repatriation of each species back to their homeland where they will thrive under the care of Mexican communities and relevant holders. To do this we need to ensure we have obtained the correct permits for export (tactic 2.1) and import (tactic 2.2). We also promote the spread of pupfish across several specialist ex situ holders (tactic 2.2) such that the population is thriving with little chance of extinction, with ample potential for harvesting for wild releases, and for educational and research purposes.

NO.	TACTIC	PRIORITY	RESPONSIBILITY	INDICATORS OF PROGRESS	WHAT WE HAVE	WHAT WE NEED
2.1	Ensure all permits for export of pupfish from source countries are obtained.	Year 1	ZSL; Vienna Zoo; San Antonio Zoo;	Export permits awarded from relevant authorities.	Animal moves officers at source zoos whose role is to obtain permits and coordinate moves.	Funds for permit application costs and staff time for processing.
2.2	Ensure all permits for import and holding of pupfish are obtained in México including permissions to disseminate pupfish across participating zoos, aquariums and universities as the ex situ population establishes.	Year 1	Aquario Inbursa (receiving lead for import); Guadalajara Zoo; Sealand Monterrey; RioVivo; Universidad Autónoma del estado de Morelos; Universidad Autónoma de Nuevo León	Import permits awarded from relevant authorities. National transfer and holding permits awarded by relevant authorities.	International Affairs Coordinating Unit at Aquario Inbursa will obtain import permits. All participating Mexican zoos, aquariums & universities have specialist staff to obtain transfer & holding permits.	Funds for permit application costs and staff time for processing.

Objective 3. Improve Mexican capacity for holding and breeding each pupfish species.

Rationale:

Effective pupfish management for breeding and releases requires specialist husbandry skills which have been developed through years of captive care provided by the three current holders based in the EU, UK, and the USA. Repatriation of fish requires that these skills be transferred effectively to Mexican aquarists (tactic 3.1) and then ensure these skills are supported by detailed standard operating procedures (tactic 3.2) which can be continually improved through critical evaluation and discussion (tactic 3.4). At the same time, we need to carefully manage the ex situ population of each species to maintain their viability and provide capacity to harvest for releases and wild recovery. Currently the global population is managed under an EAZA EEP and the coordinator of this sits at the ZSL. We propose that the growing populations continue to be managed as one global unit within the existing EEP structure (tactic 3.3).

NO.	TACTIC	PRIORITY	RESPONSIBILITY	INDICATORS OF PROGRESS	WHAT WE HAVE	WHAT WE NEED
3.1	Improve Mexican aquarist husbandry skills through hosting and training at current pupfish holding zoos in the EU, UK, and the USA as well as visits by experienced staff from current holding zoos to Mexican facilities receiving pupfish.	Year 1-2	ZSL; Vienna Zoo; San Antonio Zoo; Aquario Inbursa; Sealand Monterrey; Universidad Autónoma del estado de Morelos; Guadalajara Zoo; RioVivo; Universidad Autónoma de Nuevo León	At least one staff from each zoo, aquarium, or university planning to hold any of the pupfish species has visited and received training in required husbandry skills. Once fish arrive in México, a visit from an experienced husbandry specialist from a current holder to help guide introduction of fish to the population.	Deep and specialist husbandry knowledge at current holding zoos. Identified zoos, aquariums and universities committed to holding pupfish populations.	Funds for travel and subsistence for Mexican aquarists to visit and learn from current holding zoos. Funds for travel and subsistence for EU, UK or USA based pupfish aquarists to visit Mexican facilities receiving fish.
3.2	Provide Mexican zoos, aquariums and universities who will hold pupfish with up-to-date standard operating procedures for pupfish care and aquarium design and maintenance.	Year 1	ZSL; Vienna Zoo; San Antonio Zoo; Bristol Zoological Society	Documents revised, published and provided to Mexican aquariums, zoos and universities.	Draft and/or internal standard operating procedure documents from each current holding zoo. Keeper experience.	Human resources to allow production of a final set of standard operating procedures that draw from all zoos experience. Design, formatting and printing costs.
3.3	Transport of pupfish to México and then distribution between holders in México	Years 1-5	Aquario Inbursa; Sealand Monterrey; Universidad Autónoma del estado de Morelos; Guadalajara Zoo; RioVivo; Universidad Autónoma de Nuevo León; Zoological Society of London; San Antonio Zoo; Vienna Zoo; Bristol Zoological Society	Fish safely established as ex situ populations in México.	Aquarium and zoo specialists in moving pupfish.	Funding for international and domestic movement of pupfish.
3.4	Develop a national network for pupfish breeding management in México as an extension of the current EAZA EEP.	Years 1-5		Single global breeding management structure established that provides annual recommendations on fish moves and reproduction.	Ex situ breeding population management via EAZA EEP. EAZA EEP chaired by ZSL and EAZA Freshwater Teleost TAG chaired by Bristol Zoological Society	Funds for expanding and maintaining the scope of the current global breeding program. Online meetings for developing recommendations.

NO.	TACTIC	PRIORITY	RESPONSIBILITY	INDICATORS OF PROGRESS	WHAT WE HAVE	WHAT WE NEED
3.5	Initiate a national knowledge sharing forum in México to inspire sharing of latest best practice.	Years 1-5	Aquario Inbursa; Sealand Monterrey; Universidad Autónoma del estado de Morelos; Guadalajara Zoo; RioVivo; Universidad Autónoma de Nuevo León	Annual meeting established within México. Meeting report circulated to Mexican and international partners shortly after each meeting.	A proposed and committed list of holders interested in caring for one or more of the pupfish species	Resources to cover cost of hosting an annual meeting including travel. Meeting group size of about 10-15 people.

Goal 2: Maximise local project pride, ownership and support of pupfish recovery in the wild

Ensuring that;

- Local communities including representatives from the ejidos (communal farmlands where fish habitat exists for each species), youth groups from schools, and local politicians have pride and ownership of pupfish recovery.
- Local communities know about pupfish as an example of lost biodiversity and show excitement for the rare opportunity of restoring lost biodiversity.
- Pupfish conservation will be sustained in the long term.

Objective 4. Raise local awareness of pupfish recovery and environmental management of water resources.

Rational:

All three pupfish species were lost from their indigenous range more than 25 years ago. The causes of this loss mostly center around poor environmental management of water resources exacerbated by climate change. Locals have therefore lost any connection, and potentially memory, of these fish as a part of their local landscapes. A critical element of successful biodiversity conservation is improved awareness of local biodiversity and how to safeguard it through more sustainable practices. Extinct in the Wild species offer an additional level of empowerment given their fate is tangibly held in the hands of local communities and those who hold populations ex situ. With the right support and motivation, we believe we can empower local populations by building pride and ownership of their pupfish. This starts with awareness. By identifying local pupfish champions (tactic 4.2) and providing them resources we hope they can fast track local project ownership and create a local, and trusted, voice campaigning for improved environmental management.

NO.	TACTIC	PRIORITY	RESPONSIBILITY	INDICATORS OF PROGRESS	WHAT WE HAVE	WHAT WE NEED
4.1	Survey local public attitudes to environmental management and pupfish recovery.	Years 1, 3 & 5	Universidad Autónoma del estado de Morelos; Universidad Michoacana de San Nicolás de Hidalgo; Universidad Autónoma de Nuevo León; Local communities.	Survey designed and deployed in year one and again in year five to capture any changes in public attitude linked to the project. Survey reports published.	University partners with social science expertise.	Funding for survey materials (both online and in print) and travel. Funding for social science consultant to run or supervise surveys.
4.2	Recruit local leaders and influencers as local pupfish champions.	Years 1-5 (and beyond)	Local university, aquarium and zoo partners.	Local leaders and influencers established for each pupfish species and location.	Experience in building local leadership and ownership of freshwater fish conservation in México and abroad.	Funding for site visits and community meetings. Funding for project updates.

NO.	TACTIC	PRIORITY	RESPONSIBILITY	INDICATORS OF PROGRESS	WHAT WE HAVE	WHAT WE NEED
4.3	Develop an environmental education campaign focused on local villages for each species' release site.	Years 1-5 (and beyond)	Guadalajara Zoo; local schools and administration	Education materials for local schools produced and pupfish lessons delivered to students. Education/info materials for community events (e.g. tailored to local politicians, landowners, and religious groups) (at least four events targeted per year). All updated at least annually as project evolves.	Experience in building environmental education campaigns by Mexican zoos and aquariums.	Funding for educational materials. Funding for site visits from zoo and aquarium education specialists.
4.4	Develop a communications strategy for local and national media and local community forums including campaign materials to help 'spread the word'.	Years 1-5 (and beyond)	All partners. Local leaders and influencers.	Communications strategy published early in the project and updated at least annually as project evolves. Set of campaign materials produced in year 1 and updated as project evolves. Community forums attended and pupfish themed activities delivered.	Experience in building environmental education campaigns by Mexican zoos and aquariums.	Funding for promotional materials and media strategy. Funding for local and regional campaigns. Funding for pupfish conservation campaigns in Mexican zoos and aquariums that hold pupfish.
4.5	Develop a pupfish film that provides core messages about each species, their conservation and wider environmental awareness.	Year 1 & 5	Film media consultant and all partners as relevant	Year 1 film produced showcasing pupfish plight and environmental protection needs plus social attitudes across local, national and international communities. Year 5 film produced showcasing project success and its impact on social attitudes.	A wealth of knowledge and stock images/film.	Funds to produce two films.

Objective 5. Involve locals directly in all phases of pupfish recovery.

Rationale:

Species focused conservation projects gain strength from local leadership. We are conscious that pupfish are currently held at facilities outside México and our priorities are to bring these fish back home to the nation, but then also quickly onward to their local indigenous ranges. The best and most sustainable long-term management will be achieved if local communities are fully engaged and increasingly lead sustained recovery efforts. The successful wild recovery of these pupfish species will mean these local communities can join a select few groups of people who have successfully returned a species previously lost from the wild. Their success will be heartfelt and reverberate across México and the globe due to its significance.

NO.	TACTIC	PRIORITY	RESPONSIBILITY	INDICATORS OF PROGRESS	WHAT WE HAVE	WHAT WE NEED
5.1	Actively involve local participation in all phases of the project.	Years 1-5	Local communities and all partners	All operational tactics specified within this action plan to include offers of local representation. Locals offered training and experiential opportunities at Mexican universities, zoos or aquariums (estimated 6x locals per year).	Network of universities, zoos and aquariums with experience in training and experiential opportunities. Varied and frequent opportunities in the recovery project for local involvement.	Funds to facilitate local involvement in operational tactics of project. Funds to offer training or experiential bursaries.
5.2	Ultimately hand over long-term minimal management to local communities.	As much as possible by Year 5 and increasingly beyond.	Local communities	Local community led management of each pupfish species supported by project partners as requested.	Experience in building local leadership and ownership of freshwater fish conservation in México and abroad.	Local project leaders and volunteers.

Goal 3: Establish wild populations of each pupfish species built through cutting edge adaptive management

Ensuring that;

- We achieve successful wild population establishment of three pupfish species currently Extinct in the Wild.
- We successfully downlist each species to a lower threat category on the Red List following the ‘five-year rule’ but within 10 years of receiving project funding.
- Our method showcases cutting edge and science based adaptive management for species recovery.

Objective 6. Select the best release site/s for each pupfish species.

Rationale:

A cornerstone for successful releases (population establishment and growth) is release site selection (tactic 6.1) (Section 5 & 7 of the IUCN Guidelines for Reintroductions and Other Conservation Translocations) (IUCN 2013). Each pupfish species will require an aquatic environment which has consistent availability of water that is of sufficient quality to meet its biological needs. Importantly, habitat suitability needs to be sustained in the long term to ensure wild population viability. We know that habitats have been impacted by extensive farming including water extraction, climate change and invasive species. Some minimal management will be required (tactics 6.2 & 6.3), for example improved community water management, and identifying this will not only ensure successful pupfish recovery but also can help drive local community led environmental protection. Critically, these types of habitat management still allow a ‘wild’ designation by the IUCN Guidelines for Using Red List Categories and Criteria (Section 2.1.4; IUCN 2022).

NO.	TACTIC	PRIORITY	RESPONSIBILITY	INDICATORS OF PROGRESS	WHAT WE HAVE	WHAT WE NEED
6.1	Determine best location for release of each pupfish species based on currently available water resources, isolation from pesticides and non-native species, and ability to restore or create persistent water bodies (either or both reintroduction and assisted colonization).	Years 1-2	Hydrology consultant; IUCN CTSG; IUCN Freshwater Fish Specialist Group; Zoological Society of London; Universidad Autónoma del estado de Morelos; Universidad Michoacana de San Nicolás de Hidalgo; Universidad Autónoma de Nuevo León; University of West England; Bristol Zoological Society	Site visit completed by specialist team including fish ecologists, a hydrologist consultant, social science experts and local community representatives. Local hydrological analysis completed by consultant. Map produced of potential release sites including water availability, invasive species presence and farm proximity. Workshop with all stakeholders completed and decision on best release sites made.	Specialists in conservation translocations, freshwater fish conservation, hydrology, social sciences and invasive species management. Historical range maps.	Funding to facilitate engaging with local representatives at each potential release site for each pupfish species. Funding for consultants. Funding for site visits and a multi-day decision making workshop.
6.2	Model the best minimal management required to ensure release sites remain suitable for the short- (10 yrs) and medium-term (25 yrs).	Years 1-4	Zoological Society of London; IUCN CTSG; IUCN Freshwater Fish Specialist Group; Universidad Autónoma del estado de Morelos; Universidad Michoacana de San Nicolás de Hidalgo; Local ejidos (communal farmlands), local authorities.	Pupfish population projection model (PVA) built that projects population growth under different management options. Model projections updated annually using monitoring data following a formal adaptive management process.	Specialists to develop management options and parameterize PVA models.	Funding support for a data analyst. Identifying and engaging with local representatives at each potential release site for each pupfish species.
6.3	Model hydrological robustness of sites into the future under predicted land use and climate change (>25 yrs).	Years 1-2	Hydrology consultant; Universidad Autónoma del estado de Morelos; Universidad Michoacana de San Nicolás de Hidalgo; University of West England	Produce projected hydrological robustness over the medium to long term for each short-listed site from tactic 4.1.	Land use profiles from satellite imagery.	Funding for hydrology consultant. Identifying and engaging with local representatives at each potential release site for each pupfish species.

Objective 7. Restore or enhance water security at selected release sites.

Rationale:

A recognized key threat to successful reintroduction, and an original wild extinction driver, is desertification linked to water extraction for farming. Water shortage is further exacerbated by climate change. Whilst these threats are substantial, they are not insurmountable, and our project will focus on water security to help ensure pools where pupfish will be released are of sufficient volume (tactic 7.1) and that pupfish habitat is prioritized in water allocations (tactic 7.2). Furthermore, we will protect the quality of water in each pool by re-establishing a natural vegetation buffer from intensive farming (tactic 7.3). Water demands for sufficient pool sizes are small relative to other demands from agriculture and local communities and a combination of market value alongside our investment in environmental education is expected to secure water resources for pupfish recovery safely into the future.

NO.	TACTIC	PRIORITY	RESPONSIBILITY	INDICATORS OF PROGRESS	WHAT WE HAVE	WHAT WE NEED
7.1	Small scale engineering to prevent water loss and to store excess rainwater at each release site.	Years 1-2	Hydrology consultant - Universidad Michoacana de San Nicolás de Hidalgo; Engineering consultant; Local landowners; Local authorities; University of West England	Report produced on each sites engineering requirements. Required engineering work completed by local companies.	Land use profiles from satellite imagery. Initial site visits completed from specialist pupfish aquarist. Assessment of land ownership.	Funding for consultants. Funding for applications to local authorities for small scale engineering works. Funding for engineering works.
7.2	Small scale engineering to capture pond runoff and recycle for agricultural use.	Year 2	Engineering consultant; Representatives from Ejidos (communal farm lands); Local authorities and local pupfish champions (tactic 4.2).	A method of capturing and recycling of water runoff for agriculture implemented.		Funding to contract engineer to design and construct water capture and recycling apparatus.
7.3	Create a natural buffer around each release site to reduce risks from pesticides.	Year 2	Representatives from Mexican pupfish aquarists; Representatives from Ejidos (communal farm lands); Local authorities; Universidad Autónoma de Nuevo León	Restored vegetation buffer around each release site.		Funding to support local community led site restoration.

Objective 8. Initiate releases of each species, testing critical assumptions about how long-term captivity, and changed in situ habitat, influence survival and reproduction.

Rationale:

Successful establishment of wild populations from conservation translocation requires careful assessment of feasibility including selection of founders (tactic 8.1) (Section 5 IUCN Guidelines for Reintroductions and Other Conservation Translocations) (IUCN 2013); assessment of risks including disease (tactic 8.2), risks to source populations (tactic 8.1) (Section 6 IUCN Guidelines for Reintroductions and Other Conservation Translocations) (IUCN 2013); and establishing an appropriate monitoring and continuing management framework (tactics 8.3-8.6) (adaptive management; Section 8 IUCN Guidelines for Reintroductions and Other Conservation Translocations) (IUCN 2013).

NO.	TACTIC	PRIORITY	RESPONSIBILITY	INDICATORS OF PROGRESS	WHAT WE HAVE	WHAT WE NEED
8.1	Select appropriate individuals from each pupfish species for sustainable releases.	Years 2-4	Representatives from Mexican and International pupfish aquarists; IUCN CTSG; IUCN Freshwater Fish Specialist Group; UC Berkely	Pupfish groups created and successfully released.	Expertise in pupfish breeding management, genetic analyses and freshwater fish conservation translocations.	Sustainable harvest from the ex situ population. Funds for shipping pupfish from source aquariums to release sites.
8.2	Reduce disease risks through the release of each pupfish species.	Years 2-4	Zoological Society of London disease risk analysis experts and representatives from Mexican & International pupfish aquarists. Local veterinarians. IUCN CTSG	Disease risk analysis completed and agreed disease risk management implemented.	Expertise in disease risk analysis and management in conservation translocations.	Funding to complete analysis. Funding to implement required screening and quarantine management.
8.3	Establish a robust post-release monitoring methodology that tracks population vital rates (survival and fecundity) in closed release sites for each pupfish species.	Years 2-5	Local communities; Universidad Autónoma del estado de Morelos; Universidad Michoacana de San Nicolás de Hidalgo; Universidad Autónoma de Nuevo León; IUCN CTSG & Freshwater Fish Specialist Group (and Invasive Species Specialist Group for 8.6).	Detailed monitoring protocol method and database created. Annual reports on population vital rates produced. Integrated population projection models updated annually.	Expertise in freshwater fish population monitoring.	Identify local pupfish champions. Funding to support local community involvement in monitoring. Funding to support a Mexican pupfish project manager. Funding to support a specialist integrated population modeler.
8.4	Establish a robust monitoring methodology for tracking release site water volume and quality. Adjust if needed.	Years 2-5		Water volumes and water quality measured and reported monthly. Water filtration apparatus installed.	University laboratories to test water quality.	Funds to undertake water quality analyses. Funds to purchase and install a permanent water filter.
8.5	Establish a robust monitoring methodology for tracking pupfish foraging and anti-predator behaviours post-release. Adjust pre-release conditioning as needed.	Years 2-5		Studies published on pupfish behavioral ecology post-release. Studies published on pre-release conditioning and post-release survival. Study findings integrated within the adaptive management framework and wild population outcomes tracked via an integrated population model.	Expertise in freshwater fish population monitoring and freshwater fish experimental design.	Identify local pupfish champions. Funding to support local community involvement in monitoring. Funding to support a Mexican pupfish project manager. Funding to support a specialist integrated population modeler. Mexican student project funding for experiments.

NO.	TACTIC	PRIORITY	RESPONSIBILITY	INDICATORS OF PROGRESS	WHAT WE HAVE	WHAT WE NEED
8.6	Establish a robust monitoring methodology for presence and impact of non-native vegetation and fish competitors or predators. Remove as needed.	Years 1-5	Local communities; Universidad Autónoma del estado de Morelos; Universidad Michoacana de San Nicolás de Hidalgo; Universidad Autónoma de Nuevo León; IUCN CTSG & Freshwater Fish Specialist Group (and Invasive Species Specialist Group for 8.6).	Annual reports on presence of non-native species at each release site. Studies published of impacts from non-native species on pupfish. Non-native species removed. Monitoring protocol for incursions, and management response, published.		As in 8.5 plus funding for non-native species removal.
8.7	Track genetic diversity over time in each natural population by collecting fin-clips and sequencing the genomes of all founder individuals.	Years 2 & 5 (and beyond)	UC Berkely, contributing aquariums, zoos and universities.	Founder genomes sequenced for reference. Genomes of wild pupfish populations after three years completed to quantify genetic change. A plan for continued sampling agreed.	Expertise, methods and a genetics lab.	Funding for holder and shipping of fin-clip samples. Funding for genomic analysis.
8.8	Green status assessment for the three species.	Year 1	IUCN Freshwater Fish Specialist Group; Universidad Michoacana de San Nicolás de Hidalgo; Bristol Zoological Society; Zoological Society of London	Completed assessment for each species that includes quantification of our predicted conservation gain resulting from this project as well as longer term recovery potential.	Experience in green status assessments	Funds for staff time to gather date a and run assessments.

Objective 9. Ensure criteria for downlisting are met within life of the project.

Rationale:

Specific to the Indianapolis Saving Species Challenge the key criteria for grant application evaluation is the successful IUCN Red List downlisting of the focal species. In our case we are proposing achieving this for **three species**. There are two conditions to be met for downlisting from EW to CR, outlined in IUCN Standards and Petitions Committee (2022), which are (1) released individuals must successfully breed and their offspring recruit into the population (tactic 9.1) with at least 5 years having passed since the release, and (2) the released population must meet the criterion for being considered “wild”, i.e. not be subject to intensive management (tactic 9.2). Our approach toward minimal management still allows a ‘wild’ designation by the IUCN Guidelines for Using Red List Categories and Criteria (Section 2.1.4; IUCN 2022). Further, wild populations established outside indigenous range must comply with additional conditions, most critically that they be specially for the purpose of reducing extinction risk (overall project).

NO.	TACTIC	PRIORITY	RESPONSIBILITY	INDICATORS OF PROGRESS	WHAT WE HAVE	WHAT WE NEED
9.1	Obtain accurate estimates of fish reproduction and recruitment. Track population growth through a custom demographic model. Show survival, breeding and recruitment occurring and sustained across the 'five-year rule'.	Years 2-5 (plus additional years to meet 'five-year rule').	All project partners.	As in 8.3. Annual reports on population vital rates produced. Integrated population projection models updated annually.	Expertise in freshwater fish population monitoring and adaptive management.	As in 8.3
9.2	Establish long-term minimum management that is of low intensity such that each established pupfish population is considered "wild" under red list assessment.	Years 2-5 (plus additional years to meet 'five-year rule').	All project partners.	Minimum management established that meets criteria by year 5 of funding.		As in tactics 8.3, 8.4, 8.5 & 8.6

Goal 4: Maximise the education potential of both ex situ and in situ pupfish conservation to inspire sustainable land use and pupfish protection

Ensuring that;

- The ex situ population of Mexican pupfish provide maximum impact on public awareness.
- The ex situ population of Mexican pupfish inspire a deeper environmental awareness leading to behavior change.
- The ex situ and in situ population provides opportunity to inspire and train future Mexican conservation scientists, educators and other influencers.

Objective 10. Create educational exhibits across México's zoos, aquariums and universities.

Rational:

A thriving ex situ population in Mexican aquariums, zoos and universities (achieved through Goal 1) not only creates the opportunity for releasing pupfish back into the wild but also, critically, to reach a wide generalist Mexican audience. Reaching so many Mexicans provides a powerful means for improving awareness about each pupfish species and the wider environmental management required to recover lost Mexican biodiversity (tactics 10.1 & 10.2), pupfish and beyond! We believe a particular emphasis should be placed within the home state for all three pupfish, Nuevo León, given societal pressure for change at the regional level will best amplify efforts at the local scale (achieved in Goal 3).

NO.	TACTIC	PRIORITY	RESPONSIBILITY	INDICATORS OF PROGRESS	WHAT WE HAVE	WHAT WE NEED
10.1	Create educational exhibits across México's zoos, aquariums and universities.	Years 1-5	Aquario Inbursa; Sealand Monterrey; Universidad Autónoma del estado de Morelos; Guadalajara Zoo; RioVivo; Universidad Autónoma de Nuevo León	Educational exhibits established where pupfish are held ex situ	Aquariums, zoos and universities agreed to hold populations of pupfish and who draw large numbers of visitors and/or students.	Funding to assist in integration of pupfish and environmental education signage and interactive features linked to pupfish exhibits.
10.2	Distribute each pupfish species nationally to universities for applied research from graduate and post-graduate students.	Years 1-5	Universidad Autónoma del estado de Morelos; Universidad Michoacana de San Nicolás de Hidalgo; Universidad Autónoma de Nuevo León;	Student projects completed. Information shared with relevant partners for integration within adaptive management.	University partners with specialist skills in different aspects of environmental research and with established populations of freshwater fish.	Funding for establishment of pupfish populations held at universities. Studentship funding.
10.3	Emphasize creation of educational exhibits within the Nuevo León state including in the capital of Monterrey.	Years 1-5	Sealand Monterrey & Universidad Autónoma de Nuevo León	As in 10.1	As in 10.1	As in 10.1

Objective 11. Create ongoing opportunities for local interaction and study of pupfish in México.

Rationale:

Our project is driving a societal shift in perception of pupfish and wider environmental awareness. It becomes tangible and powerful when pupfish are visible to people and not a forgotten species held in far flung corners of the world (Goal 1). Here we focus on two societal groups. First, locally we aim to connect people directly with pupfish (tactic 11.1) by bringing pupfish directly into schools and other public spaces. Second, we aim to inspire the next generation of Mexican conservation scientists by facilitating their research training to be done with pupfish (tactic 11.2). A clear added advantage is that the science done can feed immediately back into our adaptive management approach.

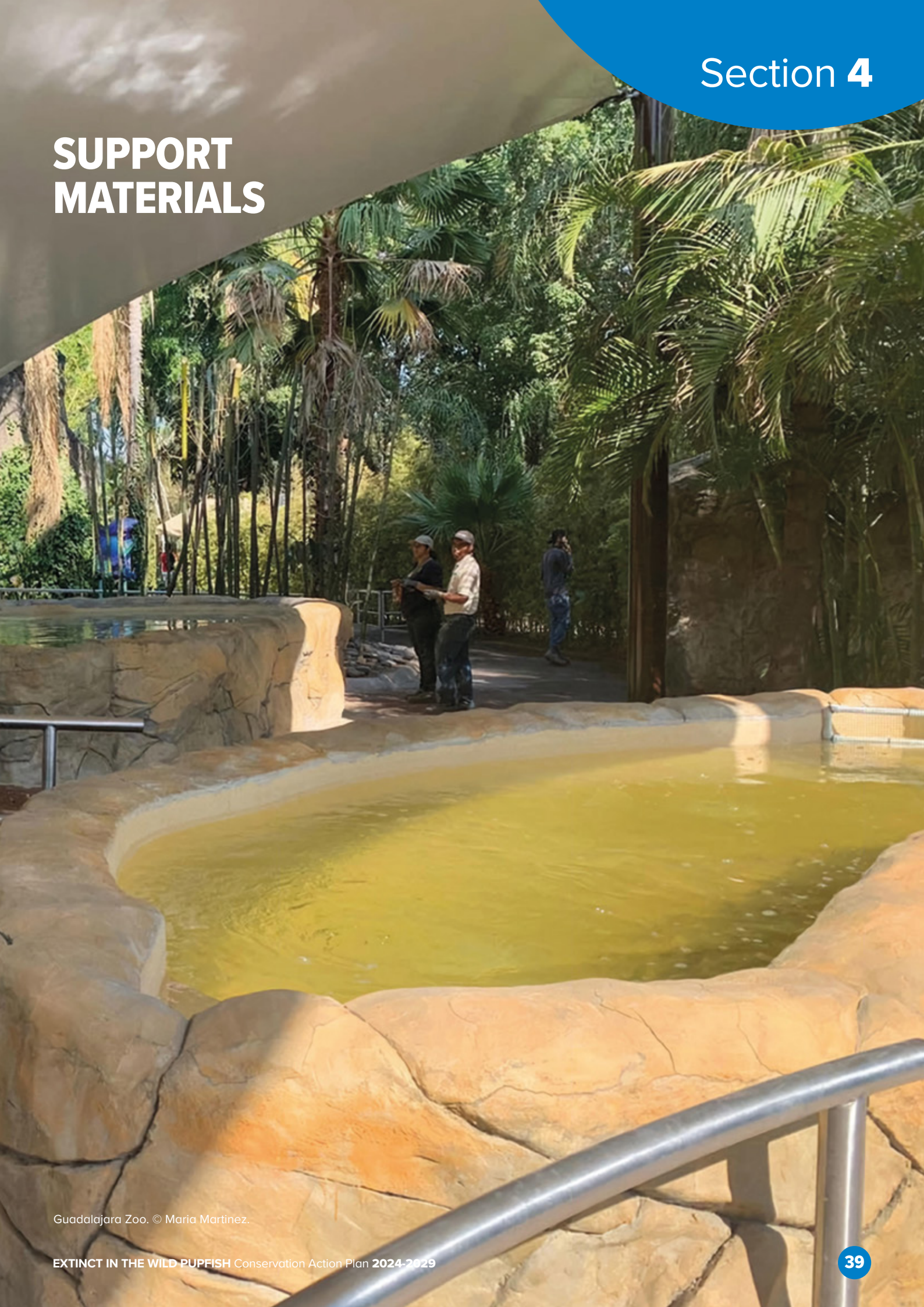
NO.	TACTIC	PRIORITY	RESPONSIBILITY	INDICATORS OF PROGRESS	WHAT WE HAVE	WHAT WE NEED
11.1	Build educational ponds or tanks at schools and/or municipal buildings local to release sites for each pupfish species.	Year 2-3	Aquario Inbursa; Universidad Autónoma del estado de Morelos; Guadalajara Zoo; Local schools and politicians.	Educational and high visibility ponds or tanks established.	Expertise in pupfish husbandry and pond/tank design.	Funding to establish ponds/ tanks and provide training in pupfish husbandry.
11.2	Partner with Mexican universities to provide community research projects for education and applied outcomes (i.e. citizen science).	Years 1-5 (and beyond)	Universidad Autónoma del estado de Morelos; Universidad Michoacana de San Nicolás de Hidalgo; Universidad Autónoma de Nuevo León	Research projects completed. School science projects completed.	University partners with specialist skills in different aspects of environmental research and with established populations of freshwater fish.	Funding for developing citizen science projects and for developing school research projects.

Budget

GOAL	BUDGET ALLOCATION ($)
Goal 1: Repatriate pupfish to México as thriving ex situ populations	455,100
Goal 2: Maximise local project pride, ownership and support of pupfish recovery in the wild	310,500
Goal 3: Establish wild populations of each pupfish species built through cutting edge adaptive management	131,500
Goal 4: Maximise the education potential of both ex situ and in situ pupfish conservation to inspire sustainable land use and pupfish protection	102,900
Total	**1,000,000**

Project site visit to Guadalajara Zoo

SUPPORT MATERIALS



Guadalajara Zoo. © Maria Martinez.

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Potosi pupfish. © Joel Sartore.