The Mohamed bin Zayed Species Conservation Fund

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للمحافظة على الكائنات الحية
The Mohamed bin Zayed SPECIES CONSERVATION FUND

Final Report

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Reversing the decline of the endangered Apennine yellow-bellied toad in Liguria (Northern Italy) through site restoration and population reinforcement

Project number: 152510524

Target species: *Bombina pachypus*

Participating organization: CeSBiN - spin-off of Genoa University

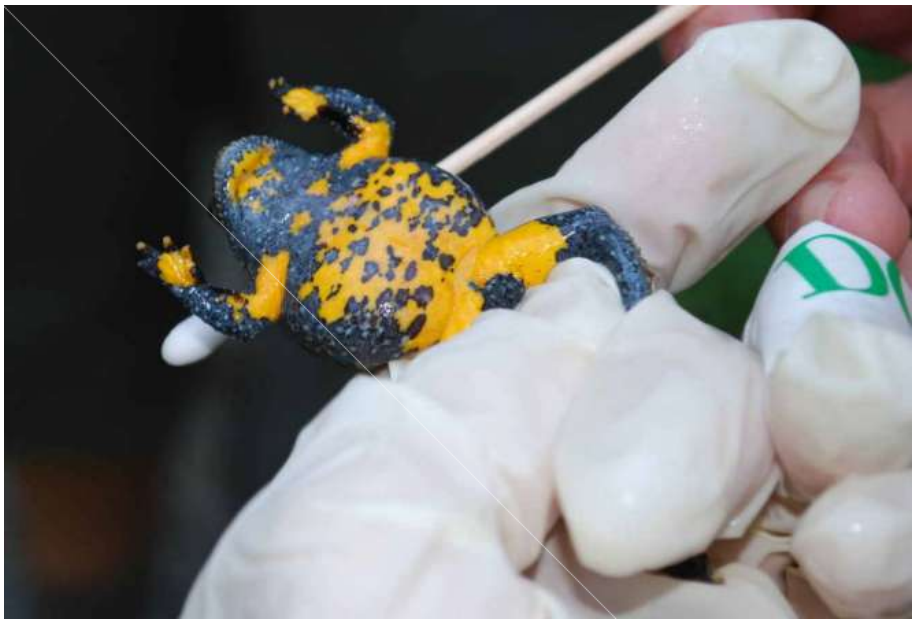
***Bombina pachypus* (Bonaparte, 1838)**

The fundamental objective of the project is to ensure the persistence of *Bombina pachypus* in Liguria (NW Italy) through habitat restoration and the establishment of new populations. In particular, we have the following set of specific objectives: 1. We will clarify the role of chytrid fungus in the decline of the species, by surveying for the presence of disease in other amphibian species at sites where *B. pachypus* has gone extinct in the past 15 years. 2. We will collaborate with experts from other Italian regions to produce a unified evaluation of management strategies. 3. We will manage extant populations in-situ by improving extant breeding sites on the basis of scientific evidence. In particular, we will reduce vegetation to increase insolation and reduce predatory pressure on tadpoles. 4. We will reintroduce captive-bred individuals at restored sites. 5. We will carry out monitoring of both the extant and reintroduced population, to compare population dynamics and optimize management actions. 6. We will train 1-2 university students in fieldwork and analysis, promote the conservation of the species in local schools and collaborate with land owners to promote traditional agricultural practices. 7. We will publicize our results to both a scientific and general audience.

The project started upon receiving funds on 15 July 2015. This date corresponds to the end of the reproductive season for many amphibian species at our latitude, including *Bombina pachypus*. For this reason, over the first six months we were able to only partially start the activities planned in 2015 (see 1st intermediate report). In the 2016 we started all planned activities adding some important actions for *Bombina pachypus* conservation.

1) Clarify the role of chytrid fungus in the decline of the species

We started the survey for the presence of the disease in other amphibian species at sites where *B. pachypus* has gone extinct in the past 15 years. In 2015 we collected a total of 31 skin swabs of six different species (*Bombina pachypus*, *Rana italica*, *Rana temporaria*, *Triturus alpestris*, *Triturus carnifex* and *Salamandra salamandra)* from eight sites. Twenty-nine samples were analyzed with real-time PCR to detect *Batrachochytridium dendrobatidis* and fortunately they were all negative. In 2016 we analyzed 25 skin swabs of *Bombina pachypus* and they were all negative. We decided to extend the area of investigation to evaluate the risk of a future in coming of the disease. These analysis confirm the high risk for the immediate future because we found 3 (7%) infected green frogs (*Pelophylax* sp.) of 34 and 4 (4%) infected *Triturus alpestris* of 110. These results highlight the importance to 1) maintain our monitoring protocol to avoid the spread of this harmful disease and 2) continue the monitoring to evaluate the its spread along time and space.



Swabbing to detect *Batrachochytridium dendrobatidis*

2) **Improve *Bombina pachypus* habitat**

In the original project this planned activity envisaged mainly a reduction of the vegetation cover to increase insolation. While carrying out field surveys during the initial six months of the project (see 1st intermediate report), we realized that habitat improvements also need to account for two additional actions. Firstly, the management of invasive aquatic vegetation through periodical removal from artificial sites; secondly, the eradication of allochthonous fishes from a very potential suitable breeding site of *Bombina pachypus*. For this reason we carried out electrofishing in the winter 2016 to avoid hurting individuals of *B. pachypus* which overwinter in terrestrial habitats. Thanks to this actions a complete eradication of fishes from the small pond was carried out and *Rana dalmatina* and *Ichthyosaura alpestris* returned immediately to breed here after four years. Moreover periodic removal of invasive plants (algae, *Equisetum* spp and *Robus* spp) was carried out from three sites.

Electrofishing and individuals of *Squalius cephalus* removed from the pond

Management of invasive vegetation through periodical removal

Before invasive vegetation removal

After invasive vegetation removal

Before invasive vegetation removal

After invasive vegetation removal

3) **Improve ex-situ center to obtain a good number of froglets for reintroduction**

We carried out periodic management of the ex-situ breeding center (vegetation cutting, water refill, etc) during the winter 2015, the spring, summer and winter 2016. In these occasions we refill the artificial ponds with water, we cut the vegetation and we install the anti-predator (dragonflies) net.

Bombina pachypus breeding center with anti-dragonflies net

Drying up of artificial pond

Artificial pond dried up

Male of *Bombina pachypus* hold in the breeding center

Replacing of the anti-predator net

4) Reintroduce ex-situ-bred individuals at restored sites

We carried out an experimental release to test alternative reintroduction methods. In May 2016, we collected a total of 240 eggs from three extant wild populations of *Bombina pachypus* in Liguria. We translocated 100 eggs to a recent, purpose-built artificial pond located near our captive breeding facility. We transferred the remaining eggs to the captive breeding facility, where they were housed in separate aquaria and reared to late stages of tadpole development. To avoid the potential transmission of pathogens, we maintained quarantine measures and only used water from the source sites in the aquaria. Extensive monitoring over the past five years has essentially ruled out the presence of amphibian chytrid fungus in the source sites (see point 1). We fed tadpoles a diet of boiled lettuce, fish flakes and, in later stages of development, protein supplements. Lighting was ensured by 3% UVB lamps specific for amphibians.

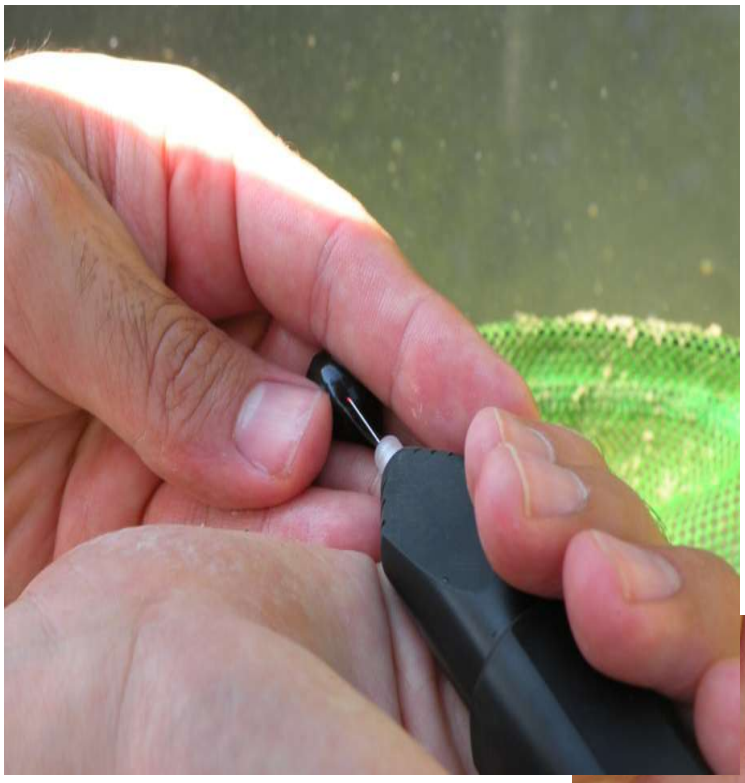
Approximately five weeks later, when tadpoles were approaching metamorphosis, showing rear and front legs, we marked them with Visible Implant Elastomers; we did the same with all tadpoles that were still present at the destination site, of those eggs released a month earlier. We obtained all required ethics permits from the Italian Ministry of Environment, and no tadpoles died or were injured as a result of marking. This allowed us to distinguish the source of individual tadpoles, assessing their survival post-release and judging whether one method performed better than the other.

Indoor raising – Eggs and hatchlings of *Bombina pachypus*

Indoor raising – tadpole of *Bombina pachypus*

Introduction of eggs of *Bombina pachypus* into the artificial site in the wild

Visible Implant Elastomers Kit



Implant of elastomer

Tadpole with elastomer

Introduction of tadpoles

5) Monitoring of both the extant and reintroduced population

We carried out periodic monitoring of seven extant populations to estimate population parameters (abundance, survival rate of differents life stage, etc). We made a photographic database (2010-2016) because individuals could be easily identified through a photo ID software. These data will be used for a paper on *Bombina pachypus* ecology and conservation where will be highlighted the Mohamed Bin Zayed species conservation fund contribution.

The survival of tadpoles in the headstarting phase was very good, approaching 90% over five weeks; conversely, only about 10% of the translocated eggs were still present as late-stage tadpoles. However, headstarted tadpoles suffered heavy predation by dragonflies in the days immediately following release. The ultimate success of the release will be demonstrated by the presence or absence of juveniles at the release sites in Spring 2017.

The results of this preliminary study suggest three things. (1) the removal of eggs from source sites in early May is unlikely to impact the extant populations; as observed annually, eggs laid during that period are washed away by late spring storms. This also happened to all eggs that we did not translocate in May 2016. (2) headstarting is relatively cheap and allows us to produce a much greater number of tadpoles for release than captive breeding, while eliminating the need for removal of adults from extant populations. (3) releases should be preceded by removal of predatory invertebrates from release sites, a task easily achievable given the small size of those sites. We plan to replicate the experiment with a greater number of eggs in 2017, to be housed at an upscaled dedicated headstarting facility made available with Mohamed bin Zayed species conservation fund.

Field survey

Bombina pachypus froglets

6) Communication (new activity)

- We continued Internet campaign by our web site with the through the creation of a web page dedicated to the project (see http://www.cesbin.it/progetti/progetto-ululone.html and http://www.cesbin.it/news/10-finanziamento-per-la-conservazione-dell-ululone.html) and via periodic news on our facebook page.

Web page

- We have presented a contribute about the conservation project at the XI National Congress of the *Societas Herpetologica Italica* that was held 22-25 September 2016 at MUSE – the Science Museum of Trento.

Oneto F., Canessa S., Ottonello D., Rosa G., Salvidio S. (2016). Conservation project of *Bombina pachypus* (Bonaparte, 1838) in Liguria (NW Italy). Abstracts book. XI National Congress of the *Societas Herpetologica Italica*, Trento, 22-25 September 2016 (see annexes 1 and 2).

Oral presentation at the XI National Congress of the *Societas Herpetologica Italica*

- We made two information boards that will be installed in the next month near the breeding center and near a breeding site to avoid bad behavior, such as the introduction of allocthnous fishes (see annex 3).

Information boards on invasive alien species (IAS)

Information board on *Bombina pachypus* conservation project

- We made available a grant for the formation of a student in *Bombina pachypus* conservation biology. The student graduated in 2016 with the thesis: Rosa G. (2016). Progetto di conservazione di *Bombina pachypus* in Liguria. Corso di Laurea in Scienze Naturali, Università degli Studi di Genova, A.A. 2015/2016.

- Thanks to the visibility achieved by the project 4 sites inside our working area were included in the Italian Herpetological monitoring network, promoted by the *Societas Herpetologica Italica* and by the Italian Ministry of the Environment and Territorial Protection and ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale)