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The restoration of the wetlands with standing waters constituting the habitat of the Italian green toad (*Bufo balearicus* Boettger, 1880)

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Abstract
The aim of the present study is the restoration of an environment suitable to the particular ecological requirements of populations of amphibians of Community interest. Ponds, pools, marshes, small standing waters, are hot spots of biodiversity. They contribute significantly to increase the ecological value of agro-ecosystems. The study here presented was required by a municipal administration because of the infringement procedure started by the European Union according to Habitats Directive (92/43 EEC) against the Municipality of Numana, in the Province of Ancona, in the Marche Region (Central Italy). This procedure has been initiated because of an alteration of the habitat of the Italian green toad, which is an endangered species included in annex IV of Habitats Directive and of the other amphibians that live in the wet micro-ecosystems near the mouth of the River Musone. The procedure required the definition of a mitigation and compensation project for the protection of the herpetofauna. It was carried out by an interdisciplinary team of experts. The phytosociological analyses of the vegetation were carried out in both the area directly interested by the project and in nearby guazzi (artificial wetlands for hunting purposes); they have shown the presence of the following associations: *Symphyto bulbosi-Ulmetum minoris* subass. *lauretosum nobilis, Cypero-Caricetum otrubae, Eleocharitetum palustris, Sparganio erecti-Veronicetum anagallidis-aquaticae, Loto tenuis-Agropyretum repentis* with the *Ranunculus sardous* variant. Moreover, the eco-physiological study of seed germination was carried out on some marsh plants (*Carex otrubae, C. pendula, C. divulsa*). These species of genus *Carex* show a strong primary dormancy that was overtaken with cold stratification at 5 °C 25/15 ° C and with a gibberellins treatment. Following the same methodological approach a study on the germination response was initiated of other typical species of these environments and it has been designed an artificial damp environment at the Botanical Garden, "Selva di Gallignano" of the Polytechnic University of Marche, to experiment with the plants to be introduced in the restoration of the habitat of *Bufo balearicus*. The investigation led to the suspension of infringement procedures by the European Commission despite the humid environment has not yet been rebuilt.

Key words: *Bufo balearicus, Carex divulsa, C. otrubae, C. pendula,* Habitats Directive, phytosociology, ecology of germination, infringement procedure, environmental restoration, vegetation.

Introduction

Ponds, pools and marshes are real resources in ecological terms because they contribute to the diversification of the landscape mosaic. Indeed, they create the appropriate conditions for the development of many species, animals and plants, which otherwise would not be able to live in those places. These little ecosystems of lentic waters are true oases of biodiversity (hot spots of biodiversity). They increase considerably the variety of natural and anthropogenic landscapes contributing significantly to increase the ecological value of agro-ecosystems (Davies *et al*., 2008). These micro-ecosystems, however, are now became rare. In fact, the urbanization and the intensive agriculture tend to delete them because they do not perform recognized economic functions although they carry out important ecosystemic functions. After all, these micro-ecosystems have no significant effect on the land occupation and in the reducing of agricultural areas due to their small surface and their location in marginal areas. They occupy small impluviums or depressions that are naturally not permeable. For these reasons, the ecosystems of "small water bodies" (“Kleingewässer” of the German-speaking scientists, according to Stoch, 2005) are now considered among the most threatened environments in Europe and particularly in Italy. Moreover, these micro-ecosystems are extremely variable over time. They are susceptible to the marked spatial changes for reasons which are both of the natural order (alternation of rainfall, presence of invasive species) and for anthropic actions, in particular for improper agricultural practices. On the contrary, these areas have a direct ecological function as they act as stepping-stones, areas of transition between aquatic and terrestrial environments although they have limited size (Biondi *et al*., 2012a). According to the classification of Gajl (1924), the small water bodies are defined “astatic” as they show sudden changes in water level. Therefore, they are marshy seasonal formations, temporary, which dry up in dry season. Even more, they can be formations linked to rainfall events which are extremely limited in time as the puddles after storms. They are ephemeral formations. These features differentiate the small water bodies from areas with perennial waters, natural and artificial. The small water bodies had not specific safeguards before the adoption of the Habitats Directive (92/43/EEC) by the European Union. The Ramsar Convention on Wetlands (adopted on February 2nd, 1971) especially refers to large are-

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as of swamps and marshes, which should be protected and managed being the primary environment for waterfowl. Therefore, they are mainly identified along their migration routes. The Habitats Directive, in Annex I, with the code 31 "Standing waters" includes many habitats which may include temporarily flooded sites, the most important of which is represented by the priority habitat: "Mediterranean temporary ponds", code 3170* (Brullo & Minissale, 1988; Bagella *et al.*, 2007, 2009; Grillas *et al.*, 2004; Alfonso, 2011; Biondi *et al.*, 2012b). Obviously, the same Directive reports other habitats with similar characteristics, although not directly related to the Mediterranean macrobioclimate or totally lentic ecosystems as related to the habitat of lakes or rivers of the code 32 (Biondi *et al.*, 2009a; Landucci *et al.*, 2013). Indeed, also habitats with a different degree of ground salt concentration of the code 31 (Biondi & Casavecchia, 2010) or even the habitats of different formations of bogs belonging to the code 71 (Gerdol & Tomaselli, 1997; Pedrotti, 1978) or to the code 72 (Mayer, 1995; Bracco *et al.*, 2000; Tomaselli *et al.*, 2006). The description of these habitats is reported in the Manual of European habitats (latest version EUR/27, European Commission, 2007) and they are interpreted for Italy by the Italian Botanical Society [English Interpretation Manual of the 92/43 EEC Habitats Directive (Biondi *et al.*, 2009b http://vnr.unipg.it/habitat/)] and updated for correlations in syntaxonomical terms (Biondi *et al.*, 2012b).

Case of study

The aim of the present study is the restoration of an environment suitable for the particular ecological requirements of some populations of amphibians of Community interest. This action was necessary because of the infringement procedure provided for the Habitats Directive (92/43 EEC) and started by the European Union against the Municipality of Numana consisting in an administrative sanction of about 9 million euros (http://ec.europa.eu). The procedure has been initiated after a complaint of a biologist concerning the alteration of the Italian green toad habitat (*Bufo balearicus* Boettger, 1880), which is a species included in the list of Annex IV of the Habitats Directive as *Bufo viridis* (Fig. 1).

Currently, the taxonomic position of *Bufo viridis sensu lato* is rather complex, in fact, the genetic studies have demonstrated how the species is actually constituted by a complex of cryptic species, morphologically indistinguishable but well separable at genetic and sometimes karyological level (Lanza *et al.*, 2007). The Italian populations are distinct from those transalpine also according to enzyme electrophoresis data and they represent at least a distinct species (Lanza *et al.*, 2007). Some herpetologists attribute this species to the genus *Pseudepidalea* (*Pseudepidalea viridis*), but this attribution is still controversial. According to the recent molecular phylogeny proposed by Stöck *et al.* (2008) (Fig. 2), we decided to adopt the name of *Bufo balearicus* Boettger, 1880, identifying the populations of mainland Italy (from Piedmont to Calabria) (Odierna & Maio, 2012).

Fig.1 - Italian green toad (*Bufo balearicus* Boettger, 1880).

The species is especially widespread along the sandy coasts and lowland floodplains. It is also found in relatively arid and anthropized environments and in various aquatic habitats, both natural and artificial. Althogh the Italian green toad is a purely terrestrial species, it moves to wet habitats, even small habitats, temporary and brackish, during the breeding season (Lanza *et al.*, 2007; Sindaco *et al.*, 2006). It is an oviparous species, that lays a large number of eggs (between 5000 and 13000) in long cylindrical cords long 2 m and more, wrapped to the aquatic vegetation. In the last decade, it seems that spawning has been adversely affected by the high use of pesticides and herbicides in agriculture that caused the decrease of the production of eggs (Ferri, 1990).

Other important herpetological species present in the area are the Italian crested newt (*Triturus carnifex*) and the Italian frog (*Hyla intermedia*) (Fiacchini *et al.*, 2003). The Common newt (*Lissotriton vulgaris*), represented in Italy by an endemic subspecies, the Common toad (*Bufo bufo*) and the Green frog (*Pelophylax bergeri - P. kl. Hispanicus*) can also be observed in the same habitats and in the same period (Caputo, 2008).

Materials and methods

Study area

The study area is located in the southern part of Conero Park (in the Marche Region, Province of Ancona) and represents the final part of the alluvial zone of the River Musone. From a bioclimatic point of view, the area belongs to the Mediterranean pluviseasonal-oceanic bioclimate and to the upper mesomediterranean

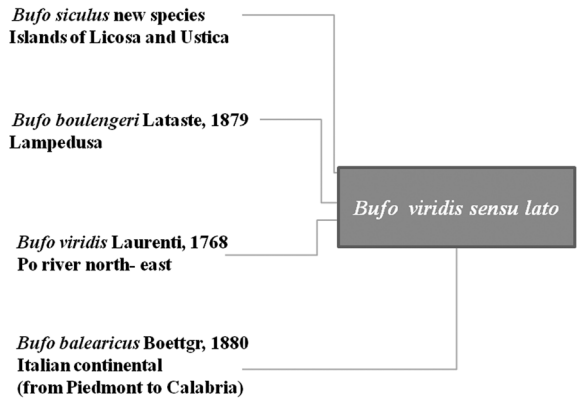

Fig. 2 - Taxonomy and distribution of *Bufo viridis* group *sensu lato*

low sub-humid bioclimatic belt (Rivas-Martinez *et al*., 1999; 2008).

Mitigation and compensation project

Mitigation and compensation project, commissioned by the Regional Conero Park, was undertaken by an interdisciplinary team of specialists (a biologist, an agronomist, a geologist, a botanist and a geometer) coordinated by an architect. Seeds and propagules of hygrophylous species indicated in the project were collected in the "guazzi" located on the right side of the River Musone and subsequently cultivated at the Botanical Garden "Selva di Gallignano". They will be used for the environmental restoration of the Italian green toad habitat. At the same time, ecophysiological studies on seed germination of these species have been carried out at the Germplasm Bank of the Polytechnic University of Marche. In addition, the mitigation and compensation project involves the replacement of a conifer forest, partially degraded, with native vegetation in order to start dynamic processes of natural colonization. The referred potential wood of this area is typical of alluvial flatzones, coastal and sub-coastal, Mediterranean and sub-Mediterranean area, of the western Adriatic. The mature stage of the series is the association *Rubio peregrinae-Fraxinetum oxycarpae* (Pedrotti & Gafta 1992), Biondi & Allegrezza 2004 (= *Lauro nobilis-Fraxinetum oxycarpae* Pedrotti & Gafta 1992 nom.ill.) found in the area in small residual nuclei. The communities participating in the creation of the forestal series and those that make up aquatic and marsh vegetation are shown in Biondi *et al*. (2002). All of these communities were used as reference for the design of mitigation and compensation project which not only provides for the reconstruction of small ponds but also for the re-naturalization of ditches, small temporarily flooded forests and other adjacent wetland habitats. In addition, an ash (*Fraxinus oxycarpa* Bieb.) wood will be realized to replace the pre-existing conifer reforestation. The ash wood represents the final stage in the succession of the mesophilous association. All plant material that will be used in the restoration work will be autochthonous. It will be multiplied from different seeds and propagules collected in the territory of the Conero Park or in the areas immediately adjacent.

Flora and vegetation analysis

Within the researches carried out for the mitigation/compensation project, several surveys were carried out from April to May 2011 in both the area directly interested by the project and in the "guazzi" in order to characterize the flora of the area and to identify any species rare or of a particular vegetation interest. For the nomenclature, the vascular flora of Conero Regional Nature Park recently published (Biondi *et al*., 2012c) has been followed.

The vegetation study was carried out following the phytosociological method of the sigmatist school of Zurigo-Montpellier (Braun-Blanquet, 1928) and lately updated by several authors (Tüxen 1977, 1979; Géhu, Rivas-Martínez, 1981; Géhu, 1991; Rivas-Martínez, 1994 and 2005; Theurillat, 1992; Biondi & Zuccarello, 2000; Biondi, 2011; Pott, 2011). Phytosociological relevés were carried out in the area designated for the wetlands and in other areas involved in the project.

Eco-physiological analysis of seed germination

The part on the study of seed germination was conducted in the laboratories of the "Seed Bank ASSB Anfiadriatic Species" at the Botanical Garden "Selva di Gallignano" Polytechnic University of Marche (Biondi *et al*., 2012d). The seeds of the most characteristic and/or rare species of the wetlands in this area and in the "guazzi" were collected. An eco-physiological study was carried out to test their germinative and reproductive capacity. These investigations are still in progress. This article will present the partial results of analyses conducted on a group of *Carex*: *C. otrubae* Podp., *C. pendula* Hudson, *C. divulsa* Stokes.

The analyses of these species have been carried out in the period August 2011 - January 2012 and the seeds were collected (July-August 2011) in the "guazzi" of the Scossicci plain located in the municipality of Porto Recanati (43° 27' N, 13° 37' E WGS84 datum) in the province of Macerata, near the area of the "Lido Azzurro" (43° 28' N, 13° 37' E WGS84 datum) located in the town of Numana, in the Province of Ancona. Following harvest, seeds were cleaned and empty and developed seeds were removed with a blower (Agriculex CB1 Column Seed Cleaner, T.A. Baxall and Co., Ltd). Afterwards, they were dried and stored in a dry room at 15°C and 15% relative humidity before being used for germination testing and morphological analysis. The germination behavior of seeds was tested at a constant (20°C) and an alternating tempera-

ture (25/15°C). Moreover, it was also investigated the effect of the following pretreatments on germination at 25/15°C: immersion for 24 hours in gibberellic acid (GA3, 1 g/l), three months of cold stratification (5°C). The photoperiod used for all the tests was of 12 hours of light and 12 hours of darkness. Four replicates of 25 seeds each were used in each germination test. The germination tests were completed after 30 days.

For all the tests, the final germination percentages were calculated and arcsine-transformed, and the levels of significance ($P < 0.05$) were obtained by analysis of variance (ANOVA) using SPSS 16.0.

Homogeneous groups were determined using Tukey's test.

Results

For the restoration of the wetlands, preliminary field researches were carried out in some artificial ponds created for hunting purpose in the Scossicci plain, on the right side of the Musone River, locally known as "guazzi" (Biondi *et al.*, 2002). These ponds are a good example of how aquatic species distribute in relation to water depth and morphology of the basin. Moreover, the plants found in these small habitats have developed spontaneously probably because of seed dispersion by birds typical of these environments. Therefore, thanks to vegetation analyses carried out in different moments of the vegetative period, it was possible to understand the ecology of each species and to collect useful information for the restoration of the wetlands of interest. Indeed, these small "guazzi" are important not only for hunting purpose but primarily because they are spots of biodiversity for agro-ecosystems supporting the development of specific habitats for plants and animals. The floristic study has clearly revealed the presence of numerous rare species including some rooting hydrophytes (pleustophytes) as: *Zannichellia palustris* ssp. *pedicellata, Potamogeton pectinatus, Ruppia maritima, Ranunculus peltatus* ssp. *baudotii* and some helophytes like *Eleocharis palustris, Juncus articulatus, Carex otrubae, Bolboschoenus compactus* and *Equisetum palustris* (Biondi *et al.*, 2002).

In the study area, there are small woods dominated, in the tree layer, by the elm (*Ulmus minor*). These are mesophilous and wet woods that, for the most part of wet seasons, are completely flooded with water that reach about 20 cm of the stem. In order to deepen the knowledge on local elm woods which, for the regional coastal and sub-coastal side, are attributed to the association *Symphyto bulbosi-Ulmetum minoris* Biondi & Allegrezza 1996, the two relevés, reported on Table 1 were done. According to these relevés it clearly appears that it is an hygrophilous wood having a strong Mediterranean characterization because of the abundant presence of laurel (*Laurus nobilis*) in the tree layer and the modest presence of *Rubia peregrina* in the herb layer. These species, based on the observations conducted on other *Ulmus minoris* formations in the submediterranean area, allow us to differentiate a new sub-association called *laureetosum nobilis* whose holotypus is the relevé 2 of tab. 1. Indeed, following the Adriatic coast to the south, the bioclimate becomes more and more Mediterranean and the vegetation is characterized by the high presence of *Laurus nobilis* and other Mediterranean species into meso-hygrophilous communities. This fact is demonstrated in the southernmost part of the Region by the presence of the following associations: *Fraxino orni-Lauretum nobilis, Rubio peregrinae-Aceretum campestris, Lauro nobilis-Populetum canescentis, Scutellario columnae-Ostryetum carpinifoliae* subass. *viburnetosum tinii, Roso sempervirentis-Quercetum virgilianae* subass. *laureetosum nobilis* (Allegrezza *et al.*, 2006; Biondi *et al.*, 2013). The meso-hygrophilous character of this elm wood is shown by the presence of *Carex divulsa*, *C. pendula* (both species being sciaphilous and hygrophilous) and *Brachypodium sylvaticum,* a species typical of mesophilous woods, not common in coastal and sub-coastal Mediterranean areas. The shrub/grass layer is also characterized by abundant ivy (*Hedera helix*). In our opinion, the conservation of these elm micro-

Tab. 1 - *Symphyto bulbosi-Ulmetum minoris* Biondi & Allegrezza 1996 subass. *laureetosum nobilis* subass. nova (holotypus rel. 2)

		Relevé number	**1**	2*
		Altitude (m a.s.l.)	3	4
		Exposition	-	-
		Slope (°)	-	-
		Cover. (%)	100	100
		Surface (m^2)	400	400
		High of tree layer (m)	15	18-20
		High of shrubs. (m)	4	3
		Charact. and diff. sp. of the ass. and of the *Sambucus nigra* variant		
P caesp	EUR.-CAUC.	Ulmus minor Miller	5.5	4.5
P caesp	EUR.-CAUC.	Sambucus nigra L.	1.2	2.2
G rhiz	SE-EUROP.	Symphytum bulbosum Schimper	.	2.2
		Diff. species of the subass.		
P caesp	STENOMED.	Laurus nobilis L.	2.3	4.4
P lian	STENOMED.	Rubia peregrina L.	1.2	+
		Charact. and diff. sp. of the ord. *Populetalia albae* and the class *Querco-Fagetea*		
P lian	EURIMEDIT.	Hedera helix L.	3.4	4,4
P scap	EUR.-CAUC.	Acer campestre L.	3.4	2.2
H caesp	PALEOTEMP.	Brachypodium sylvaticum (Hudson) Beauv.	2.2	+.2
H caesp	EURIMEDIT.	Carex divulsa Stockes	1.2	+.2
P scap	S-EUR.-S.SIB.	Fraxinus ornus L.	+	3.4
G rhiz	STENOMEDIT.	Arum italicum Miller	+	1.2
He	EURASIAT.	Carex pendula Hudson	+.2	.
H scap	CIRCUMBOR.	Geum urbanum L.	+	.
		Other species		
NP	EURIMEDIT.	Rubus ulmifolius Schott	3.3	2.3
P caesp	AVV.	Prunus cerasifera Ehrh.	1.2	1.2
NP	AVV.	Pittosporum tobira (Thunb.) Aiton fil.	+	+.2
P scap	AVV.	Acer negundo L.	+	+
H scap	EUROSIB.	Stachys sylvatica L.	+	+
P caesp	PALEOTEMP.	Crataegus monogyna Jacq.	2.2	
P caesp	SE-EUROP.	Paliurus spina-christi Miller	1.2	.
P scap		Cupressus arizonica Green	1.2	.
P scap	EURIMEDIT.	Pinus pinea L.	1.1	.
P scap	STENOMEDIT.	Pinus halepensis Miller	+	.
H scap	SUBCOSMOP.	Urtica dioica L.	.	1.2
T scap	EURASIAT.	Galium aparine L.	.	+

woods is of great importance as they represent pioneer forest formations that, as noted for the alluvial flat area of the River Potenza (Biondi *et al*., 2002), promote the restoration of flat mesophilous woods dominated by southern ash (*Fraxinus oxycarpa*). Unfortunately, adventitious and exotic naturalized species such as *Prunus cerasifera, Pittosporum tobira* and *Acer negundo* are also present in this wood. Furthermore, sick cultivated species as *Pinus pinea* and *Pinus halepensis* occur. Thus, the action to be performed in order to improve the naturality of this area should also include the elimination of adventitious and implanted species.

In all the studied area, there are several drainage canals that were excavated for an urban project that has never been realized. In such canals, different kinds of hygrophilous communities occur, following the water depth and the inclination of banks. These hygrophilous communities have been accurately studied in order to clarify their phytosociological characterization.

The first coenosis observed is represented by a *Carex otrubae* community found in a 5 cm deep drainage canal (tab. 2). It deals with a thick herbaceous community dominated by the sedges *Carex otrubae* and *C. divulsa* as well as *Juncus maritimus* and *Eleocharis palustris*. According to Venanzoni & Gigante (2000), this community belongs to the association *Cypero-Caricetum otrubae* Tüxen ex T.E. Díaz 1975. In comparison to the typical form of the association described in the Iberian Peninsula (Tüxen & Oberdorfer, 1958), the studied community is floristically impoverished. This is due to the different bioclimatic conditions and the strong environmental alteration of the area.

The community dominated by *Eleocharis palustris* (tab. 3) has been observed in some drainage canals about 10 cm deep located at the edge of grasslands. This community can be referred to the association *Eleocharitetum palustris* Schennikow 1919 largely distributed in wet areas of central-eastern Europe. This association is the subject of a recent ecological and phytosociological research carried out in Croatia (Stančić, 2008). According to this research, two distinct variants of the association occur through Europe: a continental variant, characterized by an abundant presence of species of the class *Molinio-Arrhenatheretea* and a Mediterranean variant, poorer in species. From the comparison made between our relevés and those already published in the cited work, it emerges that the community that we have found represents an intermediate condition due to the simultaneous presence of species having a Mediterranean distribution range and mesophilous Eurasian species. This can be explained considering the Sub-Mediterranean bioclimate occurring in this area, characterized by greater rainfall in respect with the more marked Mediterranean climate typical of the Croatian coast.

The community dominated by *Veronica anagallis-aquatica* (tab. 4) was found lately (at the end of May) when canals were almost completely dried up. Under these conditions, an herbaceous hygrophilous formation develops which is dominated by *Veronica anagallis-aquatica*, a species typical of stagnant waters, with *Carex otrubae* and *Alisma plantago-aquatica*. According to phtytosociological literature, communities dominated by *Veronica anagallis-aquatica* have been attributed to three main associations: *Sparganio erecti-Veronicetum anagallidis-aquaticae* Passarge 1999 described for north-eastern Germany; *Polygono hydropyperis-Veronicetum anagallidis aquaticae* Scahaminée & Weeda in Weeda *et al*. 1995 and finally the association *Veronicetum anagallidis-aquaticae* Kaiser, 1926, currently no more recognized and called "*Glycerio-Sparganion* Basalgesellschaften" (according to Floraweb) because *Veronica anagallis-aquatica* is considered as a characteristic species of the alliance. Therefore, the community found should belong to the association *Sparganio erecti-Veronicetum anagallidis-aquaticae* although floristically impoverished in comparison to the typical condition of the northeastern

Tab. 2 - *Cypero-Caricetum otrubae* Tüxen ex T.E. Diaz 1975

		Relevé number	1
		Altitude (m a.s.l.)	3
		Exposition	-
		Slope (°)	-
		Cover. (%)	80
		Surface (m2)	1x20
		Charact. and diff. species of the ass. and of the upper units	
H caesp	EURIMEDIT.ATL.	Carex otrubae Podp.	4.5
H caesp	EURIMEDIT.	Carex divulsa Stockes	2.2
G rhiz	SUBCOSMOP.	Eleocharis palustris (L.) R. et S.	2.2
G rhiz	SUBCOSMOP.	Juncus maritimus Lam.	1.2
G rhiz		Cyperus longus L. ssp. badius (Desf.) Asch. et Gr.	1.2
		Other species	
H scap	CIRCUMBOR.	Rumex acetosa L.	1.2
H bienn	CENTRO-EUROP.	Senecio erraticus Bertol.	1.1
P caesp	EUROP.-CAUC.	Ulmus minor Miller (pl.)	1.1
P caesp	AVV.	Prunus cerasifera Ehrh.(pl.)	+
NP	EURIMEDIT.	Rubus ulmifolius Schott (pl.)	+

Tab. 3 - *Eleocharitetum palustris* Schennikow 1919

		Relevé number	2	3	
		Altitude (m a.s.l.)	3	3	
		Exposition	-	-	
		Slope (°)	-	-	
		Cover. (%)	90	95	Presences
		Surface (m^2)	1x30	1,5x10	
		Charact. and diff. species of the ass. and of the upper units			
G rhiz	SUBCOSMOP.	Eleocharis palustris (L.) R. et S.	5.5	5.5	2
H caesp	EURIMED.ATL.	Carex otrubae Podp.	2.2	1.2	2
H scap	PALEOTEMP.	Lycopus europaeus L.	+	+.2	2
H scap	COSMOP.	Veronica anagallis-aquatica L.	1.2	.	1
H scap	SUBCOSMOP.	Lythrum salicaria L.	.	1.2	1
I rad	SUBCOSMOP.	Alisma plantago-aquatica L.	.	1.1	1
		Other species			
T scap	S-EUROP.	Xanthium italicum Moretti	1.2	1.3	2
H bienn	CENT-EUR.	Senecio erraticus Bertol.	2.3	.	1
H rept	CIRCUMBOR.	Agrostis stolonifera L.	2.3	.	1
H rept	PALEOTEMP.	Ranunculus repens L.	1.1	.	1
H scap	CIRCUMBOR.	Rumex acetosa L.	+	.	1
G rhiz	CIRCUMBOR.	Agropyron repens (L.) Beauv.	.	3.3	1
T scap	EURIMEDIT.	Ranunculus sardous Crantz	.	2.2	1
T scap	AVV.	Aster squamatus (Sprengel) Hieron.		1.1	1
H scap	SUBCOSMOP.	Rumex crispus L.	.	+	1

Tab. 4 - *Sparganio erecti-Veronicetum anagallidis-aquaticae* Passarge 1999

		Relevé number	4	5	
		Altitude (m a.s.l.)	3	3	
		Exposition	-	-	Presences
		Slope (°)	-	-	
		Cover. (%)	90	100	
		Surface (m2)	3	10	
		Charact. and diff. species of the ass. and of the upper units			
H scap	COSMOP.	Veronica anagallis-aquatica L.	4.5	5.5	2
I rad	SUBCOSMOP.	Alisma plantago-aquatica L.	2.2	2.2	2
H caesp	EURIMEDIT.ATL.	Carex otrubae Podp.	2.3	.	1
G rhiz	SUBCOSMOP.	Eleocharis palustris (L.) R. et S.	.	1.2	1
		Other species			
H rept	CIRCUMBOR.	Agrostis stolonifera L.	2.3	2.3	2
		Aster squamatus (Sprengel)			
T scap	AVV.	Hieron.	1.1	2.2	2
H scap	CIRCUMBOR.	Rumex acetosa L.	+	+	2

Germany. As already explained, the studied area is strongly altered by anthropic actions which largely disturb vegetation.

The canals, whose vegetation has been described above, are marginally of large areas occupied by thermo-hygrophilous grasslands where, following the project, the small lakes will be realized. This grassland shows three different aspects (table 5). The first aspect (rel. 1) refers to a slightly depressed area where the first pond will be created. In this situation, a perennial grassland has developed which is used since a long period for horse grazing; it is dominated by leguminous and grasses species and it is attributed to the association *Loto tenuis-Agropyretum repentis* Biondi, Vagge, Baldoni & Taffetani 1997 described for similar ecosystems and found in many areas adjacent to the mouth of the River Musone.

The second aspect, regarding the area where the second pond will be realized, deals with a grassland similar to the previous one, but where the dominant plants are grasses. Apparently, it is relatively dryer than the first one because, having been used for cultivation in the past, it presents a slight convex shaping in the central part and is in a slightly elevated position.

Finally, the third aspect refers to the *Ranunculus sardous* variant detected at the edge of the first grassland, in a depressed area that was submerged until the first week of May, but it was completely dry at the time of the relevé (end of May). As can be observed, it is a more pioneer community in comparison to the previous two ones and it is characterized by a significant presence of hygrophilous species such as *Mentha pulegium, Alisma plantago-aquatica* and *Carex otrubae* that show a condition of greater edaphic humidity.

In conclusion, it is possible to make some general observations on the contribution of plant communities to the phyto-biodiversity of the plain area of the River Musone and, more generally, to the territory of the Conero Natural Park. The high value in biodiversity is extremely important for the environmental restoration that will be realized as the specific recovery of the Italian green toad. Actually, it is part of a more concrete project to save its habitat and in general to focus on reconstruction of all ecosystems that it attends during the year. Therefore, the project will result in a substantial recovery of areas considered marginal within the Park territory with their riqualification in consideration of specific, eco-systemic and landscape biodiversity. As regards the conservation of biodiversity *sensu* Directive 92/43/EEC (Biondi *et al*., 2009b; 2012b) and the restoration of more natural conditions, the actions that will be carried out with the restoration project here described are focused on the restoration of habitats important for the territory of the whole Marche Region that currently are everywhere destroyed:

1410	Mediterranean salt meadows (*Juncetalia maritimi*)
3140	Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp.
3150	Natural euthrophic lakes with *Magnopotamion* or *Hydrocharition*-type vegetation
6420	Mediterranean tall humid herb grasslands of the *Molinio-Holoschoenion*

Germination tests and propagation of autochthonous germplasm

C. otrubae and *C. pendula* germinated to high percentages at alternating temperature (25/15°C) with all the treatments tested, while no seed germinated at constant temperature (20°C) (Tab. 6, Fig. 3a; Tab.7, Fig. 3b). *C. divulsa* germinated to high percentages at both constant and alternating temperatures, although the datum at constant temperature was significantly lower ($P < 0.0001$) than the others obtained at alternating temperatures with all the treatments tested (Tab. 8, Fig. 3c).

The results obtained with the control tests on *Carex pendula* with alternating temperature (99%) are consistent with other studies on *Carex* sp.pl. carried out at alternating temperatures (RBG Kew 2008; Schütz, 2000; Maas, 1989; Schütz, 1999; Esmaeili, 2009). These demonstrated a positive effect of alternating temperatures on plants and seeds of wetlands (Thompson & Grime, 1983). Whereas the results of *Carex otrubae*, in particular for the treatment with cold stratification for three months at 5°C and test temperature of 25/15°C (100%), are far better compared to the datum obtained by the RBG Kew (2008) at a similar condition (0,6% with cold stratification for six months at 4°C with 24 hours of light). Final germination percentages obtained in this study are higher than the ones reported by Grime *et al*. (1981) for the seeds of many populations of northern distribution in Britain subjected to cold stratification for twelve months at 5°C and then tested at 26°C. The results of germination tests on *Carex divulsa* seeds (100%) are similar

Tab. 5 - *Loto tenuis-Agropyretum repentis* Biondi *et al*. 1997 *Ranunculus sardous* variant

		Relevé number	1	2	6
		Altitude (m a.s.l.)	4	4	3
		Exposition	-	-	-
		Slope (°)	-	-	-
		Cover. (%)	100	100	100
		Surface (m^2)	80	80	2x20
		High of tree layer (m)	60	120	1
		High of shrubs. (m)	40	-	30
		Charact. and diff. species of the association			
H scap	PALEOTEMP.	Lotus tenuis W. et K.	4.4	2.3	1.1
G rhiz	CIRCUMBOR.	Agropyron repens (L.) Beauv.	+	1.1	3.4
T scap	S-EUROP.	Xanthium italicum Moretti	.	.	3.3
		Species of the *Ranunculus sardous* variant			
T scap	EURIMEDIT.	Ranunculus sardous Crantz	1.1	+	5,5
H scap	EURIMEDIT.	Mentha pulegium L.	.	.	2.3
I rad	SUBCOSMOP.	Alisma plantago-aquatica L.	.	.	2.2
H caesp	EURIMED.ATL.	Carex otrubae Podp.	.	.	+
		Charact. and diff. species of the upper units			
H caesp	PALEOTEMP.	Dactylis glomerata L.	3.4	3.4	.
H caesp	EURASIAT.	Poa trivialis L.	2.3	1.2	.
H bienn	PALEOTEMP.	Daucus carota L.	1.1	+.2	.
H scap	EUROSIB.	Picris hieracioides L.	2.2	1.2	.
H scap	PALEOTEMP.	Cichorium intybus L.	1.1	.	.
H bienn	CENTRO-EUR.	Senecio erraticus Bertol.	.	+.2	.
		Charact. and diff. Sp. class *Molinio-Arrhenatheretea*			
H ros	EURASIAT.	Plantago major L.	1.2	+.2	1.2
H caesp	CIRCUMBOR.	Lolium perenne L.	3.4	5.5	.
H rept	PALEOTEMP.	Trifolium repens L.	3.4	3.4	.
H ros	EUROP.-CAUC.	Bellis perennis L.	1.1	.	.
H scap	E-EUR.-PONT	Galega officinalis L.	+.2	.	.
G bulb	CENTRO-MEDIT.	Bellevalia romana (L.) Sweet	+	.	.
		Other species			
T scap	EURIMEDIT.	Medicago hispida Gaertner	1.2	1.2	.
T scap	EURIMEDIT.	Picris echioides L.	1.2	1.2	.
H ros	EURASIAT.	Plantago lanceolata L.	1.1	1.2	.
H bienn	EURIMEDIT.	Dipsacus fullonum L.	1.1	1.1	.
H caesp	PALEOTEMP.	Bromus erectus Hudson	+.2	+.2	.
NP	EURIMEDIT.	Rubus ulmifolius Schott	+	.	.
H scap	EURIMEDIT.	Mentha pulegium L.	+	.	.
T rept	PALEOTEMP.	Trifolium resupinatum L.	.	+	.
H caesp	EURIMEDIT.	Carex hallerana Asso	.	+	.
T scap	AVV.	Aster squamatus (Spreng.) Hieron.	.	.	+

to germination tests on *Carex divulsa* ssp. *divulsa* of the RBG Kew (2008; 95% at 22/10°C). According to other studies about *Carex* sp. pl. the cold stratification has a positive effect on their seed germination (Schütz, 1997; Maas, 1989; Grime *et al*., 1981; Schütz, 2000; Schütz & Rave, 1999; Baskin & Baskin, 1998). According to literature, the populations of *Carex* which had been studied showed a strong primary dormancy probably due to the high mass of the seed. The differences with dormancy and cold requirements can be linked to the thickness and the hardness of the seed coat of the different species which have been studied (Schütz, 2000). According to the percentages of germination of the literature, RBG Kew (95%); Schütz (80%) for the population of *Carex* of this work the best treatment is the cold stratification for three months at 5°C and test temperature of 25/15°C (99%). The similar results (100%) are obtained also with treatment with gibberellins.

Conclusions

The study has shown that it is possible to realize a project of compensation/mitigation to avoid the loss or complete alteration of habitats of some threatened species. In particular, it has been presented the case of the Italian green toad (*Bufo balearicus* Boettger, 1880), included among the 221 species listed in Annex IV of the Habitats Directive (92/43/ EEC), present in the southern area of the Conero Regional Park. The analyses carried out on the vegetation show that it is necessary to know the species that are part and their ecology and to interpret the evolutionary dynamics and structure of the habitat itself for a proper restoration of it. The populations of *Carex* sp. pl. that were studied did not seem to need pretreatments but rather the necessary condition for germination is the alternating temperature. The only exception is *Carex divulsa* that shows a good percentage of germination even at constant temperature, although significantly lower than that obtained at alternating temperature. The populations of *Carex* sp. pl. studied, have responded to all treatments with excellent germination percentages, so that once finished the experimental tests the seedlings, almost all in full force, were transplanted. Following the same methodological approach, a study on the response of germination of other typical species of these environments was started and a model habitat, an ar-

Tab. 6 - Summary of data on the germination of *Carex otrubae* obtained in the tests with different treatments: control at 25/15° C (1), control at 20° C (2), treatment with gibberellins 25/15° C (3) cold stratification for three months at 5°C test temperature of 25/15° C (4).

Species	Treatments	Germination (%)	P	homogeneous group
Carex otrubae	1	100	0.0001	a
Carex otrubae	2	0		b
Carex otrubae	3	100		a
Carex otrubae	4	100		a

Tab. 7 - Summary of data on the germination of *Carex pendula* obtained in the tests with different treatments: control at 25/15° C (1), control at 20 ° C (2), treatment with gibberellins 25/15 °C (3) cold stratification for three months at 5°C test temperature of 25/15° C (4).

Species	Treatments	Germination (%)	P	homogeneous group
Carex pendula	1	99	0.0001	a
Carex pendula	2	0		b
Carex pendula	3	100		a
Carex pendula	4	98		a

Tab. 8 - Summary of data on the germination of *Carex divulsa* obtained in the tests with different treatments: control at 25/15° C (1), control at 20° C (2), treatment with gibberellins 25/15° C (3) cold stratification for three months at 5°C test temperature of 25/15° C (4).

Species	Treatments	Germination (%)	P	homogeneous group
Carex divulsa	1	100	0.0001	a
Carex divulsa	2	78		b
Carex divulsa	3	100		a
Carex divulsa	4	98		a

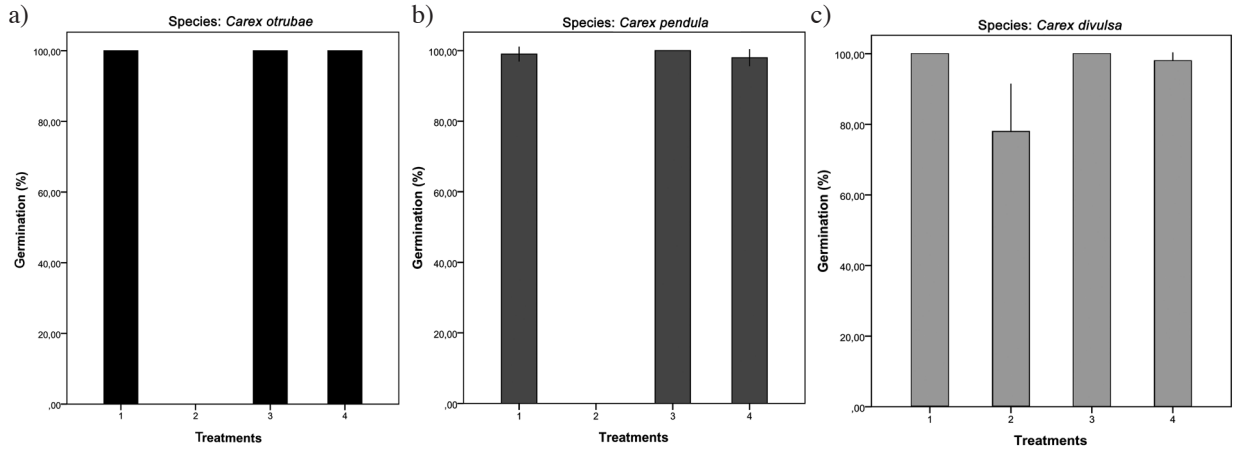

Fig. 3 - Percentage of germination of *Carex otrubae* (a), *Carex pendula* (b), *Carex divulsa* (c) obtained in the different tests. The treatments are: control at 25/15°C (1), control at 20°C (2), treatment with gibberellins (3) cold stratification for three months at 5°C test temperature of 25/15°C (4).

tificial "guazzo", was created at the Botanical Garden "Selva di Gallignano" for experiment the plants to be introduced in the restoration of the habitat necessary for the survival of the Italian green toad and other amphibians. However, the environmental restoration, is useful for many other species, both animal and plants.

In this case the experiments conducted on the "guazzi" have led to verify the significant presence of many passage and sedentary birds that visit these habitats which dry up completely in summer. It is also possible to correlate these appearances with the spread of acquatic species that are also rare. For the flora of the Marche Region, the current presence of *Ranunculus peltatus* ssp. *baudotii* is an important discovery linked to the spread of aquatic birds (Biondi *et al*., 2012c).

The project realized and the analyses have allowed to obtain the suspension of the infringement procedure, and so to avoid the sanction inflicted by the European Commission. Now, the restoration work should be realized in order to provide the Conero Park with the rare ecosystems included in the project. These could be visited by schools and tourists who crowd the area mainly during summer. Finally, the project also provides for the creation of posters and other promotional material on the above mentioned environments and on the organisms which live in them.

Syntaxonomical scheme

PHRAGMITO-MAGNOCARICETEA Klika in Klika & Novac 1941
Magnocaricetalia Pignatti 1953
Magnocaricion elatae Koch 1926
Cypero-Caricetum otrubae Tüxen ex T.E. Diaz 1975
Eleocharitetum palustris Schennikow 1919
Nasturtio-Glyceretalia Pignatti 1953
Glycerio-Sparganion Br-Bl. et Sissingh in Boer 1942
Sparganio erecti-Veronicetum anagallidis-aquaticae Passarge 1999

ARTEMISIETEA VULGARIS Lohmeyer, Preising. & Tüxen ex V. Rochow 1951
ARTEMISIENEA VULGARIS
Agropyretalia repentis Oberd., Th. Müller & Görs in Oberdorfer & al. 1967
Inulo viscosae-Agropyrion repentis Biondi & Allegrezza 1996
Loto tenuis-Agropyretum repentis Biondi, Vagge, Baldoni & Taffetani 1997
Ranunculus sardous variant

QUERCO ROBORIS-FAGETEA SYLVATICAE Braun-Blanq. & Vlieger in Vlieger 1937
Populetalia albae Br.-Bl. ex Tchou 1948
Populion albae Br.-Bl. ex Tchou 1948
Symphyto bulbosi-Ulmetum minoris Biondi & Allegrezza 1996
laureetosum nobilis subass. nova *hoc loco*

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Professor Vincenzo Caputo (Marche Polytechnic University) and Dr. Biol. David Fiacchini for their kind help in the taxonomic aspect of the Italian green toad. The technical staff of the Marche Region and the Regional Conero Park for help in information on the infringement procedure and in the definition of the restoration project.

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Appendix
Localities and dates of relevès
[Localities reference system: GAUSS-BOAGA (ROMA 1940) Est Zone]

Table 1 :
Rel. 1: Right side of the River Musone at Lido Azzurro (Numana) [43°28' N, 13°37' E], 13/04/2011; Rel. 2: Right side of the River Musone at Lido Azzurro (Numana) [43°28' N, 13°37' E], 20/04/2011

Table 2 :
Rel. 1: Right side of the River Musone at Lido Azzurro (Numana) [43°28' N, 13°37' E], 13/04/2011.

Table 3 :
Rel. 2: Right side of the River Musone at Lido Azzurro (Numana) [43°28' N, 13°37' E], 20/04/2011; Rel. 3: Right side of the river Musone at Lido Azzurro (Numana) [43°28' N, 13°37' E], 30/05/2011.

Table 4:
Rel. 4: Right side of the River Musone at Lido Azzurro (Numana) [43°28' N, 13°37' E], 20/04/2011; Rel. 5: Right side of the River Musone at Lido Azzurro (Numana) [43°28' N, 13°37' E], 20/04/2011.

Table 5:
Rel. 1: Right side of the River Musone at Lido Azzurro (Numana) [43°28' N, 13°37' E], 30/05/2011; Rel. 2: Right side of the River Musone at Lido Azzurro (Numana) [43°28' N, 13°37' E], 30/05/2011; Rel. 6: Right side of the River Musone at Lido Azzurro (Numana) [43°28' N, 13°37' E], 30/05/2011.