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The LIFE Nature and Biodiversity project "WetFlyAmphibia" - Conservation of amphibians and butterflies of open wet areas and their habitats at the Foreste Casentinesi National Park (Italy)

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Abstract
The LIFE project "Conservation of amphibians and butterflies of open wet areas and their habitats at the Foreste Casentinesi National Park" aims at improving the conservation status of three endangered amphibia (*Bombina pachypus*, *Salamandrina perspicillata* and *Triturus carnifex*) and two butterflies (*Euplagia quadripunctaria* and *Eriogaster catax*) of EC interest, inhabiting open wet areas. The project consists of different actions in 156 planned areas. Actions includes restoration of existing wetlands, creation of new wetlands, removal of trees and shrubs and the reintroduction of *B. pachypus* and *S. perspicillata* in some sites. The restoration of wetland habitats includes the creation of patches of the EC habitat interest H6430 Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels. The project started in 2015 and has a duration of 6 years. Here the challenge represented by different ecological requirements of target animals and plants is described as well as the solutions found to achieve the project aims.

Key words: amphibia reintroduction, endangered butterflies, EC H6430 habitat, habitat restoration, priority species, wetlands.

Introduction

The LIFE project "Conservation of amphibians and butterflies of open wet areas and their habitats at the Foreste Casentinesi National Park" (LIFE14 NAT/IT/000759; acronym: WetFlyAmphibia) is funded with 1,596,342.00 € and has the Foreste Casentinesi National Park as coordinator. Five other partners are involved: D.R.E.Am. Italia Ltd., the University of Pavia, the University of Bologna, Reparto Carabinieri Biodiversità of Pratovecchio and Centro Nazionale Carabinieri Biodiversità of Pieve Santo Stefano (formerly Corpo Forestale dello Stato, "forestry rangers") and the local municipal authority "Unione dei Comuni Montani del Casentino". The project started in 2015 and has a planned duration of 6 years (2015-2021). WetFlyAmphibia aims at improving the populations of three endangered European Community interest amphibia (*Bombina pachypus* Bonaparte, *Salamandrina perspicillata* Savi and *Triturus carnifex* Laurenti) and two butterflies (*Euplagia quadripunctaria* Poda and *Eriogaster catax* L.) of open wet areas of the considered national park, which is situated between Tuscany and Emilia-Romagna regions (N-Apennines, N-Italy; Fig. 1). The analysis of the population dynamics of the target species shows different conservation concerns. *Bombina pachypus* is in sharp decline at a national level and in the project area, the other target species have an irregular distribution due to several factors of disturbance and threat, mainly because of the alteration and reduction of their habitats and breeding sites. The aim is achieved through several conservation actions aimed to stop the threats for the populations of amphibians and butterflies in open wet areas and to favour the establishment of safe populations.

Planned conservation actions include population reinforcement for the target amphibia, the restoration of existing wetlands and the creation of new wetlands. The restoration of these environments also involves restoring of the aquatic and hygrophilous vegetation, including the habitat of EC interest H6430 "Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels", a process that is expected to favour the natural return of some of the target species, without any artificial release. Moreover, the restoration of the vegetation involves the population reinforcement or reintroduction of endangered plant species important for the area, specifically *Hottonia palustris* L. and *Tozzia alpina* L.

Further specific objectives of the project are: 1) improvement of the conservation status of *B. pachypus*, *S. perspicillata* and *T. carnifex* populations, through the reinforcement of populations, the improvement of the conservation status of their habitats and the

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Fig. 1 - Study area. The location of the Parco Nazionale delle Foreste Casentinesi, Monte Falterona e Campigna where the LIFE project WetFlyAmphibia is implemented.

creation of new breeding areas; 2) amelioration of the conservation status of *E. quadripunctaria* and *E. catax* populations, through the improvement of the conservation status of their habitats; 3) improvement of the conservation status of open wetlands (habitat 6430) and rare or unique plant species related to these habitats; 4) increment of the awareness of local people about the need of conservation of amphibians and butterflies species.

Expected results are: 1) detailed distribution maps of the target species including an assessment of their population size; 2) protocol of the *ex situ* breeding of *B. pachypus*; 3) protocol of the *ex situ* reproduction of selected plant species belonging to the habitat 6430; 4) improved conservation status of the target species; 5) increased local people awareness about the conservation of amphibians and butterflies in the area.

WetFlyAmphibia has just started and the first results on the effectiveness of the project will be available in the next years, as it will continue until 2021. Here, as a very preliminary outcome, only some information on the process of habitat restoration for H6430 is provided.

Methods

Study area

The area where the project is carried out is the Foreste Casentinesi, Monte Falterona e Campigna National Park. The area hosts one of the most valuable mountain forest in Europe, the heart of which is the "Foreste Demaniali Casentinesi" (Casentinesi state-owned Forests) that include the "Riserva Naturale Integrale di Sasso Fratino" (Integral Natural Reserve of Sasso Fratino), established in 1959. Small wetlands (ponds, lakes, streams) are interspersed in pastures and forests clears and represent the main habitats for the target amphibia. Here some valuable plant species are found such as *Tozzia alpina* L., *Hottonia palustris* L., *Peplis portula* L. and *Chara globularis* Thuill. Other species locally rare, like *Trollius europaeus* L., *Carex hirta* L. and *Filipendula ulmaria* (L.) Maxim., grow near the ponds and in relict patches of habitat 6430.

It is also a territory consisting of residential areas with a rich historical, artistic and architectural background. The Foreste Casentinesi National Park represents one of the oldest forests in Europe, which is composed of centuries old fir woods, beech and mountain-maple woods.

The vertebrate fauna is represented by large mammals, particularly ungulates (Deer, Fallow Deer, Roe Deer, Wild Boar and Mouflon) and Wolves, these latter are the largest predators currently present in the Park. With regard to amphibians, there are 12 species living in the Park including the species target of the project and other interesting species such as the Spotted salamander (*Ambystoma maculatum* Shaw) and the Alpine newt (*Ichthyosaura alpestris* Laurenti).

General methods

Project aims are achieved through direct actions in 156 areas within the Foreste Casentinesi National Park. These actions include the creation of new wetlands in seven areas, the restoration of the typical vegetation of habitat 6430 in 15 areas, cutting vegetation and trees in 24 wetlands, fence installation in nine areas, installation of structures to facilitates the access of the target amphibia in 46 areas, restoration or creation of fountains in 48 areas, deepening puddles in 15 areas. Additionally, it is planned to reintroduce at least 10-15 (each year) small metamorphosed individuals of *Bombina pachypus* for each area, and at least 1,000-1,500 *Salamandrina terdigitata* eggs each year. Monitoring will include the assessment of the population status of target amphibians and butterflies, the success of habitat restoration, the impact of the project on ecosystem functions and the socioeconomic effects of the project.

Restoration of H6430 and other riparian and aquatic vegetation

The restoration of the vegetation in the new or restored ponds implies the creation of three different vegetation patches encompassing the submerged part of a pond, where the amphibia will reproduce and live during their initial life stage, and the terrestrial neigh-

bours used by the amphibia as corridors between the ponds and the forest. The three vegetation types here reconstructed represent the aquatic vegetation, the hygrophilous vegetation (*Magnocaricion*) and the tall herb fringes of H6430.

The restoration of the aquatic vegetation (*Potamogeton* spp., *Myriophyllum* spp., *Chara globularis*) will be done by relocating small ramets of the target species from current areas of occurrence within the project area to the new or restored ponds. This approach will reduce the cost of production and transportation. Special attention will be paid to avoid the accidental release of non-native fauna connected to the aquatic plants. For instance, some lakes and ponds within the project area are known to host the Louisisana Crayfish (*Procambarus clarkii* Girard). These sites will be disregarded as potential source of aquatic plant material to avoid the unwanted relocation of crayfish larvae. Because the target amphibia have different requirements in terms of aquatic vegetation, the choice of plant species to be relocated in each site accounts for the target species a pond is destined to. For instance, eggs of *B. pachypus* require high amount of light, so ponds destined to this species will receive *Chara* or *Myriophyllum*. On the other hand, *T. carnifex* requires deeper ponds and broad-leaved vegetation, so *Potamogeton* spp. will be established in newt ponds (Kinne, 2004).

The initial project objective is the restoration of the riparian vegetation (*Magnocaricion*) and habitat 6430 in the new or restored ponds, achieved through the production and planting of not less than 15 herbaceous species and not less than 10,000 individual plants. A planting scheme based on the species ecological requirements has been developed. Specifically, at each planting sites the position of each species will be based on soil humidity and light requirements determined according to Ellenberg's ecological indicators (Ellenberg, 1974) modified by Pignatti *et al*. (2005). Figure 2 illustrates a general planting scheme.

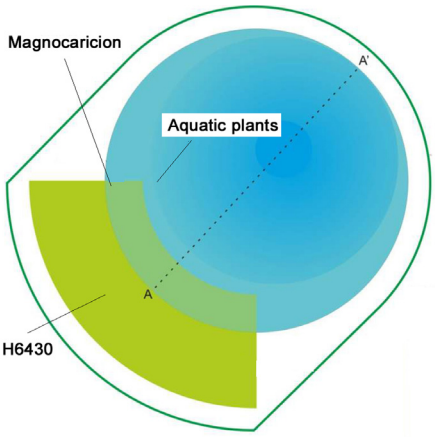

Fig. 2 - General scheme of the three vegetation belts to be created in new or restored wetlands.

Development of seed germination protocols

Ex situ reproduction of plant species belonging to the *Magnocaricion* and the H6430 is an important part of the project because large numbers of species and plant individuals will be required for restoration (Tab. 1). However, germination and cultivation requirements for most of the species selected for restoration were unknow. Thus, germination protocols for some of these species were developed at the University of Pavia to increase the success of *ex situ* propagation.

Seed germination tests were performed at the Germplasm Seed Bank of the University of Pavia sowing

Tab. 1 - List of the plant species reproduced *ex situ* for habitat restoration.

Aconitum lycoctonum L. emend. Koelle
Adenostyles australis (Ten.) Nyman
Aegopodium podagraria L.
Alchemilla xanthochlora Rothm.
Alliaria petiolata (M.Bieb.) Cavara & Grande
Angelica sylvestris L.
Arabis alpina (Willd.) Briq.
Arctium lappa L.
Caltha palustris L.
Carduus personata (L.) Jacq.
Carex cuprina (Heuff.) A.Chem.
Carex hirta L.
Carex leporina L.
Carex pendula L.
Carex pseudocyperus L.
Carex riparia Curtis
Carex strigosa Huds.
Chaerophyllum aureum L.
Chaerophyllum temulum L.
Circaea lutetiana L.
Cynoglottis barellieri (All.) Vural
Digitalis lutea L.
Epilobium hirsutum L.
Epilobium montanum L.
Epilobium parviflorum Screb.
Eupatorium cannabinum L.
Filipendula ulmaria (L.) Maxim.
Heracleum sphondylium L.
Hypericum tetrapterum Fries
Juncus articulatus L.
Juncus conglomeratus L.
Juncus effusus L.
Juncus inflexus L.
Lunaria rediviva L.
Myosotis scorpioides L.
Peplis portula L.
Petasites hybridus (L.) Gaertn.
Pimpinella major (L.) Huds.
Podospermum canum C.A. Mey
Rubus idaeus L.
Salvia glutinosa L.
Sambucus ebulus L.
Saxifraga rotundifolia L.
Senecio ovatus Willd.
Silene dioica (L.) Clairv.
Sparganium erectum L.
Thalictrum aquilegiifolium L.
Trollius europaeus L.

seeds in Petri dishes filled with 1% agar, with three replicates per tests. Seeds were then incubated in temperature- and light-controlled incubators for four weeks. Treatments differed across species and included stable temperature, alternate temperatures, cold stratification and/or the addition of 250 mg/l of Gibberellic Acid (GA_3) to stimulate germination in dormant species (Tab. 2; Baskin & Baskin, 2014). Cold stratification was performed by incubating seeds for two months at 0 °C in dark conditions.

Germination treatments were selected based on data available in the Seed Information Database (SID) of the Millennium Seed Bank of the Royal Botanic Garden, Kew (http://data.kew.org/sid/). For species not included in this database several tests were planned based on information available on other species of the same genus or on previous tests performed at the Seed Bank. Seeds were scored for germination at weekly intervals with germination defined as visible radicle emergence. At the end of each test non-germinated seeds were cut to check their viability. Seeds with fresh embryos were considered as viable seeds, while seeds with dark embryos or fungus infected seeds were considered non-viable. The final germination percentage were computed excluding non-viable seeds.

Preliminary results and discussion

The objective of the project in terms of species for the restoration of wet habitats has been largely exceeded in spring 2017. In fact, at the time of the proposal presentation it was not considered that both mountain and plain subtypes of H6430 (Biondi & Blasi, 2010) were present in the study area, so further additional species had to be produced to restore the two subtypes of H6430 depending on the elevation of each site. So far, 52 species have been produced for a total of about 12,000 individual (Tab. 1), which are ready to be released in the intervention sites. Plant production has been partially done in the premises of Carabinieri Forestali (formerly Ufficio Territoriale per la Biodiversità, UTB of Pieve Santo Stefano of the Corpo Forestale dello Stato) and at the Botanical Garden of the University of Pavia. However, most of the production has been entrusted to an external private company (Flora Conservation, Pavia), specialized in the production of the native flora. The University of Pavia provided the expertise for the production of plants, in particular seed germination and cultivation tests were performed to identify best germination protocols for the selected species.

Tab. 2 - Results of the germination tests performed on some of the species selected for habitat restoration.

Species	Temperature	GA_3	Stratification	Viability (%)	Germination (%)
Caltha palustris L.	25/15°C	no	No	78.3	29.7
	25/15°C	Yes	No	83.3	20
	25/15°C	No	2 months	73.3	52.3
Carex leporina L.	30/20°C	No	No	98.3	98.3
	25/15°C	No	2 months	96.7	100
Carex otrubae Podp.	25/15°C	Yes	No	33.3	0
	30/20°C	No	No	20	8.3
	30/20°C	No	2 months	28.3	5.8
Carex pendula L.	30/20°C	No	No	93.3	62.5
	30/20°C	Yes	No	81.6	100
Carex strigosa Huds.	30/20°C	No	No	91.6	78.1
	30/20°C	Yes	No	90	77.7
Chaerophyllum aureum L.	25/15°C	Yes	No	85	0
	5°C	No	2 months	90	11.1
Chaerophyllum temulum L.	25/15°C	Yes	No	83.3	24
	25/15°C	No	2 months	90	61.1
Digitalis lutea L.	20°C	No	2 months	100	96.6
Juncus articulatus L.	20°C	No	2 months	100	18.3
	25/15°C	No	2 months	100	65
	20°C	Yes	No	100	1.6
Juncus conglomeratus L.	20°C	No	2 months	96.6	10.3
	30/20°C	No	2 months	93.3	94.6
	20°C	Yes	No	96.6	0
Juncus effusus L.	20°C	No	2 months	100	63.3
	30/20°C	No	2 months	100	63.3
	20°C	Yes	No	100	0
Silene dioica (L.) Clairv.	20°C	Yes	No	93.3	82.1
Lunaria rediviva L.	5°C	No	2 months	97.8	0
	15/5°C	No	2 months	100	2.2
Pimpinella major (L.) Huds.	20/10°C	No	2 months	88.3	90.6
	20°C	No	2 months	88.3	79.2
Trollius europaeus L.	20°C	Yes	No	83.3	92
	20°C	No	2 months	75	4.4
Peucedanum ostrutium (L.) Koch	25/15°C	Yes	No	50	93.3
	25/15°C	No	2 months	85	50.9

Results of the germination tests are reported in Table 2. Seed viability was generally high, except for *Carex otrubae*, in which germination was very low. Viability was high, but germination very low in *Lunaria rediviva*. This species will require additional tests to identify its germination requirements. It is likely that the cold stratification has induced dormancy in the seeds instead of increasing their germination potential (Mondoni *et al*., 2017).

Best germination results were obtained at high temperatures (above 25 °C) in most *Cyperaceae* and *Juncaceae* (Tab. 2). GA_3 was essential to stimulate germination in *Peucedanum ostrutium*, *Carex pendula* and *Trollius europaeus*, but was detrimental in other species, like *Caltha palustris*, *Chaerophyllum aureum* and the genus *Juncus*. In the latter group of species, cold stratification was more effective in releasing seed dormancy (Tab. 2).

Despite the development of these protocols, the germination and consequently the production of some species (*e.g. Chamaenerion angustifolium* (L.) Scop., *Carex otrubae* Podp., *Caltha palustris* L.) resulted particularly difficult. A reason is probably the low quality of produced seeds by the very small local populations of these species in the area. So far, about 12,000 plants ready to be released in the project sites have been produced (Tab. 1).

In June 2017, a first planting trial has been carried out at the Metaleto Lake (Camaldoli, AR) in the premises of Carabinieri Forestali. This site was the first restored through a reduction of the reed community that formerly occupied about 80% of the small lake up to about 60%. Moreover, three small ponds were excavated to create a suitable habitat for the reproduction of *B. pachypus*. Here, about 500 plants of different species has been planted, according to the planting scheme described above. A recent survey (July 2017) revealed that, though the summer in the area was very dry, most plants survived and only little damages were done by ungulates. Other wetlands will be restored or created in 2017 and 2018.

WetFlyAmphibia is a highly demonstrative project because it highlights the complexity of making actions matching the ecological requirement of target animals and plants. Plants are often neglected in large conservation projects, but, on the other hand, they are essential to provide suitable habitat requirements for target animals. The restoration of existing wetlands and the creation of new ponds had to be planned according both plant and animal requirements in terms of pond depth, inclination of the shores, position within the general context, elevation and light requirements of the species.

Other interesting challenges will come from the population reinforcement of *Hottonia palustris* and *Tozzia alpina*. The first is a distylous plant, in which the frequency of the two morphs may strongly affect the long-term reproductive effort of a population and in turn the success of the reinforcement (Brys *et al*., 2007). The second is a hemiparasitic plant that requires a complete understanding of the relationships between the species and the host (*e.g.* Holzapfel *et al*., 2016).

Interestingly, the project highlighted the synergy between a private native plant company and two public institutions. The production of the target species was possible thanks to the reciprocal know-how exchange between the three actors. The seed/plant native market is becoming always more important and may also represent an important economic opportunity for biologists and naturalists.

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