Global Re-introduction Perspectives: 2016

Case-studies from around the globe

Edited by Pritpal S. Soorae

IUCN/SSC Re-introduction Specialist Group (RSG)

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Cover photo: Clockwise starting from top-left:
i. Bolson's tortoise, USA @ Turner Endangered Species Fund
ii. Wetapunga, New Zealand @ Richard Gibson
iii. Morelos minnow, Mexico @ Topiltzin Contreras-MacBeath
iv. *Silene cambessedesii*, Spain @ Emilio Laguna
v. Tasmanian Devil, Maria Island, Tasmania @Simon DeSalis
vi. Agile frog, Jersey @ States of Jersey Department of the Environment

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Re-introduction of the four leaf clover in the agricultural context of the Po River Plain, Italy

Simone Orsenigo[1], Paolo Cauzzi[2], Rodolfo Gentili[3], Graziano Rossi[1] & Thomas Abeli[1,4]

[1] - Department of Earth and Environmental Sciences, University of Pavia, I-27100, Via S. Epifanio 14, Pavia, Italy thomas.abeli@unipv.it
[2] - University of Pavia, Department of Biology and Biotechnology, Pavia, Italy
[3] - Department of Earth and Environmental Sciences, University of Milano-Bicocca, I-20126, Piazza della Scienza, Milano, Italy rodolfo.gentili@unimib.it
[4] - IUCN/SSC Re-introduction Specialist Group and Freshwater Plant Specialist Group

Introduction

The four leaf clover (*Marsilea quadrifolia* L.), is a circumboreal aquatic pteridophyte bearing four-parted leaf, floating in the water or erected in shallow water and land. It occurs in central and southern Europe, Asia and North America. It is listed in the annex II and IV of the European Directive 92/43/EEC among the species requiring special areas of conservation and it is listed in the annex I of the Bern Convention. Following (Bruni *et al.*, 2013) *M. quadrifolia* was classified as Vulnerable at the European level, where it has been facing a strong population decline at the southern edge of its distribution. For instance, in Italy where it has recently disappeared from the southern regions is classified as Endangered (Rossi *et al.*, 2013). According to Gentili *et al.* (2010), reasons for decline were identified in agricultural practices (it is currently considered a weed of rice crop), competition with invasive species (e.g. *Heteranthera reniformis*) and non-native predators like the Louisiana crayfish (*Procambarus clarkii*) and the nutria (*Myocastor coypus*). The re-introduction of *M. quadrifolia* at different sites in the Po Plain was one of the major goals of three conservation projects carried out from 2010 to 2012 (CORINAT, Life “Pianura Parmense”, RIVIVRO’).

Four leaf clover © Rodolfo Gentili

Goals

- Goal 1: Establish viable and self-sustaining populations of the four leaf clover in suitable areas of the Po Plain.
- Goal 2: Understand the threats affecting the target species and assess the impact of agricultural activity of *M. quadrifolia*.

- Goal 3: Define suitable methods of *ex-situ* propagation to increase the number of propagules for re-introduction.

Success Indicators

- Indicator 1: Long-term survival (>3 years) of the established populations.
- Indicator 2: Definition of the factors linked to the rice cultivation affecting the species survival.
- Indicator 3: Obtain enough individuals to be re-introduced, through *ex-situ* cultivation

Preparing *ex-situ* material for re-introduction
© Paolo Cauzzi

Project Summary

Feasibility: The aim of the re-introduction of *M. quadrifolia* in the context of the above mentioned projects was to establish a number of viable populations of the target species in some Special Areas of Conservation belonging to the Natura 2000 network. The alarming rate of decline of *M. quadrifolia* in Italy made the scientific community aware of the need of urgent conservation actions, to avoid the fate of other aquatic species that become extinct in the past decade, like *Stratiotes aloides* and *Aldrovanda vesiculosa*. Additionally, the conservation of this species is made mandatory by European Union legislation. However, *M. quadrifolia* is considered as a weed of rice fields, so the use of herbicide strongly reduced the possibility of natural recolonization of the historic range by the species. The degradation of habitat quality also affected the possibility to re-introduce the species in areas characterized by intensive rice cultivation. This made it necessary to highlight sites within protected areas less affected by the agricultural activity, to guarantee the persistence of re-introduced population. In one case (Bagnacavallo, Ravenna) a pond was excavated *ex-novo* to exclude alien predators like the Louisiana crayfish and to allow the regulation of the water flow.

Implementation: The main issues concerning the implementation of the re-introduction plan were the choice of the source population, the propagation of plant material and the tolerance to herbicides. Molecular analysis using AFLP markers was employed to identify the most suitable source population to obtain plant material. Both the within-population and between-population genetic diversity of *M. quadrifolia* in Italy was very low. In fact, no private alleles were identified in the analyzed populations. This, on one side, did not raise concerns about the choice of the source population, but on the other side revealed that

populations may suffer for inbreeding depression (Bruni *et al.*, 2013). Small portions of rhizoma were collected from several ramets from two source populations located in relict sites of occurrence in Northern Italy. Proven very difficult to obtain plant individuals from *in-vitro* crossing of male and female spores, plants were vegetatively propagated from rhizomas for 2 years, with excellent results. At each release site, a meta-population structure made by several sub-populations was established, to reduce the negative impact of stochastic events and to differentiate the characteristic of the microsite conditions. Such a solution was successful, as some of the subpopulations disappeared, but the population as a whole had minor damages. Tolerance tests to herbicide demonstrated the *M. quadrifolia* was quite sensitive to many common herbicides used in the cultivation of rice, thus the release sites had to be chosen within areas less impacted by the agricultural activity (Natural habitats in protected areas). Artificial floating islands were effectively used in a site with high fluctuation of the water level, that often negatively affect the species survival in artificial ponds.

Post-plantation monitoring: After 6 months from planting the species cover increased by 100%, that fell to 50% the year after the re-introduction. Such variability is an intrinsic characteristic of the species which is affected by the water level that may strongly fluctuate from year to year and by the precipitation regime, also highly variable. However, some of these fluctuation may also be due to unknown factors. After 3 years some of the sub-populations become extinct mainly as a consequence of the selection of wrong microsites (especially concerning the water level fluctuation) and predation. For instance, at the Bagnacavallo site the only population still alive is the one in the artificial pond. However, the meta-population structure buffered the damages to the single sub-populations.

Major difficulties faced

- Scarcity of suitable release sites: The use of herbicides strongly affect the species, thus release sites for the re-introduced populations were identified in small areas less affected by the cultivation of rice, or where the cultivation of rice follows practices more compatible with the species persistence, which however are very few in the whole Po Plain.
- Remove or mitigate the impact of alien species: Currently this is an unsolved problem, especially for the Louisiana crayfish, that is very difficult to eradicate or control.
- Increase the genetic variation of the re-introduced populations: The choice of different source populations partially solved this problem, leaving the remnant populations highly inbred.
- Interpretation of the re-introduced population fluctuations: Strong fluctuation was recorded during the post-release period, but reasons for strong fluctuations in the surface covered by the species at the release sites can only be hypothesized.

Major lessons learned

- When between-population genetic diversity is low, there are few concerns in the choice of the source population, but the mix of different populations may

enhance the within-population generic variation of the re-introduced populations.
- The use of herbicides is the main threat factor affecting *M. quadrifolia* as well as other aquatic species in an agricultural context.
- The meta-population approach allows to minimize the damages to the whole population even when some sub-populations disappeared.
- The use of artificial floating islands was very successful in water bodies with a high variation in the water level.

Success of project

Highly Successful	**Successful**	Partially Successful	Failure
	√		

Reason(s) for success/failure:
- Complete analysis of the threats affecting the species at a local scale.
- Understanding the ecological requirements of the species through long-term ecological studies of the remnant wild populations.
- Understanding of the tolerance to dose and types of herbicides used in the rice cultivation, allowed for the selection of suitable release sites.
- Intrinsic ability of the species for rapid growth and vegetative propagation when conditions are suitable.
- Meta-population approach.

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WORLD HEADQUARTERS
Rue Mauverney 28
1196 Gland, Switzerland
Tel +41 22 999 0000
Fax +41 22 999 0002
www.iucn.org