Effectiveness of artificial amphibian breeding sites against non-native species in a public protected area in Tuscany, Italy

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SUMMARY

The spread of non-native invasive species is among the factors thought to be responsible for the recent global declines in amphibian populations. In a Protected Natural Area of Local Interest in Tuscany, Italy, we tested approaches for preserving the local amphibian populations threatened by the presence of the red swamp crayfish *Procambarus clarkii*. The construction of artificial breeding ponds, with suitable vertical barriers, was initially effective in preventing the spread of the red swamp crayfish and created a source site for amphibians, in particular newt species. Unfortunately, five years after construction, the breeding sites were colonized by fish and crayfish, possibly due to the actions of members of the public.

BACKGROUND

Amphibians are regarded as the most endangered class of vertebrates (Gibbons *et al*. 2000, Stuart *et al*. 2004), and their global decline is matter of great concern because of its consequences for species conservation and ecosystem function (Hocking & Babbitt 2014, Cortez-Gomes *et al*. 2015). The drivers of amphibian population declines are various and well-known: habitat destruction, alteration and fragmentation; pollution; use of pesticides and fertilizers; direct capture; climate change; diseases; and the introduction of allochthonous species (Webb & Joss 1997, Kats & Ferrer 2003, Pounds *et al*. 2006, Cushman 2006, Mann *et al*. 2009). Due to their vulnerability, the European Union has included many native amphibian species in the Habitat Directive 92/43/EEC, in order to guarantee their protection by the establishment of protected areas: Sites of Community Importance (SCIs). In some cases, the implementation of correct habitat management policies may alleviate the negative effects of threats (Rannap *et al*. 2009, Kingsbury & Gibbons 2011).

Our study site was an Area Naturale Protetta di Interesse Locale (Protected Natural Area of Local Interest), "Podere la Querciola", which is part of the Stagni della Piana Fiorentina e Pratese SCI, located in Sesto Fiorentino, Florence (43°49'29'' N 11°10'24'' E). It is 35 m above sea level, extends for 55 ha and was established in 1998. Management of the area is entrusted to the local office of the environmental association Legambiente and it is also used as a public park.

The site is part of the so-called Florence Plain, originally part of a wide lake basin of Villafranchian age (1.8 million years ago), which subsequently evolved into an assemblage of marshes (Consorzio di bonifica Area Fiorentina 2005). The original landscape (marsh vegetation and wet forests) was converted by man over the centuries. As a result, the Plain today is almost totally reclaimed and consists of intensively farmed land and urban areas.

In the protected area, soils are alluvial (silt and clay) and water stagnation is frequent. When it was set up, the protected area was mainly abandoned farmland surrounded by urbanized areas (highway A11, Florence airport, industrial areas). However, many residual wetlands of high environmental importance were still present. Five species of amphibian were observed at the site: Italian crested newt *Triturus carnifex*, smooth newt *Lissotriton vulgaris*, Italian tree frog *Hyla intermedia*, Balearic green toad *Bufotes balearicus*, and Italian pool frog *Pelophylax bergeri*, together with the hybrid species *Pelophylax* kl. *hispanicus.* The latter is a hybridogenic species, produced from cross-breeding between *P. ridibundus* and the parental species *P. bergeri*. It cannot be visually discriminated from *P. bergeri*. Four of these species are protected by the Habitats Directive: Italian crested newt, Italian tree frog, Balearic green toad and Italian pool frog.

A habitat restoration project was proposed because of the presence of many threats: the increasing scarcity of breeding sites outside the protected area, infrastructure expansion and pollution, and the presence in the Plain of non-native species like red swamp crayfish *Procambarus clarkii*, mosquitofish *Gambusia holbrooki*, and topmouth gudgeon *Pseudorasbora parva*, which threaten local amphibian populations.

The target species of the intervention were Italian crested newt, smooth newt, Italian tree frog and *Pelophylax* species. In the Florence Plain all these species are linked to vegetated, semi-permanent small wetlands which are threatened by invasive alien species. In contrast, the Balearic green toad breeds in seasonal shallow pools filled with water only for limited periods. Consequently, it is much less threatened by invasive species.

Crayfish seem to have difficulties in climbing vertical surfaces (Dana *et al*. 2011), while amphibians are able to use suction to adhere by adhesive phalanx pads (tree frogs) or body gripping (frogs, newts) to vertical surfaces. The aim of the intervention documented here was to enhance local amphibian populations by building new breeding sites with suitable vertical barriers, to prevent red swamp crayfish from entering the site, while allowing both access and exit for amphibians.

ACTION

In May 2008, three new aquatic sites were designed for the area. The sites differed in depth, shape and height of the barrier, in order to compare the effectiveness of different solutions.

Site 1: a square pond with sides of length about 16 m (256 m^2 of surface), lined with rubber sheet, with a variable depth, ranging from a minimum of a few centimetres to a maximum of

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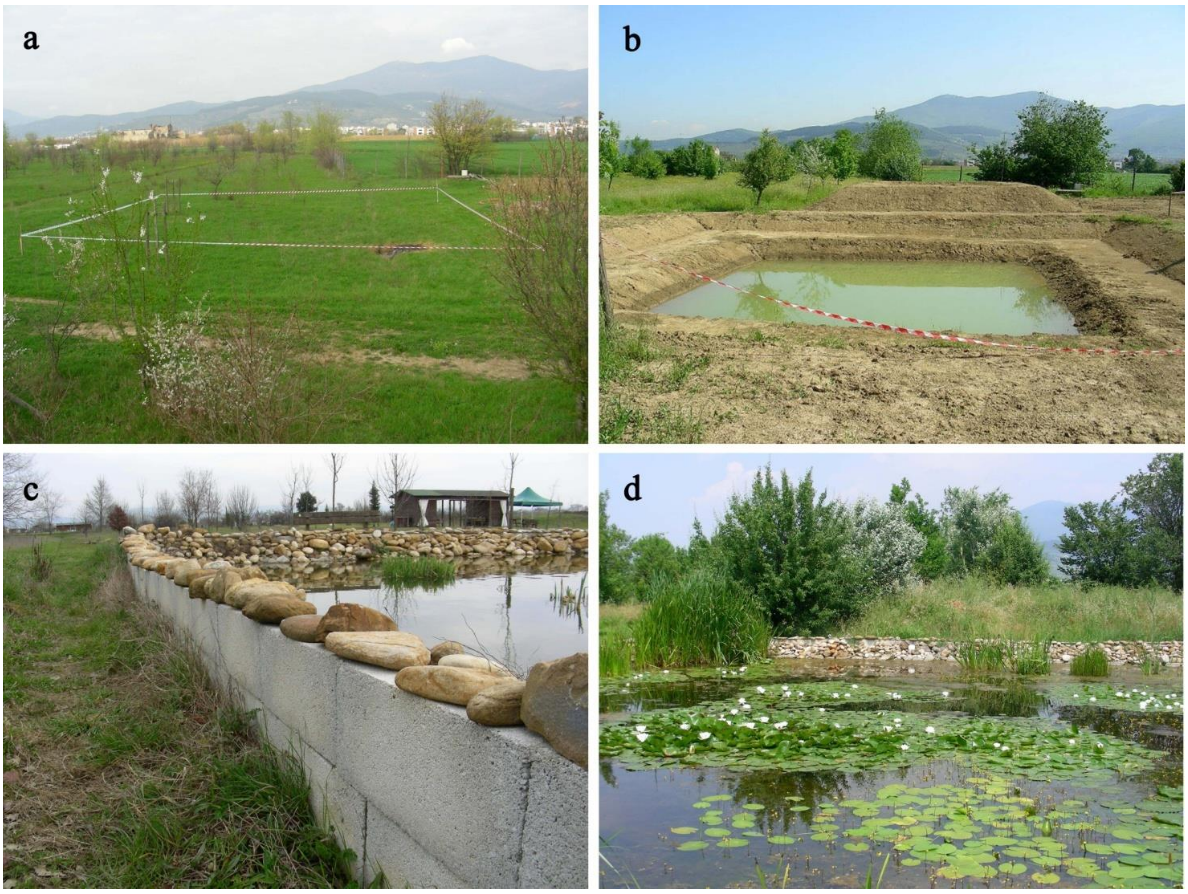

Figure 1. Site 1; a) before the intervention, b) the digging completed, c) detail of the barrier, d) spring 2012, three years after the site was created.

2 m. A 30 cm height wall of concrete bricks was built along the perimeter of the pond in order to prevent access by crayfish. This barrier extended 10 cm underground to prevent crayfish from digging under the barrier and entering the pond. To avoid any risk of access from below ground, the wall was in direct contact with the rubber sheet (Figure 1).

Site 2: a rectangular-shaped basin (400 x 200 x 80h cm) constructed with concrete blocks and waterproofed with rubber sheet. The frame was concealed with stones both for aesthetic purposes and to provide hiding places for the herpetofauna. This basin was also suitable for environmental education activities with children, who can closely observe the animals and plants inside this site without any risk (Figure 2).

Site 3: especially designed for tree frogs, and consisting of a round plastic tub (diameter 150 cm, height 95 cm), concealed with reed mat around the perimeter (Figure 3).

The ground surrounding all the sites was covered withtopsoil from excavation (approximately 20 cm depth), to protect the rubber sheet below and create a favorable habitat for microfauna and microorganisms.

Local threatened aquatic plants were introduced to all three sites after construction. The species used were: European white waterlily *Nymphaea alba*, yellow waterlily *Nuphar lutea*, yellow iris *Iris pseudacorus*, common reed *Phragmites australis*, thread-leaved water-crowfoot *Ranunculus trichophyllus*, simplestem bur-reed *Sparganium erectum*, bladderwort *Utricularia australis*, flowering rush *Butomus umbellatus*, common spike-rush *Eleocharis palustris*, broad-leaved pondweed *Potamogeton natans* and curled pondweed *Potamogeton crispus*. These were planted with the aim of providing a suitable habitat for amphibians and also new habitat to enhance conservation of the plants. As well as meeting

Figure 2. a) Site 2 b) Site 3.

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ecological requirements, the layout of the planting was planned to enhance the aesthetic impact, in order to capture the attention of visitors and maximize educational value.

Species with highly developed roots, such as waterlily and iris, were placed in submerged topsoil-filled pots. The other species were directly transplanted in the ground. Plants were collected in threatened small wetlands near the protected area, or in other parts of the Florence Plain. However European white waterlily, yellow waterlily and bladderwort were purchased from an authorized nursery selling Tuscan native plants.

The construction was completed in about one month. The excavation operations were carried out by the public institution responsible for hydrographic network management (Consorzio di Bonifica Area Fiorentina), while building operations were performed by volunteers of Legambiente. The total cost of materials amounted to €3100.

Monitoring to assess the presence of amphibians in the new sites started after the completion of the projects (January 2009). Surveys were undertaken both day and night, by visual observation and with a net. For a one-year period, between March 2012 and March 2013, further monitoring activity was carried out using deep-netting and Ortmann's funnel traps (Drechsler *et al*. 2010).

CONSEQUENCES

Only one year after the end of the habitat creation all three new habitats appeared to be thriving, with most plants growing and proliferating, especially European waterlily, yellow waterlily, yellow iris, flowering rush, curled pondweed and bladderwort.

Site 1 provided suitable habitat for both newt species and the Italian pool frog. However, only Italian crested newt and smooth newt reproduced here (Table 1). Similarly only newt species used Site 2 for reproduction (Table 2). Site 3 was used by Italian tree frog for breeding in 2009 only, with confirmed reproductive success (eggs and tadpoles). The subsequent shading of the tank at Site 3, due to the growth of surrounding shrubs, and especially the presence of temporary wetlands outside of the protected area, may have been responsible for making the site less attractive in the following years. However, adult Italian tree frogs were observed after 2009 on the plants surrounding Site 3, suggesting that a number of frogs continued to use the protected area as a refuge for resting and feeding. During monitoring of Site 1 for a year, from March 2012 to March 2013, we observed 429 different adult individuals of Italian crested newt; each individual was identified by comparing ventral patterns, which are individual-specific.

Figure 3. Injuries caused by red swamp crayfish to Italian crested newts.

At Sites 1 and 2 many individuals of Italian crested newt and smooth newt remained in the water throughout the year, resulting in paedomorphosis.

The vertical barriers around the ponds appeared to be initially effective against invasion by red swamp crayfish. No crayfish were observed in the water at any of the sites from 2008 to 2013, although some crayfish were found overnight or during rain close to the barriers and attempting unsuccessfully to climb them. No invasive fish were observed until 2013.

Unexpectedly, during March 2013, some individuals of red swamp crayfish were observed within Site 1 with evident and immediate negative impacts on the Italian crested newts (Table 1, Figure 3). The following month a group of at least six adult topmouth gudgeon were also observed at this site. The presence of predators (amphibians and insects) in high numbers was not enough to eradicate the two invasive species, which multiplied during the following two years. At the beginning of August 2015 Site 1 appeared completely compromised: amphibians had disappeared from the water (with exception of Italian pool frog or its hybrid, Table 1), the water had taken a cloudy appearance and the majority of the aquatic plants were gone (Figure 4). During June 2015, individuals of mosquitofish appeared in Site 2, the site was abandoned by all adult newts and the larvae disappeared.

In addition to the target species, many aquatic insects, such as dragonflies and damselflies (Odonata) (e.g. emperor dragonfly *Anax imperator*, scarlet dragonfly *Crocothemys erythraea*, four-spotted chaser *Libellula quadrimaculata*) successfully colonized the reconstructed habitats.

Table 1. Percentage of monthly surveys in each year of the study which detected the presence of different life stages of four target amphibian species at Site 1. Ad. = adult, Larv. = larvae, Neomet. = neometamorph.

	% detection Italian crested newt			**% detection smooth newt**			**% detection Italian tree frog**			**% detection *P.* kl. *hispanicus***			**Notes**
	Ad.	Larv.	Neomet.	Ad.	Larv.	Neomet.	Ad.	Larv.	Neomet.	Ad.	Larv.	Neomet.	
2010	100	100	42	100	100	17	8	0	0	58	0	0	
2011	100	100	42	100	100	17	0	0	0	58	0	0	
2012	100	100	33	100	100	25	0	0	0	58	0	0	
2013	100	50	0	100	33	0	0	0	0	58	0	0	Red swamp crayfish appeared March 2013. Topmouth gudgeon appeared April 2013.
2014	75	0	0	67	0	0	0	0	0	58	0	0	
2015	0	0	0	12.5	0	0	0	0	0	62.5	0	0	Site 1 appeared completely compromised

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Table 2. Percentage of monthly surveys in each year of the study which detected the presence of different life stages of four target amphibian species at Site 2. Monitoring of neometamorphosed individuals was not possible at this site because of the barrier structure.

	% detection Italian crested newt		**% detection smooth newt**		**% detection Italian tree frog**		**% detection *P*. kl. *hispanicus***		**Notes**
	Adult	Larvae	Adult	Larvae	Adult	Larvae	Adult	Larvae	
2009	50	50	50	50	8	0	50	0	
2010	67	58	58	75	8	0	50	0	
2011	83	58	100	83	0	0	50	0	
2012	92	75	100	100	0	0	50	0	
2013	83	75	100	92	0	0	50	0	
2014	100	58	100	75	0	0	42	0	
2015	75	50	75	75	0	0	25	0	Mosquitofish appeared June 2015

DISCUSSION

This experimental project tested the effectiveness of a practical model to preserve protected species (amphibians) and endangered wetlands. We suggest that these artificial breeding sites could be used to conserve local genetic strains of amphibian populations and act as a reservoir for possible reintroduction into wider areas.

The artificial breeding sites were relatively cheap and easy to construct, especially as voluntary labour was available. Two of the three sites were used by Italian crested newt and smooth newt for reproduction, leading to stable populations that were maintained for four years within the protected area. The Italian crested newt is listed in Annex II of the Habitat Directive, while the smooth newt is protected in Tuscany. Both species have declined in the Florence Plain, due to continued habitat loss (Vanni & Nistri 2005). As well as the target species, other species linked to water (e.g. insects) colonized the area, demonstrating the importance of these environments to the conservation of a large number of aquatic species.

Nevertheless, our reconstructed sites were eventually vulnerable to access by invasive, non-native species; this has highlighted the need to further improve the design of the sites, in order to prevent invasions. Based upon our experience, best design options might be to place this type of reconstructed ponds in areas not frequented by the public, or to regulate access to sensitive sites. Regular monitoring throughout the year, followed by timely eradication interventions, may also help prevent mass invasions.

Figure 4. Site 1 in August 2015 after the invasion of red swamp crayfish and topmouth gudgeon, showing cloudy water and the disappearance of the majority of aquatic plants.

We propose two hypotheses to explain the sudden appearance of the red swamp crayfish in Site 1: a) voluntary introductions by the public, since crayfish often occurred on the ground in the area surrounding Site 1 and people may be prone to put them into the water, considering this act as a good deed; b) occasional access due to vegetation growth beside the barrier after several years.

It is our opinion that the introduction of mosquitofish and topmouth gudgeon was carried out by humans. We can find no published studies demonstrating unequivocally the accidental transport of eggs or fry by water birds. Even an accidental deposition in the ponds of fish or crayfish from birds preying on these species (for example herons or common kingfisher) appears unlikely to result in a stable population of aliens, since predation by herons involves immediate swallowing and kingfisher kill prey immediately after capture. The “voluntary introduction” hypothesis is also consistent with the observation that in Site 1 several adults of topmouth gudgeon appeared simultaneously.

The other major function of the interventions was to educate the public about the importance of wetland habitats, through environmental education and direct contact with aquatic organisms, in particular amphibians. In Western culture herpetofauna enjoys little consideration among the population, because of persistent myths and folklore (Nolan *et al*. 2006), which causes additional difficulties for its preservation (Ceriaco 2012). By imitating what was seen in this protected area, and building small wetlands on private lands at little expense, citizens could make a substantial contribution to environmental conservation, allowing the genetic flow of amphibians between metapopulations through a series of stepping stones. A well-trained land owner will be able to avoid voluntary introduction of allochthonous species.

In addition, the need for a greater control of weeds along the outer edges of the barriers has emerged. It is suggested to keep a distance of at least 40-50 cm clear, to avoid facilitating access by invasive species like the red swamp crayfish. This problem could be minimised by extending the rubber sheet for at least one metre outside of the barrier, to prevent grass growth. Additionally, in cases such as Site 2 it is important to ensure that amphibians cannot become trapped inside, especially if the sites cannot be artificially filled with water. To overcome this potential problem we suggest adding soil inside the supporting structure, reducing the steepness of the sheet near the edges and giving the pond the desired shape and depth.

We also suggest conducting an awareness campaign directed at park users in order to prevent any new future introduction, by placing noticeboards and advertisements. Another part of this

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solution could be the limitation of the access to the habitats with nets or gates.

If we accept that it is impossible to completely remove the risk of voluntary introductions in public areas, Site 2 proved to be the most manageable: mosquitofish could be easily eradicated by simply emptying the entire basin and filling it again, thanks to its small dimensions. This task is more difficult in basins of major size like Site 1. It is therefore recommended, in similar contexts, to create a large number of small basins instead of constructing few larger ones.

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REFERENCES

Ceriaco L.M.P. (2012) Human attitudes towards herpetofauna: the influence of folklore and negative values on the conservation of Amphibians and Reptiles in Portugal. *J. Ethnobiol. Ethnomed*, **8**, 8.

Cortez-Gomez M., Ruiz-Agudelo C.A., Valencia-Aguilar A. & Ladle R.J. (2015) Ecological functions of Neotropical Amphibians and Reptiles: a review. *Univ. Sci.*, **20**, 229-245.

Cushman S.A. (2006) Effects of habitat loss and fragmentation on Amphibians: a review and prospectus. *Biological Conservation*, **128**, 231-240.

Dana E.D., García-de-Lomas J, González R & Ortega F. (2011) Effectiveness of dam construction to contain the invasive crayfish *Procambarus clarkii* in a Mediterranean mountain stream. E*cological Engineering*, **37**, 1607-1613.

Drechsler, A., Bock, D., Ortmann, D., & Steinfartz, S. (2010) Ortmanns funnel trap–a highly efficient tool for monitoring amphibian species. *Herpetology Notes*, **3**, 13-21.

Gibbons J.W., Scott D.E., Ryan T.J., Buhlmann K.A., Tuberville T.D., Metts B.S., Greene J.L., Mills T., Leiden Y., Poppy S. & Winne C.T. (2000) The global decline of Reptiles, Deja Vu Amphibians. *Bioscience*, **50**, 653-666.

Hocking D.J. & Babbitt K.J. (2014) Amphibian contributions to ecosystem services. *Herpetological conservation and biology*, **9**, 1-17.

Kats L.B. & Ferrer R.P. (2003) Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Diversity and Distributions*, **9**, 99–110.

Kingsbury B.A. & Gibson J. (2011) *Habitat management guidelines for Amphibians and Reptiles of the Midwestern United States.* Partners in Amphibian and Reptile Conservation, Technical publication.

Mann R.M., Hyne R.V., Choung C.B. & Wilson S.P. (2009) Amphibians and agricultural chemicals: review of the risks in a complex environment. *Environmental Pollution*, **157**, 2903-2927.

Nolan J.M., Jones K.E., McDougal K.W., McFarlin M.J. & Ward M.K. (2006) The Lovable, the Loathsome, and the Liminal: emotionality in ethnozoological cognition. *Journal of Ethnobiology*, **26**, 126-138.

Pounds J.A., Bustamante M.R., Coloma L.A., Consuegra J.A., Fogden M.P.L., Foster P.N., La Marca E., Masters K.L., Merino-Viteri A., Puschendorf R., Ron S.R., Sánchez-Azofeifa A.G., Still C.J. & Young B.E. (2006) Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature*, **439**, 161-167.

Rannap R., Lohmus A. & Briggs L. (2009) Restoring ponds for amphibians: a success story. *Hydrobiologia*, **634**, 87-95.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D.L. Fischman & R. W. Waller (2004) Status and trends of amphibian declines and extinctions worldwide. *Science*, **306**, 1783–1786.

Vanni S. & Nistri A. (2005) *Biodiversità in Provincia di Prato: Anfibi e Rettili*. Casa editrice le Balze.

Webb C. & Joss J. (1997) Does predation by the fish Gambusia holbrooki (Atheriniformes: Poeciliidae) contribute to declining frog populations? *Australian Zoologist*, **30**, 316-324.

Winandy L., Darnet E. & Denoël, M (2015) Amphibians forgo aquatic life in response to alien fish introduction. *Animal Beahaviour*, **109**, 209-216.

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