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A cost-effective model for preliminary site evaluation for the reintroduction of a threatened quillwort

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ABSTRACT

1. The choice of suitable sites for establishment of a new population of a species is the first step in a translocation programme. However, evaluation of a large number of sites can be demanding of time and money; practitioners need time- and cost-effective evaluation tools.

2. A predictive model for the preliminary evaluation of potential reintroduction sites was developed using the endangered quillwort *Isoëtes malinverniana* as a case study. The reliability of three water habitat variables (pH, conductivity, and clarity) as predictors of the presence/absence of *I. malinverniana* was tested. Three sets of logistic models were produced on the basis of the mean values of pH and conductivity measured over 3, 4 and 6 months to understand whether frequency of measurement affected the reliability of the models. Models of each set were ranked according to AIC and their reliability was then tested using data from the literature.

3. The conductivity of water and pH were the most effective predictors of the suitability of sites for the reintroduction of *I. malinverniana*. In particular there was a negative relationship between the presence of *I. malinverniana* and these variables.

4. Because of seasonal fluctuations in the variables considered, at least 4 months monitoring are required to obtain reliable results. Despite this, the method is still advantageous in terms of costs, compared with repeated measurements of a wide range of chemical characteristics at a large number of sites.

5. The main goal of this method is to limit expensive chemical analysis to a few sites chosen after the exclusion of unsuitable sites, through application of the model. Considering the similar ecophysiological features of quillworts from oligotrophic waters, the same model or conceptual framework could be applied to other quillworts and isoetid species growing in degraded areas and needing active conservation.

INTRODUCTION

Isoëtes malinverniana Cesati and De Notaris is an aquatic quillwort included in Annex II of the European Habitats Directive 92/43/EEC and listed as Critically Endangered according to the IUCN criteria (Conti *et al.*, 1997; Barni *et al.*, 2010) owing to recent contraction of its range. *I. malinverniana* is endemic to Piedmont and Lombardy (northern Italy). In the last 40 years its range has contracted by more than 80% to 12 remaining sites (Barni *et al.*, 2010). The major threats to this species are declining water quality, destructive habitat management techniques and severe competition with other native or invasive hydrophytes (Ardenghi and Abeli, 2011); these threats are common to other quillworts growing in modified ecosystems (Wen *et al.*, 2003; Chen *et al.*, 2005; Kang *et al.*, 2005). The few remaining populations of *I. malinverniana* are in highly modified agricultural catchments. This species requires urgent study and conservation action (Maunder, 1992; Guerrant *et al.*, 2004), both to protect and reinforce extant populations and to reintroduce new populations into suitable sites. However, reintroductions are expensive, high-risk options that may commit personnel to long-term management and monitoring (Falk *et al.*, 1996; Maschinski and Duquesnel, 2006). They have high rates of failure (Menges, 2008), owing to combinations of poor horticultural practices, poor ecological understanding, and lack of post-planting maintenance and monitoring (Godefroid *et al.*, 2011). In particular, the selection of suitable reintroduction sites is a crucial point for successful conservation because establishment of a new population in an unsuitable site will result in the rapid disappearance of the new population, despite aftercare (IUCN, 1998; Godefroid *et al.*, 2011). Sites for reintroduction should be identified on the basis of the ecological requirements of a species (Clewell *et al.*, 2000), but a detailed quantification of the ecological niche of a species may be expensive. Another issue to be considered is the lack of time to perform complex laboratory analysis and monitoring (e.g. EU Life + Projects allow only up to 1 year of preliminary research for reintroduction programmes). For such reasons, the development of quick and appropriate tools that can help practitioners to evaluate potential sites for reintroduction (Hauser *et al.*, 2006; Tash and Litvaitis, 2007) is considered a key issue in species conservation (Hirzel*et al.*, 2004; Rossi *et al.*, 2009), especially when resources are limited (Margules and Pressey, 2000).

Few tools with these characteristics have been developed, especially for freshwater hydrophytes (Silk and Ciruna, 2004). This is despite the fact that 200 species of *Isoëtes* (Hoot *et al.*, 2004) are known and several have been the subject of conservation measures (USFWS, 1996) such as reintroduction and habitat restoration (Rhazi *et al.*, 2004; Liu *et al.*, 2005). An exhaustive literature search failed to identify any practical tools for *in situ* or *ex situ* population management, reintroduction or monitoring.

An attempt to fill this gap has been made in this study by producing a cost-effective procedure for selecting sites for reintroduction, testing the reliability of three selected variables (electrical conductivity, pH and water clarity) as predictors of the presence or absence of *I. malinverniana* in Italy. The low number of extant populations of very rare species preclude the assembly of large datasets and application of statistical models based on a high number of variables, making exhaustive study of the ecological requirements difficult or impossible. Thus, the selection of few features is particularly useful in the case of very rare and endangered species that are often neglected in the scientific literature (Belvill and Louda, **1999**). Moreover, the selected variables were chosen because of their high ecological significance in aquatic ecosystems and the fact that they are readily measured in the field using low-cost equipment. Conductivity is a measure of the amount of solutes in the water; pH affects the nutrient and carbon availability for organisms and water clarity affects the photosynthetic process (Silk and Ciruna, **2004**). Analysis of the selected variables has enabled development of a predictive model for preliminary exclusion of unsuitable reintroduction sites. This model should be useful in allowing resources to be redirected to in-depth examination (i.e. chemical analysis) of more suitable sites. Considering the similarity of ecophysiological features of quillworts from oligotrophic aquatic environments, this model may have potential application to other quillworts and isoetid species.

METHODS

Study species

Isoëtes malinverniana Cesati and De Notaris is an endemic aquatic quillwort, growing in the western part of the Po Plain (Lombardy and Piedmont, northern Italy) (Corbetta, **1968**; Tutin *et al.*, **1993**; Figure 1). It has a three-lobed stem and usually long, hollow sporophylls with ovate ligula. Sporangia are without velum (Tutin *et al.*, **1993**) and the species is autogamous (Abeli and Mucciarelli, **2010**). Before establishment of rice fields the species probably occurred in small natural streams generated by springs. Nowadays, *I. malinverniana* occurs in running water canals used for rice field water supply. Such canals originally carried oligotrophic waters derived from the groundwater; nowadays, they carry a mixture of groundwater and river water. Historically, *I. malinverniana* has been recorded from about 50 sites. Surveys carried out since 2007 within the species range have found that it is still present in only 12 sites (Figure 1).

Figure 1. Current geographic range of *Isoëtes malinverniana* (shaded area) in the Plain of the river Po in northern Italy.

Current geographic range of *Isoëtes malinverniana* (shaded area) in the Plain of the river Po in northern Italy.

Experimental design

During 2008, all the sites known to have supported *I. malinverniana* in the past were visited, using literature and plant record observations (Corbetta, **1968**; Soldano and Badino, **1990**; Pistoja, **2007**) with the aim of identifying two types of sampling sites: (A) canals which still support *I. malinverniana*; and (B) canals which formerly supported *I. malinverniana* but from which it disappeared in the last decade. All 12 currently known populations of *I. malinverniana* were included in the present study. It was only possible to pinpoint the precise location of eight canals from which populations had been lost in the last decade and which were then classed as type-B.

The reliability of electrical conductivity (EC), pH, and water clarity as predictors of the presence or absence of *I. malinverniana* was tested to obtain a predictive model, useful for selection of potential reintroduction sites. Water pH and EC were recorded with a multi-parameter probe (HI 9811–5, HANNA Co., Italy), while water clarity was visually estimated as the ability to see the canal bottom (1) or not (0). A Secchi disk was not used because in most cases the water was only a few centimetres deep or the velocity was too great for the disk to be kept in position. These variables were measured in the centre of each canal, in three places, once a month for 6 months from March to August.

Data analysis

Water EC, pH, and water clarity were used to construct different logistic models in which the dependent variable was the type of site: A (sites with the species here growing, '1') and B (sites where the species is extinct, '0'). Akaike's Information Criterion (AIC) was applied to compare the models obtained on the basis of their loss of 'information' (Anderson, 2008). The best model within a series of models is the one that minimizes the loss of information with respect to 'reality'. Owing to the rarity of the species, the number of cases was quite low and so the corrected AIC for small sample size (AICc) was used, as recommended by Anderson *et al.* (2000). The best model was chosen by ranking the candidate models according to their Akaike weights (AICcw) (Anderson *et al.*, 2000), and choosing the one with the highest AICcw. Evidence ratios were also compared: the ratio of the AICcw of the model with the lowest AICc score to the weight of the other models. While AICcw represents the probability that the selected model is the best model in the set, evidence ratio can be used to determine whether other models can be considered equally informative or implausible.

All the possible models were obtained with different values for the variables considered. Three sets of logistic models were produced, based on the mean values of EC and pH measured over 3, 4 and 6 months to understand whether measurement time affected the reliability of the models. Because of intrinsic characteristics of EC and pH that can vary over time, models based on mean data compiled from less than 3 months were not considered. EC was log-transformed to match the normal distribution. Some preliminary data on soil and water chemistry were available (Barni *et al.* in preparation), thus the relationships between EC, pH, soil pH, nitrogen, organic carbon, P_2O_5, K_2O, and water $CaCO_3$, NO_3^-, PO_4^{3-}, Cl and NH_4^+, were tested by using Spearman correlations. Finally, the reliability of the models was tested using EC and pH values available from the literature for other species of *Isoëtes* (Rørslett and Brettum, 1989; Romero and Amigo, 1995; Wen *et al.*, 2003; Free *et al.*, 2009). Unfortunately, most of the published data referred to sites supporting growing populations and only in one case were data for an extinct site of *I. sinensis* found (Wen *et al.*, 2003).

RESULTS

Average EC and pH values after 3, 4 and 6 months are summarized in Table 1.

Table 1. Average values (standard error, S.E.) of the electrical conductivity (EC) of water and pH over 3, 4 and 6 months in the two types of site: sites supporting *I. malinverniana* (A) and sites where the species is extinct (B). Difference between sites was tested with *t*-test

	3 months		4 months		6 months	
Average values	**A (n = 12)**	**B (n = 8)**	**A**	**B**	**A**	**B**
EC(μS cm^{-1})	101.11(13.82)***	218.17(20.45)	92.9(13.6)***	208.6(21.2)	91.9(13.1)***	210.1(21.1)
pH	7.49(0.11)*	7.86(0.1)	7.45(0.1)*	7.77(0.1)	7.51(0.1)	7.72(0.08)

Significance level:
* = $P < 0.05$;
*** = $P < 0.001$.

Water was always clear in type-A sites, while only 50% of the type-B canals had clear water during the whole study period. Seven models are presented for data averaged over each of the three sets of time periods (Table 2). Model sets based on average values of EC and pH over 4 and 6 months yielded consistent results for the selected model. For both the model sets, the most informative model, having the lowest AICc and the highest AICcw, identified EC and pH as the most important predictors of the presence or absence of *I. malinverniana* (Table 2(a), (b)). The selected model across the three sets was the first in Table 2(a). This is because the model was based on average EC and pH measured across 6 months, had the lowest AICc and the highest AICcw across the three model sets; it was considered to be the most informative model. The R^2 of the selected model was 0.670. The importance of the length of the data series became more evident when the first models of each model set were directly compared with the AIC. In fact, model 1 of the 6-months model set still had the lowest AICc and the highest AICcw (0.628), the first model of the 4-months model set had AICcw equal to 0.266 and the first model of the 3-months model set had AICcw equal to 0.106.

Table 2. Three model sets based on the average values of electrical conductivity (EC) and pH over 6 months (a), 4 months (b) and 3 months (c). Models in each set were ranked according to the corrected Akaike's Information Criterion (AICc). The selected model is in bold. K is the number of variables included in the models plus constant. Likeli. = Likelihood; E. = Evidence. Models within each set are ordered from best to worst in descending order

Models	−2LogLikeli	K	AICc	AICc	AICcw	E. ratio
(a) 6 months average						
(EC, pH)	**4.771**	**3**	**12.271**	**0.00**	**0.651**	**1**
(Water clarity, pH, EC)	4.415	4	15.082	2.81	0.159	4.1
(Water clarity, EC)	8.243	3	15.743	3.47	0.113	5.8
(EC)	11.976	2	16.682	4.41	0.070	9.3
(Water clarity)	17.995	2	22.701	10.43	0.003	217
(Water clarity, pH)	17.117	3	24.617	12.35	0.001	651
(pH)	23.731	2	28.437	16.17	0.0001	6500
(b) 4 months average						
(EC, pH)	6.492	3	13.992	0.00	0.511	1
(Water clarity, pH, EC)	5.437	4	16.104	2.11	0.178	2.9
(Water clarity, EC)	8.635	3	16.135	2.14	0.175	2.9
(EC)	12.442	2	17.148	3.16	0.105	4.8
(Water clarity)	17.999	2	20.700	6.71	0.017	28.6
(Water clarity, pH)	14.338	3	21.888	7.90	0.010	51.8
(pH)	19.942	2	24.648	10.66	0.002	206.0
(c) 3 months average						
(Water clarity, EC)	7.990	3	15.490	0.00	0.315	1
(EC, pH)	8.338	3	15.838	0.35	0.265	1.2
(Water clarity, pH, EC)	5.863	4	16.530	0.69	0.223	1.4
(EC)	12.386	2	17.092	1.25	0.168	1.9
(Water clarity, pH)	14.603	3	22.103	6.26	0.014	22.9
(Water clarity)	17.995	2	22.701	6.86	0.010	30.9
(pH)	20.198	2	24.904	9.07	0.003	93.0

The selected model showed an inverse relationship between the presence of *I. malinverniana*, EC and pH (Table 3). With just 3-month average values of EC and pH, the most informative model included water clarity and EC. This model had an AICcw quite low with respect to the models of the other model sets (Table 2(c)). Moreover, due to the very low ΔAICc and evidence ratio of the first four models in this model set, it was not possible to highlight a single best model.

Table 3. Estimated parameters (B) and Standard Error (S.E.) of the logistic regressions for the first (selected) model and the second model of the first model set

Variable	B	S.E.
Model 1		
LogEC	−30.133	42.200
Water pH	−16.264	19.463
Intercept	191.202	242.762
Model 2		
LogEC	−35.009	58.793
Water pH	−16.704	26.048
Water clarity	−18.538	17889.346
Intercept	205.926	330.637

EC always significantly differed between sites of type A and B, while pH significantly differed only with 3- and 4-month average values (Table 1). Water pH was related to soil pH (Rho = 0.450; *P* = 0.047), while EC was related to water NO_3^- (Rho = 0.458; *P* = 0.043), soil C (Rho = 0.502; *P* = 0.024), P_2O_5 (Rho = 0.580; *P* = 0.007) and K_2O (Rho = 0.654; *P* = 0.002).

Testing the selected model with EC and pH values from the literature, the model did not correctly predict the absence of *I. sinensis* in extinct sites reported in Wen *et al.* (**2003**). However, it correctly predicted the presence of *Isoëtes* spp. in the other cases (Table 4).

Table 4. Model reliability with electrical conductivity (EC) and pH values from the literature. All the data referred to sites with growing *Isoëtes* spp., with the exception of the last row. Values close to 1 indicate the suitability of a site, while values close to 0 indicate the unsuitability of a site

Location and number of sites	EC(µS cm^{-1})	pH	Model result	Species treated	Reference
Ireland (159)	49	5.10	1	*I. lacustris*	Free *et al.*, 2009
Scandinavia (387)	2.66	6.55	1	*I. echinospora* *I. lacustris*	Rørslett and Brettum, 1989
S Baltic area (74)	75.3	6.90	0.99	*I. lacustris*	Szmeja *et al.*, 1997
Spain (8)	78.1	6.79	0.99	*I. longissimum*	Romero and Amigo, 1995
China: current sites (3)	60	6.70	0.99	*I. sinensis*	Wen *et al.*, 2003
China: extinct sites (7)	190	7.29	0.98	*I. sinensis*	Wen *et al.*, 2003

DISCUSSION

This work demonstrated that a simple model based on water EC and pH values can be a useful tool to select suitable sites for the reintroduction of *I. malinverniana*. This model can be easily applied because it is based on relatively simple statistics (logistic regression) and includes few variables (EC and pH) that can be quickly measured in the field with a multi-parameter probe, leaving long and expensive chemical analysis to be performed after the preliminary exclusion of unsuitable reintroduction sites. EC and pH values collected in several canals over 6 months must be included in the logistic regression equation, with the variables listed in Table 3. Results very close to 0 will suggest exclusion of a canal, results very close to 1 will suggest further analysis of the chemical composition of water and bottom sediments.

A negative relationship was found between EC and pH values and the presence of *I. malinverniana*(Table 1, 3). This might be explained by the sensitivity of *I. malinverniana* to increased nutrient and solute amounts (Barni *et al.*, **2010**). In all the type-B canals, EC was higher than in sites in which the species still grows, suggesting a role of increased eutrophication in the extinction of the species in type-B canals. An increased amount of solutes in oligotrophic environments can provide an advantage for other aquatic species, sometimes naturalized or invasive aliens, that are better competitors for nutrients in the water layer (Rhazi *et al.*, **2009**). With a few exceptions of species typical of slightly mesotrophic habitats (e.g.*Isoëtes duriei* and *Isoëtes histrix*, Grillas *et al.*, **2004**), quillworts mainly grow in nutrient-poor waters and they are, in general, highly sensitive to nutrient enrichment of the water. Wen *et al.* (2003) demonstrated that increasing nutrient concentrations, thus increasing EC, is one of the causes of the decline of *I. sinensis* in China. Furthermore, in Ireland, Free *et al.* (2009), in a survey of 68 isoetid lakes, showed that *I. lacustris* became rarer in the group of lakes with the highest mean EC value (339 μS cm^{-1}). Values of EC recorded during the present study in sites where the species is growing (type-A) are quite high (median: 76 μS cm^{-1}), compared, for example, with those of some Scandinavian isoetid lakes (median: 32 μS cm^{-1}; Rørslett and Brettum, 1989). The main reason for high EC values is probably the massive, and in some cases, excessive use of fertilizers in rice fields (Zavattaro *et al.*, 2006) – in particular, the introduction of upland farming in which the rice is cultivated without submersion has increased the nitrogen fertilization rate by 20% (Blengini and Busto, 2009). In some sites where the species is extinct, but also in a few sites which still support *I. malinverniana* populations, waste water is discharged in the channels (author's pers. obs.). These are also undoubtedly sources of nutrients and particles that may have contributed to the increase in EC.

Linked to the low amount of nutrient is the CAM metabolism typical of the genus *Isoëtes*. The total inorganic carbon is often low in very soft waters making most aquatic plants carbon-limited. The CAM metabolism ensures a consistent part in the annual carbon gain (Keeley, 1998). Very low pH values can adversely affect the development of *Isoëtes* sporelings, as demonstrated by Ctvrtlikova *et al.* (2009) and Szmeja *et al.* (1997) for *I. echinospora* and *I. lacustris*. The significant correlations between EC and pH and other water and soil chemical characteristics confirm the ecological importance of the variables included in the selected model.

The addition of water clarity to the model implies a decrease in AICcw, but the value of the evidence ratio confirms that the second model is also informative (Table 2(a)). On the other hand, light availability seems to be one of the most important factors for *Isoëtes* survival, as seen by Free *et al.* (2000) in Ireland. This feature is often linked to the degree of eutrophication, because the increase in nutrient levels can enhance the growth of epiphytic algae on sporophylls (Sand-Jensen and Borum, 1984). In some populations of *I. malinverniana* studied, plants were heavily covered by a layer of epiphytic algae.

Applicability and recommendations

The selected model is based on differences in water conditions between type-A and type-B sites, and is designed to help practitioners in the preliminary exclusion of sites that are unsuitable for the reintroduction of *I. malinverniana*. This might not be sufficient to detect entirely suitable sites but can be used for a preliminary evaluation of a large set of potential reintroduction sites because it is cost-effective and its variables are easily measurable. However, after the first screening, further analysis should be carried out on those sites initially regarded as 'suitable' (results close to 1 in the logistic regression), analysing the chemical features of water and soil and canal morphology. It should be recognized that the variables included in the model might show temporal variations, so it is essential for monitoring to be repeated over several months. However, this study has shown that 4 months may be sufficient to obtain reliable models, making the method still advantageous in cost and time, compared with monthly measurements of a wide range of chemical parameters at several sites over a year or more. The use of models based on less than 4 months average values is discouraged, even if such consideration might depend on site, species, and local conditions.

The selected model may be useful for other species, but it is less accurate when the difference between suitable and unsuitable sites is very low and its application to other taxa may need calibration. The technique applied here to *I. malinverniana* can be easily extended to species with similar ecological features such as other *Isoëtes* and isoetid species (e.g. *Eriocaulon* spp., *Stylites* spp., *Littorella uniflora* (L.) Aschers, *Lobelia dortmanna* L., *Subularia aquatica* L.), which are becoming increasingly threatened owing to water quality deterioration. The construction of models with larger datasets might make the method even more widely applicable. Finally, it is recommended that the selection of suitable sites should be only the first step in the more complicated process of reintroduction. Other considerations such as the genetic diversity of the source populations or the effective removal of the threats affecting the target species should be made (IUCN, 1998; Godefroid *et al.*, 2011). In the specific case of *I. malinverniana*, habitat restoration attempts are inconceivable in the sites from which it has become extinct because of human impact such as intensive agricultural or urban disturbance or the mechanical removal of plants. Moreover, one current population shows values of pH and EC near the upper thresholds at which the species has been observed to persist. Probably, the best candidate reintroduction sites seem to be a few streams derived from superficial springs located within the Ticino River Natural Park. Here, a new *I. malinverniana*population has recently been discovered (Paolo Cauzzi and Emanuele Vegini, University of Pavia). The model will be particularly useful, in this case to test the suitability of other places where the species might be reintroduced.

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