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Phytosociological survey as a baseline for environmental status assessment: the case of hydrophytic vegetation of a deep volcanic lake

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Abstract
Freshwater ecosystems are strongly endangered throughout Europe as they suffer from ongoing human pressure, pollution and eutrophication. Assessment and monitoring species and habitats is essential to address conservation efforts and evaluate the results of conservation policies. Thus, European Habitats Directive (92/43/EEC) and Water Framework Directive (2000/60/EC) ask for monitoring actions. Nevertheless, knowledge about hydrophytic vegetation of volcanic lakes in Central Italy is still lacking, incomplete or not updated. Herein, we analysed phytosociological data collected in 2010/2012 along six transects performed at Martignano Lake, a small volcanic lake 30 km NW from Rome, where aquatic vegetation was not previously studied. Overall we detected 17 species in 84 relevès: seven species belong to charophytes and ten are vascular plants; species richness for relevès ranges from one to eight; maximum macrophyte growing depth was recorded at 15,3 meters. The presence of eight communities were detected, three belonging to *Charetea fragilis* (*Charetum asperae, Charetum polyacanthae, Charetum globularis*) and five to *Potametea pectinati* (*Potametum denso-nodosi, Potamo-Myriophylletum spicati, Potametum pectinati, Ceratophylletum demersi, Najadetum minoris*). Limnological water variables and structural patterns of aquatic vegetation can be considered similar to patterns of other European calcareous deep-water lakes, from the Scandinavian Peninsula to the Balkans. Thus, to assess ecological condition of Martignano Lakes, the Reference Index method developed for German lakes was applied. Results indicated a good status of conservation for Martignano Lake even if, maximum growing depth revealed a negative trend in the last decades. We argue that Reference Index can be a useful method for Central Italy deep volcanic lakes.

Keywords: *Characeae*, Habitats Directive, macrophytes, Martignano Lake, Water Framework Directive.

Introduction

Aquatic vegetation impacts the geochemical functioning of freshwater ecosystem and is an important driver of freshwater biodiversity (Carpenter & Lodge, 1986; Engelhardt & Ritchie, 2001). However, human pressure, pollution and eutrophication threaten freshwater ecosystems affecting numerous ecosystem services provided by freshwaters (Sala *et al*., 2000; Dudgeon *et al*., 2006). The disappearance of macrophytes has a destabilizing effect on the aquatic ecosystem with repercussions on all the components of biota, from diatoms to fish fauna (Sheffer *et al*., 2001). Thus, they are considered to be of high conservation concern, at European Union level, by Water Framework Directive (2000/60/EC) and many aquatic vegetation types are included in the Habitats Directive (92/43/EC Annex 1; Biondi *et al*., 2009).

Together, ecosystem characteristics deeply influence aquatic plant growth and distribution (Bornette & Puijalon, 2011; Lacoul & Freedman, 2006). Therefore, macrophytes species and communities could be used as biological indicators of ecological status (see e.g. Lehmann & Lachavanne, 1999; Kolada, 2010). Several studies have shown the influence of water chemical parameters on macrophyte presence and distribution in lakes. Duarte & Kalff (1990) and Vestegaard & Sand-Jensen (2000) have shown that water chemical parameters, principally alkalinity, control the presence of species in the lakes then, ultimately, affecting the floristic difference between lakes. Other characteristics, such as depth, slope or sediment composition affect the distribution of species and community within the lakes.

Thus, the knowledge of the aquatic vegetation is of primary importance both to understand freshwater ecosystems and to implement the awareness of conservation policies, integrated in the framework of European nature management. In Italy, knowledge of aquatic flora and vegetation is scarce especially in the Central and Southern Italian peninsula. In this paper we present a contribution to the knowledge of aquatic vegetation of volcanic lakes in Central Italy, based on recent surveys (2010-2012). The focus is Martignano Lake, where aquatic vegetation was not studied and an environmental status assessment based on this component was not previously performed. Some records of aquatic plant species were only provided in limnological studies regarding zoobenthos (Stella *et al*., 1972; Mastrantuono, 1995; Mastrantuono & Mancinelli

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2003, 2005; Mastrantuono *et al*., 2008) even if, monitoring such habitats is considered extremely urgent for conservation issues and management policies (Azzella *et al*., 2013a).

In the recent years, following the inputs of Water framework Directive, several methods of evaluating the environmental quality of water bodies were proposed in Europe (see e.g. Melzer, 1999; Pall & Moser, 2009). In particular, in Italy, the Macro-IMMI method, a multimetric index useful in carbonatic lakes of Northern part of Italy, was proposed (Oggioni *et al*., 2010).

Thus, the aims of this paper are i) to describe the vegetation of Lake Martignano and ii) to assess the quality of the lakes using vegetation as an environmental indicator.

Study Area

Lake Martignano is located 30 km NW from Rome and 2 km East of larger Lake Bracciano (Fig. 1-2) that dominates the Sabatini volcanic complex. Martignano is a small sub circular lake of volcanic origin, without tributaries and emissaries, placed in an area of high nature value lacking in urban settlements. Since 1999, it has been included in the Regional Natural Park of Bracciano-Martignano and considered a “Core Area” for the land ecological network of the Province of Rome (Blasi *et al*., 2008). Catchment land cover is dominated on gentle slopes by agricultural land (arable land, fruit trees, etc.) and pastures and, on steeper slopes, by oak forests.

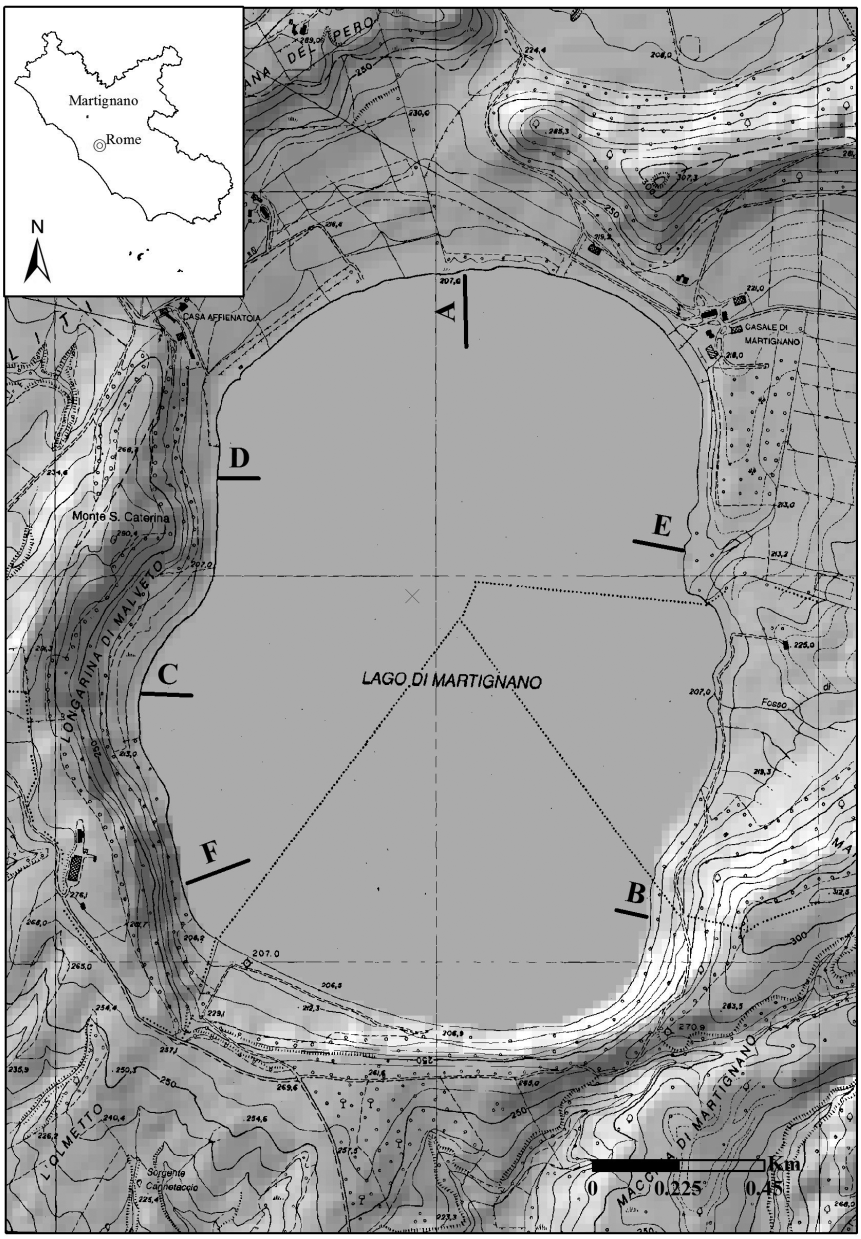

Fig. 1 - Study area and location of surveyed transects. Coordinates of transects starting and ending points are reported in Tab. 1.

Fig. 2 - A historical map (1804) printed in Venice during the Napoleonic age showing the landscape surrounding Martignano lake: the small lake of Stracciacappa was still present, unlike Baccano that was drained. Reproduction courtesy of Marisa De Castro.

Martignano is a caldera resulting from several eruptions during the last hydro-magmatic phase of the Sabatini volcanic complex, dated 80.000-40.000 years BP. The altitude of the lake is 207 m, about 50 m higher than Bracciano; the surface is 2,49 Km^2 with a drained basin of 6,49 Km^2. Maximum depth is 54 m (Limno database: Tartari *et al.*, 2004). Instead, recently Puglisi & Scarponi (2011) reported a maximum depth of 62 m and an altitude of 203 m a.s.l. Moreover, they demonstrated huge variations in water levels of the lake during the last 4000 years with a negative trend during the last century. This trend is still occurring, and we observed a water level reduction approximately of 1 m between 2010 and 2012.

The climate shows a typical Mediterranean regime. Annual precipitation amounts to 990 mm; mean annual temperature is 14,5°C. Following the regional phytoclimatic classification (Blasi, 1994) the study area is included in the mesomediterranean subhumid/humid belt of the transitional mediterranean region, as confirmed in the recent ecological land classification (Blasi & Frondoni, 2011; Capotorti *et al.*, 2012).

Martignano is a dimictic lake and, despite volcanic substrate, has oligo-mesotrophic[1] calcareous water (total phosphorous 11.4 $\mu g\ l^{-1}$; alkalinity 2.7 meq l^{-1}, pH 7.5). In the past zoological studies nine species of macrophytes were reported (Margaritora *et al.*, 2003) colonizing the substrate down to 20 m of depth.

Data and Methods

To describe qualitative and quantitative features of hydrophytic vegetation, we performed 84 phytosociological relevès during the 2010-2012 growing seasons (July) along six transects, perpendicularly placed to the lake shores (Fig. 1). Transects were selected as representative of lake vegetation according to the expert judgment of the authors, using the results of a previous

1: Limnological data refer to mean value evaluate on chemical data on water column, collected in winter (circulation) and summer (stratify water) 2010 and analyzed by IRSA-CNR Brugherio.

survey performed along 18 transects (Azzella *et al*., 2013b). The number of transects was selected according to Schaumburg (2007). Phytosociological relevès used in this paper are part of a comprehensive database regarding volcanic lakes of the Italian Peninsula (Azzella *et al*., 2012) and are available into VEGITALY database (Gigante *et al*. 2012; Landucci *et al*. 2012).

We used free diving and batiscope to detect the presence of species and cover at lower depth, underwater remotely operated cameras and a double row rake were used in deep-water. UTM 33 coordinates (datum ED50) of starting and ending points of transects are reported in Table 1. The nomenclature of vascular plants follows Conti *et al*. (2005); with regard to *Characeae* we refer to Bazzichelli & Abdelahad (2009). Syntaxonomy at alliance level follows Rivas-Martínez *et al*. (2002). Habitats are identified according to the Italian interpretation manual of Habitat (Biondi *et al*., 2009; 2012). Multivariate exploration of datasets were performed in JUICE (Tychy, 2002) using Twinspan modified clustering (Rolececk *et al*., 2009) and Redundancy analyses (RDA) for ordination. Due to the huge floristic difference between Italian Northern lakes, Macro-IMMI could not be applied to Lake Martignano. On the other hand, our previous sampling has shown the presence of 24 macrophyte species, the dominance of *Chara* species in the lake and the limnological similarities between Martignano and European calcareous deep-water lakes (Azzella *et al*., 2013b). In particular, limnological similarities between Martignano Lakes and the "Tkg13" category (Sites of carbonaceous water bodies with a stable stratification and a small catchment area in the North German Lowland) of German lakes (Schaumburg, 2007) allow the use of the Reference Index (RI) to assess trophic condition of Martignano Lake aquatic vegetation. RI was developed to assess water-lake quality evaluating the abundance of sensitive and tolerant species to environmental stressors (Schaumburg *et al*., 2004; Stelzer *et al*., 2005). Macrophytes were classified in 3 classes of tolerance: A-species (sensitive) B-species (indifferent) and C-species (tolerant). The following formula was used to calculate RI and to determine the ecological status:

$$RI = \frac{\sum_{i=1}^{n_A} Q_{Ai} - \sum_{i=1}^{n_C} Q_{Ci}}{\sum_{i=1}^{n_g} Q_{gi}} \cdot 100$$

RI = Reference Index
QA = Quantity of the i-th taxon of species group A
QC = Quantity of the i-th taxon of species group C
Qg = Quantity of the i-th taxon of all groups
A = Total number of taxa in group A
C = Total number of taxa in group C
g = Total number of taxa in all groups

Prior to performing any calculations, the nominally scaled values of plant abundance are converted into metric quantities. The Quantity was calculated from the formula: *macrophyte abundance*^{3} = *Quantity* (Schaumbur *et al*., 2007). In the original works the authors used the Kohler cover abundance scale (Kohler, 1978) to assess aquatic vegetation. The similarities between Kohler scale and Braun-Blanquet scale allow to find a correspondence between them (Tab. 2) to calculate the quantity.

The calculation of RI takes into account additional types of specific characteristics for each lake type. Only this final value can be used for the total assessment of lakes. We also used the additional characteristics suggested for TKg13-category lakes (Stelzer *et al*., 2005).

The RI ranges between minus 100 and plus 100. This range was divided into 5 classes that identify the conservation status of the lake, from bad to high status. We applied the RI at three different levels: relevè, transect and *syntaxa* level. In particular we calculated a mean RI for each vegetation type and for each transect based on single relevès to assess the ecological value of each syntaxon and of each lake sector respectively. Finally, an overall evaluation of ecological status of the lake was performed by means of transects.

Tab. 1 - Starting and ending points coordinates (UTM33 ED50) of the six transect surveyed at Martignano lake.

	START		**END**	
TRANSECT	x	y	x	y
A	278134	4666951	278146	4666805
B	278616	4665296	278536	4665315
C	277305	4665894	277421	4665860
D	277511	4666450	277591	4666427
E	278694	4666250	278613	4666267
F	277434	4665385	277536	4665422

Tab. 2 - Conversion table between Kohler scale and Braun-Blanquet scale.

KOHLER	B-B scale	**Beschreibung**
1	1 & +	very rare
2	2	rare
3	3	common
4	4	frequent
5	5	very frequent

Results

We detected overall 17 species in 84 relevès; species richness ranges from one to eight; maximum depth of macrophytic vegetation was recorded at 15,3 meters with a mean of 12,9 m. Following the results of the cluster analysis, five *Chara* vegetation types can be recognized (Figure 3): the three main clusters are each dominated by different stonewort species (*Chara glo-*

bularis, C. polyacantha, C. aspera); on the contrary, clusters in Figure 4 separate communities dominated by vascular plants (e.g. *Myriophyllum spicatum, Potamogeton perfoliatus, Ceratophyllum demersum*). Species and communities appear to respond mainly to depth and secondarily to slope (Fig. 5), even if other factors such as substratum, wave intensities and human disturbance can influence the communities presence and composition. As a result of multivariate and phytosociological analysis, eight associations, belonging to two classes (*Charetea fragilis* and *Potametea*) can be identified in the dataset as in the details described below. Some of the communities of *Potametea* cannot be properly separated in the divisive cluster analysis because they are rare in Martignano Lake and thus represented in the dataset only by one or few relevès.

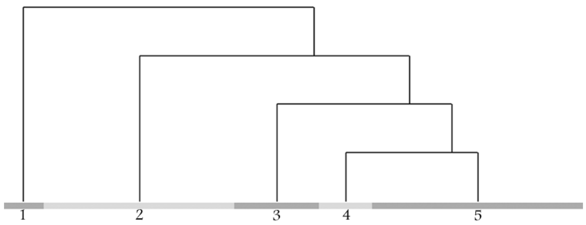

Fig. 3 - Twinspan modified cluster analysys (Rolecek *et al.* 2009) of stoneworts vegetation of Martignano lake. Pseudopspecies cut levels 0, 5, 25%; dissimilarity = total inertia. 1-*Charetum asperae*; 2-*Charetum polyacanthae*; 3-*Charetum globularis* variant with *Ceratophyllum demersum*; 4-*Charetum globularis* var. with *Nitella opaca*; 5-*Charetum globularis*.

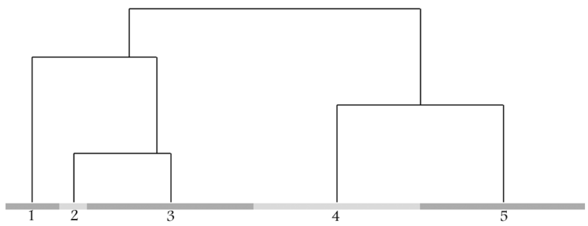

Fig. 4 - Twinspan modified cluster analysys (Rolecek *et al.* 2009) of vascular plant vegetation of Martignano lake. Pseudopspecies cut levels 0, 5, 25%; dissimilarity = total inertia. 1-*Potametum nodosi*; 2-*Potametum pectinati*; 3-*Najadetum minoris* and transition facies; 4-*Ceratophylletum demersi*; 5-*Potamo-Myriophylletum*.

Vegetation description

CHARETUM ASPERAE Corillion 1957
Tab. 3, rel. 1-4
Reference Index: 74 (Good status)

Patchy, monospecific, submerged stonewort meadows, lay down at lower depths (0,2-1 m) on sandy-stony substrata, along wind-exposed shorelines.

Characteristic and dominant species: *Chara aspera*. High frequency taxa: *Ceratophyllum demersum, Myriophyllum spicatum, Potamogeton perfoliatus*.

Contacts are with the communities dominated by vascular plants like P*otamogeton perfoliatus* and *Myriophyllum spicatum*. Rarely, *Charetum asperae* can be detected interspersed with juvenile *Phragmites australis* rhizomes, on the waterfront of the reed belt. It is completely absent where beaches are intensively used for recreation and tourism.

Chara aspera frequently occurs at lower depths in freshwater lakes where it is generally considered to be threatened by eutrophication (Krause, 1997). It is included in the Great Britain's priority list of species of conservation concern (Bryant & Stewart, 2002).

Charetum asperae is an association of oligo-mesotrophic calcareous still water, on sand or gravel, generally near the shoreline (see e.g. Ellenberg, 1988). In Italy only generic records (alliance level) were reported according to Lisy database (http://www.scienzadellavegetazione.it/sisv/lisy/index.jsp. accessed on 10-01-2013)

CHARETUM POLYACANTHAE Dambska 1966 ex Gabka et Pelechaty 2003
Tab. 3, rel. 5-27
Reference Index: 96 (High status)

Dense, monospecific, submerged stonewort meadows dominated by *Chara polyacantha*; depth ranges from 1 to 10 m. This community constitutes the vegetation belt between 3 to 7 m depth.

Characteristic and dominant species: *Chara polyacantha*.

Relevè 27 can be considered a transition towards the community characterized by *C. globularis*, founded at higher depths. Relevès 5-8 represent the transition with the contiguous communities of *Potametalia* that belt, quite uniformly, the lake at lower depths.

Communities dominated by *Chara polyacantha* were rarely observed in Europe (e.g. Pelechaty, Pukacz, 2006); in Italy, no presence of *Charetum polyacanthae* was recorded until now; instead, in Bolsena lake, in a similar environment, the presence of *Charetum hispidae* Corillion 1957 was reported by Iberite *et al.* (1995).

CHARETUM GLOBULARIS Zutshi ex Sumberova, Hrivnak, Rydlo & Ot'ahel'ova in Chytry 2011
Tab. 3, rel. 28-60
Reference Index: 4 (Good status)

Submerged, stonewort monophytic or paucispecific communities lay from 6 m down to the maximum depth of surveyed macrophytic vegetation in the lake (15.3 m).

Characteristic and dominant species: *Chara globularis*.

At higher depths, below 11 m (relevès 54-60), the community is differentiated by the presence of *Nitella opaca* (*N. opaca* variant - RI: 16); relevè 51 represents uncommon facies with *Chara intermedia*; at lower depths between 7-10 m, relevès 30-38, represent a variant with *Ceratophyllum demersum* (RI: -5); relevès 28-29 can be considered a transition towards *Charetum polyacanthae* (RI: 6).

Chara globularis is a widely distributed species with a broad ecological range; in Italy it is the second most frequently rerecorded *Chara species* after *C. vulgaris* (Bazzichelli & Abdelahad, 2009). It can be found in small ponds or lakes at different depths (0,5-18 m), in meso-eutrophic water, rich in calcium and phosphorus.

Tab. 3 - Charetea fragilis communities of lake Martignano.

Table number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Slope (degrees)	5	2	2	2	2	2	1	1	1	2	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Depth (meter)	0	0	0	0	1	2	3	4	1	3	5	5	4	4	5	5	5	6	8	5	7	6	7	6	8	10	9	7	7	8	10	8	8	8	9	10	9	7	10	10	10	9
Subtratum (S=Sand; L=Loam)	S/L	S	S	S/L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
Transect	C	F	F	F	F	F	B	A	C	F	E	A	C	B	B	F	F	B	D	C	B	F	F	C	C	D	D	A	A	A	C	E	C	B	B	F	F	E	A	C	B	A
char. species of associations and upper units (*Charion fragilis, Charetalia hispidae, Charetea fragilis*)																																										
Chara aspera	5	4	3	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Chara polyacantha	.	.	.	.	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	4	2	2	.	2	.	r	.	.	.	.	.	.	.	.	.
Chara globularis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5	5	5	4	4	5	4	2	4	3	2	5	5	4	5
Chara intermedia	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Nitella opaca	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Nitella hyalina	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
char. species of *Potametea pectinati* and other species																																										
Ceratophyllum demersum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	2	4	2	2	5	2	3	2	.	.	.	.
Potamogeton perfoliatus	2	2	2	.	2	.	3	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Myriophyllum spicatum	r	.	.	2	.	.	2	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Potamogeton pectinatus	r	r	.	r	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Potamogeton pusillus	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Elodea canadensis	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
other species																																										
Phragmites australis	.	.	.	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

A community dominated by *C. globularis* was only recently described validly, as *Charetum globularis*, by Zutshi ex Sumberova, Hrivnak, Rydlo & Ot'ahel'ova (Chytry 2011); in fact, *Charetum fragilis* Fijalkowski 1960 (*C. fragilis* = *C. globularis*) is considered nomen invalidum beacause provisionally described by the Author.

In Italy *Charetum globularis*, sub *Charetum fragilis*, was recorded in some small lakes (Pedrotti, 2004) and ponds (Lastrucci & Becattini, 2008) and recently reported also for lake Trasimeno (Landucci *et al.*, 2011).

POTAMETUM DENSO-NODOSI O. Bolòs 1957
Tab. 4, rel. 1-2
Reference Index: -83 (Bad status)

Some small patches of this community occur near the shore, in places that are not exposed to wind at depth lower than 1,5 m.

Dominant species: *Potamogeton nodosus*. Highly frequent taxa: *Myriophyllum spicatum*.

Potametum denso-nodosi is frequently occurring in meso-eutrophic water bodies, lentic or slowly flowing, at lower depths. It is widely distributed across the Europe and in Italy, coenoses referring to this syntaxon were often indicated as *Potametum nodosi* (Soò 1960) Segal 1964, *Potametum natantis* Soò1927 or *Potametum natantis* Hild 1959 (see e.g. Venanzoni & Gigante 2000). On the other hand, some Authors referred to this community as generic vegetation with *Potamogeton nodosus* (see e.g. Ceschin & Salerno, 2008 and references therein).

POTAMETUM PECTINATI Carstensen ex Hilbig 1971
Tab. 4, rel. 3
RI: -50 (Poor status)

Even if *Potamogeton pectinatus* is not a rare species in Martignano, it is frequently surveyed in several communities of *Potamion*, it rarely forms a specific community where it is the dominant species.

Dominant species: *Potamogeton pectinatus*.

In Italy, it is a widespread, frequently monospecific association, occurring in eutrophic and hypertrophic waters, with high turbidity and also anoxic conditions, pointing out low water quality. It can be replace by *Potamo perfoliati-Myriophylletum* as a result of increasing eutrophication, as observed at Trasimeno lake (Landucci *et al.*, 2011).

NAJADETUM MINORIS Ubrizsy 1961
Tab. 4, rel. 5-6
Reference Index: -99 (Bad status)

Tab. 4 - *Potametea pectinati* communities of lake Martignano.

Table number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Slope (degrees)	1	40	2	30	45	2	5	15	2	2	5	2	5	1	1	1	1	1	20	1	1	5	1	1
Depth (meter)	0,3	1	0,2	0,5	1	2,5	1	1	0,5	2,5	1	2,5	0,2	2	2	3,3	7	4	3	5	6,2	4	8,5	6
Subtratum (S=Sand; L=Loam)	L	S/L	S	S/L	S/L	L	L	L	L	L	L	L	S	L	L	L	L	L	L	L	L	L	L	L
Transect	A	B	D	C	B	A	E	D	A	B	A	E	E	B	C	A	D	D	D	D	E	E	F	D
Char. species of *Potametum denso-nodosi*																								
Potamogeton nodosus	5	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Char. species of *Potametum pectinati*																								
Potamogeton pectinatus	2	.	5	1	.	.	2	2	.	.	.	r	2	.	2	.	.	.	.	.	.	.	.	.
Char. pecies of *Najadetum minoris*																								
Najas minor	.	3	.	.	2	5	3	2	2	4	.	3	.	.	.	.	.	.	.	.	.	.	.	.
Char. species of *Potamo-Myriophylletum spicati*																								
Myriophyllum spicatum	2	r	r	r	r	r	4	3	3	3	2	4	3	3	4	5	2	3	4	3	2	2	.	.
Char. species of *Ceratophylletum demersi*																								
Ceratophyllum demersum	.	.	.	.	.	.	r	.	.	.	2	3	2	2	3	.	2	5	4	5	5	5	4	3
Char. species of *Potamion pectinati* and upper units																								
Potamogeton perfoliatus	.	.	2	2	r	.	.	4	4	3	.	4	2	4	4	3	2	2	2	2	.	2	.	2
Elodea canadensis	.	2	r	.	2	r	3	2	2	2	2	2	2	3	2	.	.	.	2	.	.	.	.	.
Potamogeton pusillus	.	3	.	.	r	r	3	2	2	2	.	.	r	.	.	.	.	.	.	.	.	.	.	.
Zannichellia palustris	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Char. Species of Charetea fragilis																								
Chara polyacantha	.	.	.	.	.	.	r	.	.	.	.	.	.	2	.	r	.	.	.	.	.	.	r	2
Chara intermedia	.	.	r	r	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.
Chara globularis	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Nitellopsis obtusa	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	3	.
Chara aspera	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Small patches of this association were surveyed within gaps of *Potamo-Myriophylletum*, at depth between 1 to 2,5 m.

Dominant species: *Najas minor*. Highly frequent taxa: *Elodea canadensis, Potamogeton pusillus*.

Najas minor is an annual aquatic plant growing in meso or eutrophic waters, rich in calcium; ecology and morphology of *Najas minor* appears quite similar to that of *N. marina*. However, communities dominated by the former were not frequently recorded in Italy (Landucci *et al*., 2011). For Volcanic lakes, at Bolsena only *Najadetum majoris* was detected (Iberite *et al*., 1995).

POTAMO PERFOLIATI-MYRIOPHYLLETUM SPICATI Rivas Goday 1964

Tab. 4, rel. 7-17

RI: -24 (Poor status).

It is the most common *Potamion* community at Martignano Lake, widespread from the shoreline down to 3 m depth, and occasionally higher. It is missing only along the beaches utilized for tourism. Frequently, it is in contact with helophytic belts dominated by *Phragmites australis*, rarely with *Charetum asperae* or *Potametum denso-nodosi*. At higher depths it is substituted by *Charetum polyacanthae*. Dominant species: *Myriophyllum spicatum, Potamogeton perfoliatus*. Highly frequent taxa: *Elodea canadensis, Ceratophyllum demersum*.

Rel. 7-9 could be considered a variant with *Potamogeton pusillus* and *Najas minor*, frequently founded around 1 m depth. (RI: -49)

The association has a broad ecological range and distribution Italy often recorded in river and lacustrine environment.

CERATOPHYLLETUM DEMERSI HILD 1956

Tab. 4, rel. 18-24

Reference Index: -29 (Poor status)

A community dominated by *C. demersum* was detected at Martignano, only in two transects, at depth ranging from 3 to 6 m and apparently interposed between the vegetation belt of *Potamo-Myriophylletum* and *Charetum polyacanthae*. Rarely, *C. demersum* patches can be found at higher depths (8-9 m), within the stonewort meadow belt. Dominant species: *Ceratophyllum demersum*. Highly frequent taxa: *Potamogeton perfoliatus, Myriophyllum spicatum*.

Cerathophyllum demersum displays a broad ecological range, tolerating also anoxic conditions and high water temperatures. *Ceratophyllum demersum* communities were frequently reported in Central Italy (see e.g. references in Landucci *et al*. 2011).

C. demersum can appear both as rooted and as a floating species so the related communities were alternatively classified by different Authors in *Lemnetea* or *Potametea* class. According to Rivas-Martínez (2002) we include this community into a specific alliance, *Ceratophyllion demersi* within *Potametalia* order, as proposed also in Venanzoni & Gigante (2000) and Landucci *et al*. (2011). We highlight the peculiar life form characteristics of ceratophyllids as *C. demersum* that in the beginning grows as a rooted submerged species then, as a consequence of changing environments can become a floating species.

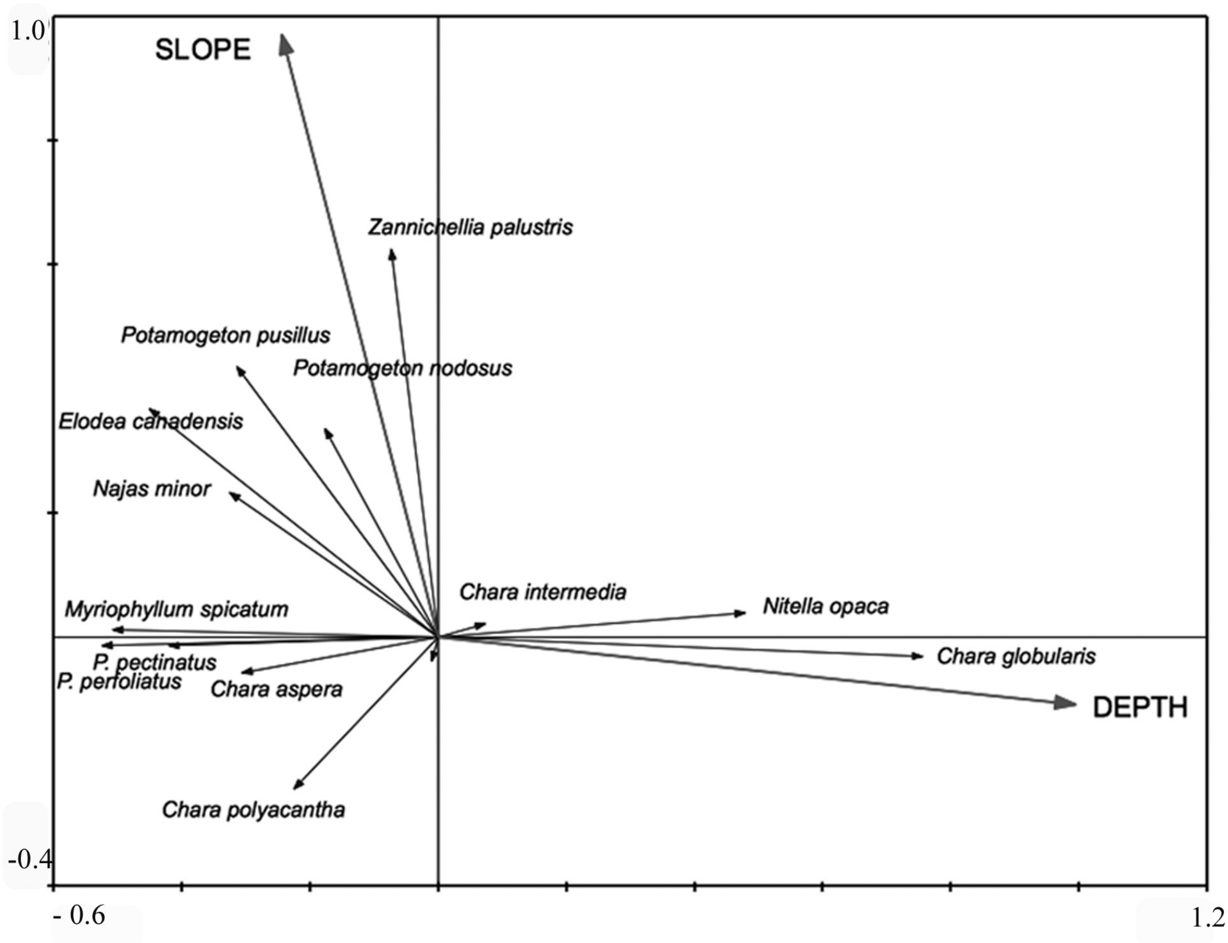

Fig.5 - RDA of hydrophytic vegetation surveyed at Martignano lake (84 releves for 17 species). Data were square root transformed. Percentage of variance explained by axis 1 = 88,4% (p = 0,002).

Vegetation pattern

Two main patterns of hydrophytic plant communities were detected (Fig. 6). The first one (transects C and F) starts with *Charetum asperae* at lower depths on sandy-stony substratum followed by *Potamion communities* generally replaced below 4-5 m depth by tall stonewort meadows (*Charetum polyacanthae; Charetum globularis*). This is the pattern with the higher transect RI, due to the dominance of charophytes from the shore down to the maximum growing depth. The second one is the most frequent and starts with *Potamion communities* (generally *Potamo-Myriophylletum*) followed at higher depths by *Charetum polyacanthae* and *Charetum globularis*. This pattern is characterized by a lower RI due to the dominance of *Potametea* community replacing the *Charetum asperae*. A third pattern type, not shown here, completely misses plant communities at lower depths and corresponds to the beaches used for recreational activities. Herein, aquatic vegetation starts directly with *Charetum polyacanthae* because vegetation of the first 2 meter depth has been completely depleted.

Reference Index

RI for transects ranges between -13,6 (poor status) to 51.9 (high status) and for syntaxa from -99 (poor status) to 96 (high status). RI values for each transect are showed in Fig. 6. The mean value for the lake calculated by mean transect values is 21,3, corresponding to an overall assessment of “good status” for the lake.

Discussion and Conclusions

During the phytosociological survey we detected the presence of 17 species that represent an amount of regional diversity of volcanic lakes in central Italy that is not negligible. Despite alien species invasion being recognized as a serious threat affecting freshwater ecosystems worldwide, only one exotic species, *Elodea canadensis*, was found in Martignano. *E. canadensis* is naturalized in several Regions of Central and Northern Italy and is considered as invasive in Latium

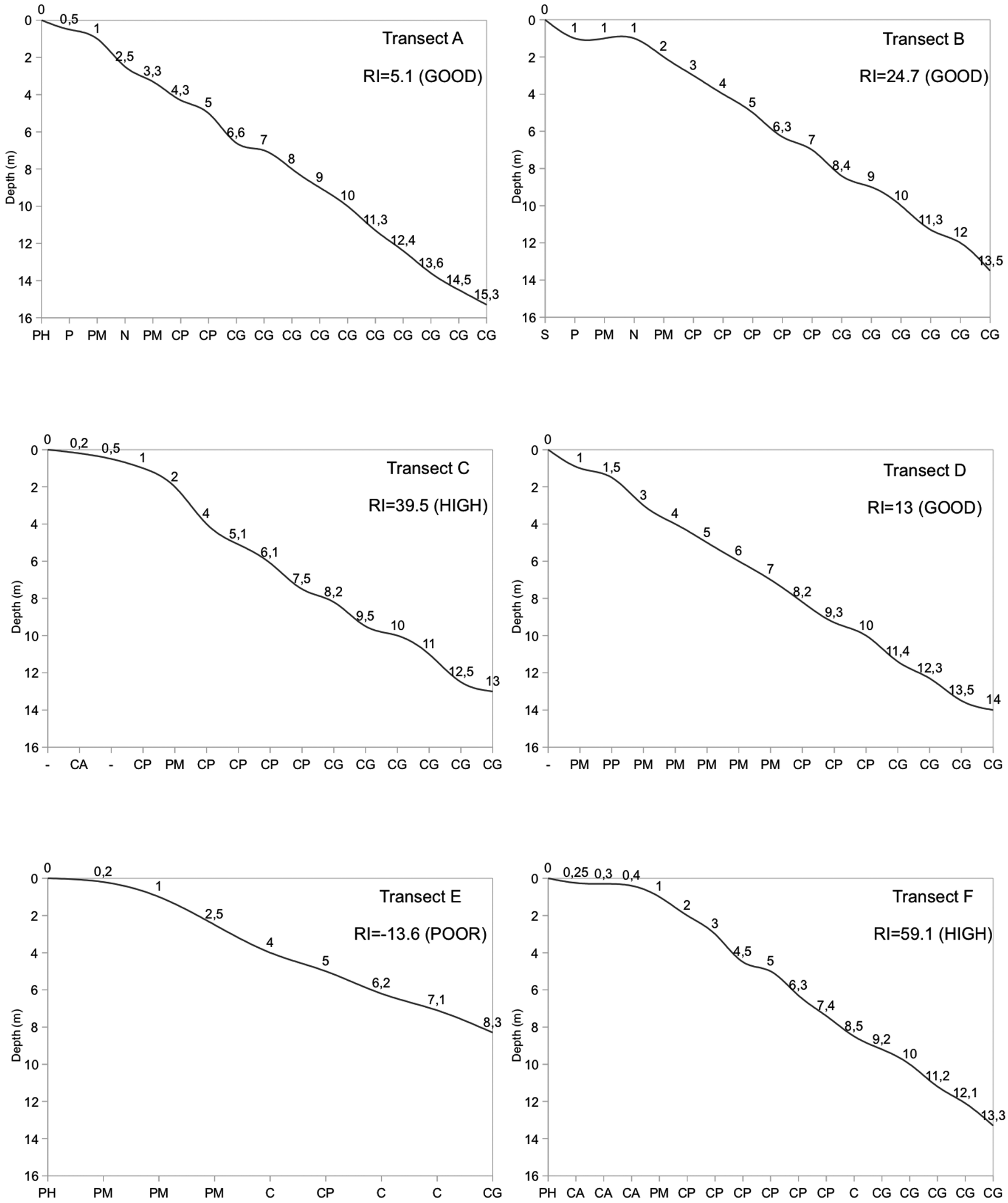

Fig. 6 - Vegetation communities and water depths at Martignano lake along the six transect reported in figure 1. C: *Ceratophylletum demersi*; CA: *Charetum asperae*; CG *Charetum globularis*; CP: *Charetum polyacanthae*; P: *Potametum denso-nodosi*; PH: *Phragmitetum*; PM: *Potamo pectinati- Myriophylletum spicati*; PP: *Potametum pectinati*; N: *Najadetum minoris*; S: *Salicetum albae*.

(Celesti-Grapow *et al*. 2010). The species has been present in the lake since 1970 (Stella *et al*., 1972) and does not apparently show recent variations in presence and abundance, thus can be now considered a stabilized component of *Potamion* communities in the lake. Comparison with previous floristic records (Mastrantuono & Mancinelli, 2003) underlines that no species disappeared during this period. On the contrary, eight species are new records (the major of these are stoneworts), probably as a consequence of a more intensive and accurate survey.

Stoneworts are generally considered as indicators of good status of conservation and the large ones (such as *Chara polyacantha*) are the first to disappear at the occurrence of pollution (Blindow, 1992; Blindow *et al*. 2002). As a consequence they are protected, not only in the Habitats Directive, but also in some nations where they are included in special red lists (e.g. Stewart & Church, 1992; Palamar-Mordvintseva & Tsarenko, 2004). The findings of *Chara polyacantha* and *Nitella hyalina* can be considered particularly interesting because *Chara polyacantha*, the dominant species in Martignano Lake, is currently classified as "endangered" in Switzerland and "critical endangered" in Germany, Norway and the Balkans. *Nitella hyalina* is very rare throughout Europe and considered "critical endangered" in the Balkan countries and Germany (Korsch *et al*., 2013; Blazencic *et al*., 2006), "extinct" in Great Britain and Switzerland (Palmer 2008, Joye & Schwarzer, 2012) and "potentially threatened" in Finland (Bjelke, 2010). In addition, considering the recent finding of the rare *Lychnotamnus barbatus* (Azzella & Abdelahad, 2011), Martignano Lake has to be considered an important conservation site for the *Characeae* at the national and European level. Then, Martignano is a lake dominated by stonewort meadows and it could be considered a typical Chara-lake (Almquist, 1929). In fact, stoneworts are able to use the dissolved carbonate as a source of CO2 and they are dominant in lakes with high alkalinity and good transparency. Furthermore, they play an important functional role in ecosystems (Kufel & Kufel, 2002; Rodrigo *et al*., 2007), for example maintaining high transparency of the water (Blidow *et al*., 2002), counteracting the proliferation of phytoplankton (Mulderij *et al*., 2003; Berger & Schagerl, 2004) or as a source of food and habitat for many animal species (van den Berg *et al*., 1997; Noordhuis, 2002). As a matter of fact, Martignano is a host to the most important *Netta rufina* (Pochard Redcrest) Italian colony, an Anatidae that eats stoneworts and finds herein its ideal wintering habitat.

In Martignano plant communities are arranged mainly along a depth gradient and differences in terms of life-forms and physiognomy reflect different ecological requirements. The hydrophytic vegetation forms a continuous belt in the lake, spanning from few cm depth to 15 m and results absent only at lower depth where the beaches are used for recreational activities. This aquatic vegetation seems to be lesser influenced by water level decrease than riparian and helophytic ones that appear, nowadays, quite fragmented and floristically altered. Probably, the slow decrease of water levels and the high lake depth allow the aquatic communities to shift towards the bottom more efficiently than riparian ones. In fact, coherent with our findings, hydrophytic species are considered more sensitive to water eutrophication and helophytes to water level fluctuation. The vegetation series along the depth gradient (Fig. 6) is quite similar to those reported for Vico Lake (Azzella & Scarfò, 2010), a volcanic lake placed to the north of Sabatini complex.

The identified plant communities can be framed in two habitats of Community interest: *Charetea fragilis* communities belong to habitat 3140 (Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp.) and some of *Potametea pectinati* communities (*Potametum denso-nodosi, Potamo-Myriophylletum spicati*) to habitat 3150 (Natural euthrophic lakes with *Magnopotamion* or Hydrocharition-type vegetation). Thus, Martignano should be considered also as a Site of Community Importance.

Currently, to assess ecological status of water bodies many indexes were proposed and the multimetric approach was preferred (Hering *et al*., 2006). Sampling along transects (Azzella *et al*., 2013b) allows us to collect data about many ecological metrics, as for example maximum growing depth or characteristic zonation. In our results *Chara* communities have positive values of Reference Index according to their capability to indicate good status of conservation. Otherwise *Potametea* communities show the lower values. It's not surprising because "*Magno-Potamion*" communities are typical of "Natural Eutrophic lakes". Then, in lakes that potentially are characterized by oligotrophyc condition the dominance of *Potametea* class points out a bad status of conservation and expresses change in the pattern of vegetation mainly due to eutrophication. The assessment of Martignano Lake suggests an increase in eutrophication in the first meters depth, probably as a consequence of intensification in agriculture and recreational use in the catchment. Coherently, the maximum growing depth recorded in this study (15,3 m) was considerably lower than reported in previous studies (20 m in Margaritora *et al*., 2003) and the lowest RI value and the lower growing depth was recorded in transect "E", the nearest to the catchment main farmland. Despite the negative trend, the quality status of the water body of Martignano can be nowadays considered "good", as pointed out by the mean of RI values for the transects.

We consider that the methodology adopted in this study has considerably increased the knowledge about vegetation compositions of "Southern Italian" aquatic regions where such features are still incomplete. In fact, despite their conservation value (Rosati *et al*., 2007), only few Important Plant Areas were based on or included freshwater ecosystems of central Italy volcanic lakes (Blasi *et al*., 2011), calling for the urgency to improve scientifically based data collection on aquatic plant communities. Indeed, our results will represent a useful baseline for future monitoring actions and to evaluate conservation efforts (Corona *et al*., 2010).

Finally, we argue that consistent results on environmental quality assessment of deep volcanic lakes can be achieved using the Reference Index methods applied to phytosociological data, in particular if surveyed along transects and joined with other metrics, as the maximum growing depth. This is the first attempt to apply RI to *syntaxa*. To improve the effectiveness of such *syntaxa* as bioindicators a larger number of such observations is desirable. Then, further investigation is necessary to test the RI method on other lakes in the Mediterranean region, in particular to define the reference conditions as requested for a satisfactory application of the European Water Framework Directive.

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Syntaxonomical scheme

CHARETEA FRAGILIS Fukarek ex Krausch 1964
CHARETALIA HISPIDAE Sauer ex Krausch 1964
Charion fragilis Krausch 1964
Charetum globularis Zutshi ex Sumberova, Hrivnak, Rydlo & Ot'ahel'ova in Chytry 2011
Charetum polyacanthae Dambska 1966 ex Gabka et Pelechaty 2003
Charetum asperae Corillion 1957
POTAMETEA PECTINATI Klika in Klika & v. Novak 1941
POTAMETALIA Koch 1926
Potamion (Koch 1926) Libbert 1931
Potametum denso-nodosi O. Bolòs 1957
Potametum pectinati Carstensen ex Hilbig 1971
Potamo pectinati-Myriophylletum spicati Rivas Goday 1964
Zannichellion pedicellatae Schaminée, Lanjouw & Schipper 1990 ex Pott 1992
*Najadetum minori*s Ubrizsy 1961
Ceratophyllion demersi Den Hartog & Segal ex Passarge 1996
Ceratophylletum demersi Corillion 1957

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