Estimating influence of environmental quality and management of channels on survival of a threatened endemic quillwort

Published version:

DOI:10.1016/j.aquabot.2013.01.009

Estimating influence of environmental quality and management of channels on survival of a threatened endemic quillwort

Elena Barni , Chiara Minuzzo , Francesca Gatto , Michele Lonati , Thomas Abeli , Cecilia Amosso, Graziano Rossi , Consolata Siniscalco

Abstract

Isoëtes malinverniana Ces. et De Not. is a narrow endemic quillwort occurring within channels used for rice fields water supply, in the lowland area of North Italy. The range of *I. malinverniana* is highly fragmented and in the last 10 years the whole population decreased by more than 80%. This strong decline is assumed to be mainly related to the intensification of agricultural practices, which led to decreasing water quality, alteration of flow regimes and mechanized management. In order to highlight which of these factors may affect population performance and fate (persistence vs. extinction) of the species, plant cover, density and leaf length were measured, and a range of water and sediment parameters were analyzed at the plot scale, within the extant populations and in eight historical sites where *I. malinvarniana* recently disappeared.

At least 4 out of 11 populations, characterized by cover values never exceeding 15% and density hardly approaching 20 plants m^{-2}, revealed scarce chance for survival, under current conditions. On the basis of discriminant analysis, nutrient enrichment did not result the main predictor of *Isoëtes* decline, as generally noticed for isoetids, since higher water nitrates, P, K and organic C content in the sediments were found in the sites where *I. malinverniana* showed the highest performance compared to sites of less viable populations. On the other hand, the high water depth, the strong increase of water flow during the rice growing season and the intensive mechanized management of channels, e.g. cutting of aquatic vegetation and dredging using excavators, were among the most important parameters explaining the disappearance of the species.

Our findings may have useful implications for the conservation or restoration of *I. malinverniana* and other rare aquatic species occurring in running waters of lowland agricultural areas. Hydrological alterations and mechanized maintenance of channels should be restricted where the species still occurs, to improve population performance and seedling survival.

1. Introduction

Around the world several aquatic species belonging to the ancient relict genus *Isoëtes*(quillwort) are rare and on the brink of extinction due to water quality deterioration and habitat destruction (Taylor et al., 1993, Leon and Young, 1996, Musselman, 2002, Rhazi et al., 2004, Chen et al., 2005, Kang et al., 2005, Chen et al., 2007 and Changkyun et al., 2008). *Isoëtes* is a cosmopolitan genus of heterosporous lycopsids with fossil records dating back to the Paleozoic (Hoot et al., 2004) and 200 or more living species (Schuettpelz and Hoot, 2006), ranging from perennial aquatics to ephemeral terrestrials (Taylor and Hickey, 1992). Considering the combination of relictual character and the pressures that act detrimentally on their habitats, *Isoëtes* species are the subject of different conservation measures (Environment Agency of Japan, 2000, Vöge, 2004,USFWS, 1996 and IUCN, 2010).

In Italy seven species of quillworts are present (Conti et al., 2005) and all of them are very rare in Italy. In particular, *Isoëtes malinverniana* is endemic to the Western Po plain and occurs, unlike most of the congenerics, within channels and ditches fed with freshwater deriving from springs and from rivers, mostly used for rice crops water supply and drainage (Soldano and Badino, 1990). Within the genus, *I.*

malinverniana has a high taxonomic significance as it is biogeographically and phylogenetically isolated from other species (Hoot et al., 2006). The range of *I. malinverniana* is highly fragmented and in the last 10 years the whole population decreased by more than 80% (Barni et al., 2010); at present, only 11 occurrences in very restricted and isolated sites are known.

For these reasons *I. malinverniana* was listed in the Italian Red Lists (Conti et al., 1997) under the IUCN category Critically Endangered (CR). Moreover *I. malinverniana* was included in annexes II and IV of the EU "Habitat" Directive 92/43. Although it was designated as a plant species of Community interest in need of strict protection, at the time only one of the extant populations is included in a Site of Community Importance.

As a first approach, the conservation of rare endemic taxa requires insights on their geographic distribution, niche characterization and particularly into limiting environmental factors and impact of activities, both past and present (Bernardos et al., 2006). Up to now, the knowledge on *I. malinverniana* ecology and causes of population reduction only relied on qualitative observations (Corbetta, 1965 and Soldano and Badino, 1990), and overall information based on field measurements is still lacking. The decline of *I. malinverniana* populations may be due to anthropogenic activities but we are still far from pinpointing which kind of habitat changes, or combined effect of them, caused their deterioration and regression. *Isoëtes* species and, to a wider extent, isoetids are reported to have morphological and metabolic strategies evolved as adaptations to unproductive oligotrophic environments (Hutchinson, 1975, Chapin, 1980 and Vitousek, 1982). Consequently, water pollution and eutrophication by contamination with domestic, agricultural or industrial effluents is generally regarded as the main predictor of *Isoëtes*and isoetid decline (Smolders et al., 2002, Arts, 2002, Wen et al., 2003, Liu et al., 2005 and Pedersen et al., 2006). Therefore, we expected that more viable populations would be found in the most nutrient poor channels with characteristics similar to those reported for most *Isoëtes* and isoetid natural habitats, and thus with higher conservation values. Besides water deterioration, the mechanized management of channels, intensively maintained by regular cutting of bankside and aquatic vegetation, dredging and re-profiling using excavators, was assumed to play an important role in the decline of*I. malinverniana* populations.

Therefore, we aimed to (i) evaluate the conservation status of the extant populations of *I. malinverniana*, (ii) investigate the relationship between population performance and habitat conditions, and (iii) identify which factors among water quality, sediment characteristic and management regime may affect the fate (persistence vs. extinction) of the species. The study of habitat characteristics and management of agricultural channels may clarify aspects of their underestimated role in hosting rare aquatic species and help in preparing technical guidelines for conservation.

2. Materials and methods

2.1. Study species

I. malinverniana Ces. et De Not. is an evergreen perennial, submerged quillwort, characterized by a short rhizomatous 3-lobed corm and numerous linear leaves, spirally arranged. Leaves can be 30–100 cm long according to Tutin et al. (1993). The ligules are triangular. Basal sporangia are without velum, megasporangia are usually on outer leaves, microsporangia on inner leaves. The megaspores are white to grey, with a mean diameter of 440–500 µ, with a rounded-triangular profile and an evident trilete mark in polar view, baculate surface, bacula on the upper face fused to form three prominent warts (Ferrarini et al., 1986 and Tutin et al., 1993). Microspores are grey, elliptic, surface granulate, with a mean diameter of

33–38 μ (Ferrarini et al., 1986). Although mega- and microsporangia have been observed at any time of the year, their decay and the consequent spore release is gathered in advanced autumn (unpublished data). The species does not spread vegetatively, thus single individuals can be easily recognized in the field. Further investigations to gain knowledge about phenology, leaf turnover and life span of the species are in progress.

2.2. Study area

I. malinverniana was first discovered by Alessio Malinverni in running water channels for rice field supply in the province of Vercelli (NW Italy) and then recognized as a species new to science in 1858 by Cesati and De Notaris. Knowledge of its distribution was then expanded to 45 sites (Mattirolo, 1912, Corbetta, 1965, Corbetta, 1968 and Soldano and Badino, 1990) in the Piedmont and Lombardy regions. The range of the species strongly decreased lately, as already referred.

Both historical and present sites are located in correspondence to the resurgence spring line between the upper and lower Po Plain (Andreis et al., 2005), where water table reaches the land surface owing to a sediment permeability decrease. Water from these springs is typically oligotrophic and has a remarkable clarity, medium temperature about 10–15 °C with limited annual fluctuations, pH slightly acid (pH = 6–6.5) and conductivity ranging from 200 to 400 μS cm^{-1} (Potenza, 2002). The spring system was exploited for irrigation by digging an extensive network of interconnected channels, used for rice cultivation, since the 15th century. Nowadays groundwater is mixed with water from rivers and from rice field drainage, during the rice growing season from April to September resulting in a deterioration of its quality and abrupt alterations of flow regime. Either semi-natural or artificial, the channels where *I. malinverniana* grows may host aquatic vegetation of *Ranunculion fluitantis* and *Callitricho-Batrachion* communities (priority habitat 3260, listed on Annex II of the Habitat Directive), where *Ranunculus fluitans Callitriche stagnalis*, *Cardamine amara*, *Fontinalis antipyretica* and *Sparganium*spp. are the most prominent species. However, where other aquatic macrophytes occur they usually display low densities and are arranged in scattered patches unmingled with*I. malinverniana* plants.

2.3. Study design

Literature and herbarium data collection, along with extensive field surveys carried out from 2005 to 2009 across the whole range of *I. malinverniana*, resulted in a comprehensive compilation of historical and present locations of the species. Hereafter two types of sample sites were identified with regard to the occurrence of *I. malinverniana*: (i) sites where the species was still present (ISO) and (ii) sites where the species previously occurred, but was not recovered during the recent surveys (EX-ISO). In order to evaluate the conservation status of 11 extant populations of *I. malinverniana*, 38 rectangular plots (0.5 × 1 m) were randomly placed with longer side parallel to the water flow at more than 3 m intervals between each other, in ISO sites. Shape and size of the plot were fixed in accordance to the channel width and to the long and narrow shape of *I. malinverniana* sub-polulations. The number of plots varied among populations from 2 to 5, depending on population size and heterogeneity.

Population performance was estimated by measuring the following structural parameters in each plot: number of individuals, here reported as density (plants m^{-2}), maximum leaf length and number of leaves per plant. Total plant cover (%) and cover of *I. malinverniana* and other macrophytes (%) occurring in each plot were also estimated.

Measurements of population performance were carried out in March and in October 2009 maintaining the same plot locations to examine plant seasonal variations. Only October data were then used in the environmental analyses because they corresponded to the maximum plant development. Using the results of the spring investigation a highly significant correlation was gained between the number of leaves and the maximum leaf length per plant in each plot ($n = 38$; $R = 0.77$; $p < 0.001$), therefore only the second parameter was measured in October.

In order to test whether the occurrence, conservation status or disappearance of *I. malinverniana* was related to environmental parameters, 22 sampling points, were randomly selected in eight historical sites and a range of environmental variables (see below) was recorded from each ISO and EX-ISO site. The number of sampling points varied among EX-ISO sites from 2 to 4, depending on site heterogeneity.

2.4. Environmental data

Water temperature (T), pH and specific electrical conductivity (EC) were measured in the field at each site, monthly, from March to October, using a portable multi parameter device (HI 9811-5, HANNA Co, Italy); water depth was measured using a folding tape. In each site three repetitions of the above mentioned measurements were performed and the mean of these three data was used in the analysis.

Moreover, two surface water samples were collected in each site in March, July and October, in the centre of each channel. A total of 114 samples of 500 ml were collected in polypropylene bottles, in order to determine the following parameters: hardness (as $CaCO_3$), nitrate (NO_3^-), ammonia (NH_3), phosphate (PO_4^{3-}), total chlorine (total Cl) and BOD_5. Analysis were performed on sub-samples using a multi parameter ion-specific photometer (HI 83200, HANNA Co, Italy) at room temperature. Only for BOD_5 a different photometer was used (Lasa 100, Hach Lange Srl, Italy).

Soil samples ($n = 60$, 500 g each) were collected in March 2009 from each ISO and EX-ISO plot for the analysis of the following parameters, according to Italian Standard methods (S.I.S.S., 1985): soil pH, percentage of sand, silt and clay, amount of organic carbon (C), nitrogen (N), available phosphorus (P_2O_5), exchangeable potassium (K_2O) and C/N ratio.

Information about direct management of the studied channels was obtained by Land Reclamation Syndicate operators and by private owners. Management potentially affecting aquatic habitats includes: manual mowing of vegetation, dredging and re-profiling using excavators, dry-out during winter. Combining intensity and frequency of the different practices, four classes were conceived according to the impact of management on aquatic habitats:

1.

No management, null impact.

2.

Manual mowing of aquatic vegetation 1–3 times per year, low impact on vegetation, no impact on the soil.

3.

Manual mowing of aquatic vegetation and/or shallow dredging with low frequency application (10–20 years), medium/high impact on vegetation, medium impact on soil.

4.

Shallow dredging with frequent applications (every 2 years) or deep dredging and re-profiling, severe impact on both vegetation and soil.

2.5. Data analysis

The three structural parameters (*I. malinverniana* percentage cover, density of individuals and average of maximum leaf length) measured in each ISO plot ($n = 38$) were used to analyze the existence of groups identified on the basis of the different population structure (at the plot level). In order to identify such groups a Hierarchical Cluster Analysis (HCA) was performed after variable standardization (*Z* scores) using Euclidean distance measure and Ward's linkage method. This cluster method attempts to optimize the minimum variance within clusters (Ward, 1963 and Anderberg, 1973). Differences in structural variables between groups were tested with one-way ANOVA. Group means were compared with the Bonferroni post hoc range test ($p < 0.05$), which takes the unbalanced replicates design into account (Soliani, 2004). *Isoëtes* percentage cover and density were examined for homogeneity of variance (Levene test) and were square root transformed before the analysis.

Water and soil parameters were compared among *Isoëtes* groups defined by the cluster analysis, including EX-ISO plots (extinct group), with one-way ANOVA followed by Bonferroni post hoc range test. Percentage variables were arcsin-transformed and the other measurements log-transformed in case of unequal variance. The relation between*Isoëtes* groups and management intensity was analyzed by a χ^2-test performed on a 4 × 4 contingency table (4 plot groups × 4 management intensity categories).

As there are likely to be several environmental factors acting in combination that affect the performance and fate of *Isoëtes* populations, an examination of these relationships is best achieved in a multivariate rather than univariate way. The role of water, soil, and management factors in discriminating among the *Isoëtes* groups was assessed using Canonical Variate Analysis (CVA) (ter Braak and Šmilauer, 2002). In order to identify the best subset of environmental factors that can discriminate among *Isoëtes* groups, the statistical significance of each factor was tested in a forward selection process by means of a Monte Carlo permutation test with 499 permutations ($P < 0.10$). Not significant environmental variables were included in the model as “supplementary environment data” in order to obtain an additional interpretation of already extracted ordination axes (ter Braak and Šmilauer, 2002). CVA was performed using 60 samples (ISO and EX-ISO plots), 12 significant environmental variables and 9 supplementary variables. All the variables included in the ordination were standardized (*Z*-scores) and then used without any transformation applied.

All analyses were performed using the SPSS v.16.0 statistical package (SPSS, 2007). CVA was performed using CANOCO version 4.5 (ter Braak and Šmilauer, 2002).

3. Results

3.1. Conservation status

The HCA performed for the 38 ISO plots (Fig. 1) identified three distinct clusters according to the measured structural traits (cover percentage, maximum leaf length and plant density). Two out of three clusters grouped plots belonging to different populations (i.e. channels) (see Appendix I), highlighting the structural heterogeneity within each population. Therefore, in order to find out the relationships between population structure and the associated environmental parameters, analyses were performed at the plot level. The first cluster ($n = 6$), which splits from the others at a lower similarity level, grouped plots where *I. malinverniana* presented the highest cover (87%), and highest density (314 plants m^{-2}) made up by stout individuals bearing 47 cm long leaves on average (group ISO-C). At a higher similarity level, a second (i.e. ISO-B) and third (i.e. ISO-A) cluster were separated, both grouping a higher number of plots ($n = 11$ and $n = 21$ respectively) with respect to the group ISO-C and both characterized by smaller plants, lower cover and densities between 5 and 30-fold lower than the plots in the first cluster.

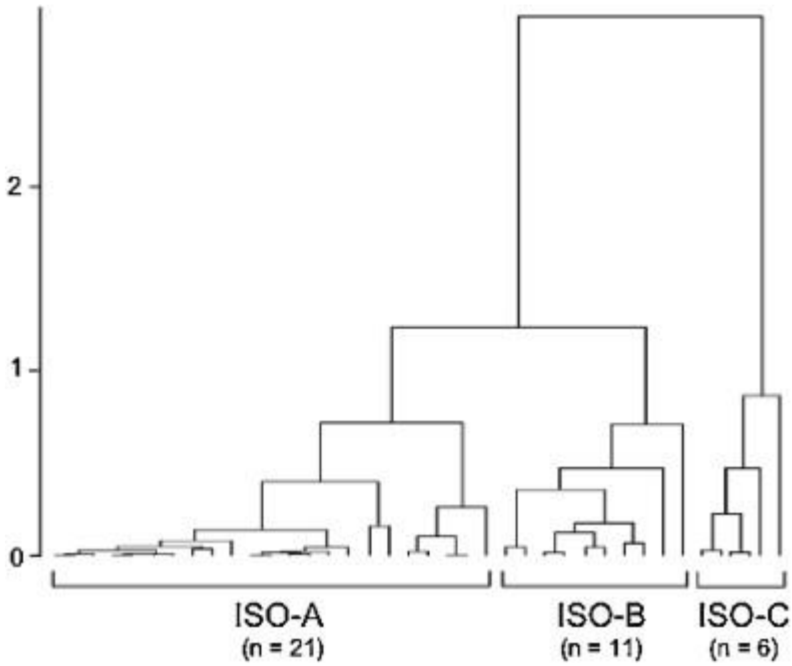

Fig. 1.

Dendrogram resulting from cluster analysis of 38 ISO plots based on *I. malinverniana* performance traits (Euclidean similarity matrix, Ward's linkage method). Number of plots in each cluster is reported in brackets.

ANOVA test showed that the means of all the three clusters differed significantly from each other for every trait (Table 1), except for plant length that did not differ between the ISO-A and ISO-B clusters. Plant length was twofold lower in ISO-A and ISO-B than in the first cluster.

Table 1.

I. malinverniana performance traits for the three clusters of ISO sites, and for the EX-ISO sites. Different letters represent significant differences between groups within each variables ($p < 0.050$; Bonferroni post hoc range test). Means ± standard error are shown.

Structural variables	ISO-A (n = 21)	ISO-B (n = 11)	ISO-C (n = 6)	F	p
Isoëtes cover (%)	6.2 ± 0.9a	46.6 ± 4.1b	82.5 ± 8.9c	121.5	<0.001
Isoëtes plant length (cm)	23.1 ± 1.5a	28.6 ± 2.2a	44.4 ± 3.2b	20.7	<0.001
Isoëtes plant density (plants m^{-2})	10.8 ± 2.4a	57.8 ± 10.3b	301.7 ± 32.6c	151.0	<0.001

Isoëtes cover resulted strongly related to the density (Fig. 2b) and separated well the three groups. The ISO-A group never exceeded 15% cover values with density hardly ever higher than 20 plants m^{-2}. The ISO-C group showed cover values always higher than 50%, combined with density ranging from 200 to about 400 plants m^{-2}.

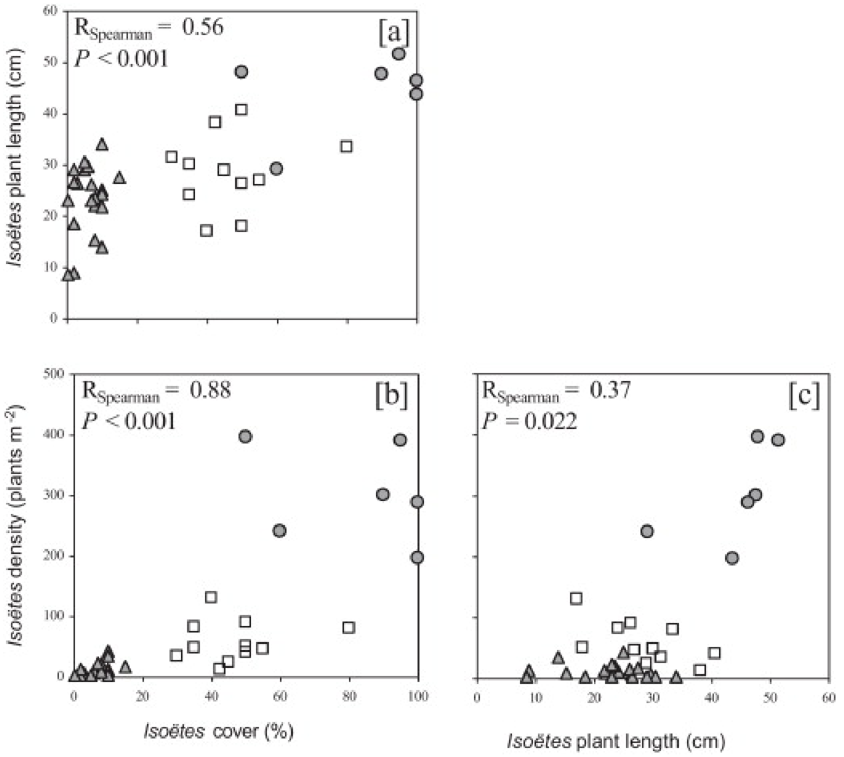

Fig. 2.
Correlation between the three structural parameters (*I. malinverniana* cover percentages, density of living individuals and average of maximum leaf length) in the ISO-A (▲), ISO-B (□) and ISO-C (•) *Isoëtes* plots.

Plant length was less closely related to both cover (Fig. 2a) and density (Fig. 2b). Although it increased among groups with increasing cover and density, it resulted more diversified within groups. In the ISO-A plots in particular, where cover and density resulted constantly low, plant length varied over a wide range (8.5–34 cm), but never reached lengths performed in ISO-C. On the contrary the ISO-C plots had an even distribution of plant length, with values in a narrower range (mainly 40–50 cm). Plant length, as well as leaf number, are structural variables that could be assumed as indicative of either plant productivity or plant age.

3.2. Habitat characteristics at the study sites

I. malinverniana growing sites in general had shallow (21.3–27.4 cm), cool (11.5–12.6 °C) and very soft (20–21.5 mg $CaCO_3$ L^{-1}) water, with low conductivity (81.3–119.6 µS cm^{-1}) (Table 2). The water pH was slightly basic (pH 7.5–7.7). Dissolved nutrient (nitrate and phosphate) concentrations were relatively high (7–10.2 mg NO_3^- L^{-1}; 0.15–0.33 mgPO_4^{3-} L^{-1}) as well as BOD_5 values, revealing more mesotrophic than oligotrophic conditions. *Isoëtes* mainly occurred on coarse substrates (86–89% sand), characterized by weakly acid pH (6.4), with low (5.3–5.2 g kg^{-1}) to moderate (10 g kg^{-1}) organic C content, that tended to accumulate (C/N ratio > 11) rather than being mineralized, due to the reduced conditions in anaerobic sediments. Nutrient content in sediments were low for nitrogen and phosphorus, while K content resulted very high (106.6–223.2 mg kg^{-1}).

Table 2
Water and sediment physico-chemical parameters for the three clusters of ISO sites, and for the EX-ISO sites. Different letters represent significant differences between groups within each parameter (p < 0.050; Bonferroni post hoc range test). Means ± standard error are shown.

Variables	EX-ISO	ISO-A	ISO-B	ISO-C	F	p
Water						
Depth (cm)	94 ± 11b	22 ± 2a	27 ± 3a	21 ± 2a	21.3	<0.001
Annual depth range (cm)	161 ± 23b	43 ± 4a	54 ± 6a	32 ± 5a	13.9	<0.001
Annual mean temperature (°C)	15.5 ± 0.3b	12.6 ± 0.2a	11.9 ± 0.3a	11.5 ± 0.1a	42.6	<0.001
pH	7.8 ± 0.0	7.7 ± 0.1	7.5 ± 0.0	7.5 ± 0.0	2.0	0.122
EC (μS cm^{-1})	192 ± 11b	105 ± 9a	81 ± 5a	120 ± 10a	24.4	< 0.001
Hardness (mg $CaCO_3$ L^{-1})	20.0 ± 0.8	19.2 ± 0.9	21.5 ± 1.3	19.8 ± 1.4	0.8	0.509
NO_3^- (mg L^{-1})	9.5 ± 0.9	7.0 ± 0.5	7.6 ± 1.0	10.2 ± 0.9	2.7	0.055
PO_4^{3-} (mg L^{-1})	0.37 ± 0.04	0.29 ± 0.04	0.33 ± 0.07	0.15 ± 0.05	2.5	0.071
BOD_5 (mg L^{-1})	3.2 ± 0.1	3.1 ± 0.3	3.9 ± 0.5	2.8 ± 0.5	1.5	0.238
Total Cl (mg L^{-1})	0.12 ± 0.00	0.13 ± 0.00	0.13 ± 0.01	0.11 ± 0.00	1.1	0.372
NH_3 (mg L^{-1})	0.01 ± 0.00	0.08 ± 0.05	0.15 ± 0.09	0.02 ± 0.01	1.7	0.175
Sediment						
pH	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.0	0.0	0.994
C/N ratio	11.4 ± 1.0	11.6 ± 0.7	9.5 ± 1.4	13.8 ± 2.5	1.3	0.286
Organic C (g kg^{-1})	11.2 ± 1.7b	6.2 ± 1.1a	5.3 ± 1.0a	10.0 ± 2.7ab	3.3	0.026
Total N (%)	0.09 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.08 ± 0.02	2.6	0.061
P available (mg kg^{-1})	15.0 ± 1.0b	8.6 ± 0.9a	10.8 ± 1.4a	13.4 ± 1.1ab	8.4	<0.001
K exchangeable (mg kg^{-1})	320 ± 29c	107 ± 14a	127 ± 23ab	223 ± 44bc	18.0	<0.001
Sand (%)	80.3 ± 3.0b	89.4 ± 1.9a	86.2 ± 2.5ab	89.2 ± 2.5ab	2.8	0.048
Silt (%)	17.0 ± 2.8b	8.9 ± 1.6a	11.1 ± 2.0ab	9.3 ± 2.4ab	2.8	0.047
Clay (%)	2.7 ± 0.3	1.7 ± 0.6	2.7 ± 0.6	1.5 ± 0.4	1.3	0.279

Values of water depth, temperature and conductivity, of soil C, P, K, sand and silt content and of impact of management generally did not differ among ISO clusters, while showed distinct differences between ISO growing sites and the EX-ISO sites.

In particular higher depth and strongly fluctuating water levels, higher values of temperature, and conductivity were all associated with the absence of *I. malinverniana*. Soil organic C and nutrients (P and K) also showed higher values in the EX-ISO sites compared with ISO sites. *Isoëtes* groups and management intensity were significantly related (χ^2 = 41.13, P < 0.001, df = 9), with the highest management intensity occurring in EX-ISO group and the recurrence of lower impact activities in the ISO-C and ISO-B groups.

Differences resulted more marked between the group C of ISO plots and EX-ISO plots, except for the soil parameters.

3.3. Relationships between environmental conditions and Isoëtes status

Canonical variates analysis (CVA) was used to discriminate among the four groups: three ISO clusters and the EX-ISO group. CVA enabled us to determine which linear combination of environmental variables best discriminated among groups and to indicate if groups were different in terms of the measured environmental factors. Eleven of the 21 environmental variables tested for discriminatory power between the groups were significant (P < 0.10, Table 3). Water temperature, water conductivity, water depth and depth range, soil K and P, and impact of management were the best discriminating variables (P < 0.01).

Table 3
Discriminatory power and statistical significance of the analyzed environmental variables on the *Isoëtes* groups. The first 12 variables ($P < 0.10$) were selected as active variables in the CVA ordination. Pearson Correlation coefficients and their significance are reported for the first CVA axes.

Variables	Performance in the model			Correlation coefficient (R)	
	Explained inertia	*F*	*P*	CVA axis 1	CVA axis 2
Water annual mean temperature (°C)	0.698	17.51	0.002	0.87^{***}	−0.14
Water EC (μS cm^{-1})	0.566	13.49	0.002	0.77^{***}	0.20
Water depth (cm)	0.533	12.52	0.002	0.77^{***}	0.05
Soil K exchangeable (mg kg^{-1})	0.491	11.36	0.002	0.69^{***}	0.38^{**}
Water annual depth range (cm)	0.427	9.62	0.002	0.69^{***}	−0.01
Management intensity	0.353	7.73	0.002	0.60^{***}	0.14
Soil P available (mg kg^{-1})	0.309	6.67	0.002	0.50^{***}	0.38^{**}
Soil organic C (g kg^{-1})	0.151	3.07	0.014	0.35^{**}	0.27^{*}
Soil sand (%)	0.131	2.64	0.036	-0.37^{**}	−0.01
Soil silt (%)	0.131	2.65	0.038	0.38^{**}	0.03
Water NO_3^- (mg L^{-1})	0.124	2.49	0.044	0.25^{*}	0.36^{**}
Water PO_4^{3-}(mg L^{-1})	0.116	2.33	0.080	0.26^{**}	-0.29^{*}
Water NH_3 (m L^{-1})	0.098	1.96	0.108	−0.19	−0.10
Soil total N (%)	0.095	1.89	0.102	0.35^{**}	0.21
Water pH	0.092	1.83	0.160	0.18	−0.17
Water total Cl (m L^{-1})	0.075	1.49	0.216	−0.04	-0.38^{**}
Water BOD_5 (mg L^{-1})	0.071	1.41	0.252	0.01	−0.10
Soil clay (%)	0.066	1.30	0.266	0.19	−0.08
Soil C/N ratio	0.065	1.28	0.279	−0.02	0.27^{*}
Water hardness (mg $CaCO_3$ L^{-1})	0.040	0.79	0.513	0.04	−0.01
Soil pH	0.001	0.01	1.000	0.08	0.07

* <0.05 probability level.
** <0.01 probability level.
*** <0.001 probability level.

Axis 1 and 2 of the CVA ordination plot are illustrated in Fig. 3. The first axis (explained variance = 59%) clearly separates the EX-ISO group from all the others ISO clusters. The length and direction of the arrows provide an indication of the relative importance of each environmental variable in the group separation (Fig. 3b). EX-ISO sites typically displayed high water depth, high water depth range, high water temperature, high EC and intensive management. Soil texture also discriminates along the first axis, with higher silt and lower sand content in the EX-ISO plots than in the ISO clusters. The second axis (explained variance = 31%) separates ISO-C plots from the other two ISO clusters. Water nitrates and phosphates, nutrient (P and K) and organic C content in the soil are the discriminating variables. ISO-C plots were characterized by higher water nitrates, higher P, K and organic C content in the soil, while higher water phosphates were associated with ISO-A plots. Water parameters traditionally analyzed to assess water quality, i.e. nitrates, phosphates, ammonia, total chlorine, BOD_5 and water pH showed a weak or null power in the group separation. The large overlap and the close proximity of the centroids in the CVA ordination plot between ISO-A and ISO-B clusters suggest that they may not be easily distinguished in terms of the measured environmental variables.

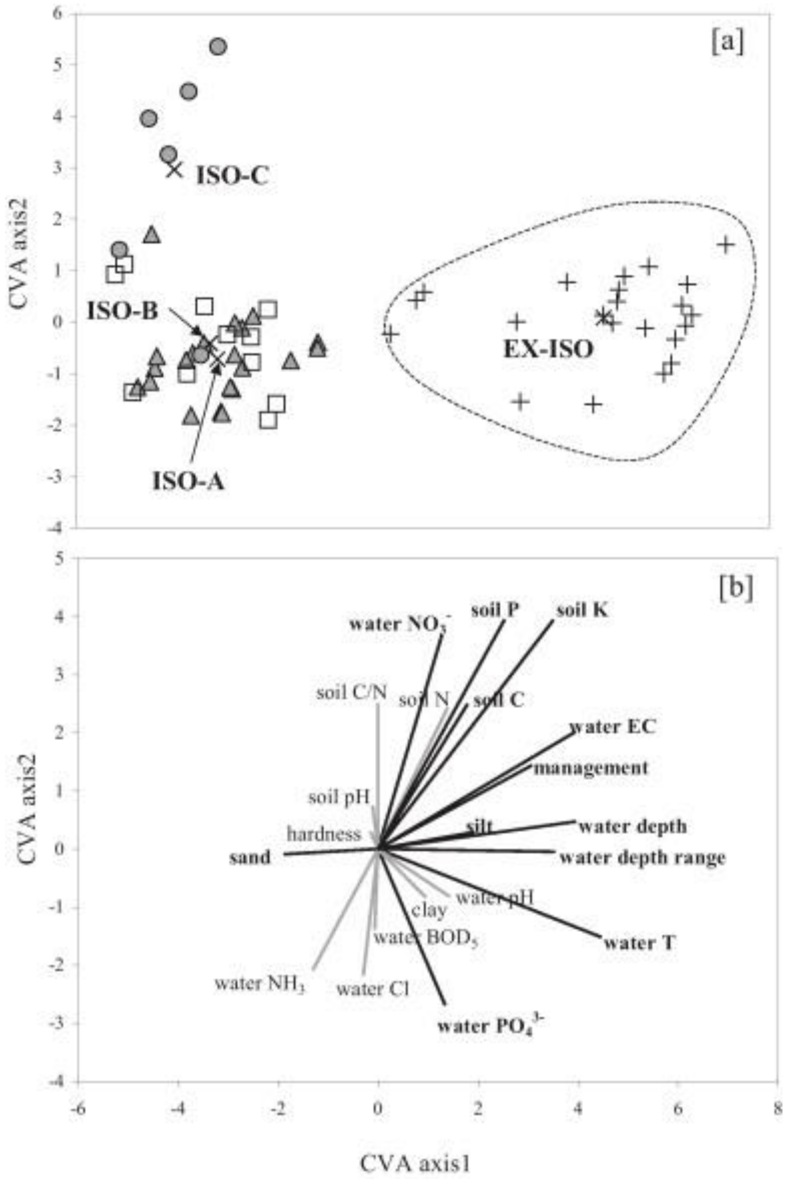

Fig. 3.

CVA diagrams based on the first two axes: (a) + = EX-ISO plots (*n* = 22); ▲ = ISO-A (*n* = 21); □ = ISO-B (*n* = 11); • = ISO-C plots (*n* = 6); X = centroids of *Isoëtes* groups; dotted lines highlight the group well differentiated along the first discriminant axis and (b) black lines: significant explanatory variables; grey lines: supplementary variables included in the model.

. 4. Discussion

Three different situations can be identified within and among *I. malinverniana* extant populations. Considering cover, density, and length as diagnostic parameters of plant and population performance (Vöge, 2003) we can assume that a decreasing health status of the plant occurs from populations with ISO-A to populations with ISO-C dominating plots.

Unfortunately we lack quantitative data revealing the *Isoëtes* growth rates in its original undisturbed habitat, severely altered or destroyed nowadays. Nevertheless, populations characterized by high cover (50–100%) with plants up to 1 m long were described before the drastic range contraction (Mattirolo, 1912 and Corbetta, 1965; 1968) and measurements on 58 herbarium specimens collected between 1858 and 1912 resulted in 45 cm as average plant length. At present, similar values are reached only in ISO-C plots, suggesting that *I. malinverniana*, if not affected by environmental constrains, tends to display these growing features. Gentili et al. (2010), assessed the genetic structure and the level of genetic variation within and among some of the extant populations of *I. malinverniana* and demonstrated that population no. 3 (most of the plots grouped in ISO-C, see Appendix I) had the highest values of genetic diversity, concluding that this would be the best candidate as a source of individuals to employ in reintroduction or reinforcement plans. On the contrary, populations with predominating ISO-A plots are also the smallest populations and, according to Gentili et al. (2010), they show the lowest values of genetic variability. The chance for survival of such very small populations, under current conditions, seems to be scarce. Therefore the understanding of factors which either negatively or positively influence the population survival results of great importance.

Contrary to our expectations, habitat conditions where *I. malinverniana* showed the highest density and productivity, were characterized by higher water nitrates, higher P, K and organic C content in the soil. Only water phosphates were lower, however they did not seem to have a significant role in the declining of *Isoëtes* populations (see CVA). The increase in water nutrients registered in sites with higher number of plants and *Isoëtes*productivity is evident but stays in the range of mesotrophic conditions (according to the Italian law D.L. 258/00). Similarly nutrient content in the sediment suggests conditions of medium-low fertility if compared to the typical agricultural soils of the surrounding plain. Therefore it seems likely that the nutrient increase, especially in the sediments, leads*Isoëtes* to faster plant growth and increased biomass, rather than "disrupting" the metabolic activity as suggestively hypothesized for other *Isoëtes* species (Wen et al., 2003). This result suggests that *I. malinverniana* can be considered relatively tolerant of eutrophication as already reported for other aquatic quillworts (Rorslett and Brettum, 1989 and Gacia and Ballestreros, 1993) and is capable of phenotypic adaptations in size as a response to habitat variations, like other congenerics (Rorslett and Brettum, 1989) and other macrophytes adapted to nutrient poor, acidic water, like *Juncus bulbosus* (Schneider et al., 2013) and *Littorella uniflora* (Bagger and Madsen, 2004).

The nutrient increase could affect indirectly *Isoëtes* by leading to enhanced growth of aquatic macrophytes and overgrowth of epiphyte algae (Sand-Jensen and Borum, 1991) that compete better for light and nutrients in the water layer. However, in the studied growing sites the cover percentage of other macrophytes resulted generally very low and significantly lower than *Isoëtes* cover particularly in ISO-C plots (12% vs. 83%) and ISO-B (10% vs. 47%), but also in ISO-A (9% vs. 6%) the cover of other hydrophytes was in no way competitive. Epiphytic algae development was rarely observed, in particular on decaying sporophylls at the end of winter, when water flow has the lowest level.

Comparison between ISO and EX-ISO sites resulted very useful in order to generate more convincing hypothesis on potential threats to *I. malinverniana*. In the CVA ordination two distinct environmental spaces were identified by the ISO plot groups (towards the left) and the extinct *Isoëtes* sites (towards the right). Environmental variables representative of water and soil physical characteristics, electrical conductivity and management type strongly characterize the suitable or inhospitable environmental space for *Isoëtes* survival.

Most of EX-ISO sites show a more southern location than ISO sites, located more close to the resurgence spring line of the Po plain. Consequently, EX-ISO sites are characterized by water temperature and electrical conductivity consistently higher than ISO sites since the lowland channel system collect relatively more water from rivers, from rice field drainage, and from rural settlements because of a wider catchment area.

Direct response of quillworts to variation in temperature is still unclear but it may limit *Isoëtes*occurrence and viability when stays under certain thresholds (Vöge, 2003). On the other hand, Gacia and Ballestreros (1994) showed that the productivity of *I. lacustris* was highest with conditions of high temperature. Although we cannot exclude the combined contribution of the increase in temperature with other environmental parameters, we lack evidence of a direct detrimental effect of water warming on *Isoëtes* occurrence. On the contrary, conductivity has been often reported as an important factor determining *Isoëtes*growth and thus was considered a useful parameter to identify habitat suitability for aquatic quillworts (Vöge, 2003, Abeli et al., 2012). Increased values of conductivity in EX-ISO sites may be both related to higher temperature values and to greater amount of ionized solutes and/or total dissolved solid, compared to ISO sites. Constant low values of water hardness allow us to exclude lime addiction as the cause of conductivity increase, while on the contrary the hypothesis of toxic pollutants in the water, needs to be deepened.

Channel systems located in the lower plain have been deepened or dredged in the past to increase channel capacity, while upper plain channels are less modified and retain small dimensions. This type of management has clearly a direct detrimental effect on aquatic plants and habitats (Hey et al., 1994), since it removes both growing plants and propagules stored in the substrate, but also implies hydrological alterations such as the deeper water and the wider fluctuating water level among seasons, that characterize EX-ISO sites. High water level and fast currents increase sediment resuspension, which affects the ability of macrophytes to survive due to the effects of abrasion and the decreased light (Biggs, 1996 and Madsen et al., 2001). *Isoëtes* species seem to be sensitive to scour (Szmeja, 1994) and to fast flow. In particular when growing in nutrient-rich sediments *Isoëtes* plants tend to increase shoot to root ratio with weaker roots (Smolders et al., 2002), and are therefore prone to be easily uprooted, owing to mechanical disturbance. Furthermore, *Isoëtes* and isoetid species are proved to be vulnerable to decreases in light levels, resulting from decreased water transparency (Sand-Jensen and Søndergaard, 1981 and Rorslett and Johansen, 1995).

Water quantity and the timing of changes in supply have a primary influence on aquatic plant communities (Haslam, 1978). The season and extent of enhanced water supply due to rice growing demand from April to September may be crucial to the occurrence and colonization of *Isoëtes* communities in these systems. Since *I. malinverniana* starts to release mature spores in late autumn (Abeli and Mucciarelli, 2010), it seems likely that the sudden inflow of great quantity of turbid water in April may heavily affect the most important phases in the demography of this species: germination, seedling establishment and initial growth.

Almost all upland sites where *Isoëtes* populations still survive are subjected to mechanized management, but with a lower impact with respect to the lowland sites. The maintenance of these channels includes at least annual manual cutting of aquatic and bankside vegetation, and shallow dredging from 2 to approximately 10-year intervals, using hydraulic excavators. Cutting does not seem to affect negatively *Isoëtes* performance, rather it seems to encourage vegetative growth in nutrient rich conditions. On the other side, dredging is not applied as deeply and extensively as in lowland channels. This allows some *Isoëtes* patches to survive in most sheltered positions, for instance under and near bridges, and therefore to maintain viable plants capable of re-colonization after dredging. Seasonal changes in water level and the associated effects on the reproductive phase occur also in upland channels, although the lower water supply and smaller channel size maintain the level variation in a narrower range.

Our findings may have useful implications for the conservation and restoration of *I. malinverniana*. Maintaining narrow channels with low mean water depth (20–30 cm) and a slow but constant flow is crucial for the conservation of the species, with particular regard at avoiding the severe increase of water flow during spring. The mechanized maintenance of channels should be restricted within reaches of channel where the species is still present, applying a buffer zone up- and downstream. On the contrary, manual mowing may be allowed, within certain limits of frequency. The above suggestions may be useful at achieving the goals of the Water Framework Directive (FWD 2000) and the “Habitat Directive (43/92/EEC), to improve sustainable conservation of *Isoëtes* and other rare aquatic species through the habitat preservation and restoration.

Acknowledgements

Researches have been carried out within the Project “Biodiversità per tutti” directed by Ente Parco Lame del Sesia and funded by Cassa di Risparmio delle Province Lombarde (CARIPLO).

The authors express their warmest thanks to Daniela Bouvet for technical support and to Adriano Soldano who introduced them into the *I. malinverniana* research.

References

Abeli et al., 2012 T. Abeli, E. Barni, C. Siniscalco, C. Amosso, G. Rossi A cost-effective model for preliminary site evaluation for the reintroduction of a threatened quillwort Aquatic Conserv.: Mar. Freshw. Ecosyst., 22 (2012), pp. 66–73

Abeli and Mucciarelli, 2010 T. Abeli, M. Mucciarelli Notes on the natural history and reproductive biology of Isoetes malinverniana Am. Fern J., 100 (2010), pp. 235–237

Anderberg, 1973 M.R. Anderberg Cluster Analysis for Application Academic Press, New York (1973)

Andreis et al., 2005 C. Andreis, S. Verde, S. Armiraglio, M. Caccianiga, B. Cerabolini Elementi per una suddivisione della Lombardia in Distretti Geobotanici Inf. Bot. Ital., 37 (2005), pp. 466–467

Arts, 2002 G.H.P. Arts. Deterioration of atlantic soft water macrophyte communities by acidification eutrophication and alkalinisation Aquat. Bot., 73 (2002), pp. 373–393

Bagger and Madsen, 2004 J. Bagger, T.V. Madsen Morphological acclimation of aquatic Littorella uniflora to sediment CO2 concentration and wave exposure Funct. Ecol., 18 (2004), pp. 946–951

Barni et al., 2010 E. Barni, C. Minuzzo, C. Siniscalco, F. Gatto, T. Abeli, C. Amosso, G. Rossi, R. Gentili, F. Pistoja, A. Soldano Isoetes malinverniana Ces. et De Not. Inf. Bot. Ital., 42 (2010), pp. 539–613

Bernardos et al., 2006 S. Bernardos, A. Amado, F. Amich The narrow endemic Scrophularia valdesii Ortega-Olivencia & Devesa (Scrophulariaceae) in the Iberian Peninsula: an evaluation of its conservation status Biodivers. Conserv., 15 (2006), pp. 4027–4043

Biggs, 1996 B.J.F. Biggs Hydraulic habitat of plants in streams Regul. Riv. Res. Manage., 12 (1996), pp. 131–144

Cesati and De Notaris, 1858 Cesati, V., De Notaris, G., 1858. Isoëtes novae descriptio. Ind. Sem. H. Bot. Gen. 1858, 1–7.

Changkyun et al., 2008 K. Changkyun, R.-N. Hye, C. Hong-Keun Genetic diversity and population structure of endangered Isoetes coreana in South Korea based on RAPD analysis Aquat. Bot., 89 (2008), pp. 43–49

Chapin, 1980 F.S. Chapin The mineral nutrition of wild plants Ann. Rev. Ecol. Syst., 11 (1980), pp. 233–260

Chen et al., 2007 J.M. Chen, R. Gituru Wahiti, X. Liu, Q.F. Wang Genetic diversity in Isoëtes yungulensis, a rare and endangered endemic fern in China Front. Biol. China, 2 (2007), pp. 46–49

Chen et al., 2005 J.M. Chen, X. Liu, J.Y. Wang, R. Gituru Wahiti, Q.F. Wang Genetic variation within the endangered quillwort Isoëtes hypsophila (Isoëtaceae) in China as evidenced by ISSR analysis Aquat. Bot., 82 (2005), pp. 89–98

Conti et al., 2005 F. Conti, G. Abbate, A. Alessandrini, C. Blasi An Annotated Checklist of the Italian Vascular Flora Palombi Editori, Roma (2005)

Conti et al., 1997 F. Conti, A. Manzi, F. Pedrotti Liste rosse regionali delle piante d'Italia Dipartimento di Botanica ed Ecologia, Università degli Studi di Camerino, Camerino (1997)

Corbetta, 1965 F. Corbetta Osservazioni relative ad una nuova stazione di Isoëtes malinvernianum Natura e Montagna, 5 (1965), pp. 57–61

Corbetta, 1968 F. Corbetta Nuovi dati sulla distribuzione di Isoëtes malinvernianum in Lomellina Giorn. Bot. Ital., 102 (1968), pp. 107–112

Environment Agency of Japan, 2000 Environment Agency of Japan Threatened Wildlife of Japan – Red Data Book, vol. 8: Vascular Plants Japan Wildlife Research Center, Tokyo (2000) (in Japanese)

Ferrarini et al., 1986 E. Ferrarini, F. Ciampolini, R.E.G. Pichi Sermolli, D. Marchetti Iconographia palynologica Pteridophytorum Italiae Webbia, 40 (1986), pp. 1–202

Gacia and Ballestreros, 1993 E. Gacia, E. Ballestreros Population and individual variability of Isoetes lacustris L with depth in a Pyrenean lake Aquat. Bot., 46 (1993), pp. 35–47

Gacia and Ballestreros, 1994 E. Gacia, E. Ballestreros Production of Isoetes lacustris in a Pyrenean lake: seasonality and ecological factors involved in the growing period Aquat. Bot., 48 (1994), pp. 77–89

Gentili et al., 2010 R. Gentili, T. Abeli, G. Rossi, M. Li, C. Varotto, S. Sgorbati Population structure and genetic diversity of the threatened quillwort Isoëtes malinverniana and implication for conservation Aquat. Bot., 93 (2010), pp. 147–152

Haslam, 1978 S.M. Haslam River Plants Cambridge University Press, Cambridge (1978)

Hey et al., 1994 R.D. Hey, G.L. Heritage, M. Patterson Impact of flood alleviation schemes on aquatic macrophytes Regul. River Res. Manage., 9 (1994), pp. 103–119

Hoot et al., 2004 S.B. Hoot, N.S. Napier, W.C. Taylor Revealing unknown or extinct lineages within Isoëtes (Isoëtaceae) using DNA sequences from hybrids Am. J. Bot., 91 (2004), pp. 899–904

Hoot et al., 2006 S.B. Hoot, W.C. Taylor, N.S. Napier Phylogeny and biogeography of Isoëtes (Isoëtaceae) based on nuclear and chloroplast DNA sequence data Syst. Bot., 31 (2006), pp. 449–460

Hutchinson, 1975 G.E. Hutchinson A Treatise on Limnology: Limnological Botany, vol. 3Wiley, New York (1975)

IUCN, 2010 IUCN, 2010. IUCN Red list of threatened species. Version 2010.1. www.iucnredlist.org.

Kang et al., 2005 M. Kang, Q. Ye, H. Huang Genetic consequence of restricted habitat and population decline in endangered Isoëtes sinensis (Isoëtaceae) Ann. Bot., 96 (2005), pp. 1265–1274

Leon and Young, 1996 B. Leon, K.R. Young Aquatic plants of Peru: diversity distribution and conservation Biodivers. Conserv., 5 (1996), pp. 1169–1190

Liu et al., 2005 X. Liu, J.Y. Wang, Q.F. Wang Current status and conservation strategies for Isoëtes in China: a case study fort he conservation of threatened aquatic plants Oryx, 39 (2005), pp. 335–338

Madsen et al., 2001 J.D. Madsen, P.A. Chambers, W.F. James, E.W. Koch, D.F. Westlake The interaction between water movement, sediment dynamics and submersed macrophytes Hydrobiologia, 444 (2001), pp. 71–84

Mattirolo, 1912 O. Mattirolo Sull'endemismo di Isoëtes malinvernianum di Cesati e De Notaris Annali di Botanica, 10 (1912), pp. 129–146

Musselman, 2002 L.J. Musselman The only quillwort (Isoetes olympica A. Braun) in Syria is threatened with extirpation Fern Gaz., 16 (2002), pp. 324–329

Pedersen et al., 2006 O. Pedersen, T. Andersen, K. Ikejima, Z.M.D. Hossain, F.O. Andersen A multidisciplinary approach to understanding the recent and historical occurrence of the freshwater plant, Littorella uniflora Freshw. Biol., 51 (2006), pp. 865–887

Potenza, 2002 A. Potenza Studio idrogeologico dei fontanili del settore orientale della pianura piemontese Tesi di Laurea in Geologia, Università di Torino (2002)

Rhazi et al., 2004 M. Rhazi, P. Grillas, A. Charpentier, F. Médail Experimental management of Mediterranean temporary pools for conservation of the rare quillwort Isoëtes setacea Biol. Conserv., 118 (2004), pp. 675–684

Rorslett and Brettum, 1989 B. Rorslett, P. Brettum The genus Isoëtes in Scandinavia: an ecological review and perspectives Aquat. Bot., 36 (1989), pp. 223–261

Rorslett and Johansen, 1995 B. Rorslett, S.W. Johansen Dynamic response of the submerged macrophyte, Isoëtes lacustris, to alternating light levels under field conditions Aquat. Bot., 51 (1995), pp. 223–242

Sand-Jensen and Borum, 1991 K. Sand-Jensen, J. Borum Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries Aquat. Bot., 41 (1991), pp. 137–176

Sand-Jensen and Søndergaard, 1981 K. Sand-Jensen, M. Søndergaard Phytoplankton and epiphyte development and their shading effect on submerged macrophytes in lakes of different nutrient status Int. Revue. Ges. Hydrobiol., 66 (1981), pp. 529–552

Schneider et al., 2013 S.C. Schneider, T. Fosholt Moe, D.O. Hessen, Ø. Kaste Juncus bulbosus nuisance growth in oligotrophic freshwater ecosystems: different triggers for the same phenomenon in rivers and lakes? Aquat. Bot., 104 (2013), pp. 15–24

Schuettpelz and Hoot, 2006 E. Schuettpelz, S.B. Hoot Inferring the root of Isoëtes: exploring alternatives in the absence of an acceptable outgroup Syst. Bot., 31 (2006), pp. 258–270

S.I.S.S., 1985 S.I.S.S. Metodi normalizzati di analisi del suolo Edagricole, Bologna (1985)

Smolders et al., 2002 A.J.P. Smolders, E.C.H.E.T. Lucassen, J.G.M. Roelofs The isoetid environment: biogeochemistry and threats Aquat. Bot., 73 (2002), pp. 325–350

Soldano and Badino, 1990 A. Soldano, A. Badino Nuove stazioni di Isoëtes malinverniana Cesati e De Notaris nel Vercellese. Tipificazione (Pteridophyta Isoëtaceae) Riv. Piemont. Stor. Nat., 11 (1990), pp. 65–69

Soliani, 2004 L. Soliani Manuale di statistica per la ricerca e la professione Uni Nova, Parma (2004)

SPSS, 2007 SPSS SPSS for Windows, Rel. 16. 0. 1 SPSS Inc., Chicago (2007)

Szmeja, 1994 J. Szmeja Effects of disturbances and interspecific competition in isoetid populations Aquat. Bot., 48 (1994), pp. 225–238

Taylor and Hickey, 1992 C.W. Taylor, J.R. Hickey Habitat evolution, and speciation in Isoetes Ann. Missouri Bot. Gard., 79 (1992), pp. 613–622

Taylor et al., 1993 W.C. Taylor, N.T. Luebke, D.M. Britton, R.J. Hickey, D.F. Brunton Isoëtaceae Reichenbach Flora of North America Editorial Committee (Ed.), Flora of North America, vol. 2, Pteridophytes and GymnospermsOxford University Press, New York (1993) pp. 64–75

ter Braak and Šmilauer, 2002 C.J.F. ter Braak, P. Šmilauer CANOCO for Windows Version 4.5 Wageningen, The Netherlands (2002)

Tutin et al., 1993 T.G. Tutin, N.A. Burges, A.O. Chater, J.R. Edmondson, V.H. Heywood, D.M. Moore, D.H. Valentine, S.M. Walters, D.A. Webb Flora Europaea (Second ed.), vol. 1Cambridge University Press, Cambridge (1993)

USFWS, 1996 USFWS Recovery Plan for Louisiana quillwort (Isoëtes louisianensis Thieret) U.S. Fish & Wildlife Service, Atlanta, Georgia (1996)

Vitousek, 1982 P.M. Vitousek Nutrient cycling and nutrient use efficiency Am. Nat., 119 (1982), pp. 553–572

Vöge, 2003 M. Vöge Environmentally related demography: field studies on Isoëtes lacustris L. (Lycophyta, Isoëtaceae) in Europe S. Chandra, M. Srivastava (Eds.), Pteridology in the New Millennium, Kluwer Academic Publishers, The Netherlands (2003), pp. 233–260

Vöge, 2004 M. Vöge Non-destructive assessing and monitoring of populations of Isoëtes lacustris L Limnologica, 34 (2004), pp. 147–153

Ward, 1963 J. Ward Hierarchical grouping to optimize an objective function J. Am. Stat. Assoc., 58 (1963), pp. 236–244

Wen et al., 2003 M.Z. Wen, X.A. Pang, Q.F. Wang, W.C. Taylor Relationship between water chemistry and the distribution of the endangered aquatic quillwort Isoëtes sinensis Palmer in China J. Freshw. Ecol., 18 (2003), pp. 361–367