Macrophytes in Aquatic Ecosystems: From Biology to Management

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Macrophytes in Aquatic Ecosystems: From Biology to Management

Proceedings of the 11th International Symposium on Aquatic Weeds, European Weed Research Society

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TABLE OF CONTENTS

BIOLOGY AND SPECIES ECOLOGY

Genetics and biological traits

Physiology and production

Ecology and ecotoxicology

COMMUNITY AND BIOINDICATION

Relationships between plants and other organisms

Plant community ecology

Bioindication

SOLVING PROBLEMS?

Management

Control

Invasion

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Preface: Organising knowledge on aquatic plants: the European Weed Research Society and scientific networks

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Since the publication of the last EWRS symposium on aquatic plants in Lisbon (Caffrey et al., 1999), scientific knowledge on the topic has increased substantially, for example, global change and plant invasions and the problems they cause are increasing. Some events have occurred that increased the research effort on macrophytes, such as the European Directive on Water Quality (OJEU, 2000), and some standards have been produced dealing with aquatic plants: examples include the European standard on river plant studies (CEN, 2003) and the French standard presented in the present issue (AFNOR, 2003) to enhance the application of the Directive.

Problems caused by the proliferation of native and exotic aquatic plants in aquatic ecosystems in France have been occurring for several decades. Standing water bodies have been worst affected. Management by cutting goes back at least to the 1920s, primarily in ponds used for fish production. Over the last three decades aquatic plant management problems have increased sharply, especially in waters used for leisure activities. This has resulted for several reasons:

- quantitative and qualitative changes in the water bodies (e.g., changes in availability of resources, eutrophication and various amenities)
- changing human uses of aquatic ecosystems (particularly the development of multi-use management)
- increased sensitivity of users to plant-associated problems in water-bodies.

These changes have resulted in an increasing reluctance to accept the natural constraints on use of a water-body typically generated by the natural functioning of the aquatic ecosystem (such as the presence of a large standing crop of aquatic vegetation): users expect a certain quality related to the pursuit of their water-based leisure activities, particularly if they are paying. This drives the managers of water-bodies to set up regular management activities in an attempt to meet users' expectations. As a result, active aquatic plant management programmes are increasingly common in French lakes and other waters used for leisure activities.

In Europe, the first efforts aimed at coordination in this field, by specialist meetings, occurred in the1960s, with the setting up of the "European Weed Research Council", which subsequently became the "European Weed Research Society". Since then this association has organized a series of symposia dedicated to aquatic plant management and to the biology and ecology of aquatic plants. EWRS symposia on aquatic weeds take place every three or four years in different European countries. The first, in 1964, was in France, in La Rochelle, then Germany in 1967, Britain in 1971, Austria in 1974, the Netherlands in 1978, Yugoslavia in 1982, again in Britain in 1987, Sweden in 1991, Ireland in 1994 and Portugal in 1998. Proceedings of all symposia have been published. Selected papers from the last three symposia have been published in special issues of the international journal "Hydrobiologia".

Several members of the Aquatic Plants Group of EWRS also contributed to a major review of aquatic plant biology and management (Pieterse & Murphy, 1990), which was distributed worldwide to scientists and technicians involved with aquatic plant management. At the symposium in Lisbon (September 1998), a decision was taken to organize the current symposium in France.

In France, the EWRS collaborated with COLUMA, an association involved in techniques of weed control in agriculture. Within this forum, an "aquatic plants" group was established in the early 1970s, which wrote and published in 1973 the first French document dedicated to the control of aquatic plants. This document included keys to the identification of most aquatic plants, drawings, information on the biology and ecology of the plants and a synthesis on aquatic plant control techniques. A new edition of this document was produced in 1988 (COLUMA, 1988) but it is now sold out.

Subsequently, some 15 French scientists and researchers on aquatic plants created a Group of Scientific Interest "Inland Water Macrophytes" in order to coordinate research into the biology, ecology and management of aquatic plants in France. During the past decade this group has undertaken a series of joint studies on aquatic plant communities in rivers, produced a bibliographic review of those aquatic plants prone to causing weed problems in France, on macrophytes as bio-indicators, and has put forward a Macrophyte Index for use in French rivers.

Over the past three decades in France, only two major symposia dedicated to aquatic plants have been held (although relevant scientific communications have been presented at other meetings). The first was in Alsace in 1994, on the biology and the ecology of aquatic plants (Trémolières & Muller, 1995) and the second was a seminar organized in 1996 by the French Ministry of the Environment on introduced species (animals and plants) in continental aquatic water bodies (Bergot & Vigneux, 1997).

The 11th International Symposium on Aquatic Weeds, held in Moliets et Maâ (Département des Landes) in 2002 was attended by 160 delegates from more than 25 countries (mainly from Europe, but also Mexico, Brazil, New Zealand, Egypt, Iran, India and the United States). The meeting was a resounding success with 110 contributions, 37 of which have been reviewed and edited for publication in this present volume.

The wide range of study, scale and aims of these papers led us to name this special issue : "Macrophytes in aquatic ecosystems : from biology to management", following the old paradigm : "knowledge to achieve management and management to achieve knowledge".

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BIOLOGY AND SPECIES ECOLOGY

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Invasive river plants from Portuguese floodplains: What can species attributes tell us?

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Key words: species traits, invasion potential, alien species, invasiveness, freshwater systems

Abstract

Biological traits of aquatic and riparian plants were compared among invasive and non-invasive groups of species from both native and exotic *taxa* in Portuguese fluvial systems. Twenty-six riparian and aquatic species were selected and their frequency of occurrence and foliar percentage cover was analysed at 401 sampling sites on the main catchments of Central and Southern Portugal. Canonical correspondence analysis and a hierarchical clustering were applied to the species traits data set. No typical invader profile emerged from the analyses. However, some specific attributes were clearly related to the alien and native plant invasiveness, such as vegetative reproduction, fragment viability and life span. The results of the present work could help with the early detection of invasive plant species in Portuguese fluvial corridors.

Introduction

Many research studies on the management of alien plant invasions in freshwater ecosystems have been traditionally directed towards distribution and control, rather than to the prediction of invasions (Wade, 1997). However, in recent years research efforts have been focused on assessing the risk of alien plant species becoming invasive and on developing ecological models that will predict plant invasiveness. The relation between traits of organisms and ecosystem features is a central theme in plant ecology (Greulich & Bornette, 1999), and simple biological characters could usefully explain the invasion process (Rejmánek & Richardson, 1996; Rejmánek, 2003). Indeed, risk assessment for aquatic (Willby et al., 2000) and riparian plants (Pollock et al., 1998) has been focused on the biological traits, including physiological, genetic and demographic features, dispersal strategies (Kean & Barlow, 2000) and life-history traits, related to competition and reproduction (Greulich & Bornette, 1999), of successful invaders (Ashton & Mitchell, 1990). Because individual features alone are insufficient (Mack, 1996), invasive species performance in other regions or habitats were compared (Williamson & Fitter, 1996; Müller & Okuda, 1998) in addition to the climatic profiles in their native ranges (Lonsdale, 1999).

An analysis of the alien invaders' attributes has not yet been performed in Portuguese Mediterranean-type rivers (Ferreira & Moreira, 1995). Thus, as a first approach, the present study aims to explore whether the biological traits commonly used in the literature could be helpful in explaining the invasion patterns in Mediterranean-type rivers, in order to prevent future invasions and to control invading species at the earliest stage in the invasion process.

Material and methods

Species selection and sampling analysis

The principal aquatic and riparian invasive species from Portuguese river corridors were selected, based on previous studies (Aguiar et al., 1996; Ferreira et al., 1998, Ferreira & Moreira, 2000; Aguiar et al., 2001), and compared with non-invasive species from the same habitats.

Floristic data from the fieldwork performed at 401 sites on river corridors in Central and South Portugal during the late spring/early summer of 1994–2002 was used in the analysis. The basins surveyed present a typical Mediterranean climate with an important intra and inter-annual hydrological variability. Each sampling site comprised a 100 m-long stretch, and lateral limits for the sampling area were the approximate maximum annual wetted width of the river corridor. An analysis of the frequency of occurrence and of the percentage cover (six cover classes) of the selected species, together with the revision of Moreira et al. (2002) and an expert judgement, permitted the definition of the invasive/non-invasive species status). For the purposed of this study an invasive species is considered as a species with superficial cover higher than 50% in at least four (1%) of the total sampling sites.

Biological traits

Twenty-six species traits were analysed for each of the selected species, according to Willby et al. (2000) for European river plants, and complemented with the information given on The European Floras (Tutin et al., 1964–1993), Iberian floras [Valdés et al., (1987), Franco (1971; 1984), Franco & Rocha-Afonso (1994; 1998; 2003)], and with Vasconcellos (1970), Bornette et al. (1994), Pautou & Arens (1994), Townsend & Hildrew (1994), Haury & Pattée, (1997), Peltre et al. (1997), Chicouène (2002) (See Electronic Supplementary Material[1]).

Data treatment

A data set with the species traits and respective categories was used in the multivariate analysis. This data was classified using NTSYS-pc 2.0 (Rohlf, 1993), with an asymmetrical similarity coefficient (Jaccard, 1908) to define species groups with similar attributes. A Canonical Analysis (CA) was carried out (ter Braak & Smilauer, 1998) in order to select the most important species attributes that explain the invasion potential of the species.

Results

Table 1 presents the frequency of occurrence at all sites for each of the selected aquatic and riparian species. It also shows the proportion of sites with species whose superficial cover is >50% and >25% in the surveyed area. The invasive status was also indicated in Table 1 for the two approaches (the fieldwork and the bibliographic revision). All of the nine exotic *taxa* were considered invasive by one of these approaches. These were the knotgrass (*Paspalum paspalodes* (Michx.) Scribn.), the giant reed (*Arundo donax* L.), the pacific mosquitofern *Azolla filiculloides* Lam., the parrotfeather (*Myriophyllum aquaticum* (Vell.) Verdc.), the water hyacinth (*Eichhornia crassipes* (Mart.) Solms-Laub.), *Cyperus eragrostis* L., *Aster squamatus* (Sprengel) Hieron., *Bidens frondosa* L. and *Xanthium strumarium* L.

The dendrogram of the hierarchical clustering of the studied species is presented in Figure 1. Four groups of species were obtained at a cut-off point of 0.31 (Jaccard's similarity coefficient). Group 1 is composed mainly by graminoid-type plants, and includes important alien invasive plants of riparian corridors, such as the giant reed and the water hyacinth (Aguiar et al. 1996). Group 2 includes widely distributed aquatic and riparian plants, and mainly non-invasive native species, whereas group 3 comprises strictly aquatic plants (e.g. hydrophytes) with a strong invasion potential. These include the exotic parrotfeather, and the natives: coontail (*Ceratophyllum demersum* L.), sago pondweed (*Potamogeton pectinatus* L.) and Eurasian watermilfoil (*Myriophyllum spicatum* L.). Group 4 includes two invasive hydrophytes – the alien pacific mosquito fern, and the native duckweed (*Lemna gibba* L.).

The first axis of the CA (Fig. 2a; eigenvalues for Axes I and II = 0.20; 0.13) accounts for some of the differences between the aquatic and the riparian species. This differentiates, in the negative part of the axis, between the majority of the native

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[1] Electronic Supplementary Material is available for this article at http://www.dx.doi.org/10.1007/s10750-006-0155-7

Table 1. Frequency of occurrence (%) of the selected aquatic and riparian species at all sites and percentage of sites where a given species presents cover >50% and >25% in the surveyed area

Acronym	Species	Frequency of occurrence (%)	Sites with species cover (%)		Invasive status attributed		Concordance between the two previous columns
			> 50%	> 25%	Field-work[a]	Bibliography	
Pasppa	*Paspalum paspalodes*	69.6	15.5	40.6	ai	ai	X
Arundo	*Arundo donax*	26.4	6.7	11.9	ai	ai	X
Oenacr	*Oenanthe crocata*	58.9	3.5	13.2	ni	nni	
Ranupe	*Ranunculus peltatus*	22.9	3.0	7.5	ni	nni	
Apiuno	*Apium nodiflorum*	53.4	2.5	14.9	ni	ni	X
Azolfi	*Azolla filiculoides*	5.7	2.0	2.7	ai	ai	X
Panire	*Panicum repens*	9.2	1.5	7.0	ni	ni	X
Phraau	*Phragmites australis*	12.5	1.5	4.7	ni	ni	X
Lotuul	*Lotus uliginosus*	11.0	1.2	2.5	ni	nni	
Mentsu	*Mentha suaveolens*	71.8	0.7	6.2	nni	nni	X
Myriaq	*Myriophyllum aquaticum*	5.5	0.7	3.7	ani	ai	
Myrisp	*Myriophyllum spicatum*	11.0	0.7	1.5	nni	ni	
Callst	*Callitriche stagnalis*	14.0	0.5	1.7	nni	nni	X
Cypeer	*Cyperus eragrostis*	33.7	0.5	4.2	ani	ai	
Eichcr	*Eichhornia crassipes*	2.2	0.5	1.5	ani	ai	
Baldra	*Baldellia ranunculoides*	10.0	0.2	1.7	nni	nni	X
Carepe	*Carex pendula*	12.2	0.2	3.5	nni	nni	X
Cerade	*Ceratophyllum demersum*	11.2	0.2	3.2	nni	ni	
Xantst	*Xanthium strumarium*	17.2	0.2	2.0	ani	ai[b]	
Alisla	*Alisma lanceolatum*	21.4	0	2.2	nni	ni	
Astesq	*Aster squamatus*	13.7	0	0	ani	ai	
Bidefr	*Bidens frondosa*	30.4	0	1.2	ani	ai	
Glycfl	*Glyceria fluitans*	6.0	0	1.2	nni	nni	X
Lemngi	*Lemna gibba*	9.7	0	1.5	nni	ni	
Potape	*Potamogeton pectinatus*	1.7	0	0.5	nni	ni	
Scroau	*Scrophularia auriculata*	23.4	0	1.5	nni	nni	X

The invasive status attributed by fieldwork and by the revision of Moreira et al. (2002) is noted with 'ai' for alien invasive species, 'ni' for native invasive species, 'nni' for native non-invasive species and ani for alien non-invasive species.

[a] Invasive species - species with percentage cover > 50% in at least 4 sites.

[b] The alien status of this species is controversial in the bibliography consulted.

and exotic invasive hydrophytes (groups 3 and 4) and the riparian species of group 2 on the positive side of the axis. However, invasive species of group 1 occur on both sides of the first axis, and no clear trends were detected for the second axis.

Figure 2b shows the distribution patterns of the biological traits. The first axis clearly opposes the vegetative propagation strategies and related attributes on the negative part (e.g., strong viability of fragments; strong phenoplasticity, etc.) to the sexual reproduction and related attributes (e.g. annual or biannual species, weak phenoplasticity, small seeds, etc.) on the positive part. The second axis accounts mainly for extreme differences in morphological features (e.g., plant size, growth form, etc.).

Discussion

Some divergence was found between the two approaches used to establish invasive status. Three

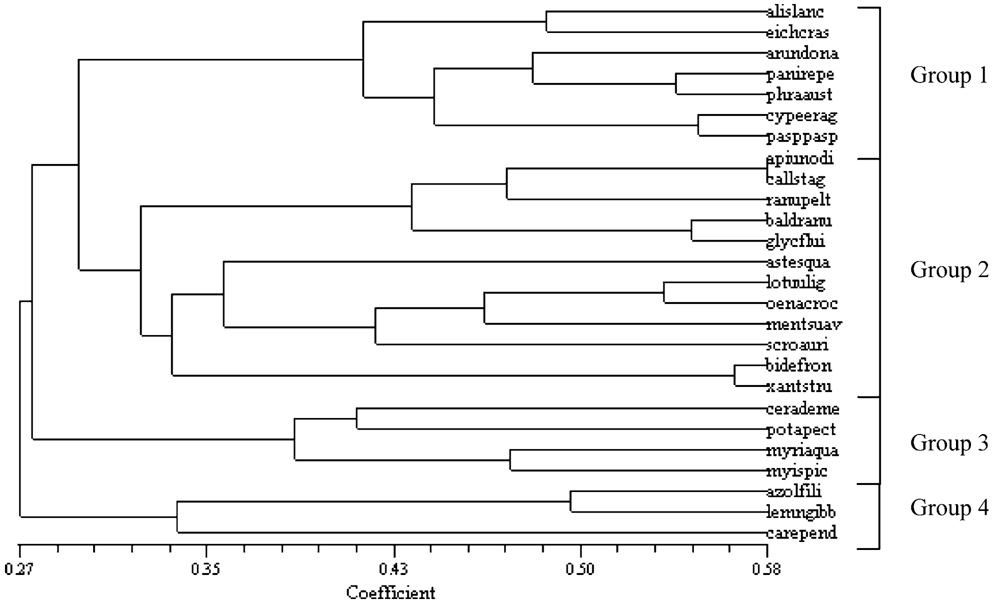

Figure 1. Hierarchical grouping of species using the Jacard similarity coefficient on the species traits data. A 31% of similarity level was taken as the cut-off point. Acronyms are given in Table 1.

explanations are offered: (i) the spatial scale (for instance, the water-hyacinth has a thermally-constrained distribution in Portugal whereas the giant reed is a widespread invasive species); (ii) the type of habitats surveyed (for example, canals and ponds create favourable habitats for the excessive growth of certain native and exotic hydrophytes (Moreira et al., 1999), such as parrotfeather or duckweed, and were not included in this study), and (iii) the time factor (some *taxa* such as *Bidens frondosa* and *Aster squamatus* could be at the earlier stages of the invasion process).

The invasive species differed greatly in their biological traits. No typical invader profile could be delineated for Portuguese fluvial corridors, as was emphasized by Roy for the generality of invader plants (1990). However, the majority of the invasive plants are located at the periphery of the CA biplot (e.g. pacific mosquitofern, giant reed, parrotfeather), whereas the non-invasive *taxa* (e.g. *Carex pendula, Callitriche stagnalis, Baldellia ranunculodes, Mentha suaveolens*) occupy a more central position (Fig. 2a). This means that a higher distinction of species traits exists for the invasive species groups. Nevertheless, some alien species, like *Bidens frondosa* and *Aster squamatus*, were positioned at the positive extreme of the first CA axis, and are not clearly invasive species in Portugal. These, however, have been identified as invasive in Northern Europe (Pysek et al., 1995; Gruberová et al., 2001; Grapow et al., 2001). This could be related to the particular geomorphological and climatic conditions of our fluvial ecosystems or to the fact that these species could be in a lag phase *sensu* Wade (1997). Although the 'ideal invader' was not defined, the most important biological attributes of the invasion process were identified, and led to the prime ecological strategies *sensu* Grime et al. (1990) i.e. reproduction, growth and resource acquisition.

The biological traits analysis should be complemented with ecological traits, and should also comprise the relationships between species assemblages and environmental conditions (Townsend & Hilldrew, 1994; Bernez et al., 2004) and the spatial and temporal vegetation changes (Starfinger, 1998). The results obtained should also be compared to similar studies in other Iberian or Mediterranean climate regions, and enlarged to other species and biological traits, thus contributing to the construction of a predictive model of species risk assessment.

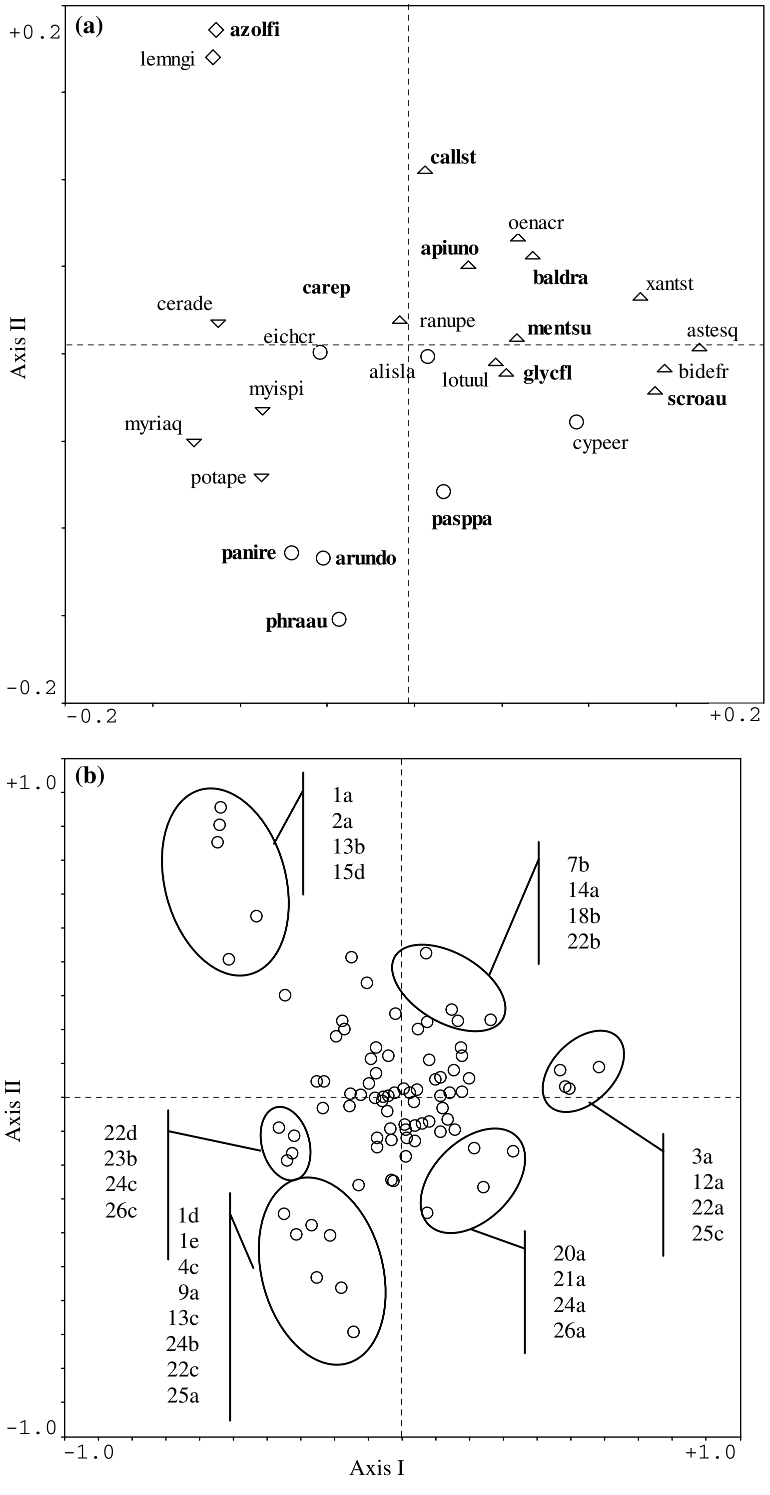

Figure 2. (a). Canonical correspondence analysis biplot for the species. Symbols indicate the species groups obtained on the classification analysis: ○ – group 1, Δ – group 2, ∇ – group 3, ◇ – group 4. Species acronyms in bold represent the concordance between the fieldwork data and the bibliographic revision. (b). Canonical correspondence analysis biplot for the species traits. Acronyms for species and for species traits, are given in Table 1 and in Appendix, respectively.

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Relationships between macrophytic vegetation and physical features of river habitats: the need for a morphological approach

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Abstract

The aim of this paper is to study the relationships between the physical features of rivers and the distribution of macrophyte vegetation. Field work was undertaken at 207 stations along the Scorff River and its tributaries, a salmon river system in southern Brittany (western France). The physical features were considered using a principal component analysis (PCA). Stepwise multiple regression models made it possible to assess their relationships with the botanical data. The first five axes of the physical PCA (used as explicative variables) were initially linked to the most frequently surveyed species, then to their eco-morphological types, and, finally to Arber's (1920. Water Plants. A Study of Aquatic Angiosperms. Cambridge Univ. Press, Cambridge, 414 pp) morphological classification. It was concluded that plant morphology was closely related to these environmental factors. This could contribute to the development of predictive models for plant distribution and could increase the knowledge of reference vegetation related to bioindication systems.

Introduction

The sensitivity of aquatic macrophytes to chemical pollution in rivers has been amply demonstrated by many authors (Kolher, 1971; Haslam, 1987; Trémolières et al., 1994; Daniel & Haury, 1995; Ali et al., 1999). However, the properties of a good indicator of water quality must involve not only its sensitivity to pollution, but its selectivity as well (Murtaugh, 1996). This second aspect has received much less attention in bio-indicator surveys based on aquatic macrophytic vegetation (Kelly & Whitton, 1998). Studies on the subject generally aim at either establishing degradation sequences (Carbiener et al., 1990), or elaborating a synthetic index, based on specific scores (Haury et al., 1996; Holmes et al., 1999). Both approaches need to establish reference situations for plant communities, or for the distribution of species. It is neither obvious nor simple to define references situations in running waters. The degradation sequences usually proposed follow the longitudinal gradient of the river. However, it is not possible for such vegetation references to be the same in the upstream section and in the downstream section of the river. It is also difficult to integrate smaller scale variations in physical features: for example, pool – riffle sequences, or local variations in incident light.

Butcher (1933) had already observed that the chief factor governing the distribution and abundance of aquatic macrophytes in English streams was water current. Others factors that he recognised as important included bottom substrate and light availability. In a survey of 17 Florida streams (with low current velocity and relatively homogeneous substrate), Canfield & Hoyer (1988) observed that nutrients were not related to the abundance of aquatic macrophytes. Instead, shading by riparian vegetation seemed to be the dominant factor. These physical factors are even capable of hiding the effects of heavy point pollution on aquatic plant communities (Daniel & Haury, 1996b; Demars & Harper, 1998; Bernez et al., 2001). Thus, a reliable bioindication system for water quality requires characterisation of the relationships between the physical features of the river, and its macrophytic vegetation.

We hypothesised that this relationship was mainly determined by the morphological traits of the aquatic macrophytes. The aim of this paper is to test the relationships between the physical features of running waters and macrophytic vegetation and to assess the relevance of considering the morphological characteristics of the aquatic plants for a better understanding of these relationships.

Materials and methods

The study was based on field work along the Scorff River and its tributaries, a salmon river system in southern Brittany (France). Schist and granite are the predominant geological substrata in the river basin (Daniel & Haury, 1996a). In total 207 stations (each a 50 m stretch) were sampled in order to represent the variability of the physical features of the river, with a stratified sampling plan based on stream order (from 1 to 5), light and water current. Vegetation relevés (with an estimation of cover) were recorded from within each station in July 1994. Aquatic macrophytes studied included all the plants growing in the usually submerged part of the channel (Holmes & Whitton, 1977): macroalgae, bryophytes (taxonomy according to Smith (1978) for the mosses and Smith (1990) for the liverworts) and spermatophyta (taxonomy according to Tutin et al. (1968–1993)). Physical features were recorded on the same date for each station, and included depth, width, size of substrate (visual cover estimation), water velocity, water surface appearance (laminar, riffle or turbulent) and incident light (using the canopy cover estimation).

Taxa were classified according to the ecomorphological types proposed by Den Hartog and Van Der Velde (1988), with the addition of two other classes: helophytes (Raunkiaer, 1905) and bryides (Mäkirinta, 1978). The morphological classification proposed by Arber (1920) was adapted to the flora studied (Table 1) and used in the analysis as well.

A principal component analysis (PCA) was made to assess the general structure of the physical variables of the habitat – software: SPAD (CISIA, 1999). Stepwise multiple regression models – software: SYSTAT (Wilkinson, 1997) – were then established in order to quantify the relationships between the physical features and the aquatic macrophyte taxa, the ecomorphological classification and the Arber morphological classification, successively. For these analyses, the first five factorial axes of the previous PCA were used as predictive variables. The factorial axes were integrated into the models one at a time and selected according to the Fisher statistic (probability limit of acceptance: 0.10). The coefficients of regression made it possible to compare the models. The major advantage of this approach was the statistical independence of the explicative variables (the factorial axes of the PCA).

Results

Physical features of the habitat analysis

The first five axes of the PCA (with eigenvalues greater than 1) together accounted for 75% of the total inertia of the data set (Fig. 1). The first axis (26% inertia) corresponded to the water velocity

Table 1. Biological classification adapted from Arber (1920)

Without roots	A
Live unattached in the water	*Lemna* sp.
Rooted in the soil and all leaves submerged	B
Rooted in the soil and floating leaves differentiated	C
Rooted in the soil and sometimes with aerial leaves	D

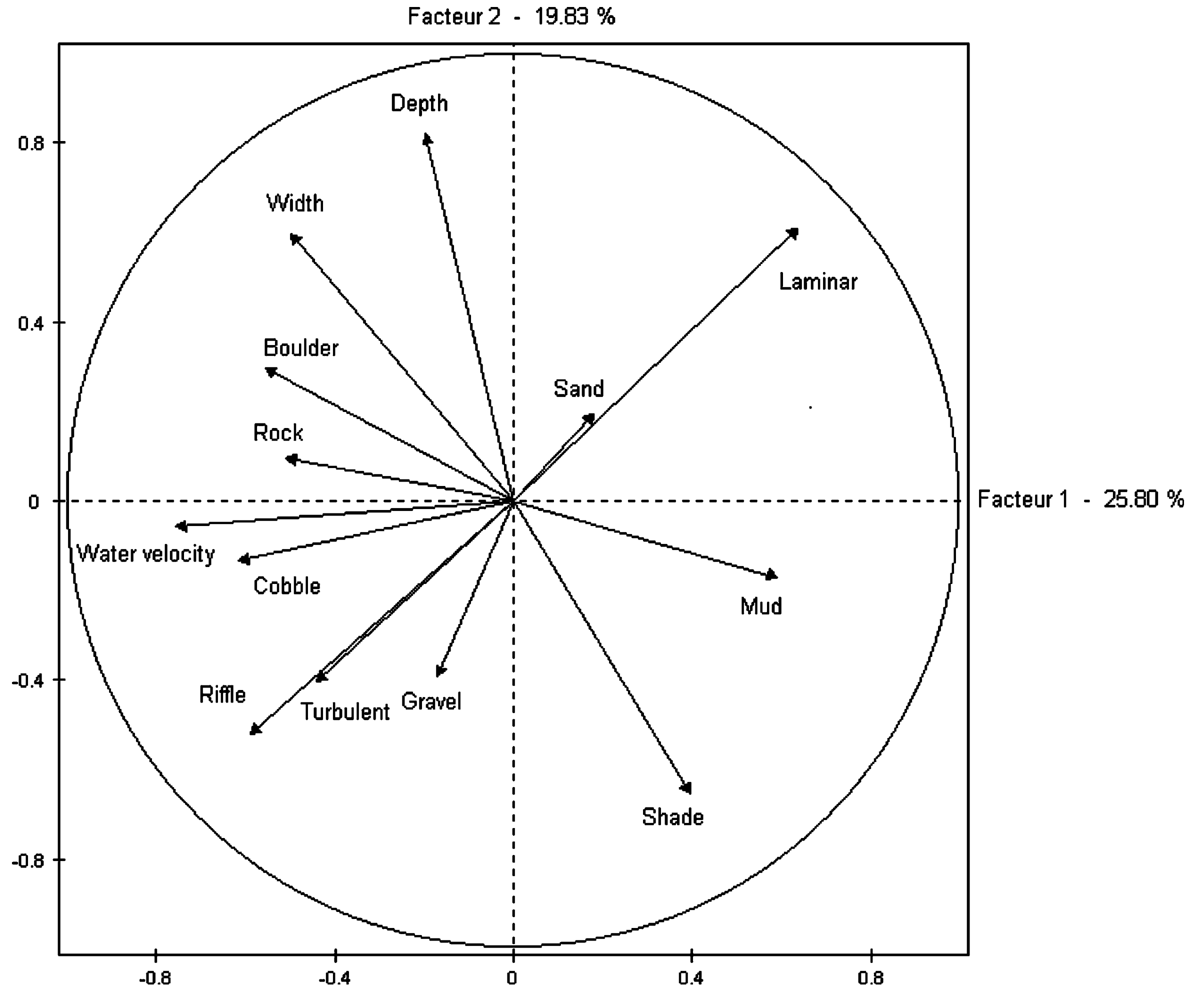

Figure 1. Representation of the first factorial plan of the principal component analysis involving the physical features of the habitat as active variables.

(factorial coordinate: −0.75) and the laminar appearance of the water (+0.63). The second axis was mainly explained by several variables (depth – factorial coordinate: + 0.82, incident light – + 0.65 and width – + 0.60) corresponding to the longitudinal gradient of the river. The predominant bank materials were moderately correlated to these first two axes, whereas the specific size substrates appeared on the next factors. Sandy substrate stations contrasted with boulder and cobble substrate stations with the 3rd and 4th axes, respectively. The 5th axis was mainly explained by a differentiation of the mud substrate stations.

Regression models for the aquatic macrophytes taxa

Stepwise multiple regression models using factorial axes of the PCA were performed for the 40 taxa recorded with a frequency greater than 5%. Several models appeared significant (Table 2). The plants growing in swiftly flowing water had generally the best-fitted statistical models, for example: *Fontinalis antipyretica, Rhynchostegium riparioides, Callitriche hamulata.* The first axis corresponding to water flow was used in almost all the cases, except for riparian plants (such as *Iris pseudacorus*) and those growing in deep stations (*Nuphar lutea*).

Table 2. Stepwise multiple regression models using factorial axes of the PCA for the 40 taxa

Taxa	*R*	*F*1	*F*2	*F*3	*F*4	*F*5
Lemanea sp.	0.58**	−0.94**		−0.76**		
Fontinalis antipyretica	0.57**	−1.37**	0.67**			
Rhynchostegium riparioides	0.57**	−1.84**	−0.43*	−1.13**		
Vaucheria sp.	0.56**	−1.28**	0.75**			0.78*
Callitriche hamulata	0.56**	−1.98**	1.11**		1.15**	
Phalaris arundinacea	0.55**	−0.33**	1.6**			0.53*
Fissidens gr. *pusillus*	0.54**	−0.45**		−0.6**	−0.66**	
Sparganium emersum	0.54**		1.03**			
Chiloscyphus polyanthos s.l.	0.52**	−1.1**	−0.32*	−0.83**	−0.62**	
Octodiceras fontanum	0.51**	−0.48**	0.63**		0.55**	1**
Ranunculus penicillatus	0.49**	−4.5**	2.98**	2.1**	2.06*	
Other macro-algae	0.43**	−1.04**	1.87**			
Callitriche obtusangula	0.43**	−0.67**	0.98**			
Potamogeton alpinus	0.43**	−0.91**	0.64**		0.51*	
Amblystegium fluviatile	0.42**	−0.31**		−0.29**	−0.17*	−0.24**
Mentha aquatica	0.4**	0.28**		−0.31**	0.29*	−0.42**
Nuphar lutea	0.39**		0.7**		−0.39**	
Amblystegium riparium	0.38**	−0.93**				
Apium nodiflorum	0.37**	1.5**	−1.48**	1.57**		
Riccardia chamaedryfolia	0.35**	−0.39**	−0.35**	−0.29*	−0.38*	0.5**
Glyceria fluitans	0.31**	1.07**				−1.14**
Galium palustre	0.31**	0.27**		−0.19**		
Myriophyllum alterniflorum	0.3**	−0.37**	0.52**			
Equisetum fluviatile	0.3**	0.03**			−0.05**	−0.09**
Porella pinnata	0.3**	−0.45**				0.43*
Lemna minor	0.28**		0.74**	0.48*		
Scapania undulata	0.27**		−0.48**	−0.95**		
Callitriche stagnalis	0.26**	0.36**				
Polygonum hydropiper	0.24**		0.04	0.05**	0.06*	
Callitriche platycarpa	0.23**		0.63**			
Sparganium erectum	0.22**	0.46*			0.76*	−1.12**
Hildenbrandia sp.	0.21**	−0.35**				
Myosotis gr. *Scorpioïdes*	0.21**	0.24**			0.31*	
Oenanthe crocata	0.2**	−0.7*	−0.93**			
Alisma plantago-aquatica	0.19**	−0.02**			0.03*	
Ranunculus peltatus	0.13*		0.43*			
Iris pseudacorus	0.12*				0.14*	
Batrachospermum sp.	/					
Brachythecium rivulare	/					
Apium inundatum	/					

R: regression coefficients; *F*1–*F*5: equation parameters; **: $p < 0.05$; *: $0.05 < p < 0.10$; empty case: $p > 0.10$.

The second factor (correlated mainly with water depth) was usually integrated into the models as well. Its positive values were associated with the downstream hydrophytes and its negative values, either with the rheophilous bryophytes (*Riccardia chamaedrifolia*), or with some helophyte, widely distributed in small brook riffle (*Oenanthe crocata*, *Apium nodiflorum*). The 3rd and 4th axes were integrated into the models in the case of some bryophytes associated with large-size substrata and for plants associated with sand substrata (*Ranunculus penicillatus*, *Lemna minor*, *Apium nodiflorum*). The 5th factor was rarely integrated; it led to a better determination of the abundance of species usually associated with fine substrates (e.g. *Phalaris arundinacea*, *Glyceria fluitans*).

Use of the morphological classifications

Table 3 presents the same calculations for the ecomorphological types. Nearly all the models were significant ($p < 0.05$). The first factor was used in all the cases. A good fit was possible for the bryids with only two factorial axes. However, the abundance of the helophytes could not be significantly predicted by any model.

Finally, the use of the Arber classification (adapted to our data) appeared to be the most suitable for this approach. Significant models ($p < 0.05$) were calculated for all the classes (Table 4). The comparison of the regression coefficients obtained at each step of the study confirmed the relevancy of this simple morphological typology (Fig. 2).

Discussion and conclusion

The principal variation axes of the physical features provided the main predictive variables used in the regression models. These results conform to the recognised influence of the physical river habitat on macrophytic vegetation (Butcher, 1933) and the interest of an morphological approach (Dawson et al., 1999). The results are also in line with the findings of Chambers (1987) for lake macrophyte vegetation. She suggested that the physical environment primarily determines the growth-form composition of aquatic plant communities. Thus, a simple morphological classification appears to be an effective tool for predicting a large part of the distribution of macrophytic vegetation in running waters.

These results confirm the necessity of studying not only the biological traits of the aquatic macrophytes but their links to ecological factors as well (Bornette et al., 1994; Ali et al., 1999; Willby et al., 2000). Such a morphological approach can help to determine the macrophyte carrying capacity of running water stations. Further calibration studies in other streams could lead to the development of a system defining the

Table 3. Stepwise multiple regression models using factorial axes of the PCA for the ecomorphological types

Ecomorphological types	*R*	*F*1	*F*2	*F*3	*F*4	*F*5
Bryides	0.73**	−7.66**		−4.27**		
Peplides	0.52**	−2.45**		2.87**	1.28*	
Batrachiides	0.50**	−4.82**	3.41**	2.2**	2.21*	
Elodeides	0.25**	−0.45**	0.66**			
Nympheides	0.29**	1.11**	0.59*			
Helophytes	/					

R: regression coefficients; *F*1–*F*5: equation parameters; **: $p < 0.05$; *: $0.05 < p < 0.10$; empty case: $p > 0.10$.

Table 4. Stepwise multiple regression models using factorial axes of the PCA for the biological classification adapted from Arber (1920)

Biological classification (Arber)	*R*	*F*1	*F*2	*F*3	*F*4	*F*5
A	0.77**	−10.8**	2.58**	−5.78**		
B	0.56**	−2.43**	1.77**		1.43**	
C	0.60**	−6.15**	6.55**	2.21*	2.47*	
D	0.25**	2.35**			2.42*	

R: regression coefficients; *F*1–*F*5: equation parameters; **: $p < 0.05$; *: $0.05 < p < 0.10$; empty case: $p > 0.10$.

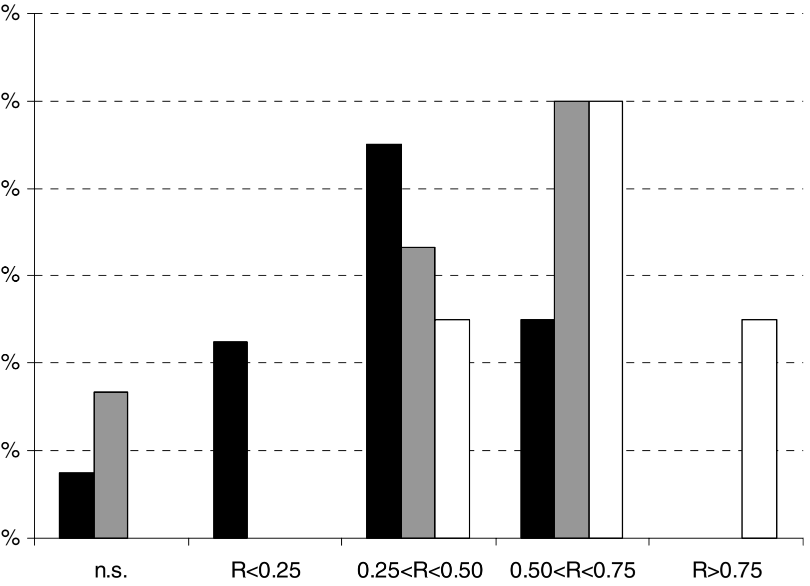

Figure 2. Frequency of the regression coefficient obtained in the stepwise multiple regression models. (Black: with the 40 taxa, grey: with the ecomorphological classification, white: with Arber classification.)

morphological groups of aquatic plants related to the physical features of the stations, which may complement and extend the capabilities of existing bioindication systems.

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Morphological variation in *Eichhornia azurea* (Kunth) and *Eichhornia crassipes* (Mart.) Solms in relation to aquatic vegetation type and the environment in the floodplain of the Rio Paraná, Brazil

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Key words: morphological traits, river, aquatic plants

Abstract

Eichhornia azurea and *E. crassipes* are the most frequent dominants of aquatic vegetation in the floodplain of the Upper Rio Paraná in Brazil. Morphological traits of samples collected at sites where they dominated the vegetation were measured and compared between vegetation types identified in the floodplain. Total leaf weight, specific leaf area and the leaf to root weight ratio of *E. azurea* and total leaf dry weight, total leaf area and total plant dry weight of *E. crassipes* differed significantly between vegetation types from 1999. Pearson correlation coefficients indicated a number of significant relationships between morphological traits and environmental variables. Five *E. azurea* traits increased linearly with water depth and four with water clarity. The leaf to root weight ratio also increased linearly with sediment iron and calcium content. *E. crassipes* total root dry weight and total plant dry weight were respectively quadratically related to sediment calcium and the euphotic proportion of the water column. Total leaf dry weight and total plant dry weight increased linearly with water depth while remainder dry weight decreased linearly with sediment phosphorus content.

Introduction

During vegetation surveys conducted between 1998 and 2001 in the floodplain waterbodies of the Rio Paraná in southern Brazil, it was observed that *Eichhornia azurea* (Kunth) and *E. crassipes* (Mart.) Solms were present at the majority of sites sampled (Murphy et al., 2003). *E. azurea* was the most frequent dominant species recorded, followed by *E. crassipes*. This was despite wide-ranging physical and chemical properties of the waterbodies themselves, and variation in the plant communities, which they supported. Environmental gradients in the floodplain result from variations in topography and the effect of the Rio Paraná and its tributaries, which influence water chemistry and water table level during flood events (Junk et al., 1989; Thomaz et al., 1997). However, the natural hydrological cycle of the floodplain has been increasingly disturbed by the construction of hydroelectric dams upstream (Agostinho & Zalewski, 1996). This has led to alterations in aquatic communities (Agostinho et al., 2000) and biological production and successional processes (Ward & Stanford, 1995; Thomaz et al., 1997). Such natural and human-induced influences on floodplain environments have consequences for growth patterns of aquatic macrophytes (Benassi & Camargo, 2000; Rubim & Camargo, 2001; Thomaz et al., 2002). The importance of the two *Eichhornia* species across the range of habitats found in the Paraná illustrates their plasticity and ability to adapt to different growth conditions (e.g., Gopal, 1987; Camargo & Esteves, 1996). For example, *E. crassipes* is a component of every one of the Paraná floodplain aquatic plant community types discussed by Neiff (1986) while *E. azurea* occurs in all but one. As conditions vary across the floodplain due to both natural processes and anthropogenic impacts, it is interesting to investigate how *E. azurea* and *E. crassipes* respond to different habitat types by analysing their morphological responses to vegetation type and environmental factors at locations where their success is indicated by their predominance in the plant community.

Methods

During springtime between 1999 and 2001, vegetation community composition data, environmental information and measurements of morphological traits of dominant species were collected from a total of 99 randomly selected sites within the waterbodies of the floodplain of the Upper Rio Paraná. Sites included open and closed lagoons, backwaters and flowing channels, located within a radius of approximately 40 km of the field station of the Universidade Estadual de Maringá which is situated on the left bank of the river, close to the town of Porto Rico in Paraná state, Brazil. Lists of species and their abundances were compiled, a set of environmental variables was measured *in situ*, or samples collected for later laboratory analysis, and a randomly selected individual of the dominant species (the species with greatest frequency of occurrence in quadrat samples) was collected. For *E. crassipes* a single colonial individual (with any daughter plants still attached) comprised the sample. *E. azurea* is bank-rooted with floating stems which reach out several metres into the water with roots and emergent leaves at each node. Samples were taken by counting back 15 (1999: average sample length 2.2 m) or 10 leaf-nodes (2000 and 2001: average sample length 1.45 m) from the distal end of the stem and cutting off the specimen at this point. All measurements were subsequently standardised, for analysis of the full 3-year dataset, by dividing values where appropriate by the number of nodes per sample. On returning to the laboratory, total leaf area of the sample of the dominant species was estimated by taking a sub-sample of leaves and measuring their area using a portable scanner attached to a laptop computer and Delta-T-scan software for image analysis. The leaf sub-sample, leaf remainder, root and remaining (stem plus any inflorescence) portions were separated and dried before weighing. The leaf sub-sample area was converted to an estimate of total area by multiplying by a factor calculated as the ratio of the total leaf weight to the weight of the leaf sub-sample. Morphological traits used in the analysis were: total leaf dry weight, total root dry weight, total remainder (all other plant structures combined) dry weight, total plant dry weight, total leaf area, specific leaf area (a measure of leaf thickness) and the ratio of leaf weight to root weight.

Water pH and conductivity and sediment redox potential were measured in the field using portable metres and probes. Light attenuation in the water column was measured using two light sensors, one at the water surface and the second fixed a known vertical distance below, connected to a hand-held metre. The light extinction coefficient was calculated as $k = \log_e I/I_0 \times 1/z$ where I = light intensity recorded by the submerged probe, I_0 = light intensity recorded by the surface probe, and z = distance between the two probes. Water depth was taken as the average of three measurements from the surface of the sediment to the water surface within the vegetation stand being sampled. Sediment samples were collected using a Petersen grab, oven-dried to a constant weight and ground in a mortar and pestle. ADAS total extractable phosphorus, total Kjeldahl nitrogen, total iron and total calcium content were determined. Water samples were frozen as soon as possible after collection and then later analysed for total nitrogen and total phosphorus concentrations.

Species presence-absence data collected in 1999, for which the biggest single-year dataset was available, were analysed using TWINSPAN (Hill, 1979) to classify sites into groups containing similar species assemblages. Using analysis of variance, the morphological traits of *E. azurea* and *E. crassipes* were compared between the different vegetation types in which they occurred as dominant species. Variables were tested for normality using Ryan–Joiner tests and for equivalence of variance in groups, then transformed if necessary. Variables without a normal distribution were compared between TWINSPAN groups using Kruskal–Wallis tests. Differences were considered significant at $p \leq 0.05$. All analyses were conducted using MINITAB 13.30.

After transformation to normalise variables as necessary, Pearson product-moment correlation coefficients were calculated between pairs of trait and environment variables. In a few cases, quadratic or cubic functions were more appropriate for describing relationships. Following linear regression analysis, the resulting models were assessed for compliance with the assumptions of this analysis (residuals normally distributed with uniform variance along the regression line) and rejected where necessary. Relationships were considered significant at $p \leq 0.05$.

Results

TWINSPAN analysis of the 1999 species data revealed 3 major vegetation types occurring within aquatic habitats of the floodplain. The characteristics of these groups are compared and discussed in detail by Murphy et al. (2003).

Group A consisted of 9 sites and was indicated by abundant *Salvinia* sp., moderately abundant *Cyperus diffusis* and the presence of *Limnobium laevigatum*. Group B was indicated by a low abundance of *Cyperus diffusis*, a high abundance of *Salvinia* sp. and the absence of *Limnobium laevigatum*. This was the largest group with 30 sites. Group C was made up of 6 sites and was indicated by the absence of *Salvinia* sp.

In all groups, *Eichhornia azurea* was a strong dominant, present at most stations at high abundance. Group A consisted of sites with water that tended to be deeper and clearer than in the other groups, with low conductivity, alkalinity and phosphate concentration. Sediment Ca, Fe and P were low, but total N was high compared to other sites. This suggests that the group represents habitats with deep water and good light availability but in general, poorer availability of nutrients. *E. azurea* was a dominant component of the vegetation, while high abundances of free-floating species were also characteristic of this group. *E. crassipes* was present at all but one site in this group, in moderate abundance. In the larger group B, sites had very variable water and sediment properties and therefore did not tend to differ significantly from other groups. *E. azurea* was in high abundance, again with frequent floating species, but the emergent *Polygonum* species and *Panicum prionitis* were absent. *E. crassipes* was present at just over half the sites in this group, mainly at low abundance. Group C sites were shallow with generally cloudier water than the other sites. Water and sediments were nutrient-rich. *Nymphaea amazonum* in combination with submerged species such as *Myriophyllum aquaticum* and *Cabomba caroliniana* were commonly present in sites of this group while the abundance of *E. azurea* was conspicuously reduced, although it still tended to be the most frequent species at each site. *E. crassipes* was absent.

The range of trait measurements recorded for *E. azurea* and *E. crassipes* are indicated in Tables 1 and 2, respectively. All variables measured showed wide ranges, representing a large sample of the variation present in the floodplain.

Total leaf weight ($p = 0.021$), leaf thickness (SLA $p < 0.001$) and the ratio of leaf weight to root weight ($p = 0.008$) of *E. azurea* were significantly greater in group B (means = 65.79 g, 48.88 cm^2/g, 1.54, respectively) than group C (means = 23.35 g, 106.9 cm^2/g, 1.25 respectively) (Fig. 1). Leaves were also significantly thicker in group A (mean = 47.33 cm^2/g) than group C. *E. azurea* therefore had greater abundance, greater frequency of dominance and more developed leaves in group B, the most common vegetation type identified, than in group C. In group C, conditions were more favourable for the growth of submerged species than in the other groups in

Table 1. Median, minimum and maximum measurements of *E. azurea* traits per 15 nodes sample in 1999

Trait	Median	Minimum	Maximum
Total leaf dry weight (g)	52.16	6.74	218.20
Total leaf area (cm^2)	3123	228	14514
Specific leaf area (cm^2/g)	66.47	13.24	155.70
Total root dry weight (g)	21.54	2.90	119.70
Leaf weight/root weight	2.14	0.29	33.34
Remainder dry weight (g)	98.05	8.90	350.30
Total plant dry weight (g)	181.2	25.6	538.5
Maximum stem length (m)	2.27	0.94	3.60

Table 2. Median, minimum and maximum measurements of *E. crassipes* traits per individual in 1999

Trait	Median	Minimum	Maximum
Total leaf dry weight (g)	12.37	1.60	40.20
Total leaf area per (cm^2)	1221	99	5506
Specific leaf area (cm^2/g)	98.7	52.2	630.1
Total root dry weight (g)	4.20	0.11	30.50
Leaf weight/root weight	2.07	0.32	90.97
Remainder dry weight (g)	2.50	0	9.00
Total plant dry weight (g)	24.10	1.81	79.70
Maximum stem length (m)	0.420	0.15	1.61

which emergent or floating life-forms dominated. Shallow water, although it tended to be cloudy, allowed sufficient light to penetrate for underwater photosynthesis so that the aerial leaves of *E. azurea* may not have created as much of a survival advantage as in other habitats. When competing with species with similar requirements for water surface area or bank rooting positions, *E. azurea* tended to be extremely successful. However, when it occurred where the environment permitted the growth of species with different growth strategies, it could not maintain the same degree of dominance over the other species. The species clearly has wide environmental tolerances but still showed a reduction in its success when growing near the limits of these tolerances.

Although much less abundant than *E. azurea*, *E. crassipes* is also important due to its widespread presence over most areas of the floodplain. It is also usually the dominant species when *E. azurea* is not. *E. crassipes* plants had significantly greater total leaf dry weight ($p = 0.009$), total leaf area ($p = 0.018$) and total plant dry weight ($p = 0.008$) in group A (means = 25.17 g, 2704 cm^2, 42.64 g, respectively) than in group B (means = 4.49 g, 484 cm^2, 8.64 g, respectively). No other measured traits differed significantly between the two groups. The greater success of *E. crassipes* in group A coincides with lower *E. azurea* abundance compared to group B and so could be due to the greater suitability of the sites for the free-floating rather than the bank-rooted species. The generally lower nutrient status of sediments at group A sites may have prevented *E. azurea* from maximising its growth thus permitting greater development of *E. crassipes*. The deeper water of group A sites may also have favoured the free-floating form of many of the species in this group.

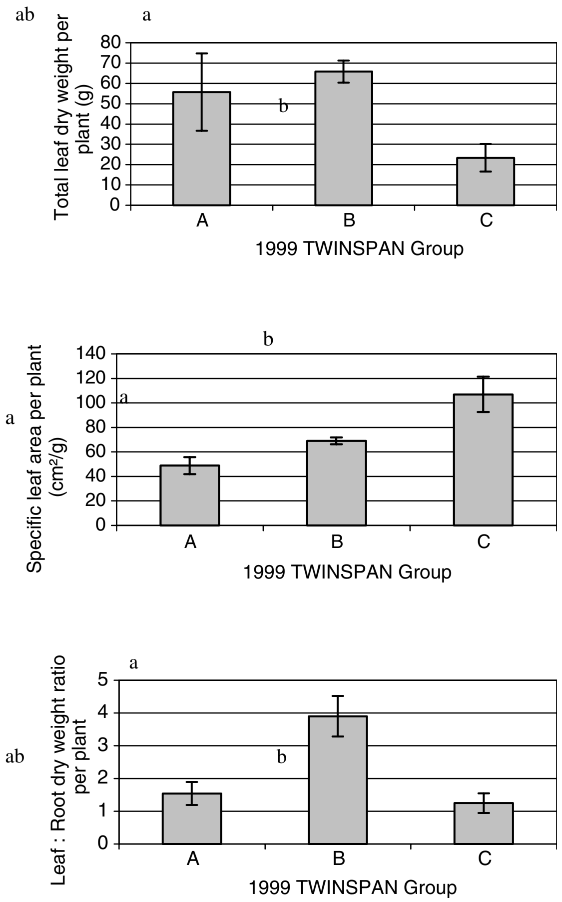

Figure 1. Means and standard errors of three morphological traits of *E. azurea*, which differed significantly between TWINSPAN vegetation communities. Groups without a letter in common were significantly different.

Significant Pearson correlation coefficients for relationships between *E. azurea* trait variables and environmental variables are given in Table 3. The results of the correlation analysis show that $\log_e$ leaf:root weight ratio increased linearly with Fe and $\log_e$ Ca (Table 3). When sediment was rich in Fe or Ca, investment in roots was low in relation to leaves. A possible explanation for this is that high levels of reduced iron (Fe^{2+}) in anoxic sediment can produce toxic conditions for roots and restrict growth (Snowden & Wheeler, 1993).

Variation in root biomass with seasonal variation in a floodplain lake of the Rio Mogi-Guaçu has been shown by Camargo & Esteves (1996).

Table 3. Pearson product-moment correlation coefficients (*r*) and probability (*p*) for linear relationships between *E. azurea* trait variables and environment variables (1999–2001)

E. azurea trait variable	Environmental variable	*r*	*p*
$\log_e$ leaf weight/root weight	Sediment Fe	0.222	0.039
$\log_e$ leaf weight/root weight	$\log_e$ sediment Ca	0.271	0.011
√Total leaf dry weight	$\log_e$ water depth	0.250	0.018
$\log_e$ total leaf area	$\log_e$ water depth	0.320	0.002
maximum stem length	$\log_e$ water depth	0.278	0.008
√Remainder dry weight	$\log_e$ water depth	0.288	0.006
√Total plant dry weight	$\log_e$ water depth	0.293	0.005
$\log_e$ total leaf area	$\log_e k$	−0.225	0.036
Maximum stem length	$\log_e k$	−0.352	0.001
√Remainder dry weight	$\log_e k$	−0.269	0.012
√Total plant dry weight	$\log_e k$	−0.261	0.014

E. azurea root biomass reached its maximum in September when water TKN and TP were lowest, and its minimum in January when TKN and TP were highest. During the low temperatures, water levels and nutrient conditions of September, rhizomes and leaves were at their lightest. The nitrogen and phosphorus contents of rhizomes and leaves reflected the concentrations of these nutrients in the water (Camargo & Esteves, 1996). The results showed that seasonal variation in morphology and tissue nutrient content of *E. azurea* were closely tied to the influence of the annual flood pulse.

In this study, spatial variation in water depth was a very important factor influencing *E. azurea* traits. Total leaf weight, total leaf area, maximum stem length, total remainder weight and total plant weight all increased linearly with water depth (Table 3). Individual *E. azurea* plants were therefore generally larger in deep-water habitats. This could be an effect of reduced competition because plants with submerged life forms may have been excluded from these habitats due to low light availability in deep water, while emergent species may not have been able to tolerate the depth of flooding. Such deep water habitats are suitable for floating species, but *E. azurea* has an advantage over these in being able to use the sediment as well as the water as a source of nutrients.

E. azurea biomass per unit area has also been shown to vary with the annual fluctuations in water level in the floodplain (Bini, 1996). The greatest amount of living material was found during the period of March to June when water levels were at their highest. During July to November, total *E. azurea* phytomass reached a maximum, but a greater proportion was comprised of dead material.

E. azurea growth was also increased at sites with higher water clarity. Total leaf area, maximum stem length, remainder weight and total plant weight all decreased with $\log_e k$ (Table 3). As the species does not photosynthesise underwater, there is not an obvious explanation for this but water clarity depends on phytoplankton concentration and suspended and dissolved substances, which may in turn affect the growth of *E. azurea*.

In *E. crassipes*, $\log_e$ water depth was positively correlated with $\log_e$ total leaf dry weight ($r = 0.665$, $p = 0.007$) and total plant dry weight ($r = 0.520$, $p = 0.047$). Log_e remainder dry weight was negatively correlated with sediment phosphate concentration ($r = -0.554$, $p = 0.032$). In addition to the linear correlations, there were two quadratic relationships:

$$\text{Total root dry weight (g)} = 31.6168 - 0.0423861\,\text{Ca} + 1.48 \times 10^{-5}\,\text{Ca}^2 \tag{1}$$

$r = 0.558$, adjusted $r^2 = 0.478$, $p = 0.011$. (Calcium units were mg/kg).

$$\text{Total plant dry weight (g)} = 33.2920 - 24.8630\,(\log_e \text{Zeu/d}) + 9.27379\,(\log_e \text{Zeu/d})^2 \quad (2)$$

$r = 0.713$, adjusted $r^2 = 0.419$, $p = 0.020$

E. crassipes total root weight initially declined with sediment calcium content, reached a minimum at approximately 1500 mg/kg and then increased with increasing calcium (Equation 1). Total plant weight initially decreased with $\log_e$ Zeu/d and then levelled off at higher values of $\log_e$ Zeu/d before beginning to increase again (Equation 2). Zeu/d represents the proportion of the water column in which there is sufficient light for photosynthesis.

The cubic relationship between total root weight and sediment Ca shows that total root weight was maximised either at low or high levels of Ca and minimised at intermediate levels. This could be explained if proportionally greater investment is made in roots when Ca is in short supply, in order to maximise uptake, than when levels are intermediate. High Ca levels may generally stimulate plant growth resulting in heavier roots again.

Total plant weight initially decreased with $\log_e$ Zeu/d and then slightly increased again. Although *E. crassipes* does not rely on underwater photosynthesis, it might be affected by the influence of water clarity on other species. For example, clearer water would make a habitat more available to species, which do photosynthesise underwater and might provide greater competition in these circumstances. The $\log_e$ Zeu/d was also correlated with sediment Ca, which at intermediate levels was associated with increased root growth.

The strong positive relationship of leaf weight and total plant weight with water depth indicates a general advantage of deeper water to *E. crassipes*. Shallow water limits the volume of water available into which roots can expand. It could also encourage growth of emergent species or submerged-rooted species, which might compete with *E. crassipes*.

The decrease in remainder weight with sediment P may be explained if high levels of P stimulate production of daughter plants resulting in a lower average stem weight per plant.

Conclusion

Comparison of *E. azurea* traits between vegetation communities has shown that this species is likely to be responding to spatial variation in environmental and plant interaction conditions by showing plasticity in its leaf morphology. This was detected as varying total leaf weight, specific leaf area and leaf to root weight ratio. *E. crassipes* also showed variation in leaf morphology (leaf area, leaf biomass and total biomass) providing further evidence for leaf adaptations to variations in conditions experienced by the macrophyte communities occurring within the river floodplain. The vegetation types identified by TWINSPAN are highly likely to reflect underlying physical and environmental pressures determining plant communities. Each vegetation type exists due to a combination of species tolerances for environmental factors and disturbance and competition effects. Correlation and regression analysis of individual pair-wise relationships between dominant plant traits and environmental variables showed weak but highly significant relationships and it is likely that many factors interact to determine the success of the *Eichhornia* species. Both *Eichhornia* species are clearly capable of adapting to natural changes in habitat as well as those, which are likely to be underway due to river regulation. However, water clarity and depth and nutrient cycling are important factors in structuring populations of *E. azurea* and *E. crassipes* and so spatial and temporal changes in their growth can be anticipated. The analysis of *Eichhornia* morphology in relation to growth conditions across sites where it is not necessarily dominant would provide a fuller picture of its environmental tolerances.

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Changes in the allocation of some chemical compounds in structures of *Oryza glumaepatula* (Steud) in an Amazonian lake subjected to an anthropic impact (Lake Batata, Porto Trombetas)

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Key words: Amazon, aquatic macrophyte, chemical composition, nutrients, bauxite tailings, anthropic impact

Abstract

Lake Batata is a typical clear water lake of the Amazon, located at the right margin of the Trombetas River, at the town of Porto Trombetas (PA). It is subjected to a large and predictable variation of the water level, greater than 6 m, being in the floodplain of the Trombetas River. For 10 years (1979–1989) Lake Batata has received the tailings of bauxite processing from the activities of Mineração Rio do Norte S.A. These tailings impacted about 30% of its total area with an effluent of fine granulometry. Nowadays, two different areas can be observed at Lake Batata, one impacted by the bauxite tailings and one where the natural characteristics have been preserved. The aquatic macrophyte *Oryza glumaepatula* (Steud), known as wild rice, is one of the species found in the two areas, impacted and natural. The goal of this research was to evaluate the concentration of organic matter, organic carbon, N, P, lipids, soluble carbohydrates, and cell-wall fraction in three structures of *O. glumaepatula* (leaves and culm, adventitious roots, and basal roots), as well as to identify the possible differences in the allocation of these compounds between individuals in the natural and impacted areas. While the differences between the different structures of the plant are preliminary data for studies on the detritus chain and herbivory, the differences between the natural and impacted areas show the probable impact of the tailings on *O. glumaepatula*.

Introduction

Most aquatic ecosystems in the amazonian region are located in the floodplain of the great rivers found there. In these ecosystems, the community of aquatic macrophytes is one of the most important, being found in igapó and várzea areas and exhibiting adaptations to the marked seasonal water level fluctuation. The life cycle of the aquatic macrophytes in this region is determined by the annual fluctuation of the water level (Junk & Piedade, 1993).

The community of aquatic macrophytes has a major role in the lacustrine environment, since it influences the water chemistry, is one of the basis of the food chain, acts as a nutrient reservoir and provides shelter to several species of animals as well as substrate for the periphyton (Wetzel, 1993). The chemical composition of aquatic macrophytes is a particularly important subject of study, allowing inferences on the herbivory chain and on their role as nutrient reservoirs (Esteves et al., 1984). Besides, Junk & Piedade (1993) underline the role of these plants in the nutrient cycling in amazonian aquatic environments, especially due to their high productivity.

Aquatic macrophytes have differing concentrations of the variables studied in each of the many structures. This is observed because these structures have different functions – while some are essentially supporting organs, rich in lignin and cellulose, such as roots, the leaves and culm have a high concentration of soluble carbohydrates and therefore a small cell-wall fraction. The distribution of these variables in the plant is dependent in the fenological status of the plant, and impacts, anthropic or otherwise, may influence this distribution.

Lake Batata stands apart from the other lakes in the floodplain of the Trombetas River by having received an anthropic impact of great proportions. For 10 years 50.000 m^3/d of effluent of bauxite tailings were dumped in its margins, impacting close to 30% of the total area of the lake. This environment is densely colonized by the aquatic macrophyte *Oryza glumaepatula* (Steud) (wild rice), which is the most conspicuous among the aquatic macrophytes of this ecosystem, occupying about 3% of the total surface of the lake. Rooted to the substrate and adapted to survive in well-lit areas, *O. glumaepatula* may reach a length of 8 m (Rubim, 1995). The primary productivity of *O. glumaepatula* is high, reaching 14.6 g DW/m^2/day in Lake Batata (Enrich-Prast, 1998). This species may be found in both the impacted and the non-impacted area in Lake Batata.

The goal of this research was to evaluate the strategy of allocation of some chemical compounds among differing structures of *O. glumaepatula* and to evaluate whether the bauxite tailings had any influence on this resource allocation. Therefore we determined the concentrations of nitrogen, phosphorus and organic carbon, the percentages of organic matter, lipids and soluble carbohydrates, and the cell-wall fraction, in three structures (leaves and culm; adventitious roots; basal roots) of individuals of *Oryza glumaepatula* found in two areas of Lake Batata: natural and impacted.

Study area

Lake Batata is located on the right margin of the Trombetas River, in its floodplain. Both are considered clear-water ecosystems, according to the classification proposed by Sioli (1950). Lake Batata is connected to the Trombetas River by a main channel and several smaller channels, and in the flood period connection is even less restricted. For 10 years (1979–1989) this ecosystem received the dumping of effluents from the process of bauxite extraction in a nearby quarry, operated by Mineração Rio do Norte (MRN). This material, composed by fine particles (<50 μm), with 6–9% of suspended solids discharge, was dumped directly over the lake margin, landfilling about 30% of its area (Esteves et al., 1990). The margins of lake Batata are colonized by some aquatic macrophytes, and distinctive among them is *O. glumaepatula*. Lake Batata is subjected to a marked fluctuation of the water level throughout the year, with a direct influence on the development of *O. glumaepatula*. This aquatic macrophyte germinates in the low water, when the sediment is exposed. After flooding, its growth follows the rise of the water level. When their culms are submersed the individuals of *O. glumaepatula* develop adventitious roots allowing them to absorb nutrients directly from the water column.

Material and methods

Samples of *O. glumaepatula* were collected in March 1996, when the water level is rising, following the method of squares proposed by Westlake (1963) in two stations at Lake Batata with different characteristics. One station was located in an area with natural characteristics preserved and the other station was located where the impact by bauxite tailings was noticeable. In each station three squares of 0.25 m^2 were collected. The material was transported to the laboratory, washed, and separated according to structure: leaves and culm; basal roots; adventitious roots. These structures were dried at a stove, weighed and ground for the following chemical analyses:

Organic carbon

The concentration of organic carbon was determined by the application of the coefficient of 46.5% to the results of organic matter, according to Westlake (1963). The amount of organic matter was determined by ignition in a muffle furnace at 550 °C, for four hours, with the percentage of organic matter being estimated as the difference in weight before and after ignition.

Nitrogen

The concentration of nitrogen was determined through digestion at high temperatures, followed by the addition of NaOH and distillation with H_2SO_4. This method was proposed by Allen et al. (1974).

Phosphorus

The concentration of phosphorus was obtained by strong digestion, according to the method proposed by Fassbender (1973).

Lipids

The concentration of lipids (ethanol-soluble material) was obtained by gravimetry after extraction, according to the method proposed by Folch et al. (1957).

Soluble carbohydrates

The concentration of soluble carbohydrates was obtained by the phenol-sulfuric acid method described by Dubois et al. (1956).

The percentage of cell-wall fraction was determined after dilution of the protoplasmatic fraction, according to Van Soest & Wine (1967).

All results were subjected to statistical analysis. For the concentrations of organic carbon, nitrogen and phosphorus and for the cell-wall fraction we employed the parametrical test "*t*" of Student ($\alpha = 0.05$). For the comparison of the results of soluble carbohydrates and lipids we employed the non-parametrical Mann–Whitney analysis (*U* test), with $\alpha = 0.05$ or 0.10, varying with the amount of replicates (when one of the compared pairs of data has only two replicates, the smallest α found in standard tables is 0.10).

Results

In the comparison between the concentrations of organic carbon we observed that in the natural area adventitious roots had concentrations similar to those found in leaves and culm (Fig. 1a). On the other hand, these structures as well as basal roots had similar values in the impacted area. The culm and leaves had high concentrations of organic carbon in both areas, and the basal roots had low concentrations, also in both areas. In all structures the concentration found in individuals from the impacted area was lower compared to individuals from the natural area. The Student "*t*" test found significant differences between basal roots and the other structures in the natural area, as well as between leaves and culm and the other structures in the impacted area. Comparing the same structure between the two areas, significant differences were observed in leaves and culm and in adventitious roots.

The concentration of nitrogen found in adventitious roots is higher than the concentration observed in basal roots (Fig. 1b). These two structures have high values of nitrogen concentration compared to leaves and culm. These findings hold for both the natural and impacted areas. The Student "*t*" test found significant differences between all pairs of structures. When we compared the same structure through the two areas we consistently observed higher values in the natural area. The Student "*t*" test found significant differences only between the concentrations of the adventitious roots through the two areas, though.

In the natural area, the values of phosphorus concentration in adventitious roots were higher than in basal roots (Fig. 1c). Leaves and culm had values similar to those of basal roots. In the impacted area the concentrations observed in leaves and culm show a significant marked decrease. The Student "*t*" test showed significant differences between the values observed in the adventitious roots and those of other structures in both areas, and between the values observed in leaves and culm and those of basal roots in the impacted area. The comparison of the same structure between the two areas revealed higher values in the natural area. The Student "*t*" test detected significant differences only in the already mentioned case of leaves and culm, where there was a reduction in the impacted area from the values found in the natural area.

The results of lipid concentration in both kinds of roots were similar in the natural area, while in the impacted area we observed an increase in the values observed in the basal roots, which presented values markedly higher than those observed in adventitious roots (Fig. 1d). Both kinds of roots had smaller values than those observed in leaves

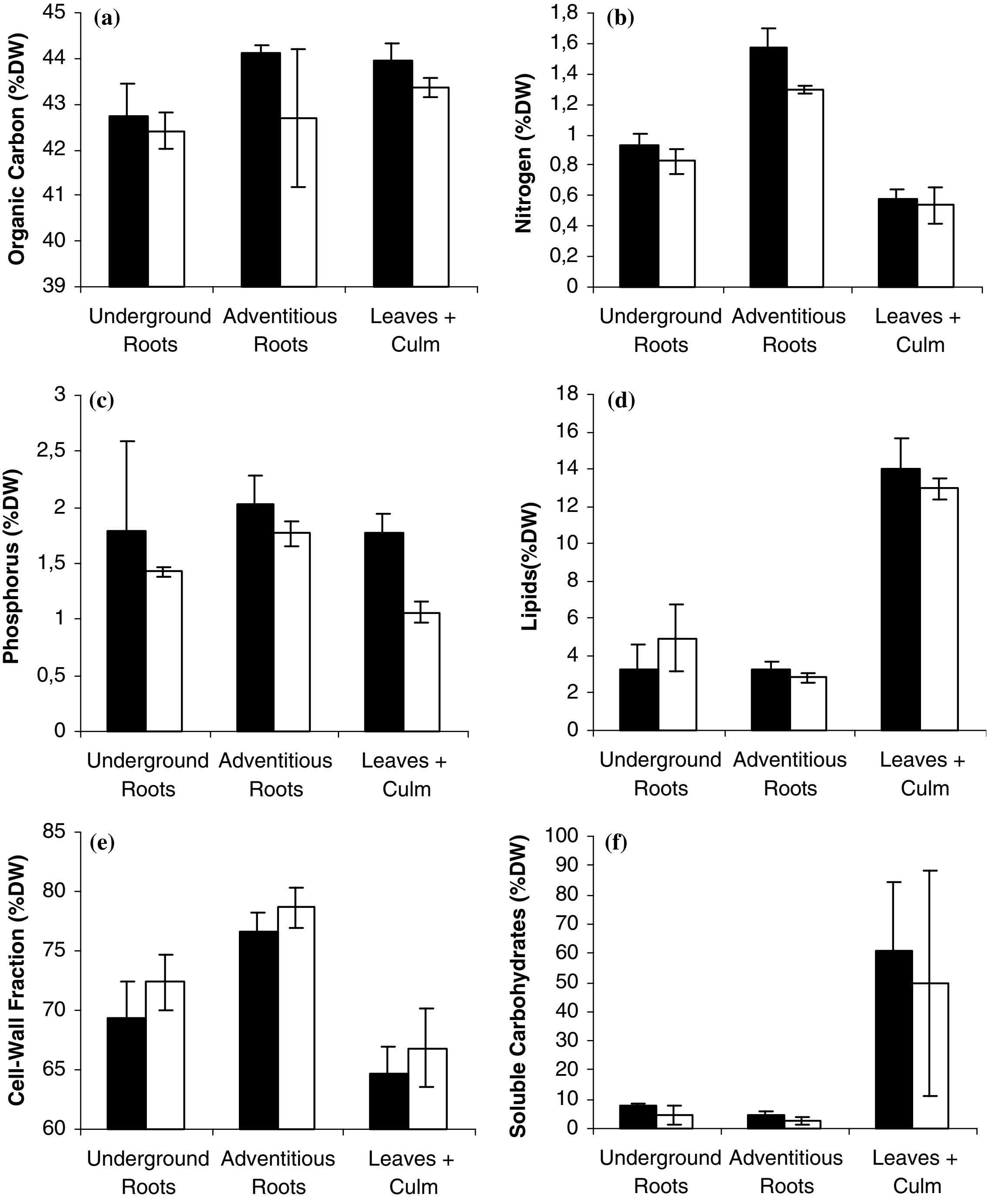

Figure 1. Concentrations of Organic carbon (a), nitrogen (b), phosphorus (c), lipids (d), and cell-wall fraction (e), as well as soluble carbohydrates (f), of three structures of *O. glumaepatula* at natural and impacted areas of Lake Batata (PA) (□ natural area, ■ impacted area).

and culm, in both areas. The Mann–Whitney *U* test showed significant differences between the values of leaves and culm and the values of the two kinds of roots in the two areas, as well as between adventitious and basal roots in the impacted area. Comparing the same structure between the two areas we found that adventitious roots and leaves and culm had higher values in the natural area, and basal roots had higher values in the impacted area. The Mann–Whitney *U* test did not detect any significant difference between values of the same structure in two different areas.

Regarding the percentage of cell-wall fraction, adventitious roots had values higher than those of basal roots in both areas (Fig. 1e). Leaves and culm had lower values than both kinds of roots in the two areas. The Student "*t*" test confirmed the significance of these findings. When we compared the same structure at the two different areas we observed higher values in the natural area. The Student "*t*" test did not confirm the significance of this finding.

Leaves and culm had higher values of soluble carbohydrates compared to the roots, while basal roots had higher values than those of adventitious roots (Fig. 1f). The Mann–Whitney *U* test did not detect significant differences between the values of adventitious and basal roots in the impacted area, but found a significant difference in the natural area. The differences between leaves and culm and both kinds of roots were significant. Again, the natural area had higher values compared to the natural area, but the Mann–Whitney *U* test did not detect any significant difference.

Discussion

The individuals at the natural area had higher values of almost all analyzed compounds, with the exception of cell-wall fraction, an indication that the bauxite tailings had a negative influence on the chemical composition of the individuals of *O. glumaepatula* in the impacted area. Cell-wall fraction stands for the amount of structural, hard-to-digest material, such as lignin and cellulose, and its complement, the protoplasmatic fraction, stands for the easy-to-digest compounds (carbohydrates, proteins, lipids, etc.) (Van Soest & Wine, 1967). Therefore there is an inverse relationship between the two fractions. The differences between the two areas were statistically confirmed only in a few instances, as follows: adventitious roots and leaves and culm, regarding concentration of organic matter; adventitious roots, regarding concentration of nitrogen; and leaves and culm, regarding concentrations of phosphorus. In spite of the absence of statistical confirmation, we must take note of the fact that in all analyses, with the sole exception of the concentration of lipids in the basal roots, this trend was observed: individuals at the impacted area had lower values for the compounds analyzed in comparison to individuals at the natural area.

The smaller concentrations of lipids, soluble carbohydrates, protoplasmatic fraction (the complement of cell-wall fraction) and organic carbon could be the result of a deficiency in basic nutrients (N and P), which are the main limiting factors for most aquatic macrophytes (Esteves, 1988). The sediment of Lake Batata has a pronounced gradient from the natural to the impacted area, regarding concentrations of total-N and available-P. The sediment of the natural area has higher concentrations of these elements than the sediment of the impacted area (Roland, 1995). The sediment is the section of the lake ecosystem with the highest concentration of these elements, according to Nogueira & Esteves (1990). Lower concentrations of these elements in the sediment and in the water column of the impacted area may act as a negative factor to the population of *O. glumaepatula*, forcing it to consume more lipids and soluble carbohydrates (with a side effect on the protoplasmatic fraction).

There is a similar gradient from the natural to the impacted area regarding concentrations of total-N in the water column, and this section of the ecosystem is also exploited by *O. glumaepatula*, at this season of the year, due to the absorptive capacity of its adventitious roots (Rubim, 1995). The great fluctuation of the water level in the area is reflected in a great variation of the concentration of total-N throughout the year, but the gradient was observed in the high water season, when the individuals were sampled.

The percentages of nitrogen were lower than those found by Howard-Williams & Junk (1977) in individuals of *O. glumaepatula*, in Central Amazon, and only the adventitious roots had values higher than those observed by those authors. As the major contribution to the total mass of the plant is made by the leaves and culm, on the whole individuals of Lake Batata had lower concentrations of nitrogen than individuals at Central Amazon. We must remember, however, that the ecosystem studied by those authors is a white water environment, with a quite higher nutrient load than that found in Lake Batata. Rubim (1995), studying also *O. glumaepatula* in a white water environment, found values of nitrogen concentration quite higher, but the highest values were observed in the leaves, unlike the results of our research, where adventitious roots had the highest concentrations of N. Possibly the source of the difference is in the methodologies employed – in the research of Rubim (1995) the leaves were separated from the culm, unlike our methodology. As most of the total mass of the set "leaves plus culm" is provided by the culm, the high values of the leaves could have been masked by the low values of the culm in our results. Rubim (1995) found high concentrations of nitrogen at the end of the terrestrial phase, but as the water rises the concentrations in the culm fall. As our research is focused on the high water period (March), the values of nitrogen concentration in the culm are probably at their lowest, and this could explain the low value of the set "leaves plus culm". Only a specific research that takes apart leaves and culm can answer this question conclusively.

The highest concentration of phosphorus and nitrogen in the adventitious roots is probably a result of the function of these structures, which absorb nutrients from the water column. The concentration of these nutrients in the individuals of *O. glumaepatula* is several times higher than that observed in the sediment and in the water column by Roland (1995) at Lake Batata.

The high concentration of organic carbon in the adventitious roots at the natural area is a probable result of the great development of these structures at this area. In the sampling period, with high waters, the individuals have well developed adventitious roots, used for the absorption of nutrients from the water column. In the impacted area the concentration of organic carbon in adventitious roots is much lower, and is located at intermediate values between basal roots (lower) and leaves and culm (higher). Possibly the bauxite tailings interfere with the absorption of nutrients from the water column, and this, coupled with the smaller amount of available nutrients, reduces the value of adventitious roots for the organism, resulting in a decrease of the investment of the plant in these structures.

The high concentrations of soluble carbohydrates and lipids are a reflection of the photosynthetic activity at the leaves and culm, which exhibit concentrations of these compounds quite high in comparison to the other structures.

The cell-wall fraction, representing compounds of difficult digestion, is a measurement of the nutritive value of the plant. So, it is not surprising to observe that the cell-wall fraction is smaller in the leaves and culm, as this plant has a yearly life cycle and does not reproduce by seeds (Rubim, 1995), and therefore has no energetic reserves at its roots.

Concluding, we may say that a trend of decrease in the analyzed compounds between the natural and impacted areas was observed. Although this trend was not statistically significant, later studies with more numerous samples may confirm whether it is a real feature of the population or just a methodological artifact. We may also say that the concentration of each analyzed compound is different in each of the structures of the plant. Also, the patterns of distribution of analyzed compounds among the many structures are constant, both in the impacted area and in the natural area, the exceptions being the concentration of organic matter (and organic carbon, which is linked to organic matter).

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Primary production of *Utricularia foliosa* L., *Egeria densa* Planchon and *Cabomba furcata* Schult & Schult.f from rivers of the coastal plain of the State of São Paulo, Brazil

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Key words: *Cabomba furcata*, *Egeria densa*, primary production, *Utricularia foliosa*

Abstract

Seasonal variation in gross primary production (GPP) of *Utricularia foliosa* Linnaeus, *Egeria densa* Planchon and *Cabomba furcata* Schult & Schult.f. in rivers of the coastal plain of the state of São Paulo, Brazil was examined in relation to water physico-chemistry. These three species do not affect the multiple uses of the streams and are present throughout the year. The most productive was *U. foliosa* (maximum production 24.7 $mgO_2\ g^{-1}$ DW h^{-1}), while *C. furcata* had an intermediate GPP (maximum production 17.5 $mgO_2\ g^{-1}$ DW h^{-1}) and *E. densa* was lowest at 5.6 $mgO_2\ g^{-1}$ DW h^{-1}. Despite the low amplitude of seasonal variation in this south tropical area, the three species showed seasonal variation in the primary production: GPP was positively correlated with photosynthetic active radiation for *U. foliosa* and *E. densa*, and there was a negative correlation for *C. furcata*. For *U. foliosa*, GPP was positively correlated with temperature and dissolved inorganic carbon and the GPP of *C. furcata* was positively correlated with dissolved inorganic carbon.

Introduction

In the coastal plain of the south of São Paulo State (Brazil) there are many streams with low current velocity that drain very different areas in terms of geology and vegetation. As a result, these streams exhibit different types of water, classified as black, white and clear waters (Camargo et al., 1996; 1997). The stream waters are little impacted by human activities because most of the region is included in a conservation unit (Parque Estadual da Serra do Mar). The climate of this south tropical region has a low amplitude of seasonal variation, with daytime air temperature varying between 15.2 and 30.1 °C and monthly precipitation between 244.7 mm in summer (December, January, March) and 98.3 mm in winter (June, July, August) (Lamparelli & Moura, 1998).

The streams are colonised by many species of aquatic macrophytes of different ecological types. The most abundant submerged species are *Utricularia foliosa* Linnaeus, *Egeria densa* Planchon and *Cabomba furcata* Schult & Schult.f. The three species do not affect the multiple uses of the streams and are present during all the year. *U. foliosa* is a non-rooted submerged of carnivorous habit, abundant in black water streams. *E. densa* and *C. furcata* are submerged rooted species which grow in clear water streams. These species play an important ecological role in these streams, providing favourable conditions for animals (as holdfast sites, surfaces for egg deposition, food source) and contribute to primary production.

The objective of this work was to compare the seasonal variation of the gross primary production of these three species and to determine the relationship between primary production and physical and chemical characteristics of the water.

Materials and methods

Measurements of primary production were conducted in summer (February), autumn (May), winter (August) and spring (November) 1999. Clear (97% transparency) flasks with ca. 2000 ml capacity were filled with stream water using a siphon. Apical branches of each species with ca. 15 cm length were placed in each flask, using triplicate incubations. For further correction of the primary production of the macrophyte, the production and respiration of the phytoplankton was measured from one clear and one dark flask that did not contain the plant. The incubations were made for a period of 4 h (ca. 10:00–14:00 h). Afterwards the water from the flasks was siphoned to 150 ml flasks for measurement of dissolved O_2 ($mg\ l^{-1}$) using Winkler's method (Golterman et al., 1978). The titration was made by an electronic burette (Scohtt Gerate, model T 80/10). Dry weight of the apical branches used in the incubations was obtained by drying at 80 °C until constant weight.

Gross and net primary production and respiration were estimated using the equations of Vollenweider (1974):

$$\mathrm{NPP} = (c - i)v/(t \cdot \ \mathrm{DW})$$

$$R = (i - d)v/(t \cdot \ \mathrm{DW})$$

$$\mathrm{GPP} = \ \mathrm{NPP} + R$$

NPP being the net primary production ($mgO_2\ g^{-1}$ DW h^{-1}), R is the respiration ($mgO_2\ g^{-1}$ DW h^{-1}), GPP is the gross primary production ($mgO_2\ g^{-1}$ DW h^{-1}), c is the O_2 concentration in the clear flask and d in the dark flasks ($mg\ l^{-1}$), i is the initial O_2 concentration in the flasks ($mg\ l^{-1}$), v is the flask volume (L), t is the incubation time (h) and DW is the plant dry weight (g).

At the beginning of the incubation, values of pH, electrical conductivity ($\mu S\ cm^{-1}$), temperature (°C), salinity (%) and turbidity (NTU) were taken in triplicate, using a Horiba (model U10) Water Quality Checker. Photosynthetically-active radiation (PAR) ($\mu mol\ m^{-2}\ s^{-1}$) was measured with a LiCor light meter (Model 189) coupled to an underwater Licor quantum sensor (Model 192). A 1.0 l water sample was collected for further measurement of the total alkalinity ($meq\ l^{-1}$), by titration, and dissolved inorganic carbon (DIC) ($mM\ l^{-1}$), according to Mackereth et al. (1978), was sampled from the site where the incubation had been carried out.

Approximately 0.5 l of the water sample was filtered (Whatman GF/C) in the field laboratory, kept in polyethylene flasks and frozen to −20 °C. Non filtered samples (0.5 l) were also frozen in the same manner. The concentration of ammoniacal nitrogen (ammoniacal-N) ($\mu g\ l^{-1}$) (Koroleff, 1976), nitrite (NO_2–N) ($\mu g\ l^{-1}$), nitrate (N–NO_3–N) ($\mu g\ l^{-1}$) and total dissolved nitrogen (TDN) ($mg\ l^{-1}$) (Mackereth et al., 1978) as well as total dissolved phosphorus (TDP) ($\mu g\ l^{-1}$) and orthophosphates (PO_4–P) ($\mu g\ l^{-1}$) (Golterman et al., 1978) were measured from the filtered samples in the Laboratory of Aquatic Ecology of the Department of Ecology of UNESP/Rio Claro-SP. The non-filtered water samples were used to determine total nitrogen (TN) ($mg\ l^{-1}$) (Mackereth et al., 1978) and total phosphorus (TP) ($\mu g\ l^{-1}$) (Golterman et al., 1978).

For the analysis of variance (ANOVA) the statistical software SYSTAT (version 5.03) was used (Wilkinson, 1990). For the analysis of variance differences were considered significant when $p < 0.05$. A Newman–Keuls post-hoc test was used for mean-separation with statistically-significant relationships.

Results

Figure 1 shows the mean values and standard deviations of the primary production of the three species in the four seasons of the year. GPP of *U. foliosa* showed marked seasonal variation, with mean values significantly higher in summer (24.7 mg O_2 g^{-1} DW h^{-1}) intermediate values in spring (17.6 mg O_2 g^{-1} DW h^{-1}) and significantly lower values in autumn (8.5 mg O_2 g^{-1} DW h^{-1}) and winter (7.2 mg O_2 g^{-1} DW h^{-1}). For *E. densa* significantly higher values of GPP occurred in autumn (5.6 mg O_2 g^{-1} DW h^{-1}) than in spring (4.9 mg O_2 g^{-1} DW h^{-1}), summer (3.8 mg O_2 g^{-1} DW h^{-1}) or winter (3.0 mg O_2 g^{-1} DW h^{-1}). The GPP of *C. furcata* also varied seasonally, with significantly higher values in summer (17.5 mg O_2 g^{-1} DW h^{-1}), intermediate values in autumn (11.3 mg O_2 g^{-1} DW h^{-1}) and winter (10.7 mg O_2 g^{-1} DW h^{-1}), and significantly lower values in spring (6.5 mg O_2 g^{-1} DW h^{-1}). The differences between the primary production of the three species is clear: *U. foliosa* is the most productive, *C. furcata* has an intermediate GPP and *E. densa* had the lowest GPP during the year.

Analysing the relation between GPP and physical and chemical variables, of the three species, some correlations are evident. GPP of *U. foliosa* and *E. densa* is positively correlated with PAR and GPP of *C. furcata* is negatively correlated with PAR. For *U. foliosa*, GPP is correlated positively with temperature and concentrations of DIC. The GPP of *C. furcata* is correlated with concentrations of DIC (Fig. 2). The other abiotic variables, such as TN and TP, were not significantly correlated with the primary production of the three species.

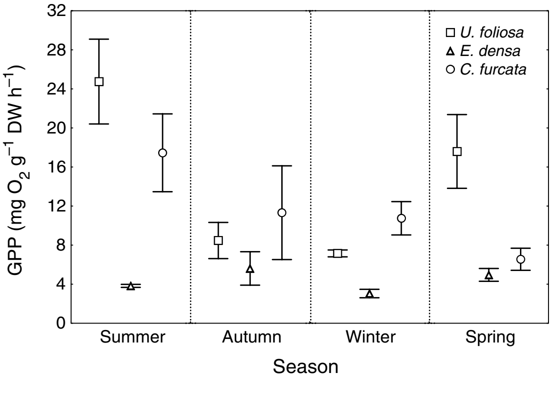

Figure 1. Gross primary production (GPP) of *U. foliosa*, *E. densa* and *C. furcata* in four seasons of the year.

Discussion

In temperate regions aquatic macrophytes present a seasonal variation of primary production that is a function of temperature and photoperiod (Payne, 1986). In tropical regions seasonal variation of primary production is related to rainfall and level of water, usually produced by a flood pulse (e.g., Camargo & Esteves, 1995). In the south coast region of São Paulo State the climate is homogeneous in terms of temperature and rain, which are well distributed during the year and a flood pulse does not occur (Camargo et al., 1997). However the three species still showed evidence of seasonal variation in primary production. The seasonal maxima and minima GPP differed between the three species. For *U. foliosa* the highest GPP was measured in summer and the lowest in winter, for *C. furcata* highest in summer and lowest in spring, and for *E. densa* highest in autumn and lowest in winter.

Light intensities, temperature and concentration of DIC are important variables that control the primary production of submerged aquatic macrophytes (Sand-Jensen, 1989). Probably, these variables are important factors that control the primary production of the three species in rivers of the São Paulo coastal plain, because the results showed correlations between these variables and primary production. In fact, there is evidence that the low values of PAR, temperature and DIC in winter are limiting to primary production of *U. foliosa* in this season. The lower values of PAR, in winter, appear to limit the production of *E. densa*. On the other hand the higher values of PAR, and lower values of DIC, in winter and spring limited the production of *C. furcata*.

The season of higher and lower GPP for the three species is different, probably due to the characteristics of the species and the physical and chemical characteristics of the water. These characteristics of the water depend of the alternation between sunny and rainy days, in all seasons of the year.

The most productive species in rivers of the target area is *U. foliosa*, a submerged non-rooted species. The carnivorous habit is an important additional source of nutrients for this species (Pott & Pott, 2000) and probably for this reason the GPP is not limited by the low total

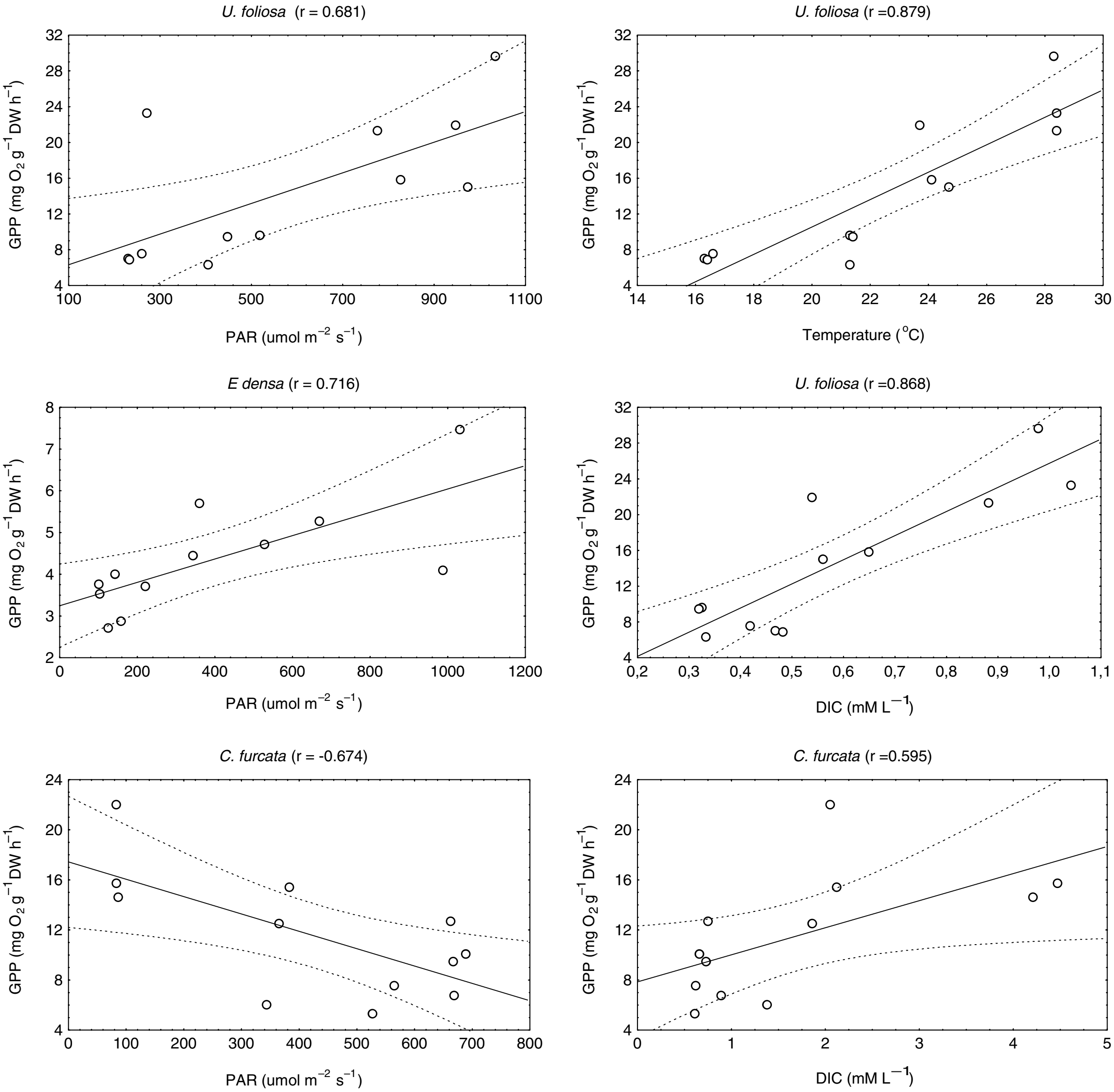

Figure 2. Relation between gross primary production (GPP) and photosynthetic active radiation (PAR), temperature and dissolved inorganic carbon (DIC).

nitrogen and total phosphorus concentration in water.

Although *E. densa* has a tendency to form large detached masses of vegetation which interfere with the utilisation of water resources in many aquatic ecosystems (Barreto et al., 2000), but in the streams of the south coast of São Paulo state, it does not causes problems. Probably, the low light intensities restrict the GPP of *E. densa* and consequently this species does not interfere with the uses of aquatic resources in the region.

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An experimental study of the plastic responses of *Ranunculus peltatus* Schrank to four environmental parameters

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Key words: *Batrachium*, spreading macrophyte, morphological traits, current velocity, light intensity, water depth

Abstract

The impact of four environmental parameters (water depth, type of substratum, current velocity and light intensity), on *Ranunculus peltatus* morphology and reproduction was tested in four 1 month semi-controlled experiments. Four development stages were underlined from April to August 2001 in *R. peltatus*: an elongation stage (April–June), a flowering stage (May–June), a fragmentation stage (June–July) and a potential regenerative stage (July–August). Water depth was therefore tested on *R. peltatus* elongation, type of substratum on *R. peltatus* elongation and flowering, current velocity on *R. peltatus* fragmentation and light intensity on its possible regeneration. The maximum development was measured for a 32 cm water depth. Current velocity did not have a significant effect on *R. peltatus* fragmentation. Regeneration depended strongly on light availability. This stage occurred only for unshaded or 50% shaded plants. Darkness prevented plants from regrowing.

Introduction

Phenotypic plasticity in plants, either morphological or reproductive, is one or their most important adaptations to temporal and spatial environmental variability (Sculthorpe, 1967; Grime et al., 1986; Sultan, 2000). This ability to adapt its phenotype to suit the habitat allows plastic species to compete for a wide range of habitats. Plastic species are indeed able to improve their resource acquisition, their resistance and adaptability to stress or disturbance (Grime et al., 1986). Plasticity gives therefore a distinct competitive advantage over other species that lack this attribute which explain the high plasticity found in spreading aquatic macrophytes (Ashton & Mitchell, 1989).

Ranunculus peltatus Schrank is a very plastic species which can dominate open sections of streams (Cook, 1966). This plasticity promotes its local spreading in French hydrosystems and especially in the Vosges mountains (Thiébaut & Muller, 1999). For several aquatic macrophytes, and especially rooted submerged macrophytes, morphological plasticity is induced by physical parameters (Westlake, 1973). In *Ranunculus* species, biomass studies show that their growth are sensitive to light (Dawson & Kern-Hansen, 1979), current velocity, substratum (Boeger, 1992) and water depth (Dawson, 1976). No data are however available on *Ranunculus* morphological variation according to these parameters. Besides, environmental factors are closely inter-related in river situations. No clear correlation between environmental parameters and plants morphology can be well underlined with field studies (Westlake, 1973).

In the present study, semi-controlled experiments were used in order to isolate one factor and test it, the other remaining constant. This type of experimental studies are furthermore close to field conditions. The aim of this study was to test the effect of four physical parameters, water depth, type of substratum, current velocity and light intensity, on the growth, regeneration and colonisation abilities of *R. peltatus*. Two main questions were tackled:

(i) Is *R. peltatus* plasticity dependent on physical parameters?
(ii) Are reproduction and vegetative growth dependent on similar physical parameters?

Study area

The study was performed in the Northern Vosges Biosphere Reserve (49° 1′ N, 7° 23′ W, NE France). The experimental site is located in the upper reach of the Schwartzbach stream and was selected for its physical characteristics. This moderately shaded stream (70% of light intensity), is 3–4 m wide, 40–50 cm deep and is characterised by a 0.2–0.4 m/s current velocity. A 0.7 m/s maximum velocity was noticed during the experimental period. The hydraulic regime is relatively stable year round. This stream flows on eroded sandstone substrate and is characterised by oligotrophic waters.

Methods

Chemical surveys

About 500 ml of water were collected weekly in mid-stream from April 15 to August 15 in 2001 (16 sampling dates). Acid Neutralising Capacity (ANC) was determined by Gran's titration (NF T 90-035, AFNOR, 1986). Conductivity and pH were measured using a combined glass electrode and corrected for temperature (25 °C). Reactive Soluble Phosphorus and ammonia were analysed using spectrophotometry (NF T 90-023, NF T 90-015, AFNOR, 1986).

Experimental design

Nine 1.50 m×0.5 m tanks were built and placed directly in the river bed in a zone considered as homogeneous according to light and current velocity. A 5 cm-layer of substrate was added at the bottom of each tank for *R. peltatus* to anchor. Adjustable floats located on each side of the tank controlled the water depth at which the tank was located from the water surface. The different modalities were assigned randomly to tanks following a bloc-experimental design. Four environmental parameters, water depth, type of substratum, current velocity and light intensity, were tested one at a time during four 1-month-experiments. This short experimental duration was chosen in agreement with *R. peltatus* growth rate. Less than one month is generally necessary for this species to double its initial biomass (Sand-Jensen & Madsen, 1991; Madsen & Brix, 1997). Such duration was therefore considered as sufficient for highlighting potential plastic adjustments. For each experiment, one factor was tested, the other remaining constant. Each physical parameter was randomly associated to a different one-month-period (Table 1). Three modalities were selected for each parameter according to *R. peltatus* ecological requirements.

Table 1. Characteristics of each 1-month experiment

	Period of test	Factor tested	Modalities		
First experiment	April 15–May 15	Water depth (cm)	17	32	48
Second experiment	May 15–June15	Substratum (mean particle diameter, D, cm)	Sand (D < 0.2)	Gravel (0.5 < D < 4)	Pebble(4 < D < 15)
Third experiment	June 15–July 15	Current velocity (m/s)	0.2	0.3	0.4
Fourth experiment	July 15–August 15	Light intensity	I(100%)	I(50%)	I(0%)

D: Particle diameter

The three water depths were obtained by adjusting the floats for each tank. The different substrata were collected in the same geological area. The three different current velocities were obtained by increasing artificially the initial stream velocity. Two dams were therefore built so as to double the initial current velocity at the end of the second dam. The three different light intensities corresponded to 100% shaded tanks with dark plastic films (I(O%)), 50% shaded tanks with white cloth (I(50%)) and unshaded tanks (I(100%)). Three replicates were made for each modality. The control tanks were characterised by the following combination of physical parameters: 17 cm-water depth, sand, 0.2 m/s current velocity and I(100%). The phenology of *R. peltatus* was therefore studied in plants transplanted in these control tanks.

Morphological measures

Eight traits were selected: A, plant weight; B, main stem length; C, maximum branch length; D, number of internodes; E, number of buds; F, number of flowers; G, percentage of cut plants; H, percentage of washed away plants. These traits characterise either vegetative growth (traits A, B, C, D), sexual reproduction (traits E, F) and fragmentation (traits B, G, H). Morphological traits were measured on transplanted plants at the beginning and at the end of each experiment, and their variation was studied. Transplanted plants were 50–70 cm long, collected with viable apex and developed adventitious roots. They were planted in a 10×10 cm^2 pots filled with sand and covered with plastic films. Three pots were fixed in each tank. Washed away plants were not included in statistical analyses except for the fragmentation study.

Results

Chemical surveys

The water was oligotrophic ($[PO_4^{3-}\text{-P}] = 26 \pm 15$ μg/l, $[NH_4^{+}\text{-N}] = 48 \pm 19$ μg/l), neutral ($pH = 6.56 \pm 0.23$), weakly mineralised (Conductivity = 58 ± 3 μS/cm) and poorly buffered (Alkalinity = 235 ± 33 μeq/l). During the experimental period, these chemical characteristics were stable and no significant change in trophic conditions appeared ($n = 16$; *F*-test, $p > 0.1$).

R. peltatus *phenology*

A significant time effect was underlined on the variation of all morphological traits. Main stages of *R. peltatus* development were determined using a selection of five morphological traits (Table 2). The mean increase in length of the main stem and branch was highest from April 15 to June 15 which underlined an elongation stage (*F*-test, $p < 0.05$). Flowering appeared essentially from May 15 to June 15, preceded by buds production. The percentage of cut stems was the highest during the third experiment and the increase of main stem was negative. This stage corresponded to a fragmentation stage. Finally, a regrowth was noticed corresponding to *R. peltatus* regenerative stage.

Impact of water depth on elongation stage

The highest increase of the main stem length and weight was found for 32 cm-water depth, and the lowest for 17 cm-water depth (Fig. 1; *F*-test, $p < 0.05$). There was no significant changes in the variation of branch lengths (*F*-test, $p > 0.1$). The development of new internodes tended to be the highest for 32 cm-water depth and the lowest for 17 cm-water depth (*F*-test, $p < 0.1$).

Table 2. Mean values and standard deviation for the variation of five morphological traits ($n = 9$)

	April 15–May 15	May 15–June 15	June 15–July 15	July 15–August 15	*F*-test
Variation of main stem length (cm)	75.9 ± 39.6	56.2 ± 41.9	−25.8 ± 29.8	50.6 ± 16.7	$p < 0.5$
Variation of maximum branch length (cm)	49.3 ± 41.9	40.3 ± 13.8	3.1 ± 2.6	22.6 ± 16.1	$p < 0.01$
Variation of bud number	3.0 ± 4.1	0.9 ± 1.3	0	0	$p < 0.01$
Variation of flower number	0	1.7 ± 1.7	0	0	$p < 0.01$
% Of uncut stems	100	77.8	44.4	100	

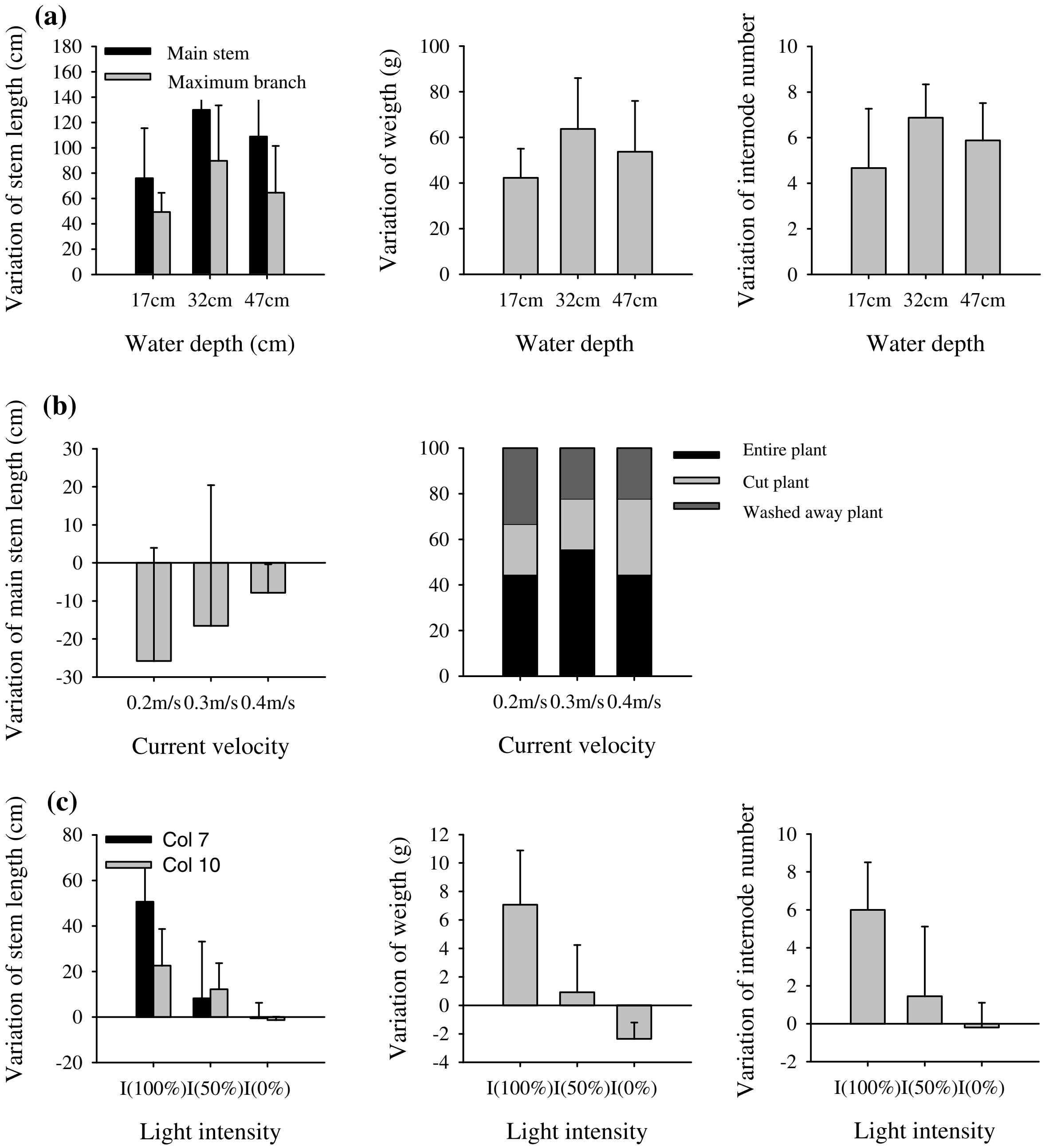

Figure 1. Variation of *R. peltatus* morphological traits in 1 month according to environmental parameters. a: water depth, b: current velocity, c: light intensity.

Impact of category of substratum on flowering and elongation stage

The absence of plant anchorage in substratum did not allow us to conclude on the role of this parameter on *R. peltatus* flowering and elongation.

Impact of current velocity on fragmentation

There was no significant differences in the variation of stem length according to current velocity (*F*-test, $p > 0.1$). A higher current velocity did not seem to induce a higher percentage of damaged stems (Fig. 1b).

Impact of light intensity on regeneration

The highest length increase of the main stem was found for unshaded tanks (I(100%)) (Fig. 1c) (*F*-test, $p < 0.05$). In darkness (I(O%)), the length variation of the main stem and secondary branch was negative. Similar results were found for traits "number of internodes" and "plant weight" (*F*-test, $p < 0.05$).

Discussion

R. peltatus plasticity depended on physical parameters. Clear impact of water depth and light intensity on *R. peltatus* morphology was indeed underlined. However, we were not able to conclude on the effect of substratum. The duration of experiment was certainly to short for *R. peltatus* anchorage. Four distinct life stages were besides underlined but only the impact of water depth on *R. peltatus* growth, of current velocity on its colonization abilities and of light intensity on its regeneration abilities could be assessed through this study.

Optimal development of *R. peltatus* by the elongation of main stems and branches occurred for medium water depth. Submerged aquatic plants tend to elongate their stems in order to reach water surface for achieving their photosynthesis the most efficiently as possible. Besides, *R. peltatus* needs to reach water surface to flower (Cook, 1966). This can explain the differences of elongation between low and medium water depth. Two hypothesis can be suggested to explain the results obtained in deepest water. The first one concerns the experiment duration. One month was a too short period for *R. peltatus* to reach water surface. This hypothesis seems quite improbable as *Ranunculus* species are characterised by very short doubling times in the beginning of summer (Eichenberger & Weilenmann, 1982). The second hypothesis is the high pressure on stems by water flow. The longer stems are, the more resistant they need to be. An intermediate development might be a trade-off between energy devoted to strengthen stems and to biomass production. Current velocity had no impact either on length of remaining stems or on fragmentation rate of *R. peltatus*. These results were quite surprising as strong current velocities such as after storms are known to accelerate fragmentation of *Ranunculus* stems and favour their dissemination (Dufayt, 2000). Our results may be explained by the relatively low current velocities tested. Though *R. peltatus* is less commonly found in current velocities higher than 0.5 m/s, it may be therefore interesting to test the impact of such current velocities on its fragmentation. Light intensity had a great impact on *R. peltatus* regeneration. Darkness prevented *R. peltatus* to regenerate. 50% of shading defavoured markedly its regrowth. Such impact was already underlined for *R. calcareus* development in the early summer which had give birth to reducing-light-availability management methods (Dawson & Kern-Hansen, 1979). Plants if regenerate, will be able to survive more easily during winter and insure the stand survival. Available light at the end of summer is therefore conditioning *R. peltatus* growth the year after.

Few hypothesis can be formulated taking into account *Ranunculus* species life-cycle: Light may have also an impact on the other development stages. Current velocity may furthermore influence stem elongation such as for *Myriophyllum spicatum* (Strand & Weiss, 2001). Water depth may also influence flowering. Furthermore, *R. peltatus* morphological plasticity was well explained by water depth or light intensity but part of the variability noticed may also be linked with other physical parameters. For instance, temperature induced some morphological plastic adjustements in several spreading species (Barko et al., 1982) as well as turbidity. Besides, plasticity can be induced by chemical factors such as mineralization (Chatenet et al., 2002) or trophic levels (Barko et al., 1988). On-going experiments should enable to complete this work and test the hypothesis formulated.

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The response of *Ceratophyllum demersum* L. and *Myriophyllum spicatum* L. to reduced, ambient, and enhanced ultraviolet-B radiation

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Key words: *Ceratophyllum demersum*, *Myriophyllum spicatum*, Ultraviolet-B radiation, UV-B absorbing compounds, Photochemical efficiency of PS II, Respiratory potential

Abstract

The response of *Ceratophyllum demersum* and *Myriophyllum spicatum* to three levels of UV-B radiation – reduced (*ca.* 50% reduction), ambient and enhanced UV-B radiation, simulating 17% ozone depletion – is discussed. The research revealed that UV-B stimulated the production of UV-B absorbing compounds in *C. demersum*, but not in *M. spicatum*. The relative amount of UV-B absorbing compounds was about four times lower in *C. demersum*. Enhanced UV-B also affected respiratory potential in *C. demersum* (on average 3.7 mg O_2/gDM/h), but no effect on *M. spicatum* (on average 5.5 mg O_2/gDM/h) was detected. Increased need for energy revealed that UV-B radiation exerted stress in *C. demersum*. No changes in chlorophyll *a* and no disturbance to photochemical efficiency due to UV-B were observed in either species.

Introduction

Solar UV-B radiation on the Earth's surface has increased during the last two decades as a consequence of the degradation of the stratospheric ozone layer (Häder et al., 1998). Exposure of organisms to enhanced UV-B radiation could cause damage to DNA, proteins, membranes and the photosynthetic apparatus (Björn, 1999). Plants have evolved different mechanisms and structures, which protect them from UV-B radiation or which repair any damage. The majority of research into UV-B and higher plants was conducted in the terrestrial environments. The potential of terrestrial plants to cope with UV-B radiation depends on growth form, life span, radiation environment and position in the phylogenetic tree (Les, 1988; Les et al., 1991; Rozema et al., 1997; Bjőrn, 1999; Gaberščik et al., 2001; Gaberščik et al., 2002). Aquatic plants are flexible and continuously change their position in the water column. Therefore, the exposure time of a given assimilation area to UV-B radiation is variable. Terrestrial plants are more or less rigid and their form and branching depend to the large extent on the radiation environment (Rozema et al., 1997). Because of these differences we presume that the strategy of aquatic higher plants in coping with the qualitative changes of radiation is possibly different to that of terrestrial plants.

In the present study, we examined the effects of reduced, ambient and enhanced UV-B radiation (simulating 17% ozone depletion) on two submersed macrophytes – *Ceratophyllum demersum* L. and *Myriophyllum spicatum* L. *C. demersum* and *M. spicatum* are cosmopolitan species, exhibiting a similar life form and colonising mainly eutrophic stagnant and flowing waters (Martinčič et al., 1999). They grow rapidly, tolerate disturbance and stress and are successful competitors (Nichols & Shaw, 1986). In spite of many similarities there are two important features that could influence their response to UV-B radiation: (1) *M. spicatum* is rooted into the sediment and *C. demersum* is free floating in the water column or anchored into the substratum, and (2) they have different positions in the phylogenetic tree. Paleobotanical evidence has shown that the family Ceratophyllaceae has vestiges of ancient angiosperms that diverged early from the evolutionary line, leading to the most modern taxa (Les, 1988; Les et al., 1991). Therefore, it was evident that the genus *Ceratophyllum* was successful, not only spatially but also temporally, throughout the evolution. We hypothesised that mechanisms to overcome UV-B stress in *C. demersum* and *M. spicatum* could help to account for the wide distribution and success of both species in aquatic systems.

Materials and methods

C. demersum and *M. spicatum* were exposed to different UV-B levels under outdoor conditions. *C. demersum* was taken from a pond in the Botanical Garden (Ljubljana: 320 m asl, 46 °35′ N, 14 °55′ E, Slovenia) and *M. spicatum* from Lake Bohinj (547 m asl, 46 °17′ N, 13 °54′ E, Slovenia). The plants were cultured under natural conditions in the Botanical Garden one year before starting the irradiation experiment. Plastic containers of 0.50 × 0.70 × 0.45 m were used to grow the plants. The water depth was 30 cm. The plant samples for analyses were taken just below the water surface, in order to ensure that UV-B doses were only slightly weakened by the water column. An UV-B supplement system was designed, as described by Björn & Teramura (1993). Three different treatments were applied: simulation of 17% ozone depletion (UV-B (+)) was performed using Q-Panel UV-B 313 lamps, filtered with cellulose diacetate foils, which block the UV-C range (wavelengths lower than 280 nm). In the control plot, plants were exposed to the radiation produced by Q-Panel UV-B 313 lamps filtered using Mylar foil, which cuts out wavelengths below about 320 nm. The lamps were fixed 1 m above the plants. An area of 1 × 2 m was irradiated for the first and second plots. In the third plot, (UV-B (–)) the same area was covered with a Mylar foil filter positioned 0.80 m above the plants to obtain an average 50% reduction of UV-B. Ambient UV-B was monitored using ELDONET (European Light Dosimeter Network) (ENV4-CT 96-0191). The supplemental doses were calculated and adjusted weekly, using the program published by Björn & Murphy (1985) based on the generalised plant action spectrum by Caldwell (1968). The systems were timer controlled. UV-B biologically effective doses (UV-B_{BE}) are shown in Fig. 1.

All analyses and measurements were made on fresh material from the first fully developed leaf. Chlorophyll *a* was extracted with 90% acetone. Extracts were centrifuged in a refrigerated ultracentrifuge (2K15, Sigma, Osterode, Germany) at 15,000 Hz for 3 min at 4 °C. Absorbance was measured at 647, 664 and 750 nm with a UV/VIS spectrometer system (Lambda 12, Perkin-Elmer, Norwalk, CT, USA). The amounts of pigment were calculated, as described by Jeffrey & Humphrey (1975). Total methanol-soluble UV-B absorbing compounds (UV-B AC) content was estimated following the procedure by Caldwell (1968). UV-B AC were extracted with methanol:distilled water:HCl 79:20:1 by volume. After 20 min of incubation, the samples were centrifuged in a refrigerated ultracentrifuge. The extinctions of the supernatants were measured in the range from 280–320 nm at intervals of 1 nm with the UV/VIS spectrometer, integrated and calculated per the dry mass (DM) of the sample. Leaves were dried at 105 °C.

The optimal photochemical efficiency of photosystem II (PS II), defined as the variable to maximal fluorescence ratio (Fv/Fm) and the effective photochemical efficiency (Y) were measured

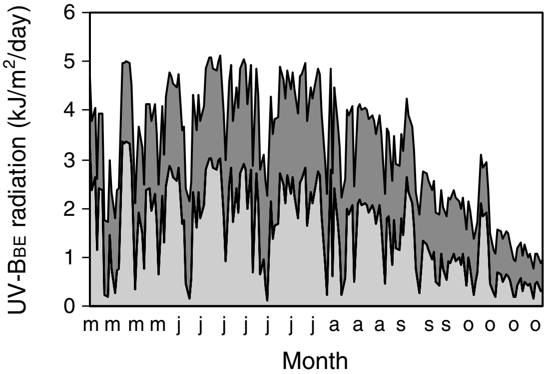

Figure 1. Natural (the lighter area) and supplemental UV-B_{BE} (biologically effective ultraviolet B) radiation (darker area) between spring and autumn 2000.

using a modulated fluorometer (OS-500, Opti-Sciences, Tyngsboro, MA, USA) (Schreiber et al., 1995). Respiratory potential was estimated by measuring the potential electron transport system (ETS) activity of mitochondria, as reported by Packard (1971) and modified by Kenner & Ahmed (1975). Fresh plant material was weighted and homogenised for enzyme analysis in ice-cold homogenisation buffer (<2 °C) and sonicated in an ultrasound homogeniser (20 s; 40 W, 4710 Cole-Parmer, Vernon Hills, Illinois, USA). The homogenate was centrifuged at 10,000 Hz for 4 min at 0 °C in a refrigerated ultracentrifuge. Substrate solution and iodo-nitro-tetrazolium-chloride (INT) were added to three replicates of the supernatant and incubated at 20 °C for 40 min. After 40 min the reaction was stopped and the formazan absorption at 490 nm was determined. Electron transport system (ETS) activity was measured as a rate of INT reduction, which was converted to the amount of oxygen, utilised per unit weight of dry matter of leaves per hour.

The significance of differences between the UV-B(−)/control treatments and the UV-B(−)/UV-B(+) treatments was tested by two-way *t*-test (** ≤ 0.01,* ≤ 0.05; $n=3$–5).

Results and discussion

The response of the majority of primary producers to UV-B is the production of UV-B absorbing compounds which provide a protective screen filtering harmful UV-B rays (Olsson, 1999; Germ et al., 2002a). The increase in the amount of these compounds under enhanced UV-B radiation was significant in *C. demersum*, but negligible in *M. spicatum*, while the total amount produced was much higher in *M. spicatum* (Fig. 2). The amount produced in *M. spicatum* was comparable to that in terrestrial plants (Gaberščik et al., 2002). This is possibly a consequence of the fact that the genus *Myriopyllum* is phylogenetically younger. As it has been reviewed by Rozema et al. (1997), the complexity of UV-B absorbing compounds has been increasing during evolution. On the other hand, land plants evading the aquatic environment show a reduction in the range and quantity of phenolic substances, which is accompanied by a reduction of morphological complexity (Les, 1988; Les et al., 1991; Webster, 1991). The lower amounts in *C. demersum* appeared to be associated with longer evolutionary development of genus *Ceratophyllum* in the aquatic environment. The study of two more "modern" species, with amphibious characteristics (*Batrachium trichophyllum* and *Potamogeton alpinus*) revealed higher amounts of UV-B absorbing compounds and only slight influence of UV-B on production of UV-B absorbing compounds (Germ et al., 2002b).

The effects of UV-B on chlorophyll contents are varied (Rozema et al., 1997). The results in this study showed a slight increase in *C. demersum* and no change in *M. spicatum* (Table 1). Different responses were obtained in the respiratory potential, which reflected the need for energy (Amthor, 1995). In *C. demersum*, ETS activity increased with increasing UV-B doses (Fig. 3). This additional energy enabled the production of UV-B absorbing compounds and other protective mechanisms. However, *M. spicatum* exhibited no differences in respiratory potential at different UV-B levels,

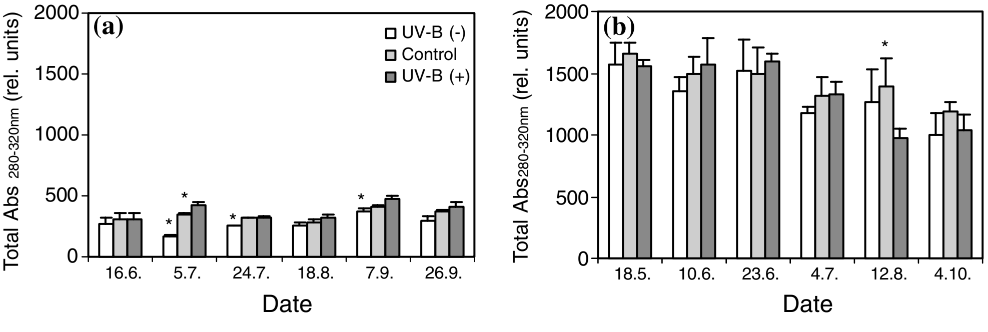

Figure 2. Relative amounts of total UV-B absorbing compounds (from 280 nm to 320 nm) per dry mass (DM) in *C. demersum* (a) and *M. spicatum* (b) growing under different UV-B levels.

Table 1. Seasonal mean values of chlorophyll *a* content and potential (Fv/Fm) and effective quantum yield of PS II (Y) in *C. demersum* and *M. spicatum* exposed to three levels of UV-B radiation with standard deviations (in brackets); (DM = dry mass)

Species	Treatment	Chl *a* mg/g DM	Fv/Fm	*Y*
C. demersum	UV-B (−)	1.46 (0.51)	0.76 (0.03)	0.26 (0.04)
	Control	1.67 (0.75)	0.75 (0.02)	0.25 (0.05)
	UV-B (+)	1.73 (0.69)	0.74 (0.03)	0.22 (0.03)
M. spicatum	UV-B (−)	3.75 (1.40)	0.74 (0.04)	0.40 (0.09)
	Control	3.84 (1.23)	0.71 (0.11)	0.42 (0.05)
	UV-B (+)	3.87 (1.19)	0.73 (0.05)	0.32 (0.06)

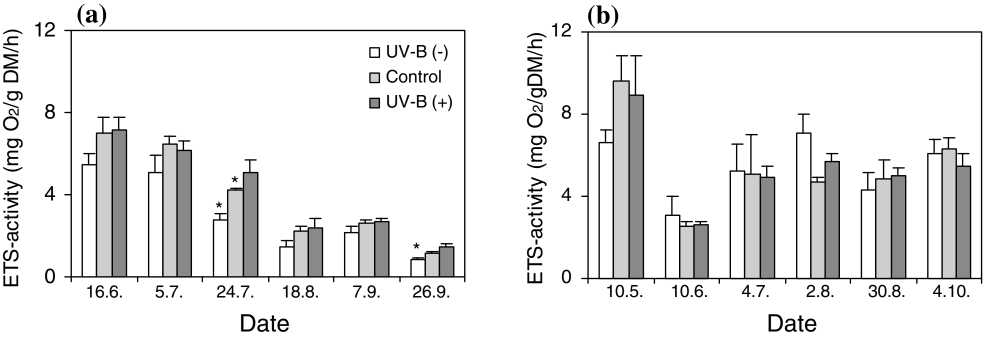

Figure 3. ETS activity compounds per dry mass (DM) in *C. demersum* (a) and *M. spicatum* (b) growing under different UV-B levels.

indicating that the UV-B doses applied presented only minor stress. Variability in the results obtained during the experiment in both species was probably the consequence of changing temperatures during the growth season and due to ontogenetic development. The potential photochemical efficiency of PS II remained at the similar level in plants under all UV-B treatments (Table 1). The values were close to optimal (0.83), which revealed undisturbed photosynthetic apparatus (Schreiber et al., 1995), even though the photosynthetic apparatus has been recognised as one of the important targets of UV-B radiation (Xiong, 2001). The effective quantum yield was lower (compared to optimal quantum yield) with no respect to the treatment, that could be explained as temporal photoinhibition (Schreiber et al., 1995).

On the basis of the results from this study, we concluded that *C. demersum* and *M. spicatum*, treated with UV-B radiation corresponding to 17% ozone depletion, exhibited different responses, even though they were colonising similar habitats and have similar growth forms. *C. demersum* increased the production of UV-B absorbing compounds and ETS activity. The amount of UV-B absorbing compounds in *M. spicatum* was relatively higher and it was possibly saturated. No damage to PS II was detected in either species. It is likely that the life strategy of both species would support successful competition in aquatic ecosystems even under enhanced UV-B radiation.

Acknowledgements

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Effect of reservoir drawdown on biomass of three species of aquatic macrophytes in a large sub-tropical reservoir (Itaipu, Brazil)

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Key words: aquatic macrophytes, reservoir, water level, water hyacinth, aquatic weeds, Upper Paraná River, *Egeria najas*, *Eichhornia crassipes*, *Salvinia herzogii*, growth dynamics

Abstract

The biomass and maximum depth of colonization ($Z_{\max}$) of *Egeria najas* Planchon (submerged), and the biomass and area covered by a stand of *Eichhornia crassipes* (Mart.) Solms and *Salvinia herzogii* Raddi (floating species) were assessed to measure the effects of a 5 m drawdown in water level of the Itaipu Reservoir (Brazil–Paraguay), which lasted about 3 months. The frequency of occurrence of the two first species, and of *Salvinia* spp was also assessed in the main arms of the reservoir. A conspicuous decrease of *E. najas* biomass was observed and this attribute did not reach the previous values even 10 months after the water level had returned to normal. Rapid growth of free-floating species (surface $t_{\text{doub}} = 2.9$ and 3.2 days for total biomass of *S. herzogii* and *E. crassipes*, respectively, and 2.3 days for surface area covered by both species) was recorded immediately after the water level returned to normal. This fast growth was related to phosphorus increases in water. A clear succession was observed over a period of 103 days, during which *S. herzogii* was slowly substituted by *E. crassipes*. The effects of water level drawdown were also observed on *E. najas* frequency, given that this species occurred in 38% of the stands investigated before the water drawdown, but in only 9 and 6% of stands following 1 and 10 months of water level recovery, respectively. Water drawdown did not affect the frequency of occurrence of the floating species, which remained approximately constant, and lower than 15% in several of the reservoir arms investigated.

Introduction

Aquatic macrophytes typically increase the complexity of environmental structure, and are of ecological importance in many freshwater ecosystems (Esteves, 1998). Stimulation of aquatic macrophyte colonization has been considered as a strategy to increase biodiversity and fisheries in reservoirs (Agostinho & Gomes, 1997). Although such a strategy has still not been adopted in Brazil, several native species of aquatic macrophytes grow in most large Brazilian reservoirs. Among them, some species (e.g., *Egeria densa*, *Egeria najas*, *Eichhornia crassipes*) have become a nuisance in the last decade, affecting leisure activities, navigation and energy production (Thomaz & Bini, 1999). Few studies on the ecology of these species have been undertaken in their native habitats, and relatively little is known about how these plants respond to management measures under such conditions.

The Itaipu Reservoir, a major impoundment of the Upper Paraná River, located on the Brazil–Paraguay border, is colonized by a rich assemblage of aquatic plants (62 species, including 42 euhydrophytes: Bini et al., 1999; Thomaz et al., 1999).

The water level of this reservoir is relatively constant, usually fluctuating less then 1.0 m year^{-1}. However, during the unusually dry year of 1999, the reservoir water level rapidly decreased after August 1999, reaching 5 m below its average water level (which is at 219–220 m above sea level: a.s.l.), from November 1999 to February 2000, after which it recovered again (Fig. 1). We took the opportunity to measure the effects of water level drawdown on the following attributes of three of the dominant and most widely distributed aquatic plant species in Itaipu: biomass, $Z_{\max}$ and frequency of ocurrence of the submerged *Egeria najas*; and biomass and area covered by the free-floating species *Eichhornia crassipes* and *Salvinia herzogii*. The frequency of occurrence of the first two species, and of *Salvinia* spp. (*Salvinia auriculata*, *S. herzogii* and *S. minima*: not distinguished during the survey) were also assessed in eight arms of the reservoir.

Study area

This investigation was carried out in the Itaipu Reservoir, a large (1350 km^2), deep (mean depth = 22.5 m), and dendritic reservoir located on the Upper Paraná River, along the Brazil–Paraguay border (between latitudes 24°05′S and 25° 33′ S; longitudes 54° 00′ W and 54° 37′ W). The theoretical residence time is ca. 40 days but only ca. 29 days in the main water body, being greater in the side-arms. Aquatic plants are restricted to the sheltered, shallow upper portions of inlets (arms) along the reservoir margins (Thomaz et al., 1999). Although the main body is oligo-mesotrophic, the arms fluctuate from mesotrophic to eutrophic, according to local phosphorus and nitrogen concentrations (Bini et al., 1999). A detailed map and limnological description can be found elsewhere (Bini et al., 1999; Thomaz et al., 1999).

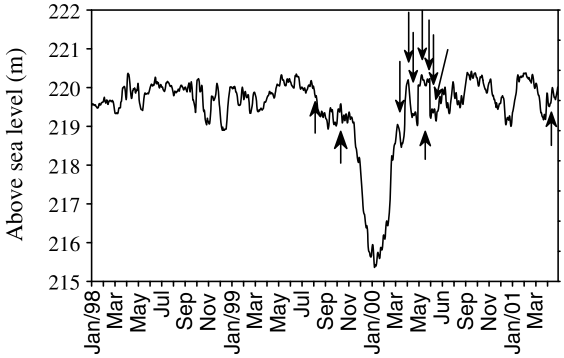

Figure 1. Water level fluctuation in the Itaipu Reservoir; the lower arrows indicate *Egeria najas* sampling dates and the upper ones sampling dates for *Eichhornia crassipes* and *Salvinia herzogii.*

Methods

E. najas biomass was measured on the following dates: (i) 9–11 August 1999: before the water level decreased; (ii) 7–9 October 1999: after the water level had decreased by about 0.5 m; (iii) 8–10 May 2000: 58 days after the water level had recovered from a 5 m reduction in level; and (iv) 15 April 2001: ca. 14 months after the water level had recovered to normal (Fig. 1). Samples of biomass were taken from 9 stands in three arms along the eastern (Brazilian) shore of the reservoir, formed by the rivers São Francisco Falso, São Francisco Verdadeiro and Ocoí.

Above-ground biomass was assessed at two depths (0.5 and 1.0 m below ambient water level, hereafter labelled ‘shallow’ and ‘deep’) in August 1999. From these depths, and in relation to the reservoir absolute water level, we estimated the sampling isobaths (in terms of height a.s.l.) colonized by *E. najas*. Subsequently samples were taken from the same isobaths, regardless of current water level. Samples were collected using a rake from quadrats of 0.5 × 0.5 m and returned to the lab, washed, and dried at 105 °C to constant weight.

For the first three dates (9–11 August 1999, 7–9 October 1999 and 8–10 May 2000), $Z_{\max}$ was recorded in 9, 10 and 7 sites, respectively. The isobaths colonized by *E. najas* were also estimated from $Z_{\max}$, which were obtained by sampling the stands with rakes down to the depth-limit of colonization.

The effect of water level on *E. najas* shallow and deep biomass was assessed by an Analysis of Variance with repeated measurements. The test was applied only to the results from August and October, since in May 2000 and April 2001 zero biomass was recorded. The effects of water level drawdown on $Z_{\max}$ was not assessed statistically because the data obtained in May 2000 were not from the same stands sampled previously (the reason for this was that plants had by then disappeared from the locations originally supporting the stands of *E. najas*).

Following the return of the water level to ca. 219 m a.s.l., in March 2000 (see Fig. 1), a rapid growth of floating plants (*E. crassipes* and *S. herzogii*) occurred in one reservoir arm (Ocoí). On 21 March 1999, floating plants were almost absent from this arm and this was considered as time zero ($t = 0$) for calculation of doubling times. The total biomass and surface area covered by both species were measured during development of the stand after $t = 40, 49, 67, 77, 91$ and 103 days. Biomass samples were collected inside a floating quadrat (0.5×0.5 m) in four different regions of the stand. Plant material was washed in the field and dried at 105 °C to constant weight. The stand limits were demarcated with a GPS (15 m positional accuracy) and plotted on a map (1:10 000 scale) to provide estimates of total surface area covered by floating vegetation.

Doubling times ($t_{\text{doub}} = \ln(2)/r$; where r = instantaneous growth rate) were measured for surface area (m^2) and total biomass (kg DW). Values of r were calculated by linear regression between time, in days, and ln of surface area covered by plants or ln of total biomass. Total biomass was estimated by multiplying surface covered by plants (m^2) by their biomass (kg DW m^{-2}).

The frequency of occurrence of *E. najas*, *E. crassipes* and *Salvinia* spp was assessed on a wider spatial scale in Itaipu. Since there are three species of *Salvinia* (*S. auriculata*, *S. herzogii* and *S. minima*) colonizing the reservoir, and they were not discriminated during the large scale surveys, their frequency of occurrence were considered together and the results are shown to the genus level. A total of 235 stands of aquatic macrophytes, distributed in the eight main arms were investigated in April 1999, that is, 4 months before the beginning of the water level decrease. The number of stands in which each species was present were used to estimate the frequencies of occurrence. These stands were demarcated by GPS and used in subsequent surveys, which were carried out in 111 stands in April 2000, in 229 stands in January 2001, and in 235 stands in June 2001 and January 2002. Thus, frequencies were obtained at about 1, 10, 15 and 21 months after the water level had recovered to normal.

Results

The response of *E. najas* biomass to water level fluctuation was highly significant, with different effects on the biomass at both depths, as can be seen by the significance of the time × depth interaction (Table 1). The shallow biomass had already decreased by October, after the initial 0.5 m reduction in water level (Fig. 1). Biomass at both depths dropped to zero in all 9 sites investigated after the 5.0 m water level reduction and no recovery was observed even 14 months after the water level had returned to normal (Fig. 2a).

The water level drawdown apparently also affected *E. najas* $Z_{\max}$, since this plant was found colonizing deeper isobaths, at least in some sites, after the water level had recovered (Fig. 2b). In other words, in some locations, *E. najas* had probably moved to deeper places in the reservoir, which were not colonized when the water level was high.

A conspicuous growth of *E. crassipes* and *S. herzogii* began to develop immediately after the water level of Itaipu had returned to normal (Figs 3a, b). These species covered an area of 17.7 ha in the Ocoí arm within 68 days, producing an estimated total maximum standing crop of 58.9 tonne (DW) 79 days after growth commenced. The fast growth is indicated by values of t_{doub} obtained in the initial stage of growth (first 40 days), which was of 2.3 days for surface area covered by both species, and of 2.9 and 3.2 days for total biomass of *S. herzogii* and *E. crassipes*, respectively.

Table 1. Results of ANOVA with repeated measurements to assess the effect of months (effect 1) in two depths (effect 2) on biomass of *E. najas*. Only August and October 1999 were considered in the analysis. Depth at which the biomass were measured were 0 and 0.5 m

Effects	d.f. effect	d.f. error	*F*	*p*
1	1	15	3.86	0.069
2	2	30	61.06	0.000
1*2	2	30	5.93	0.007

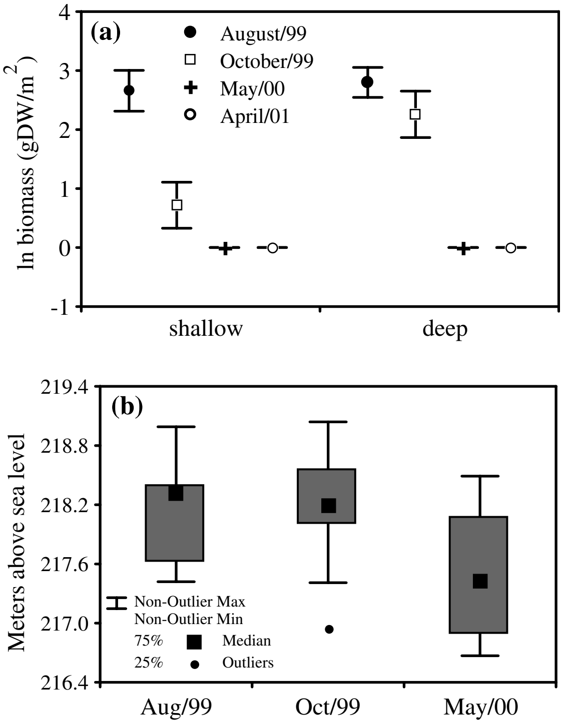

Figure 2. (a) Biomass of *Egeria najas* recorded from two depths before water level drowdown (August 2000), after an initial decrease of. 0.5 m (October 2000); and after water level had returned to normal following a 5 m decrease (May 2000 and April 2001). (b) *E. najas* $Z_{\max}$ recorded in August and October 1999 and May 2000.

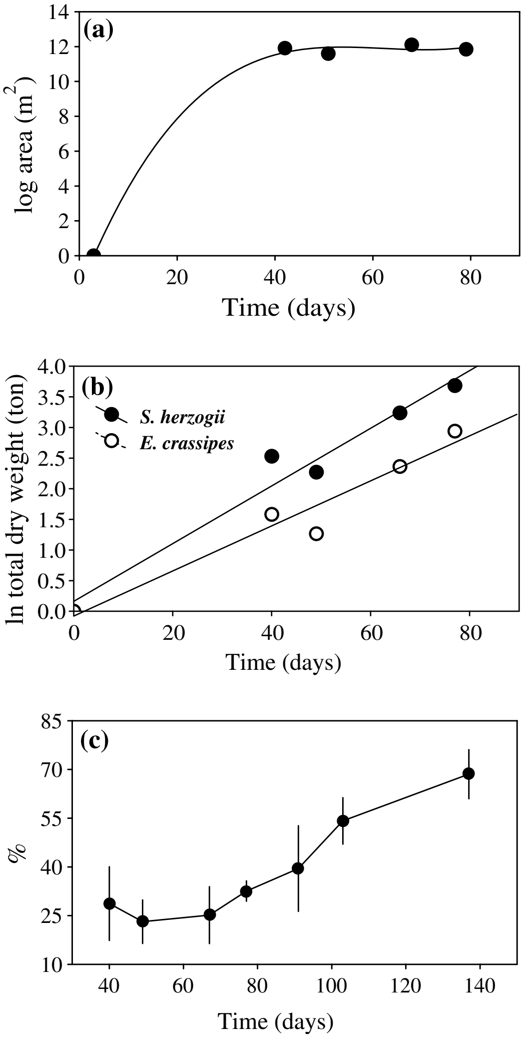

Figure 3. (a) Surface coverage of a mixed stand of *S. herzogii* and *E. crassipes*; (b) total biomass of *S. herzogii* and *E. crassipes* recorded after water level recovery; (c) percentage of *E. crassipes* biomass (± SE) in relation to total biomass (*S. herzogii* + *E. crassipes*) during the growth of both species. In (a) and (b), data are shown only until the 80th day because the stand dispersed after that, making it impossible to measure cover and total biomass.

In the first days of growth, free-floating *Pistia stratiotes* L. was observed at low biomass (less than 5%) inside the stand, but it was not found after the 50th day. During this phase, the stand was clearly dominated by *S. herzogii*, whose biomass accounted by 69% of the total biomass. Nevertheless, a succession was observed and *S. herzogii* was slowly being substituted by *E. crassipes* (Fig. 3c). The biomass of this species, in relation to the first, increased from 28% in the 40th day to 57% in the 103rd day (Fig. 3c).

The response of frequency of occurrence to water level drawdown was also different for each group of aquatic macrophytes. Before the water level drawdown, *E. najas* was found in 38% of the stands investigated, although a wide range of values occurred: from 17% in Passo Cuê arm to 87% in São João arm (Fig. 4a). Its occurrence decreased to 9% (from 0% at São Vicente to 13% at Ocoí arm) 1 month after water level had recovered and it remained at low levels even 10 months later, when *E. najas* was found in only 6% of the stands (from 0% at Passo Cuê, Pinto, São Francisco Verdadeiro and Arroio Guaçu arms to 20% at São João) (Fig. 4a). In general, the frequency of occurrence of this species recovered slowly in all arms, except at Arroio Guaçu, as can be seen from the data for June 2001 and January 2002 (Fig. 4a).

The frequencies of floating species were apparently less affected by water drawdown.

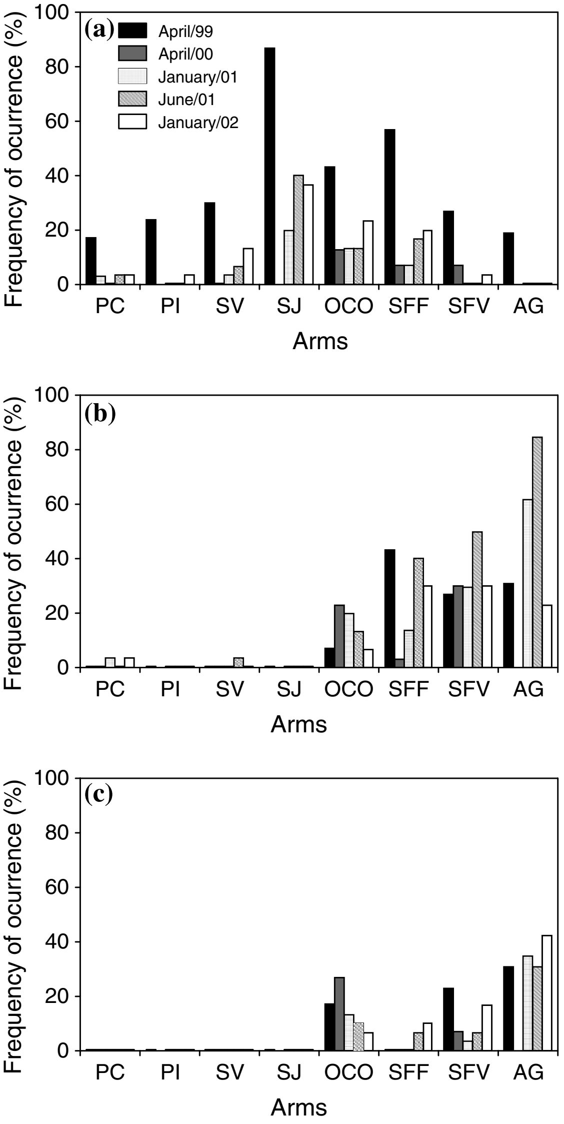

Figure 4. Frequency of occurrence of (a) *E. najas*, (b) *E. crassipes* and (c) *Salvinia* spp before (April 1999) and after the water level drawdown in Itaipu. Symbols on the horizontal axis represent arms of the Brazilian (left) shore of the reservoir. PC, Passo Cuê; PI, Pinto; SV, São Vicente; SJ, São João; OCO, Ocoí; SFF, São Francisco Falso; SFV, São Francisco Verdadeiro; AG, Arroio Guaçu.

Despite the variation among arms (Fig. 4b), *E. crassipes* was found in 13% of all stands in April 1999 and from 12 to 24% in the subsequent surveys. Similarly, *Salvinia* spp. occurred in 9% of the stands in April 1999 and 2000, and from 4 to 9% in the following surveys (Fig. 4c).

Discussion

Due to the high turbidity (Secchi disc depth usually < 1.5 m) of Itaipu, *E. najas* primarily colonizes water usually shallower than 3.0 m in this reservoir (Thomaz & Bini, 1999). Thus the decrease in water level of 5 m is highly likely to have affected the entire normal area of colonization of *E. najas*. Although *E najas* biomass did not recover in the 9 sites investigated, it was not eliminated from the reservoir, and it was found colonizing other sites 1 month after the water level recovery (see Fig. 4a).

Water level drawdown caused a decrease in the frequency of occurrence of *E. najas* (from 38% in April 1999 to less than 10% in April 2000 and January 2001). Although occurring at a reduced number of sites when compared to data from before the drawdown, the presence of *E. najas* 1 month after the water level had recovered indicates that this species has a strong ability to regenerate from the stress caused by short-term drought (up to 3 months). There are several possible explanations for this. First, propagules may remain viable in the dried-out sediments, germinating once the water level had recovered, and inundated the sediments again. In this case, it is probable that only the deeper sites (3–5 m), which were dry for a shorter period of time, supported viable propagules. Second, propagules exported to deeper sites before the drawdown would have encountered favourable conditions to germinate when such sites became shallower and more light reached the sediment. Third, plants already established may have moved to deeper sites, by lateral spreading, remaining there while the water level remained low. These last two possibilities are not mutually exclusive and they could also be an explanation for the lower isobaths (deeper water) colonized by *E. najas*, as indicated by $Z_{\max}$, after the water level recovery (see Fig. 2b). A fourth possible mode of recolonization, from distant sources upstream or downstream of the target areas (e.g., via current, wind or animal vectors) also represents a potential source of propagule reinvasion but is considered less likely to be of importance than recolonization from in-site or nearby sources of propagules.

Other studies have shown the importance of water level fluctuation in influencing submerged macrophyte dynamics in regulated lakes (e.g., Rørslett, 1984; Machena, 1989; Rørslett & Agami, 1987; Murphy et al., 1990; Thomaz et al., 2000). Besides enabling the colonization of deeper sites, as observed in Itaipu, short-term drops in water levels may also increase submerged plant biomass in deeper waters (Hudon, 1997). On the other hand, exposure to air may quickly kill the aboveground biomass of submerged species, and this can be the basis of a useful operational management strategy for controlling this group of plants in regulated waterbodies (Murphy & Pieterse, 1990). Our results indicate that sediment exposure for a period of 3 months substantially reduced *E. najas* biomass for at least 10 months and *E. najas* frequency for at least 21 months in Itaipu. Nevertheless, based on the data obtained in January 2002, it seems that the frequency of this species is slowly increasing again. Surveys are currently underway to determine the full recovery period needed by the plant.

Despite the negative effect of water level drawdown on biomass of *E. najas*, a fast growth of floating species was observed after water level recovery. The fast growth obtained in the initial stage of growth (t_{doub} = 2.9 and 3.2 days for total biomass of *S. herzogii* and *E. crassipes*, respectively, and 2.3 days for surface area covered by both species), are characteristic of the range obtained elsewhere in tropical regions for floating macrophytes (Talling & Lemoalle, 1998; Rubim & Camargo, 2001).

Rapid growth of floating species is usually associated with an increase in water nutrients (Gopal, 1987; Rubim & Camargo, 2001) and in the Itaipu Reservoir the distribution of these species is positively related to N and P concentrations (Bini et al., 1999). The fast growth observed in our investigation was probably related to an increase in phosphorus concentration in the water after the water level recovery, derived from P release from re-flooded hydrosoils, and decomposition of terrestrial vegetation that had developed on the exposed sediments. For nearly 3 months (November 1999 to January 2000) the shoreline hydrosoils of the reservoir were exposed to air (see Fig. 1) and terrestrial grasses grew over the exposed sediment. The flooding of these areas was followed by an increase in nutrients, especially phosphorus, the concentration of which increased from 24 μg l^{-1} in February to 71 μg l^{-1} in April. Low rainfall during this period (<140 mm in March and April 2000, compared to >300 mm in January and February 2000) was also important for accumulation of biomass in this arm, given that an increase in water turnover would have helped to disperse the biomass of floating species. As a consequence of the rapid growth of these floating plants, light probably limited algae development and algal blooms were not a feature in this arm during this period.

Our results suggest that the process of macrophyte recovery from medium to long-term exposure of the littoral zone to air, produced by water level drawdown, resembles the successional process usually observed in newly created tropical reservoirs. This often involves early development of floating species, in response to nutrient increases in the water column, followed by the development of a more-structured submerged community (Machena, 1989). Fast growths of floating plants, such as that observed in Itaipu following the water level recovery have been described during the filling phase of several tropical reservoirs (e.g., Esteves, 1998; Talling & Lemoalle, 1998; Walker et al., 1999; Thomaz & Bini, 1999).

On a wider spatial scale, our data for eight arms of Itaipu showed that the frequency of occurrence of submerged and floating species was differently affected by water level drawdown. While *E. najas* was consistently found in fewer stands after the water level recovery, *E. crassipes* and *Salvinia* spp frequencies remained about the same in the reservoir, although the occurrence of these species increased in some arms and decreased in others. Different from submerged species, massive losses of free floating species usually occur in response to increases in water turnover, as observed in Itaipu following the rains of June 2000. In this reservoir floating species found favourable habitats in areas protected from wind, which can explain the maintenance of their frequency, even after water level recovery, or fast growth, as observed in Ocoí arm.

The succession observed during the floating species growth phase in Itaipu showed an initial dominance by *S. herzogii*, following by elimination of *P. stratiotes*. After the 50th day, *S. herzogii* was gradually substituted by *E. crassipes*, which dominated the stand by the 103rd day. The high competitive ability of *E. crassipes* and *S. molesta* relative to *P. stratiotes* has been demonstrated in laboratory experiments by others (e.g., Agami & Reddy, 1990; Benassi & Camargo, 2000). Among the factors that caused this tendency along the succession, the initial biomass of each species is important (Benassi & Camargo, 2000). In addition, the initial fast growth of *S. herzogii* may be associated with its architecture, characterized by more horizontal than vertical growth. When conditions are favourable, this species can rapidly cover large surfaces. On the other hand, *E. crassipes* occurred in low densities from the beginning of the stand growth, but this plant initially grows upright rather than horizontally over the water surface. This architecture provides an exposed surface area for light capture and increases photosynthetic efficiency in a competitive situation (Reddy, 1988; Agami & Reddy, 1990). Thus, had not the stand been dispersed after 103 days, *E. crassipes* would probably have further increased its dominance over *S. herzogii*.

Despite the fast growth observed, the area colonized by floating species (ca. 0.2 km^2) represents less than 0.02% of Itaipu's total area. In addition, growth of these plants was observed in only one arm and the reasons why they did not grow in other arms are not clear, given that the water level drawdown would have affected the entire reservoir simultaneously.

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Populations of *Myriophyllum alterniflorum* L. as bioindicators of pollution in acidic to neutral rivers in the Limousin region

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Key words: *Myriophyllum alterniflorum*, bioindication, river, experimental enrichment, morphology, nitrate-reductase

Abstract

Myriophyllum alterniflorum D.C. (Haloragaceae) was studied in the acidic to neutral rivers Vienne and Gartempe (Limousin region, France). Two complementary levels of organisation were evaluated to determine their usefulness for pollution assessment: morphological traits of river populations, and eco-physiological responses to ammonium enrichment in indoor experiments. Inter-node length, stem diameter and leaf length were measured and their ratio calculated for 100 selected plants collected in 20 homogeneous areas. Using multidimensional analysis, their ecological significance for trophic assessment was demonstrated: stem diameter was linked to conductivity, and leaf length/stem diameter ratio to phosphates. Nitrate reductase activity was measured in three populations (upstream, median and downstream populations) after experimental enrichment with ammonium. Ammonium enrichment decreased activity starting at very low concentrations. However, inhibition levels depended on tested populations, with upstream populations being less sensitive due to natural ammonium content in water. The differences could be explained by an eco-adaptation to the trophic level of water. The relationships between the two levels of organisation are discussed. These results could help to distinguish different ecotypes using *Myriophyllum alterniflorum*.

Introduction

Increase and diversification of aquatic populations has led researchers and authorities to examine biological sentinels, such as macrophytes (plants visible to the naked eye), as a indicators of water quality degradation. Many studies have thoroughly characterized species ecology (Butcher, 1933; Haslam, 1978; Holmes & Whitton, 1977), plant communities (Kohler, 1971; Wiegleb, 1984; Newbold & Holmes, 1987; Haury & Muller, 1991; Riis et al., 2000) and created indices of environmental quality (Dawson et al., 1999; Haury et al., 2000). Differential reactivity between populations of the same species is an additional consideration. A population under environmental constraints can, therefore, develop specific morphological characteristics (Murphy & Ali, 1998; Demars & Harper, 1998) or show a contrasted physiological response, for example, resistance to ammonium pollution (Rolland et al., 1999). These two latter approaches were used to investigate *Myriophyllum alterniflorum* D.C., a species that grows in water with a low mineral content.

Study site

The study zone, as previously described (Chatenet et al., 2000), was situated in the Massif Central

Table 1. Linkage coefficients between variable groups of multiple factorial analysis (100 individuals).

	1	2	3	4
1: Plant morphology	**1.60**			
2: Water quality	0.28	**1.74**		
3: Substratum	0.36	0.29	**1.67**	
4: Runoff	0.11	0.14	0.48	**1.42**

Bold coefficient indicate the internal variability of the compartment; other coefficient are usual correlation ones; underlined ones point out the more significant ones.

eastern border, located in the Limousin region (France). Prospected catchment basins (Vienne and Gartempe) drain exclusively crystalline substrates. Water was generally oligotrophic, except downstream of urban areas where point source eutrophication was visible.

Materials and methods

Myriophyllum alterniflorum is a hydrophyte with submerged leaves, whorled by three or four, divided into more than eight segments and small flowers that appear above water. It is widely distributed in running water from the head of the catchment basin to downstream sections, in well illuminated and shallow (maximum 40 cm), sections of river.

A biometric study was performed after methodically sampling approximately ten urban areas (pollution of human origin). Twenty sites were designated: ten upstream and ten downstream from point source pollution. For each, five clumps of plants were sampled at random in homogeneous stream flow. The longest stem of each was used for further analysis (100 stems analysed for all sites). For each stem, the studied part was chosen between the upper third or quarter of each stem under the growth zone and without senescent leaves. The length (precision 0.001 mm) of five internodes, stem diameter and length of one of the four leaves of the upper node were measured with a slide calliper. Local environment (depth, light, flow conditions, granulometry of substratum and water chemistry) were described for homogeneous streams by using pre-established parameters (Ombredane et al., 1995). Morphological data were treated by multivariate analysis. Correlations were tested by variance analysis (Statistica logiciel©, ANOVA test).

The eco-physiological impact of pollution on *Myriophyllum alterniflorum* populations was evaluated by quantification of nitrate reductase activity (NRA). The principle is based on the blockage of nitrate reductase by anoxia and darkness. Nitrites accumulate in extracellular medium and are quantified by a colorimetric reaction (Griess reaction), as proposed by Robin et al. (1983) and adapted to aquatic plants by Rolland (1995). NRA was calculated based on concentration differences between incubated and control samples. According to the differential reactivity hypothesis of populations to ammonia pollution, spring time plant sampling was undertaken in upstream, median and downstream areas. Five days after acclimatization in a controlled environment (plexiglass aquaria filled with low mineral content aerated river water maintained at 18°C under horticulture neon lighting: 3000 lux; 14 h day/10 h night), plants were exposed to ammonia enrichment (0–5 mg l^{-1} N-NH_4). A one day exposure was selected following preliminary experiments to determine maximum inhibition of NRA. Statistical significance was evaluated by interaction variance analysis (Statistica logiciel©, ANOVA test).

Results

Morphological characteristics of Myriophyllum alterniflorum *and environment*

The results showed only small differences for diameter (1.09 ± 0.14 mm) and internode (6.22 ± 0.93 mm), but they were greater for leaves and leaf/stem diameter ratios. Preliminary multivariable analysis (Table 1, Fig. 1) showed that *Myriophyllum alterniflorum* morphology depended primarily on substrate granulometry, associated with flow conditions. Secondly, the increase in

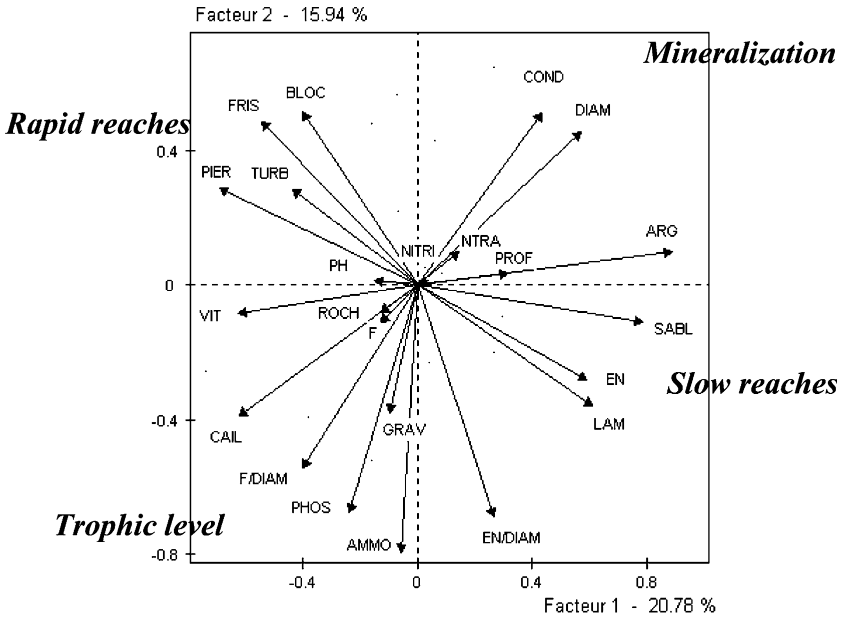

Figure 1. Distribution of environmental factors and morphological traits in the first multiple factorial analysis plane. (*Legend:* COND: conductivity, NTRI: nitrites, NTRA: nitrates, AMMO: ammonium, PHOS: orthophosphates, PH: pH, VIT: leakage rate, LAM: laminar runoff, FRIS: irregular runoff, TURB: turbulent runoff, ARG: clay, SABL: sand, GRAV: gravel, CAIL: flint, PIER: stone, BLOC: block, ROCH: rock, EN: internode, DIAM: stem diameter, F: leaf).

water trophic level (ammonium and phosphate factor) determined morphological traits, such as internode/diameter ratio. Conductivity also appeared determinant.

Variance analysis results are presented Fig. 2. Fine substrate and weakly running reaches (homogeneous stream flow) increased stem diameters and internode length. For water chemistry, there were clear correlations between conductivity and stem diameters and between orthophosphate content and leaf/stem diameter ratios (Table 2).

Nitrate reductase activity and ammonia enrichment in Myriophyllum alterniflorum

The results obtained from the three populations treated with two ranges of concentrations of ammonium (0–1 and 1–5 mg l^{-1}) and corresponding interaction variance analysis are summarized in Table 2. In median and downstream populations even very low concentrations (0.1 mg l^{-1} N-NH_4) induced significant NRA inhibition which was more pronounced in downstream populations.

Upstream populations reacted less consistently. Analysis of variance validated the difference between treated and control plants but the pollutant effect on NRA was not significant.

Discussion

Use of biological traits in Myriophyllum alterniflorum

Provided simple traits are pertinent to assess ecological parameters, ratios between organs seem more accurate; it means that organs are not independent and that it could be useful to model these relationships between organs. That *Myriophyllum alterniflorum* morphology depends primarily on substrate granulometry has been already shown by Aiken (1981) who studied very variable substrata (from peat to sand). In Limousin rivers, with much more homogeneous sediments, such a gradient appeared too, indicating that measurements on sediments should be undertaken when studying precise plant ecology. The increase in stem diameter and internode lengths with finer substrates and slow flowing reaches revealed the absence of shearing forces related to current as well as elevated turbidity and depth, which induced plant

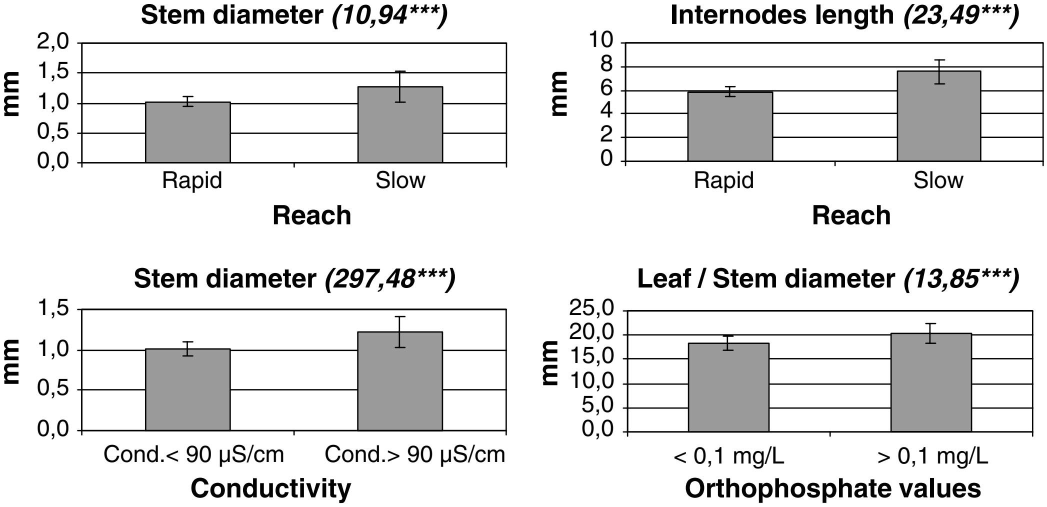

Figure 2. Variation of morphological traits under environmental conditions (F: Fisher test, *** $p < 0.001$).

Table 2. Results of ANOVA test (interaction variance analysis)

Pollution :	Upstream populations		Median populations		Downstream populations	
	0–1 mg l^{-1}	1–5 mg l^{-1}	0–1 mg l^{-1}	1–5 mg l^{-1}	0–1 mg l^{-1}	1–5 mg l^{-1}
Control-incubated difference	529.7 ***	206.5 ***	329.8 ***	124.7 ***	309.6 ***	251.7 ***
Concentration effect	16.0 ***	0.3 N.S	13.9 ***	8.3 ***	43.5 ***	12.5 ***
Interaction effect	1.2 NS	4.8 **	13.0 ***	9.7 ***	29.0 ***	12.7 ***

F: Fisher test (F ratio) ,*** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.1$ or NS: $p > 0.1$.

chlorosis by restricting light penetration. The response to trophic level can be explained by stimulation of plant growth when nutrient quantities increase. In any case, these biological traits should be measured as a complement to plant surveys to obtain a better diagnosis of rivers reaches.

Physiological responses of Myriophyllum alterniflorum

Differences in Nitrate Reductase Activity between populations can be explained by the physical chemistry of the natural environment. Upstream populations grow in water that is naturally rich in ammonium. This could result in reduced reactivity towards artificial ammonia pollution. On the other hand, downstream populations live in nitrate rich eutrophic waters. This could stimulate NRA physiologically and amplify ammonia inhibition in a controlled environment. These results are comparable with those obtained with *Elodea canadensis*, where plant reactivity was different according to harvesting area (Rolland et al., 1999). In our study as well, *Myriophyllum alterniflorum* reactions differed in the same catchment area. These different populations should be checked to point out either differences in physiological plasticity only or genetic differentiation leading to true ecotypes.

Conclusion and perspectives

This study shows that morphological or eco-physiological variability between different populations of the same species can provide information concerning water quality. Other traits such as branching, adventitious roots, chlorophyll content could also be considered as indicative for stress due to pollution and/or eutrophication and to perturbation such flooding or drying. In bioindication systems, it would be fitting to consider the ecological form and not only species in order to specify the diagnosis. Furthermore, these ecotypes could be used to precisely quantify Nitrate Reductase Activity by culturing cells, which would provide year-round homogeneous living material.

Due to the determining influence of physical factors (substratum, light, current), Nitrate Reductase Activity results were not reproducible in plants immediately after their collection. A rigorous method for sampling must be perfected in order to reduce environmental influences on morphological analysis and biomarker expression. Exact relationships between morphology or physiology and physico-chemical quality of the water could then be established.

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Aquatic plant bioassays used in the assessment of water quality in German rivers

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Key words: macrophytes, *Lemna minor*, bioassay, water quality, sediments, sediment toxicity assessment

Abstract

A novel aquatic bioassay with *Lemna minor* (duckweed), a representative of higher plants, was applied to pore waters (interstitial waters) from river sediments. The results have been used for the ecotoxicological assessment of sediments in several German rivers. The aim of the present study is to demonstrate the usefulness of a macrophyte bioassay, as a supplement to the standardised algae bioassays, with regard to the ecotoxicological assessment of aquatic systems. Results relating to the development of a sediment contact test (using the whole sediment) with *Lemna minor* are also presented.

Introduction

Chemical and biological testing methods are used for pollution analysis of wastewater, surface water, landfill leachate, etc., but also for sediments and dredged material. Chemical testing methods, like priority pollutants analysis and non-target screening, give information regarding the quality and quantity of pollution. However, not all types of contaminants can be identified. Biological testing methods can give a qualitative description of the presence and strength of toxicity, even though the identification of the pollutants is not possible. Utilisation of several different toxicity tests is necessary to estimate potential hazard. Aquatic organisms of all three trophic levels (producers, consumers, decomposers) have long been used in ecotoxicological evaluation of pollution in aquatic systems and their sediments. To date, the standard procedure applies algae (*Desmodesmus* (*Scenedesmus*) *subspicatus*, German standard DIN 38412 L33, 1991), bacteria (*Vibrio fischeri*, EN ISO 11348-3, 1998) and crustacea (*Daphnia magna*, German standard DIN 38412 L30, 1989). For some years, international scientific fora have discussed whether unicellular algae, being structurally and systematically simple organisms, are suitable representatives of all autotrophic life forms (e.g. Wang & Freemark, 1995; Fairchild et al., 1998; Praszczyk et al., 1999). Moreover, the European Water Framework Directive (EU-WRRL, 2000) considers macrophytes as indicators of water pollution at the same level as macrobenthos, fish and phytoplankton.

The estimation of the toxicity potential of the bio-available contaminants in sediments takes place at different levels (whole sediment, pore water, aqueous elutriate). In the routine procedure for each sample, the pore water and an elutriate produced particularly for this purpose were examined with different standardised bioassays. The biological effectiveness of sediment-bound pollutants should be examined by sediment contact tests. To date, no standardised (or at least widely accepted) contact tests (bulk sediment test) using higher plants for the assessment of aquatic sediments have been developed. The phytotoxic effects are up to now assessed only by conclusions derived from aquatic tests. Sediment contact tests are necessary to assess the ecotoxicological potential of sediments. They show high ecological relevance, reproductive effects can be detected, and several specific exposure pathways can be included. Additionally, in the context of the EC regulations on hazardous substances, the integration of a primary producer (plant) exposed by its roots in a test battery is demanded (Riedhammer et al., 1999).

This paper presents the application of a novel aquatic bioassay with *Lemna minor*, a representative of higher plants, to pore waters of river sediments. The results have been used for the assessment of sediments of several German rivers. The aim of the present study was to demonstrate the necessity and value of a macrophyte bioassay, as a supplement to algae bioassays, with regard to the assessment of aquatic systems. In addition to the aquatic plant tests, first approach to sediment contact tests with *Lemna minor* were developed and their results presented here.

Material and methods

Sediment samples

The sediment samples were collected from waters in the period from 1997 to 2002 with a Van Veen grab sampler from the sediment surface (0–10 cm), and with a corer from the deeper sediment (10–200 cm). The sampling sites are specified in Table 1.

Pore water

The pore water was extracted by centrifuging the sediments (17 000 *g*; 20 min; 281 K).

Aquatic Lemna test (duckweed test)

The aquatic duckweed test was performed following a procedure described by Feiler & Krebs (1999).

Algae test

The algae test was made according to DIN 38412 L33 (1991), using *Desmodesmus* (*Scenedesmus*) *subspicatus*.

Parameter

For the determination of growth changes (%), the number of leaf segments (fronds) in the aquatic duckweed test and the fluorescence in the algae test, were used as measure of biomass.

Sediment contact test

Sediment contact tests were performed according to Feiler & Krebs (2001). The test organism used was *Lemna minor*. The parameter of growth yield was the number of fronds. Lighting and temperature as well as evaluation criteria were the same as in the aquatic duckweed test. During sediment contact tests, the plants were in direct contact with the whole sediment.

Table 1. Description of the sampling sites

River basin	River km*	Location (acronym)	Sediment sample	Sampling time
Moselle, barrage	195,88	Trier (T)	Surface	June 1997
Moselle, marina	197,5	Trier-Monaise (TM)	Core	October 1997
Saar, barrage	66,5	Lisdorf (L)	Surface	August 1997
Saar, old arm	68,8	Wadgassen (W)	Core	October 1997
Elbe, groyne field	319,4	Magdeburg (M)	Surface	April 2001
Elbe, old arm	252,0	Klieken (K)	Surface	April 2001
Oder, groyne field	655,0	Hohenwutzen (H)	Surface	April 2001
Rhine, barrage	333,5	Iffezheim (I)	Core	May 2001

*Kilometer mileage of the Federal Waterways.

Table 2. Ecotoxicological classification of sediments according to Krebs (2000)

Highest dilution level without effect	Dilution factor	pT-value	Toxicity classes (7-level system)	Toxicity categories (4-level assessment system)
Original sample	2^{0}	0	0	Blue
1:2	2^{-1}	1	I	Green
1:4	2^{-2}	2	II	
1:8	2^{-3}	3	III	Yellow
1:16	2^{-4}	4	IV	
1:32	2^{-5}	5	V	Red
≤(1:64)	$\leq 2^{-6}$	≥6	VI	

pT-value method

The sediment assessment followed the pT-value method according to Krebs (2000, 2001). The pT-value (*potentia toxicologiae*) is the negative binary logarithm of the first non-toxic dilution factor in a dilution series in geometric sequence with a dilution factor of two. Accordingly, the pT-value gives an indication of how many times a sample must be diluted in the ratio 1:2 to reach a stage of no toxic effect. Non-toxicity was assumed below a threshold of 20% inhibition. The pT-value reached with the most sensitive organism in a test battery is used for ecotoxicological classification of waters and sediments. The toxicity classes are marked by Roman numerals (Table 2).

Results and discussion

Application of the aquatic Lemna test for the evaluation of the toxicity potential of sediments of different German river basins

Sediments from the rivers Moselle, Saar, Elbe, Oder, and Rhine were examined for their ecotoxicological potential. The aquatic duckweed test was employed, along with the methods of the standard test battery (standardised DIN tests using algae, luminescent bacteria, and *Daphnia*). In the aquatic duckweed test, the different growth behaviour of *Lemna minor* indicated the presence of contaminants (Figs. 1 and 2). Typical reactions of *Lemna minor* indicating toxicity were: decrease of fronds (low growth rate), bleaching of fronds (loss of chlorophyll, inhibition of photosynthesis), necrosis, declining colonies, small daughter fronds, loss of or very long roots, and/or gibbous form of the fronds. By performing dilution series, the pT-value method could be used to determine the toxicity potential of sediment (Table 2, Fig. 2).

Figures 1 and 2 show examples selected as representative results from the aquatic duckweed tests in pore waters from surface sediments. These are compared with the respective results of the algae tests. Striking differences were observed between the results of the duckweed tests and the algae tests with pore waters. While no toxic effect at all was noted in the samples from Trier (T) by the algae test, the duckweed test showed a high growth inhibition (Fig. 1). Further, after considering the dilutions (pT-value method), a higher pT-value in the duckweed test was determined (Fig. 2). In the samples from Lisdorf (L), Klieken (K) and Hohenwutzen (H) the differences in growth changes between algae and *Lemna* were not so distinct in the original solution (Fig. 1). The inhibition in the aquatic duckweed test in the dilution series, however, resulted in a pT-value of 2 in the samples from Lisdorf and Klieken and pT 3

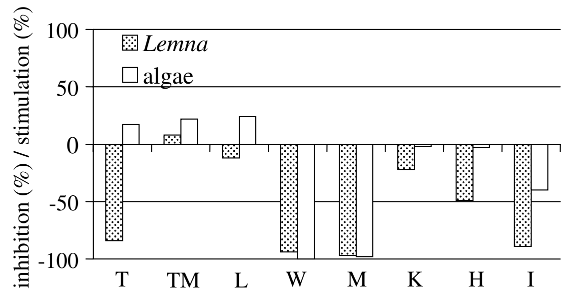

Figure 1. Comparison of the results from pore water studies using the aquatic duckweed test (*Lemna minor*) with those of the algae test (*Desmodesmus subspicatus*). Growth changes (%) in pore water (Abbreviations see Table 1).

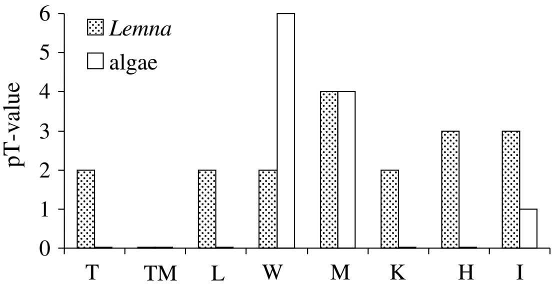

Figure 2. Comparison of the pT-values from pore water studies using the aquatic duckweed test (*Lemna minor*) with those of the algae test (*Desmodesmus subspicatus*) (Abbreviations see Table 1).

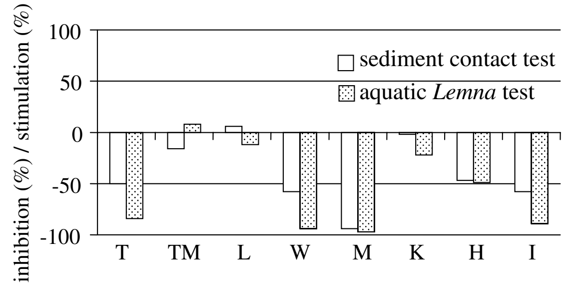

Figure 3. Comparison of sediment contact tests (bulk sediment test) with the aquatic duckweed test in pore water (Abbreviations see Table 1).

in those from Hohenwutzen (Fig. 2). In all four cases, the duckweed test was highly sensitive, in contrast to the algae test (pT 0). The results of the sediment samples from Iffezheim (I) were markedly different. Here, the sensitivity of *Lemna* resulted in a pT-value of 3 vs pT 1 in the algae test. Another noteworthy difference occurred in the tests with the sediment core from the old arm of the River Saar at Wadgassen (W). Here, the pore water produced in both tests nearly the same inhibition percentage (Fig. 1). In the dilution series, however, the inhibiting effect persisted much longer in the algae test (pT 6), indicating an extremely high toxic load (Fig. 2). Inhibition in the duckweed test resulted in a pT-value of 2. An example where both tests show the same results is demonstrated by the sample from Magdeburg (M). Both tests yielded a high growth inhibition and high pT-values indicating a distinct toxic load in the sediment from Magdeburg.

These results showed that using the aquatic duckweed test, different toxicity classes could be distinguished. The comparison of the duckweed and algae tests revealed differences between both tests, showing that the algae test was unable to detect all contaminants that are detrimental to higher plants. This proves that one test alone does not indicate all phytotoxic contaminants. Ultimately, the test battery should therefore be supplemented by a plant bioassay.

Sediment contact tests with higher plants

In addition to the aquatic plant test, initial steps using sediment contact tests with *Lemna minor* were developed. Figure 3 presents selected representative results from the sediment contact tests with *Lemna minor* on material from the rivers Moselle, Saar, Elbe, Oder, and Rhine, compared with the results of the respective pore water studies with *Lemna minor*. The findings show that *Lemna minor* is able to grow in direct contact with the whole sediment. Distinct differences in the growth behaviour were observed, indicating sediment contamination. Significant toxic effects occurred in the contact tests for those samples for which strong growth inhibitions were also measured in the aquatic tests. Only in one case, in the sample from Trier-Monaise (TM), was the sediment contact test more sensitive than the aquatic test. The results achieved so far suggest that the aquatic duckweed test indicates contamination with the same sensitivity or higher than the sediment contact test. Despite this unexpected finding, it appears reasonable to integrate the sediment contact test in monitoring programmes because of its potential to respond to adsorbed contaminants and thus its ecological relevance.

Conclusion

The findings suggest that an extension of the test battery, using macrophyte bioassays, may be necessary. A research project (BMBF 02WU0240) is attempting to develop a sediment contact test using higher plants as indicators. As with the available duckweed test for the studies of the aqueous phase (meanwhile in the standardisation procedure for ISO/CN 20079), a macrophytic sediment contact test would supplement the existing concept of sediment assessment (Chapman, 1986; Ahlf, 1995; Heininger et al., 1998) by introducing a further component for the indication of phytotoxic effects in whole sediment samples.

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The relationship between *Callitriche* L. clones and environmental variables using genotyping

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Key words: *Callitriche*, environment, monitoring, multivariate analysis, RAPD, water quality

Abstract

The genetic variation and clonality in *Callitriche* L. populations from 16 headwater rivers in Belgium were determined by random amplified polymorphic DNA (RAPD). It was shown that samples of morphological *C. obtusangula* can be separated into at least two genetically distinct groups. Both gene pools occurred within each considered river basin, i.e. Nete and Meuse. A multivariate analysis showed that the variability among different clones, as characterized by their genotypes, can be explained through ecological indicator values such as diatom saprobity and environmental variables related to water quality type. This study showed that multilocus genotypes or gene pools might have a non-random distribution in the rivers and in their particular environments.

Introduction

Species of *Callitriche* L. (Callitrichaceae) exhibit considerable diversity in chromosome numbers, pollination systems and ecology. Most species are amphibious and the vegetative plasticity of the leaf shape as well as the absence of mature fruits, render taxonomic identification difficult. Aspects of the morphological and chromosomal variation were studied by Schotsman (1967, 1982). In running water, clonal patches of most *Callitriche* species possess floating rosettes of spatulate leaves that develop when stems reach the surface. However, submerged leaves and even all leaves on a plants can be linear shaped. Although *Callitriche* taxa are widespread in rivers of various water qualities and often represent the only tolerant macrophyte in more polluted waters, this vegetative plasticity limits the practical use of *Callitriche* taxa as bio-indicators. A phylogenetic analysis at species level (Philbrick & Les, 2000) using morphological character states as well as maternally inherited chloroplast DNA (sequences of *rbcL*) resulted in weak differentiation of, e.g. *C. platycarpa* Kütz. from *C. stagnalis* Scop., *C. hamulata* Kütz. Ex Koch from *C. brutia* Petagna but in a clear separation of *C. hermaphroditica* L. whereas *C. obtusangula* Le Gall. was not considered. Identification at species level is a necessity for field monitoring, however, DNA sequencing is more laborious than the amplification of random amplified DNA products as obtained from random amplified polymorphic DNA (RAPD) or amplified fragment length polymorphism (AFLP). The advantage of the latter techniques is their relevance at different hierarchical levels from the individual clone in populations to the species level. RAPD as a tool was used in several macrophytes to clarify the taxonomic status, to assess the degree of clonality and expansion of clones (Vanderpoorten 1999; Keller 2000; Koppitz & Kühl 2000), to detect hybrids (De Greef & Triest 1999) or to identify origins of introduced weeds (Hofstra et al. 2000).

The objective of this study was to investigate the genotypes of *Callitriche* populations in headwaters of rivers in Flanders (Belgium) and to estimate the clonality among headwaters from two basins (Nete and Meuse) as well as the relationship of the clones and their genotypes with environmental variables.

Material and methods

A total of 25 *Callitriche* populations from 16 headwater rivers in Flanders were sampled for DNA extraction and PCR-based analysis of the following RAPD primers: OPA4, OPA5, OPO6, OPO7 and OPO19 (amplification products for each primer are abbreviated as A4a, A4b, etc. in Figure 2). All steps of the RAPD analysis were performed as described in De Greef & Triest (1999) for *Scirpus*. The taxonomic identity on basis of leaf morphology was assumed to be *C. obtusangula* for 15 rivers and *C. stagnalis* for one river (V47).

The presence and absence of amplified products after RAPD were scored for multivariate analysis with NTSYSpc 2.1 (principal coordinate analysis, PCOORDA, using the simple matching coefficient; Neighbour Joining tree using Manhattan distance coefficient). Water quality variables were temperature, pH, conductivity, %O2, DO, BOD, alkalinity, T-hardness, Ca-hardness, Mg-hardness, Si, Cl, *o*-PO_4, TP, NO_2, NO_3, NH_4. Substrate quality variables were T-Kjehldal N, TP and particle sizes in six classes. Water and substrate quality variables were measured after American Public Health Association (1989) during a sampling campaign in September 1999. Habitat descriptors were indices of diverse organisms. For the macrophyte vegetations, indices were calculated as weighted means after Haury et al., (1996), after Ellenberg et al., (1992) using *N*-values and thereof derived British modified *N*-values and *R*-values (Hill et al. 1999). Other indices were based on diatoms (a saprobity value after Van Dam et al. 1994), the Belgian Biotic index on macroinvertebrates and the Flemish index of biotic integrity on fishes (unpublished). Correlations (Pearsons product-moment) between the first three axes of the PCOORDA and environmental variables (chemical variables of water and substrates; habitat descriptions) were done with STATISTICA and multivariate analysis of genotypes and environmental variables with CANOCO. A canonical correspondence analysis (CCA) was performed with forward selection using Monte Carlo permutation test, after checking the gradient length in a detrended correspondence analysis (DCA).

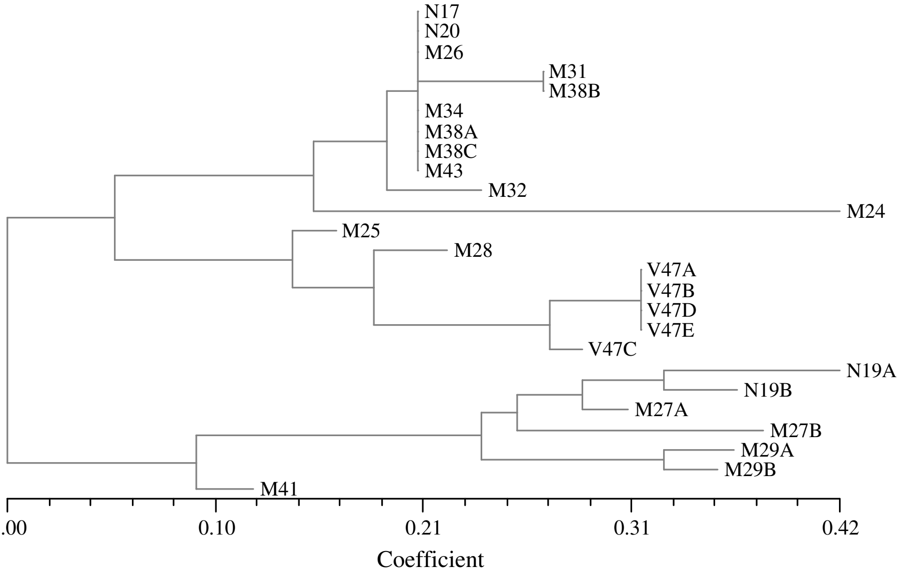

Figure 1. Neighbour Joining Tree (Manhattan distances) of RAPD genotypes in *Callitriche* populations, indicating clonal diversity.

Results

The 25 *Callitriche* populations revealed clonality and could be reduced to 21 samples for further data analysis using a selection of 16 diagnostic amplified DNA products. A Neighbour Joining tree (Fig. 1) combined with a CCA (Fig. 2) revealed at least three groups with a separation along the first axis of *Callitriche* from the 'Warmbeek' (M27, M29), 'Achterste Nete' (N19) and another group of clones from as well the Nete basin (N17, N20) as the Meuse basin (M26, M31, M34, M38, M43). Along the second axis are the related clones from more alkaline water in the 'Voer' (V47). The clustering tree placed emphasis on separation of single clones but a similar grouping was obtained than with a CCA.

The correlation of multilocus genotypes with the environmental variables (Table 1) revealed a highly significant relationship of the first axis (PC1) with the saprobity of the water as was estimated from benthic diatom assemblages. A higher number of significant relationships of water and soil variables with PC2 is due to the limited number of samples from more alkaline water (PC1).

To investigate further the genotype-environment relationship, a DCA that revealed a unimodal distribution (gradient length of 2.7) was followed by a CCA (Fig. 2). This direct gradient analysis showed the following significant variables: the saprobity index (as estimated by diatom assemblages), the index of biotic integrity (based on fishes), total Kjeldahl nitrogen of the sediment, alkalinity, sediment particle size and macrophyte scores (modified Ellenberg *R*-values of UK and the scores as presented for France by Haury et al., 1996). The cumulative percentage variances of the genotypes-environment relation were 41, 68 and 86% for axes 1–3.

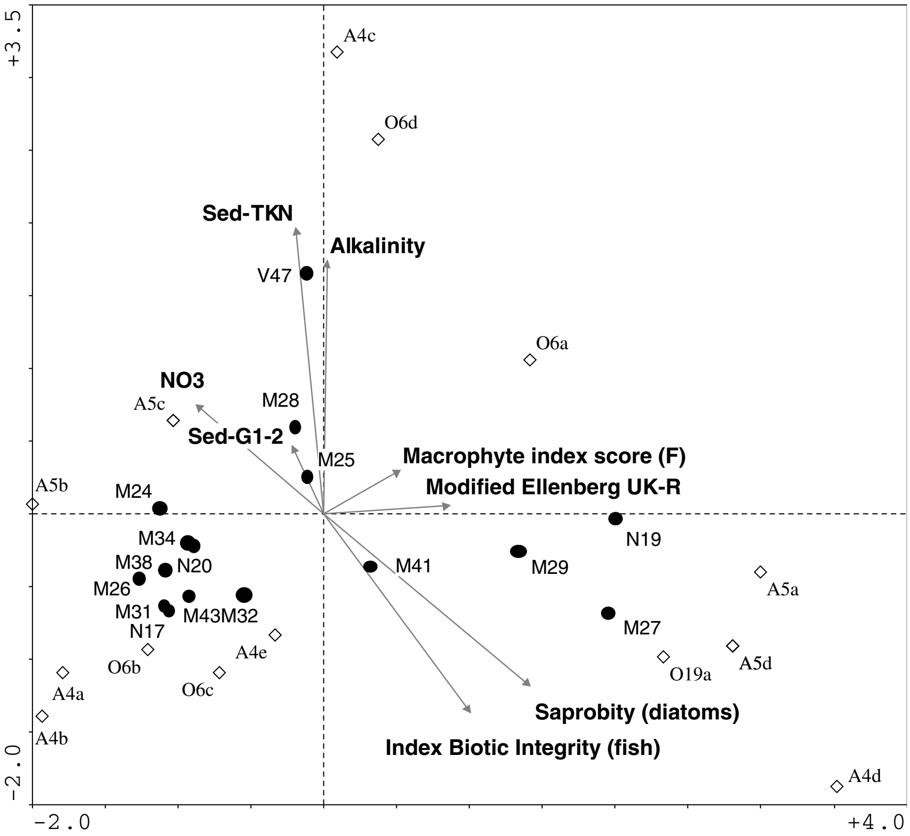

Figure 2. Canonical correspondence analysis (CCA) of RAPD genotypes in *Callitriche* populations and environmental variables (water and soil quality and ecological indicator values).

Table 1. Correlations and their significance level of the RAPD based principal coordinate axes (PC1, 2 and 3) with environmental parameters

	PC1	PC2	PC3		PC1	PC2	PC3
Conductivity	– p, ns	−0.67 $p = 0.000$	–	Sed-TKN	–	−0.9 $p = 0.000$	–
Alkalinity	–	−0.76 $p = 0.000$	–	Sed-TP	–	−0.46 $p = 0.023$	–
T-hardness	–	−0.81 $p = 0.000$	–	Sed-G > 2mm	0.4 $p = 0.050$	–	–
Ca-hardness	–		0.41 $p = 0.044$	UK-N	–	–	0.69 $p = 0.000$
Mg-hardness	–	−0.79 $p = 0.000$	–	Macro (F)	–	–	−0.6 $p = 0.002$
Si	–	−0.75 $p = 0.000$	–	UK-R	–	–	0.52 $p = 0.008$
Cl	–	0.69 $p = 0.000$	–	Nr Fish	–	0.47 $p = 0.019$	–
o-PO4	–	−0.69 $p = 0.000$	–	Nr Macroinv	–	–	–
TP	–	−0.79 $p = 0.000$	–	Saprobity	−0.53 $p = 0.008$	0.69 $p = 0.000$	–
NO3	–	−0.42 $p = 0.039$	–	IBI score	–	0.7 $p = 0.000$	–

Discussion and conclusion

Callitriche populations from rivers are difficult to identify morphologically to the species level when flowering structures or fruits are not available during most of the season and therefore are considered less suitable for macrophyte monitoring. Random DNA fragments reveal clones within and among rivers, as well as their degree of diversity. This study showed that multilocus genotypes or gene pools might have a non-random distribution in the rivers and in the particular environments. However, the observation that there is no clear relationship along a first axis (PC1) between a gene pool and a particular water or soil quality parameter is compensated by the highly significant relationship with more general indicators of ecological quality such as the diatom saprobity index and fish index. Indeed, these indices integrate water and habitat quality over longer periods and are an advantage over the punctual water quality estimations. Therefore, the latter might not be sensitive enough to elucidate relationships of gene pools with the environment. Nevertheless, genotypes from harder waters showed clear differences along the second axis (PC2) suggesting that the degree of alkalinity can be an important factor for understanding the distribution of genotypes and taxa. Whether the distribution of *Callitriche* genotypes in rivers of different water quality are determined primarily by their random clonal spread, rather than being the result of a selection of adapted genotypes through the environment is yet unclear. In general, RAPD-based values for between-population diversity increases with increasing distributional range (Nybom & Bartish 2000). In this study, the *Callitriche* populations did not cover all taxa or all biogeographical regions in Belgium. However, it was shown that samples of morphological *C. obtusangula*, can be separated in at least two genetically distinct groups. Both gene pools occurred within each considered river basin, i.e. Nete and Meuse. Samples from a larger geographical area, including more pronounced differences in water quality types will lead to a better understanding of the ecological relevance of clonal and non-clonal distribution patterns.

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Assessing and predicting the success of *Najas flexilis* (Willd.) Rostk. & Schmidt, a rare European aquatic macrophyte, in relation to lake environmental conditions

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Key words: *Najas flexilis*, plant traits, fitness, macrophyte, conservation, lake

Abstract

Najas flexilis (Willd.) Rostk. & Schmidt is a submerged annual macrophyte, rare in Europe, which is protected under the EC Habitats Directive. *N. flexilis* appears to be decreasing in the British Isles, its main stronghold in Europe. We outline the environmental conditions required for *N. flexilis* growth, comparing between present and recently extinct sites for the plant. Plant traits (leaf area/shoot length; and reproductive number/shoot length) can be used to assess *N. flexilis* population success, and models are produced that can predict this. Both the comparison between present and extinct sites, and the models, suggest that eutrophication and acidification of lakes are the main threats to *N. flexilis*. Acidification appears to reduce the ability of *N. flexilis* to produce seeds (potentially fatal for an annual). On the other hand, eutrophication leads to conditions where *N. flexilis*, an obligate carbon dioxide utiliser, cannot photosynthesise due to the predominance of bicarbonate rather than dissolved carbon dioxide in lake water.

Introduction

Najas flexilis (Willd.) Rostk. & Schmidt is a rooted submerged macrophyte, of elodeid growth form (Den Hartog & Segal, 1964). However, unlike many other elodeids *N. flexilis* never reaches the water surface to flower and is relatively short (rarely >0.3 m in height). *N. flexilis* is an annual and has not been reported to possess any method of vegetative reproduction (Hutchinson, 1975). If there is no long-term seed bank this reliance on yearly seed production could have important repercussions for *N. flexilis* conservation in many of its threatened European habitats.

N. flexilis grows in mesotrophic lakes and is an obligate dissolved carbon dioxide utiliser (Hough & Wetzel, 1978; Hough & Fornwall, 1988). It has been reported from depths of 0.2–14.0 m (Pip and Simmons, 1986). The phosphorus requirement of the plant is supplied primarily from the sediment (Carignan and Kalff, 1980; Moeller et al., 1988).

N. flexilis has a circumpolar, boreal-montane distribution, but is found much more frequently in North America than in Europe and Asia (Hultén & Fries, 1986; Preston & Hill, 1997). It is rare enough in Europe to be listed in Annexes II and IV of the EC Habitats Directive (92/43EEC). *N. flexilis* has its European stronghold in Scotland, with 35 lake sites at which positive records have been made during 1998–2000 ("present" sites). There are eight sites in Scotland and Ireland where the plant has been recorded in the last century, but searches during 1998–2000 have failed to find it ("extinct" sites).

Due to the high conservation status of the plant and this apparent trend of extinctions, knowledge of the environmental conditions controlling plant success is required if its future conservation is to succeed. An effective method of assessing plant success within a site is also required, so that at-risk sites can be identified and appropriate action taken to prevent further extinctions.

Methods

N. flexilis *presence and absence*

During the course of this study, 42 lakes were investigated in Scotland (during July–September of the years 1998–2000), together with 10 lakes in Ireland (August 2000). This included all the post-1980 records for *N. flexilis* in Scotland along with all mainland Scotland pre-1980 records and a geographically extensive range of the Irish sites. At each site *N. flexilis* presence or absence was recorded, together with a set of environmental factors likely to be relevant to the ecology of *N. flexilis* in Britain and Ireland. *N. flexilis* was searched for using snorkel, boat and shoreline searches.

Conductivity and pH were measured with Hanna portable meters, and photosynthetically active radiation (PAR) was measured with a Skye SKP210 twin-sensor meter, on site. The PAR readings were taken at the water surface and 0.5 m beneath the water surface. From these readings, the light extinction coefficient was calculated using the standard formula (Kirk, 1994). One water sample per lake was collected at a sub-sample of the sites ($n = 35$) and analysed by the Scottish Environmental Protection Agency for the environmental variables listed in Table 1. These measurements were made after filtration, to exclude particulates. Carbon dioxide concentration was estimated using the method in Wurts & Durborow (1992). One sediment sample per lake (collected by Ekman grab) was taken in the immediate vicinity of *N. flexilis* stands (present lakes), or at a randomly selected deep-water location (extinct lakes). All samples were collected from approximately the same depth in the sediment (approx. 0–5 cm). The sediment was then dried, ground and sieved to remove solid particulates greater than 2 mm in size, then analysed by the Scottish Agricultural College for the environmental variables listed in Table 1.

The environmental conditions at present sites (where *N. flexilis* was found during 1998–2000) were compared to extinct sites (where *N. flexilis* was not found during 1998–2000) to determine the environmental tolerances of the species. The extinct sites were split into two groups. The first group contains the more oligotrophic extinct sites, and the second the more eutrophic extinct sites. The environmental variables of both the extinct groups were compared to those where *N. flexilis* was present, using a Mann–Whitney *U* test. This test was chosen due to the small sample size in the more oligotrophic extinct group, and the non-parametric nature of the data (due to the detection limit imposed on many of the environmental variables).

N. flexilis *fitness and lake environmental conditions*

A method for assessing *N. flexilis* success is required to assist a monitoring programme aimed at identifying at-risk sites, to prioritise action to prevent further extinctions. A model predicting the success of *N. flexilis* could also be used to determine when sites are at risk from extinction, or when extinct sites may return to a condition suitable for reintroduction. Species abundance is often used to determine the success of a species at a particular site. However, it is difficult to measure *N. flexilis* abundance accurately. Preliminary surveys showed that *N. flexilis* could not be seen at all sites by snorkelling at the water surface. This was due to the deep water typically inhabited by *N. flexilis* and the coloration of the water (often peat-stained). Tests in clear water agreed with comments in the literature (Capers, 2000) that values of abundance obtained by grapnelling corresponded poorly with the real abundance of *N. flexilis*: the plant is consistently under-recorded by this method, as many individuals ‘‘escaped’’ the grapnel due to the plants’ slender and fragile form. Scuba diving, or the use of underwater video cameras, is an option, but time and resource factors usually preclude the use of these methods for routine, extensive monitoring.

Instead, measures of plant attributes were used in this study to determine quantitative relationships between the environmental factors (which

Table 1. The range of environmental variables measured at *Najas flexilis* present and extinct sites, (a) in the sediment, (b) in the water

	Oligotrophic extinct	Present	Eutrophic extinct
(a)			
Available P (mg/l)	0.9–2.9 ($n = 2$)	0.3–17.3 ($n = 41$)	5.3–72.9* ($n = 7$)
Median	1.9	2.6	21.8
Available K (mg/l)	–	4–166 ($n = 31$)	28.3–89.2 ($n = 4$)
Median	–	35.1	77.1
Extractable Mg (mg/l)	–	16.3–388 ($n = 31$)	54.1–189 ($n = 4$)
Median	–	87	146
Extractable Ca (mg/l)	145–5250 ($n = 2$)	198–331000 ($n = 41$)	805–25000 ($n = 7$)
Median	2697.5	950	3140
Total Fe (mg/kg)	27 600–20600* ($n = 2$)	94.4–168000 ($n = 41$)	123–14500 ($n = 7$)
Median	24 100	1250	216
Total N (mg/kg)	320–6220 ($n = 2$)	240–142000 ($n = 41$)	600–11400 ($n = 7$)
Median	3270	3320	1540
Extractable Na (mg/l)	–	8.3–300 ($n = 29$)	20.5–72.5 ($n = 4$)
Median	–	52.4	50.05
(b)			
PH	6.46–6.98* ($n = 2$)	6.62–8.3 ($n = 42$)	7.45–9.01* ($n = 7$)
Median	6.72	7.46	8.64
Conductivity (μS/cm)	54.9–116* ($n = 2$)	55–447 ($n = 42$)	158–410 ($n = 7$)
Median	85.45	235	215
Light extinction coefficient	1.35–4.4 ($n = 2$)	0.53–7.32 ($n = 42$)	0.91–5.18 ($n = 7$)
Median	2.88	1.91	1.83
Alkalinity $CaCO_3$ (mg/l)	4.91–5.7* ($n = 2$)	6.71–69.71 ($n = 29$)	41.5–98.66* ($n = 3$)
Median	5.3	23.45	72.65
Ca (mg/l)	1.32–2.02* ($n = 2$)	2.06–33.4 ($n = 30$)	14.22–24.72* ($n = 3$)
Median	1.67	9.59	21.7
Fe (mg/l)	0.2–0.16 ($n = 2$)	$<$0.03–1.69 ($n = 30$)	0.04–0.35 ($n = 3$)
Median	0.18	0.21	0.18
Mg (mg/l)	1.54–2.12 ($n = 2$)	1.34–8.8 ($n = 30$)	6.4–12* ($n = 3$)
Median	1.83	4.8	8.8
N-NH3 (mg/l)	$<$0.04 ($n = 2$)	$<$0.04–0.1 ($n = 33$)	$<$0.04–0.28 ($n = 4$)
Median	$<$0.04	$<$0.04	0.07
Total oxidised N (mg/l)	$<$0.1 ($n = 2$)	$<$0.1–1.369 ($n = 33$)	$<$0.1–2.646 ($n = 4$)
Median	$<$0.1	$<$0.1	$<$0.1
Dissolved reactive P (mg/l)	$<$0.003 ($n = 2$)	$<$0.003–0.045 ($n = 33$)	$<$0.003–0.074 ($n = 4$)
Median	$<$0.003	$<$0.003	0.012
Total P (mg/l)	0.012 ($n = 2$)	$<$0.003–0.056 ($n = 30$)	0.014–0.16* ($n = 3$)
Median	0.012	0.0095	0.017
K (mg/l)	0.3–0.54* ($n = 2$)	0.16–2.82 ($n = 33$)	0.3–2 ($n = 4$)
Median	0.42	1.2	1.4
CO_2 (mg/l)	1.32–4.52 ($n = 2$)	0.40–10.18 ($n = 29$)	0.037–9.07 ($n = 3$)
Median	2.92	2.21	2.69

*Denotes a significant difference between the extinct group with the * and the present group, $p \leq 0.05$, using a Mann–Whitney U test.

appeared to differ between present and extinct sites) and the success of *N. flexilis*. Plant traits have often been used to look at genotypic effect on plant fitness (eg. Dudash, 1990; Ouborg & Van Treuren, 1995; Fischer & Matthies, 1998). Plant traits have also been used to compare the fitness of species within the same genus to different environmental regimes (Sultan, 2001). The ideal fitness measure might include seed production, probability of seed germination, probability of survival to adulthood and reproductive number of those individuals that survived to adulthood (Dudash, 1990). This is not always possible. Instead, probability of the progeny reaching maturity and reproducing can be substituted by reproductive weight or vegetative success of the reproducing parent, as this represents the resources allocated, or available, to the offspring (Sultan, 2001). Such fitness traits are phenotypically plastic within genotypic constraints. This plasticity will be determined by the way in which the genotype of the organism interacts with its environment. It is this environmentally driven plasticity which can be used to determine the realised niche of *N. flexilis* in Britain and Ireland, as the patterns of fitness response (along with the growth traits that underlie them) determine the range of conditions in which species may survive and successfully reproduce, and hence their ecological niche-breadth (Sultan, 2001). Phenotypic plasticity has been successfully related to environmental variables in the field including specific leaf area and depth as a function of light (Spence et al., 1973).

Three whole-plant samples were randomly collected from 17 Scottish populations, and trait analysis was undertaken on the fresh plants. Leaf area was measured for each plant using the method of Ali et al. (1999). Leaf area measurements are known to be a good surrogate for the photosynthetic success of plants, and hence the resources available to be allocated to the progeny. Number of reproductive structures was also recorded, to represent the reproductive success of the individuals. A problem with relating wild population plant traits to environmental factors is that they can be affected by the age of the individual, which is often unknown in wild perennial populations. However, this problem does not exist when measuring plant traits of an annual, such as *N. flexilis* the established-phase of which lives only for a maximum of one year. To account for the differences found in plants collected at the beginning, to those at the end of the field season all traits were divided by shoot length, a function of age.

Data were tested for normality using Ryan–Joiner test procedures, and appropriate transformations were applied to normalise individual data sets. Multiple regression procedures were used to relate *N. flexilis* fitness to the suite of environmental variables listed in Table 1.

Results

N. flexilis *presence and absence*

The Mann–Whitney *U* tests showed a significant difference between both extinct groups and the present group (Table 1).

N. flexilis *fitness and lake environmental condition*

In unfavourable conditions for *N. flexilis* growth, the two elements of fitness used, reproductive number and leaf area, did not reduce uniformly together. In more oligotrophic lakes ($pH < 7.0$) leaf area was intermediate, but the number of reproductive structures reduced dramatically. At one site only one seed was found in three mature plants. This agrees with the results of the experiments by Titus & Hoover (1993). In other environmentally similar lochs, only one or two plants were found, so they could not be sampled for fitness. It is probable that this low frequency of plants and low seed frequency among plants is related. At the more eutrophic end of the range ($pH > 8.0$), plants appeared to have longer internodes, which reduced the total leaf area of the plants as there were less leaf nodes and therefore less leaves per shoot length (overall shoot length did not appear to increase in plants with extended internodes). The lack of nodes also reduced the number of reproductive structures as these develop in the leaf nodes. Both of these conditions could possibly lead to population collapse. This is illustrated in Figure 1a & b, where each fitness component is regressed against pH. Both of the plots are noisy (as pH is not the only factor controlling fitness). However they illustrate the unequal decline in the two components of plant fitness. As

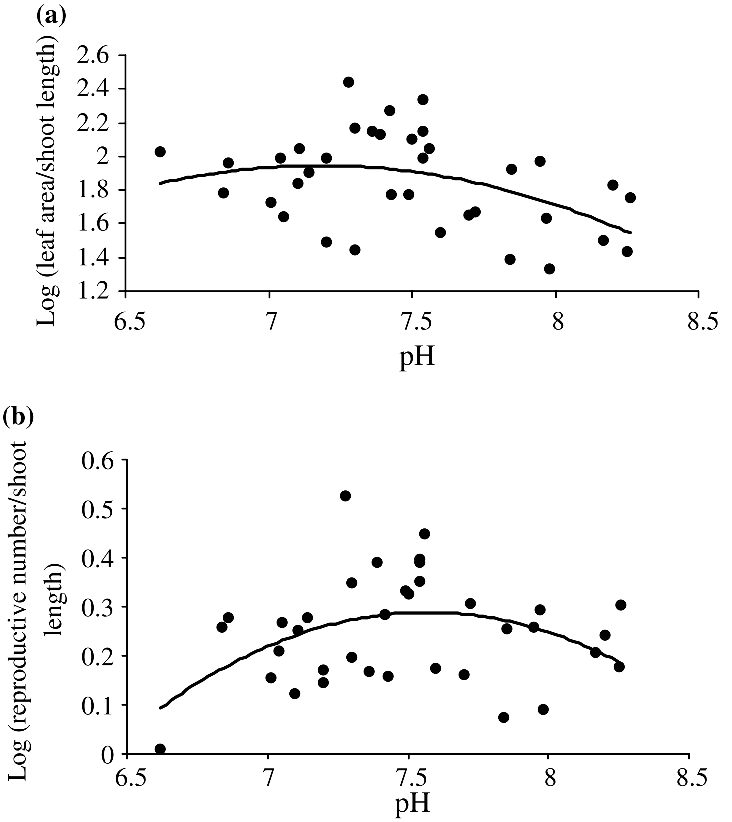

Figure 1. (a) The effect of pH on Log (leaf area/shoot length) $r^2 = 0.181$, $p < 0.05$. (b) The effect of pH on Log (reproductive number/shoot length) $r^2 = 0.165$, $p = 0.052$.

a result of this situation (reproductive number/shoot length) × (leaf area/shoot length) is used as the measure of fitness and is the measure predicted by the resulting models.

Predicting N. flexilis *fitness*

The models for leaf area/shoot length and number of reproductive structures/shoot length are intrinsically similar. This is to be expected as the leaves provide the resources for the reproductive structures. Light extinction coefficient is a good indicator for predicting photosynthetic success as leaf area/shoot length. However, it is not as good for predicting the number of reproductive structures produced. The quantity of calcium in the sediment is a better indicator. This is probably because it will affect the pH of the water, which appears to affect reproductive success.

Model 1: $r^2 = 0.886$ $p < 0.005$
$\text{logLa/sl} = 0.515 + (1.276\text{logAlk}) - (1.012\text{logPs}) + (50.775\text{TPw}) - (0.0921\text{Mgw}) - (0.599\text{logLec})$
Model 2: $r^2 = 0.672$ $p < 0.005$
$\text{Rep/sl} = 0.993 + (2.154\text{logAlk}) - (1.179\text{logPs}) + (44.801\text{TPw}) - (0.132\text{Mgw}) - (0.856\text{logCas})$
Model 3: $r^2 = 0.768$ $p < 0.005$
$\text{LogFit} = -0.628 + (2.263\text{logAlk}) - (1.810\text{logPs}) + (84.067\text{TPw}) - (0.156\text{Mgw}) - (0.919\text{logLec})$
Model 4: $r^2 = 0.743$ $p < 0.005$
$\text{LogFit} = 0.642 + (2.738\text{logAlk}) - (1.392\text{logPs}) + (59.509\text{TPw}) - (0.199\text{Mgw}) - (0.649\text{logCas})$

Where:
La/sl = Leaf area/shoot length
Fit = (Leaf area/shoot length) × (Reproductive structure number/shoot length)
Rep/sl = Number of reproductive structures/shoot length
Alk = Alkalinity mg/l

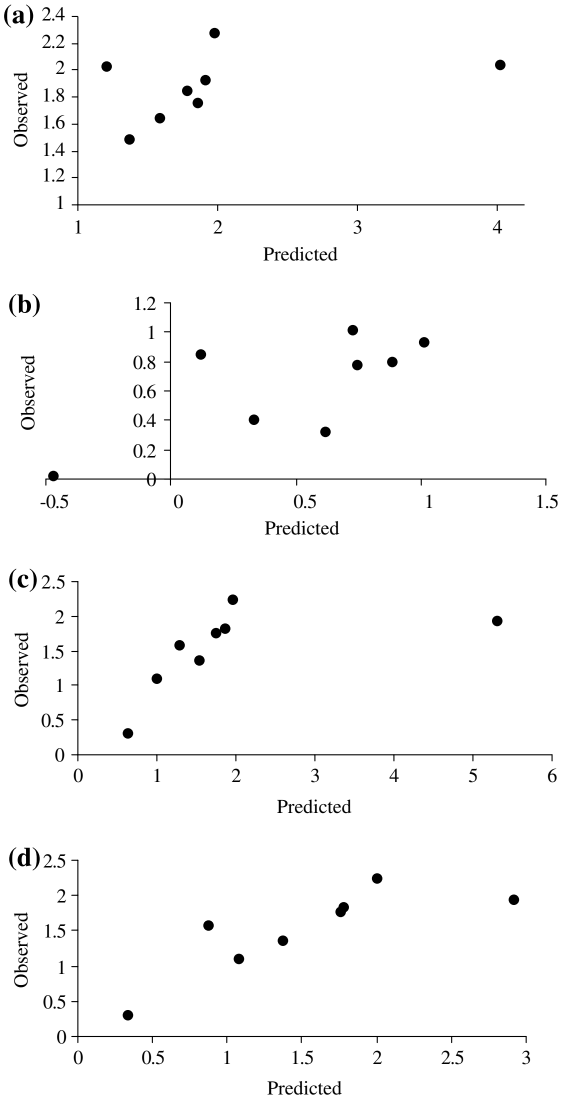

Figure 2. The observed and predicted fitness values of *Najas flexilis* for four Irish sites and four additional Scottish sites, three in S. Uist and one in Kintyre, (a) = model 1, (b) = model 2, (c) = model 3, (d) = model 4.

Ps = Extractable phosphorus in the sediment mg/l
TPw = Total phosphate in the water mg/l
Mgw = Magnesium in the water mg/l
Cas = Calcium in the sediment mg/l
Lec = Light extinction coefficient

The models created were tested using four Irish sites and four Scottish sites not previously used in the creation of the models. The predicted values were plotted against the observed values (Fig. 2). The model predicting reproductive success alone works poorly. All the other models appear to predict the fitness of *N. flexilis* quite well. The major exception from the test data is from Lough Leane in Ireland where the fitness of the plant is consistently predicted to be greater than the observed fitness. Lough Leane is presently becoming increasingly eutrophic. This recent change may well be affecting the plants, but it is possible that phosphate will not yet have accumulated in the sediment, hence the over-prediction of plant fitness. These results show that, with the exception of Lough Leane, the models can equally be applied to the Irish and Scottish *N. flexilis* populations. Therefore they could be used to predict which *N. flexilis* sites are at risk and which have recovered to a point where reintroduction is possible.

Discussion

There is an overlap between the ranges of the environmental variables of the present and extinct site groups. This is because the population at the extinct site could have become extinct at any time since the last record, in some cases a period of over 100 years. Since the time of extinction, the lake quality may have improved to a state where *N. flexilis* could theoretically survive, but recolonisation may not have occurred. Another possible reason why the ranges for the present and extinct site groups are not discrete is that the environmental variables do not affect *N. flexilis* independently and extinctions will be brought about by a number of contributing factors.

The water chemistry measurements are a snapshot of the lake water chemistry. The nutrient levels in the samples are low, as they were taken in summer, when most of the nutrients will be incorporated in the growing plant and/or cyanobacterial material. However, it is within this time period that *N. flexilis* grows, so this is an appropriate time period to take the measurements. Many of the dissolved reactive phosphate, total oxidised nitrogen and NH_3 concentrations were below the detection limit, which undoubtedly contributed to the fact that no significant difference was found between these nutrient levels in the present and extinct groups, and their effect on the fitness of *N. flexilis* could not be determined.

Carbon dioxide concentration was not found to be significantly different between the present and extinct groups. This is probably due to the carbon dioxide concentration values being only estimates, as well as the reasons given above for lack of significant differences between the present and absent groups. The concentration of carbon dioxide would have to be measured directly to give a better indication of its true effect on *N. flexilis* growth. However, pH and alkalinity are often measured by the environmental protection agencies in the British Isles and can give an indication of the carbon dioxide availability for plant growth.

The models, and the present extinct site comparisons, suggest that eutrophication and acidification are the main threats to *N. flexilis*. They both suggest that phosphate concentration in the water and sediment are important, as are calcium and alkalinity. The conditions that predominate in the more oligotrophic sites appear to affect the reproductive success of the plants, but not their photosynthetic capacity. Unless the plant is preferentially allocating resources to photosynthetic tissue instead of the reproductive structures (which is an unlikely strategy for an annual plant), this suggests that these environmental conditions are inhibiting reproductive development alone. Experiments on floral development and pollen viability in these conditions are required to confirm this.

Alkalinity, pH and calcium levels in the water are all indicators of the concentration of dissolved inorganic carbon in the water. Higher levels of these measures indicate that the predominant form of inorganic carbon in the water is bicarbonate. These measures were all found to be significantly higher in the eutrophic extinct group than the present group. The pH, alkalinity and calcium levels and also the inorganic carbon equilibrium will be determined, in part by, the characteristics of the catchment, but they will also shift in accordance with biological activity within the lake. High levels of photosynthesis by either algae or macrophytes within a lake will tend to shift the equilibrium towards a bicarbonate dominated system, at least in daytime when photosynthesis is occurring. *N. flexilis* is an obligate CO_2 user and cannot utilise bicarbonate (Hough & Fornwall, 1988).

High levels of photosynthesis in aquatic plants require high nutrient levels to sustain them. As nitrogen and phosphorus often limit primary production in mesotrophic lakes (where *N. flexilis* grows) an increase in these nutrients would increase photosynthetic production, which would in turn decrease available dissolved CO_2 concentrations. This would seriously limit the growth of *N. flexilis*, since in such an environment it would be incapable of fixing carbon. The significantly higher levels of phosphate in the water and the sediment and the higher pH, alkalinity and calcium levels, in the more eutrophic extinct sites, suggest that this may be the cause of *N. flexilis* extinction in these lakes. The extinct eutrophic sites in this study are all found in the more intensive agricultural areas, suggesting that phosphate inputs from agriculture in the catchments of these lakes may be a serious threat to the species.

Models 1 and 3 show that underwater light availability is also important for predicting plant success. The quantity of PAR reaching *N. flexilis* plants will decrease as algal and macrophyte growth increases, because these will intercept the light before it reaches the relatively low-growing *N. flexilis*. However, *N. flexilis* naturally grows at low light levels and there is no significant difference in the light extinction coefficient between present and absent sites. This suggests that light would have to be severely limited to cause *N. flexilis* extinction.

Conclusions

The models and analysis of present and extinct sites, have suggested that the major threats to this species in Scotland are those related to eutrophication and acidification. Both of which can cause population extinction. The fitness measures developed here can be used to monitor and assess the present *N. flexilis* populations and elucidate which populations are at risk. The models can be used to calculate the predicted fitness of *N. flexilis* at extinct sites to determine if the lake quality is suitable for reintroduction of *N. flexilis*.

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Seasonal variability in the palatability of freshwater macrophytes: a case study

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Key words: aquatic plants, dry matter content, herbivory, *Lymnaea stagnalis*, phenology

Abstract

The pond snail *Lymnaea stagnalis* (L.) was used for a laboratory assessment of seasonal variation in palatability of three freshwater macrophytes: *Potamogeton lucens*, *Elodea canadensis* and *E. nuttallii.* For each species, 2–5 populations were investigated in spring and in summer. Preliminary results showed that the feeding rate of similarly-aged snails bred under standard conditions was stable over time. In contrast, snail feeding rate on the three macrophyte species decreased from spring to summer, which was therefore interpreted as a decrease in plant palatability. This decrease was probably due to tissue maturation, as suggested by the concomitant increase in the dry matter content of leaves of the three species. The high palatability of the species studied during the spring may prove detrimental in cases of strong herbivore pressure, and could have consequences for macrophyte distribution among aquatic habitats.

Introduction

The last decades have seen an increasing number of studies on herbivory of freshwater macrophytes (for a review, see: Lodge, 1991; Newman, 1991; Lodge et al., 1998). These have demonstrated that the magnitude of losses due to herbivory is of the same order in freshwater as in other ecosystems (Jacobsen & Sand-Jensen, 1994; Lodge et al., 1998; Cebrian, 1999). Moreover, differential grazing pressure by invertebrate herbivores on different plants can influence their competitive interactions and may thus play a key role in the structuring of macrophyte communities (Sheldon, 1987; Van et al., 1998; Gross et al., 2001).

The palatability of macrophyte species to generalist consumers integrates all the chemical and physical properties of the plant likely to modulate grazing rate (Bolser et al., 1998; Pennings et al., 1998; Cronin, 1998), and is therefore a trait of ecological importance. Previous studies have attempted to relate palatability to different life-history strategies that will influence macrophyte distribution in the field (Elger et al., 2002; Elger & Willby, 2003).

To date, the intraspecific variability in freshwater macrophyte palatability to generalist herbivores has been poorly studied, although it may be an important issue in understanding the interactions between plants and herbivores in aquatic systems. The aim of the present study was to develop a method for the assessment of seasonal variation in macrophyte palatability and to test this method on three macrophyte species.

Materials and methods

Plant collection

Three macrophyte species were studied: *Potamogeton lucens* L., *Elodea canadensis* Michx and *E. nuttallii* (Planch.) St John. All of them are widely distributed in temperate regions of the northern hemisphere, and the latter two are North American species invasive in Europe.

These species were collected in cut-off channels of the River Rhône floodplain (S.E. France) at an early stage of their annual growth cycle and later in the season, when they had established a dense canopy. This corresponded respectively to June and September 2000 for *Elodea* species, and to May and July 2001 for *P. lucens*.

Each species was sampled at several sites on each date to integrate inter-population variability into the analysis. Hence, two populations of each *Elodea* species and five populations of *P. lucens* were investigated.

Assessment of seasonal variations in plant palatability

Seasonal variation in the palatability of the three macrophyte species was assessed through laboratory non-choice feeding experiments with a generalist consumer, the pond snail *Lymnaea stagnalis* (L.). Numerous qualities make this snail suitable for laboratory feeding experiments (Elger & Barrat-Segretain, 2002), and it has been previously used with success for similar studies (Smits, 1994; Elger et al., 2002). Successive cohorts of snails were bred on a lettuce-based diet, in filtered water maintained at constant temperature (22 °C) and photoperiod (L:D ratio = 12:12). The breeding was designed to provide individuals of similar size and age (30–32 mm shell length, 12 weeks old) for each feeding experiment.

To ensure that the grazing rate of snails from such successive cohorts was stable through time, a feeding experiment was performed with lettuce leaves, at three dates during spring and summer 2000 (May 14th, June 21st and August 2nd). A similar experiment was carried out on each macrophyte sample collected in 2000 and 2001. Apical shoots of *Elodea* species (about 6 cm length), and young leaves of *P. lucens* were used after removal of marl coverage by gentle brushing under running water. All the fragments used were green, devoid of grazing or necrosis and, as only apical parts were used, periphyton development was very sparse.

For each plant sample, paired reference (ref.) and treatment (tre.) trials were performed. Small plastic containers were filled with 750 ml of water and kept at 22 °C. Snails were placed individually in the treatment containers, and remained starved for the first 24 h. The reference containers were left grazer-free. On each date, 15 paired replicates for lettuce, 20 for each *Elodea* species and 50 for *P. lucens* were performed. Fresh fragments of each plant species were distributed separately in the containers after weighting (WM0). The snails were removed after 24 h of grazing and the plant fragments remaining in each container were dried (48 h at 70 °C) and the dry mass remaining (DM1) determined.

The linear relationship between $\mathrm{DM1}_{\mathrm{ref.}}$ and $\mathrm{WM0}_{\mathrm{ref.}}$ was assessed separately for each sample. For each pair of containers, the estimated coefficients were used to calculate $\mathrm{DM1'}_{\mathrm{ref.}} = \mathrm{a}\times \mathrm{WM0'}_{\mathrm{ref.}} + \mathrm{b}$, where $\mathrm{WM0'}_{\mathrm{ref.}} = \mathrm{WM0}_{\mathrm{tre.}}$, and the dry mass consumed was determined by $\mathrm{DM1'}_{\mathrm{ref.}} - \mathrm{DM1}_{\mathrm{tre.}}$.

Assessment of macrophyte dry matter content

The mean dry matter content (DMC) of each macrophyte sample was also determined to relate it to its palatability. Fresh plant fragments were weighed (WM), dried for 48 h at 70 °C, and then reweighed (DM). The DMC of fragments was then calculated as 100×DM/WM. On each date, 20 fragments for each *Elodea* species and 50 fragments for *P. lucens* were used.

Results

The experiment with lettuce showed that mean (±SE) daily consumption by snails amounted to 31.3±1.3 mg (Fig. 1). This rate did not vary significantly between the successive cohorts of snails (ANOVA, $F_{2,42} = 1.46$, $p = 0.24$).

The dry mass consumed daily by a snail averaged 3.1±0.5 (±SE) mg for *E. canadensis*, 3.5±0.6 mg for *P. lucens* and 6.0±0.5 mg for *E. nuttallii*. Nevertheless, the three species experienced important seasonal differences in snail consumption. All of them were consumed less in summer than in spring (Fig. 2). This difference was highly significant for *P. lucens* and *E. nuttallii* (*t*-test, $p < 0.001$), but not significant for *E. canadensis* (*t*-test, $p = 0.24$).

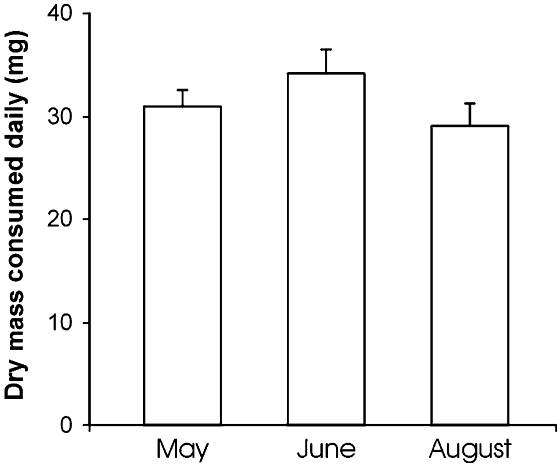

Figure 1. Dry mass (mean ± SE) of lettuce consumed daily by an individual of *Lymnaea stagnalis* at three dates from May to August.

The DMC of the macrophytes studied ranged from 10.1 ± 0.2% (for *E. nuttallii* in spring) to 21.7 ± 0.4% (for *P. lucens* in summer). This parameter also varied seasonally (Fig. 3), increasing significantly for all three species from spring to summer (*t*-test, $p < 0.001$ for *E. canadensis* and *P. lucens*, $p = 0.014$ for *E. nuttallii*).

Discussion

Seasonal variations in macrophyte palatability

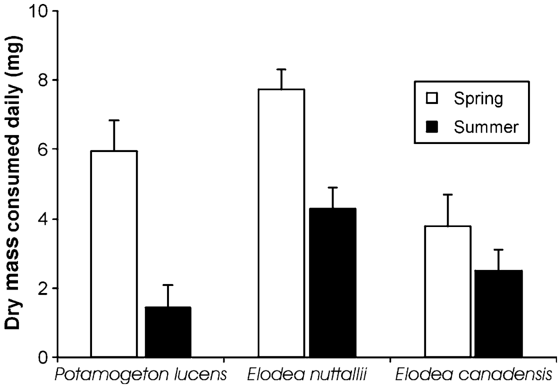

Figure 2. Dry mass (mean ± SE) of *Elodea nuttallii*, *E. canadensis* and *Potamogeton lucens* consumed daily by an individual of *Lymnaea stagnalis* in spring and in summer. The results obtained for the different populations of each species on each date were pooled.

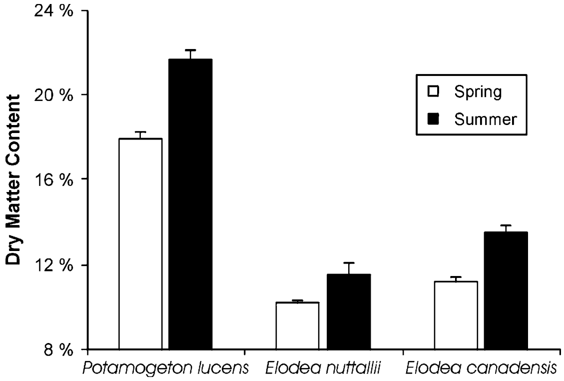

Figure 3. Dry matter content (mean ± SE) of *Elodea nuttallii*, *E. canadensis* and *Potamogeton lucens* in spring and in summer. The results obtained for the different populations of each species on each date were pooled.

As shown by the experiment with lettuce, the intrinsic feeding rate of successive cohorts of *L. stagnalis* bred under standard conditions is stable through time, which legitimises the use of this snail to assess seasonal variations in macrophyte palatability. Hence, the differences in feeding rate recorded within the three macrophytes studied can be regarded as a genuine decrease in their palatability from spring to summer.

Although we consistently used apical leaves or shoots, due to seasonal and interspecific differences in growth rate it was impossible to standardise the age of tissue that was used. Consequently the decrease in macrophyte palatability was probably due to tissue maturation, and conforms to the pattern observed for terrestrial or marine plants (Feeny, 1970; Cronin & Hay, 1996; Alonso & Herrera, 2000). The palatability of freshwater macrophytes is mainly ruled by tissue toughness and by the amount of deterring chemicals (Bolser et al., 1998; Lodge et al., 1998). Nutritive quality of tissues can also modulate plant palatability, but is of secondary importance in herbivore preferences (Newman et al., 1996). All of these features are likely to vary seasonally, and such variations may explain the observed pattern in palatability.

In spring, rapidly growing tissues have high concentrations of proteins (Feeny, 1970; Bryant et al., 1983). Later in the season, the nitrogen content generally decreases as growth slows and tissues mature (Mochnacka-Lawacz, 1974; Speiser & Rowell-Rahier, 1991). Moreover, resource allocation to the synthesis of anti-herbivore chemical defences is presumably minimal in spring, due to the high metabolic demand for growth (Herms & Mattson, 1992). Data from terrestrial and marine habitats confirm that the plant concentration of carbon-based secondary defence compounds (*e.g.* phenolics, terpenoids) is low at this period, but increases during the growing season (Feeny, 1970; Muller-Riebau et al., 1997, Dumay et al., 2002). Lastly, differentiation processes tend to follow cell division and enlargement, and effective structural defences are only acquired by plants once the growth peak is over (Herms & Mattson, 1992).

The increase in DMC during tissue maturation seems to be a general pattern, also observed in terrestrial plants (Mattson, 1980; Speiser & Rowell-Rahier, 1991; Herms & Mattson, 1992). DMC, or close correlates such as specific leaf area, have been suggested as indicators of the relative investment of the plant between growth and defence processes (Westoby, 1998; Wilson et al., 1999). Moreover, tissue DMC might be linked to structural adaptations, such as lignin, fibre and mineral contents, that directly act as feeding deterrents (Mattson, 1980; Grime et al., 1996). The ability of this trait to predict the palatability of freshwater macrophytes, both at inter- and intra-specific level, is discussed in detail in a previous paper (Elger & Willby, 2003).

Ecological consequences of seasonal variation in palatability

Herbivory of submerged macrophytes by freshwater invertebrates has been demonstrated under natural conditions (Sand-Jensen & Madsen, 1989; Jacobsen & Sand-Jensen, 1994). High plant palatability implies increased risk of grazing. Therefore, the three species studied are potentially more susceptible to grazing losses in spring. Although established macrophytes are rarely killed by invertebrate herbivores, intense herbivory on a plant species is detrimental as its success relative to less palatable competitors can be compromised by grazing (Sheldon, 1987; Van et al., 1998; Gross et al., 2001).

The density of invertebrate grazers can be locally reduced by hydraulic disturbances (Newman, 1991; Holomuzki & Biggs, 1999) or by the top-down effect of fish predation (Sheldon, 1987), which allows the persistence of some macrophyte species despite their high palatability during part or all of the season (Sheldon, 1987; Elger et al., 2002). Under high herbivore pressure, such species could persist only if they can compensate for grazing losses by active regrowth (Wallace & O'Hop, 1985). However, possibilities of compensation are reduced in resource-limited habitats (Jacobsen & Sand-Jensen, 1992). Invertebrate herbivory could therefore effectively govern the distribution of high-palatability species in the field by precluding, for instance, their long-term persistence in the most oligotrophic sites.

Conclusion

In the present study, feeding experiments provided an integrative expression of plant susceptibility to grazing, and revealed major seasonal variation in palatability. Variations in DMC indicated that tissue maturation was significant from spring to summer, although we consistently used apical tissue. The next step would be to determine other traits that are also modified during leaf maturation and that may therefore contribute to a seasonal decrease in macrophyte palatability. The possibility of a relationship between seasonal patterns in the palatability of various macrophyte species and their distribution over a wide range of habitats also merits further investigation.

Acknowledgements

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Recovery of *Potamogeton pectinatus* L. stands in a shallow eutrophic lake under extreme grazing pressure

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Key words: submerged macrophytes, restoration, exclosures, fish herbivory

Abstract

In shallow lakes, submerged macrophytes contribute to the stabilization of the clear water state. If lost, a number of mechanisms prevent re-colonization. Lake Müggelsee (730 ha) lost its submerged vegetation due to increasing eutrophication and switched to phytoplankton dominance in 1970. After the reduction of nutrient loading in 1990, *Potamogeton pectinatus* L. started re-colonizing the lake. During the following years, it spread at a mean rate of 2.5 ha per year to all available areas <80 cm depth. Between 1993 and 1999, decreasing maximum biomass indicated hampered growth. Exclosure experiments revealed that herbivory reduced the aboveground biomass by more than 90%. Both waterfowl and fish were found to contribute to the grazing pressure despite a low abundance of the known herbivorous fish species and waterfowl in spring and summer. Protection of stands against grazing resulted in higher biomass of shoots, whereas shoot and tuber density did not change. Both shading by phytoplankton and periphyton, as well as grazing pressure, prevented the submerged vegetation of Lake Müggelsee from developing back to a dense zone that contributed to the reduction of turbidity.

Introduction

Eutrophication due to anthropogenic impacts caused a decline of submerged vegetation in many shallow, north temperate lakes (e.g. Körner, 2002). This reduction is mainly explained by the shading effects by phytoplankton and epiphyton (Phillips et al., 1978). Besides turbidity and sediment re-suspension, it has been proposed that grazing by herbivorous birds hampers the re-colonization after reductions of external nutrient loads and/or biomanipulation (e.g. Lauridsen et al., 1993; Søndergaard et al., 1996). Perrow et al. (1997), however, state that there is little evidence in the literature to support this hypothesis and Van Donk et al. (1994) and Mitchell & Wass (1996) could not find a significant effect of waterfowl grazing on the (re-) establishment of submerged plants. Other investigations do suggest an impact of fish, usually rudd (*Scardinius erythrophthalmus*) on macrophyte growth (Prejs, 1984; Van Donk et al., 1994). Several authors proposed that grazing might be critical for macrophyte development in lakes at the nutrient threshold where both a clearwater and a turbid state may occur (Søndergaard et al., 1996) and when light or other conditions for macrophyte growth are marginal (Mitchell & Wass, 1996).

In the following study the re-establishment of *Potamogeton pectinatus* L. stands in a eutrophic shallow lake was investigated a decade after starting the reduction of external nutrient loads. Conditions were appropriate for macrophyte growth, but nutrient concentrations around

0.1 mg P l^{-1} still caused summer cyanobacteria blooms (Körner, 2001) and high periphyton biomass (Roberts et al., 2003). It was hypothesised that the impact of grazing on the development of submerged plant stands might be crucial during this stage of recovery.

Description of sites studied

Lake Müggelsee is a eutrophic, shallow lake in Berlin (Germany) with an area of 7.3 km^2 and a mean depth of 4.9 m (description in Driescher et al., 1993). At the beginning of the 20th century, a diverse submerged vegetation covered one third of the lake. At least 20 species occurred in the lake, with *Potamogetonaceae* dominating the shallower areas and *Fontinalis antipyretica* forming a belt in the deeper regions. Increasing eutrophication lead to the total disappearance of submerged plants around 1970 and a switch to phytoplankton dominance (Körner, 2001). *P. pectinatus* probably did survive in small stands during the 1970s and 80s. Since 1990, external nutrient loads have been reduced, resulting in a spring clearwater phase and re-appearance of submerged macrophytes, mainly *P. pectinatus* (Körner, 2001). Between 1999 and 2001 nutrient (total phosphorus: 112 ± 16 (standard error) $\mu g\ l^{-1}$, NH_4^+–N: 0.12 ± 0.02 mg l^{-1}, NO_3^-–N: 0.29 ± 0.08 mg l^{-1}) and chlorophyll concentrations (27 ± 4 $\mu g\ l^{-1}$) and Secchi depths (April–June: 2.3 ± 0.3 m, July/August: 1.2 ± 0.1 m) did not change significantly.

Material and methods

The submerged vegetation of Lake Müggelsee was mapped and biomass determined in 1993 (Awe & Körner, unpublished), 1999 and 2000 (for methods see Körner, 2001). The development of selected *P. pectinatus* stands has been followed during the vegetation period of 1999 and 2000 at the wind-exposed northern (N) and sheltered western (W) shore in 0.5 m water depth. Effective fetch for the prevailing wind directions W/SW of the exposed and sheltered site was 0.43/1.44 km and 0/0 km, respectively. Biomass of *P. pectinatus* was harvested at monthly intervals using a tube corer (0.181 m^2, four replicates). Aboveground shoots, tubers, roots and rhizomes were dried separately at 60 °C until weight constancy.

In order to determine the influence of herbivorous birds and fish on *P. pectinatus* growth, two sets of exclosure cages with 2 cm mesh size were placed at 0.5 m water depths at the N and W shore in the lake in April 2001. One set of cages (four at each location) was fully closed to protect macrophytes against all birds and fish above 2 cm body width ($1 \times 1 \times 0.5$ m), whereas the other was open for 25 cm above the sediment to allow fish to enter ($1 \times 1 \times 0.25$ m). Biomass in the cages and the unprotected surrounding was harvested in June (one sample per cage, four samples at randomly selected unprotected locations) and dry weight, length and number of aboveground shoots, rhizomes and tubers were recorded. Means were compared separately for the exposed and sheltered site using a one-way analysis of variance (ANOVA) ($p < 0.05$) and a subsequent multiple comparison using Tukey's test (SPSS). Exponential rates of net plant aboveground biomass increase (d^{-1}) were calculated for the interval 23rd of March (biomass approximately 1 g dw m^{-2}) until the harvesting as $(\ln dw_2 - \ln dw_1)/t$, where dw_2 and dw_1 are the final and initial mean biomass and t is time (days). Grazing rates of fish and birds were calculated from the differences between growth rates of differently protected stands. Herbivorous water birds were counted between May 2000 and May 2001 every 2–4 weeks.

Results

The area colonized with submerged macrophytes in Lake Müggelsee increased between 1993 and 1999 from 6 ha to 22 ha (Fig. 1), resulting in an average colonization rate of 2.5 ha per year. Total area covered, maximum and average colonization depth (1.5 m and 0.8 m, respectively) and colonization density did not change from 1999 to 2000. *P. pectinatus* was the dominating species, covering 99% of the colonized area. It germinated from tubers at the end of March and became senescent in August. Plants did not reach the water surface and did not flower. Maximum biomass at the sheltered W shore was significantly higher than at the exposed N shore (Fig. 1). Values declined between 1993 and 1999 at the N shore and between

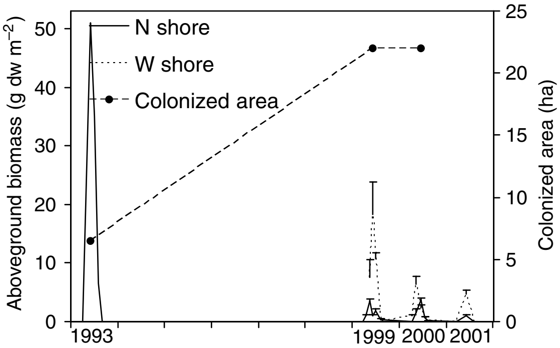

Figure 1. Development of aboveground biomass (+ standard error) of *P. pectinatus* in 0.5 m water depth at the wind-exposed northern (N) from 1993 to 2001 and at the sheltered western (W) shore from 1999 to 2001 and colonized area in Lake Müggelsee.

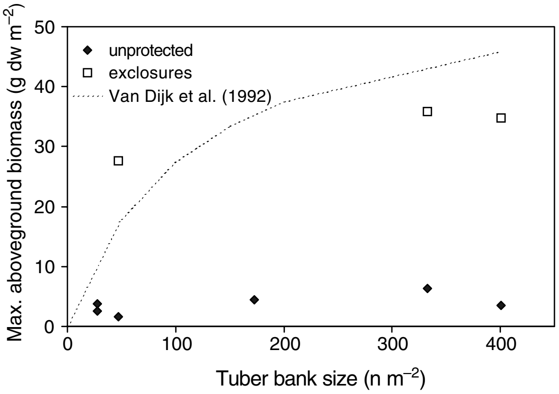

Figure 2. Data pairs of mean maximum aboveground biomass of unprotected stands and stands in exclosures and mean size of tuber bank of *P. pectinatus* in Lake Müggelsee in 1999 and 2000. The dotted line shows the relationship derived for *P. pectinatus* in Lake Veluwe in 1987 by Van Dijk et al. (1992).

1999 and 2001 at the W shore (Fig. 1). Tubers germinated in April and above-ground shoots disappeared in August. Average maximum shoot length (June, 1999–2001) was significantly lower at the exposed shore (5.0 ± 0.1 cm) compared to the sheltered shore (8.0 ± 0.3 cm). Shoot densities (730 ± 220 at N and 1350 ± 320 at W) did not differ significantly between shores. Sheltered stands had significantly more tubers compared to the exposed stands at the beginning (333 ± 76/ 28 ± 13) as well as at the end of the growing season (157 ± 23/21 ± 10, data from 2000). Significantly less tubers at the end of the growing season compared to the beginning were found at the exposed shore in 1999 (401 ± 99/3 ± 2). The maximum aboveground biomass of the stands was independent of the tuber bank size (Fig. 2). Mean individual tuber weight was significantly higher at the sheltered shore at the end of the growing season in 2000 (7.2 ± 0.5/0.8 ± 0.6).

Protection of *P. pectinatus* stands against herbivory from fish and waterfowl resulted in significantly higher biomass and longer shoots compared to the unprotected stands at both shores (Fig. 3). Shoot biomass of stands in cages protected against bird herbivory only (half closed) resulted in an intermediate biomass at the sheltered stand, but was not significantly different from the unprotected stand at the exposed shore. Shoot length differed significantly between differently protected stands with longest plants in the fully closed cages, intermediate in the half closed and lowest in the unprotected stands. A significant reduction of the rhizome biomass in unprotected stands was only found at the exposed site, whereas a reduction of the total tuber biomass could only be found at the sheltered site. Grazing by birds and fish did not reduce shoot and tuber densities and individual tuber weight (Fig. 3).

Potentially herbivorous birds present were swans (*Cygnus olor*), coots (*Fulica atra*), mallards (*Anas platyrhynchos*), tufted ducks (*Aythia fuligula*), pochards (*Aythia farina*) and goldeneyes (*Bucephala clangula*). Coot numbers increased from about 1 coot ha^{-1} from May to July to 6 coots ha^{-1} in December. Numbers of diving ducks increased from 0.06 ha^{-1} in summer to 0.85 ha^{-1} in winter. Total numbers of swans varied between 0.01 and 0.04 ha^{-1}, with more swans at the W compared to the N shore.

Discussion

In Lake Müggelsee, *P. pectinatus* spread rapidly between 1993 and 1999 to all available areas shallower than 80 cm depth. Re-colonization occurred probably from remaining stands by vegetative propagation as plants do not flower and extensive seed-baring stands are lacking upstream (unpublished). Although nutrient concentrations and therefore shading by phytoplankton and periphyton did not increase, maximum biomass declined between 1993 and 2001. Only a few studies report such low values for *P. pectinatus*, whereas shoot

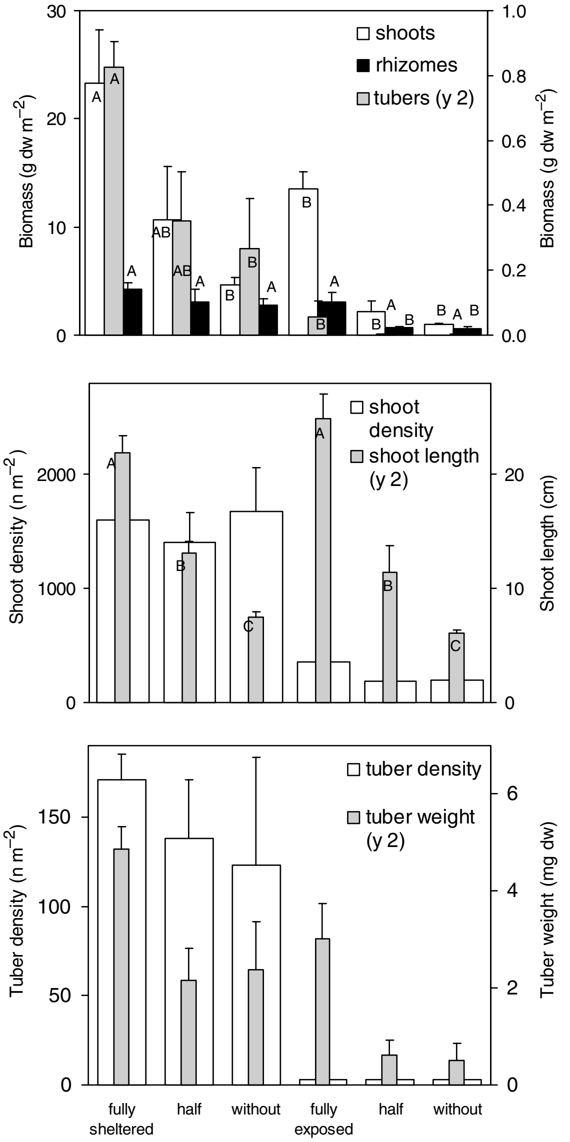

Figure 3. Biomass (+ standard error) of shoots, rhizomes and tubers, shoot density and length, and tuber density and weight of *P. pectinatus* stands in 0.5 m water depth at the sheltered western (W) shore and the wind-exposed northern shore in Lake Müggelsee in June 2001. Stands were protected against herbivory from birds and fish (fully), birds only (half) or not protected (without). Different letters indicate significant differences at $p < 0.05$ (one-way ANOVA with subsequent multiple comparison test (Tukey)). Shoot and tuber density and tuber weight were not significantly different.

densities were high (e.g. Ozimek et al., 1986). Due to the low shoot length *P. pectinatus* did not reach the water surface and could therefore not escape turbid conditions during cyanobacteria blooms in July and August. Growth of stands at the exposed and sheltered site probably differed due to differences in wave stress (Ozimek et al., 1986) and/or grazing pressure, as the contents of organic matter in the sediment were comparable (unpublished) and periphyton densities were not significantly different (Roberts et al., 2003).

Contrary to the findings of Van Donk et al. (1994), macrophyte consumption by vertebrate grazers did negatively affect the recovery of submerged macrophytes in Lake Müggelsee, as protection resulted in significantly higher biomass. Exclosure experiments revealed that fish have a higher impact than waterfowl although the known herbivorous fish species like rudd (*S. erythrophthalmus*) or carp (*Cyprinus carpio*) were not abundant in the lake. Plants were obviously not totally pulled out of the sediment as e.g. by grass carp (Prejs, 1984), as shoot densities were not reduced. An impact of young roach (*Rutilus rutilus*) was suggested from studies using underwater videos (Körner & Dugdale, 2003). Surprisingly, tuber density and individual tuber weight were also not significantly reduced, although tuber production is positively correlated to the aboveground biomass (Van Dijk et al., 1992; Figure 2). Tuber densities at the end of the vegetation period in August were, however, lower than at the beginning in some cases in the unprotected stands.

The net growth rate of fully protected *P. pectinatus* in 2001 was significantly higher at the sheltered (0.070 d^{-1}) compared to the exposed shore (0.051 d^{-1}). Both were lower than the optimum growth rate reported by Nielsen & Sand-Jensen (1991) (25% or 46%, respectively). Still, protected stands reached the water surface and contrary to Mitchell & Wass (1996) it is therefore concluded that grazing pressure has a high impact on the re-establishment of submerged vegetation even if growth conditions are appropriate. Fish grazing rates were higher at the exposed shore (0.046 d^{-1}) and comprised 92% of total grazing compared to the sheltered shore (0.027 d^{-1}), where bird grazing rates of 0.012 d^{-1} contributed 30% to total grazing. The bird grazing rates were higher than those reported by Mitchell & Wass (1996) for black swan at densities of up to 25 ha^{-1}. The calculated grazing rates probably overestimate the ingested material as plant growth is supposed to be slower with grazing.

It was concluded that the impact of grazing on the development of *P. pectinatus* in shallow lakes can be crucial when conditions for macrophyte growth are only appropriate in shallow areas due to summer cyanobacteria blooms and periphyton growth. Grazing probably hampers the growth of *P. pectinatus* resulting in high calculated grazing rates of fish and birds despite low abundances of waterfowl and the apparent absence of herbivorous fish.

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Shoreline vegetation of Lake Nubia, Sudan

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Key words: High Dam Lake, water level, plant strategy, moisture zones

Abstract

The paper reports on the flora along the banks of Lake Nubia and the long-term changes that have taken place since the formation of the Aswan High Dam Lake 30 years ago. The study also aims to determine the main factors that govern plant distribution in this area. Shoreline vegetation was studied from Debeira at the Sudanese–Egyptian border to the 'Dal' Cataract at the south end of the lake, in Sudan. The shoreline was classified into four moisture gradient zones depending on the period of inundation, namely: wet zone (frequently inundated and recently exposed); moist zone (periodically inundated); semi-dry zone (rarely inundated); and dry zone (never inundated). Plants characterising each zone were identified. Soil texture and thickness of the deposits were measured for each zone at each site. According to Grime's strategy, the study revealed that species of the wet and the moist zones are mainly disturbance-tolerant; species observed in the 'semi-dry' zone are competitive-stress-tolerant; and those of the 'dry' zone are stress-tolerant. The present study indicates that elevation above mean sea level, and period of inundation and texture of deposits play an important role in governing the distribution of the shoreline vegetation.

Introduction

The construction of the Aswan High Dam in Upper Egypt created one of the largest man-made lakes in Africa, which extends for 500 km south of the dam to the cataract at Dal in Sudan. The major portion of the lake lies in Egypt and is known as Lake Nasser. On the Sudanese side (20°27′–22°00′ N latitudes and longitudes 30°35′–31°14′, Fig. 1), it is referred as Lake Nubia.

Although many botanical studies have been carried out on Lake Nasser (e.g. Boulos, 1966; Abdallah & Sa'ad, 1972; El-Hadidi, 1976; Springuel et al., 1991; Ali, 2000), the literature contains very few floristic records for Lake Nubia (Ali, 2003). Before the construction of the lake, Ahti et al. (1973) reported botanical findings made during the Finnish Biological Expedition to Nubia in 1962 and Pettet et al. (1964) carried out a bio-climatic floral and faunal survey of the Wadi Halfa area in the same year.

The water level in the reservoir increases in July and reaches its maximum in November and December of each year, owing to an increase in the flow from the Blue Nile and Atbara River, which come from the summer rainfall on the Ethiopian Plateau. Levels decrease gradually until July (dry period). When the flood water reaches the reservoir, the level starts to rise again. In November 1999, the water level reached its highest level to date (181.6 m above mean sea level (MSL)).

The present project aims to study the shoreline vegetation of Lake Nubia, to detect the temporal variation in the vegetation that may have occurred after the construction of the lake 30 years ago, and to determine the main factors that govern current plant distribution.

Materials and Methods

Fifteen sites (24 transects) were located along the lake shore, from Debeira at the Sudanese–Egyptian boarder (337.5 km south of the Aswan High Dam) to the 'D' Cataract at the south end of the lake (500 km south of the Aswan High Dam, Fig. 1).

Using data on water level during the past few years and a base levelling Theodolite, four moisture gradient zones were recognised in each transect: (1) *Wet zone* (frequently inundated and recently exposed): the main part of the zone is only exposed (1–2 months) and remains too wet to support any vegetation (175.9–176.9 m above MSL); (2) *Moist zone* (periodically inundated): most of the zone is exposed for a longer period (3–4 months) than the wet zone (176.9–180.3 m above MSL); (3) *Semi-dry zone* (rarely inundated): the lower edge of the zone was inundated twice in 1998 and 1999 for short periods, it was exposed subsequently (180.3–184.8 m above MSL); and (4) *Dry zone* (never inundated): <184.8 m above MSL.

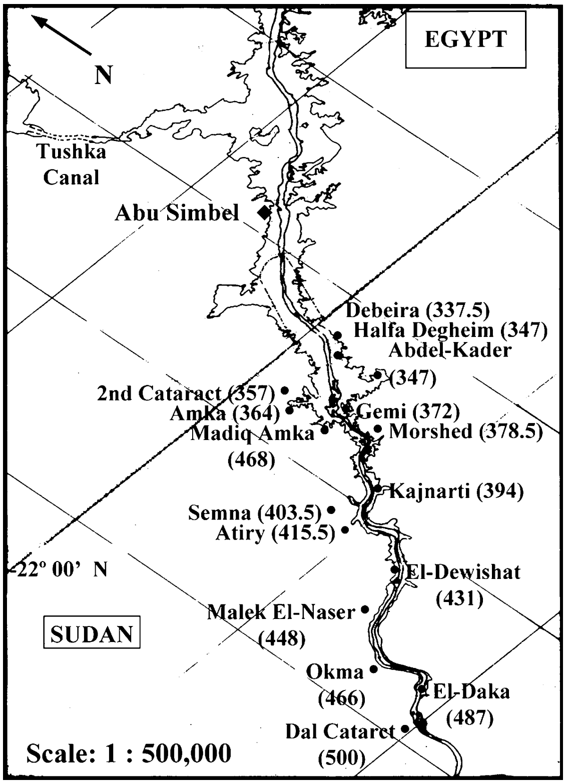

Figure 1. Sampling sites, Lake Nubia, Sudan. (distances (km) south of the Aswan High Dam are given in brackets).

Transects were located perpendicular to the water's edge. In each transect vegetation was sampled from different elevation zones. In each zone, a stand of five 1 m^2 quadrats was randomly located. In total, 83 stands were surveyed. Frequency (F) and mean cover (%C) were determined for each species per stand. Also, average cover (%AC) for each species in each of the recognised moisture zones was calculated. Soil texture and thickness of the deposits were measured for each zone at each site.

Data were analysed using canonical correspondence analysis (CCA)-CANOCO for Windows Version 4.0 (Ter Braak & Smilauer, 1998). Species recorded only once (usually as individuals) were excluded from the analysis. The environmental factors are variables represented by dummy classes. The texture of the soil surface type (with five classes) was analysed with using multiple regression (Montgomery & Peck, 1982) by defining five dummy environmental variables: 'silt', 'loam', 'sandy loam', 'sand' and 'gravely sand'. Thickness of the sediments comprises two dummy variables: 'thin' (<0.5 m) and 'thick' (>0.5 m). Elevation and period of inundation are represented by four dummy variables of the above described moisture zones: 'wet', 'moist', 'semi-dry' and 'dry'. For example, the variable 'silt' takes the value 1 when the soil texture is silt and the value 0 otherwise.

Results

Sixty-one species were recorded in 83 stands of 24 transects located in 15 sites along the Lake Nubia shores. Species with high cover abundant (C) and frequencies (F) were *Tamarix nilotica* (Ehrenb.) Bunge (C 5–100%, F 32.5%), *Persicaria lanigera* (R. Br.) Soják (C 5–100%, F 19.3%), *Persicaria senegalensis* (Mein.) Soják (C 5–100%, F 10.8%), *Crypsis schoenoides* (L.) Lam. (C 1–90%, F 16.9%), *Eragrostis aegyptiaca* (Willd.) Delile (C 1–90%, F 15.7%), *Glinus lotoides* L. (C 1–80%, F 43.4%), *Portulaca oleracea* L. (C 1–80%, F 12.0%), *Phragmites australis* (Cav.) Trin. ex Steud. (C 1–70%, F 19.3%), *Hyoscyamus muticus* L. (C 1–60%, F 39.8%), *Crypsis aculeata* (L.) Aiton (C 1–60%, F 13.3%), *Heliotropium supinum* L. (C 1–50%, F 27.7%), *Cyperus michelianus* (L.) Delile (C 1–40%, F 33.7%), *Cyperus alopecuroides*

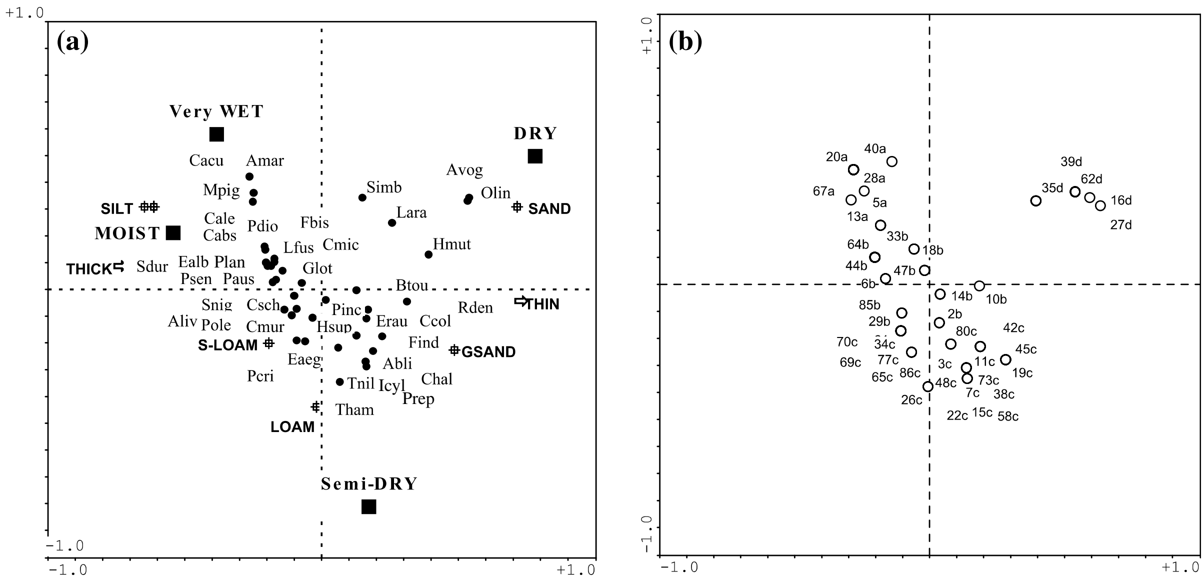

Figure 2. (a) CCA of Lake Nubia shoreline vegetation (●) in relation to the nomial environmental classes: (■) moisture zones : a: wet, b: moist', c: semi-dry and d: dry; deposits texture type ( ): 'silt', 'loam', 'sandy loam', 'sand' and 'gravely sand'; and deposits thickness (≡): 'thin' and 'thick'. Shoreline species: Tnil = *Tamarix nilotica*, Hmut = *Hyoscyamus muticus*, Plan = *Persicaria lanigera*, Psen = *Persicaria senegalensis*, Chal = *Cardiospermum halicacabum*, Ccol = *Citrullus colocynthis*, Lfus = *Leptochloa fusca*, Paus = *Phragmites australis*, Icyl = *Imperata cylindrica*, Cabs = *Coccinia absyssinca*, Find = *Fagonia indica*. Simb = *Salsola imbricata*, Pcri = *Pulicaria crispa*, Mper = *Mimosa pigra*, Prep = *Panicum repens*, Abli = *Amarnthus blitoides*, Glot = *Glinus lotoides*, Amar = *Ambrosia maritima*, Pdio = *Pluchea dioscoridis*, Pinc = *Pulicaria incisa*, Aliv = *Amaranthus viridis*, Avog = *Astragalus vogelii*, Btou = *Brassica tournefortii*, Cmur = *Chenopodium murale*, Cacu = *Crypsis aculeata*, Csch = *Crypsis schoenoides*, Calo = *Cyperus alopecuroides*, Cmic = *Cyperus michelianus*, Erau = *Echium rauwolfii*, Ealb = *Eclipta alba*, Eaeg = *Eragrostis aegyptiaca*, Fbis = *Fimbristylis bisumbellata*, Hsup = *Heliotropium supinum*, Lara = *Lotus arabicus*, Olin = *Oligomeris linifolia*, Pole = *Portulaca oleracea*, Rden = *Rumex dentatus*, Saeg = *Senecio aegyptius*, Snig = *Solanum nigrum*, Sdur = *Sorghum dura*, T'gla = *Trigonella glabra*. (b) CCA of Lake Nubia shoreline sites. Site numbers followed by the letter 'a' are from the 'wet zone', 'b' from the moist zone, 'c' from the 'semi-dry zone' and 'd' from the 'dry zone'.

Rottb. (C 5–40%, F 12.0%) and *Fimbristylis bisumbellata* (Forssk.) Bubani (C 1–30%, F 25.3%).

Tamarix nilotica and *G. lotoides* dominated the Lake Nubia shores from Debeira (337.5 km south of Aswan High Dam–AHD) to El-Daka (487 km south of AHD). The presence of *H. muticus* extended from Debeira (337.5 km south of AHD) to El-Dewishat (431 km south of AHD). *P. lanigera* was mainly present in the southern section of the lake from Semna (403.5 km south of AHD) to Okma (466 km south of AHD). *P. senegalensis* was also present in southern part at three sites; Semna, Malek El-Nasser and Okma (403.5, 448 and 466 km south of AHD, respectively). The presence of *H. supinum* was restricted to the northern section of Lake Nubia from Debeira (337.5 km south of AHD) to Morshed (378.5 km S. AHD).

CCA ordination diagrams representing species and nominal environmental variables are presented in Figure 2(a), and those representing sites are presented in Figure 2(b). Stands located within the wet zone (labelled 'a') and the moist zone (labelled 'b') were grouped at the top-left corner of the diagram (Fig. 2(b)). Stands belonging to these two zones are characterised by thick silt sediments that can hold water for long periods. Species which characterised the 'wet' zone have low %AC (e.g. *C. aculeata* (5.6%), *G. lotoides* (4.1%), *P. lanigera* (2.9%), *C. schoenoides* (2.8%), *Ambrosia maritima* L., *C. alopecuroides*, *C. michelianus* (2.6%) and *Mimosa pigra* L. (0.4%). Denser vegetation was recorded in the 'moist' zone, characterised by *G. lotoides* (15.2%), *P. senegalensis* (11.7%), *P. lanigera* (10.5%), *P. australis* (5.3%), *H. supinum* (4.3%) and *T. nilotica* (4.0%) and *Coccinia absyssinca* (Lam.) Cogn. (3.2%)).

Stands placed within the elevation limits of the 'semi-dry' zone (labelled 'c') are situated at the bottom-half of the CCA diagram (Fig. 2a and b). Stands at the bottom-middle of the CCA diagram, characterised by loamy deposits, supported abundant populations of *T. nilotica* (AC 34.6%), were occupied by *H. supinum* (AC 8.6%) and *E. aegyptiaca* (AC 4.3%), and *Amaranthus blitoides* S. Watson and *Echium rauwolfii* Delile (AC 1.8%). Stands located at the bottom-left of the CCA diagram, characterised by thick sandy-loam deposits, supported *G. lotoides* (AC 9.8%), *C. schoenoides* (AC 6.1%), *P. lanigera* (AC 5.5%), *P. senegalensis* (AC 4.6%), *P. oleracea* (AC 3.8%) and *P. australis* (AC 2.6%) were observed. Other stands at the bottom-right of the CCA diagram characterised by thinner deposits of gravely-sand texture, where *H. muticus* (AC 8.6%), *Citrullus colocynthis* (L.) Schrad. (AC 0.7%) and *Fagonia indica* Burm. (AC 0.4%) were common. Some of the 'b' stands penetrated to the lower part of the diagram where 'c' stands were located (Fig. 2(b)).

Stands situated within the elevation limits of the 'dry' zone (labelled 'd', at the top-right corner of the CCA diagram, Fig. 2(b) were characterised by shallow sand deposits and by abundant growth of *H. muticus* (AC 37.7%) and very low average cover of *Astragalus vogelii* (Webb) Bornm. (0.3%) and *Salsola imbricata* Forssk. (0.1%). Also, *Oligomeris linifolia* (Hornem.) J.F. Macbr. (AC 5.5%), *G. lotoides* (AC 4.5%), *H. supinum* (AC 4.1%), *Lotus arabicus* L. (AC 3.8%), were common. These stands were clearly separated from the rest of the stands (Fig. 2(b)).

Discussion

Ahti et al. (1973) reported 119 species (30 cultivated species and 13 agricultural weeds) in the area from Wadi Halfa to Gemi (Fig. 1). This is far more than the number of species recorded during the present survey and more closely approximates the 64 species recorded by Pettet et al. (1964) in the same area. Sernson's similarity index between the two studies (Ahti et al., 1973 and Pettet et al., 1964) was 69%. This may be due to variation in the sampling site locations (e.g. close to cultivated land or cities), although the two studies were in the same area at the same time (in 1962). In the present study 27 species are new records to the area. Similarity indices of the plants recorded in this study to the above two studies were 34% and 39%, which indicate that the flora of the area has greatly changed since the dam was commissioned.

Stands from the wet and the moist zones were similar in terms of their species composition and type, and the depth of deposits. Therefore, CCA could not sharply separate between them. But stands sampled from the dry zone were clearly separated from the other zones, because they contained arid and semi-arid desert vegetation elements (e.g. *H. muticus*, *S. imbricata*, *A. vogelii* and *O. linifolia*) growing on sand deposits that were not observed in the other zones. Also, some stands from the moist zone were quite similar in term of their species composition to stands from the semi-dry zone. These stands are intermediate transitional sites between the two zones.

The study revealed that stands of the semi-dry zone can be subdivided into three sub-zones, according to type of deposits and species distinguishing them: (a) sub-zone with loam deposits, characterised by high AC% of *T. nilotica*, and lower AC% of *H. supinum*, *A. blitoides* and *E. rauwolfii* (mainly in sites from the middle sector). Also, this sub-zone was distinguished by the presence of *P. repens*, *I. cylindrical* and *T. glabra*, which can tolerate high salts on the soil surface that produced by *T. nilotica* (Springuel et al., 1991); (b) sub-zone characterised by sandy-loam deposits and dominated by amphibious and/or short lived perennials of lower AC% than those of the previous zone, (e.g. *G. lotoides*, *P. senegalensis*, *P. lanigera* and *P. australis* (mainly in sites from the southern sector)); and (c) sub-zone distinguished by thin gravely-sand deposits, where species with the lowest AC% were recorded, e.g. *H. muticus*, *F. indica* and *C. colosynthis* (mainly in stands from the northern sector). These indicate that deposit type is the most important factor affecting species composition and abundance. Similar findings were obtained by Springuel et al. (1991), who studied the vegetation gradient of Lake Nasser's shoreline in Egypt and by Friedel et al. (1993) in arid Australian environments.

Looking at the strategies of the plants present at each of the four recognised moisture zones, some plant traits can be used directly to associate them with Grime's strategy (Grime et al., 1986; Murphy, 1990; Murphy et al., 1990). Species recorded in the wet and the moist zones are mainly disturbance-tolerant with little stress evidence (disturbance traits: e.g. annuals and a few short-living perennials, rapid growing, early flowering, producing large vegetative propagules and/or vigorous seed production). Most of the species in the 'semi-dry' zone are competitive-stress-tolerant, (stress traits: e.g. perennials, deeply rooted; competitive traits: e.g. high biomass, high water requirement). Species that characterised the 'dry' zone are stress-tolerant, (stress traits: e.g. xerophytic (rigid waxy leaves or very small-sized leaves), perennials, deeply rooted).

The present study indicated that elevation above the MSL, period of inundation and texture of deposits play an important role in determining the distribution of the shoreline vegetation. It is recommended that a long-term study is undertaken to follow the development of the plant populations and the role of sediment transportation in this process. Also, monitoring of plant communities with a high degree of ecological preservation is needed to enable future comparisons is recommended.

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Stoneworts (*Characeae*) and associated macrophyte species as indicators of water quality and human activities in the Pays-de-la-Loire region, France

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Key words: stoneworts, Characeae, Pays-de-la-Loire, water quality, pond, gravel pit

Abstract

The distribution of *Characeae* vegetation from the Pays-de-la-Loire region was studied in ponds and standing waters (e.g. gravel pits, quarries, oxbows) in relation to water quality, other existing algae or macrophyte competition, and human activities. The potential of using stoneworts to characterise the type of biotope is presented and stoneworts show some possibility for use as general bioindicators of trophic quality in ponds and other still waters.

Introduction

The family *Characeae* is the largest and most structurally complex of the green algae (Corillion, 1975; Moore, 1986; Soulié-Märsche, 1989, Krause, 1997), and occupies a wide diversity of habitats (Stewart & Church, 1992; Bornette et al., 1994; Lambert-Servien, 1995; Grillas & Tan Ham, 1998; Vestergaard & Sand-Jensen, 2000; Auderset Joye et al., 2002). Being fast colonizers (Beltman & Allegrini, 1997), stoneworts are often the first plants to grow in newly dug ponds, gravel pits and quarries at the end of the exploitation or after lake restoration (Van den Berg, 1999). Their bioindicator value has been examined in different studies (Krause, 1981; Guerlesquin, 1985; Grillas, 1990; Haury et al., 2002). In the Pays-de-la-Loire, their distribution has been recorded by Corillion (1986) and Guerlesquin & Lambert-Servien (1999).

The objective of the study is to update knowledge about charophyte communities in the Pays-de-la-Loire (Fig. 1) and to survey the extension and the development of the European Habitat (n° 3140 in Annexe I from the Habitat Directive): "Calcareous oligo-mesotrophic waters with *Chara* spp. benthic vegetation"(Lambert-Servien & Guerlesquin, 2002), in relation to human impacts. Competitive relations with other algae or macrophytes were also taken into account.

Materials and methods

Site description

About 140 ponds, sand and gravel or clay-pits, quarries and river cut-off (oxbow lake) were surveyed (Table 1). They are located on the Massif Armoricain, the Bassin Parisien or in the river Loire floodplain, principally in the department of Maine-et-Loire.

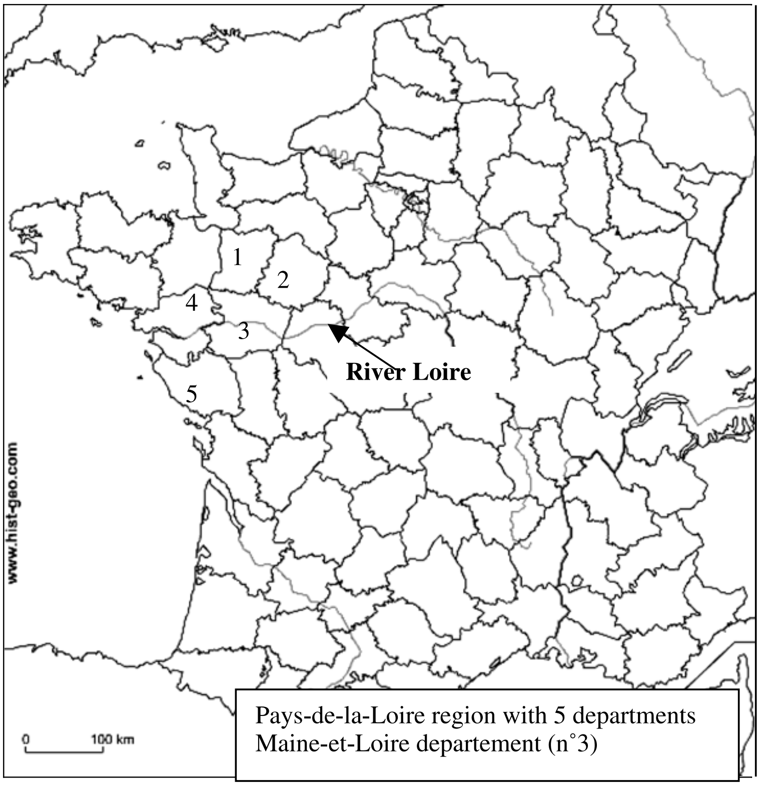


Figure 1. Study area.

Table 1. Data set of the water bodies studied in Pays-de-la-Loire

	68 Ponds	24 Gravel pits,	3 Quarries	15 Clay pits	30 Oxbows
Area (ha)	$3 \times 10^{-3} \rightarrow 60$	$6 \times 10^{-4} \rightarrow 35$	3×10^{-2}	$12 \times 10^{-3} \rightarrow 1.2$	15×10^{-2}
Depth (m)	0.3 → 4 m	0.2 → 4 m	0.5 → 1.5 m	0.5 → 1.3 m	0.5 → 1 m
Origin	Man made often regulated	Man made	Man made	Man made	Natural
Activities	Fishing, bird protection, aquatic activities, water supply	Fishing, bird protection	–	Fishing, bird protection, aquatic activities	Cattle watering
Calcium (mg/l)	2.3/35/91.2	19.5/38.1/88.2	30.4/67.6/111	1.9/13.8/42.3	18.7/37.7/62.7
Total phosphorus (mg/l)	0.02/0.02/2.45	< 0.02/0.07/0.25	< 0.02/0.07/0.13	< 0.02/0.02/0.73	0.15/0.7/1.8
Orthophosphate (mg/l)	–	< 0.02/0.02/0.05	< 0.08	< 0.08/0.14/0.89	< 0.08/0.43/2.17
Nitrate (mg/l)	0.09/2.4/3.2	< 0.1/0.8/2.2	0.09/5.5/20	0.09/0.7/2	0.09/1.15/3
Ammonium (mg/l)	–	0.006/0.04/0.1	< 0.02	< 0.02/0.18/2.04	< 0.02/1.29/5.62
Nitrites (mg/l)	–	0.015/0.07/0.18	< 0.05/0.11/0.3	< 0.05	< 0.05/0.14/0.58
Conductivity (μS/cm)	43/320/779	150/274/418	–	135/304/404	–
pH	6.3/8.4/9.7	7/8.4/10.1	7	6.47/7.9/8.7	–

For the parameters of water quality, the figures are: minimum/mean/maximum.

Macrophytes

A survey of charophyte vegetation was undertaken from April to August/September, between 1985 and 2001. Depending on the location of the Characeae populations (scattered or more widespread within the system), the samples were collected from homogeneous habitats, or from vegetation transects distributed perpendicular to the shore, in order to assess species composition, frequency of occurrence and estimate relative abundance. A visual estimation of the percentage of each taxon was recorded, following the usual Braun-Blanquet methods (Braun-Blanquet et al., 1952). The hydrophyte vegetation (i.e. all submerged and floating-leaved species), growing with the Characeae, was also recorded with the same criteria.

Environmental conditions

The distribution patterns of the Characeae were studied in relation to environmental variables, particularly water chemistry, following the standarts methods: calcium and total phosphorus (NF EN ISO 11885), orthophosphates (NF EN ISO 10304-1), nitrates and nitrites (NF EN ISO 10304-2), ammonium (NF T 90015-2) in mg/l or using field apparatus as thermometer for water temperature, conductimeter and pH-meter (Modele WTW – Multiline F/SET-3-400327) for conductivity and pH, meter and Secchi-disk for depth and turbidity.

Data processing

As a first step we used Correspondence Analysis with SPAD 5.0 Software (Cisia-Ceresta, 2001) to analyses the samples × species dataset (presence/absence data only), for all the investigated water bodies, with or without stoneworts. The environmental data included location parameters, types of waterbodies (qualitative variables) and chemistry (quantitative variables) as illustrative variables. The analysis was then repeated using only those waterbodies, which supported stoneworts.

Results

Species richness and communities (Table 2)

The European Habitat n° 3140 is represented by three genera *(Chara*, *Nitella*, *Tolypella)* and 22

Table 2. Characeae observed in Pays-de-la-Loire

Chara (12)	CF: *C. aspera* Deth. ex Willd., 1809 (CHAS), *C. delicatula* Ag., 1824 (CHDE), *C. globularis* (=*fragilis*) Thuillier, 1799 (CHGL), *C. hispida* L., 1753 (CHHI)
	CV: *C. vulgaris* L. (CHVU), *C. (vulgaris* var.*) longibracteata* (Kütz in Reich.) H. et J. Gr. (CHVL), *C. (vulgaris* var.*) hispidula* A. Br.(CHVH), *C. (vulgaris* var.*) contraria* Braun ex Kützing, 1845, p.p.(CHVC), *C. denudata* Braun, 1847 (CHDN), *C. connivens* Salzmann ex Braun, 1835 (CHCO)
	NF: *C. braunii* Gmelin, 1826 (CHBR), *C. fragifera* Durieu de la Maisonneuve, 1859 (CHFR)
Nitella (9)	CV: *N. confervacea* (Bréb.) A. Br., 1863 (NICO)
	NF: *N. flexilis* Agardh, 1824 (NILF), *N. gracilis* (Smith) Agardh, 1824 (NIGR), *N. hyalina* (DC.) Agardh, 1824 (NIHY), *N. mucronata* (A. Br.) Miquel, 1840 (NIMU), *N. translucens* (Pers.) Agardh, 1824 (NITR)
	NST: *N. opaca* Agardh, 1824(NIOP), *N. syncarpa* (Thuill.) Chev., 1827 (NISY), *N. tenuissima* (Desv.) Kutz., 1843 (NITE)
Tolypella(1)	CF: *T. prolifera* (Ziz. Ex Braun) Leonhardi, 1863 (TOPR)

CF: *Charion fragilis* Krausch 1964, CV: *Charion vulgaris* (Krause ex Krause & Lang 1977) Krause 1981, NST: *Nitellion syncarpo-tenuissimae* Krause 1969, NF: *Nitellion flexilis* Segal ex Krause 1969, each Characeae species is followed by the abbreviations used in Fig. 1.

stoneworts species. Mostly comprising summer-growing species (exceptionally earlier-growing species), it gathers four phytosociological alliances: *Charion fragilis*, *Charion vulgaris*, *Nitellion syncarpo-tenuissimae* and *Nitellion flexilis*.

Distribution

Stonewort communities are spread through approximatively 79% of the gravel-pits and 28% of the ponds (Fig. 2a) surveyed in the target region. Charophytes have also been observed in 53% of the surveyed clay-pits and 13% of the oxbows but as these waterbodies are very numerous and heterogeneous, only a small percentage have been visited during the last years so stoneworts are probably under-recorded. Only three flooded quarries were surveyed, but all had stoneworts.

Most water bodies individually supported only one charophyte species (Fig. 2a). The highest density of charophytes was observed in quite new waterbodies (gravel pits or ponds dug less than 10 years ago) and few older ponds where stoneworts maintain with phanerogams. In clay pits and ponds, the genus *Chara* and *Nitella* were well represented (Fig. 2b) but the genus *Nitella* has not been found in gravel pits or quarries. The genus *Tolypella* was only recorded in a river cut off (oxbow). The species distribution shows that cosmopolitan Characeae, such as *Chara globularis*, *C. vulgaris* and *C. vulgaris* var. *contraria* are spreading in the different biotopes (Fig. 2c).

The first Correspondence Analysis (Fig. 3a) suggested that eutrophication led to lack of stoneworts, except in one oxbow, where a spring-growing species (*Tolypella prolifera*) was found. The second phenomenon was the distinction of pioneer and cosmopolitan species. The third group mainly comprised *Nitella* communities linked to high mineralization.

For the second analysis (Fig. 3b) only including standing waters with *Characeae*, the first axis showed the contrast between gravel pits and ponds, as well as the opposition between "calcareous, basic meso to mesotrophic water communities" and more "neutral, acidic water communities" where *Nitella* species could grow. The third axis distinguished nutrient-rich water bodies such as oxbows, gravel pits and quarries. As already partly shown by Clemenceau (2000), Lambert-Servien (1995) and Lambert-Servien et al. (1998), the locations of the different genera were linked to the type of pond or gravel. Table 3 completes this description of the waterbody types versus trophic status, linked to the different stoneworts species occurrences (trophic classification here follows Håkanson et al., 2000 and Matthieu, 1979, pers. comm. A. Cazaubon, Univ. Marseille).

Competition with other algae and flowering plants

Different species of the Potamion have been observed within the Characeae communities: *Potamogeton trichoides*, *P. crispus*, *Myriophyllum spicatum*, *Najas major*, *N. minor*, *Utricularia australis*, *Zannichellia palustris*. Some species of the *Littorelletea* were also present such as: *Littorella uniflora*, *Baldellia ranunculoides*. The development of algae and Cyanobacteria among the stoneworts can lead to a situation of increasing competition as the season advances. Some examples of competition have been observed mostly against *Chara globularis* and *C. vulgaris*. The competitors were: *Oscillatoria* sp., epiphytic diatoms, *Cosmarium* sp., *Zygnema* sp., *Spirogyra* sp., *Mougeotia* sp., *Hydrodictyon reticulatum*, *Cladophora* sp., *Rhizoclonium* sp., *Oedogonium* sp.

Discussion

The occurrence of *Characeae* vegetation distribution seems to be linked to different gradients. The first one is a time effect. Most of the newly dug gravel pits have stonewort communities, but in some of them, competition between these pioneer algae and flowering plants is beginning. In ponds, pioneer stages have usually disappeared, and helophytes have colonised the shorelines. Thus, depending on the characteristics of pond shoreline, some stonewort communities are maintaining themselves, while in other cases, they are only point communities and they are decreasing. In most cases, we observed a progressive increase of *Myriophyllum* and/or *Potamogeton* communities, or a replacement by helophytes in shallow areas, while stoneworts disappeared. Competition with filamentous green algae occurred, especially in

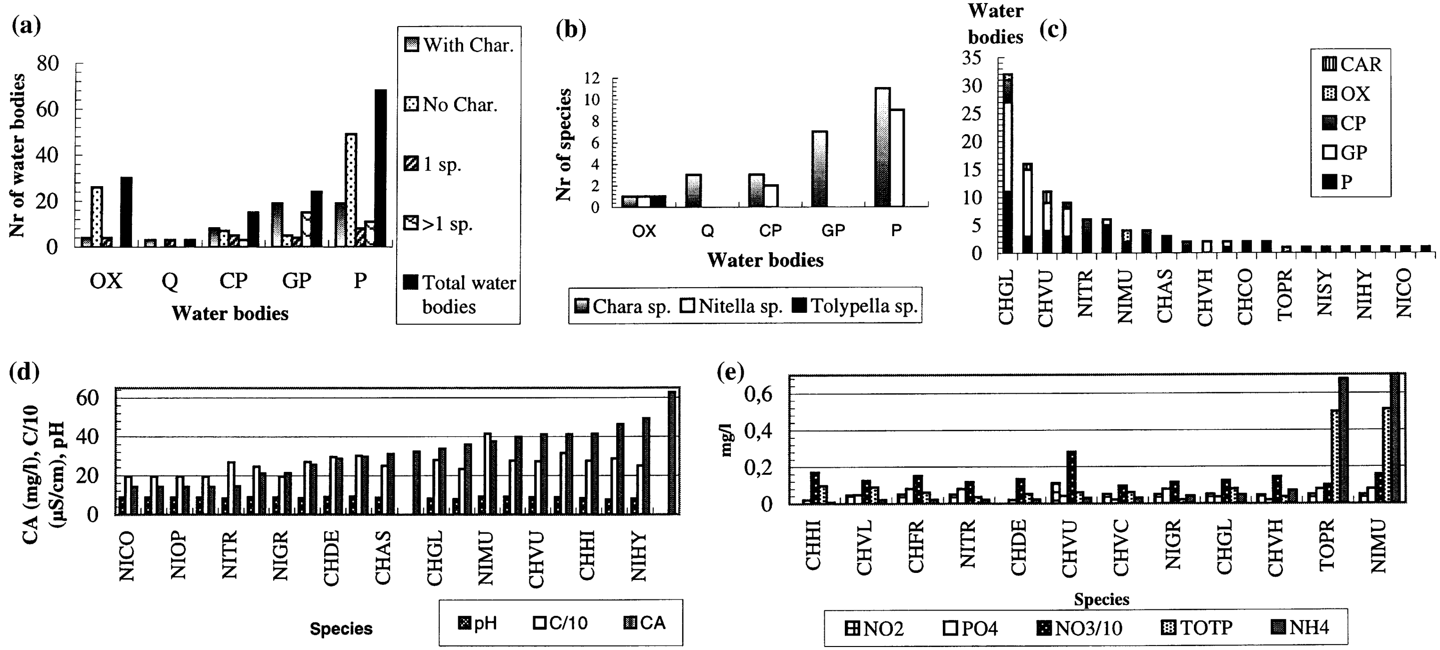
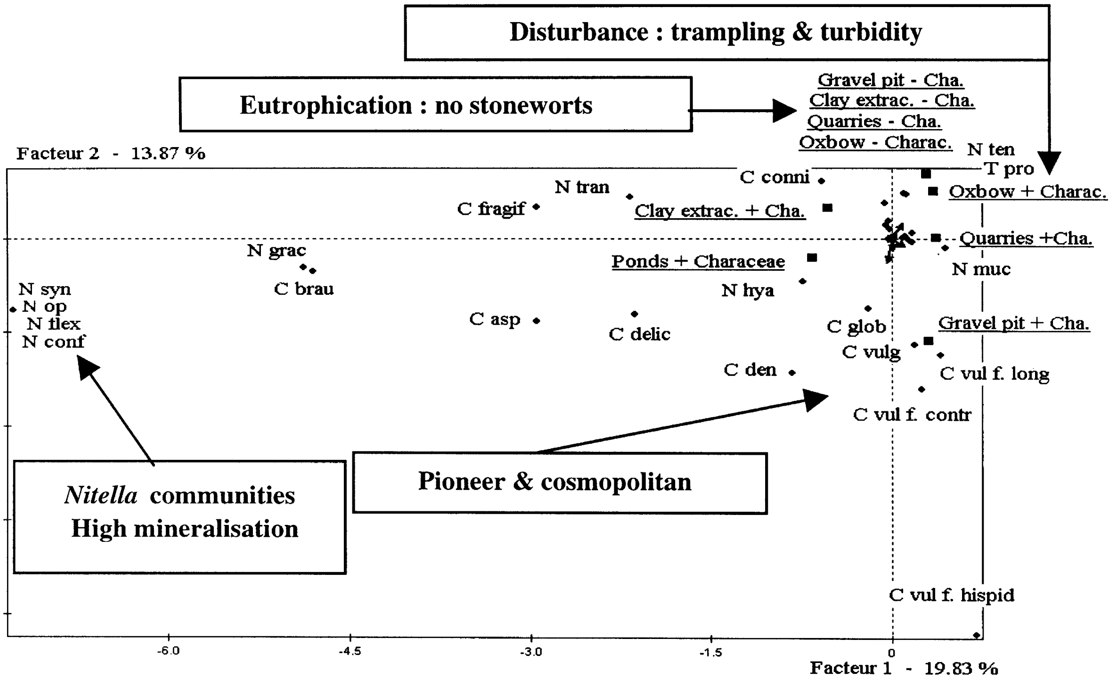


Figure 2. Charophyte distribution according to the different types of water bodies and their physico-chemistry. (a) Presence, absence, species diversity of Charophytes in the water bodies; (b) Number of Charophytes species in the water bodies; (c) Charophytes distribution in the water bodies; (d) Mean values of physical parameters for all the Charophytes species (1); (e) Mean values of physical parameters for some Charophytes species (2); OX: Oxbows; Q: Quarries; CP: Clay-pits; GP: Gravel-pits; P: Ponds With Char.: With Charophytes; No Char.: Without Charophytes; 1 sp.: only one species of Charophyte; > 1 sp.: more then one species of Charophytes; Total water bodies: quantities of the different types of water bodies. C/10: conductivity/10; CA: Calcium (mg/l). CHAS: *Chara aspera*, CHDE: *C. delicatula*, CHGL: *C. globularis (=fragilis)*, CHHI: *C. hispida*, CHVU: *C. vulgaris*, CHVL: *C. vulgaris* var. *longibracteata*, CHVH: *C. vulgaris* var. *hispidula*, CHVC: *C. vulgaris* var. *contraria*, CHDN: *C. denudata*, CHCO: *C. connivens*, CHBR: *C. braunii*, CHFR: *C. fragifera*, NICO: *Nitella confervacea*, NILF: *N. flexilis*, NIGR: *N. gracilis*, NIHY: *N. hyalina*, NIMU: *N. mucronata*, NITR: *N. translucens*, NIOP: *N. opaca*, NISY: *N. syncarpa*, NITE: *N. tenuissima*, TOPR: *Tolypella prolifera*.


Disturbance : trampling & turbidity
Eutrophication : no stoneworts
Gravel pit - Cha.
Clay extrac. - Cha.
Quarries - Cha.
Oxbow - Charac.
Facteur 2 - 13.87 %
N ten
T pro
C conni
Oxbow + Charac.
N tran
C fragif
Clay extrac. + Cha.
0
N grac
Quarries +Cha.
Ponds + Characeae
C brau
N muc
N syn
N op
N flex
N conf
N hya
C asp
C delic
C glob
Gravel pit + Cha.
C vulg
C den
C vul f. long
C vul f. contr
-1
-2
-3
-4
Nitella communities
High mineralisation
Pioneer & cosmopolitan
C vul f. hispid
-6.0
-4.5
-3.0
-1.5
0
Facteur 1 - 19.83 %

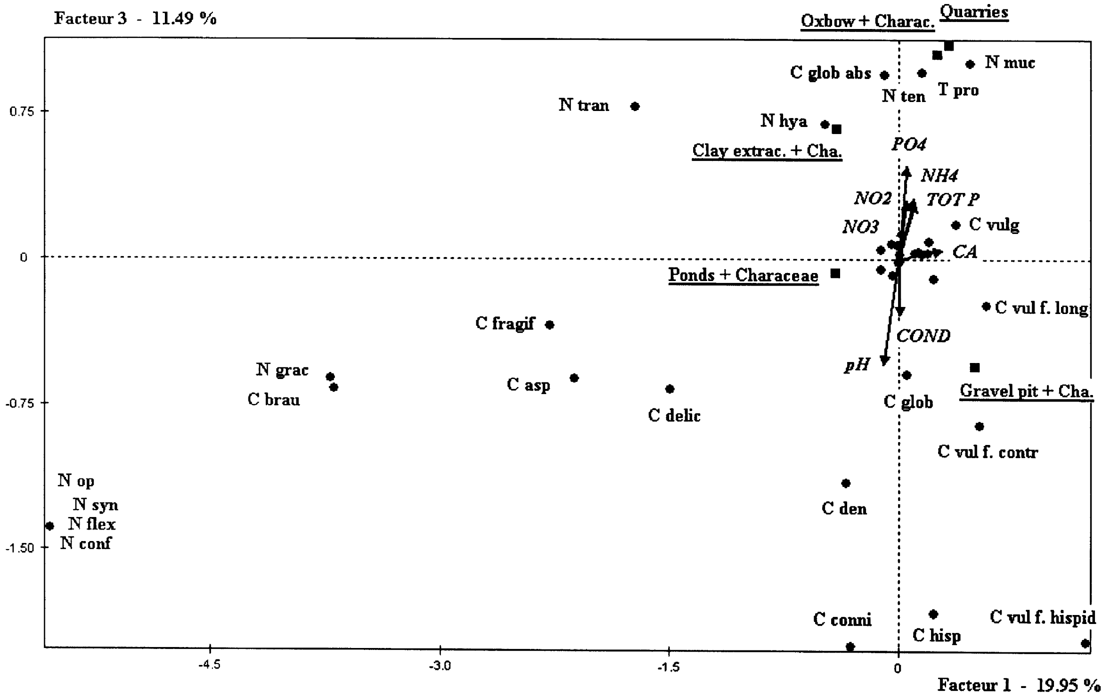

Facteur 3 - 11.49 %
Oxbow + Charac.
Quarries
N muc
C glob abs
N ten
T pro
0.75
N tran
N hya
Clay extrac. + Cha.
PO4
NH4
NO2
TOT P
NO3
C vulg
CA
0
Ponds + Characeae
C vul f. long
C fragif
COND
pH
N grac
C asp
Gravel pit + Cha.
C brau
C glob
-0.75
C delic
C vul f. contr
N op
N syn
N flex
N conf
C den
-1.50
C conni
C hisp
C vul f. hispid
-4.5
-3.0
-1.5
0
Facteur 1 - 19.95 %

Figure 3. Typology with Multiple Component Analysis considering presence–absence of stoneworts + chemistry and location. (a) Multiple Component Analysis: presence–absence of stoneworts + chemistry and location (82 water bodies); (b) Multiple Component Analysis presence of stoneworts + chemistry and location (53 water bodies) C asp: *Chara aspera*, C delic: *C. delicatula*, C glob: *C. globularis (=fragilis)*, C glob abs: no *C. globularis (=fragilis)*, C hisp: *C. hispida*, C vulg: *C. vulgaris*, C vulg f. long: *C. vulgaris* var. *longibracteata*, C vulg f. hispid: *C. vulgaris* var. *hispidula*, C vulg f. contr: *C. vulgaris* var. *contraria*, C den: *C. denudata*, C conni: *C. connivens*, C brau: *C. braunii*, C fragif: *C. fragifera*, N conf: *Nitella confervacea*, N flex: *N. flexilis*, N grac: *N. gracilis*, N hya: *N. hyalina*, N muc: *N. mucronata*, N tran: *N. translucens*, N op: *N. opaca*, N syn: *N. syncarpa*, N ten: *N. tenuissima*, T pro: *Tolypella prolifera* Ponds + Characeae, Clay extraction + Characeae, Oxbow + Characeae, Quarries, Gravel pit + Characeae pH, COND: conductivity, NO_2: Nitrites, NO_3: Nitrates, NH4: Ammonium, CA: Calcium, TOT P: Total phosphorus, PO4: Orthophosphate.

shallow areas where summer water temperature can increase up to 25 °C.

The data show evidence of a trophic gradient within the waterbodies studied (Table 3; Fig. 2d, e) with a decrease from oxbows, quarries, clay pits, ponds to gravel pits. In this region, no oligotrophic shallow lakes or gravel pits occur. Mesotrophic systems were represented by few waterbodies, in which 6 charophyte species occurred when turbidity was not too high. *Nitella hyalina* was restricted to clear mesotrophic waters. The waterbodies of this region were mostly eutrophic. Fourteen stoneworts were present in these systems (with *Chara denudata*, *C. hispidula*, *Nitella tenuissima* solely restricted to this type). Different water bodies are included in the hypereutrophic category ($Pt > 0.09$ mg/l), but the mean value of total phosphorus is important to take into account: gravel pits and quarries: 0.07 mg/l, ponds and clay pits: 0.2 mg/l, oxbows: 0.7 mg/l. Only rarely Charophytes occurred at high Pt (around 0.25 mg/l): mostly *Chara globularis*, *C. contraria*, *C. fragifera*, characteristics of this system: *Chara hispida*, *C. connivens* and *Tolypella prolifera*. Four species (*Chara globularis*, *C. vulgaris*, *C. contraria* and *C. longibracteata*) were recorded at all trophic levels (the last two ones exceptionally in hypereutrophic waters). The results show that some stoneworts can be interesting indicators of water quality as some Characeae were known to be good indicators of water quality for

Table 3. Stonewort distribution according to aquatic surroundings and trophic levels (total phosphorus) in the Pays-de-la-Loire region

Trophic level (P_t mg/l)	Aquatic surroundings (% among the measured water bodies)	Stonewort species comments
Oligotrophic <0.01	One exceptionnel pond record	*C. delicatula*, *C. aspera*, *C. braunii*, *C. fragifera*, *Nitella flexilis*, *N. confervacea*, *N. opaca*, *N. translucens*, *N. gracilis*, *N. syncarpa* Most of them disappeared when increasing phosphorus.
Mesotrophic 0.01–0.02	None oxbows, ponds 6%, clay pits 6%, gravel pits 16%, quarries 25%	*Chara globularis*, *C. vulgaris*, *C. contraria*, *C. longibracteata*, *C. aspera*, ***Nitella hyalina***
Eutrophic 0.02–0.09	None oxbows, quarries 25%, clay pits 46%, ponds 48%, gravel pits 62%	*Chara vulgaris*, *C. globularis*, *C. contraria*, *C. longibracteata*, ***C. hispidula***, *C. delicatula*, *C. fragifera*, *C. aspera*, *C. braunii*, ***C. denudata***, *Nitella translucens*, *[N. mucronata]*, *N. gracilis*, ***N. tenuissima***
Hyper-eutrophic > 0.09	Gravel pits 21%, ponds 45%, clay pits 46%, quarries 50%, all the oxbows	Generally no stonewort. In some few cases: *Chara globularis*, *C. vulgaris*, ***C. hispida***, ***Chara connivens***, *[Nitella mucronata]*, ***[Tolypella prolifera]*** Very seldom: *Chara contraria*, *C. fragifera*, *C. longibracteata*

Stonewort species linked to a trophic level are in **bold**; those found only in pit or quarry are underlined, those found only in pond are double underlined and those found in oxbow are between brackets [].

phosphorus (Guerlesquin, 1985), though earlier findings of phosphorus toxicity to *Chara* have not been confirmed in later papers (Kufel & Kufel, 2002). The particular response of *Tolypella prolifera* and *Nitella mucronata* to high content of total phosphorus and ammonium, regarding the regional results must be pointed out. In the particular case of the eutrophic oxbow with heavy cattle trampling, *Tolypella prolifera* avoided disturbance thanks to its early development, and disappeared when turbidity became too high. The particular response of *Nitella mucronata* to higher calcium content than other *Nitella species* (Kufel & Kufel, 2002) has been observed. The apparent response of *Nitella hyalina* and *Nitella tenuissima* to high calcium needs further study as these species were only recorded twice.

The third main factor is human use: pond management and maintenance. Control of water level influences the distribution of these algae by uncovering the shorelines where they can grow, or by limiting the depth of water, which increases the water temperature in summer. Natural increase of silting over the years or dredging creates a modification of the substrate and of the water transparency, with the environment becoming less favourable for *Characeae*. Anthropic activities such as fishing can have different consequences: destruction of stonewort vegetation when fishermen are wading in the *Chara* communities, modification of the species or extinction when there is a calcium input to increase pH for fish production.

Conclusion

In the Pays-de-la-Loire region, stonewort species are both numerous and widely distributed. Their biological interest for conservation, assessed by the Habitat Directive, is enhanced by some rare species such as *Tolypella prolifera*, which was proposed as a ‘‘determining species’’ by Guerlesquin & Lambert-Servien (1999). Stoneworts show some possibility for use as general bioindicators of trophic quality in ponds and other still waters. The differing sensitivity of some species to disturbance due to water pollution or physical habitat alteration might provide the basis for building a simple bioindication scheme for standing waters. Stoneworts are also useful to characterise the type of biotope, as well as its age and to provide some idea of the dates of creation or dereliction of the waterbody. Distribution versus water chemistry contents should be studied more precisely, with seasonal variations, to settle the proper value of a regional scale, and to compare them to literature. In addition, the relationships of stonewort communities with the general functioning of these still water areas, such as temperature cycles, phytoplankton development and chemical cycles needs further study. The success and stability of stonewort communities, and their functioning in competition with filamentous algae and higher plants also needs further work in order to determine the mechanisms of stonewort community dynamics.

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Habitat variability of the *Littorelletea uniflorae* plant communities in Polish *Lobelia* lakes

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Key words: indicators of habitat conditions, *Littorelletea uniflorae*, *Lobelia* lakes, oligotrophy, soft water

Abstract

A comparative analysis of the habitats of six plant communities of the *Littorelletea uniflorae* Br.-Bl. et R.Tx. 1943 class (*Ranunculo-Juncetum bulbosi* (Nordh. 1921) Oberd. 1957, *Lobelietum dortmannae* (Osvald 1923) Tx. ap. Dierss. 1972, *Myriophyllo-Littorelletum* Jeschke 1959 (variant with *Littorella uniflora* and variant with *Myriophyllum alterniflorum*), *Luronietum natantis* Szańkowski 1998 n.n. and *Isoëtetum lacustris* Szańkowski et Kłosowski 1996 n.n.) was conducted in Poland. The communities differ considerably from each other with respect to water and substrate properties, and attain their optimum development under specific habitat conditions. The present study reveals that, in Poland, the substrate properties of the above communities, with the exception of calcium content, vary to the same extent as those of most communities typical of eutrophic lakes.

Introduction

Among the large number of natural postglacial bodies of water in Europe, the *Lobelia* lakes have been given particular attention. The latter are distinguished from other lakes by their soft waters, poor in calcium and nutrients, and by the presence of rare, relict and endangered species (e.g., *Lobelia dortmanna*, *Littorella uniflora*, *Isoëtes lacustris*, *Luronium natans*, *Sparganium angustifolium*, *Juncus bulbosus*, *Myriophyllum alterniflorum*, *Ranunculus reptans*, *Baldellia ranunculoides*), which form the plant communities of the *Littorelletea uniflorae* Br.-Bl. et R.Tx. 1943 class (Dierßen, 1975; Pietsch, 1977; Mäkirinta, 1989; Szmeja & Clément; 1990; Schaminée et al., 1992). It is estimated that there are about 150 *Lobelia* lakes in Poland, which are located mainly in the Pomerania Lake District (Szmeja, 1996). However, their number has been declining steadily. The changes in the flora and vegetation structure of the *Lobelia* lakes are mainly connected with the influence of eutrophication and acidification (Szmeja, 1997).

Until the mid 1980's studies of the communities of the *Littorelletea uniflorae* class in Poland were concerned mainly with their floristic composition, phytocoenose structure and distribution along the lake littoral (Dambska, 1965; Rejewski, 1981; Boiński & Boińska, 1988). The above communities were regarded as indicators of soft oligotrophic waters and mineral substrates (Pawłowski & Zarzycki, 1972). In 1986, habitat studies of the *Littorelletea uniflorae* vegetation were initiated in Poland (Kłosowski, 1994a, b; Szańkowski, 1998; Szańkowski & Kłosowski, 1996, 2001). The above investigations showed that *Isoëtes lacustris*, *Juncus bulbosus*, *Littorella uniflora*, *Lobelia dortmanna*, *Luronium natans and Myriophyllum alterniflorum* formed the communities of the *Littorelletea* class in Polish *Lobelia* lakes and their ecological amplitudes and optimum occurrence differed considerably from each other.

The present study summarizes the habitat data of six communities of the *Littorelletea uniflorae* class in *Lobelia* lakes in Poland: *Ranunculo-Juncetum bulbosi* (Nordh. 1921) Oberd. 1957, *Lobelietum dortmannae* (Osvald 1923) Tx. ap. Dierss. 1972, *Myriophyllo-Littorelletum* Jeschke 1959 (variant with *Littorella uniflora* and variant with *Myriophyllum alterniflorum*), *Luronietum natantis* Szańkowski 1998 n.n. and *Isoëtetum lacustris* Szańkowski et Kłosowski 1996 n.n.

Material and methods

The survey was carried out between 1986 and 1997, at the height of the growing season (July–August). The habitats of 28 *Lobelietum dortmannae* (from 18 lakes), 22 *Isoëtetum lacustris* (from 17 lakes), 18 *Ranunculo-Juncetum bulbosi* (from 15 lakes), 13 *Myriophyllo-Littorelletum* – variant with *M. alterniflorum* (from 8 lakes), 16 *Myriophyllo-Littorelletum* – variant with *L. uniflora* (from 12 lakes) and 18 *Luronietum natantis* phytocoenoses (from 8 lakes) were investigated. The patches were selected on the basis of dominance of species forming the particular communities. In each phytocoenosis, one phytosociological relevé (Braun-Blanquet, 1951) was recorded and the properties of water and substrate were analyzed. The floristic composition of the phytocoenoses studied is demonstrated in Table 1. The study involved diving when samples were collected in waters deeper than 1 m. Substrate samples were collected by means of a tubular bottom sampler and transferred to plastic bags. They were a mixture of a few random subsamples taken within a phytocoenosis from the root layer. Water samples were taken from an intermediate depth at which a given phytocoenosis occurred and transferred to two 1000-ml plastic containers. Half of the water samples were preserved by the addition of 1 ml of H_2SO_4 for NH_4^+, PO_4^{3-} and COD analyses. Both substrate and water samples were stored at 4 °C and analyzed within 5–10 days. Water depth was measured in the shallowest and deepest point of each patch along the slope of the lake bottom. In total, eight properties of water and eight properties of substrate were considered in the present study.

The following determinations were carried out in both water and substrate samples: pH (using pH-meter), PO_4^{3-} spectrophotometrically by the molybdate method, Ca^{2+} and K^+ with a flame spectrophotometer. In addition, water samples were analyzed for electrolytic conductivity (EC), chemical oxygen demand (COD) as consumption of $KMnO_4$ in acid medium, NH_4^+ by Nessler's method and Na^+ with a flame spectrophotometer. Substrate samples were analyzed for total Fe (spectrophotometrically by the rhodanate method) and NO_3^- (spectrophotometrically by the cadmium reduction method). Concentrations of Na^+ and K^+ were determined in 100 ml solutions extracted with 2 ml 1:1 HCl from evaporated and ignited 100 ml samples of water. Hydration of substrate samples was determined by drying them at 105 °C to constant weight. Organic matter content was assessed by ignition of dry samples at 550 °C for 1.5 h. The ion contents were determined in solutions extracted by addition of 100 ml of demineralized water to a substrate sample containing 1 g of dry matter. In the case of solutions used for determinations of Ca^{2+}, PO_4^{3-} and total Fe, each substrate sample was first dissolved in 20 ml 1:1 HCl within 2 h. After 24 h the extracted solutions were filtered with 3 m-paper, rinsed with small amounts of demineralized water and the final quantities of the solutions were adjusted to 250 ml. Contents of K^+ in substrate were determined in 100 ml water solutions extracted with 2 ml 1:1 HCl from ignited samples.

The multivariate species–habitat relationships were demonstrated by means of Canonical Correspondence Analysis (CCA) using CANOCO for Windows Version 4.0 (Ter Braak & Šmilauer, 1998). Two sets of data were analyzed separately: species–water properties and species–substrate properties. The points of six dominant species representing the ecological optima of the associations studied were displayed in CCA diagrams. Moreover, the habitat conditions of the associations studied were compared with respect to each property using quartiles.

Results

From Figure 1 it is clear that the communities of the *Littorelletea* class develop in a wide range of depth in Polish *Lobelia* lakes. *Juncus bulbosus* develops in the shallowest waters of the littoral zone (0.1–0.4 m). The deepest zone of the lake littoral is inhabited by *Isoëtes lacustris* (1.0–2.5 m). However, dense patches of this species sometimes occur at a depth of 0.6–5.0 m. Since the

Table 1. A synoptic table of the phytocoenoses studied. JB: *Ranunculo-Juncetum bulbosi*; LD: *Lobelietum dortmannae*; LU: *Myriophyllo-Littorelletum*, variant with *Littorella uniflora*; MA: *Myriopyllo-Littorelletum*, variant with *Myriophyllum alterniflorum*, LN: *Luronietum natantis*; IL: *Isoëtetum lacustris*. Constancy classes: V: 100-81%, IV: 80-61%, III: 60-41%, II: 40-21%, I: 20-11%, +: 10-6%, r: 5% or less

Community type	JB	LD	LU	MA	LN	IL
Number of relevés	18	28	16	13	18	22
Number of lakes	15	18	12	8	8	17
LITTORELLETEA						
Ranunculus reptans	II$^{(+-2)}$	I$^{(+)}$	.	$+^{(+)}$	.	.
Juncus bulbosus	V$^{(4-5)}$	II$^{(+-1)}$	.	$+^{(1)}$	.	.
Lobelia dortmanna	IV$^{(+-3)}$	V$^{(3-5)}$	IV$^{(+-4)}$	II$^{(+-1)}$	II$^{(+-1)}$	II$^{(+)}$
Littorella uniflora	$+^{(2)}$	II$^{(+-3)}$	V$^{(4-5)}$	II$^{(+-3)}$		II$^{(+-1)}$
Myriophyllum alterniflorum	$+^{(1)}$	II$^{(+-2)}$	III$^{(+-3)}$	V$^{(4-5)}$	I$^{(+-1)}$	III$^{(+-3)}$
Luronium natans	I$^{(+)}$	II$^{(+)}$	$+^{(+)}$	II$^{(+)}$	V$^{4-5}$	$+^{(+-1)}$
Isoëtes lacustris	$+^{(+)}$	III$^{(+-1)}$	IV$^{(+-1)}$	I$^{(+)}$	III$^{(+-1)}$	V$^{(3-5)}$
Eleocharis acicularis	$+^{(+)}$	.	I$^{(+-1)}$	I$^{(+-1)}$	.	.
Chara delicatula	$+^{(+)}$	I$^{(+-1)}$	.	$+^{(+)}$	$+^{(+)}$	$+^{(+)}$
Sparganium angustifolium	I$^{(+)}$	.	.	.	II$^{(+)}$	.
Nitella flexilis	.	.	$+^{(+)}$	.	I$^{(+-1)}$	II$^{(+-1)}$
Elatine hydropiper	.	.	$+^{(+)}$	.	.	.
POTAMETEA						
Polygonum amphibium	I$^{(+)}$	I$^{(+)}$	II$^{(+)}$	.	.	r$^{(+)}$
Potamogeton natans	I$^{(+)}$	I$^{(+)}$	.	I$^{(+)}$	.	r$^{(+)}$
Elodea canadensis	.	r$^{(+)}$	I$^{(+-2)}$	III$^{(+-2)}$	$+^{(+)}$	r$^{(+)}$
Myriophyllum spicatum	.	.	.	II$^{(+-1)}$	.	.
Nuphar lutea	.	.	$+^{(+)}$	.	$+^{(+)}$	.
Nuphar pumila	.	.	.	I$^{(+)}$	$+^{1}$	.
Myriophyllum verticillatum	.	.	.	$+^{(+)}$	I$^{(+-1)}$.	.
Callitriche hamulata	.	.	.	$+^{(+)}$	$+^{(+)}$	r$^{(2)}$
Ceratophyllum demersum	.	.	.	I$^{(+)}$	.	.
Potamogeton obtusifolius	.	.	.	.	$+^{(+)}$.	.
Potamogeton bertholdii	.	.	.	.	.	r$^{(+)}$
PHRAGMITETEA						
Galium palustre	I$^{(+)}$	.	.	.	.	.
Alisma plantago-aquatica	$+^{(r)}$	.	.	.	.	.
Glyceria fluitans	I$^{(+-1)}$	.	.	.	.	.
Lysimachia thyrsiflora	II$^{(+-1)}$	I$^{(+)}$	.	.	. .	
Carex rostrata	I$^{(+-1)}$	r$^{(+)}$	$+^{(+)}$	.	.	.
Eleocharis palustris	IV$^{(+-1)}$	II$^{(+-2)}$	III$^{(+-1)}$	$+^{(+)}$	.	.
Phragmites australis	I$^{(+-1)}$	r$^{(+)}$	.	$+^{(+)}$	$+^{(+)}$	.
Equisetum fluviatile	.	I$^{(+)}$	I$^{(+)}$	$+^{(+)}$	.	.
Phalaris arundinacea	.	r$^{(+)}$	.	.	.	.
Scirpus lacustris	.	.	.	$+^{(+)}$	.	
SCHEUCHZERIO-CARICETEA						
Hydrocotyle vulgaris	III$^{(+-2)}$	.	.	.	.	.
Agrostis stolonifera	II$^{(+-2)}$	.	.	.	.	.

Continued on p. 120

Table 1. (Continued)

Community type	JB	LD	LU	MA	LN	IL
Agrostis canina	$+^{(2)}$	.	.	.	.	.
Ranunculus flammula	$\mathrm{I}^{(2)}$	$\mathrm{I}^{(+)}$	.	.	.	.
Carex lasiocarpa	$\mathrm{I}^{(2)}$	$\mathrm{I}^{(+-1)}$	.	.	.	.
Calamagrostis stricta	$+^{(+)}$	.	.	.	.	.
Eriophorum angustifolium	$+^{(+)}$	.	.	.	.	.
Bryophytes						
Sphagnum cuspidatum	$+^{(+)}$	.	.	.	.	.
Sphagnum denticulatum	$\mathrm{II}^{(+-3)}$	$+^{(+)}$	.	.	$\mathrm{I}^{(+-1)}$	$\mathrm{II}^{(+-2)}$
Warnstorfia fluitans	$\mathrm{I}^{(2-3)}$	.	.	.	.	$\mathrm{II}^{(+-3)}$
Warnstorfia exannulata	$\mathrm{I}^{(+-1)}$	.	.	.	.	$\mathrm{r}^{(1)}$
Chiloscyphus polyanthos	$+^{(1)}$	.	.	.	.	$\mathrm{r}^{(3)}$
Fontinalis antipyretica	.	$\mathrm{r}^{(+)}$	$\mathrm{I}^{(+)}$	.	.	$\mathrm{II}^{(+-2)}$
Drepanocladus tenuinervis	.	.	$+^{(1)}$	.	.	.
Riccardia multifida	.	.	.	.	.	$\mathrm{r}^{(3)}$
Fontinalis dalecarlica	.	.	.	.	.	$\mathrm{r}^{(3)}$
Other species						
Juncus effusus	$\mathrm{II}^{(+-1)}$	.	.	.	.	.
Lycopus europaeus	$\mathrm{I}^{(+)}$	.	.	.	.	.
Mentha aquatica	$+^{(+)}$	.	.	.	.	.
Sparganium × diversifolium	$+^{(+)}$	.	.	.	.	$\mathrm{r}^{(+)}$
Lemna trisulca	.	.	.	$\mathrm{I}^{(+-1)}$	.	.
Chara fragilis	.	.	.	$+^{(+)}$	.	.
Utricularia minor	.	.	.	.	$+^{(+)}$	.

limits shown in the diagram are medians of depth data recorded in the shallowest and deepest point of each patch, only the most typical scenario is demonstrated. *Lobelia dortmanna*, *Littorella uniflora*, *Myriophyllum alterniflorum* and *Luronium natans* occupy an intermediate position. *L. dortmanna* usually grows at the same depth as *L. uniflora* (0.4–0.8 m) as well as in very shallow waters (0.4–0.1 m) as in the case of *J. bulbosus*. *M. alterniflorum* occurs in a wider depth range than *L. uniflora*; its patches are found at the depth ranging from 0.5 to 1.2 m. The depth range of *L. natans* is narrow (0.8–1.2 m) and often overlaps the zone of *I. lacustris*. During the survey the patches of the communities of the *Littorelletea* class were never recorded above the water line.

The layout of *Littorelletea* associations along the depth gradient relates to the big differences in some parameters of substrate (Figs 2 and 3). The CCA diagram demonstrates that *L. natans* is distinguished from the other species by the high content of organic matter in substrates (20–40% of dry matter, Fig. 3), which are highly saturated with water (about 75–85%). Since the variables are strongly correlated with the first ordination axis, the organic matter content and hydration play a most important role in differentiating the substrates. *I. lacustris* grows on substrates richest in PO_4^{3-} (0.5–1.0 g/kg of dry matter, Fig. 3), K^+ (0.2–0.9 g/kg) and total Fe (1–4 g/kg). These parameters are highly correlated with each other. The substrates of *L. natans* and *I. lacustris* are most acidic (the values of both medians of pH are 5.2, Fig. 3). *J. bulbosus*, *L. dortmanna*, *L. uniflora* and *M. alterniflorum* are found mainly on sandy substrates with a small content of organic matter (0–5%). Moreover, they are poor in K^+, PO_4^{3-} and total Fe but differ considerably with respect to NO_3^-. Like *L. natans* and *I. lacustris*, *J. bulbosus* and *L. uniflora* grow on substrates containing higher amounts of NO_3^-, whereas the substrates of *L. dortmanna* and *M. alterniflorum* contain lower

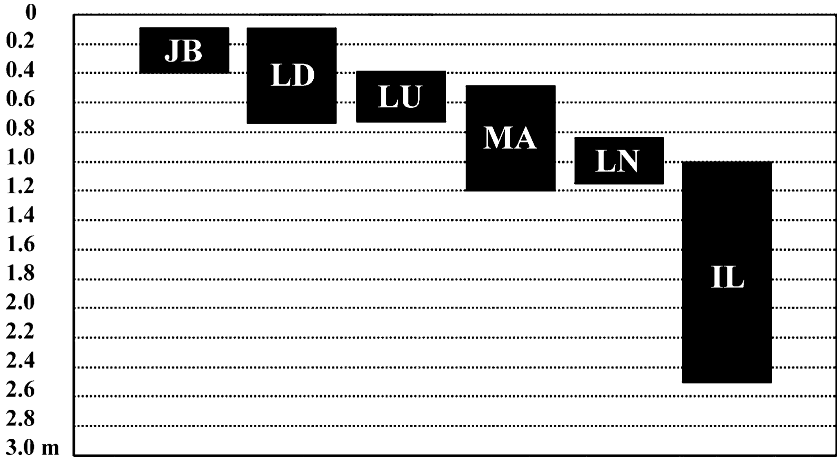

Figure 1. Distribution of the communities studied along the gradient of depth in Polish *Lobelia* lakes. The limits are medians of depth recorded in the shallowest and deepest point of each patch. JB – *Ranunculo-Juncetum bulbosi*; LD – *Lobelietum dortmannae*; LU – *Myriophyllo-Littorelletum*, variant with *Littorella uniflora*; MA – *Myriopyllo-Littorelletum*, variant with *Myriophyllum alterniflorum*, LN – *Luronietum natantis*; IL – *Isoëtetum lacustris*.

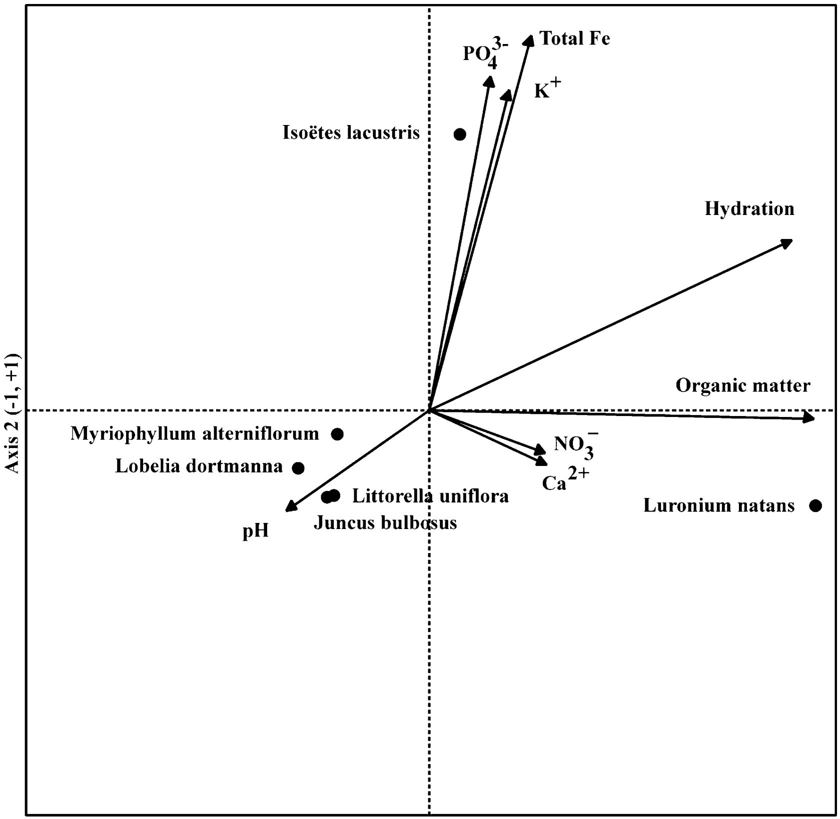

Figure 2. CCA ordination diagram of 1st and 2nd axes obtained from 8 parameters of substrate and 115 relevés. The character species of the six associations studied were plotted. Eigenvalues: $\lambda 1 = 0.77$, $\lambda 2 = 0.50$, $\lambda 3 = 0.19$, $\lambda 4 = 0.11$, $\Sigma\lambda = 6.46$.

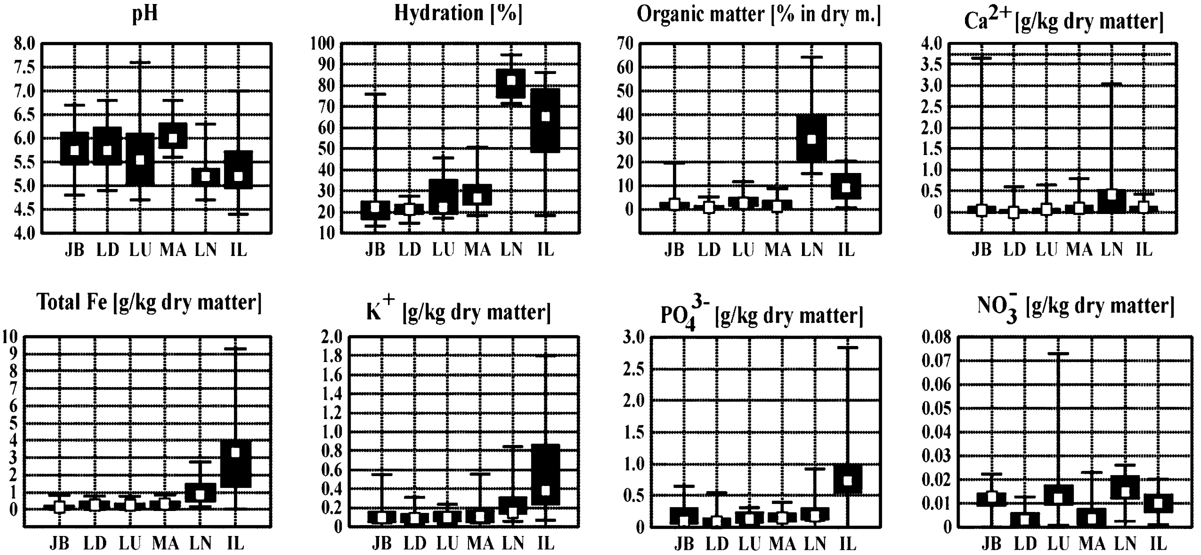

Figure 3. Variability of the phytocoenoses studied in relation to 8 parameters of substrate. Black boxes show 25–75% interquartile ranges of values and white boxes show the medians. JB – *Ranunculo-Juncetum bulbosi*; LD – *Lobelietum dortmannae*; LU – *Myriophyllo-Littorelletum*, variant with *Littorella uniflora*; MA – *Myriopyllo-Littorelletum*, variant with *Myriophyllum alterniflorum*, LN – *Luronietum natantis*; IL – *Isoëtetum lacustris*.

levels of nitrate. Compared with the other species, *L. natans* shows preference for substrates richer in Ca^{2+}.

Considerable differences between the phytocoenoses studied were also found regarding their waters (Figs 4 and 5). From the ordination diagram (Fig. 4) it is clear that Ca^{2+} is the main factor differentiating the waters of *Littorelletea* communities in Poland. *M. alterniflorum* develops in waters with the highest amount of Ca^{2+} (12–18 mg/l), whereas *L. natans* grows in very soft waters containing 1–2 mg of Ca^{2+}/l. A relatively high amount of calcium is recorded in the waters of *L. dortmanna* (7–12 mg/l) and *I. lacustris* (6–8 mg/l). The waters of *J. bulbosus* and *L. uniflora* contain 2–5 mg of Ca^{2+}/l. *M. alterniflorum* is also distinguished from the other species by higher values of pH, EC and COD (Figs 4 and 5). It develops in neutral and slightly alkaline waters with pH exceeding 7.5 (Fig. 5), whereas *I. lacustris* prefers slightly acidic waters with pH 5.8–6.3. The waters of *M. alterniflorum* are characterized by the highest values of electrolytic conductivity (85–150 μS/cm), which in the case of *L. uniflora* and *L. dortmanna* reach 85 μS/cm. The EC of waters in the patches of *J. bulbosus*, *I. lacustris* and *L. natans* ranges from 40 to 60 μS/cm. However, 50% of the *L. natans* phytocoenoses develop in waters with EC 40 μS/cm. Compared with the other communities studied the patches of *M. alterniflorum* develop in waters with a higher amount of dissolved organic matter (COD varying between 6 and 10 mg O_2/l). The lowest COD (2 mg O_2/l) was recorded in the waters of *L. natans*. This species grows in waters with relatively high concentration of Na^+ (5.5–6.5 mg/l, Fig. 5). The other species, with the exception of *L. uniflora*, develop in waters with a low concentration of Na^+ (2–4 mg/l). It is notable that *I. lacustris* grows in waters with the highest concentration of PO_4^{3-} (about 0.04 mg/l) and NH_4^+ (up to 0.35 mg/l, Fig. 5).

Discussion

A considerable amount of work has been published on the *Littorelletea uniflorae* class (see Murphy 2002). However, most of the research was focused on its phytosociological characteristics and phytocoenose structure (e.g., Den Hartog & Segal, 1964; Schoof-van Pelt & Westhoff, 1969; Schoof-van Pelt, 1973; Dierßen, 1975; Pietsch, 1977; Markirinta, 1989; Szmeja & Clément, 1990; Schaminée et al., 1992; Rodwell, 1995). The asso-

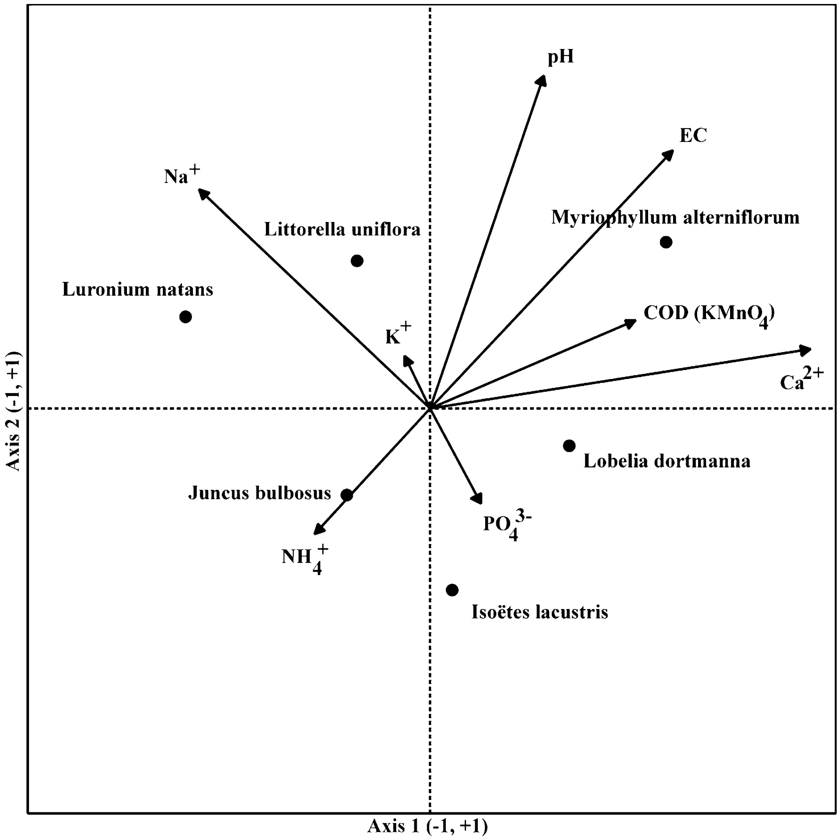

Figure 4. CCA ordination diagram of 1st and 2nd axes obtained from 8 parameters of water and 115 relevés. The character species of the six associations studied were plotted. Eigenvalues: $\lambda 1 = 0.57$, $\lambda 2 = 0.50$, $\lambda 3 = 0.28$, $\lambda 4 = 0.11$, $\Sigma\lambda = 6.457$.

ciations of the *Littorelletea* class are regarded as indicators of soft and very soft waters ranging from oligotrophic, oligo-dystrophic to mesotrophic (Murphy, 2002) with Ca^{2+} concentrations below 15 mg/l (Murphy 2002) and alkalinity below 2 meq/l (Arts 2002). They are associated with mineral substrates poor in calcium (Schoof-van Pelt, 1973; Arts et al., 1990; Arts & Buskens, 1998; Smolders et al., 2002 etc.).

The present results were compared with the ecological data reported by other authors from other regions of Europe. Earlier observations which point to calcium-poor waters as optimal for the development of communities of the *Littorelletea* class have been confirmed by this study. In the waters of most of the phytocoenoses analyzed in the study the Ca^{2+} concentration did not exceed 12 mg/l. Only in the waters of *Myriophyllo-Littorelletum* – variant with *M. alterniflorum* the Ca^{2+} concentration often reached 18 mg/l. Compared with the soft waters inhabited by isoetid vegetation in different regions of Europe, e.g., Finland, Norway and Great Britain (Arts, 2002) the range of Ca^{2+} in the waters of the *Littorelletea* communities in Poland is relatively wide (0–20 mg/l). The present study showed that the communities of *Littorelletea* differed from each other with respect to other properties of water as well (Na^+, NH_4^+, electrolytic conductivity, COD–$KMnO_4$, pH). It appears, however, that calcium is the crucial property in differentiating the waters of the associations studied. Considerable differences were found between the waters of *Luronietum natantis*, containing the lowest amount of Ca^{2+}, and those of *Isoëtetum lacustris*, *Lobelietum dortmannae* and *Myriophyllo-Littorelletum* – variant with *M. alterniflorum*, which were richer in Ca^{2+}. The association of *M. alterniflorum* phytocoenoses with waters containing higher levels of Ca^{2+} has already been established (Pietsch, 1977, 1982, 1984

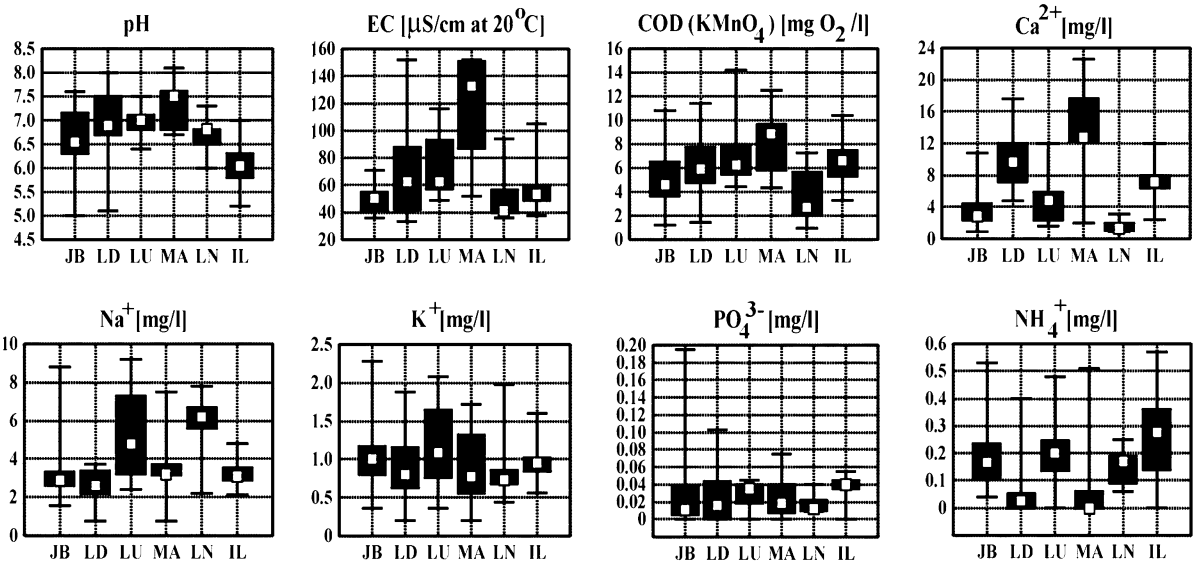

Figure 5. Variability of the phytocoenoses studied in relation to 8 parameters of water. Black boxes show 25–75% interquartile ranges of values and white boxes show the medians. JB – *Ranunculo-Juncetum bulbosi*; LD – *Lobelietum dortmannae*; LU – *Myriophyllo-Littorelletum*, variant with *Littorella uniflora*; MA – *Myriopyllo-Littorelletum*, variant with *Myriophyllum alterniflorum*, LN – *Luronietum natantis*; IL – *Isoëtetum lacustris*.

& Vöge, 1993). However, it is interesting to note that the patches of *Lobelietum dortmannae* are found to occur at higher Ca^{2+} concentration than those of *Myriophyllo-Littorelletum* – variant with *L. uniflora*. The data obtained by other authors from Western Europe indicate that *Littorella uniflora* develops in waters with higher concentration of Ca^{2+} (Pietsch, 1982) and alkalinity (Farmer & Spence, 1986, Vestergaard & Sand-Jensen, 2000). It seems that the narrow amplitude of Ca^{2+} that lies within low values in the waters of *Luronietum natantis* is characteristic only of the *Lobelia* lakes in Poland. In Western Europe, *L. natans* occurs in waters with a wider range of Ca^{2+} (Pietsch, 1982; Greulich et al., 2000).

The present study has also confirmed that the communities of *Littorelletea uniflorae* are associated with substrates poor in Ca^{2+}. In most of the phytocoenoses studied, the Ca^{2+} content did not exceed 0.2 g/kg of dry matter and only in the case of *L. natans* reached 0.5 g/kg. The following properties proved to be more crucial in differentiating the substrates of the *Littorelletea* communities studied in Poland: organic matter content, hydration, PO_4^{3-}, K^+ and total Fe. The above properties varied considerably in the littoral zone of the *Lobelia* lakes. The extent to which they varied was comparable to that of substrates of most communities typical of eutrophic waters (see Kłosowski, 1994b). The present results and the findings of other authors (Roelofs, 1983; Van Katwijk & Roelofs, 1988; Arts et al., 1990; Arts & Buskens, 1998) indicate that some of the communities associated with the shallow part of the littoral zone (*Ranunculo-Juncetum bulbosi*, *Lobelietum dortmannae* and both variants of *Myriophyllo-Littorelletum*) are confined to mineral substrates. However *Luronietum natantis*, which occurs in Poland at intermediate depths, attains its optimum development on very thick (60–100 cm), highly hydrated organic sediments, whereas the deep-water community of *Isoëtetum lacustris* shows preference for rather thin (5–20 cm) mineral–organic substrates, with higher PO_4^{3-}, K^+ and total Fe contents. The results obtained for the latter two communities are inconsistent with the data reported from other regions of Europe, indicating that *L. natans* develops best in sites where the organic matter content does not exceed 20% (Greulich et al., 2000), whereas *Isoëtes lacustris* shows preference for nutrient-poor sandy sediments (Arts & Buskens, 1998 after Schoof-van Pelt, 1973).

Although the substrates of the communities studied contained small amounts of NO_3^-, they differed considerably with respect to this parameter. Nitrate is especially important since the isoetids are mainly dependent upon it for their nitrogen-source (Schuurkers et al., 1986; Arts, 2002; Smolders et al., 2002).

Conclusions

The present study reveals that the communities of *Littorelletea uniflorae* class differ from each other with respect to their habitat conditions not only between the different regions of Europe but also within an individual area as in the case of the *Lobelia* lakes in the Pomerania Lake District. It appears that nearly every plant community of the *Littorelletea* class attains its optimum development under specific habitat conditions. The *Littorelletea* associations may, therefore, be good indicators of the littoral habitat conditions in soft water lakes.

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The possibility of submerged macrophyte recovery from a propagule bank in the eutrophic Lake Mikołajskie (North Poland)

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Key words: submerged macrophytes, eutrophication, seed, oospores, propagule bank, lake littoral

Abstract

Changes in submerged macrophyte communities in the eutrophic Lake Mikołajskie have been studied for the last 40 years. As the recent commissioning of a number of sewage treatment plants within the complex of the Mazurian Great Lakes has led to an improvement in water transparency, it was expected that species composition would start to return to the state present before intensive eutrophication. The role of seed and oospore banks in the reconstruction of submerged macrophytes is analysed on the basis of laboratory experiment. Cores of sediments (9 cm long) were collected from the littoral of Lake Mikołajskie. The cores were divided in 3 cm layers and were incubated under laboratory conditions. Five species of submerged macrophytes were germinated from the propagule banks. The richest in viable propagules was the deepest layer and poorest in active propagules was the shallowest layer of sediment. The recolonization of the littoral zone of Lake Mikołajskie by species, which occurred at a earlier period in the lake's history is possible because viable propagules have persisted in deeper sediments.

Introduction

The changes in submerged macrophyte communities in the littoral of Lake Mikołajskie are perhaps among the best-documented examples of the long-term changes of this community in temperate eutrophic lakes due to advancing eutrophication. Previous papers (Ozimek & Kowalczewski, 1984; Kowalczewski & Ozimek, 1993) clearly indicated a decline in macrophyte species and a changing floristic type – from *Chara* type through a *Potamogeton* type to a *Myriophyllum–Potamogeton* type. Between 1990 and 2000 all Characeae species disappeared completely (Ozimek unpubl.).

Rapid eutrophication of Lake Mikołajskie has taken place between 1970s and 1990s because large amounts of artificial fertilisers from agricultural land and untreated sewage from town and villages were washed into the lake. The town and villages located on the lake-shore up to 1996 had no proper sewage systems, and untreated wastes went directly to the lake. Lake water showed an increase in nutrient concentrations and a deterioration of water transparency, especially during spring and summer, i.e. during the period critical for plant development. The Secchi depth decreased from 4.5 to 2.0 m in spring and from 2.5 to 0.5 m in summer over the period 1970–1995. Light measurements in the littoral zone indicated that 1% of surface light penetrated to a depth of 6.0 m in 1975, to a depth of 3.5 m in 1980 and only to a depth of 2.0 m in 1990 (Ozimek, unpubl.).

After political changes in Poland, many farms were closed for economic reasons, and their land remained unused. In 1997 the town of Mikołajki opened its modern sewage treatment plant. These positive changes limited the inflow of nutrients and resulted in significantly improvement transparency of the water (Kufel & Kufel, 1999).

The propagule bank is an important factor ensuring the recolonization of submerged macrophytes in lake littoral (Crawford, 1979). The recovery of macrophytes is needed to ensure the persistence of the clear water state (Hosper, 1997).

The working hypothesis assumed a relationship between reestablishment of a diverse submerged macrophyte community from the propagule bank and improvement of light conditions in temperate eutrophic lake. In order to verify these hypotheses laboratory experiments on germinating plants from propagules located in different layers of sediment were carried out.

Material and methods

Lake Mikołajskie is holomictic and has a surface area of 460 ha, a mean depth of 11 m and maximum depth of 27.8 m. The area occupied by the submerged macrophytes was about 44 ha in 1971, 30 ha in 1980, 25 ha in 1995 (Kowalczewski & Ozimek, 1993).

Sediments were collected in the littoral of Lake Mikołajskie from three sites at which the species composition, distribution and biomass of submerged macrophytes had previously been carried out in July each year between 1975 and 2001. Samples were collected by tubular Kajak bottom sampler (surface area of 10 cm^2) at 0.5 m water depth intervals, between 0.5 and 3.5 m inclusive. The depth 3.5 m was the maximum depth-range of submerged macrophytes in the last two decades. Cores of sediment 9 cm long were taken (ten cores from each depth). Each core was divided into three layers of 0–3, 3–6 and 6–9 cm. The organic matter content in dry mass of sediment was determined from 5 replicates in each layer. Dry samples were incinerated in a muffle furnace for 5.5 h at 550 °C (Hermanowicz et al., 1976).

The germination of plants from propagules (seeds and oospores) present in sediments was conducted in 1.5 l chambers in the laboratory. The mean temperature of water in chambers was 20.0 ± 0.1 °C, and light intensity was 40 $\mu m\ s^{-1}\ m^{-2}$. Light intensity has checked using a LI-COR 21–189 photometer. Photoperiod was 16 h of light and 8 h of dark. Each chamber was filled by 40 ml of sediments and 1.0 l of water. To compensate for evaporation volume 0.2 l of water was added daily to each chamber. Schematic diagram of experiment is shown on Fig. 1. There were five replicates for germination of propagules from each layer of sediment of each depth. Incubation of sediments was lasted 61 days. Counts of germinating plants being made daily during 30 days and after 61 day of experiment.

Results

The organic matter contents in the littoral sediments used in experiments was low (not exceeding 2%) and was distributed evenly. There were thus no significant differences in organic matter contents either between successive layer of sediment from given depth, or between the same layers taken from different depth (Fig. 2). This is mainly a reflection of the carriage of matter to deeper parts of lake, as well as the rapid mineralization of organic matter.

Five species were germinated from propagule bank during the 61 days of experiment. The germination rate for propagules differed in relation to the species and layer from which sediments had come. The most rapid onset of germination was found for *Chara fragilis* in the middle (3–6 cm) layer of sediment. The prevailing plants to germinate from all layers were *Myriophyllum spicatum*, *Potamogeton pectinatus* and *P. perfoliatus*, while oospores of *Chara* capable of germination were mainly found in the deepest (6–9 cm) layer (Table 1). The distribution of viable propagules at different depths varied with no plants emerging from sediments taken from depths of 1.0 and 1.5 m, irrespective of which of the three layers was involved. Furthermore, irrespective of the depth from which sediments had been collected, the layer shown to be richest in viable propagules was the deepest (6–9 cm) layer, while that poorest in propagules is the shallowest (i.e., the first 3 cm of sediment, Fig. 3).

Discussion

The recolonization of the littoral zone by species, which occurred earlier in the lake is therefore possible, if their germinable propagules have persisted in sediments (Grillas et al., 1993; Bonis

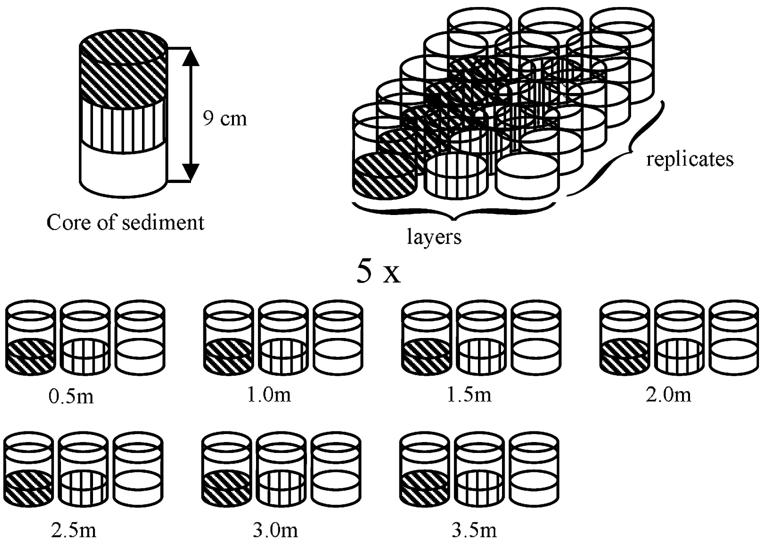

Figure 1. Schematic diagram of experiment on propagules germination from sediment of Lake Mikołajskie.

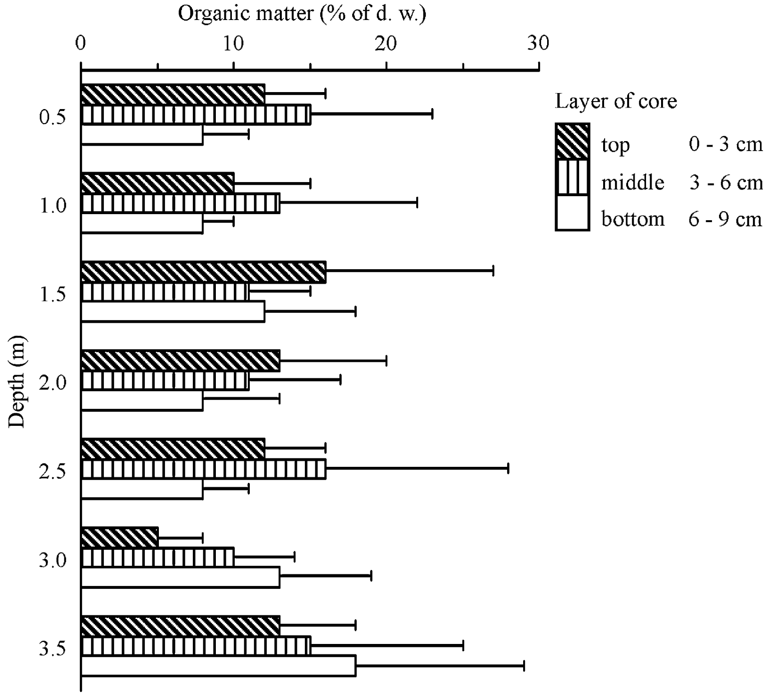

Figure 2. The organic matter content (mean ± SD, *n* = 5) in the successive layer of sediments taken from different depth of littoral of Lake Mikołajskie (July, 2001).

et al., 1995). During restoration of hypereutrophic lake the reestablishment of submerged macrophytes from seed banks has been indicated by various authors (Meijer et al., 1994; Simons & Nat, 1996). Stoneworts play an important role in this process since they produce a large number of propagules. As reported Van der Berg (1999) in the *Chara* 6 years old stands about 1.6×10^6 oospores per m^2 had accumulated in the top layer (1–7 cm) of sediments.

Table 1. Onset of germination of propagules and percent of particular species to total number of germinated plants after 61 days of experiment in successive layers (1–top, 2–middle, 3–bottom) of sediment of the littoral of Lake Mikołajskie (July 2001)

Species	Layers of sediment					
	1	2	3	1	2	3
	Onset of germination (day of experiment)			Contribution (%)		
M. spicatum L.	11	12	20	69	62	6
P. pectinatus L.	20	32	60	20	11	1
P. perfoliatus L.	60	60	60	11	8	4
P. tamogeton lucens L.	60	60	60	0	3	3
C. fragilis Desvaux	0	3	12	0	16	86

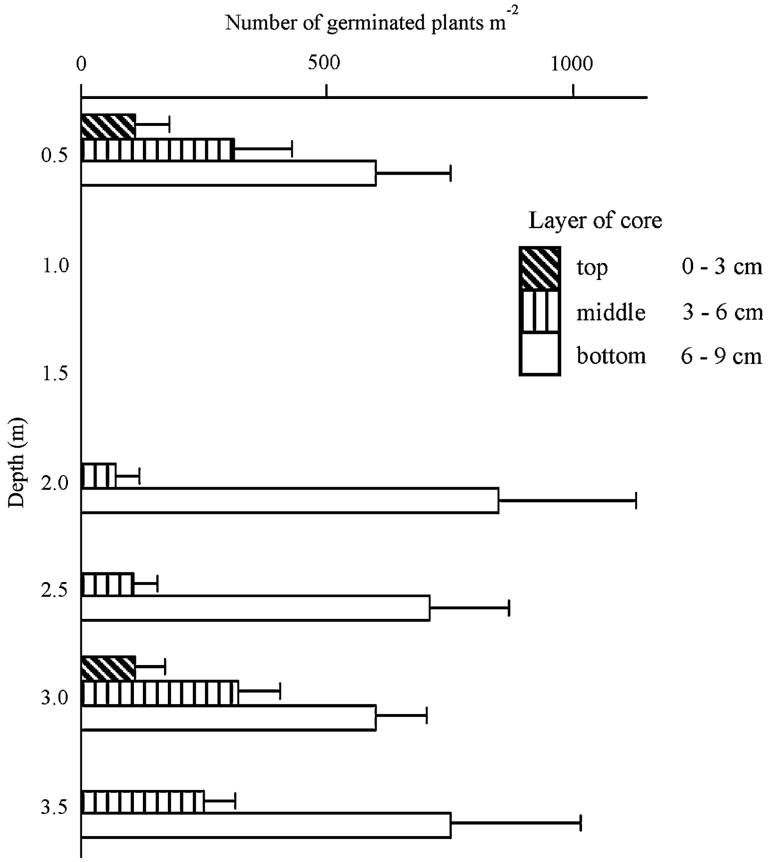

Figure 3. Number (mean ± SD, $n = 10$) of germinating propagules per m^2 from successive layer of sediments taken from different depth of littoral of Lake Mikołajskie after 61 days of cultivation (July, 2001).

Under field conditions a large part of oospores and seeds may undergo dormancy. This may result in an accumulation of oospores in sediment, in ecology called "storage effect". The "storage effect" is demonstrated in sediments in Lake Mikołajskie. Historical records of plant distribution at the site are useful because the seeds of desired species will be present where they had the densest growth in the past. *Chara* having been present only in vegetative form from 1972 and it disappeared from the site of Lake Mikołajskie in 1978 (Ozimek & Kowalczewski, 1984).

According to Van der Berg (1999), ca. 15% of the oospore generated in any given year go on to germinate in the next year. Thus the active oospores in the superficial layer had been exhausted during the years in which there was renew of stoneworts every year, but no restoration of the oospore bank had occurred due to the species confinement to the vegetative form. That can be the reason of oospores absent in top-layer of sediments of Lake Mikołajskie.

No plants emerging from sediments taken from depths of 1.0 and 1.5 m. This result of experiment has not to mean that on this depths sediments in whole littoral are lack in macrophyte propagules. On the depths 1–1.5 m on sites from which sediments were collected *Elodea canadensis* and *Batrachium circinatum* have dominated during the last decade. *E. canadensis* does not produce seeds. For seeds of *B. circinatum* probably in laboratory conditions there were not properly environmental signals (light, temperature?) to start of germination.

M. spicatum, *P. perfoliatus* and *P. pectinatus* have been noted every year during the last 50 years in Lake Mikołajskie on a investigated site. Their seeds germinated from all layer of core sediment. The seeds of most herbaceous wetland species are capable of persisting more than a year in sediments, and some persist for many years (Leck & Simpson, 1995). The present results confirmed that.

It is essential to know the conditions required for germination. Germination of persistent species often responded positively to light, aeration, low organic matter content in sediment (Leck & Simpson, 1995).

The organic matter content in the littoral sediment of Lake Mikołajskie cannot limit propagule germination due to its low content. The experiment showed that light stimulated germination of propagules. After improvement of light conditions the reestablishment of a diverse community of submerged macrophyte from propagules bank is possible in the littoral of Lake Mikołajskie. Stoneworts play an important role in this process because they can recovery from oospores bank of several decades old.

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Factors influencing the distribution of aquatic plant communities in Irish canals

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Key words: management, weed control, TWINSPAN, CCA

Abstract

The aquatic plant communities in two Irish canals were examined between 1990 and 1994 to investigate the factors that influence plant species distribution and community structure. Four main aquatic plant groups were identified by TWINSPAN. Two groups were characterised by bankside vegetation, including *Carex* sp., *Juncus bulbosus* and *Scirpus lacustris*. Large mixed assemblages of emergent, floating leaved, free floating and submerged plant species characterised the third group. The fourth group was species poor and was dominated by *Cladophora* sp. Canonical Correspondence Analysis identified weed control history, total oxidised nitrogen, water colour, distance from feeder stream and distance from summit as the factors that potentially influenced the distribution of plant species. The results indicated that ongoing management practices did not significantly alter the overall structure and abundance of the plant communities during the study period.

Introduction

Irish canals are man-made, mesotrophic to eutrophic aquatic systems (Caffrey & Allison, 1998). Aquatic conditions in the canals favour the development of abundant and diverse macrophyte communities (Caffrey, 1991). These features include shallow (< 2 m), relatively clear water with a stagnant or slow flow, deep and soft mud substrates, little bankside shading and a minimum of instream disturbance (Caffrey et al., 1998). Proliferation of macrophytes in many canal sections interferes with amenity exploitation, principally navigation and angling (Caffrey, 1993a). It is, therefore, necessary to implement weed control programmes that will meet the requirements of the different user groups.

There was no structured, aquatic plant management strategy operating on the canals during the 1970s and 1980s. A combination of mechanical cutting and herbicide treatment (predominately with dichlobenil) was used to remove vegetation from bank to bank. Problems with rapid weed regrowth and colonisation with herbicide-resistant plant species were encountered and satisfaction among waterway users was low. In 1990 an integrated weed management programme was introduced with the objective of maintaining an open navigable central channel, fringed on both sides with an abundant and diverse flora (Caffrey, 1993a, b).

In order to effectively manage aquatic plant communities it is important to understand their biological and ecological requirements and the factors that influence their distribution. Between 1990 and 1994 macrophyte investigations were conducted throughout the operational canal network (then 274 km). Contemporaneous data pertaining to the physico–chemistry, macroinvertebrate and fish fauna and any other environmental or anthropogenic factors that might influence aquatic plant community structure and distribution were compiled. The data were analysed using two multivaritate techniques, Two Way Indicator Species Analysis (TWINSPAN) and canonical correspondence analysis (CCA).

The objective of the study was to define the principal aquatic plant communities in the canals and to identify the factors that potentially influenced their composition and distribution.

Study area

The canals studied were the Grand Canal, which included the Circular and Edenderry Lines, the Barrow Line and the Royal Canal (Fig. 1). From its summit level at Lowtown the Grand Canal flows east to Dublin, west to the River Shannon and south to the Barrow River (Barrow Line) The principal water supply to the Grand Canal is the Milltown Feeder, which enters the canal near Lowtown. The Royal Canal flows east to Dublin and west to the River Shannon from its summit level in Mullingar. It receives its principal water supply from Lough Owel which is a clean, unpolluted oligotrophic lake. The physical, physicochemical and biological characteristics of these canals are described in Caffrey et al. (1998). The water level in the canals is regulated by the lock system and there is very little fluctuation in water height for one season to the next. The water is more or less static.

The full length of the Grand Canal (133 km) and the Barrow Line (45 km) was investigated during the present study. On the Royal Canal, the channel from Lock 26 east to Dublin city (96 km) was studied (Fig. 1). The western sector of this canal was excluded because of ongoing reconstruction operations.

The three canals were subdivided into 90 sections for the purpose of this study. Most of these sections corresponded with canal levels, which are the areas of channel contained between two sets of lock gates. Canals were coded as follows: G = Grand Canal, C = Circular Line,

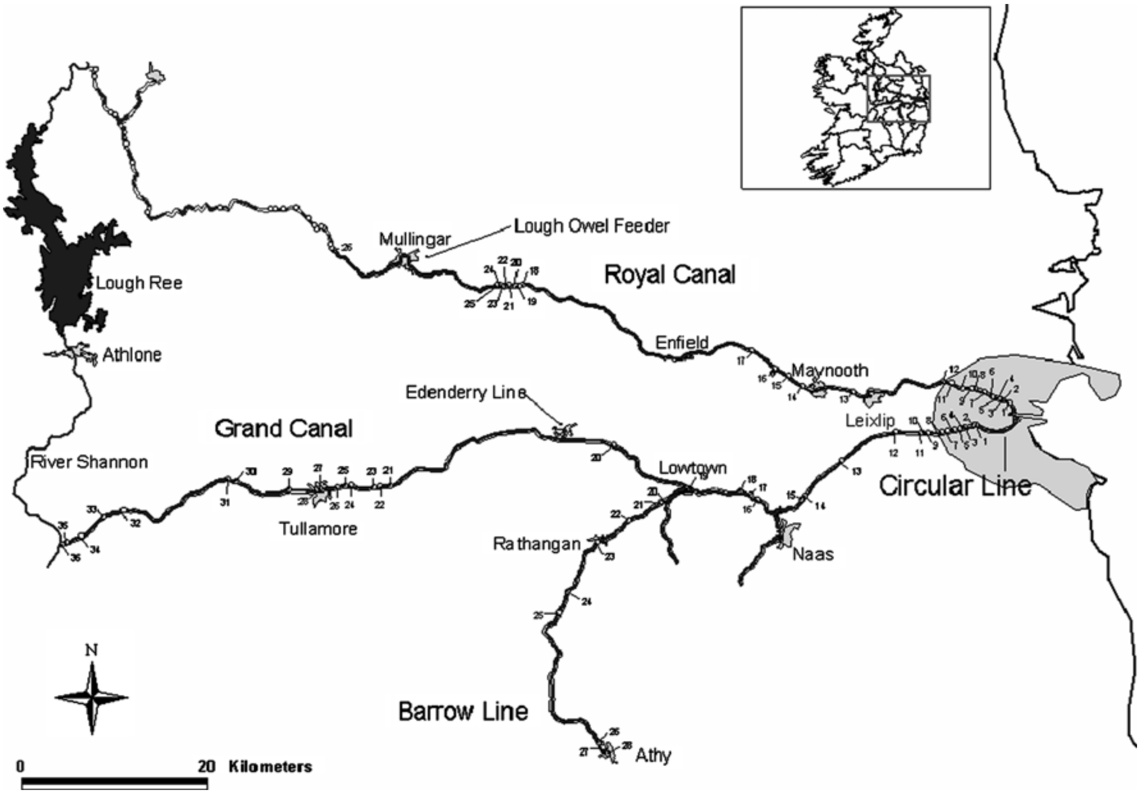

Figure 1. Map showing the location of the Royal, Grand and Barrow Canals. The numbers show the locations of sets of lock gates along the canals. The section of Royal Canal from Lock 26 west was under reconstruction during the study.

B = Barrow Line, EL = Edenderry Line and R = Royal Canal. Sections were coded according to canal level (for example, G5 = Grand Canal, level 5). Where obvious changes in aquatic plant community composition or abundance occurred within a long (> 10 km) uninterrupted canal level, this was subdivided into an appropriate number of subsections. Subsections were designated by the suffix 's' (viz. G20s1–G20s4).

Materials and methods

Detailed taxonomic inventories and estimates of percentage plant cover were compiled for each section in April, June/July and September from 1990 to 1994, inclusive. An eight-pronged grapnel was used to collect submerged macrophyte species. Surveys were conducted from a boat or while walking the bankside. Within each section relevés were recorded from representative 100 m lengths of canal on each sampling occasion. The percentage cover occupied by each species was determined visually and converted to a simple scale for subsequent analysis, as follows: 1 ≤ 5%; 2 = 6–25%; 3 = 26–50%; 4 = 51–75%; 5 = >75%. This is the scale developed by Braun-Blanquet. Cover is defined as the percentage surface area of the canal bed occupied by the vertical projection of the aquatic plants (Best, 1981). The same personnel always collected the field data, thus ensuring that the estimates were standardised.

Water samples were collected from each section on each sampling date and analysed for a wide range of physico-chemical parameters. Information on canal maintenance and management regimes, and other factors that might influence macrophyte growth, community composition and vegetation abundance (e.g. water quality, weed control history, substrate) were also collected.

Field data on plant species cover abundance were classified using TWINSPAN (Hill, 1979). The species-environment data sets, comprising 68 plant species and 31 environmental variables, was analysed using CCA (ter Braak, 1989).

Results

Sixty-eight plant taxa were recorded and used in the analyses. Of these only six taxa were present in more than 50% of sections. These were *Glyceria maxima* (Hartman) Holmberg, *Nuphar lutea* (L.) Sibth. + Sim., *Phragmites australis* (Cav.) Trin. ex Steudel, *Cladophora* sp. (cf. *glomerata*), *Myriophyllum verticillatum* L. and *Scirpus lacustris* L.

Four recognisable plant Groups (A–D) were produced by TWINSPAN. For the purposes of this study those classifications produced for the September 1990 and September 1994 data sets are examined (Fig. 2a, b). Two obvious changes in community distribution occurred between September 1990 and 1994. In the former period six of the seven sections on the Circular Line (C1–6) were assigned to Group D. In the second period five sections were assigned to Group C (C1–5). This change was attributed to the new records of *Elodea canadensis* Michx. in the Circular Line in 1994. Contiguous sections on the western sector of the Grand Canal (G21–26 and G28) moved from Group B in September 1990 to Group A in 1994 (Fig. 2a, b). This change was attributed to the disappearance of *Potamogeton pectinatus* L., *P. lucens* L. and *Sparganium emersum* Rehmann from these sections.

Groups A–D are characterised below based on the classification derived from the autumn 1994 dataset, which represents the current status of plant communities (King & Caffrey, 1998).

Group A

Twenty-four of the 90 sections examined were assigned to Group A (Fig. 2b). This Group occupied 87 km on the Royal and 19 km on the Grand Canal. All of the sections assigned to this group supported an abundant and diverse bankside flora. *Juncus* spp., *Carex* spp., *Nasturtium officinale* L. and *Ranunculus lingua* L. were commonly recorded in this group but were uncommon or absent from the other three groups. *Nuphar lutea* and *Potamogeton natans* L. were also well represented here. Dense, obstructive stands of *M. verticillatum* were present in many sections assigned to Group A.

Group B

Twenty-seven canal sections were assigned to Group B (Fig. 2b), a group characterised by dense marginal stands of *Phragmites australis* and locally dense stands of *Potamogeton pectinatus*,

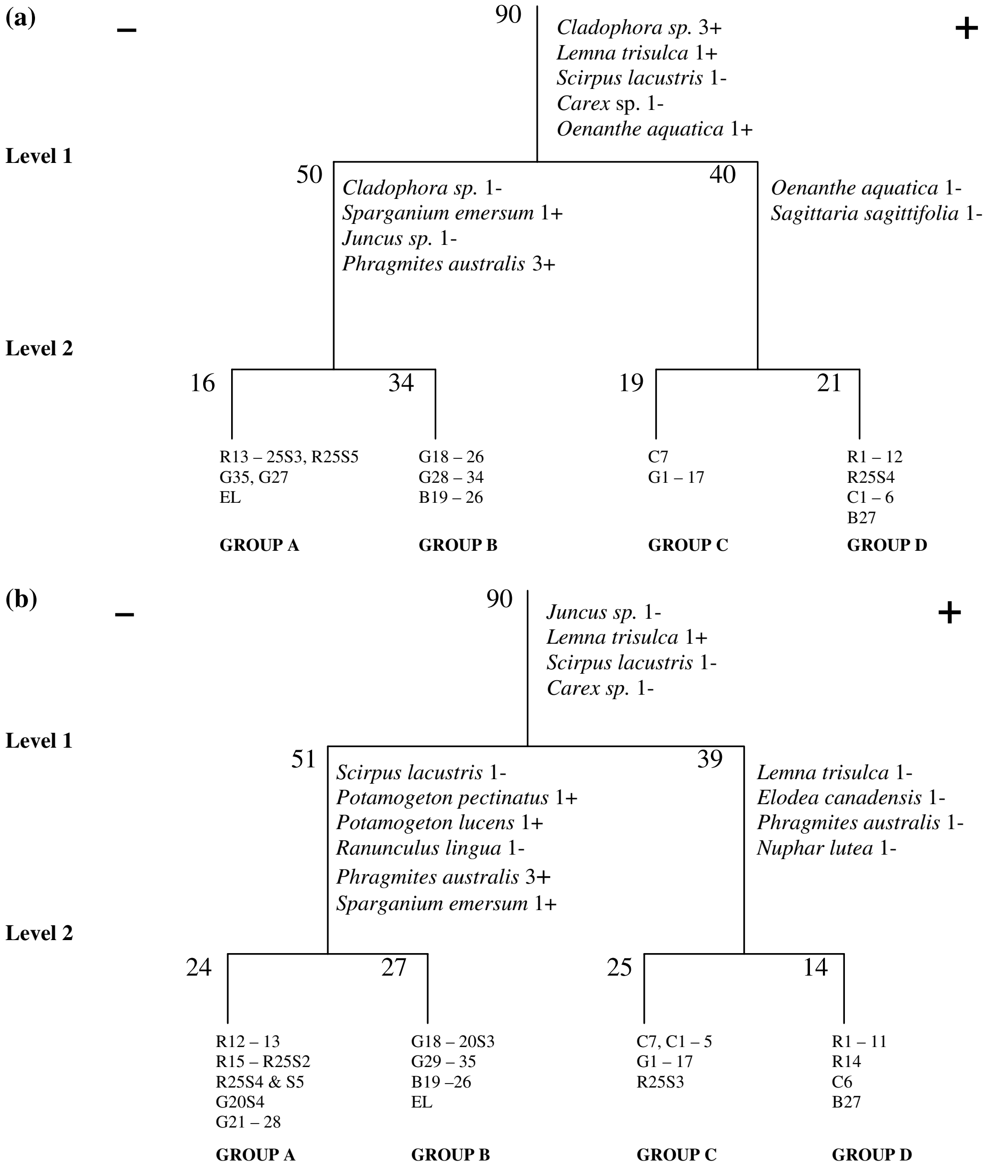

Figure 2. (a, b) TWINSPAN analysis of the September vegetation data collected in (a) 1990 and (b) 1994. The indicator species with their pseudospecies cut levels are given for each division and expressed as dendrograms with their group preference specified by + or –. Group A, B, C and D are the designated final group clusters.

S. emersum and *P. lucens*. These species rarely formed mixed vegetation assemblages. Total percentage plant cover during the summer/autumn in sections assigned to Group B was relatively low (< 20%), although localised weed problems did interfere with the amenity exploitation of the waterway.

Group C

Twenty-four of the 25 canal sections assigned to Group C were located on the Grand Canal (Fig. 2b). This community was characterised by large mixed assemblages of emergent, floating leaved, free floating and submerged plant species.

Prominent among these, and poorly represented in other canal sections, were *Sagittaria sagittifolia* L., *Oenanthe aquatica* L. Poiret and *Lemna trisulca* L. Other species that were recorded with relatively low abundance in the remaining groups but proliferated here were *Elodea canadensis*, *N. lutea*, and *Oenanthe fluviatilis* (Bab.) Coleman. The filamentous green alga *Cladophora* sp. (cf. *glomerata*) and *Sparganium emersum* were also commonly represented in Group C. Total percentage plant cover during the summer season in sections assigned to Group C was generally high (>75%). Mechanical weed control was used to maintain an open navigable channel in these sections. No chemical treatment was permitted because the water in this eastern sector of the Grand Canal is used to supply a major brewery in Dublin city.

Group D

In terms of length of canal occupied, Group D was the smallest group defined by TWINSPAN. This group comprised 14 canal sections (Fig. 2b) and occupied a total channel length of 13 km. The indicator species for this group was *Cladophora* sp. (cf. *glomerata*). This group was species poor and the sections occupied were subjected to periodic disturbance, including dewatering, dredging and/or pollution.

CCA Ordination

Of the 31 environmental variables used in the CCA species – environmental analysis, only 12 were selected for subsequent analysis (Fig. 3). These

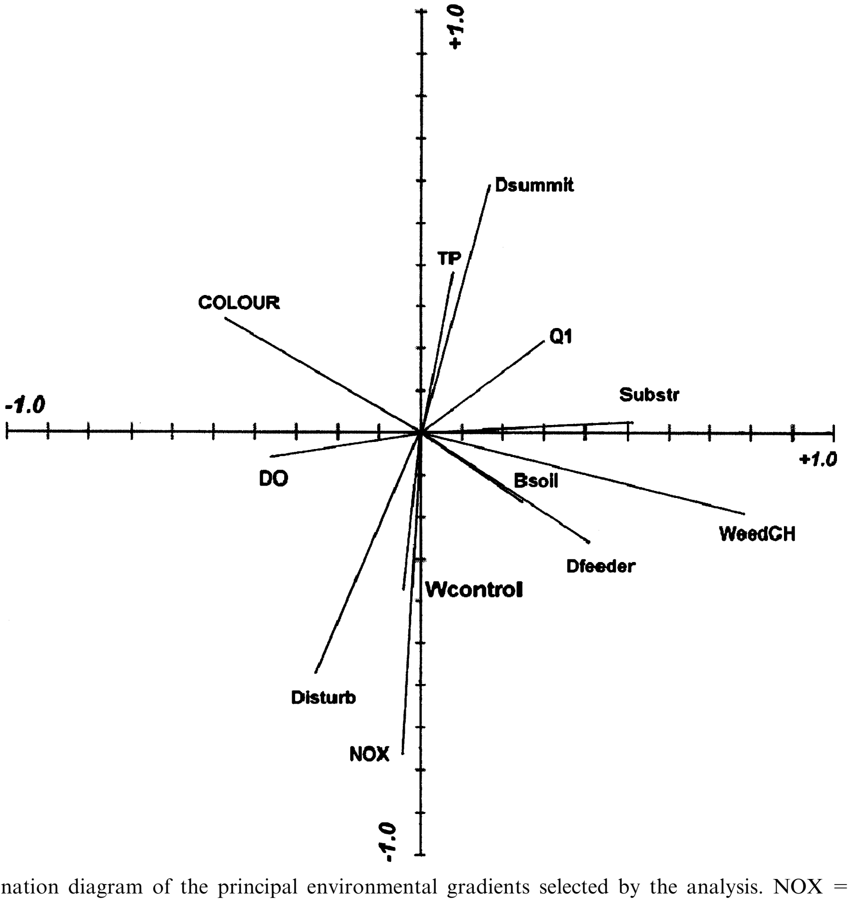

Figure 3. CCA ordination diagram of the principal environmental gradients selected by the analysis. NOX = Oxidised nitrogen, DO = Dissolved oxygen, TP = Total Phosphorus, QI = Colour, Water quality, Dfeeder = Distance from feeder stream, Dsummit = Distance from summit, Bsoil = Substrate Bank soil, Wcontrol = Weed control, WeedCH = Weed control history, and disturb = Disturbance.

accounted for 43.7% of the variance in the data. CCA produced two dominant ordination axes, axis I and axis II, with eigenvalues of 0.29 and 0.208, respectively. Eigenvalues indicate the amount of inherent variability accounted for along a given principal axis. The first axis of this analysis was statistically significant (Monte Carlo Test, $p < 0.05$). Weed control history, substrate and colour were strongly aligned with axis I. Group C sections were strongly associated with these environmental gradients. Oxidised nitrogen, distance from summit and disturbance were strongly aligned with axis II (Fig. 3). Group D sites were most closely associated with this axis.

Discussion

Studies in the UK have indicated that canals support macrophytes species that are rare or uncommon elsewhere in the country (Palmer & Newbold, 1983; Hanbury, 1986). This is also the case in the Grand and Royal Canals where nationally rare species (e.g. *Groenlandia densa* (L.) fourr. and *Ceratophyllum demersum* L.) are locally abundant. Another submerged species whose distribution in both Ireland and Britain has contracted in recent years (Preston & Croft, 1997), but which has expanded its range considerably in canals, is *M. verticillatum*. This is one of the most common submerged plant species present in the canals studied and accounts for a high percentage of the weed problems reported each year (Caffrey & Monahan, 2002).

Factors which govern the distribution of plant communities in watercourses often act synergistically and simple correlations between environmental and/or anthropogenic factors and the occurrence of these communities are rarely encountered (Caffrey, 1990a). The situation may be further complicated in man-made canals where external factors, including inchannel and bankside maintenance, dredging, aquatic weed control, water flow regulation and boat traffic, influence plant community composition and distribution.

Weed control history was selected by CCA as the primary factor discriminating between Groups defined by TWINSPAN. In the Grand Canal and Barrow Line (representing 178 km of channel) two Groups predominated. The composition of these reflected the different methods used to control obstructive weeds in sections occupied by these Groups. Group B was more or less restricted to canal sections where herbicide treatment, using dichlobenil, was the primary weed control method. The flora in these canals was characterised by dense stands of *P. pectinatus*, *S. emersum* and *P. lucens*. The first two species are resistant to the activity of dichlobenil (Caffrey, 1993b) and obviously availed of the competitive advantage that the continued use of this herbicide provided. The prevalence of *P. lucens*, a species that is sensitive to the activity of dichlobenil (Caffrey, op. cit.), relates to the fact that this is a 'late species' (Haslam, 1978) and normally emerges in late May. This is normal in Irish canals 4–6 weeks after herbicide application and has little adverse effect on the growth of *P. lucens* (Caffrey, 1993b).

By contrast, in those canal sections where dichlobenil was never applied (because of a perceived risk to potable water supply), Group C was best represented. In these channels weed control was mechanically operated, which probably explains the prevalence of disturbance-tolerant species, including *E. canadensis*, *L. trisulca*, *S. sagittifolia* and *O. aquatica* (Haslam, 1978; Hroudova et al., 1992).

The presence of an abundant and diverse marginal aquatic flora, including *R. lingua*, *Carex* spp. and *Juncus* spp., distinguished Group A from the other Groups. The presence of soft peaty banks along the canal sections occupied by this Group may have influenced the successful establishment of these species. Dense stands of *M. verticillatum*, a species that spreads rapidly by specialised buds or turions (Caffrey & Monahan, 2002), were also a common feature of this Group.

Group D was restricted to canal sections that had been subjected to physical disturbance or pollution. In addition, these sections exhibited extremely low total oxidised nitrogen (TON) levels. These factors probably explain the low plant species diversity recorded in canal sections occupied by this group and the dominance of the opportunistic alga *Cladophora* sp., a species that is adapted to disturbance, physical damage and highly altered physico-chemical conditions (Pitcairn & Hawkes, 1973; Haslam, 1978; Murphy & Eaton, 1983; Caffrey, 1986, 1990b).

The four groups distinguished by TWINSPAN appear to be relatively stable and suggest that ongoing weed control practices, as they are currently operated, will not significantly alter the current structure or relative abundance of the plant communities. This concurs with the findings of Fox & Murphy (1990a, b) who reported that, for river vegetation, any alteration in the plant community caused by management resulted in less variation than already existed between sites. Most of the weed control procedures operated in Irish canals achieve only short-term (3–4 months) control and are followed by vigorous regrowth of the plant species that were present before treatment (Wade, 1982; Caffrey, 1990b).

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Evaluation of the status of lakes located in the City of Olsztyn (Masurian Lake District, N-E Poland) by the macrophytoindication method (MPhI)

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Key words: phytocenotic diversity index, colonization index, succession process, synanthropization process, synanthropization index

Abstract

Urban lakes belong to various groups, for example from "young" to "old" with regard to development, and from "natural" to "anthropogenic" with respect to transformations due to human activity. The majority of these lakes are eutrophic and polytrophic, but special attention should be paid to mesotrophic ones, with relatively unchanged vegetation, with species of the class *Charetea*, e.g. Lake Redykajny (43.3% of the phytolittoral) or Lake Tyrsko (44.0% of the phytolittoral).

Introduction

The Macrophyto indication method (MphI) method of water ecosystem evaluation, based on analysis of aquatic and emergent vegetation, was elaborated through studies on postglacial lakes in Poland (Rejewski, 1981). Analysis of the structural and spatial arrangement of littoral vegetation allows us to determine both their successional development, and anthropogenic transformation in the process of synanthropization. The method involves lakes of a trophic series, characterized by both harmonious and disharmonious development (poly-, hypertrophic). The main assumption underlying this method is that lakes, as ecosystems, are oligotrophic at the moment of their formation, and an increase in trophy is a simple function of time. In the case of naturally evolving systems, an increase in their complexity and gradual organizational-functional complication may be observed (Margalef, 1968, 1975; Odum, 1971).

In the MPhI method the measure of vegetation anthropogenic changes is a departure from the maximum value of the phytocenotic diversity index in relation to the actual value of this index (Rejewski, 1981).

It would be difficult to determine the degree of anthropogenic transformations by means of the occurrence of seed-producing invasive weed species because, in Poland, only two alien species can be found in lake littoral, i.e.: *Acorus calamus* and *Elodea canadensis*. The presence of *A. calamus* communities indicates considerable anthropogenic changes in the habitat, but *E. canadensis* can be an indicator of mesotrophic environments.

Site description

The city of Olsztyn (54°62′ E, 20°66′ N, N–E Poland) is characterized by a high level of lake density – over 8.0%. The lakes examined show various morphometric features (Table 1); most of them are flow-through water bodies.

Methods

Littoral vegetation surveys were conducted between 1990 and 2001, employing a common phytosociological method (Braun-Blanquet, 1951). The areas occupied by particular communities

Table 1. Morphometry of the urban lakes in Olsztyn

	Lake name	Lake area (ha)	Maximal depth (m)	Mean depth (m)	Length of the shoreline (m)
1.	Redykajny	29.9	20.6	8.0	2725
2.	Tyrsko	18.6	30.4	9.6	1680
3.	Sukiel	20.80	25.0	6.6	2100
4.	Podkówka	6.9	6.0	2.9	1380
5.	Czarne	1.50	6.5	3.1	700
6.	Starodworskie	6.99	23.0	7.9	900
7.	Pereszkowo	1.80	4.5	2.2	1000
8.	Krzywe (total)	412.00	43.0	10.6	22500
	Gutkowo Part	119.8	43.0	13.2	4925
	upsztych Part	85.4	32.0	10.1	5700
	Przejma Part	88.4	40.0	12.7	2700
	Olsztyn Part	118.4	20.0	10.6	6500
9.	Kortowskie	89.70	17.2	5.9	4800
10.	Skanda	51.10	12.0	5.8	4500
11.	Trackie	52.80	3.8	2.1	4800
12.	Dugie	26.80	17.3	5.3	4080

were determined using a bathometric map (Institute of Inland Fishery 1964).

The method (MPhI) was used to establish the level of the lake succession development and to evaluate the anthropogenic changes taking place in the process of synanthropization (Rejewski, 1981). This method enables one to differentiate between two processes characterized by a similar course, but indicating different directions of transformation: succession and synanthropization. "Natural" succession results in more complex structures and spatial, while synanthropization – in simplified ones.

Having determined the phytocenotic diversity index (*H*) – from the Shannon-Weaver formula – and the colonization index (Z), it is possible to determine the so called "succession product" or "succession index" (I_s – reflecting the developmental "age" of a lake ecosystem) and synanthropization index of aquatic vegetation (W_s – reflecting structural changes in lake vegetation caused by human activity).

$$H = -\Sigma \mathrm{ni}/N \times \ln \mathrm{ni}/N$$

where:

H – phytocenotic diversity index (the Shannon–Weaver index);

ni – area covered by the phytocenoses of a given plant community, expressed as a percentage of the total phytolittoral area;

N – phytolittoral area (100%).

The colonization index (Z) was determined as the quotient of the phytolittoral area (*N*) and the water-table area limited by an isobath of 2.5 m:

$Z = N/\mathrm{isob.}\ 2.5$

The value of the phytocenotic diversity index (*H*) is the lowest for oligotrophic lakes, as very few phytocenoses (single-species clusters) form phytolittoral there. However, due to high water transparency they occupy large areas, so the value of the colonization index (*Z*) is highest in such lakes (Rejewski, 1981). The value of the phytocenotic diversity index (*H*) rises with an increase in trophy, because there are more phytolittoral-forming communities (*S*). The value of the colonization index (*Z*) decreases, due to shading. The trophogenic zone diminishes. The indices *H* and *Z* are correlated negatively from oligo- to eutrophy (harmonious development). However, a further increase in trophy results in a decrease in the values of both indices (*Z* and *H*) – positive correlation. Poly- and hypertrophy (disharmonious development) result in the elimination of plants, especially immersed ones. In extreme cases it leads to almost total disappearance of the phytolittoral.

The phytocentic diversity index (H) is then the lowest, and the value of the colonization index (Z) also approaches zero.

Process of succession

$$I_s = H \times Z$$

Taking into consideration the values of the above indices, the lakes examined were classified to appropriate groups, determining the level of their development in the process of succession (Table 2).

At each natural stage of development, lake ecosystems may be exposed to anthropopressure. The effects of man-induced changes depend mainly on the morphometric characteristics of the lakes and nature of the catchment

Process of synanthropization

$$W_s = \exp(-H \times Z / H_{\max})$$

In order to evaluate the range of anthropogenic changes in the vegetation of a lake ecosystem, it should be confronted with the actual possibility of maximum development of its spatial structures (Rejewski, 1981). It follows that it is necessary to know the maximum value of the phytocenotic diversity index. This index shows a tendency to reach its highest values where there are no factors limiting the development of lake plant cover. It assumes its maximum in a theoretically possible situation when all plant communities forming the littoral zone of a lake ecosystem are co-dominants, i.e. occupy the same area. (Table 3).

In such a case the Shannon-Weaver formula takes a simplified form: $H_{\max} = \ln S$, where $H_{\max}$ – maximum value of the phytocenotic diversity index; S – number of plant communities forming the phytolittoral.

The synanthropization index (W_s) fulfils the requirements included in Directive 2000/60/EC (OJEU, 2000), concerning ecological quality indicators used for the purposes of ecological status classification. In order to make it possible to compare different monitoring systems, such indicators must be expressed by numerical values from zero to one, with very good ecological state being expressed by values close to one, and bad ecological state – by values close to zero. The values of the synanthropization index also vary from one to zero, but very good ecological state is expressed by values close to zero, whereas bad ecological state – by values close to one. The values of the synanthropization index (W_s) also vary from one to zero, but very good ecological state is expressed by values close to zero, whereas bad ecological state – by values close to one. However, this should not diminish the general suitability of this index for lake ecological status evaluation.

Detailed descriptions of particular groups (succession and synanthropization) will be included in the English elaboration of the MPhI method.

Results

Taking into account their developmental advancement and changes resulting from anthropopressure, four polytrophic lakes situated in the city of Olsztyn were classified as "old" and "anthropogenic", respectively. One of them is Lake Dugie (the most degraded lake in Olsztyn),

Table 2. Lake ecosystem classification with respect to the degree of succession in the littoral vegetation (acc. to Rejewski, 1981)

	Lake groups	Phytocenotic diversity index (H)	Colonization index (Z)	Succession index (I_s)
1.	"Very young lakes"	0.5–1.5	>2.0	> 3.0
2.	"Young lakes"	1.6–2.0	1.5–2.0	± 3.0
3.	"Mature lakes"	>2.0	(0.3) 0.5–1.5	> 0.5
4.	"Ageing lakes"	1.5–2.0	(0.3) 0.5–1.5	0.5–1.5
5.	"Old lakes"	<1.4	<1.5	0.5–1.5

Table 3. The variability ranges for the synanthropization quotient and synanthropization index (acc. to Rejewski, 1981; Ciecierska, 1997)

	Groups of lakes according to the degree of anthropogenic transformation	Synanthropization index (W_s)
1	"Natural lakes"	0.00–0.39
2	"Weakly synanthropic lakes"	0.40–0.59
3	"Moderately synanthropic lakes"	0.60–0.69
4	"Synanthropic lakes"	0.70–0.79
5	"Anthropogenic lakes"	0.80–1.00

whose synanthropization index was the highest among the lakes examined, and close to the maximum value 1.0 ($W_s = 0.907$). This lake is characterized by a small number of plant communities, among which the most important role is played by phytocenoses *Acoretum calami* and *Elodeetum canadensis* (60.9% and 17.1% of the phytolittoral, respectively), and the lowest value of the colonization index (Z – Table 4) – the phytolittoral occupies only 1.5% of the lake area. The other lakes belonging to this group are also characterized by small phytolittoral zones.

The other city lakes belong to various groups, from "young" to "old" with regard to development, and from "natural" to "anthropogenic" with respect to anthropogenic transformations. The majority are eutrophic lakes, but special attention should be paid to mesotrophic ones, with relatively unchanged vegetation, surrounded by meadows with species of the class *Chareta*, e.g. Lake Redykajny and Lake Tyrsko. Due to a high number of plant communities, both these lakes, and the North Part of Lake Krzywe (sometimes considered a separate lake), are characterized by the highest values of the phytocenotic diversity index (*H*) and the colonization index (*Z*). Their phytolittoral constitutes 31.4% (Lake Redykajny), 32.8% (Lake Tyrsko) and 19.5% (North Part of Lake Krzywe) of their total areas. They were classified as "young" with regards to their development advancement, and "natural" as concerns the effects of anthropopressure (Table 1).

Discussion and conclusions

In oligotrophic lakes the colonization index (*Z*) reaches the highest values (3.0–4.0, Rejewski, 1981), because their trophogenic zones cover the largest areas. The values of the phytocenotic diversity index (*H*) are low in these lakes because phytolittoral, although extensive, is formed by a few plant communities only. Taking into account their development in the process of succession and anthropogenic transformation, such values are typical of "very young lakes" and "natural lakes" respectively (Rejewski, 1981). An increase in lake trophy is accompanied by phytolittoral reconstruction – vegetation with lower habitat requirements is replaced by eurytopic vegetation (Hutchinson, 1975; Sculthorpe, 1985). The colonization index (*Z*) decreases, because the trophogenic zone becomes smaller. The phytocenotic diversity index (*H*) increases as a consequence of a growing number of plant communities and a higher level of ecosystem complexity (Margalef, 1968, 1975). According to the MPhI method, mesotrophic lakes - usually characterized by a high number of phytocenoses of the class *Charetea* – are classified as "young" as regards their development and "weakly synanthropic" or "moderately synanthropic" with respect to the effects of anthropopressure. Some deep lakes located in the city of Olsztyn were also classified in this way: Redykajny, Tyrsko, the North Part and the West Part of Lake Krzywe. Plant communities of the class *Charetea* cover large areas in the phytolittoral zones of these lakes, especially the first two ones. The best state of water in Lakes Redykajny, Tyrsko and the North Part of Lake Krzywe was also confirmed by hydrochemical analyses (Lossow et al., 1991; Mientki et al., 1996), although in the 1980s their quality deteriorated considerably.

Theoretically the values of the colonization index (*Z* – the lowest) and the phytocenotic diversity index (*H* – the highest) should reach constant levels in lakes characterized by dynamical stabilization, indicating the end of succession typical of eutrophic lakes in the temperate zone (Lampert & Sommer, 1993). According to the

Table 4. Values for the morphometry and indices characterising biocenotic and spatial structure of the vegetation in urban lakes of Olsztyn

	Name lake	Area of 2.5 m isobath (ha)	Phytolittoral area (ha)	Number of phytolittoral communities (S)	Phytocenotic diversity index (H)	Max. phytocenotic diversity index ($H_{\max}$)	Colonisation index (Z)	Succession Index (I_s)	Effect of succession	Synanthropization index (W_s)	Effect of synanthropization
1	Redykajny	29.9	9.4	27	2.63	3.30	1.69	4.44	"young"	0.26	"natural"
2	Tyrsko	18.6	6.1	19	2.04	2.94	1.55	3.16		0.34	
3	Sukiel	20.8	4.9	11	1.47	2.40	1.02	1.50	"ageing"	0.56	"weakly synanthropic"
4	Podkówka	6.9	3.0	17	1.93	2.83	1.03	1.99		0.90	
5	Czarne	1.5	1.0	7	1.54	1.95	1.00	1.54		0.45	
6	Starodworskie	7.0	1.7	9	1.36	2.20	0.82	1.12	"old"	0.60	"moderately synanthropic"
7	Pereszkowo	1.8	0.6	3	0.99	1.10	0.31	0.31		0.76	"synanthropic"
8	Krzywe (total)	412.0	70.8	30	1.50	3.40	1.39	2.09	"ageing"	0.54	"weakly synanthropic"
	North Part	119.8	23.4	20	1.78	3.00	1.58	2.83	"young"	0.39	"natural"
	West Part	88.4	17.7	19	1.23	2.94	1.63	2.00		0.51	
	Part "Mia"	15.5	8.2	12	0.90	2.48	1.78	1.60	"old"	0.53	"weakly synanthropic"
	Central Part	84.6	8.9	19	0.92	2.94	1.18	1.09		0.69	"moderately synanthropic"
	South Part	103.7	12.6	17	0.99	2.83	0.95	0.96		0.72	"synanthropic"
9	Kortowskie	89.7	8.8	10	0.98	2.30	0.45	0.44		0.83	"anthropogenic"
10	Skanda	51.1	2.7	14	1.10	2.64	0.37	0.41		0.86	
11	Trackie	52.8	8.3	14	1.16	2.64	0.34	0.40		0.87	
12	Dugie	26.8	0.4	8	1.22	2.10	0.07	0.09		0.96	

MPhI method, these lakes are considered "mature", with the highest level of phytocenotic organization. However, due to the allogenic character of water ecosystem evolution, water bodies become poly-, or even hypertrophic. In such a situation the values of both (*H*, *Z*) indices decrease and the phytolittoral area diminishes. The ecological groups of immersed plants belonging to the *Potamion* alliance and plants with floating leaves belonging to the *Nymphaeion* alliance vanish first. According to the MPhI method, these lakes are "old" and "anthropogenic", characterized by disharmonious development. Four lakes situated in the city of Olsztyn were classified to this group, including Lake Dugie whose synanthropization index was the highest among the lakes examined (Table 1). This is the most degraded lake in Olsztyn (Lossow et al., 1991). Reclamation work has been carried out there for many years.

The MPhI method may be applied to evaluate the status of lakes (trophic series), taking into consideration both their developmental advancement and anthropogenic transformation. The threshold value of the synantropization index – 0.8 is here of primary importance, as lakes in which it is exceeded become highly polytrophic. Low costs and labor consumption of this method should also be emphasized.

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Aquatic plants as environmental indicators of ecological condition in New Zealand lakes

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Key words: submerged macrophytes, indicators, lake health, monitoring, LakeSPI

Abstract

Submerged aquatic plants can act as measurable indicators of ecological conditions occurring within a lake, and they need only be monitored once a year or even less. Historically in New Zealand there has been a reliance on water quality sampling for monitoring the health of lakes and these methods can be complex and costly involving multiple site visits and chemical analysis of water samples. As a result, lake monitoring has been irregular, or not done at all. LakeSPI or 'Lake Submerged Plant Indicators' is a new management tool that uses aquatic plants to monitor and assess ecological condition in a wide range of lake types. The method generates three indices: a Native Condition Index (extent and diversity of native plants) and an Invasive Condition Index (extent and impact of alien weeds), which are generated from scores allocated to carefully selected vegetation features; and an integrated LakeSPI Index which is largely derived from components of the other two indices and provides an overall indication of lake ecological condition. The LakeSPI method can be used to assess the status of lakes and monitor trends occurring within them, and it is expected that the use of LakeSPI will facilitate regular monitoring and reporting on a much wider range of lakes than has been possible using traditional water quality methods. By utilizing submerged aquatic plants the method focuses on lake littoral margins where there is greatest public interaction and interest.

Introduction

Assessment of environmental condition in aquatic ecosystems has traditionally revolved around physico-chemical measurements of water. Methods have often been based on quite simple methods such as Secchi disc to measure water clarity, while more comprehensive measurements have focused on a variety of physico-chemical parameters, such as pH, conductivity, vertical temperature and DO profiles through the water column, chlorophyll *a*, and measurement of dissolved and total nitrogen and phosphorus.

Aquatic macrophytes have been widely surveyed, described and monitored, however their use to assess water quality and/or lake condition has been limited. In the United Kingdom and Europe macrophytes have been used to characterise the trophic status of a range of waterbodies. Palmer et al. (1992) reported on the TRS (Trophic Ranking Score) based on macrophyte species in standing water bodies, while Holmes et al. (1990) reported on the MTR (Macrophyte Trophic Rank) that uses macrophyte species in streams and river habitats. Melzer (1999) developed a 'macrophyte index' using aquatic macrophytes as indicators of water pollution in European lakes. These scoring systems are based on the frequency and cover of species present and how each species characterises the trophic state of a water body based on their individual tolerance to eutrophic water condition. Nichols et al. (2000) also used aquatic macrophytes to develop an AMCI (Aquatic Macrophyte Community Index) in

Wisconsin lakes, which characterises the quality of aquatic plant communities.

It is clear from studies where aquatic macrophytes have been used that common universal principles exist, such as the premise that the maximum depth of submerged plant growth directly reflects water clarity (Sculthorpe, 1967). On the other hand it is also apparent that there are differences in the way particular species (sensitive and invasive) respond to environmental conditions (e.g., trophic status) and geographic influences. For example, in New Zealand reported aquatic plant biomass levels do not reliably reflect the trophic status of a waterbody (Howard-Williams et al., 1987). Furthermore, the relatively recent introduction of invasive macrophytes into New Zealand during the last one hundred and fifty years has meant that many invasive species have still not reached their potential distribution limits.

In New Zealand, due to these reasons we have developed a new management tool that uses selected features of submerged aquatic plants to measure the condition of a waterbody. We have called this method LakeSPI (pronounced "Lake Spy") or 'Lake Submerged Plant Indicators'. It is intended that LakeSPI will provide managers with an effective and economic method to assess the ecological status of lakes and to monitor trends occurring within them. The incentive for devising such a method followed from the New Zealand government support of the Rio Convention 1992. Agenda 21 of this convention obligates governments to develop, among other things, "indicators of sustainable development" by: "developing methodologies with a view to undertaking systematic sampling and evaluation on a national basis of the components of biological diversity identified by means of country studies" and to measure and monitor "the status of ecosystems and establish baseline information on biological and genetic resources, including those in terrestrial, aquatic, coastal and marine ecosystems...". Cabinet directed the New Zealand Ministry for the Environment (MFE) to develop a national programme of indicators to monitor performance in environmental management. A number of national indicators were identified by MFE, of which two were particularly relevant for the development of LakeSPI. These were change in biological condition for selected ecosystems and change in the distribution and relative abundance of weed species. Both of these indicators have been incorporated into the LakeSPI method and will contribute to the implementation of a wider programme led by MFE that seeks to establish environmental performance indicators for regional and national reporting purposes in New Zealand.

Assessment of lake condition using aquatic plants

There are many advantages of using submerged plants as indicators of lake condition. For example, submerged plants are predominantly rooted or anchored to the bed of lakes and therefore represent a readily observable and measurable feature and they reflect environmental conditions impacting plant growth over reasonably long time frames (prior to sampling) (Schwarz et al., 2000). This is in contrast with many other biological indicators that may be highly mobile such as fish, difficult to sample or identify such as plankton, or that have high temporal and spatial variability thus requiring frequent measurements throughout the year. Because submerged plant indicators need only be monitored once a year or even less, they can be cost effective and avoid the expense of often frequent chemical laboratory testing of water samples. Another key difference is that while water quality monitoring methods emphasise profiles in the lake centre, submerged plant indicators emphasise the littoral zone around a lake, where the greatest public interaction and observation occurs.

Another advantage of using submerged plants is that they can tolerate short periods of reduced water clarity over time. Because net plant growth is an integration of average water clarity over ecologically meaningful time-scales, the maximum depth to which plants grow tends to be relatively stable throughout any one-year and often over much longer time frames (Schwarz et al., 2000). This contrasts with physical light or water clarity readings, which can be highly variable over short time frames. Even on any one-day, Secchi disc readings can vary between observers and can be affected by cloud cover and time of day.

LakeSPI concepts

In New Zealand lakes, like in many other countries where submerged plants dominate the aquatic vegetation, there are two major factors that affect ecological condition relative to pristine state. Firstly, the water quality and clarity has often been reduced due to increases in sediment and nutrients associated with surrounding land use activities (Vant, 1987). The resulting reduced light zone in the lake further reduces the available habitat for aquatic plant growth and in severe cases can result in the decline and loss of all submerged plant growth (Scheffer et al., 1993). Secondly, in many lakes there has been a significant invasion of native plant communities by alien invasive plant species. This has lead to reduced biodiversity (de Winton & Clayton, 1996) and can result in the loss of virtually all native plant species from a lake (Howard-Williams et al., 1987).

These influences provided the key concepts for the development of LakeSPI (Edwards & Clayton, 2002). The first method concept is based on the principle that a plant requires light to photosynthesise and grow. The maximum depth to which submerged plants can grow in a lake often shows a close relationship to water clarity or the depth to which sufficient light for photosynthesis penetrates the water surface (Sculthorpe, 1967). In the absence of other determining factors, the deeper the submerged vegetation the better the water clarity is. As a result, the depth of vegetation growth can be accordingly interpreted as a measure of ecological condition, where a higher value represents better condition. The second concept is based on the principle that native species are preferable to invasive species and that high native biodiversity is preferable to low native biodiversity. This concept is really comprised of two elements that need to be independently assessed; native vegetation status, and degree of invasive species impact. Developing a method that adequately characterised 'native condition' (inclusive of biodiversity) and 'invasive condition' (status and impact), as well as macrophyte response to water clarity (reflected in depth limits) therefore became the key components in the development of LakeSPI method.

LakeSPI methodology

The LakeSPI method has been developed for use in all New Zealand lakes where submerged plant growth exceeds a ten percent cover at any given site. It is not suitable for lakes where submerged plants are rare or non-existent or where salinity, alkalinity, acidity, altitude or size prevents the development of normal submerged vegetation communities. Five sites have proven sufficient to characterise both the native condition and the invasive condition of vegetation in most lakes by using SCUBA divers to individually assess and measure the structural and compositional features of the lake vegetation. These features are then represented by a numerical score used to construct a Native Condition Index and Invasive Condition Index.

The 'Native Condition Index' aims to capture the native character of vegetation in a lake based on the diversity and quality of indigenous plant communities present. Scores are attributed to each of five key characteristics that include measures of native plant depth, the presence of sensitive native species/genera or communities and the cover, ratio and distribution of littoral area occupied by native plants. The 'Invasive Condition Index' focuses on the changed vegetation structure and composition of a lake arising from the influence and degree of impact by alien invasive plant species. For this index particular focus is given to eight invasive plant species that are ordered from lowest to highest invasive impact based on their known potential for undesirable ecological impact in New Zealand lakes (Champion & Clayton, 2001):

Juncus bulbosus L., *Ranunculus trichophyllus* Chaix
Potomogeton crispus L.
Elodea canadensis Michaux
Vallisneria gigantea (Graeb)
Lagarosiphon major (Ridley) Moss
Egeria densa Planchon
Hydrilla verticillata (L.f.) Royle
Ceratophyllum demersum L.

It should be noted that this ranking would be different for other countries. For example, *Ceratophyllum demersum* in New Zealand forms dense surface reaching weed beds from depths of 6 m, with less tall but still dense beds displacing and smothering native submerged vegetation to depths of 10 m and sometimes even deeper. In comparison, this same species is a native of the United States of America and is non-weedy, quite benign in its growth habit and would not be ranked as an exotic or an invasive weed species in that country.

Once both the Native and Invasive Condition Indices have been generated, components from each of these indices combine to generate an overall LakeSPI Index. This final index represents the overall biological or ecological status of a lake based on submerged plant vegetation information obtained. A key assumption of the LakeSPI method is that higher native scores indicate better lake condition and they will proportionally increase the value of the Lake SPI index (positive scores). In contrast, any invasive influences upon a lake will decrease the LakeSPI value (negative scores).

The LakeSPI Index can be used to detect changes in lake condition over time and to make direct comparisons between lakes. Moreover, the separate generation of Native Condition Indices and Invasive Condition Indices can be used to specifically clarify the current ecological condition of a waterbody or even the nature of deterioration within waterbodies. To facilitate comparison of lake indices, a baseline score has also been established that depicts the maximum potential LakeSPI score for any given lake based on lake depth, and an absence of human associated impacts (such as invasive species and eutrophication).

LakeSPI case studies

Over 50 lakes have been surveyed as part of the development and testing of the LakeSPI method, with lakes chosen to represent a diversity of lake types and conditions found throughout New Zealand.

To demonstrate one use of aquatic plants as indicators for comparing lake ecological condition, an evaluation was made of the LakeSPI method by comparing two lakes (Tarawera and Okataina) from the same region (Rotorua lakes, central North Island of New Zealand) based on their vegetation changes over a period of several years. Both lakes are oligotrophic and have had moderately stable water quality conditions and water clarity for more than twenty years (Burns et al., 1997; Wells et al., 1997). Burns et al. (1997) reported that Lake Okataina had not undergone significant change in trophic condition (based on nutrient and chlorophyll concentrations, dissolved oxygen depletion rates and algal composition) from 1971 to 1994, although their data did show a reduction in mean Secchi depth of 0.9 m for this same period.

Current LakeSPI scores based on five survey sites within each of Lakes Okataina and Tarawera were obtained during the development of the LakeSPI method. These scores were then compared to historic vegetation survey data obtained from traditional full lake surveys (Clayton, 1983) by selecting the five nearest corresponding survey sites. The Lake Okataina comparison is based on twenty years (i.e., 1981 vs 2001), while the Lake Tarawera comparison is based on fourteen years (i.e., 1988 vs 2002).

Lake Okataina has undergone little change over a twenty-year time frame and the LakeSPI results have reflected this (Fig. 1). The Native Condition Index has declined marginally, while the Invasive Condition Index has also increased marginally. Both of these factors have a negative

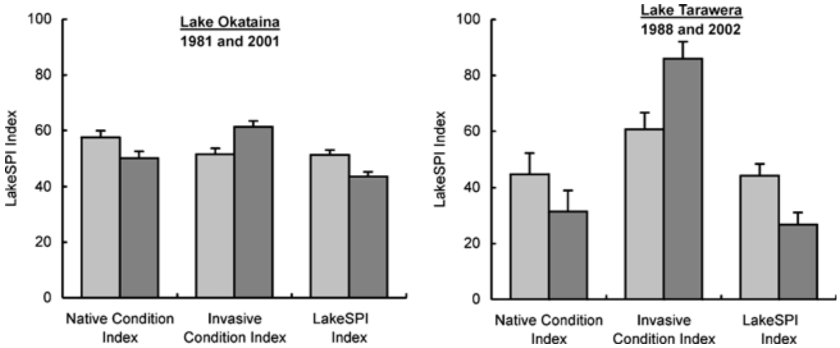

Figure 1. LakeSPI Indices for Lakes Okataina and Tarawera. Stippled bars represent historic data, solid bars represent most recent data. Error bars are +1 standard error of the mean.

influence on the most recent LakeSPI index and results suggest that there has been a slight reduction in mean water clarity between the two survey periods. This is consistent with Secchi data reported in Burns et al. (1997) even though the total combination of trophic indicators he reported showed no significant change.

In contrast to Lake Okataina, Lake Tarawera has more variable vegetation characteristics, with standard errors for Native Condition, Invasive Condition and LakeSPI indices being much higher than those for Lake Okataina. Nevertheless, Lake Tarawera has undergone significant change in the LakeSPI scores over the last fourteen years. The Native Condition Index has reduced and the Invasive Condition Index has increased resulting in the LakeSPI score being almost halved. There is no evidence from water quality monitoring data that water clarity has declined in the last 10 years, however the LakeSPI score suggests a small reduction in vegetation depth limits over the last fourteen years. It is quite possible that this case study is demonstrating the sensitivity of plants to pick up subtle changes in water clarity that are not being picked up by irregular Secchi disc measurements.

Conclusion

Environmental performance indicators must be practical, meaningful, repeatable and sensitive to moderate change while representing the ecological and biological condition of a water body. Advantages provided by using submerged plants as environmental indicators include their year round presence, ease of sampling, characterization of lake littoral margins, and integration of environmental conditions impacting plant growth over quite long time frames. These benefits help reduce the costs associated with expensive and frequent laboratory testing of water samples. The LakeSPI method described in this paper can be used to help assess, monitor and better manage lake condition. Although the method has been developed for use in New Zealand lakes, it is suggested that with appropriate modifications it could be adapted for application to lakes in other countries where submerged vegetation prevails.

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A new method to assess water trophy and organic pollution – the Macrophyte Biological Index for Rivers (IBMR): its application to different types of river and pollution

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Key words: macrophyte, index, water quality, eutrophication, methodology, rivers

Abstract

The paper presents a new index for assessing water trophy and organic pollution. It is based on only true aquatic macrophytes – being calculated on species score, coefficient of ecological amplitude and degree of cover. The method was tested in an acidic lowland river and an alkaline mountain river, and is shown to be validated by bio-indication scales based on macrophyte communities. The practical interest is discussed regarding the Water Framework Directive.

Introduction

Numerous papers dealing with mineralisation, trophy, and organic pollution in rivers have already demonstrated the possibility of establishing biotypologies based on flora (Grasmück et al., 1995) or phytosociological communities (Carbiener et al., 1990; Muller, 1990), and of developing indices to assess river water quality (e.g., Newbold & Holmes, 1987; Haslam, 1987). An alternative method would be to take biological traits into account (e.g., Ali et al., 1999). Such approaches to the subject have been reviewed by Haury et al. (2000) and confirmed the possibility of using macrophytes for the assessment of water quality in different rivers or ecoregions. Three main methods are, thus, currently available for assessing eutrophication in running waters: Mean Trophic Rank (MTR: Dawson et al., 1999; Holmes et al., 1999), Trophic Index with Macrophytes (TIM: Schneider, 2000) and the present Macrophyte Biological Index for Rivers (IBMR or MBIR). The latter represents an improvement of an earlier system published by the Group of Scientific Interest 'Macrophytes of inland waters' (Haury et al., 1996).

In order to assess its particular value, the index was calculated for different rivers impacted by fish-farms and domestic effluent. The results obtained were compared with the bio-indication scales of the trophic level based on the macrophyte communities established in both alkaline and acid watercourses by Robach et al. (1996). Future developments in the use of river macrophytes to implement the Water Framework Directive (WFD) are discussed.

Study sites

The River *Scorff* is a lowland river in Brittany, flowing over granite and schists bedrocks. On the main river, sources of pollution consist of urban effluent from a village, a sewage treatment plant, and two fish-farms. On its *St Sauveur* tributary, another treatment plant and a smaller fish-farm are present. The River *Nive des Aldudes* is a Pyrenean mountain water course flowing over alkaline substrata, and with pollution coming from seven fish-farms and two sewage treatment plants. Surveys were conducted in 2000 on 25 stretches of the *Scorff* and its tributary, and on 32 stretches of the *Nive des Aldudes*. Water analyses showed increases in ammonium and orthophosphate concentrations downstream from the fish-farms, the treatment plants and the village effluent sources.

A macrophyte-based classification of bio-indication was already available from two other areas: (i) the streams on the sandstone hills of the Northern Vosges (maximum altitude: 580 m) present an afforested catchment with slightly mineralised acidic stream water (pH: 4.5–7.2; conductivity: 40–130 μS/cm); and (ii) Rhine plain of French Alsace where groundwater-fed streams flow through a calcareous floodplain with highly mineralised bicarbonated stream water (pH: 7.5–8.2; conductivity: 400–1000 μS/cm).

Materials and methods

European literature and floras, in addition to our own studies of river vegetation, provided a provisional list of French river macrophytes (which included cyanobacteria, fungal growth and bacteria clumps in sewage effluent, macroalgae, bryophytes, pteridophytes and higher plants). There were certain difficulties encountered in selecting appropriate species. An initial attempt included hygrophytes, helophytes and subaquatic bryophyta (Haury et al., 1996), although this resulted in poor relationships with water quality (Bernez et al., 2001). Following detailed consideration, some 206 aquatic macrophytes were retained.[1]

Haury et al. (2000) indicated the properties that seemed to be important in assessing the aptness of the main taxa and plant communities to act as bio-indicators. However, comparisons between ecoregions have shown that, depending on whether the water is acid or alkaline, species or communities can react to different concentrations of N and P. The same is true along continental gradients (Robach et al., 1996). It is therefore, only possible to devise relative bio-indication scales. Such scales are based on ecological profiles provided by a National data base, on previously described biotypologies (Trémolières et al., 1994; Robach et al., 1996; Haury et al., 2001), and on the judgements of various experts.

Two criteria were considered for each macrophyte taxon. The **scores** (**CSi**) ranged from 0 (heavy organic pollution and heterotrophic taxa) to 20 (oligotrophy). They gave the overall value for «water quality» as determined by two nutrients – orthophosphate and ammonium, and heavy organic pollution, as indicated by the presence of sewage fungi. The species distribution range across four trophy classes (oligotrophic, mesotrophic, eutrophic, hypertrophic) was recorded using a **coefficient of ecological amplitude** (**Ei**): Coefficient 1, representing wide amplitude, covered three classes of trophy, and coefficient 3, representing a very limited amplitude, was restricted to just one class.

Surveys should be conducted by trained surveyors in the appropriate season. They should be conducted along 50–100 m-long stretches, which include both swift and slow flowing habitats. Percentage cover (exact numeral) is estimated in the field for all the macrophyte taxa (**i**), and classed

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[1] Electronic supplementary material is available for this article at http://dx.doi.org/10.1007/s10750-006-0175-3 and accessible for authorised users.

according to a **scale of cover** (**Ki**), going from 1 to 5 (1: $<0{,}1\%$; 2: $0{,}1-<1\%$; 3: $1-<10\%$; 4: $10-<50\%$; 5: $\geq 50\%$), so enabling inclusion in the subsequent calculation of the index.

$$IBMR = \frac{\sum_i Ei * Ki * CSi}{\sum_i Ei * Ki}$$

By comparison with the previous French index (Haury et al., 1996), macrophyte scores were modified to fit to a scale of five levels of trophic status (decreasing from oligotrophic to hypertrophic): $\text{IBMR} > 14$ = Very good; $14 \geq \text{IBMR} > 12$ = Good; $12 \geq \text{IBMR} > 10$ = Moderate; $10 \geq \text{IBMR} > 8$ = Poor; $8 \geq \text{IBMR}$ = Bad.

The ranking system for bio-indication was based on both botanical censuses and physico-chemical analyses of the stream water. Statistical analyses enabled us to propose an alphabetic system (A–F for hard water and A′–D′ for acidic water) based on ammonium and phosphate ion concentrations (Robach et al., 1996: Table 1). The trophic level, expressed as soluble reactive phosphorus of the water, was usually higher in acidic than in alkaline water.

Results

Application of IBMR to the rivers Scorff *and* Nive des Aldudes

The IBMR showed changes at and below all point sources of pollution (Fig. 1). For the *Scorff* a general degradation of water quality was apparent from upstream to downstream. For the *Nive des Aldudes*, the general trophic level remained the same, while heavy organic pollution was observed downstream the first fish-farm, giving rise to bacterial clumps.

The IBMR and the ranking system for bio-indication

In hard water the IBMR index varied from 15.9 (corresponding to Group A) to 6.8 (Group F), whist in acidic water the range was from 15.6 to 10.5 (Table 2). In acidic water the index showed a significant difference between oligo-mesotrophic (A′B′) and meso-eutrophic communities (C′D′). In alkaline water the index distinguished three groups: the first corresponded to an oligotrophic level (IBMR > 15); the second grouped

Table 1. Bio-indication ranking system for eutrophication in the running hard water of the Alsace plain (A–F) and soft water of the Northern Vosges mountains (A′–D′): macrophyte communities and trophic level (P- PO_4^{3-}, N-NH_4^+) (from Robach et al., 1996)

Dominant or characteristic species	Steps	Trophic level	N-NH_4^+ (μg/l)		P-PO_4^{3-} (μg/l)	
			Mean	SD	Mean	SD
Potamogeton coloratus	**A**	Oligotrophic strict	14	7.3	7	1.7
Berula erecta	**B**	Oligo-mesotrophic	22	13.8	13	5.5
Callitriche obtusangula, B. erecta, Elodea Canadensis	**C**	Mesotrophic	45	27.8	15	6.8
Zannichellia palustris, Groenlandia densa Nasturtium officinale	**D**	Meso-eutrophic	34	31.3	29	23.6
Oenanthe fluviatilis, Ceratophyllum demersum, Ranunculus fluitans	**E**	Eutrophic	61	40	40	33
Potamogeton nodosus *P. lucens, P. pectinatus*	**F**	Hypertrophic Meso-saprobic	255	107	191	116
Potamogeton polygonifolius	**A′**	Oligotrophic	43	13	20	26
P. polygonifolius, Ranunculus peltatus, Callitriche hamulata, C. platycarpa	**B′**	Oligo-mesotrophic	49	14	22	11
C. hamulata, E. canadensis, O. fluviatilis, Myriophyllum alterniflorum, P. alpinus	**C′**	Mesotrophic	86	35	53	19
C. hamulata, C.obtusangula	**D′**	Eutrophic	170	109	139	46

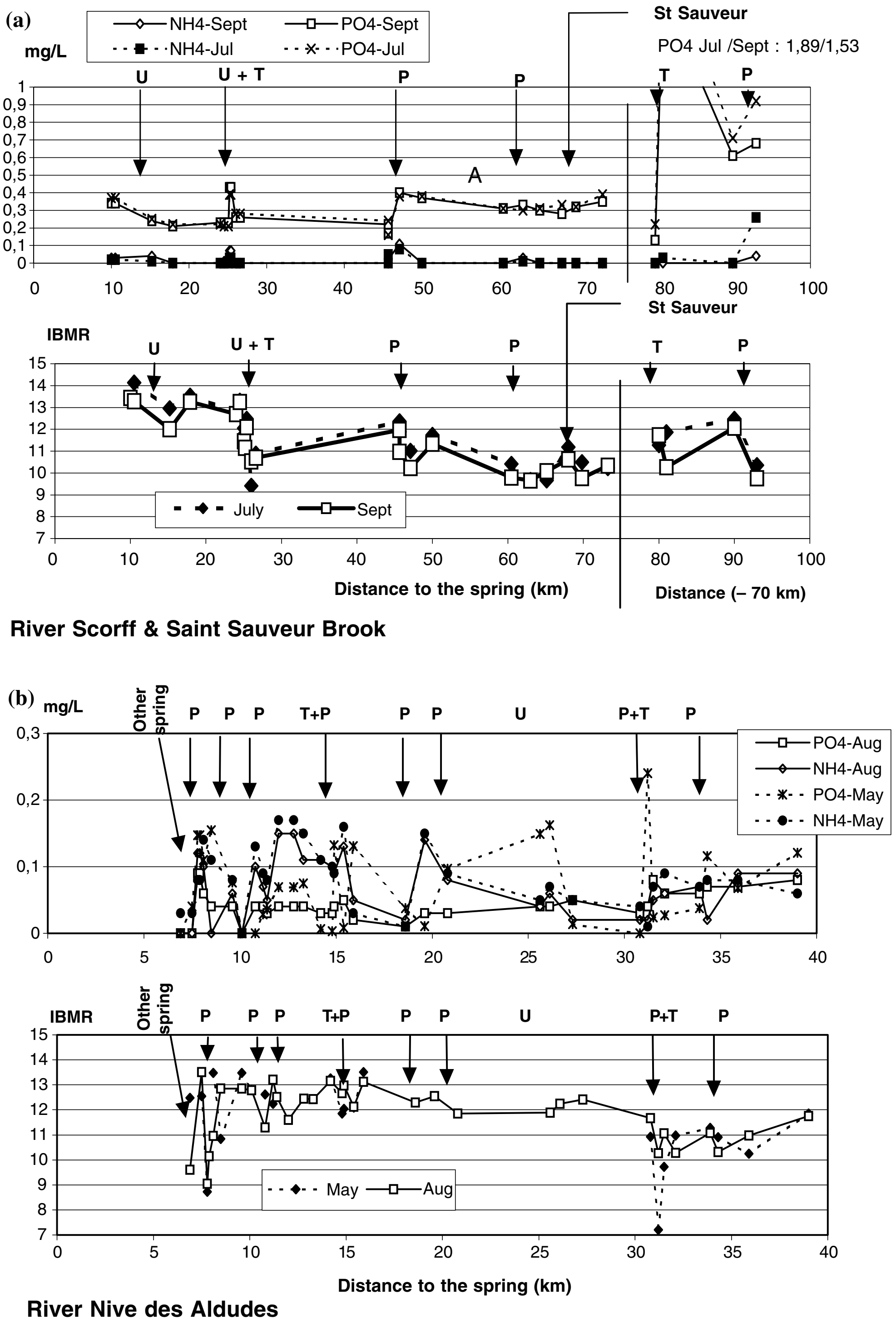

Figure. 1. Ammonium, orthophosphates and IBMR on the two rivers Legend: P: Pisciculture (fish-farm); U: urban sewage; T: sewage treatment plant. *For River Scorff and Saint Sauveur Brook, vertical line shares the tributary and broken arrow assesses the joining point. For River Nive des Aldudes, important secondary spring joins the main course not very far from the upper spring.*

Table 2. Mean and standard deviation of IBMR calculated on the macrophytes communities of the bio-indication ranking system in hard and soft water

Community	A	B	C	D	E	F
IBMR Alsace plain	15.9	12.4	10.9	9.5	8.1	6.8
SD	1.8	1.1	0.5	1.2	1.2	0.8
Community	A′	B′	C′	D′		
IBMR Northern Vosges	15.6	13.9	11.0	10.5		
SD	1.1	0.7	0.9	0.5		

meso-eutrophic communities together (B, C, even D with the IBMR circa 10); while the third group characterised eutrophic communities E and F (IBMR *circa* 7.5).

Discussion and conclusions

As demonstrated for both rivers, the IBMR effectively assesses not only trophic disruption, but also heavy organic pollutions (where heterotrophic organisms begin to be observed). This simple method can be used by field surveyors, provided they have some guidance and training from senior scientists. They will also need help in taxonomic determinations. The IBMR was widely tested throughout France in 2001 and 2002 by more than 10 separate teams of surveyors on more than 100 sites.

A comparison of the IBMR values calculated for macrophyte communities did not show any significant difference (*t* test) in alkaline and acidic water between A and A′, C and C′ and D and D′. We did, however, find a slightly significant difference between the B and B′ communities. The difference could be explained by the floral composition and the high score of the oligotrophic *Potamogeton polygonifolius* in B′, whereas B community contained only oligo-mesotrophic aquatic plants.

Some limitations are nevertheless apparent. For example, in certain rivers flowing over mobile substrata where macrophytes are scarce, the surveyed stretch ought to exceed 100 m. When only a few taxa are present, these must be strong indicators for the IBMR to be used with confidence. In deep water, the methodology must be adapted by using pin-point sampling (Dutartre et al., 1999; Dutartre, unpublished data).

The method is being standardised with the aim of setting up a method in France for assessing river quality (SEQ) which can be used for the WFD. The IBMR can already be considered to be a good index for estimating whether or not a river is affected by nutrient inputs (eutrophication) and/or heavy organic pollution. It cannot, however, be considered as the complete answer for comparing type-specific macrophyte communities. It is a relevant indicator of disturbed situations when in good agreement with the bio-indicator ranking system based on reference macrophyte communities. To get a better fit with the WFD requirements, references from unpolluted rivers should be taken into account wherever and as far as they still exist. Such reference phytocoenoses and degradation sequences still remain to be assessed in most French regions – as indeed the differences between expected and observed vegetation. Intercalibration with the MTR, Austro–German methods (Kohler & Janauer, 1995; TIM: Schneider, 2000) and the Saprobic Index (Husàk et al., 1989) ought to be the next step in implementing the WFD using macrophytes.

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Comparison of different biological indices for the assessment of river quality: application to the upper river Moselle (France)

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Key words: chemical survey, macroinvertebrates, macrophytes, indices, water quality

Abstract

The aim of our study was to assess the water quality of the upper Moselle river by using biological indices. Simultaneous physico-chemical surveys were also undertaken from May 1999 to April 2000. Twelve sampling sites were selected in order to provide a wide range of potential pollution. Chemical analysis did not reveal any major problem of pollution. However a lower water quality resulting from domestic pollution was established for some sampling sites. A biological monitoring combining both macroinvertebrates and macrophytes was performed. Biological indices based on plant community structure and macrophyte composition were not pertinent tools, whereas simple indices based on taxonomic richness of particular groups of macroinvertebrates were strongly correlated with several chemical parameters, showing that such simple biological variables should represent powerful indicators of ecosystem degradation.

Introduction

Biological monitoring often appears to be more appropriate in the assessment of pollution of aquatic ecosystems than traditional chemical evaluation of water quality. As aquatic organisms may integrate effects of perturbations, numerous methods have been proposed to assess both water and biological quality. In particular, methods based on benthic macroinvertebrate communities have been frequently used. Water quality monitoring using aquatic macrophytes has been developed in Europe over the past two decades (Harding, 1981; Holmes & Newbold, 1984; Carbiener et al., 1990, Robach et al., 1996; Dawson et al., 2000; Haury et al., 2002). Six approaches were identified to assess the quality of aquatic ecosystems through macrophyte communities (Demars & Harper, 1998): (1) identification of community assemblages, (2) biomass measurements, (3) classification based on the drainage order combination, (4) ecomorphology, (5) phytosociology and (6) identification of communities using weightings to indicator species. Even though diversity indices based on macroinvertebrate communities are widely used (Guérold, 2000), they have rarely been employed to assess the impact of water pollution on macrophyte communities (Small et al., 1996; Baattrup-Pedersen & Riis, 1999; Thiébaut et al., 2002).

The aim of the study was to assess the pollution of rivers by using biological approaches based both on macroinvertebrate and aquatic macrophyte communities and to compare their response to water pollution.

Description of sites studied

The study area is located in the upper Moselle river (North Eastern, France) which is the main tributary of the Rhine river. The catchment of the upper Moselle river is 3706 km^2. The river drains granite and metamorphic bedrocks. Twelve sampling sites were selected along a longitudinal gradient, in order to provide a diversity of potential polluted sites. Eight sites (sites: Bussang, Fresse, Rupt, Mitreuches, Eloyes, Archette, Igney, Velle) were located on the upper part of the Moselle river and four sites (sites: Bresse, Saulxures, Zainvillers, Cleurie) along its main tributary, the Moselotte river. Sites were nearly shadeless and their substrate was composed of peebles. The mean current velocity of these twelve sites was 0.63 m/s and the mean water depth was 0.52 m.

Materials and methods

Chemical analyses

At each site, 500 ml of water was collected seven times in the year at different flow conditions. Oxygen, conductivity and pH were measured in the field using a multiparameter instrument (WTW 340i). The following parameters were analysed in laboratory (within 24 h of sample collection). Alkalinity was measured by titration. Main cations (Ca, Mg, Na, K) were analysed using atomic absorption spectrophotometry. Sulfate, chloride, nitrite-nitrogen (NNO_2) and nitrate-nitrogen (NNO_3) were determined in the laboratory by ion chromatography. Ammonium nitrogen (NNH_4) and soluble reactive phosphorus (SRP) were analysed using spectrophotometry. Nitrogen (N) (Kjeldahl) and total phosphorus (TP) were determined after digestion with acid and analysed using standard method. The biological oxygen demand (BOD) and the chemical oxygen demand (DCO) were measured following the procedures described in the French norms (respectively NFT 90-103 and NFT 90-101).

A Principal Components Analysis (PCA) based on the mean physico-chemical variables was realised to test for space and time variability (software ADE-4, version 2001, CNRS Lyon).

Biological monitoring and aquatic macrophyte communities

The botanical survey was conducted in June and September 1999. A standard length of watercourse (100 m) was selected. All macrophytes present were recorded, together with the estimated percentage cover of each species. Organisms were identified to species or to the lowest practical taxonomic level. A Canonical Correspondence Analysis (CCA) using CANOCO, was established between aquatic macrophytes and the main chemical variables. The following indices were tested on macrophyte data: taxonomic richness S, abundance Q, Shannon diversity index (Shannon & Weaver, 1963), Margalef's diversity index (Margalef, 1958), Simpson's index (Simpson, 1949). Pearson's correlation coefficient was used between indices and physio-chemical parameters using Statistica (Version 5.5, StatSoft).

Biological monitoring and macroinvertebrate communities

At each site, four samples of macroinvertebrates were taken in May 1999, using a modified Surber sampler (0.084 m^2, 353 μm mesh). Only organisms belonging to Epheromeptera, Plecoptera and Trichoptera orders (EPT) were taken into consideration. Organisms were identified to the lowest practical taxonomic level. Different indices based on macroinvertebrate biodiversity were tested: Ephemeropta richness, Plecoptera richness, Trichoptera richness, Ephemeroptera, Plecoptera, Trichoptera richness (EPT), Shannon–Wiener and Margalef diversity indices applied to EPT. Pearson's correlation coefficient was used between indices and physio-chemical parameters using Statistica (Version 5.5, StatSoft).

Results

Physico-chemical composition

The PCA performed on chemical analysis showed that the Moselle river and its main tributary, the Moselotte river, was classically characterised by an increasing gradient of mineralisation from upstream to downstream as the first axis explains 55.6% of the variance. The second axis which explains 34.8% of the variance, corresponded to a domestic pollution gradient (Fig. 1a). Chemical analysis did not reveal any major problem of pollution except during high flow in October (Fig. 1b)

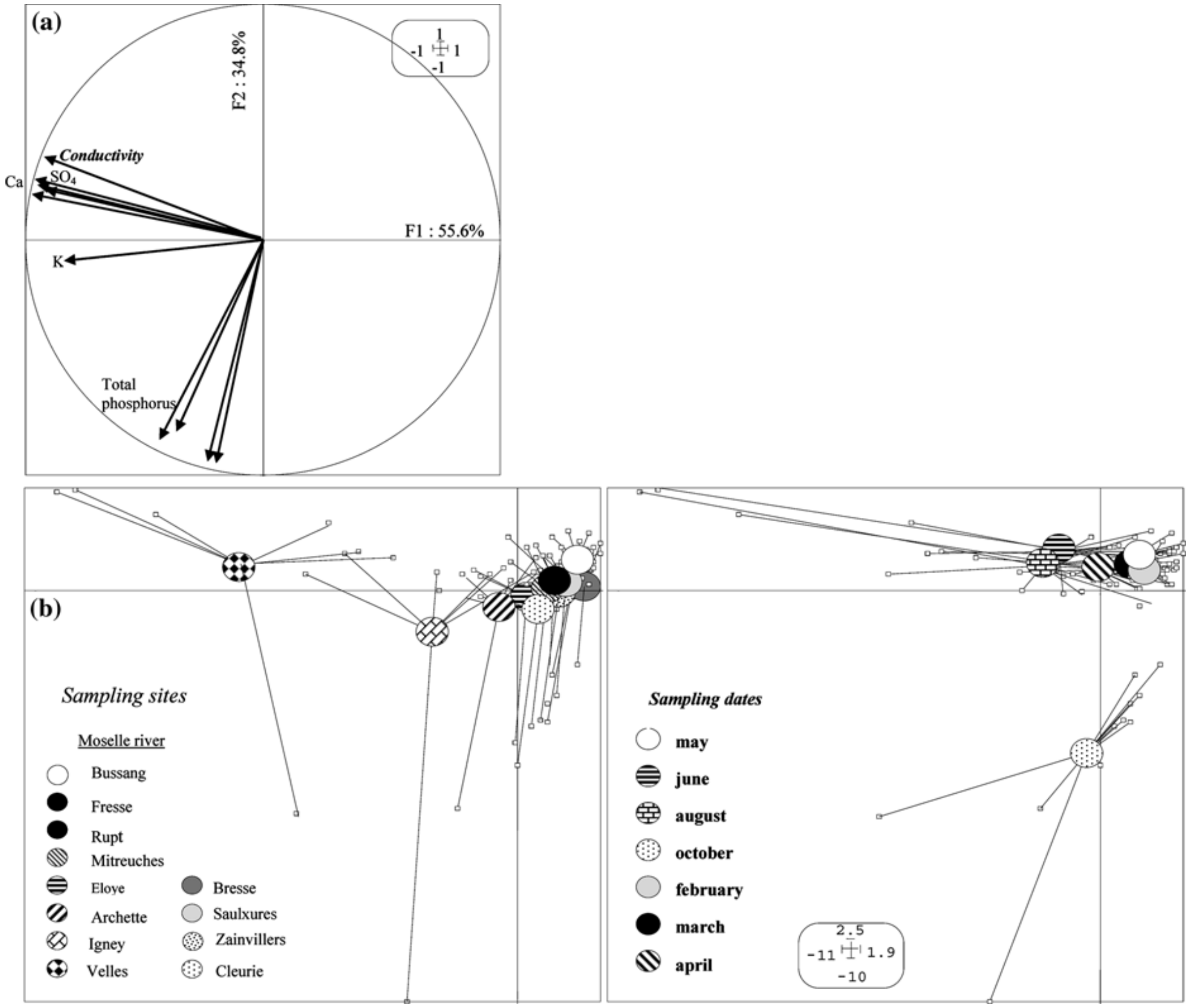

Figure 1. Principal component analysis (PCA) of physico-chemical variables. (a) Correlation circle showing the position of the variables on the F1F2 plane. (b) Ordination of the 12 selected sites on the F1F2 plane. (c) Position of the sampling date on the F1F2 plane. Circles denote the weighted average of all sample taken from a given sampling site. Line link sample (small square) to weighted average.

Aquatic plant communities

The first axis of CCA is a mineralisation gradient. From the CCA ordination diagram (Fig. 2), it can be seen that alkalinity and conductivity are strongly correlated with the first CCA axis. Species with a high positive score (*Myriophyllum spicatum*, *Cladophora* sp.) on that axis were therefore restricted to downstream site (Velle) with high alkalinity and conductivity. The second axis can be interpreted as being related to the ratio of ammonium to phosphate, the idea being that these variables have on the second axis about equal canonical coefficients of opposite sign (0.51 and −0.55). The second axis is still a contrast between ammonium and phosphate concentrations. No significant correlations were established between macrophyte diversity indices and chemical variables, except for Simpson index.

Macroinvertebrate communities

EPT richness was severely depressed at some sampling sites showing that some taxonomic

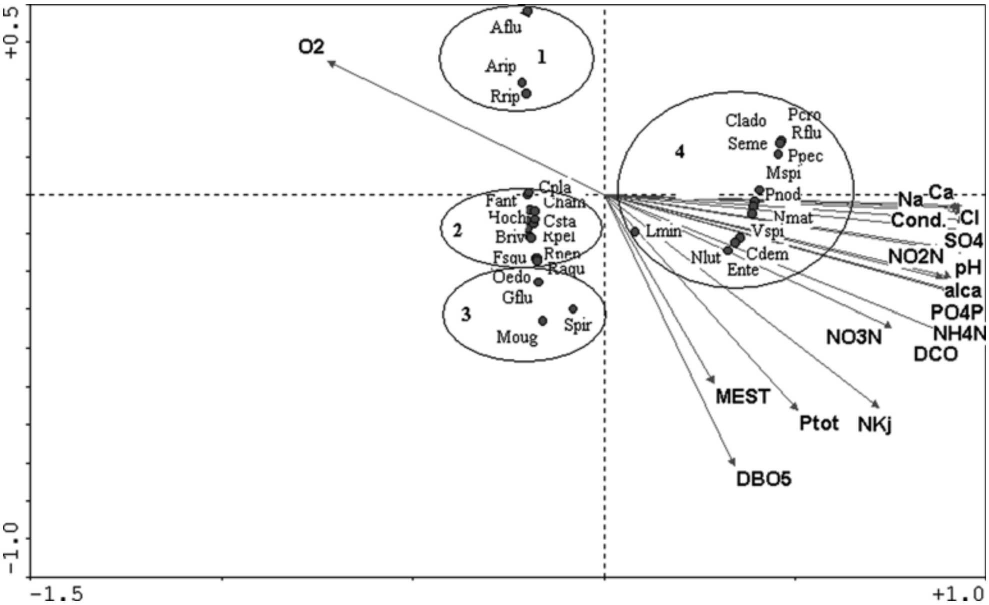

Figure 2. Canonical Correspondence Analysis between aquatic plants and chemical variables. Bryophyte: *Amblystegium fluviatile* – Aflu; *Amblystegium riparium* – Arip; *Brachythecium rivulare* – Briv; *Fontinalis antipyretica* – Fant; *Fontinalis squamosa* – Fsqu; *Hygrohypnum dilatatum* – Hdil; *Hygrohypnum ochraceum* – Hoch; *Hyocomium armoricum* – Harm; *Rhacomitrium aciculare* – Raci; *Rhynchostegium riparioides* – Rrip; *Scapania undulata* – Sund; Aquatic Vascular plants: *Callitriche stagnalis* – Csta; *Callitriche hamulata* – Cham; *Callitriche platycarpa* – Cpla; *Elodea Canadensis* – Ecan; *Glyceria fluitans* – Gflu; *Lemna mino* – Lmin; *Myriophyllum spicatum* – Msp; *Ranunculus aquatilis* – Raqua; *Ranunculus peltatus* – Rpel; *Ranunculus penicillatus* – Rpen; *Sparganium emersum* – Seme; Algae: *Cladophora* sp. – Clado; *Lemanaea fluviatilis* – Lflu; *Melosira* sp. – Melo; *Mougeotia* sp. – Moug; *Nitella flexilis* – Nflex; *Oedogonium* sp. – Oedo; *Oscillatoria* sp. – Osci; *Spirogyra* sp. – Spir; *Vaucheria* sp. – Vauc.

groups were strongly affected. For example, no species of Plecoptera were recorded from four sampling sites. Correlation analyses between biological variables and indicators of domestic pollution revealed several significant relationships between the richness of EPT, Plecoptera, Trichoptera (Table 1). For example the richness of ETP and each taxonomic group was significantly negatively correlated with kjedahl nitrogen concentrations. In this sense, the more highly significant relationships were found with EPT and Trichoptera richness. On the contrary, diversity indices were poorly correlated with chemical parameters, showing that this metrics were less powerful indicators of pollution than richness.

Discussion

Vegetation is assumed to be more linked to the instability than to the nutrients inputs of the Moselle river (running water from the tributary and flood disturbances). The absence of correlation between diversity indices based on aquatic plants and chemical variables corroborates this situation. This is in contradiction with another study previously realised, which established a significant correlation between diversity indices (Shannon's index, Margalef's index) and phosphate and between Margalef's index and nitrogen in stable habitats of streams in the Vosges mountains (Thiébaut et al., 2002). In our study area, factors such as depth, substrata, shading, width, bed stability, singly or in some combinations, have a stronger influence on the floristic community than the water chemistry in the upper Moselle catchment. In the literature, significant correlations have been established between physical and floristic parameters (Baattrup-Pedersen & Riis, 1999). Macrophyte species diversity in streams also increases as the spatial heterogeneity and/or diversity of habitats increases. The number of species and abundance also depend on biotic variables (herbivory). Use of diversity indices

Table 1. Correlation matrix between several chemical indicators of domestic pollution and biological variables based on macroinvertebrate communities

Biological variables	N Kj		NO_3		NO_2		P tot		PO_4	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Richness										
EPT	−0.938	0.000	−0.758	0.011			−0.800	0.005	−0.788	0.007
Plecoptera	−0.771	0.009	−0.909	0.000	−0.714	0.020	−0.837	0.003	−0.777	0.008
Trichoptera	−0.928	0.000					−0.659	0.038	−0.675	0.032
Ephemeroptera	−0.698	0.025								
Diversity indices										
Shannon & Wiener							−0.676	0.032		
Margalef	−0.882	0.001								
Simpson										

based on macrophyte communities did not allow us to assess pollution status of the Moselle river, because the influence of factors other than a change in trophic status is deemed significant.

Since several decades methods based on macroinvertebrate communities have become more and more popular (De Pauw & Vanhooren, 1983; Resh & Jackson, 1993; Barbour et al., 1996; Thorne & Williams, 1997). If perturbation conditions are present for a period sufficient to induce detrimental effects at a population and consequently at a community level, it becomes possible to evaluate changes in the community composition by using simple biological variables such as richness and diversity indices. Such metrics may be simply an estimation of the taxonomic richness or diversity indices which combine abundance and richness. As previously reported by Norris & Georges (1993) they are seen as a useful way to condense complex data, making interpretation easier. In the present study, estimation of the richness of Plecoptera, Trichoptera or ETP appeared more accurate biological parameters to reveal domestic pollution than diversity indices. The use of these three taxonomic groups is interesting as many species are polluo-sensitive, relatively easy to identify at a genera or species level. In several countries such as in France, these three groups are also the best known. Contrary to other biological methods such as the IBGN index (AFNOR, 1992) commonly used in France (that need only determinations at the family level), genera/species richness evaluation provides a more important information because it represents a direct evaluation of the (bio)diversity of macroinvertebrates and its erosion when ecosystems are polluted. In this sense, it is necessary to stress that the more precise are the determinations of invertebrates the more pertinent are the biological variables (Guérold, 2000).

Conclusion

Chemical analysis did not reveal a major perturbation. However a lower water quality resulting from domestic pollution was established at some sampling sites. A number of factors, including ecological conditions, can affect macrophyte communities in streams undergoing water pollution that are not evaluated by diversity indices. In this study diversity indices based on aquatic macrophytes were not pertinent tools to assess water quality, whereas the three taxonomic groups of macroinvertebrates (EPT), appeared to be more relevant to assess domestic pollution of running waters.

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A comparison of macrophyte indices in headwaters of rivers in Flanders (Belgium)

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Key words: Macrophytes, monitoring, scores, environment, multivariate analysis, EU-directive

Abstract

Macrophytes were monitored in 48 headwater rivers in Flanders (Belgium). Only 26 sites had macrophyte vegetation, which was used to calculate five different indices and scores. These indices originated from UK, France, Czech Republic and Germany. Macrophytes were significantly correlated with a number of habitat variables, such as transect size, river width, slope, Huet zonation, distance to source, shadowing and average depth. For the substrate there were significant negative correlations with the fine particle sizes and a positive correlation with the coarse particles. A multivariate analysis of the macrophytes in these headwaters showed three groupings, essentially separated on basis of water hardness and pH.

Introduction

Monitoring the biological quality and ecological status of running waters can be carried out using diverse groups of organisms. Many European authorities have adopted a macro-invertebrate saprobic or biotic index system for regular monitoring, whereas fishes, macrophytes, phytoplankton and phytobenthos (diatoms) were not systematically included (De Pauw et al., 1992). The EU-directive on 'water' (EU, 2000), however, implements also a monitoring scheme for the latter groups of organisms and a comparative monitoring will be needed to obtain an improved assessment of the ecological quality of running waters (Triest et al., 2001).

Macrophytes were not only studied in the context of lake and ditch trophic systems (Seddon, 1972; de Lange & van Zon, 1983; Lehmann & Lachavanne, 1999), alkalinity (Vestergaard & Sand-Jensen, 2000) or acidification (Arts et al., 1990) but also as indicator species or vegetations in running waters (Kohler, 1975; Wiegleb, 1981; Caffrey, 1986; Muller, 1990; Grasmück et al., 1995; Haury et al., 1996; Thiébaut & Muller, 1998; Riis et al., 2000). A macrophyte index scheme comparable to the concept of a macroinvertebrate biotic index was adopted for the Irish waters (Caffrey, 1986) using the relative abundance of four different sensitivity groupings. A more profound use of macrophytes as indicators of organic pollution can be based on the 10-point scale of saprobic valency for more than 100 taxa of Czechoslovakia (Husák et al., 1989). The advantage is that every taxon has a maximum of 10 points in each of the considered five classes of limnosaprobity (xeno-, oligo-, beta-meso-, alpha-meso- and polysaprobity) and that this follows a Gaussian distribution curve. The calculated indicative weight as well as an individual saprobic index of each taxon could be worked out on the basis of Czechoslovakian field data. In French rivers (Haury et al., 1996), a check list of more than 240 taxa is worked out with specific scores from 0 to 10 (poor to high water quality).

However, if hyporheic riparian vegetation has to be included in the monitoring, a nearly complete list of plant indicator values is available from Ellenberg et al. (1992). Ellenberg indicator values for moisture, nitrogen status, soil reaction (acidity/lime content), chloride concentration, light regime, temperature and continentality were based on field experience with more than 2700 Central European vascular plant species. These values do not refer to conditions at a specific moment but present an integration over time. Calculations of weighted average indicator values can be considered as an effective form of calibration. Though designed for Central European vegetations, they have been used outside that region. Schaffers & Sýkora (2000) reviewed the uses, checked the reliability of the indicator values for moisture, nitrogen and soil reaction and concluded that the Ellenberg indicator system provides a valuable tool for habitat calibration, even in more western European vegetations. The observation that functional groups of aquatic vegetation and wetlands are consistent across a broad geographical range of Europe (Hills & Murphy, 1996) further supports the use of Ellenberg values in macrophyte monitoring.

In the case of mountain streams or of different substrata, moss species and a wide diversity of aquatic macrophytes can be very reliable indicators of water quality and typology. However, lowland rivers in Belgium show a narrower range of their macrophyte composition due to the lack of a xenosaprobic zone and to the short stretches of oligosaprobity, often located in forested areas with severe light limitations for macrophyte growth. Aquatic bryophytes were not present in the forested headwaters because of the soft substrate conditions. In this study, we investigated macro-

Table 1. Overview of the calculated macrophyte indices on basis of earlier published lists with scores

		a	b	c	d	e	#taxa
Breiloop	N17	5.0	5.8	4.6	2.1	7.2	5
Desselse Nete	N18	5.3	5.8	4.1	1.7	6.9	7
Achterste Nete	N19	7.5	6.1	4.9	2.2	7.3	7
Asbeek	N20	5.8	6.0	4.0	1.3	6.9	3
Laambeek	D21	5.0	6.0	4.0	–	6.0	1
Laambeek	D22	–	–	6.0	–	–	1
Bollisserbeek	M24	5.0	4.0	6.0	1.5	6.0	1
Dommel	M25	8.4	7.6	3.2	2.1	6.9	4
Gielisbeek	M26	6.8	6.0	4.0	1.2	6.5	3
Warmbeek	M27	6.1	6.2	4.6	1.5	6.7	10
Warmbeek	M28	6.3	5.7	4.8	1.6	7.1	5
Warmbeek	M29	5.5	5.9	4.6	1.7	7.0	11
Warmbeek	M30	6.2	5.8	4.6	1.7	7.0	5
Abeek	M31	7.0	6.2	4.0	1.0	7.0	2
Bullenbeek	M32	6.2	5.7	4.9	1.3	6.6	6
Wijshagenbeek	M34	6.3	6.2	4.6	1.6	6.8	7
Bezoensbeek	D35	6.0	6.0	5.5	–	6.0	2
Zuurbeek	M38	6.1	6.1	3.8	1.0	6.9	5
Asbeek	M39	4.5	4.5	5.7	1.0	6.5	2
Ziepbeek	M40	5.0	5.0	6.8	–	5.7	3
Schaachterzijp	M41	6.7	6.1	4.0	1.2	6.9	5
Zanderbeek	M43	5.4	5.9	5.1	1.8	6.5	10
Zanderbeek	M44	5.3	5.6	5.3	1.8	6.4	10
Voer	V45	6.0	6.5	5.5	–	6.5	2
Voer	V46	6.2	6.4	4.8	1.6	7.0	4
Voer	V47	5.7	5.9	4.6	1.6	6.8	10

a: Ellenberg-N (Centr. Eur*)*; b: British MV-N (UK); c: Haury et al. (France); d: Husak (Czech Rep.); e: British MV-R (UK).

phytes in 48 headwaters of presumed best water quality in Flanders and compared different indices.

Methods

A total of 48 headwaters in Flanders were monitored for macrophytes by using the frequencies of aquatic plant taxa (including riparian emergent macrophytes) in river wide transects of 20 m length. Indices were calculated as weighted means after Ellenberg et al. (1992) using *N*-values and derived British modified *N*-values, British modified *R*-values (Hill et al., 1999); after Husák et al. (1989) and Haury et al. (1996). Correlations (Spearman rank order) between different macrophyte scores and environmental variables (chemical variables of water and substrates; habitat descriptions) were done with STATISTICA and multivariate analysis with CANOCO. A canonical correspondence analysis (CCA) was performed with forward selection using Monte Carlo permutation test, after checking the gradient length in a detrended correspondence analysis (DCA). Water quality variables were temperature, pH, conductivity, %O2, dissolved oxygen (DO), biological oxygen demand (BOD), alkalinity, T-hardness, Ca-hardness, Mg-hardness, Si, Cl, o-PO4, total phosphate (TP), NO2, NO3, NH4. Substrate quality variables were T-Kjeldahl N, total phosphate (TP), particle sizes in six classes. Habitat descriptors were transect surface, river width, slope, fish zonation, distance to source, altitude, average depth, stream velocity, transparency, meandering, pool-riffle, natural structure, habitat score, habitat quality assessment of river and margins, shading. Water and substrate quality variables were measured after American Public Health Association (1989).

Results and discussion

Macrophyte taxa and indices

A total of 35 macrophyte taxa were observed in 26 of the 48 monitored headwaters. In 22 headwaters no macrophytes were observed due to either shading or intense agricultural practices. The number of taxa per river varied from 1 to 11 with an average of 4.6. Most frequently observed were *Callitriche obtusangula* in 15 and *Polygonum hydropiper* in 14 sites. Common were *Glyceria maxima*, *Lycopus europaeus*, *Myosotis scorpioides*, *Ranunculus penicillatus* var. *penicillatus* and *Rorippa nasturtium-aquaticum*, each from more than five locations, whereas the following taxa were observed only in one river: *Apium nodiflorum*, *Berula erecta*, *Callitriche stagnalis*, *Equisetum fluviatile*, *Glyceria fluitans*, *Groenlandia densa*, *Potamogeton crispus*, *Potamogeton polygonifolius*, *Potamogeton pusillus*, *Ranunculus penicillatus* var. *calcareus* and *Sagittaria sagittifolia*.

The calculation of macrophyte indices (Table 1) on basis of the weighted averages was done after Ellenberg et al. (1992) using *N*-values, and derived British modified *N*-values and British modified *R*-values (Hill et al., 1999); after Husák et al. (1989) and Haury et al. (1996). The former four can be interpreted especially towards trophic status or eutrophication, whereas the last one can be relevant for the ecological indication of more acidic versus more alkaline headwaters. Nevertheless, the *R*-values in this study do not sufficiently differentiate, though the most acidic river (Ziepbeek = M40) results in the lowest value with $R = 5.7$.

Regarding those indices that are more related to the trophic status and eutrophication, there is no clear differentiation between most of the localities. Only the Dommel (M25) shows a higher

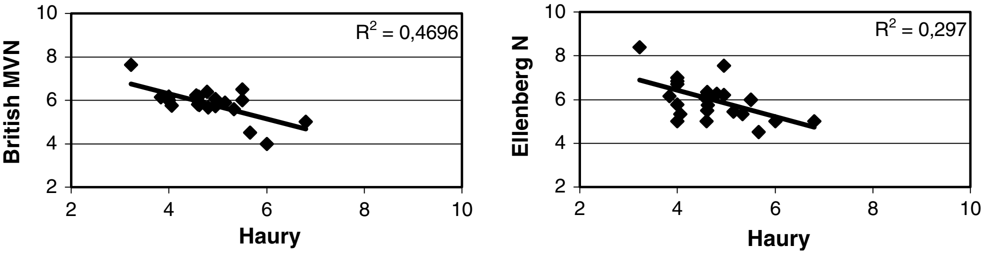

Figure 1. Comparison of the macrophyte scores (Haury et al., 1996) with *N*-values of Ellenberg (Ellenberg et al., 1992) and the British modified *N*-values (Hill et al., 1999). There is a large representation of macrophyte scores in the middle zone and only a few at the extremes.

trophic status for each of the considered indices. Those indices that tend towards a lower trophic status on basis of the macrophytes are the Bolliserbeek (M24), Asbeek (M39) and Ziepbeek (M40).

The correlation between the British modified *N*-value and the macrophyte scores from France (Haury et al., 1996) is the most significant among all comparisons (Fig. 1). A method for the conversion from macrophyte scores to a scale of five classes is not yet validated and available. Extreme values are rare and the number of taxa as well as the number of vegetated sites were not

Table 2. 'Spearman rank order' correlations between five macrophyte indices (see text) and environmental parameters

Index	N-Ellenberg (D)	N-Ellenberg (UK)	Haury et al. (F)	Husak et al. (CZ)	R-Ellenberg (UK)
T	0.53***	0.52***	0.44**	0.41**	0.44**
pH	–0.47***	–0.36**	–0.48***	–0.46***	–0.46***
Cond	–0.42**	–0.35*	–0.53**	–0.34*	–0.40**
%O2	–	–	–	–	–
DO	–	–	–0.34*	–	–
BOD	–	–	–	–	–
Alk	–	–	–	–	–
T-hard	–	–	–	–	–
Ca-hard	–	–	–	–	–
Mg-hard	–	–	–	–	–
Si	–0.51***	–0.46***	–0.46***	–0.59***	–0.56***
Cl	0.42**	0.34**	–	0.51***	0.46***
o-PO$_4$	–	–	–	–	–
TP	–	–	–	–	–
NO2	–0.33*	–0.33*	–	–	–
NO3	–	–	–	–	–
NH4	–	–	–	–	–
Sed-TKN	–	–	–	–	–
Sed-TP	–	–	–	–	–
TS	0.46***	0.55***	0.45***	0.52***	0.57***
RW	0.44**	0.53***	0.44**	0.51***	0.55***
SL	–0.47***	–0.45***	–0.50***	–0.53***	–0.56***
HZ	0.50***	0.43**	0.46***	0.52***	0.54***
DS	0.46***	0.50***	0.48***	0.47***	0.50***
AL	–	–	–	–	–
AD	–	0.23	–	0.43	0.34
SV	–	–	–	–	–
ME	–	–	–	–	–
PR	–	–	–	–	–
NS	–	–	–	–	–
HS	–	–	–	–	–
HQA	–	–	–	–	–
SH	–0.55***	–0.54***	–0.41**	–0.66***	–0.60***

***$p < 0.001$;**$p < 0.01$; *$p < 0.05$: T = temperature; pH; Cond = conductivity; %O2; DO = dissolved oxygen; BOD = biological oxygen demand; Alk = alkalinity; T-hard = total hardness; Ca-hard = Ca-hardness; Mg-hard = Mg-hardness; Si = silicate; Cl = chloride; o-PO$_4$ = orthophosphate; TP = total phosphate; NO2 = nitrite; NO3 = nitate; NH4 = ammonia; Sed-TKN = total Kjeldahl nitrogen in sediment; Sed-TP¨ = total phosphate in sediment; TS = transect surface; RW: river width; SL = slope; HZ = Huet zonation; DS = distance to source; AL = Altitude; AD = average depth; SV = stream velocity; ME = meandering; PR = pool-riffle patterns; HS = habitat structure; HQA = habitat quality assessment; SH = shadowing.

sufficiently large to try out the effect of other conversions. The adaptation of a macrophytes index to e.g. the fish zonation could be a possibility, as well as a calibration according to a realistic range of scores within a river stretch.

Correlations between macrophyte indices and environment

Macrophytes were absent in the headwaters of presumed best water quality of 21 of the 48

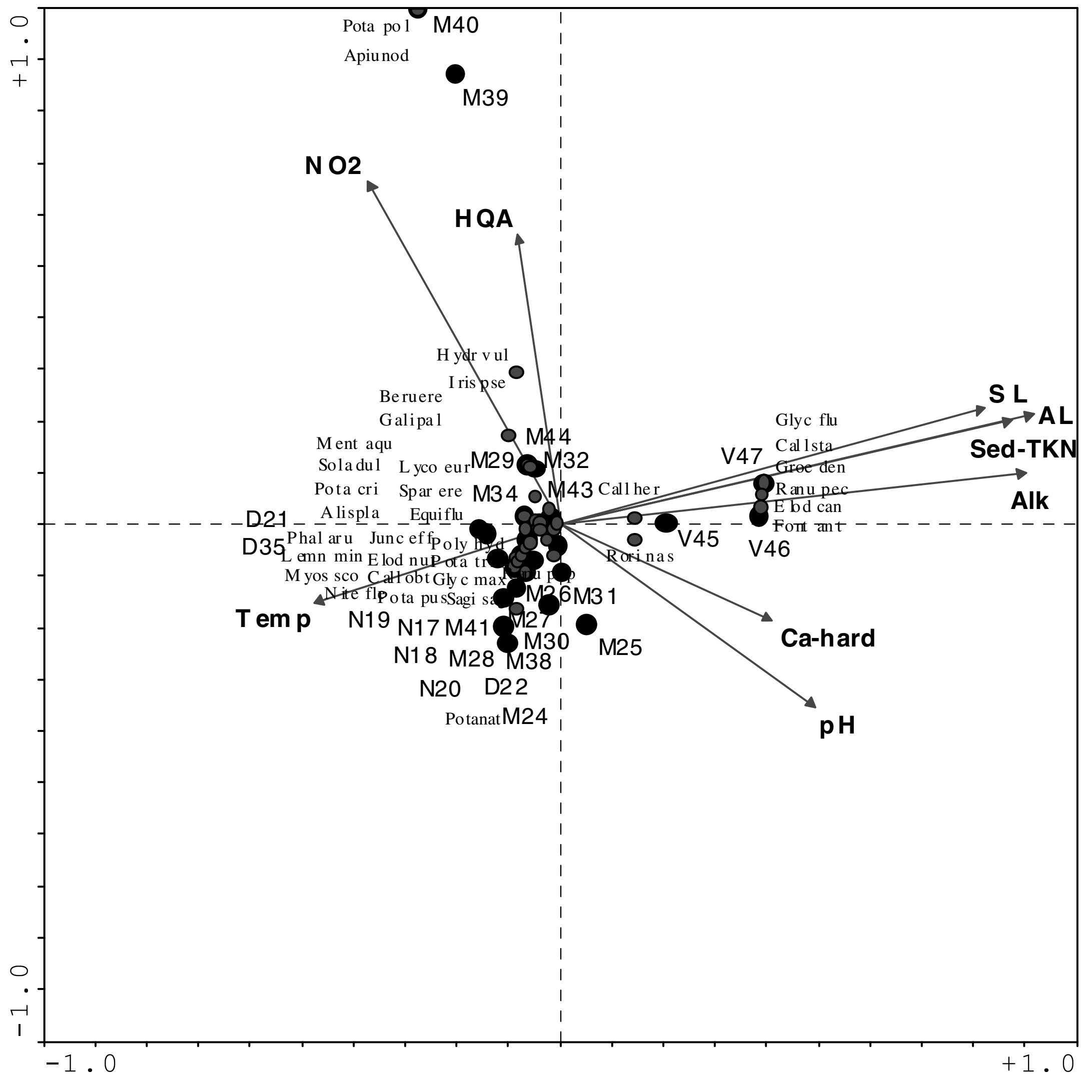

Figure 2. Canonical correspondence analysis on basis of macrophyte taxa and significant environmental variables. Fourteen percentage and 27% were explained by axis 1 and 2. (HQA = overall Habitat Quality Assessment of river area; NO_2 = nitrate; Temp = temperature; SL = slope, AL = altitude; Alk = alkalinity; Sed-TKN = Total Kjehldahl nitrogen in sediment, Ca-hard = Ca-hardness, pH). *: sites; ♦: species names. For plant species names, see Results and Discussion section, plus Alis pla = *Alisma plantago-aquatica*, Calli her = *Callitriche hermaphroditica*, Cerat dem = *Ceratophyllum demersum*, Elod can = *Elodea canadensis*, Elod nut = *Elodea nuttallii*, Font ant = *Fontinalis antipyretica*, Gali pal = *Galium palustre*, Hydr vul = *Hydrocotyle vulgaris*, Iri pse = *Iris pseudacorus*, Junc eff = *Juncus effusus*, Lemn min = *Lemna minor*, Ment aqu = *Mentha aquatica*, Nite fle = *Nitella flexilis*, Phala aru = *Phalaris arundinacea*, Pota nat = *Potamogeton natans*, Sola dul = *Solanum dulcamara*, Spar ere = *Sparganium erectum*.

rivers. Macrophytes were significantly correlated (Table 2) with a number of habitat variables (transect size, river width, slope, Huet zonation, distance to source, shadowing and average depth). For the substrate there are significant negative correlations with the fine particle sizes (SED-C = Vol.% clay < 2 μm as percentage of the fraction < 1 mm: SED−FL = Vol.% fine loam 2–16 μm as percentage of the fraction < 1 mm) and a positive correlation with the coarse particles (SED−S = Vol.% sand 250–500 μm as percentage of the fraction < 1 mm: SED−CS = Vol.% coarse sand 500–1000 μm as percentage of the fraction < 1 mm). In relation to the sediment features, there is a negative correlation with silicate. However, very few chemical variables showed significant correlations (chloride, conductivity and pH).

A DCA on basis of the abundance of all macrophyte taxa was considered. On basis of the resulting gradient length of 7.12 a unimodal regression was performed with the combination of chemical variables and habitat characteristics. Three major groupings were obtained with a CCA (Fig. 2): headwaters from sandy soils and more acidic waters (*Potamogeton polygonifolius* and *Apium nodiflorum*); headwaters from limestone rich areas (*Groenlandia densa*, *Fontinalis antipyretica*, *Ranunculus penicillatus* var. *calcareus*) and an undifferentiated group in waters of moderate pH and moderate nutrient levels.

The CCA on basis of macrophyte taxa and significant environmental variables resulted in 14 and 27% explained by axis 1 and 2. According to the first axis, the following variables explain most of the variation: SL = slope, AL = altitude; Alk = alkalinity; Sed-TKN = Total Kjehldahl nitrogen in sediment. According to the second axis the overall Habitat Quality Assessment of river area (HQA) and nitrite are explaining the variation significantly. According to this analysis, the sandy and more acidic headwaters in nature reserves (high HQA scores) are separated from the harder waters of the Voer (V45, V46, V47). All other headwaters remain as a third indifferent group.

Compared to other bio-indicator organisms, macrophyte indices were most related to the index of biotic integrity (fishes) and less with diatom indices of saprobity or macro-invertebrate based biotic indices. The latter differences were also found by Triest et al. (2001).

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SOLVING PROBLEMS?

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The prediction of macrophyte species occurrence in Swiss ponds

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Key words: aquatic plants, environmental variables, predictive models, small waterbodies, Switzerland

Abstract

The study attempted to model the abundance of aquatic plant species recorded in a range of ponds in Switzerland. A stratified sample of 80 ponds, distributed all over the country, provided input data for model development. Of the 154 species recorded, 45 were selected for modelling. A total of 14 environmental parameters were preselected as candidate explanatory variables. Two types of statistical tools were used to explore the data and to develop the predictive models: linear regression (LR) and generalized additive models (GAMs). Six LR species models had a reasonable predictive ability (30–50% of variance explained by the selected predictors). There was a gradient in the quality of the 45 GAM models. Ten species models exhibited both a good fit and statistical robustness: *Lemna minor, Phragmites australis, Lysimachia vulgaris, Galium palustre, Lysimachia nummularia, Iris pseudacorus, Lythrum salicaria, Lycopus europaeus, Phalaris arundinacea, Alisma plantago-aquatica, Schoenoplectus lacustris, Carex nigra.* Altitude appeared to be a key explanatory variable in most of the species models. In some cases, the degree to which the shore was shaded, connectivity between water bodies, pond area, mineral nitrogen levels, pond age, pond depth, and the extent of agriculture or pasture in the catchment were selected as additional explanatory variables. The species models demonstrated that it is possible to predict species abundance of aquatic macrophytes and that each species responded individually to distinct environmental variables.

Introduction

Hydrophytes play an important role in the freshwater ecosystem functioning of many shallow waterbodies: as primary producers, by providing structure in the habitat of many animal species, and provide shelter and food to invertebrates (e.g., Castella et al., 1984; Bänziger, 1998; Antoine, 2002) and fish (e.g., Rossier, 1995). The factors controlling the distribution of macrophytes in bodies of standing water have been investigated by many authors. The distribution of macrophytes is often related to water chemistry, especially as influenced by eutrophication (e.g., Lehmann & Lachavanne, 1999). The significance of parameters such as morphometry, water levels, perturbations and disturbances, composition of bottom substrates, land-use in the catchment and surroundings, connectivity between water bodies, as well as interactions with the fish fauna has also been demonstrated (e.g., Jupp & Spence, 1977; Rørslett, 1991; Wright et al., 1992; Bornette & Amoros, 1996; Lehmann et al., 1997; Lougheed et al., 2001; Oertli et al., 2002). Many of these studies are concerned with the relationships between species richness of aquatic macrophytes and environmental variables but few involve study of a high number of individual species (e.g., Heegaard et al., 2001; Bio et al., 2002).

The analysis of species–environment relationships is a central issue in ecology and provides the baseline information needed for habitat distribution modelling (see Scott et al., 2002). A wide range of models has been developed to cover aspects as diverse as biogeography, conservation biology and climate-change research (Guisan & Zimmermann, 2000). The development of predictive models for the occurrence of individual species is currently viewed as a way to increase the efficiency of habitat assessment and as tools for the management of endangered or invasive species (e.g., Lehmann et al., 2002; Overton et al., 2002). The species richness of different taxonomic groups of fauna and aquatic plants recorded in a range of 80 ponds in Switzerland was modelled with a set of environmental variables (Oertli et al., 2000). The selected model predicted that the species richness of aquatic plants involved altitude, area, mean depth and nitrogen concentration levels. Here, we explore the extent to which the abundance of individual aquatic plant species recorded in the ponds can be modelled. The task is to identify variables that have the highest explanatory potential. The results may aid pond managers in identification of parameters upon which they could act to enhance or limit certain species.

Study areas

A previously established inventory of 8000 ponds (Borgula et al., 1994) provided the baseline for a stratified sample of 80 ponds distributed fairly evenly with respect to altitude (210–2757 m.a.s.l), area (6–94000 m^2) and biogeographic regions (Jura, Swiss Plateau and Alps). Of these ponds 31 were known to have a natural origin with an age exceeding 4000 years (since the end of the last glaciation). The other 49 had various ages and were man-made (e.g., for gravel or clay extraction, fishing or nature conservation). The main pond characteristics are listed in Table 1.

Table 1. Mean values and ranges of preselected variables characterising the 80 ponds

	Variables		Units	Mean	Minimum	Maximum	Median
Local scale	Morphometry	Log10 (area)		3.31	0.78	4.98	3.26
		Area	m^2	8817	6	94346	1834
		Mean depth	cm	172	26	850	114.5
		Shoreline development[a]		1.50	1.02	3.27	1.34
	Physical and chemical variables	Water transparency	cm	43	4	60	51
		Conductivity	$\mu S\ cm^{-1}$	383	6.2	1367	396
		pH-class (1 = acid, 2 = neutral-basic)		1.9	1	2	2
		Eutrophic class P[b] (total P classes, according to Wetzel, 1983)		–	1	4	2
		Eutrophic class N[b] (Nmin classes, according to Wetzel, 1983)		–	1	4	2
	Others	Age	Years	–	1	>4000	100
		Altitude	m. asl	1008	210	2757	733
		Extent of shade cover by trees on the shoreline	%	3.1	1	6	3
Larger scale	Catchment area	Proportion of agriculture in the catchment area	%	30	0	100	7.5
	Surroundings	Fraction of the surroundings (within 50 m of the pond) forested	%	35	0	100	32
	Connectivity[c]	Within a radius of 1 km		3.22	0	7.72	4.06

[a] Ratio of the length of the shoreline to the circumference of a circle of area equal to that of the pond (Wetzel, 1983).

[b] Eutrophic class P and N: 1 = oligotrophic, 2 = mesotrophic, 3 = eutrophic, 4 = hypertrophic, Nmin = Inorganic nitrogen (sum of nitrate, nitrite and ammonia).

[c] Measure of pond isolation. This measure takes into account the number and size of ponds within a radius of 1000 m and their distance from the studied pond. Large values indicate low degrees of isolation.

Methods

Survey of flora

Using quadrat samples (0.5×0.5 m), floristic composition was assessed in the 80 ponds during the summer months (1996–1999). Quadrats were positioned in the water at 5 m intervals along transects perpendicular to the longest axis of each pond. Transects were located every 5 m for small ponds and every 20 m for large ponds. In each quadrat sample the abundance of submerged, floating-leaved and emergent macrophytes was assessed using five classes. The number of quadrats sampled was proportional to the pond surface. A mean abundance was calculated (sum of abundance in the quadrats/number of quadrats) for the species recorded in each pond. Mean species abundances were used as a response variable in the analyses. Plants considered here as aquatic are the 254 phanerogams listed in the highest humidity class (=5) of Landolt (1977): this includes true aquatics (species submerged or with floating leaves) and most of the emergents. To this "aquatic" species pool, a set of 22 of the most frequently recorded helophytes (listed by Landolt under class 4), Bryophyta and 8 taxa of Characeae were added. Species nomenclature follows Aeschimann & Heitz (1996) and Corillion (1975).

Environmental variables

In total, 14 environmental variables were used to characterise each pond (Table 1). These predictor variables were preselected according to their potential importance to aquatic vegetation.

Data analysis

Linear Regression (LR e.g., Draper & Smith, 1981) and Generalised Additive Models (GAM: Hastie & Tibshirani, 1990) were used to model the relationship between species and the environmental variables. Linear Regression is a very popular tool and is often used for modelling species occurrences and distributions. It estimates only one statistical parameter for each variable in the model, the slope, which is an advantage with small sample size. A stepwise procedure was used in the LR regression and the explanatory variables were selected using the Fischer test with a threshold of $p = 0.01$. This method relies on linear relationships between species and ecological variables. Nevertheless, species responses in their natural environment might differ from frequently assumed linear relationships. The Generalised Additive Model (Hastie & Tibshirani, 1990) is a more powerful tool because the non-parametric characteristics of the GAM allow modelling of any shape of response curve (e.g., sigmoid, plateau-shaped, etc.) without having to assume particular relationship between the dependent (plant species) and the independent variables (environmental variables). This technique has been used with success in many studies in the last decade (e.g. Austin & Meyers, 1996; Lehmann, 1998; Bio et al., 1998, 2002).

The quality of the models was evaluated through the explained deviance (D^2) and its stability by simple correlation (r_1) and five-fold cross-validation correlation (r_2). The sample was randomly split into five approximately equal-sized groups. Models were recalculated for four of these groups and validated on the fifth. The higher the r^2-value, the higher is the stability of the models. The GAMs were performed using S-PLUS (Mathsoft) and a set of functions developed for generalised regressions and spatial predictions (GRASP; Lehmann et al., 2002). A cubic-spline smoother was used as a function to smooth the environmental variables (X_j), with three degrees of freedom (d.f.). Mean species abundances were transformed in order to be able to use a quasi-binomial distribution (0 = absence; abundance 1 = 0.2; 2 = 0.4; 3 = 0.6; 4 = 0.8; 5 = 1.0). A forward stepwise procedure was used. The explanatory variables were selected using the Fischer test with a threshold of $p = 0.01$.

Results

Although the 80 ponds studied represented only a very small fraction of the number of ponds estimated to occur in Switzerland, 154 aquatic plant species were recorded, representing about 54% of the Swiss aquatic plant flora. Only 45 species occurred in more than 10 ponds. The majority of them were helophytes and a few species were floating or submerged macrophytes (*Chara globularis*, *C. vulgaris*, *Lemna minor*, *Nymphaea* *alba*, *Potamogeton alpinus, P. natans*, *P. pectinatus*, *P.* gr. *pusillus, Ranunculus trichophyllus*). Most of these species are considered as common and non-threatened in Switzerland. The most frequent plants recorded were *Phragmites australis* (51%), *Caltha palustris* (34%), *Lythrum salicaria* (34%), *Mentha aquatica* (34%), *Carex rostrata* (33%), *Typha latifolia* (33%) and *Carex nigra* (31%).

Models LR

The LR models for 45 individual species had explained variance (R^2) ranging from 0 to 51%. Only six species had 32–51% of the variance explained (Table 2). Altitude, the variable the most frequently selected in LR, was involved in five of the six species models and had a negative effect on the abundance of two species. The rise of pond area increased the abundance of *Phragmites australis* and *Lythrum salicaria* and the extent of shade cover of the shore had a negative effect on their abundance. High values of total phosphorus content in the water increased the abundance of *Juncus filiformis* and *Typha latifolia*. Some other variables were involved positively or negatively, depending of the species, in a few models.

Models GAM

There was a gradient in the quality of the Generalised Additive Models. *Calliergonella cuspidata*, *Carex flava*, *Equisetum palustre*, *Glyceria fluitans* and *Polygonum amphibium* could not be modelled with the environmental variables tested. In total, 40 species models had 16–99% of total deviance in abundance explained and a cross-validation coefficient r_2 varying between 0 and 0.82. A set of 10 models had 87–99% deviance explained and very high simple-validation coefficients. These models included many predictor variables ("over-fitting") and have very low confidence (cross-validation coefficients). *Chara vulgaris*, *Nymphaea alba*, *Potamogeton alpinus* and *P. pectinatus* for example belong to this group and were modelled with low accuracy. Models incorporating one (generally altitude) or two variables had generally low deviance in abundance explained (*Chara globularis*, *Potamogeton* gr. *pusillus* or *Mentha aquatica* for instance). Ten models (Table 3), including three to five variables, showed a high degree of explained variation (more than 50% of the deviance explained) and relatively high stability (cross-validation above 0.46). Five other models were close to these arbitrary limits (*Alisma plantago-aquatica*, *Lycopus europaeus*, *Lysimachia vulgaris*, *Potamogeton natans* and *Typha latifolia*).

In the GAMs, altitude was involved in 90% of the best models and was the main explanatory variable for the majority of the species (Table 3). The extent of shade on the shore and connectivity were concerned with more than half of the models. Area, mineral nitrogen and total phosphorus content of the water, and pond age were involved in about one-third of the models. Shore sinuosity and pH were not included in the best species models.

Table 2. Standardised contribution coefficients of LR selected explanatory variables to the six best models (more than 30% explained variance ($p < 0.05$))

	Freq %	Alt	Area	Shore shade	Ptot	Age	Shore dev	pH	Nvar	R^2
Carex nigra	25	0.72							1	0.51
Phragmites australis	41	−0.64	0.34	−0.26			0.19		4	0.49
Juncus filiformis	12	0.47			0.29			−0.30	3	0.37
Lythrum salicaria	27	−0.65	0.28	−0.42					3	0.37
Eriophorum angustifolium	10	0.63						0.29	2	0.35
Typha latifolia	26				0.25	−0.51	−0.28		3	0.32

alt: altitude; area: $\log_{10}$(area); shore shade-class: percentage of the shore shaded; shore dev: shoreline sinuosity; Ptot: eutrophication class P; age: age-class; shore dev: shore sinuosity index. Number of selected predictors in species model (Nvar), percentage of explained variance (R^2).

Table 3. Contribution of GAMs selected explanatory variables to the 10 most accurate models and validation diagnostic

Species	Freq %	Alt	Area	Depth	Shore shade	Nmin	Ptot	Connect	Agri catch	Forest env	Age	Cond	Trans	Nvar in GAM	Null dev	Resid dev	%D	r_1	r_2
Lemna minor	26	10.9		4.3	4.3			5.7						4	49.5	22.9	54	0.74	0.46
Phragmites australis	51	18.3	9.4		6.8						4.5			4	75.2	33.0	56	0.79	0.67
Galium palustre	28	6.8				7.3	3.5							3	30.3	11.9	61	0.83	0.67
Lysimachia nummularia	20	3.2				3.1	1.7	1.7						4	20.1	7.4	63	0.75	0.55
Iris pseudacorus	29				3.7	3.6				3.6	1.9			4	30.5	10.6	65	0.80	0.59
Lythrum salicaria	34	7.4	3.2		3.5				2.0					4	24.0	8.1	66	0.83	0.62
Phalaris arundinacea	28	7.2	2.0		4.8		4.5	2.5						5	35.9	9.6	73	0.90	0.60
Schoenoplectus lacustris	18	18.9		9.5					4.5	9.2	3.5			5	45.1	9.6	79	0.92	0.66
Carex nigra	31	10.6			3.8			2.6				2.0		4	46.2	9.3	80	0.90	0.72
Eriophorum angustifolium	13	7.5						2.5					1.6	3	17.8	3.4	81	0.94	0.82

Number of selected predictors in species model (Nvar in GAM), null deviance (null dev), residual deviance (resid dev), percentage of explained deviance (% D), simple validation coefficient (r_1) and cross-validation coefficient (r_2). All models were selected with threshold $p < 0.01$.

alt: altitude; area: $\log_{10}$(area); depth: mean depth (m); shore shade-class: percentage of the shore shaded; Nmin: eutrophic class N; Ptot: eutrophic class P; connect: connectivity within a radius of 1 km; agri catch: agricultural catchment; forested env: % of forested surface in the close surrounding of the pond (50 m); age: age-class; cond: conductivity; trans: transparency of the water.

Comparison of the Phragmites australis *models*

Four variables were included in the LR and GAM models. They explained respectively 49% of the variance and 56% of the deviance. Three variables were common to both models: altitude, size and shade and gave the same level of contribution. Age, the fourth and the least contributive variable in the GAM was not integrated in the LR. In contrast, the shore development was included in the LR but not in the GAM. The response curves in the GAM showed that relationships between *Phragmites australis* abundance and the four variables selected were not linear but fitted plateau-shape or sigmoid curves. This close fit to the data explain why GAMs gave more information than LRs and included different variables, although the main contributive variables were the same in both models. The GAM model indicated that high abundance of *Phragmites australis* would occur in big ponds older than 40 years, located at low altitude and with low shade on the shore.

Discussion

Linear regression gave relatively low prediction of species abundance, with only six models explaining more than 30% of the variance. As expected, GAMs described and quantified better than LR the relation between individual plant species and characteristics of their habitat. Indeed, a higher number of species were successfully modelled by GAMs and the four species modelled by both methods had better accuracy with GAMs than LRs. GAMs can produce accurate models, as revealed by the good fits and the high cross-validation coefficients. The best models are the ones that simultaneously account for the most variation in the data with the fewest terms (Burnham & Anderson, 1998). Ten models showed a high level of explanation of species abundance (>50% explained deviance) including two to five variables and high stability (cross-validation >0.4). Four other models were close to these arbitrary limits (*Lycopus europaeus*, *Lysimachia vulgaris*, *Potamogeton natans* and *Typha latifolia*) and could be improved with additional data. Species with lower frequencies were usually unsuccessfully modelled and the chance for a species to be modelled with accuracy increased with its frequency. However, species like *Mentha aquatica* and *Caltha palustris* (present in 34% of ponds) failed to be modelled with accuracy. The reasons might be insufficient occurrences in each abundance class (*C. palustris* is present only at low densities) and/or lack of sensitivity of the species to the environmental variables tested in this study.

As the models were cross-validated they do not need to be re-evaluated by an independent dataset. A cross-validation on five subsets is like the sum of five independent evaluations but has the advantage of using, in the final model, the entire information available.

According to Stockwell & Peterson (2002), the number of ponds sampled in this study (80) was reasonable enough to ensure the accuracy of prediction. These authors explored sample size needs for accurate modelling for three predictive modelling methods and found that logistic regression (data in presence/absence) can develop accurate models based on about 50–100 samples. Moreover, abundance data contain more information than presence/absence data so our models based on 80 units should be more accurate than those based on occurrence data only.

Each species responded individually to distinct combination of environmental variables. Species reacted to local characteristics of their habitat (nitrogen, phosphorus, conductivity, water transparency, pond age, pond area, depth of the water, shade cover and sinuosity of the shore) but also to variables performing at other spatial scales (altitude, land-use in the surroundings and in the catchment area and pond connectivity). Altitude appeared as a key explanatory variable for most species and was generally the variable contributing most in the models. This result is not surprising because altitude summarises climatic components (temperature, light, precipitation), known to be major factors for plant growth.

Statistically good models tell us that the variables selected in the final model are related to the abundance of the species but cannot prove that the models are ecologically significant. The results showed that the model of *Phragmites australis* abundance was valid statistically. Examination of the response curves revealed that the model was also quite ecologically significant. For the other species statistically accurately modelled, the ecological influence of some of the selected variables must still be validated. Therefore, expert knowledge remains essential to analyse the ecological pertinence of the model.

Conclusion

The species models generated demonstrate that it is possible to predict species abundance of aquatic macrophytes. GAMs were able to produce statistically valid response models for 11 species. Species with robust models are among the most frequent plants, but generally it is the most rare species that give rise to conservation concerns. In that context it would be desirable to produce models to manage endangered or invasive species. Two species threatened in Switzerland were present in our data set but none was significantly explained by the variables tested (*Nymphaea alba* (18%) and *Potamogeton* gr. *pusillus* (*P. pusillus* and *P. berchtoldii*) (16%)) (Moser et al., 2002). Reliable models for species like *Phragmites australis* and *Lythrum salicaria* or *Myriophyllum spicatum* would also help the development of strategies to control these plants, native in Europe but behaving as invasive in North America (e.g. Buchan & Padilla, 2000, Blossey et al., 2001; Tewksbury et al., 2002). Statistical tools like GAMs show considerable potential in construction of models for application in the management of the habitat of particular species. However, in the absence of observational data, expert knowledge can be used to predict species occurrence and abundance (e.g., Keddy, 2000; Willby et al., 2000).

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Risk assessment method for submerged weeds in New Zealand hydroelectric lakes

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Key words: aquatic plants, weed potential, risk assessment, weed management

Abstract

The latent potential for problematic weed growth in a hydro-lake is proportional to water level fluctuation, water clarity, lake shape, littoral gradient and exposure to wave action. These five factors were used to assess a score for measuring the latent potential for weed impact and each factor was allocated a score of 1–5, with a theoretical maximum of 25. Any hydro-lake scoring above 15 could be expected to present potential inconvenience to power generation, given the presence of suitable submerged weed species. Assessment of potential impact arising from weed invasion must firstly determine what species are already established, and whether it is possible for a weed species of greater potential impact to be introduced. The risk of this occurring is dependent upon a number of factors, including public accessibility, proximity to sources of those species and the desirability of the waterbody to potential weed vectors. Once these factors are quantified, appropriate surveillance strategies in high-risk hydro-lakes may then be developed.

Introduction

New Zealand has an abundance of rivers, lakes and an oceanic climate providing year-round moderate rainfall. These have all contributed to the development of a network of hydroelectric lakes that supply around 80% of the nation's electricity. The potential for any new hydro-lake to create suitable habitat for weed growth has often received only cursory regard, insufficient to warrant modification of station design prior to lake filling. Nearly all weed problems at power stations over the last 30 years or more can now be attributed to species that are not native to New Zealand. These weeds have resulted in world record biomass, significant loss of generation potential, and environmental and utilitarian impacts (Clayton, 1982, 1996; Howard-Williams et al., 1987). In order to assist power station managers to better appreciate the level of risk posed by weeds for their hydroelectric lakes, a simple largely subjective scoring system was devised.

Unlike many tropical or subtropical countries floating plant species such as salvinia (*Salvinia molesta* D. Mitch.) and water hyacinth (*Eichhornia crassipes* (Mart.) Solms-Laub.) do not thrive in New Zealand on account of the cool temperate climate. In addition, an effective national weed management strategy has virtually eliminated these two species from public waterbodies where they have previously occurred.

Erect marginal species (e.g., phragmites (*Phragmites australis* (Cav.) Steudel) and Manchurian wild rice (*Zizania latifolia* (Griseb.) Stapf)), and sprawling species (e.g. alligator weed (*Alternanthera philoxeroides* (C. Mart.) Griseb.), reed sweet-grass (*Glyceria maxima* (Hartman) Holmb.), parrot's feather (*Myriophyllum aquaticum* (Vell.) Verdc.), watercress (*Nasturtium officinale* R. Br.) and primrose willow (*Ludwigia* *peploides* (Kunth) Raven)) potentially pose a weed management problem for small hydro-lakes. However, lake size and wave action result in limited suitable habitat apart from protected backwaters and sheltered embayments. Overall, these plant species tend not to be a problem to hydro-lake managers and are unlikely to become problematic in the foreseeable future.

Submerged aquatic plants have the most immediate and future relevance to New Zealand hydro-lake operations. Historically, we had no tall-growing, canopy-forming submerged native plant species and this has allowed a range of introduced species with alternative growth strategies to displace many native communities (Howard-Williams et al., 1987). *Elodea canadensis* Michaux and *Ranunculus trichophyllus* Chaix, two of the earliest aquatic plant arrivals, are widespread and now close to reaching their full potential impact. Fortunately they present minimal concern to managers of hydro-lakes, since they grow less dense and are less competitive than other less widely distributed weed species. *Egeria densa* Planchon, *Lagarosiphon major* (Ridley) Moss and *Ceratophyllum demersum* L. (hornwort) have proven to be the most problematic weed species on account of their substantial surface-reaching growth forms. *C. demersum* was the first weed species to temporarily close down a hydro-electric power station in 1965 (Widgery, 1967) and this same species continues to dominate in many North Island hydro-lakes. These species can tolerant low water clarity and can survive ice-cover during winter (Haramoto & Ikusima, 1988; Spencer & Wetzel, 1993). Hornwort does not produce roots so it is readily dislodged and can be found impaled on submerged obstacles, such as drowned or fallen trees or caught on hydro-lake booms and screen intakes, where it will keep growing until removed. Waikato hydro-lakes (a chain of eight lakes on the longest river in the North Island) have from 10 to 53% of their littoral margin supporting submerged weed growth. The larger the area of lake and the higher the proportion of vegetated area, the greater the likelihood of a weed problem. Nevertheless, the variation between stations on the same river system is considerable and the quantity of weed removed from any one station can differ significantly from year to year. For example, one hydro-lake (Whakamaru) in one year had 7286 m^3 of weed removed from the station intakes during four Autumn months of peak efflux, yet during the following 2 years only 728 m^3 and 1,259 m^3 were removed for the same period. Other potential weed species in hydroelectric lakes include *Hydrilla verticillata* (L.f.) Royle, currently restricted to one geographically isolated region, and *Myriophyllum spicatum* L., which fortunately has not yet made an appearance in New Zealand.

Methodology

Aquatic plants will only grow where they have access to light, nutrients and in most cases a suitable substrate for anchorage. The following factors collectively determine the vulnerability or latent potential of any waterbody to support problematic weed growths if invaded by a submerged weed species.

Water level fluctuation

Hydro-lakes with large routine (e.g. daily) or annual fluctuations in water level tend to support significantly less submerged plant growth compared to waterbodies with small fluctuations; however, disturbance tolerant species such as *C. demersum* and *Najas marina* L. can persist and show rapid recovery following changes in water level (Springuel & Murphy, 1991; Ali et al., 1995). In the 1970s, routine water level fluctuations of c. 5 m were used to avert nuisance growths of weeds on the Waikato River hydro-lakes. In these lakes problematic surface-reaching growths are normally restricted to water <4 m depth, while sub-surface weed beds may still occur between 4–10 m depth. If water level fluctuation exceeds the 5–6 m depth range then nuisance weed growth would normally be avoided, even when clear water may still permit significant growth of non-vascular species in deeper water (e.g. Lake Hawea). Alternatively, where fluctuations exceed the plant photic zone there will be no vegetation present (e.g. Cobb Reservoir). Small ranges of frequent water level fluctuation (2–3 m) can increase habitat diversity by creating an amphibious zone that favours either emergent species or prostrate turf-forming species that are tolerant of periodic exposure and immersion.

Water clarity

The depth of water that plants can grow in is directly related to water clarity. Glacial fed lakes (Lake Pukaki and Tekapo) can have high inorganic turbidity from fine colloidal glacial flour that severely restrict light penetration into water thereby preventing any weed growth in the lakes or their interconnecting canals. Lakes with low inorganic suspensoids, high fertility and long residence times typically inhibit submerged weed beds by supporting dense phytoplankton populations, but these pose no problem to power generation since microscopic plants pass through intakes, turbines and cooling pumps without blockage. Dissolved colour from plant tannins can also reduce light available for submerged weed growth either from natural catchment inputs (e.g. forested catchments) or from industrial discharges (e.g. pulp and paper mills). Although this can help to reduce weed problems, the effect of low water clarity can be partially mitigated by species such as hornwort that are tolerant of low light conditions.

Shape of hydro-lake shoreline

Size and shape of a hydro-lake will affect the extent of weed growth and the likelihood of dislodgment from prevailing wind direction, wind fetch and wave action. Weed growth in natural lakes used for power generation is often less prone to interfere with power generation since uprooted plants can be deposited in deep water or along the lake shoreline, rather than being drawn into station intakes. A hydro-lake in a flooded channelised valley will often have its long axis in line with the siting of the power station, so that wind and water movement will effectively channel dislodged weed directly towards station intakes, with little opportunity for shoreline stranding or deposition. In such cases, weed growth may still present a management problem even if the area of growth only represents a small area relative to open water. Station operation and design can partially mitigate the severity of weed problems experienced.

Littoral gradient and substrate

Steep shoreline gradients reduce the habitat available for weed growth. For example, two hydroelectric lakes (Matahina and Aniwhenua) in close proximity to each other on the same river (Rangitaiki) have the same weed species (*C. demersum*), but with widely different weed problems. One lake (Matahina) has littoral margins with mostly steep gradients and minimal sediment accumulation, while upstream the other lake (Aniwhenua) has shallow shoreline gradients with significant habitat for problematic weed growth.

Exposure to wave action

Exposure is primarily a function of lake size. Howard-Williams & Davies (1988) reported that where wave fetch perpendicular to shorelines exceeded 4 km only non-nuisance growths of submerged species occur, such as scattered patches and clumps <1 m in height. As wave fetch reduces, the risk of weed bed uprooting from periodic storm events increases. This is particularly relevant for shoreline areas close to power station intakes if they provide suitable habitat for weed growth.

Using the above factors it is possible to generate a score of the potential impact from submerged weeds within New Zealand hydro-lakes. The following factors are considered on a 1–5 scale, where 1 represents minimal risk of impact and 5 represents maximum impact. Although the scores have a subjective element, they have the advantage of being simple to understand and apply without the aid of specialist instrumentation or scientific expertise. These scores describe the latent potential for weed impact based on the physical characteristics of the waterbody.

A. *Level fluctuation*: Score = 1 (Fluctuation > 6 m); Score = 2 (Fluctuation 4–6 m); Score = 3 (Fluctuation 2–4 m); Score = 4 (Fluctuation 1–2 m); Score = 5 (Fluctuation < 1 m)
B. *Water clarity*: Score = 1 (Turbid – Secchi < 0.5 m); Score = 2 (Secchi 0.5–2 m); Score = 3 (Secchi 2–3.5 m); Score = 4 (Secchi 3.5–5 m); Score = 5 (Clear water – Secchi > 5 m)
C. *Shoreline shape*: Score = 1 (Open lake); Score = 3 (Relatively open lake with outlet receiving prevailing winds); Score = 5 (Channelised)

D. *Littoral gradient and substrate*: Score = 1 (Steep, rocky gradients); Score = 3 (Moderate gradients with sand/silt sediments); Score = 5 (Shallow shelving shoreline with silty sediments)

E. *Exposure to wave action*: Score = 1 (Prevailing wave fetch > 4 km over 50% of shoreline); Score = 3 (Prevailing wave fetch < 4 km); Score = 5 (Sheltered small lake < 1 km max dimension, low wind/wave impact).

Any hydro-lake scoring 2 or less in either factors A or B (water level fluctuation and water clarity) would have no potential submerged weed problem, since conditions would not be suitable for any significant growth irrespective of how high any of the other factors (C–E) scored. For example, Tekapo, Pukaki, Ohau and Benmore hydro-lakes (Table 1) present no significant risk from submerged weed species. All of these lakes are strongly influenced by glacial melt-water, which maintains extended periods of turbid and highly reflective water. Even if they have minimal water-level fluctuations (e.g. Ohau) turbid water would inhibit submerged weed growth.

Any hydro-lake scoring > 15 has the potential to support significant growths of submerged weeds and could present some inconvenience to power generation, given the presence of suitable submerged weed species. Manapouri has a stable water level (score = 5) and good water clarity (score = 5) with *E. canadensis* growing to 9 m depth, but other features (C–E) restrict the potential for problematic weed growth (score = 13). Even if *C. demersum* were to invade any impact is likely to be localised and of no concern to station operation. On the other hand, this same species could still have a significant ecological impact by displacing native vegetation in deeper water and reducing biodiversity.

Assessment of current weed status

Lakes can be characterised based on existing weed species and their potential for more problematic species to invade. We have developed an Aquatic Weed Risk Model (Champion & Clayton, 2001) specifically for aquatic species that can be used to assess the severity of potential impact caused by a species. This assessment is specific to each country based on environmental conditions in which those species would grow and the management activities that they are likely to impact. For example, *C. demersum* is rated as one of the worst submerged weed species in New Zealand, yet it would score lowly as a nuisance weed species in USA where it is an indigenous species that rarely causes problems. A similar situation occurs with *H. verticillata* which ranks as the worst submerged species in

Table 1. Score of suitability of habitat and growth conditions (latent potential) for submerged weeds in selected New Zealand hydro-lakes

LAKES	A	B	C	D	E	TOTAL	Potential Problem
Tekapo (L)	2	1	–	–	–	–	No
Pukaki (L)	2	1	–	–	–	–	No
Ohau (L)	4	2	–	–	–	–	No
Benmore (R)	3	1	–	–	–	–	No
Waitaki (R)	3	2	–	–	–	–	No
Manapouri (L)	5	5	1	1	1	13	Yes
Matahina (R)	4	3	4	2	5	18	Yes
Dunstan (R)	5	5	3	3	3	19	Yes
Karapiro (R)	4	3	5	3	5	20	Yes
Whakamaru (R)	4	3	5	5	5	22	Yes
Aniwhenua (R)	5	3	4	5	5	22	Yes

A: level fluctuation; B: water clarity; C: shoreline shape; D: littoral gradient; E: exposure to wave action.
Lakes – L: natural lake; R: dammed river valley.

New Zealand, and would probably also be so in USA, however in Australia, where this species is native, its growth is rather benign.

Based on the Aquatic Weed Risk Model (Champion & Clayton, 2001) the following weed species are ranked in order of severity (low to high) of known nuisance value to hydroelectric power generation for New Zealand conditions (N.B., although the Champion & Clayton (2001) Weed Risk Model ranks *H. verticillata* higher than *C. demersum* due to its currently restricted distribution, production of perennating structures and competitive ability in still water, in this paper *C. demersum* is scored higher for nuisance value on account of its propensity to fragment and its ability to form detached floating mats that threaten hydroelectric power generation activities). A 10-point scale is used to provide a relative estimate of the impact potential using examples of naturalised invasive species.

Score = 1 (*Juncus bulbosus* L.); Score = 2 (*Potamogeton crispus* L., *Ranunculus trichophyllus*); Score = 5 (*Elodea canadensis*); Score = 6 (*Lagarosiphon major*, *Egeria densa*); Score = 7 (*Vallisneria* spp); Score = 8 (*Hydrilla verticillata*); Score = 10 (*Ceratophyllum demersum*)

Lakes with the highest ranked weed species (*C. demersum*) have already realised their full potential for weed problem expression and there is no merit in undertaking a Weed Introduction Risk Assessment. On the other hand, any lakes with lower ranked weed species can be prioritised using the Weed Introduction Risk Model based on the probability of a worse weed being introduced.

Discussion

Assessment for potential risk is based on the assumption that lakes do not yet have the worst possible weeds established. Any lake already scoring > 20 (Latent Impact score + Weed Status score) is likely to have an existing weed problem; however the maximum potential score may increase should a more problematic species establish. Thus the maximum potential score for a lake = Latent Impact score + 10 (based on the assumption that *C. demersum* is present). The difference between this and the actual score represents the additional risk posed by weed species not yet introduced into the lake.

There are many mechanisms by which weed species can spread between water bodies. These include natural spread (e.g. water currents, wind-borne seed, wildlife), recreational craft (especially boats and trailers), fishers/nets, diggers/harvesters, deliberate planting, accidental escape (e.g. ornamental pond overflow) and aquarium release. The most problematic submerged weeds reproduce asexually, so spread is primarily human-mediated movement of plant fragments. The risk of transfer of these propagules to an unimpacted hydro-lake is therefore essentially the probability that one or more of the vectors noted above will move plant fragments from an infested water body to the unimpacted lake. The variables to be considered are; firstly, the proximity of sites with more problematic weed species in relation to the unimpacted lake; secondly, how accessible the weed site(s) and unimpacted lake are to the potential vector; and thirdly, how desirable the weed site(s) and unimpacted lake are to the potential vector (e.g. are both waterbodies well stocked with eels that may be fished by commercial and recreational fishers).

In each case the probability of introduction can be assessed as high or low. For naturalised aquatic weed species, knowing their geographical distribution and their primary mechanism(s) of spread can be used to assess the likely risk of introduction to a new catchment or waterbody within a catchment, as well as provide a basis for considering suitable preventative strategies.

Conclusion

The risk posed by aquatic weeds to hydro-lakes should be considered individually with a management strategy determined for each lake. Hydro-lakes can be ranked in priority order, based on the their potential for problematic weed growth. Any lakes with a Latent Impact score below 15 should be considered of low or minimal risk and no further management response strategy is likely to be required. For lakes scoring above 15, these should be prioritised in relation to their score, with an appropriate weed introduction risk assessment undertaken and a management strategy developed.

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Predicting interactions between wetland vegetation and the soil-water and surface-water environment using diversity, abundance and attribute values

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Abstract

This study investigated the response of freshwater wetland vegetation to hydrological driving factors by assessing collective vegetation variables, traits of dominant plant populations and hydrological and hydrochemical variables, repeat-sampled within wetland sites across Scotland and northern England. Sampling was conducted at 55 permanent sample stations located along 11 independent transects. Eco-hydrological interactions were investigated using a regression-based modelling approach. Facets of the water-table dynamic (e.g., level of drawdown, level of fluctuation), along with vegetation abundance (e.g., biomass, stem density) and diversity (e.g., species richness) values, were used to build predictive models. Of the models predicting vegetation characteristics, the greatest predictive power was $R^2 = 0.67$ ($p < 0.001$) for a model predicting stem density (m^{-2}). Conversely, vegetation variables proved useful for predicting characteristics of the water-table environment. In this instance, the greatest predictive power was $R^2 = 0.79$ ($p < 0.001$) for a model predicting minimum water table level (i.e. maximum level of drawdown). The models were tested using data collected during 2000 from repeat sites and independent sites. This approach might be successfully applied for the purposes of integrated eco-hydrological management and monitoring of freshwater wetland vegetation.

Classical approaches to vegetation classification are important for understanding and managing various habitats, freshwater wetlands included (e.g. Denny, 1985). However, floristic gradations in wetlands are often complex, making community separation difficult. The limitations of classical methods in monitoring temporal changes in plant communities are also well known (e.g. Rodwell, 1991).

Keddy (1992a) stated that "assembly rules provide one possible unifying framework for community ecology", and that with the environment acting as a filter for certain traits (or combinations of traits), principles should be generally applicable to systems with differing taxonomies. Also, that "the need for general predictive models grows" as "ecology matures, and the world's environmental problems continue to multiply" (Keddy, 1992b).

Functional classifications (e.g. Grime et al., 1988; Keddy, 1992b) have been successfully applied (e.g. Diaz et al., 1998; see also Duckworth et al. 2000 for a recent review). Examples exist for wetland and aquatic ecosystems (e.g. Hills et al., 1994; Willby et al., 1997; Daoust & Childers, 1998; Ali et al., 1999). Such approaches have potential for use as components of tools for environmental monitoring (e.g. Murphy et al., 1994; 2003; Kennedy & Murphy, 2003).

Wheeler & Giller (1982) described predictive relationships between species richness and attributes of above-ground plant material in fen systems of the Norfolk Broads, England. The findings generally supported the idea that increases in biomass are often associated with a reduction of species density. Later work (Wheeler & Shaw,

1991) produced similar findings over a wider geographical area (lowland England and Wales). Willby et al. (1998) developed a minimal linear model incorporating plant community attribute measures to predict plant α-diversity in Scottish riverine floodplain wetlands. The model has good predictive power ($R^2 > 0.9$), but due to the large number of predictors used, this is within a strictly limited envelope of applicability. Similarly, Murphy et al. (2003) identified environmental predictors of three primary aquatic vegetation parameters (assemblage, α-diversity, and abundance) in the *varzea* plant communities of the Upper Rio Paraná, Brazil. This study also evaluated functional attributes of the vegetation itself as qualitative markers, or quantitative predictors, of these parameters for modeling purposes.

The main aims of this study were to: (i) provide a series of general and specific predictive equations describing eco-hydrological relationships within the wetland vegetation studied, and (ii) test the predictive capacity of these models using test data from independent and repeat sample stations.

Materials and methods

Seven sites were sampled. These included (with UK Grid references for transect start and finish points: T): Glen Moss, Central Scotland (T1: NS367696-368698; T2: NS366697-366699); Insh Marshes Northern Scotland (Invertromie Fen (T1: NH812023-805029), Insh Fen (T2: NH775001-774005) and Balavil Fen (T3: NH793022-796019)); Lochwinnoch (Aird Meadow), Central Scotland (T1: NS364585-365587; T2: NS361585-362585); Nether Whitlaw Moss, South East Scotland (T: NT506294-511295); Tarn Moss, Northern England (T: NY398274-402276). Fixed stations were established at intervals of approximately 30-50 m along each transect and were visited monthly during May–August 1999. This was repeated during 2000 at Insh Fen, and two new sites: Endrick Marshes, Central Scotland (T: NS438873-438877); Wood of Cree, South West Scotland (T: NX375719-376717). In total 55 stations along 11 transects were monitored.

Water level range gauges (see Bragg et al., 1994) were used to measure minimum and maximum water levels relative to ground surface during periods between sampling, and water levels were measured using dipwells installed alongside the gauges. pH and electrical conductivity (μS/cm) were measured using Hanna meters. Soil redox potential (mV) was measured at a sub-soil depth of 2–3 cm using a Hanna meter with a self-referencing platinum electrode probe. Mixed water samples were taken from the dipwells. These were filtered through 0.5 μm Whatman GF/C glass fibre filters to remove suspended materials. Cl, F, NO_3^-, and SO_4^{2-} were determined from sub-samples using a DIONEX ion chromatograph. Samples were eluted with sodium hydroxide ($Na_2CO_3/NaHCO_3$) solution and conductivity was suppressed with dilute sulphuric acid (H_2SO_4). Detection limits were 0.02 mg l^{-1}. K and Na were determined using flame photometry. Ca, Mg and Mn were determined using flame atomic absorption spectrometry (AAS); samples were dosed with strontium nitrate ($Sr(NO_3)_2$) solution (0.4%) to suppress interference for Ca and Mg. Fe was determined by graphite furnace AAS. Samples were diluted where appropriate and the detection limit was 0.01 mg l^{-1}. PO_4^{2-} was determined during 2000 using the method of Murphy & Riley (1962), to 0.01 mg l^{-1}.

Species presence and abundance was recorded at each visit using a 1 m^2 quadrat, within a 1 m radius of each station. Nomenclature followed Stace (1997), Smith (1978) and Watson (1994). Dominant species (a maximum of three) were recorded as those with an abundance of ≥65%.

For each dominant species, canopy area was assessed using a transparent 10 cm by 10 cm acetate sheet, marked with a 1 cm grid; a score, to a maximum of 100%, was noted where the grid intersections projected down onto the dominant species. Number of leaves was also recorded for three random ramets. These ramets were then oven-dried at 60 °C for 1 week. Stem, leaf, and reproductive structure total biomass were recorded. For the whole species assemblage, stem and reproductive structure density per m^2 were estimated from the average of three random counts, conducted using a 10 cm by 10 cm quadrat. Nearest neighbour distance was measured between three random pairs of stems and scored on a 1–5 scale ($1 = 0$–2 cm; $2 = 2.1$–4 cm; $3 = 4.1$–6 cm; $4 = 6.1$–8 cm; $5 \geq 8$ cm). Total biomass was estimated per m^2 from a sample harvested at ground level within a 10 cm by 10 cm quadrat. Necromass was removed, and the sample oven-dried. Monthly measurements were made from different areas within a 1 m radius of fixed sampling stations due to the destructive nature of the sampling.

Models were constructed from the 1999 dataset. The response curve of each variable (i.e. linear, cubic, quadratic) was assessed in relation to independent variables using SPSS 9.0. Stepwise regressions were then conducted, starting with full models. Variables were culled where they offered no contribution to the model. Predicted scores were compared with observed values by calculation of the product-moment correlation coefficient, and residuals were checked for normality by the construction of normal probability plots. Models were tested using data collected in 2000 from independent sites and repeat sites.

Results

Species richness (S) was strongly predicted ($R^2 = 0.64$; $p < 0.001$: Equation 1 – Table 1) from five predictor variables. Environmental predictors were redox potential (RED) and Cl content of the water (CL). The cubic function of both variables suggests they increased initially relative to increasing species richness, decreased, and then increased again. Stem density (STDE) increased linearly relative to increased species richness, whilst dominant species reproductive structures dry weight ($\log_e$RamDWR) appeared to decrease linearly. Number of leaves per ramet ($\log_e$RamLV) took a quadratic function, suggesting an initial decrease followed by an increase in the values relative to increasing species richness.

The model was relatively specific due to the large number of predictor variables employed, and would therefore be expected to have a limited envelope of applicability. Using the entire 2000 test data set, two values (Insh Marshes 7 and 8) were poorly predicted. The correlation between predicted and observed values was low ($r = 0.08$) as a result (Table 2). However, due to a general drying of the stations along Insh Marsh transect 1 (Kennedy, 2001), several highly oxidising redox values were recorded. Therefore, samples with redox values falling outwith the parameters of the model construction data were removed from the analysis. The correlation between observed and predicted values was slightly increased ($r = 0.19$). In addition, values for Wood of Cree stations 1–5 were generally under-predicted. This may be due to the species rich nature of the site (see Kennedy, 2001), coupled with other variable values which are generally more comparable to the other sites sampled. Removal of these samples (Table 2) increased the correlation between predicted and observed values ($r = 0.49$). Values for the independent Endrick Marsh sites were well predicted, suggesting a more general applicability of the model within a poor fen wetland type.

The model predicting stem density ($\log_e$STDE) used fewer predictor variables and had a predictive power of $R^2 = 0.55$ ($p < 0.001$: Equation 2 – Table 1). Average water level had a quadratic response expressed relative to increasing stem density, with an initial decrease followed by an increase (i.e. greatest stem density in wetter sites). Level of water table fluctuation was expressed as the opposite of this (with an initial increase relative to increasing stem density). The relationship between pH and stem density was linear, with increasing stem density as water samples became less acidic. When tested, the model gave a relatively good correlation ($r = 0.44$) between predicted and observed values (Tables 1 and 2).

Average water table level relative to ground surface level (WAT) was strongly predicted ($R^2 = 0.77$; $p < 0.001$: Equation. 3 – Table 1) from five basic measures of the dominant population(s), hence the model was relatively specific. An increase in water table level was predicted by a quadratic response in the number of leaves per ramet ($\log_e$RamLV), characterised by an initial decrease in number of leaves. Canopy area (RamCA), and ratio of dry weight of stems to dry weight of leaves (DWS:DWL) also characterised an increase in number of leaves via a cubic function, but this time with an initial increase in the variables. Dry weight of reproductive structures per ramet (RamDWR) and number of reproductive structures per ramet ($\log_e$RamRE) were both characterised by an increase followed by a decrease (quadratic function) in their values, relative to increasing water table level. The predicted values correlated well with the observed values ($r = 0.72$: see Table 2).

Table 1. Different models

Models	Regression equations	Statistics
(1) Species richness (S)	$\mathrm{S} = -13.871 + 0.01991(\mathrm{RED}) + 11.466(\log_e\mathrm{CL}) + 2.940(\log_e\mathrm{STDE}) - 3.498(\log_e\mathrm{RamLV}) + 0.0001887(\mathrm{RED}^2) - 0.00000128(\mathrm{RED}^3) - 5.014(\log_e\mathrm{CL}^2) + 0.594(\log_e\mathrm{CL}^3) + 0.816(\log_e\mathrm{RamLV}^2) - 0.7381(\log_e\mathrm{RamDWR})$	($F = 5.489$; df $= 10$; $R^2 = 0.64$; $p \le 0.001$)
(2) Stem density (STDE)	$\log_e\mathrm{STDE} = 3.352 - 0.0278(\mathrm{WAT}) + 0.0003852(\mathrm{WAT}^2) + 0.469(\mathrm{PH}) - 1.072(\log_e\mathrm{FLU})$ $0.282(\log_e\mathrm{FLU}^2)$	($F = 8.930$; df $= 5$; $R^2 = 0.55$; $p \le 0.001$)
(3) Average water level (WAT)	$\mathrm{WAT} = -20.838 - 23.368(\log_e\mathrm{RamLV}) + 1.372(\mathrm{RamCA}) + 6.732(\log_e\mathrm{RamRE}) + 6.605(\log_e\mathrm{RamDWR}) - 11.491(\log_e\mathrm{RamLV}^2) - 0.0284(\mathrm{RamCA}^2) + 0.001287(\mathrm{RamCA}^3) - 4.0(\log_e\mathrm{RamRE}^2) - 0.849(\log_e\mathrm{RamDWR}^2) - 0.845(\log_e\mathrm{RamLV}^3) - 18.266(\mathrm{DWS{:}DWL}) + 19.305(\mathrm{DWS{:}DWL}^2) - 5.106(\mathrm{DWS{:}DWL}^3)$	($F = 6.504$ df $= 13$; $R^2 = 0.77$; $p \le 0.001$)
(4a) Minimum water level (specific model) (MIN)	$\mathrm{MIN} = -33.154 + 5.344(\mathrm{S}) + 1.531(\mathrm{RamCA}) + 13.274(\log_e\mathrm{RamRE}) - 0.856(\mathrm{S}^2) + 0.03442(\mathrm{S}^3) - 0.0295(\mathrm{RamCA}^2) + 0.0001534(\mathrm{RamCA}^3) - 6.535(\log_e\mathrm{RamRE}^2) - 1.864(\mathrm{DWS{:}DWL}) + 9.391(\mathrm{DWS{:}DWL}^2) - 3.398(\mathrm{DWS{:}DWL}^3)$	($F = 10.08$; df $= 11$; $R^2 = 0.79$; $p \le 0.001$)
(4b) Minimum water level (general model) (MIN)	$\mathrm{MIN} = -567.438 + 5.543(\mathrm{S}) - 1.031(\mathrm{S}^2) + 0.04672(\mathrm{S}^3) + 122.854(\log_e\mathrm{STDE}) - 0.844(\log_e\mathrm{STDE}^2) - 0.00979(\mathrm{BT})$	($F = 6.99$; df $= 6$; $R^2 = 0.55$; $p \le 0.001$)
(5) Level of water fluctuation (FLU)	$\log_e\mathrm{FLU} = 0.160 - 1.753(\mathrm{NENE}) + 0.407(\mathrm{NENE}^2) + 0.01581(\mathrm{BT}) - 0.0000234(\mathrm{BT}^2) + 0.00000001025(\mathrm{BT}^3) - 0.924(\log_e\mathrm{RamDWR}) + 0.149(\log_e\mathrm{RamDWR}^2) + 0.321(\log_e\mathrm{REPR}) - 0.283(\mathrm{DWS{:}DWL})$	($F = 5.95$; df $= 9$; $R^2 = 0.63$; $p \le 0.001$)

BT: total biomass per m^2; CL: Cl content of the water; DWL: dry weight of leaves; DWS: dry weight of stems; FLU: average level of water table fluctuation; MIN: minimum water table level; NENE: nearest neighbour; PH: pH; RamCA: canopy area; RamDWR: reproductive structures dry weight per ramet; RamLV: number of leaves per ramet; RamRE: number of reproductive structure per ramet; RED: redox potential; REPR: number of reproductive structures per m^2; S: species richness; STDE: stem density; WAT: average water level.

Table 2. Correlation coefficients between ranked scores of observed 2000 data and values predicted from the various models constructed using 1999 data

Model	2000 Test data used and correlation coefficient (r)
(1) Species richness (M^2)	• All test data; $r = 0.08$ • Sites with extreme redox values removed (E1 and I6-8); $r = 0.19$ • Sites with extreme redox values (E1 and I6-8) and Wood of Cree sites removed; $r = 0.49$
(2) Stem density (M^2)	•All test data; $r = 0.44$
(3) Average water level	•All test data; $r = 0.72$
(4a) Minimum water level (specific model)	• All test data; $r = 0.65$
(4b) Minimum water level (general model)	• All test data; $r = 0.39$
(5) Level of water fluctuation	• All test data; $r = 0.10$

A specific model predicting minimum water table level (MIN) ($R^2 = 0.79$; $p < 0.001$: Equation 4a – Table 1) utilised one collective vegetation variable, species richness (S: cubic function, with an initial increase in relation to less drawdown), and three dominant population traits as predictor variables. The dominant population traits were, canopy area (RamCA: again with a cubic function with an initial increase), number of reproductive structures per ramet ($\log_e$RamRE: quadratic function, increasing, and then decreasing in relation to more permanently inundated conditions), and ratio of dry weight of stems to dry weight of leaves (DWS:DWL). This final variable exhibited a cubic relationship, with an initial decrease in "leafiness" of individuals in relation to wetter conditions. A more general model ($R^2 = 0.55$; $p < 0.001$: Equation 4b – Table 1) utilised three collective variables alone: species richness (S) took the same form as for the specific model. Stem density (STDE) characterised increased minimum water table levels by increasing, and then decreasing. Total biomass per m^2 (BT) predicted decreased drawdown as values decreased linearly.

The values predicted by the specific model for the full test data set were well correlated with the observed values ($r = 0.65$: see Table 2). The figures predicted by the general model for the complete test data set were less well correlated with the observed values ($r = 0.39$: see Table 2).

Average level of water table fluctuation ($\log_e$FLU) was well predicted ($R^2 = 0.63$; $p < 0.001$: Equation 5 – Table 1) from three collective vegetation variables: nearest neighbour (NENE), total biomass per m^2 (BT) and number of reproductive structures per m^2 ($\log_e$REPR), and two dominant population variables: dry weight of reproductive structures per ramet ($\log_e$RamDWR) and ratio of dry weight of stems to dry weight of leaves (DWS:DWL). Both nearest neighbour distance and dry weight of reproductive structures per ramet exhibited a quadratic response, with an initial decrease, and then an increase in values relative to increasing fluctuation. Biomass exhibited a cubic response, suggesting an initial increase in relation to increasing fluctuation, followed by a decrease, and finally increasing again. The number of reproductive structures overall increased relative to greater water level fluctuation, while the amount of dry weight of stems to leaves decreased (i.e. the plants comprising the dominant populations became relatively more "leafy").

Average level of water table fluctuation was well predicted from the observed values for a number of samples, but the correlation between predicted and observed scores was relatively low (Table 2).

Discussion

The wetland vegetation sampled can be defined in relation to environmental pressures acting on plant survival which form gradients driving assemblage and structure, including level of inundation, degree of water table fluctuation, hydrosoil redox and pH. Vegetation attributes have been used to indicate the trophic status of various wetland community types (e.g., Wheeler & Proctor, 2000; Kennedy, 2001). In addition, vegetation height has been used to predict species competitive ability (Keddy & Shipley, 1989), and to characterise hydrochemical parameters (Willby et al., 1997).

Species comprising various associations will inevitably change over spatial scales (Duckworth et al., 2000; Keddy, 1992a, b). However, generic attributes will usually be present, and therefore have the potential to override the limitations of floristic approaches in predicting vegetation–environment interactions (e.g., Hills et al., 1994; Ali et al., 1999).

The methodologies adopted have enabled good predictions of eco-hydrological relationships in the wetlands sampled. In addition, model validation gave generally good correlations between predicted and observed values, and there is also evidence for a wider applicability of the outputs of the work to equivalent systems (i.e. predicted values were often good for data collected from entirely independent sites).

Our findings show that biomass appears to increase as disturbance (in the form of water table fluctuation) increases. Whilst this appears counter to the findings of Wilson & Keddy (1986), who illustrated a relationship between low levels of disturbance and increased biomass along a lake shore, our finding may be explained by considering the relative level of disturbance, which is likely to be lower (and less frequent) in most wetland systems than in vegetation fringing open water.

In this study, various biomass values were important for the construction of models predicting level of drawdown and level of water table fluctuation. In agreement with the findings of Willby et al. (1998), redox potential of the substrate proved to be a good indicator of species diversity in wetland vegetation, as did stem density. Murphy et al. (2003) also used redox potential as a predictor of diversity, for aquatic habitats within a wetland complex, but the predictive power of the model was relatively low. This suggests that the value of using substrate redox potential as a predictor of diversity (and other variables) may be limited to wetland habitats with rooted vegetation, where substrate redox values have a direct influence on the plants present. It also appears that redox may be of most use in stable wetland systems where substrates are anoxic. Where drawdown occurred for a number of stations, resulting in more aerobic substrate conditions, redox values were not well predicted. Another factor confounding the ability to predict species richness may be the history of the sites. The Wood of Cree samples used as test data were relatively more species rich than those for the other fen habitats sampled (including those used for model construction), despite comparable substrate and groundwater conditions. A number of comparable systems (e.g., Endrick Marshes, Glen Moss, and Nether Whitlaw Moss) have been subject to activities including major landscaping, controlled winter flooding, and marl digging (Kennedy, 2001), all of which may have impacted upon diversity. However, due to lack of detailed records previous to these workings, and of limited peat deposits which might yield pollen evidence, this is a hypothesis which may remain untested. In addition, Wood of Cree fen is groundwater fed, but is also subject to periodic inundation from the main channel of the River Cree (Paul Collin, RSPB., pers. comm., 1999). In cases where flooding is intermediate, nutrients and hydrophyte seed sources are maintained at favourable levels (Junk et al., 1989; Abernethy & Willby, 1999).

A number of intuitively likely eco-hydrological relationships have also been described. For example, stem density appears to increase initially in relation to greater water table fluctuation, and then fall off again as fluctuations become more extreme. Frequent fluctuations may allow mineralisation of nitrogen to nitrates, and increase their availability in generally nutrient-poor wetland soils (Patrick & Mahapatra, 1968). However, higher levels of fluctuation will cause a stressful environment (Grime et al., 1988), and therefore may preclude many species from establishment. Higher levels of fluctuation still may favour stress tolerators, and competition and stem density may be reduced as a result.

Biological systems are inherently noisy, and models will often have "noisy limits" (Murphy & Hootsmans, 2002). Wetlands are dynamic systems, and therefore no exception to this rule. Within this study a number of stations at Insh Marshes were subject to a relatively large degree of drawdown over a period of three seasons, from 1998 to 2000 (Kennedy, 2001). Some of the models constructed appeared to be sensitive to this phenomenon. Although they included data from a number of such sites in their predictive equations, the predicted values for repeat sample data (from the following year) were not always reliable. However, a prolonged drawdown in any wetland system would potentially lead to conversion of the vegetation towards a more terrestrial community type. In addition, these same models consistently produced good predicted values for samples from independent test data sites (e.g. for Endrick Marsh stations), and from repeat stations (e.g. Insh Marshes) which remained relatively stable over the 3 years in terms of their average water table levels. Grieve et al. (1995) identified areas within the Insh marshes, away from the main channel of the River Spey, which were less reliant upon riverine input, and were largely maintained by groundwater (telluric) inputs. It therefore seems that a number of the models produced may be better applied to freshwater wetland systems with major groundwater inputs. In this instance, variables such as hydrosoil redox potential, which proved to act as a good predictor of variation in several state variables, would remain stable. While the models may be applicable to less stable riverine-influenced systems, this will undoubtedly be within "noisy" limits. Further testing of the models to assess their wider applicability would therefore be desirable.

In conclusion, this work goes some way to answering questions in wetland vegetation management (e.g. predicting the effects of drawdown on wetland species: Newbold & Mountford, 1997), and in the use of vegetation as a hydrological monitoring tool (Wheeler & Shaw, 1995).

Vegetation traits have been used as successful predictors of the interaction between vegetation and the water-table dynamic. Conversely, characteristics of the hydrology have been predicted from factors of the vegetation. This knowledge may aid the application of wetland vegetation as a rapid assessment tool in the eco-hydrological management of wetland reserves. In association with initial scoping studies of major hydrological inputs and balances in wetlands (i.e. riverine or telluric), the use of a number of "useful" trait measurements may be utilised as part of an informative and coherent assessment of potential water level change impacts upon wetland vegetation (e.g., changes in diversity or productivity etc., which a certain course of water table management might produce). In the same way, this approach can inform us of current hydrological regimes (e.g., how dynamic a water table is, average level of inundation etc.). An increased understanding of such eco-hydrological interactions can therefore further aid the sensitive management of wetlands.

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Richness and structure of plant communities in temporary pools from western Morocco: influence of human activities

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Key words: temporary pool, Morocco, human activities, species richness, flora, plant communities

Abstract

Temporary pools are numerous in coastal plains of Atlantic Morocco and have a rich and diverse flora. These habitats are increasingly under pressure by man impact through grazing by domestic livestock and the development of annual crops in their catchments. The objectives of this work were to evaluate the species richness and the species composition of the vegetation of a sample of 30 pools in this region, in order to assess the structure of the vegetation within pools and to evaluate the role of environmental and anthropogenic factors in their richness and species composition. The results highlighted the species richness of the pools with 300 species found among which annual species were heavily dominant. The structure of the vegetation varied within pools according to a topographical/flooding gradient and between pools in relation with the land use in the surrounding areas. Three vegetation belts were recognized from the centre to the periphery of the pool. The total species richness per pool was not found significantly related to environmental or anthropogenic factors. The species characteristics of the pools were found mostly in the centre and their number affected by hydrological factors and land use in the pool and in the surrounding areas. The species characteristics of woodland habitats and of agriculture crops were found mostly in the peripheral zone. Temporary pools maintain specific communities of both aquatic and amphibious species, probably because of the selection induced by water level variations. The peripheral zone, although often dominated by terrestrial species, is clearly interpreted as part of the pool. It contains amphibious species highly characteristic of the pool for which the irregular flooding is a key environmental factor which decreases competition.

Introduction

Temporary pools (locally called “dayas” in Morocco), are depressions that fill with water during the rainy season and dry out most frequently along spring or early summer. There pools are numerous in the Northwest of Morocco (Thiery, 1987) and they are unique habitats, especially in terms of the number of rare or endangered plant and animal species they harbour (Metge, 1986; Rhazi et al., 2001a). The diversity of the vegetation of these temporary pools has been long recognized in Europe and in North-Africa (e.g. Braun-Blanquet, 1936; Chevassut & Quézel, 1956; Metge, 1986). The biological diversity of the temporary pools has been maintained over the centuries although subjected to extensive human activities. More recently these pools have been facing increasing human pressure by urban, industrial and agricultural development (Quézel, 1998). In addition to the threat of destruction by infrastructural development, temporary pools are subject to increasing human pressure mainly due to agriculture and diverse disturbances ranging from the drainage of the pools to the extraction of fine sediments or graveling. These changes could lead to changes in the species richness of the vegetation of the temporary pools (Hill & Keddy, 1992; Rhazi et al., 2001a; Crosslé & Brock, 2002) through diverse mechanisms (e.g. trampling, erosion, filling-in, selective grazing, nutrient enrichment and pesticide intrusion).

The objectives of this study were (1) to identify the organisation of the pool vegetation (2) to evaluate the species richness and the effects of environmental variables and human activities on this floristic richness of the pools (3) to quantify the contribution of neighbouring ecosystems to the vegetation.

Description of the study sites

The pools that were studied are located in the province of Benslimane (33° 38′ N, 7° 07′ W, 268, 000 ha), which is situated between Rabat and Casablanca. This region has a Mediterranean climate, sub-humid in the coastal part and semi-arid in the continental part. Annual rainfall is 462 mm, concentrated mainly in winter (Zidane, 1990).

Temporary pools, which are numerous in this region, make up 2% of the total area of the province (Rhazi, 1990). They are found in two main types of habitat: cork oak woodland and agricultural land. They vary in size, depth, nature of substrate and length of submersion period. These pools are endoreic and fed by water only through direct rainfall and runoff.

Materials and methods

In the Benslimane Province 30 pools were selected by means of a stratified sampling system based on the bio-climate (sub-humid/semi-arid), the geology (quartzite, sandstone, schist, limestone, alternating sandstone and pelite, alternating sandstone and quartzite) and location (in woodland or an agro-pastoral environment). Seven pools were situated in woodland and 23 in agricultural environment. These pools were visited in spring and summer 1997–1998. During these visits 330 phyto-ecological surveys were carried out along transects through homogenous vegetation units. These surveys consisted in a vegetation releve, (noting the cover and the growth form for each species), the measure of environmental variables (texture of the soil, the parent rock, the bio-climate, the maximum depth of water, the diameter of the water body and the duration of flooding) and of man-use variables, (the presence of drains, the occurrence or not of domestic grazing, the land use by grazing or annual crops of the area surrounding the pool). The percentage of each type of growth form for all 30 pools and the total species richness per pool were then calculated. The species found in the pools were split in 4 groups on the basis of the literature (Jahandiez & Maire, 1931–1934; Maire, 1952–1987; Fennich, 1990; Zidane, 1990; Rhazi et al., 2001a): Pool-species, Forest-species, Agriculture-species and Ubiquitous-species. Each group was made of species recognized as characteristics of one type of habitat except the last one (Ubiquitous) gathering opportunistic species found in all types of habitats. The contribution of the species from each group to the total richness of the pools was quantified. The effect of the environmental variables on the total species richness per pool was tested by linear regression and the number of species per group (Pool-, Forest- and Agriculture-species) was tested by linear regression and variance analysis (ANOVA) followed by comparison of means using the Tukey–Kramer test. The richness per vegetation belt in typical species of pools, woodland, agricultural land and the ubiquitous group was tested by χ^2. A correspondence analysis was conducted using the vegetation data from all the 330 vegetation releves made at the 2 sampling dates. The CA was conducted with only 200 species retaining only species that were present in more than 6 releves.

Results

Growth form

A total of 300 species was found during the 2 field visits in the 30 pools studied. Among the various growth form (Fig. 1) a very clear predominance of therophytes (70%) appeared whereas other growth forms were found uncommon.

Organisation of the vegetation

The first 2 axes of the CA (Fig. 2) explained respectively 25.8% and 19% of the total variance. Axis 1 separated the aquatic species (*Ranunculus peltatus*, *Callitriche bruti*a, *Glyceria fluitans*, etc.) from the terrestrial (e.g., *Leontodon taraxacoides*, *Plantago coronopus*, *Trifolium campestre*). Axis 2 separated the Forest-species (e.g., *Cistus spp*, *Cynara humilis*, *Asphodelus microcarpus*) from the Agriculture-species (e.g., *Silene gallica*, *Ormenis mixta*, *Raphanus raphanistrum*). The coordinates of the orthogonal projection of the vegetation releves on axis 1 were significantly correlated to the maximum depth of the water recorded in these surveys ($r^2 = 0.53$; $p < 0.05$; $n = 330$). Axis 1 was therefore interpreted as an hydrological gradient. This gradient along axis 1 (Fig. 2) corresponds to a topographical gradient from the external margin of the pools (terrestrial), an amphibious zone and the inner (aquatic) part of the pool. These three zones were designated as "vegetation belts".

Species richness

The total species richness per pool (Table 2a, b) was not significantly correlated ($p > 0.05$) with the size of the pool (diameter of the water body), maximum water depth, nor soil characteristics, nor anthropozoogenic factors nor type of bio-climate ($p > 0.05$).

Within pools the number of species from the Pool-, Forest- Agriculture- and Ubiquitous-groups were significantly different between the 3 vegetation belts (Table 1, $\chi^2 = 218.94$; $p < 0.0001$). The inner and intermediate belts contained more Pool-species than the peripheral belt which was richer in species from the Woodland-, Agriculture and Ubiquitous-groups (Table 1).

The number of species from the Pool-group was significantly different according to the type of environment surrounding the pools. The number of Pool-species was higher within forested environment (28.00 ± 1.57) than for pools within agricultural environment (23.26 ± 1.13). The number of Pool-species was also significantly higher for pools in a grazed environment (forest and pastures) (26.38 ± 1.12) than for pools in an environment made of fields of annual crops (19.66 ± 0.95) (Table 2a,). The number of Pool-species was significantly correlated with the hydrological factors (depth, diameter of the water body, Duration of flooding, Table 2b) but not with other environmental factors. Similarly the number of Forest-species was significantly different according to the land use surrounding the pool. It was higher for pools within a forested environment (15.14 ± 2.38) than for pools within an agricultural environment (7.78 ± 0.76). The number of Forest-species was not correlated with any other environmental and human factors (Table 2a, b).

The number of Agriculture-species (Table 2) was significantly higher in pools within an agricultural environment (21.91 ± 1.61) than for pools established in forests (13.42 ± 1.81). There was a significant negative correlation between the number of Agriculture-species and the diameter of the water. The number of Agriculture-species was higher in drained (25.91 ± 2.11) than non-drained pools (17.44 ± 1.53). The richness in species characteristic of agricultural lands was not significantly correlated with other environmental factors ($p > 0.05$) (Table 2a, b).

The richness in Ubiquitous species was not significantly correlated to the type of land use surrounding the pools ($p > 0.05$) (Fig. 3).

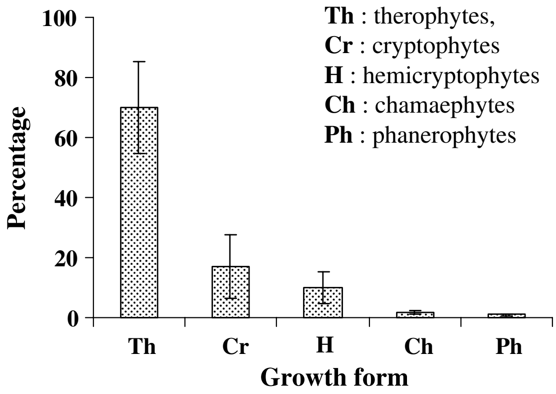

Figure 1. Relative frequency of the various growth forms of the plant species found on the 30 pools (bars = SD).

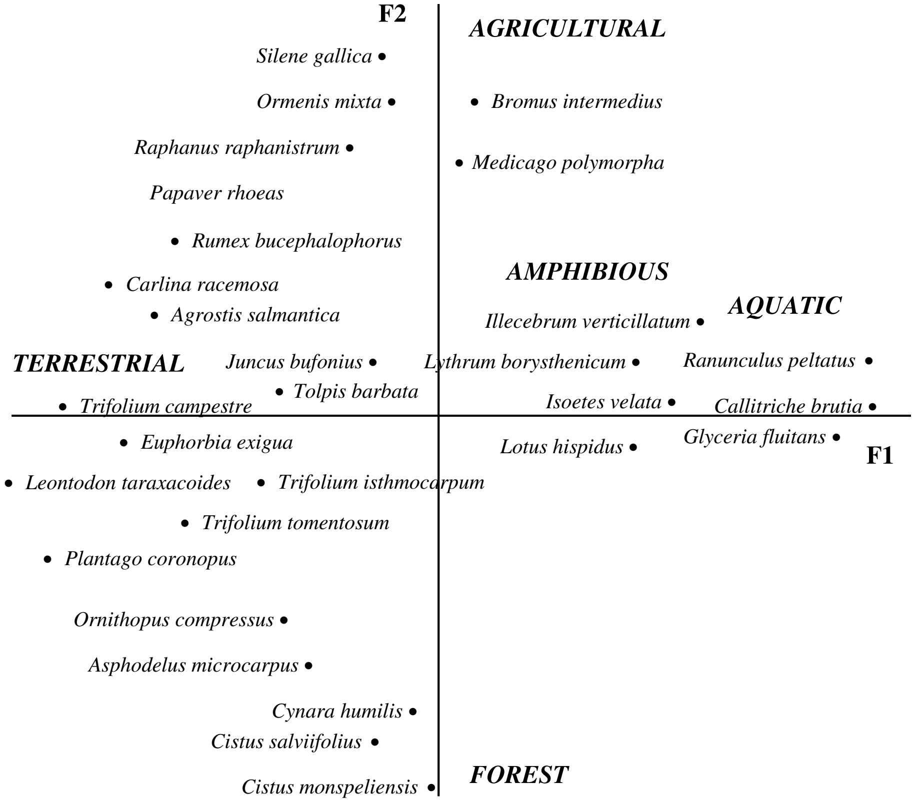

Figure 2. Plot ½ of the correspondence analysis (CA) on the floristic survey of the 30 pools. Species oflimited contribution, located in the centre of the diagram, are not shown to facilitate interpretation of the graph.

Discussion and conclusions

The predominance of annuals (70%) in the flora of the pools is a result of adaptation to harsh environmental conditions (Médail et al., 1998). The unpredictability of the filling and drying-up periods of the pools, which is linked to the Mediterranean climate, the occurrence of severe droughts that alternate with floods, and the intra- and inter-annual variations in water depth, leads to the selection of short-cycle species which invest in sexual reproduction to the detriment of their vegetative development. The predominance of therophytes has also been observed by Médail et al. (1998) for pools in France and by Zedler (1987) for vernal pools in California. The survival of species from one year to another depends mainly on germination from the seed bank (e.g. Bonis et al., 1995; Rhazi et al., 2001b, Brock & Crosslé, 2002).

The vegetation of the pools investigated (CA) is arranged in 3 belts that follow the hydrological gradient (axis F1) : inner, intermediate and peripheral belts dominated by aquatic, amphibious and terrestrial species respectively. A similar organisation of vegetation has been observed in the vernal pools of California (Bauder, 2000). In

Table 1. Richness in typical species of pools, forests, agricultural land and the ubiquitous group in the three vegetation belts

Species	Pool	Forest	Agricultural	Ubiquitous
Inner Belt	15	0	2	1
Intermediate Belt	59	2	3	1
Peripheral Belt	9	55	95	58

Table 2. (a) Results of variance analyses (ANOVA) of the total species richness, the richness in species characteristic of Pools, Forests and Agricultural land according to the land use (woodland or agriculture) outside the pool, the type of utilisation of the pool (agriculture or pasture), the drainage, the bio-climate, the nature of the rock and the texture of the surface soil horizon. (b) Results of linear regressions between the total species richness, the number of species characteristic of Pools, Forests and Agricultural land and the maximum depth of water, the diameter of the water surface recorded in spring, the length of the submersion period ($n = 30$, result significant when $p < 0.05$)

	Total Richness			Pool species			Forests species			Agriculture species		
(a)	ddl	*F*	*p*	ddl	*F*	*p*	ddl	*F*	*p*	ddl	*F*	*p*
Land use (outside)	1	3.29	0.080	1	4.43	**0.044**	1	15.22	**0.001**	1	18.70	**0,018**
Utilisation (inside)	1	1.29	0.264	1	13.3	**0.001**	1	2.73	0.109	1	2.12	0.150
Drainage	1	2.85	0.102	1	1.58	0.210	1	0.01	0.940	1	11.03	**0.003**
Bio-climate	1	0.12	0.720	1	0.81	0.370	1	0.46	0.500	1	0.09	0.756
Nature of the Rock	5	1.44	0.240	5	1.35	0.270	5	0.24	0.940	5	2.17	0.090
Texture	5	1.93	0.120	5	1.31	0.290	5	3.70	0.120	5	1.06	0.400
(b)		R^2	*p*		R^2	*p*		R^2	*p*		R^2	*p*
Maximum Depth of Water		0.03	0.370		0.31	**0.001**		0.01	0.490		0.08	0.140
Diameter of Water		0.01	0.790		0.22	**0.009**		0.02	0.440		0.16	**0.030**
Length of Submersion		0.02	0.480		0.17	**0.023**		0.01	0.530		0.03	0.320

wetlands this zonation has been attributed to the distribution of species according to their flooding tolerance (Brewer et al., 1997; Lenssen et al., 1999). However in temporary pools in dry regions the intensity of summer drought could also play an important additional role in this zonation (Rhazi et al., 2001b). This structuring of the vegetation in belts has been found also in the seed stock in these temporary pools (Rhazi et al., 2001b).

The inner and intermediate belts contain typical pool species (Table 1) whose richness is linked to hydrological factors but independent of pedological and geological factors. These typical pool species are more abundant in pools located in woodland than in those located in agricultural environments. This result suggests a negative effect of the agricultural practices in the neighbouring areas, possibly through the accumulation via runoff of pesticides or nutrients (nitrates and sulphates) from fertilizers (Anderson & Vondracek, 1999; Rhazi et al., 2001a). These typical pool species do not appear to be affected to the same extent by grazing and even by the drainage of the pools.

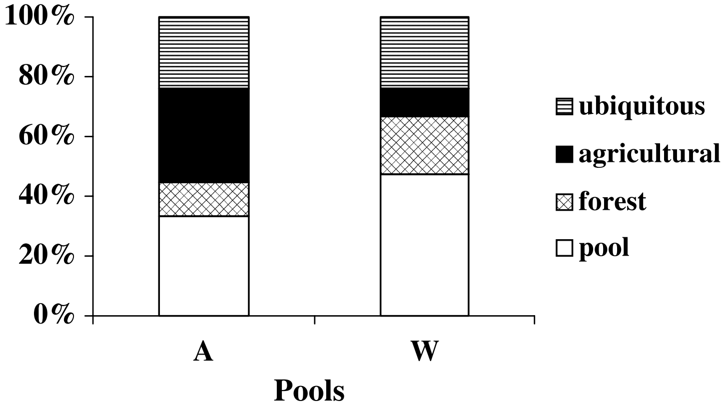

Figure 3. Percentage of species typical of pools, forests, agricultural land and ubiquitous in pools with agricultural catchments (A) and in pools with woodland catchments (w).

The ecotonal peripheral belt is the richest (Table 1) which is attributed to lower hydromorphy (e.g. Gough & Grace, 1998). The species of this belt can be found mostly in to the Ubiquitous-, Forest- and Agriculture-species but contain also amphibious Pool species. The external belt thus bears the marks of neighbouring woodland and agricultural ecosystems, which contribute 14% and 20% respectively to the total richness of the pools. The invasion of the pool margins by species from surrounding terrestrial environments is facilitated by the wind and the frequent movements of livestock (Metge, 1986) capable of transporting seeds from one place to another. The terrestrial species find suitable habitats in the peripheral belt taking advantage of the low frequency of flooding. However irregular flooding seems to be an important environmental factor selecting annual species. The first year following a high water level is characterised by a high proportion of annuals in the vegetation which decreases gradually during the following years (Rhazi et al., 2001b and unpublished data). In the centre of the pool, the long submersion period and the diameter of the water surface prevents successful establishment by terrestrial species from the margins, thus enabling the pool to maintain its typical and original aquatic and amphibious flora. According to Metge (1986), this raises a problem concerning the limits of these ecosystems: should the peripheral belt be included in the pool ecosystem, or is it an interface between the aquatic environment and surrounding terrestrial environments? The results of this study, together with previous studies (Rhazi et al., 2001a), indicate that the peripheral belt should be included in the pool ecosystem. Although the species composition of the peripheral belt can exhibit high similarity with adjacent terrestrial habitats, the species composition is strongly affected and aquatic and amphibious species dominate. Furthermore this belt is the most favoured habitat for rare and unstable species which are characteristics of the Mediterranean temporary pools (e.g. *Damasonium stellatum*, *Pilularia minuta* or *Elatine brochoni*). These species although abundant in the seed stocks (Rhazi et al., 2001b) appears only during the most wet years when they beneficiate from the suitable water regime and the displacement by flood of competitive species.

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Ecological management of aquatic plants: effects in lowland streams

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Key words: ecological management, macrophytes, backwater, lowland stream, flume experiments, field survey

Abstract

Recently, a significant increase in macrophyte growth has been observed in many lowland rivers in Flanders, mainly due to eutrophication and an improvement of the water quality. This growth strongly influences the channel roughness (Manning-*n*). The first purpose of the project was a better understanding of the complex relationship between biomass development and discharge capacity in lowland rivers. In order to avoid the backing up of water upstream, the whole vegetation body is usually mowed annually. This project also investigated a lighter ecological management of aquatic plants in which only a part of the vegetation is removed in separate and alternating blocks, seeking a compromise between sufficient discharge and conserving large parts of the macrophyte vegetation with all its functions. Beside laboratory experiments, field survey was undertaken in the Grote Caliebeek, a tributary of the Kleine Nete in Flanders, Belgium. The results indicated that the presence of macrophytes in lowland rivers slowed down the waterflow and resulted in a raised water level. The upstream water level followed biomass development at a certain discharge level. The mowing experiments and the field survey indicated that alternating weed cutting patterns can reduce fall in an effective way. This research emphasizes the possibilities of alternating weed cutting patterns in order to deal with water flow problems. In the long term there will be the need for a better understanding of the ecological relationships in the search for a sustainable integrated method of controlling aquatic vegetation.

Introduction

Aquatic macrophytes are important in the functioning of the water body. They offer food and shelter for many organisms and promote habitat diversity. Despite many benefits, they can limit the discharge capacity of lowland rivers. In bare streams only the channel roughness, expressed as Manning's roughness coefficient (*n*), limits discharge. This Manning-*n* is increased by the development of aquatic macrophytes, in a complex interaction with discharge and mean velocity. Roughly speaking, at a certain discharge an increasing biomass will result in an increasing Manning-*n*, an increasing fall and a decreasing discharge capacity (Soulsby 1974; Brookes, 1986; Watson, 1987). In many field situations the aquatic vegetation consists of a mixture of growth-forms: emergent plants rising above the water surface, floating plants and submerged plants (Stephens et al., 1963). Research on the hydraulic effects of these mixed vegetations is poorly understood (Hearn et al., 1994; Riis et al., 2000).

To overcome flooding the whole vegetation body is usually mowed annually in a mechanical way, using a hydraulically operated bucket. This complete removal restores discharge capacity but has a negative ecological impact on many aquatic organisms.

In this paper we want to discuss the hydraulic effects of a mixed vegetation. We also focus on a quantitative basis for a more sensitive ecological method of mowing the aquatic vegetation in separate and alternating blocks. Both field survey and laboratory experiments were carried out.

Materials and methods

Typical aquatic vegetation of a transect of the Grote Caliebeek, a tributary of the Kleine Nete in Flanders (Belgium), consisting of submerged, floating and emergent macrophytes, was placed in an aluminium tray (length (*L*): 0.77 m; width (*W*): 0.56 m; height (*H*): 0.08 m) in an artificial flume (*L*: 35.78 m; *W*: 0.56 m; *H*: 0.75 m). The biomass fresh weight was determined and a series of seven discharges was applied to the macrophytes at high and low water level, controlled by a weir at the end of the flume. Fall was measured by two point gauges, up- and downstream of the embedded macrophytes (Fig. 1a) and plotted against the upstream mean current velocity. Velocity was determined by means of discharge *Q* and wetted section A. Because of the seasonal changes in biomass the experiments were repeated in June, July and August.

Fresh weight biomass was also determined on the field in a transect of the Grote Caliebeek from June to September, before the period of weed cutting. This lowland stream has a length of approximately 21.5 km and a mean width of 3.5 m. Discharge and water level were measured out with an ultrasonic doppler instrument, respectively a pressure probe from July to September. The water level upstream of the vegetation was plotted in time to investigate the link with the biomass development.

In the second experimental (Fig. 1b) setup a mixed vegetation of the Grote Caliebeek was placed in an artificial flume (*L*: 25 m; *W*: 3 m; *H*: 0.8 m). The macrophytes were embedded in wooden trays (*L*: 0.75 m; *W*: 0.50 m; *H*: 0.08 m),

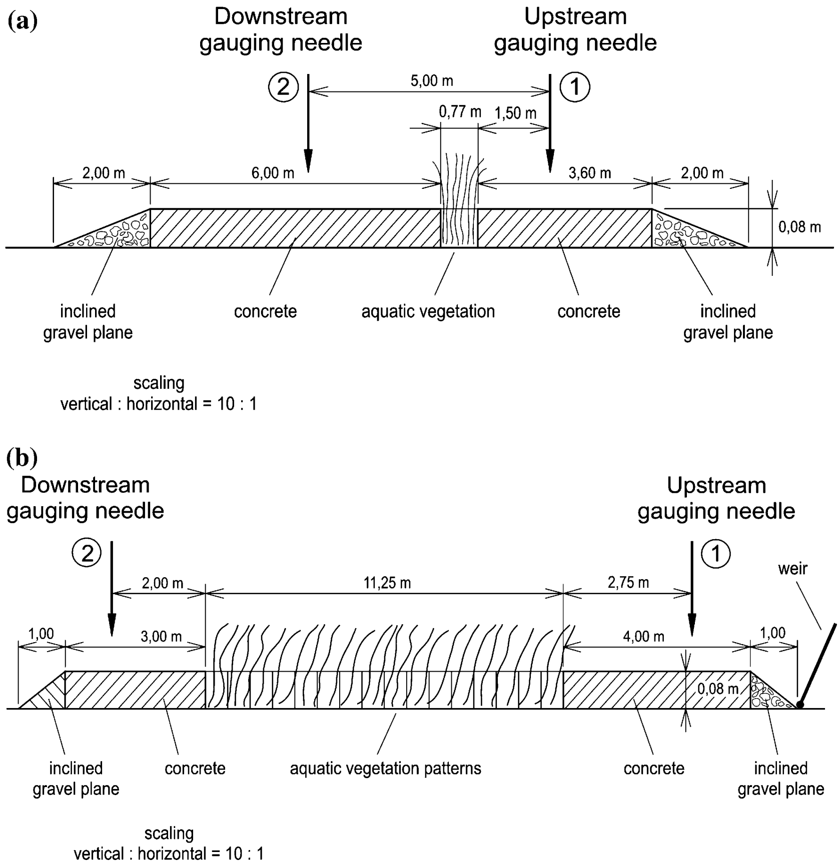


Figure 1. (a) Schematic plan of the first experimental setup in an artificial flume (*L*: 35.78 m; *W*: 0.56 m; *H*: 0.75 m). An aluminium tray (*L*: 0.77 m; *W*: 0.56 m; *H*: 0.08 m) filled with a mixed vegetation is placed in an artificial flume. (b) Schematic plan of the second experimental setup in an artificial flume (*L*: 25 m; *W*: 3 m; *H*: 0.8 m). Wooden trays (*L*: 0.75 m; *W*: 0.50 m; *H*: 0.08 m) filled with a mixed vegetation are placed in an artificial flume according to the different patterns of Table 2.

arranged according to six vegetation patterns and two reference patterns (Table 1). The effect of different mowing regimes on the water level was investigated by applying a series of six discharges while the up- and downstream water level were measured with point gauges. The selection of patterns was based on Viaene & Vereecken (2001) where ecologically valuable patterns are characterized by lower cutting percentages, a micromeandering flow behavior and longer vegetation blocks. Manning-*n* coefficient is calculated via the dynamic equation of gradually varied flow in prismatic channels (Chow, 1959):

$$\frac{dh}{ds} = \frac{S_0 - n^2 \frac{P^{4/3}}{A^{10/3}} Q^2}{\sqrt{1 - S_0^2 - \frac{Q^2 B}{g A^3}}}$$

in which dh = change in waterlevel (m), ds = change in distance in the longitudinal direction (m), S_0 = bottom slope (–), n = Manning-*n* coefficient (s/m$^{1/3}$), P = wetted perimeter (m), A = wetted section (m^2), B = storage width (m), Q = discharge (m^3/s) and g = 9.81 m/s^2.

A two-way Analysis of Variance (ANOVA) mixed model '$Y = AB$' is used in order to evaluate the effect of the fixed pattern (A) and random factor discharge (B) on Manning-*n* number (Y).

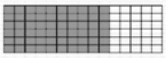
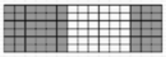
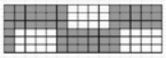
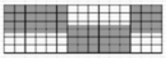
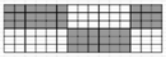
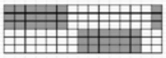
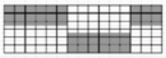
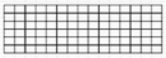
To evaluate patterns in practice, a transect of the Grote Caliebeek was cut according to pattern 5.

Results

The vegetation used in the first experimental setup comprised of *Sparganium emersum*, *Potamogeton natans*, *P. pectinatus* and *P. trichoides*. Due to determination problems the biomass of *Potamogeton pectinatus* and *P. trichoides* were combined (*Potamogeton* spp.). Figure 2 shows the evolution in biomass as fresh weight per species and total fresh weight from June to August. Under the tested circumstances:

- mean current velocity is directly proportional to the fall
- at a constant velocity and total biomass, the fall is higher in shallow streams
- if total biomass increases, an increasing velocity will lead to an even more pronounced fall
- if water level rises, the fall will increase more slowly with an increasing velocity (Fig. 2).

Table 1. Mean fall, percentage reduction in fall, mean Manning-*n* number, mean % reduction in Manning-*n* and weed cutting percentage for the different tested patterns

	Pattern no.	Mean fall (cm/m)	Mean % reduction in fall compared to pattern *R*1	Mean Manning-*n* Number (s/m$^{1/3}$)	Mean % reduction in Manning-*n* compared to pattern *R*1	Weed cutting %
	*R*1	0.376	0	0.27196	0	33
	1	0.362	4	0.26467	3	40
	**2**	**0.229**	**40**	**0.19811**	**27**	**40**
	**3**	**0.153**	**60**	**0.15778**	**42**	**42**
	**4**	**0.102**	**74**	**0.12417**	**54**	**50**
	**5**	**0.066**	**82**	**0.10200**	**62**	**57**
	**6**	**0.081**	**79**	**0.10967**	**0**	**58**
	*R*2	0.008	97	0.03644	87	100

Each cell on the left corresponds to a wooden trail (length: 0.75 m; width: 0.50 m; height: 0.08 m). Filled cells indicate aquatic macrophytes, empty cells indicate mowed vegetation. Alternating patterns are highlighted in the table. **Grouped patterns on the left indicate no significant differences in Manning-*n* number** ($P \leq 0.05$).

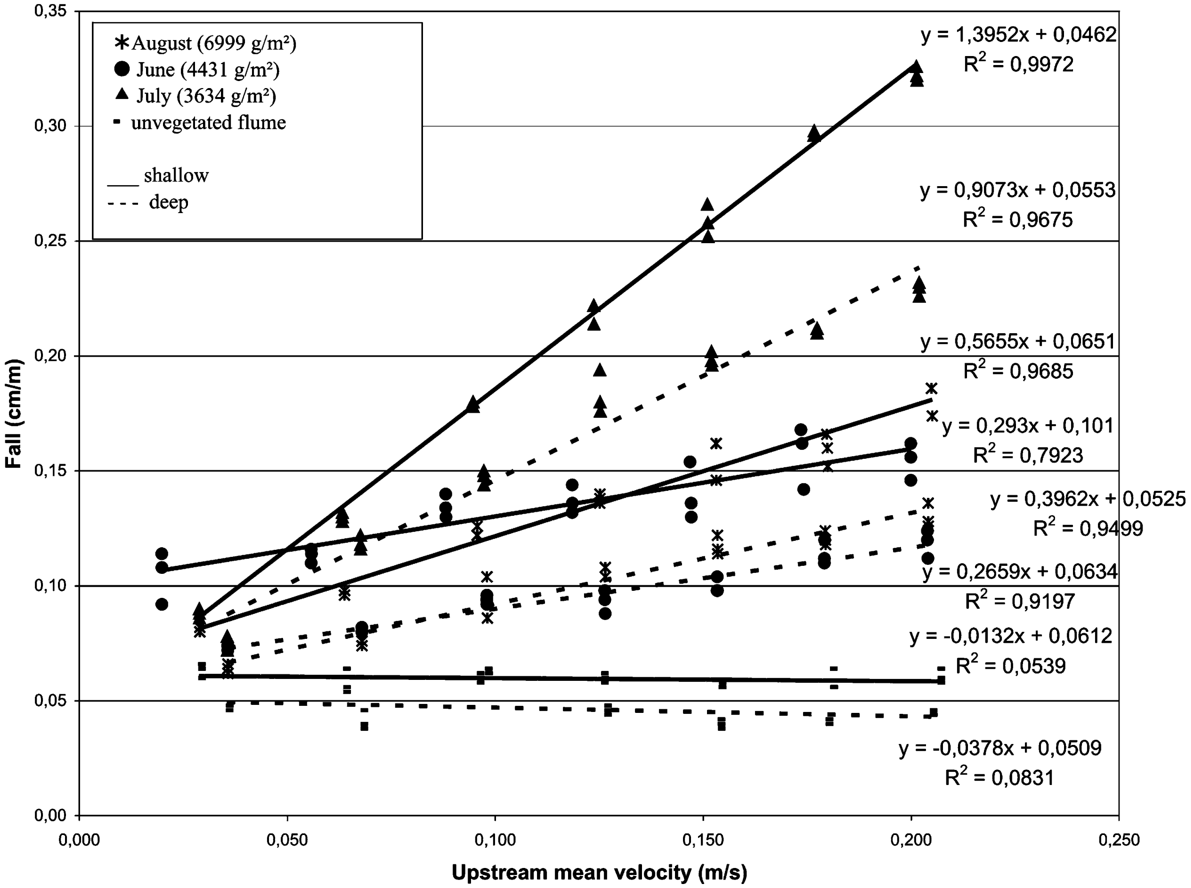


Figure 2. Experimental relation fall/mean current velocity for the vegetation of the Grote Caliebeek.

On the field, the upstream water level in a transect of the Grote Caliebeek follows the total biomass development at a certain discharge level during July (1.719 kg/m^2), August and the first half of September (2.555 kg/m^2) (Fig. 3).

For all tested patterns in the experimental setup, the measured fall rises with an increasing discharge. The difference in fall between the patterns increases with an increasing discharge. The most dense pattern (*R*1) leads to the highest fall. Less dense patterns with a higher cutting percentage or less meandering patterns have a lower fall (Table 1). Results of ANOVA indicate significant main effects for discharge ($P \leq 0.0001$) and pattern ($P \leq 0.0001$) and a significant interaction effect discharge*pattern ($P \leq 0.0001$). Grouped patterns in Figure 1 indicate no significant differences in Manning-*n* number ($P > 0.05$).

In the Grote Caliebeek, after a weed cutting regime according to pattern 5 (Sep. 02, Oct.), the upstream water level reaches acceptable heights, even at much higher discharges, compared to the period before the weed was cut (Fig. 3).

Discussion

We expect the approximately linear curves of Figure 2 to bend down at higher velocities, as the vegetation is pushed towards the bottom of the flume and the ‘open’ water above the vegetation increases. The less dense or the more flexible the vegetation, the less the backing up of water upstream is pronounced. This is known as the drowning out of roughness elements (Watson, 1987). Due to washing out of the macrophytes the discharge was limited to an upstream mean velocity of approximately

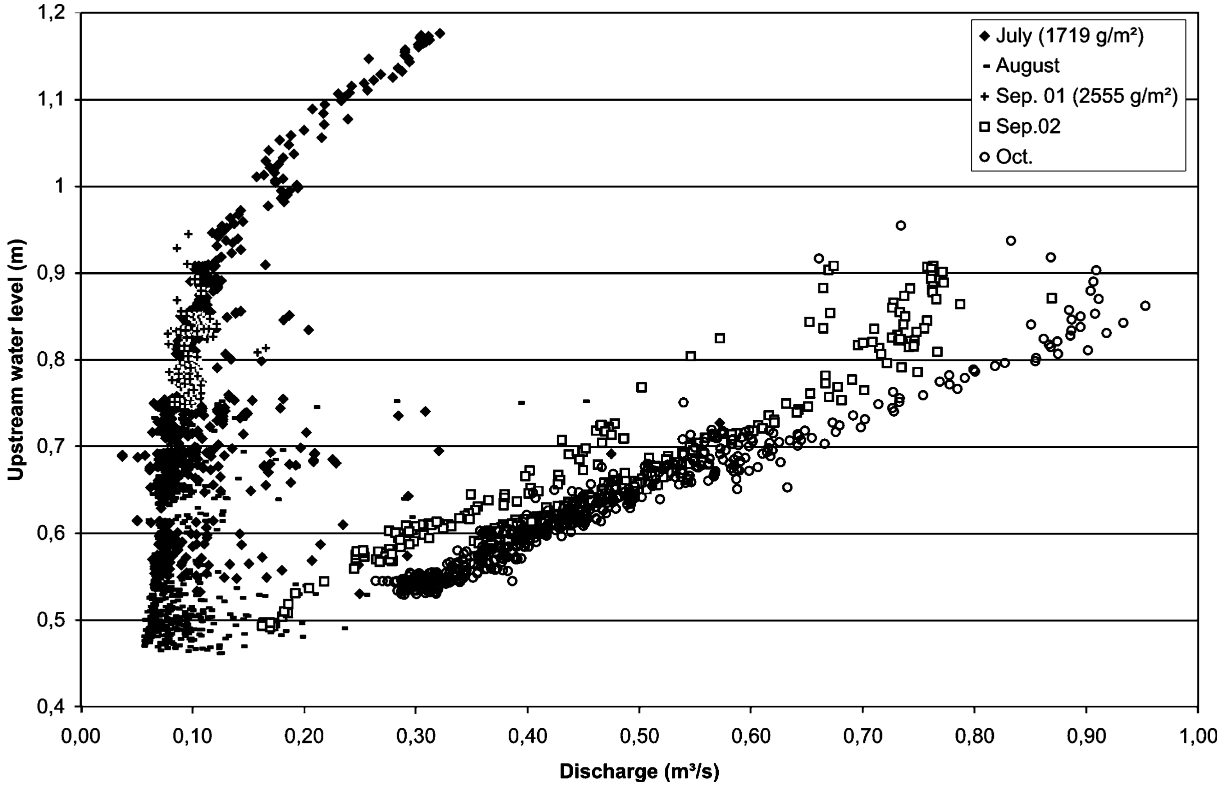


Figure 3. Upstream water level in relation to discharge (hourly means) for a transect of the Grote Caliebeek from July until October. The index 01 and 02 means respectively before and after weed cutting in October.

0.2 m/s. Therefore the point of bending down was not reached during these experiments.

In shallow waters the 'open' part of the section is smaller compared to deeper ones. Similarly this results in a higher resistance and a higher fall at comparable current velocities.

Biomass development in August was not measured. Based on Figure 2 one can state that in August total biomass had to be smaller than in July (1.719 kg/m^2) and during the first half of September (2.555 kg/m^2).

Under the tested circumstances, alternating weed cutting patterns can reduce fall from 40–82% (Table 1). This percentage is higher when the pattern is less dense or less meandering.

Field measurements in the Grote Caliebeek indicate the effectiveness of alternating weed cutting schemes in order to prevent flooding during periods of high discharge levels. A first indication of good practice is a minimal block length of 5–7 times the stream width, based on the principle of meandering streams, whereas one meander has the dimensions of 10–14 times the width (Madsen, 1995).

Conclusion

This research emphasizes the possibilities of alternating weed cutting patterns in order to deal with water flow problems in the short term. In the search for a sustainable integral method of controlling aquatic vegetation there will be the need for a better understanding of the ecological relationships. Important features with this are control of river nutrient load, management methods like shading and period of weed cutting.

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Control of *Myriophyllum verticillatum* L. in Irish canals by turion removal

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Key words: weed control, harvesting, amenity canals

Abstract

Myriophyllum verticillatum L. is the single most obstructive aquatic plant in Irish canals. Because of the importance of these canals as national amenities, considerable resource has been directed towards the long-term control of this aggressive plant. A combination of mechanical harvesting and chemical treatment has traditionally been employed to control *Myriophyllum* growth but these methods have only provided short-term control. *Myriophyllum verticillatum* reproduces by producing turions between September and November each year. These over-wintering propagules sink to the canal bed where they remain dormant until February. At a trial site on the Royal Canal turions were harvested in November 1994 and 1995, significantly reducing the number of propagules present. Biomass and plant cover with *M. verticillatum* throughout the 1995 and subsequent growing seasons was dramatically reduced. The implications for weed control programmes, habitat development and amenity usage are discussed.

Introduction

Sexual propagation among submerged macrophytes is considered to be of limited importance, while vegetative or asexual propagules are generally accepted as the most important mechanism for propagation and dispersal among this plant group (Sculthorpe, 1967; Hutchinson, 1975). Vegetation propagules include viable plant fragments, winter buds, tubers and turions. Most submerged macrophytes spread by fragmentation, where viable portions of stem, stolon or rhizome are torn or shed from the parent plant and form the nucleus of a new population. A few macrophytes, notably *Sagittaria sagittifolia* L. and *Potamogeton pectinatus* L., produce tubers as a means of asexual propagation and dispersal (Kautsky, 1987, 1990; van Wijk, 1988). The production of turions (specialised vegetation buds which function as propagules and as over-wintering structures) is characteristic of a number of species of *Potamogeton* (van Wijk & Trompenaars, 1985; Preston, 1995) and of *Myriophyllum verticillatum* (Weber & Nooden, 1974, 1976).

Myriophyllum verticillatum is the principal constituent of the submerged macrophytes flora in *circa* 30% (100 km) of Ireland's navigable canals. Water quality and habitat conditions in these man-made waterways favour the development of extensive and obstructive macrophyte stands (Caffrey & Allison, 1998; Caffrey et al., 1998). These growths are a feature of the canals during spring, summer and autumn and can seriously impact the beneficial use of these heritage waterways (Caffrey, 2001). *Myriophyllum verticillatum* is a submerged perennial macrophyte whose distribution in Irish watercourses is rather limited but which proliferates in the shallow, productive and relatively still waters of these canals. By May each year the plant is well established and dense monodominant stands occupy long canal sections. Between September and November the plants produce large numbers of turions, which serve as a mechanism for surviving unfavourable conditions while also acting as propagules for short- and long-distance dispersal.

Mature turions are club-shaped and range in length from 2 to 6 cm. Between 4 and 12 turions are produced by an individual plant (Caffrey & Monahan, 1995). When mature, generally in mid- to late November, the individual turions abscise from the dead or dying parent stem and float on the water surface, often in clusters, for a number of days. Many are attached to short stem segments which provide buoyancy for the propagules. It is at this floating stage that long-distance dispersal can occur, with large numbers of turions moving, under the influence of wind or flow, along the canal corridor. Within a few days the density of the turions increases and they sink to the canal bed (Weber & Nooden, 1974). As water temperatures increases in February the turions germinate and individual plants to *circa* 10 cm high are established by early April (Caffrey & Monahan, 1995).

Traditionally, obstructive growths of *M. verticillatum* in the canals are treated using a combination of mechanical (cutting) and chemical (dichlobenil) control. These methods are costly, labour intensive and ecologically disruptive, and yet provide only relatively short-term control. The present study investigates the impact that turion removal has on the long-term control of *M. verticillatum* at a trial site on the Royal Canal, and the implications for annual weed control programmes, habitat development and general amenity exploitation.

Materials and methods

A 4.5 km long section of the Royal Canal was selected for study. The canal at this location is approximately 13 m wide and has a design depth of 1.55 m. The water is typically clear and boat traffic density is light (Caffrey et al., 1998). This section was typical of many other sections of the Royal and Grand Canal and supported dense stands of *M. verticillatum*. These commonly occupied >90% surface cover and produced a dry weight biomass in excess of 200 g m^{-2}. To maintain a navigable channel the canal was treated annually with dichlobenil (as Casoron G SR) in May and, because of vegetation regrowth, often received a mechanical cut in August. Turions were physically removed from the channel in November 1994 and 1995 using a boat-mounted weed harvester.

Vegetation samples for biomass analysis (g dry weight m^{-2}) were collected at the study section on six occasions each year between 1993 and 1996. The sampling regime was designed to monitor the effects of the various weed control operations on biomass development of *M. verticillatum* throughout the growing season. A stove pipe sampler of 23 cm internal diameter was used to collect the vegetation (Weber, 1973; Caffrey, 1990). On each occasion, 10 replicate samples were taken. The stove pipe was also used to determine the number of turions present (no. m^{-2}) at 10 replicate sites along the study section before and after turion removal.

Results

The pattern of biomass development for *M. verticillatum* at the study site during the four-year study is presented in Figure 1. The typical reaction of the vegetation to traditional weed control practices, as operated in 1993 and 1994, is clearly demonstrated. Dichlobenil effectively suppressed plant growth for 10–12 weeks post-treatment. This was followed by a period of rapid regrowth which culminated in the establishment of dense, obstructive vegetation stands by early to mid-August. Mechanical cutting at this time temporarily removed this vegetation, although regrowth through September and October was often sufficient to present further impediments to navigation and other amenity use (Fig. 1).

Turion counts conducted in the study section in November 1993 and 1994 revealed 863 (±102) and 914 (±88) turions, respectively. The majority of the turions were attached to moribund, leafless, rooted stems, although a number had already abscised and lay on the canal bed. Following turion harvesting in November 1994 a significant reduction in turion density, to 154 (±14), was recorded.

Biomass production of *M. verticillatum* in the 1995 growing season, following turion removal in the previous November, was dramatically reduced. By May of that year, when >200 g m^{-2} of vegetation might normally be expected, only 15 g m^{-2} was present (Fig. 1). Because of the reduced biomass present no dichlobenil was applied in April 1995. By July only 39 g m^{-2} of *M. verticillatum* was recorded.

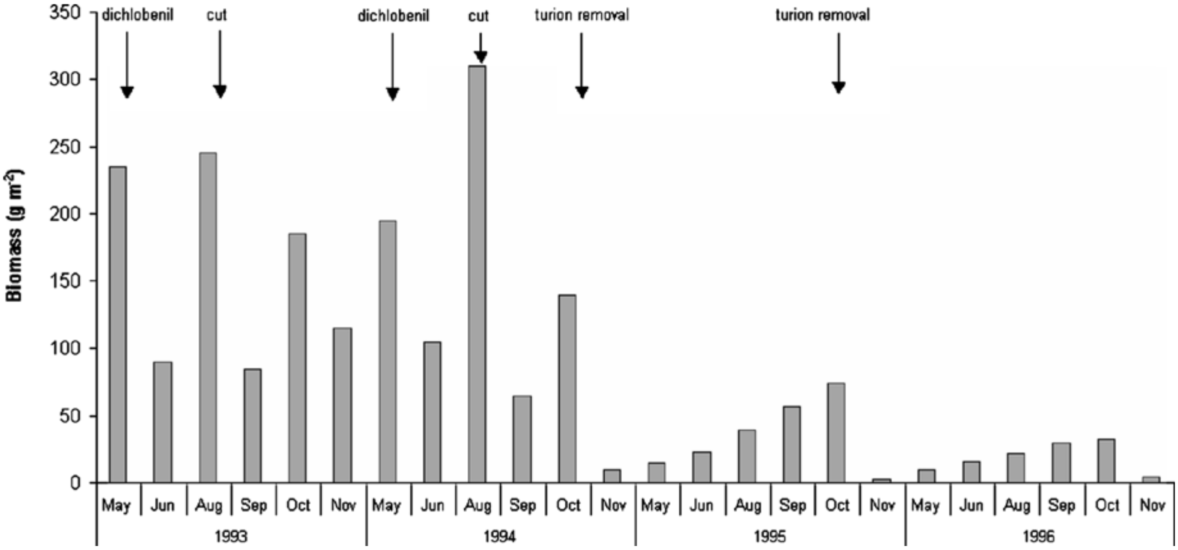

Figure 1. Effects of dichlobenil treatment, mechanical cutting and turion removal on the biomass development of *Myriophyllum verticillatum* on a section of the Royal Canal between 1993 and 1996.

This level of biomass posed no threat to amenity use of the waterway and, hence, no mechanical cut was applied. Maximum biomass was recorded in October 1995, when 81 g m^{-2} was present.

Sampling in early November 1995, prior to turion removal, revealed a propagule density in the study channel of 223 ± 32. This was reduced to 34 ± 14 following the turion harvest. Biomass production of *M. verticillatum* in the 1996 season was further reduced on that recorded in 1995 (Figure 1) and no problems for navigation, angling or other water-based activities were reported. There was, therefore, no necessity to apply dichlobenil or to mechanically cut. Only 48 (± 13) turions were recorded in this channel in November 1996. During the period of the trials contiguous canal sections continued to experience problems with *M. verticillatum* growth. In long sections of untreated canal plant stands grew vigorously during the spring and summer months, and commonly filled the water column. In these sections all amenity pursuits were restricted and users expressed considerable dissatisfaction.

During the 1996 growing season a number of macrophytes species which had hitherto been unrecorded at this site, or which were present with low abundance, were observed. Most prominent among these were *Chara* spp., *Fontinalis antipyretica* and *Potamogeton lucens* L.

Discussion

The true amenity potential of long sections of the Royal and Grand Canals is impacted for many months each year by dense, obstructive vegetation stands. Prominent among the weed species involved is *Myriophyllum verticillatum*. Traditional control methods, which include annual treatment with dichlobenil, often followed by mechanical cutting, have been unsuccessful in providing long-term control of this weed problem. By contrast, the physical removal of turions from the channel in November has significantly reduced the growth of *M. verticillatum* in the following growing season and appears to have the potential to provide long-term control of this weed species in the canals.

As a strategy for control of a major aquatic weed problem in Irish canals, turion removal has a number of important logistical, social and economic benefits over traditional approaches. With turion removal only one weed treatment *per annum* is required. This compares with two separate operations where traditional control is operated. The savings in respect of man-power, transport and equipment costs are considerable. Further significant savings are made as there is no requirement for costly herbicides to be applied to these canals sections.

Turion removal is conducted in November when plant biomass is naturally reduced and when there are fewer demands by the waterways managers on manpower and equipment. Traditional treatment involves herbicide application in May and subsequent weed cutting in July/August. It has been demonstrated that weed harvesting conducted during the summer months can remove large numbers of macroinvertebrates and juvenile fish from a watercourse (Dawson et al., 1991; Unmuth et al., 1998). Studies conducted on Irish canals, however, have shown that the policy of removing obstructive vegetation from only the central navigation channel, while preserving the marginal fringes, minimises this impact (Monahan & Caffrey, 1996). The potential impact on faunal communities in the canals is further reduced when harvesting is conducted in November, when water temperatures are low and faunal activity is limited (Cowx, 2001).

The results from the present study have demonstrated that not only can turion removal effectively control constructive aquatic weed growth in the canals but it can also increase plant species diversity within the channel. The reduced vegetation biomass and the increased macrophytes diversity creates inchannel conditions that favour the establishment of enhanced invertebrate and fish populations (Caffrey, 1993).

In November the canals are closed to navigation and the pursuance of other water-based amenity activities is limited. Weed harvesting conducted at this time, therefore, has little impact on amenity exploitation. By contrast, herbicide application and weed harvesting conducted at the height of the summer season can seriously impact on recreational use of these amenity waterways.

Turion removal for the long-term control of *M. verticillatum* in canals is being adopted by waterways managers in Ireland as a valuable and accepted weed control strategy. Its application over the coming seasons should dramatically reduce weed problems in the canals while also enhancing their appeal among all user groups.

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The potential for biological control of invasive alien aquatic weeds in Europe: a review

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Abstract

A retrospective analysis shows that invasive, alien, free-floating and emergent aquatic weeds in Europe are good targets for classical biological control, and that genus-specific chrysomelid and curculionid beetles offer the most potential. *Ludwigia* spp., *Azolla filiculoides*, *Lemna minuta*, *Crassula helmsii* and *Hydrocotyle ranunculoides* should be prioritised as targets. Fungal pathogens have been under-utilised as classical agents but, whilst they may have some potential against free-floating weeds, they appear to be poor candidates against submerged species, although the suitability of arthropod agents against these difficult targets still merits investigation. The use of indigenous pathogens as inundative agents (mycoherbicides) shows some promise.

Introduction

Biological control can be defined in its simplest form as the use of living organisms for the control of pests. There are several strategies for such control, namely: (1) the inoculative strategy (also known as classical biological control) involving the one-off introduction of a co-evolved, host-specific agent from the area of origin of the invasive pest to provide sustainable control, (2) the augmentative approach, which augments the numbers of a native agent, usually an arthropod, to produce an attack higher than that normally endured by the target, and (3) the inundative strategy, which is mainly applicable to microbial agents and involves the use of a mass-produced formulated product in much the same way as a chemical pesticide. Most products are based on fungal agents and termed mycoherbicides.

This paper deals with classical biological control of invasive aquatic vascular plants (excluding riparian species) which is the strategy usually considered against alien plants (“neophytes”). Presently, classical biological control is facing two challenges. In addition to the obvious need to select effective agents, there is increasing concern that biological control agents (BCAs) may attack non-target indigenous plants. The risks and benefits need to be clearly assessed and consequently the indiscriminant use of non-specific or generalist agents, such as the grass carp and other fishes against aquatic weeds worldwide, cannot be considered as biological control neither in the classical nor the scientific sense. What often is not highlighted is the remarkable safety record of this approach to weed control combined with the high degree of success with both arthropod and fungal agents (McFadyen, 1998; Evans et al., 2001). Only four of the 350 species of insects and pathogens used against 133 alien weed species around the world are known to have established self-sustaining populations on non-target plants and this potential was known and duly assessed at the time of introduction (Blossey et al., 2001). In an analysis of 117 biological control agents used against 55 alien weed species in Hawaii, the continental

United States and the Caribbean since 1902, Pemberton (2000) showed that virtually all the non-target, native plant species that have been attacked by biological control agents are closely related to the target weed species and that their host range was predictable using current protocols. *Teleonemia scrupulosa* Stal, the only insect to be recorded to attack a native plant not related to its host, was released in Hawaii in 1902 without host specificity testing (Pemberton, *loc.cit.*). This would have been revealed had the modern centrifugal phylogenetic protocol been applied (Wapshere, 1974; Goettel et al., 2001). From these and other analyses, herbivorous biological control agents appear to be stable over evolutionary time. The inherent safety of co-evolved natural enemies has also been emphasised by Marohasy (1996) for arthropod agents. A critical analysis of over 600 releases of classical agents revealed that evidence of host expansion or host shift was negligible, and with little or no environmental or economic impact. Moreover, all of these were predictable behavioural responses and not the result of genetic change. Whereas chemical pesticides have been described as "evolutionary evanescent", and consequently not sustainable, classical biocontrol is genetically stable and thus persistent. For arthropod agents, non-target effects have been restricted to close relatives usually within the same genus and this was, in most cases, predicted by host specificity tests. Therefore, an effective way to further minimise the risks is to select target plants for which there are no, or very few, native congeneric species in the area of potential introduction. Of the dozen invasive species each in different genera, which are mentioned here, only five species groups, namely *Lemna minuta* Kunth, *Crassula helmsii* A. Gerger, *Ludwigia* spp., *Hydrocotyle ranunculoides* L.f. and *Myriophyllum aquaticum* (Vell.) Verdc. have any native congeneric species in Europe (Table 1). The number of critical plant species, and therefore the risk, is minimal for water weeds in Europe. Hence, there is considerable potential for safe biological control for these most important and troublesome alien aquatic weeds.

Selection and assessment for biological control

Prioritisation of weeds for biological control requires a balance between the severity of the problem, measured by the ecological, economic and social impacts of the weed, and the potential for its biological control. In turn, the selection of

Table 1. Invasive alien aquatic plants in Europe and congeneric European native species (Tutin et al., 1964)

Invasive alien species	Area of origin	European congeneric species
Free-floating species		
Eichhornia crassipes (Mart.) Solms	South America	None
Salvinia molesta Mitchell	South America	*S. natans* (L.) All.
Azolla filiculoides Lam.	South America	None
Lemna minuta Kunth	North America	*L. gibba* L., *L. minor* L., *L. trisulca* L.
Emergent species		
Ludwigia spp.	South America	*L. palustris* (L.) Elliott
Crassula helmsii A. Berger	Australia/New Zealand	*C. tillaea* Lester-Garland, *C. aquatica* (L.) Schönl., *C. vaillantii* (Willd.) Roth
Paspalum paspalodes (Michx) Schribner	Pantropical	None
Hydrocotyle ranunculoides L.f.	North America	*H. vulgaris* L.
Myriophyllum aquaticum (Vell.) Verdc.	South America	*M. alternifolium* DC., *M. spicatum* L., *M. verticillatum* L.
Submerged species		
Elodea canadensis Michx	North America	None
E. nuttallii (Planch.) St. John	North America	None
Lagarosiphon major (Ridley) Moss	South Africa	None
Egeria densa Planch.	South America	None

agents for biological control of weeds has often been a subject of debate, but no set criteria have been widely adopted. The task of defining satisfactory and practical selection criteria, applicable to a range of potential agents and weeds in different eco-climatic zones, is very difficult. However, in a retrospective application of the rating systems designed principally for arthropod agents of terrestrial weeds, and further modified to select new biological control agents for aquatic weeds, Forno & Julien (2000) found that irrespective of the criteria set, the same agent received the highest rank for each weed and that all of these, except those on *Hydrilla verticillata* (L.f.) Royle, have been effective control agents. The criteria used were: damage by adults and immature stages, duration of attack and the number of generations per year. Not surprisingly, beetles in the family Chrysomelidae and Curculionidae received the highest scores and were the dominant taxa in successful programmes against biological control of aquatic weeds, as they are for terrestrial herbaceous plants (Crawley, 1989; Gassmann, 1995). Whilst no such comparative analysis has been undertaken with respect to plant pathogens, no doubt due to their novelty and thus, the relatively small number of agents screened, rusts and smuts would undoubtedly receive the highest score because of their narrow host range, efficient dispersal mechanisms and the high profile damage inflicted on their hosts, as exemplified by cereal rusts in agricultural systems.

Nearly 20 years ago, Wapshere (1985) emphasised the importance of ecological similarities between the native and proposed introduced ranges when selecting potential BCAs. It has been argued that there is probably less variation in the climate of surface and submerged water habitats between climate zones than for terrestrial habitats over the same range (Forno & Julien, 2000). However, other ecological factors may play an important role such as water nutrient status, seasonal variations and local hydrology. For example, the larvae of the weevil species *Bagous affinis* Hustache feed on *Hydrilla verticillata* tubers only when water recedes to expose the plants, a situation that occurs in Pakistan where the weevil was collected, but is rare in Florida where it was released, so that it was difficult to establish field populations (Forno & Julien *loc.cit.*). This emphasises the importance of comprehensive field observations of both the target plant as well as the potential agent, when surveying in the area of origin.

The post-release impact of BCAs is almost as difficult to assess as their potential effectiveness prior to release. As stressed by Harris (1991), it is important to distinguish between biological success, impact on the host plant population and control success. Biological success is in increasing order, a measure of the establishment of the agent, a measure of how the agent is using the plant resources and its ability to sustain high population densities. The impact on the host plant population is a measure of the decrease in the reproduction or biomass of the weed population at sites favourable to the agent whilst, control success reflects economic, environmental and human gains following a reduction in the weed population. Assessment of overall success rates is even more difficult and can vary by a several-fold magnitude when the geographical scale is considered, as for example programme assessment at a regional, national or global scale (Crawley, 1989; Lawton, 1990; McFadyen, 1998).

Biological control of invasive alien aquatic plants

Classical biological control of invasive alien aquatic plants began in the early 1960s with programmes against *Alternanthera philoxeroides* (Mart.) Griseb. and *Eichhornia crassipes* (Mart.) Solms. It is clear that the success rate with invasive aquatic plants has been very high. Past experience shows that both floating and emergent weeds are good targets for successful control as evidenced by the programmes against *Salvinia molesta* Mitchell, *Pistia stratiotes* L., *Azolla filiculiodes* Lam., *Eichhornia crassipes*, and *Alternanthera philoxeroides*, the latter being the sole emergent species targeted until now. However, the submerged species seem to be more difficult targets (i.e., *Hydrilla verticillata*, *Myriophyllum aquaticum* and *M. spicatum* L.). A survey of pathogens of *M. spicatum* in Europe yielded inconclusive results (Harvey & Varley, 1996). Experience also shows that the most important successes have been reported with insects of the weevil and leaf beetle families. The augmentation approach using native or adventive insect species, as well as fungal pathogens as mycoherbicides, has been used against Eurasian watermilfoil, *M. spicatum* and *Hydrilla verticillata* in North America and *Ludwigia ascendens* (L.) Hara in Thailand with some occasional and local success (Napompeth, 1990; Verma & Charudattan, 1993; Creed & Shelton, 1995; Harvey & Varley, 1996; Shearer, 1998). It is interesting to note that the North American weevil *Euhrychiopsis lecontei* (Dietz) may be an important agent in promoting the decline of Eurasian watermilfoil in its most northern range after its natural host shift from the native North American northern watermilfoil *Myriophyllum sibiricum* Kom. This is a rare example of natural control by a native agent which has an impact on an exotic species for which there is evidence of better performance than on the native host (Newman et al., 1997).

The following section details some biological control programmes against aquatic weeds worldwide and highlights the potential for biological control of such species in Europe in the light of previous experience and knowledge of the natural enemy complex known for selected species. *Eichhornia crassipes*, water hyacinth is a free-floating species native to the Amazon basin. It has become widespread throughout the tropical and subtropical regions of the world and is described as the world's worst aquatic weed (Hill & Cilliers, 1999). It is now spreading to more temperate areas such as in Europe and South Africa. Successful control against *E. crassipes* has been recorded at some sites in 12 out of 32 countries (Forno & Julien *loc.cit.*). To date, water hyacinth has been best controlled in areas where the weed is free to sink once damaged, and not subjected to regular removal by periodic or annual flows, or mechanical and herbicide treatments. Five insects and one mite have been released against water hyacinth around the world (Julien, 2001). Where biological control of water hyacinth has been successful it was either as a result of the activities of the South American weevils *Neochetina eichhorniae* Warner or *N. bruchi* Hustache or both, with little or no contribution from the other four arthropods released. *Neochetina eichhorniae* is less dependent on good quality plants for development than *N. bruchi* which is well adapted to nutrient-enriched water. This species has also been shown to be cold-tolerant, highlighting the potential importance of *N. bruchi* for future programmes in Europe. Biological control of water hyacinth has not been successful in all of the weed's habitats and other potential agents are being considered, including the use in Africa of indigenous pathogens in the form of a mycoherbicide (Julien *loc.cit.*; http:/www.IMPECCA.net), as well as co-evolved pathogens from South America (Evans & Reeder, 2000). It is important that management strategies are developed integrating biological control with other control techniques and using biological control as the base component to underpin the programme.

Azolla filiculoides is a free-floating fern native to South America. With the exception of Europe, several generalist herbivorous insect species have been recorded in the exotic range of the plant worldwide, e.g. in the USA, India, or the Philippines (Hill, 1999), but it appears that little work has been done on *Azolla* in its native range in tropical America. Two genus-specific insects have been discovered in North America. The weevil *Stenopelmus rufinasus* Gyll. is indigenous to the southern and western USA where it occurs on *A. caroliniana* Willd. and *A. filiculoides* (Hill, *loc.cit.*), however, it has also been collected on *A. filiculoides* in South America. This weevil, imported both from Florida and Argentina, has been very successful against *A. filiculiodes* in South Africa where it has been able to survive the cold winters of the eastern Free State (Hill, *loc.cit.*). The flea beetle *Pseudolampsis guttata* (LeConte) is also native to southern USA. It was not released in South Africa because of concerns about attack on native *Azolla* species in the country and because of the successful control with *S. rufinasus*. This is not a concern in Europe where no native *Azolla* species occur. The weevil *Stenopelmus rufinasus* is adventive in UK but it does not appear to have had the same impact on the target plant as it does in South Africa. However, a massive outbreak of the weevil and the subsequent decline/eradication of the fern in more southerly regions of the UK in 2002 does belie this perception. Further genetic analysis of both the insect and the host plant would help in understanding the origin and type of association between these organisms in Europe.

With the exception of the shore-fly *Lemnaphila scotlandae* which has been reported as attacking *Lemna* species in Florida (Buckingham, 1989), little is known about the natural enemies of the free-floating duckweed *Lemna minuta* in its natural range in North America. The weevil, *Tanysphyrus lemnae* Fab., which is already present in the British Isles, creates transparent leaves similar to those caused by *L. scotlandae*. Since *L. minuta* has been observed replacing *A. filiculoides* in the field (Janes, 1995), it would be appropriate to target both species at the same time.

The taxonomy of *Ludwigia grandiflora* (Mich.) Greut. & Burd., a native of South America, is apparently confusing. The plant is described under various names, or it may consist of a complex of species. *Ludwigia peploides* (Kunth) Raven from North America is also naturalised in Europe. *Lysathia ludoviciana* (Fall.) (Chrysomelidae) has been identified as a potential biological control agent of *L. grandiflora* in the southeastern USA (McGregor et al., 1996) but little is known on the natural enemies associated with *Ludwigia* spp. in their native range.

Myriophyllum aquaticum is native to South America and produces both submerged and emergent leaves. It is invasive not only in Europe but also to a certain extent in North America and in South Africa (Cilliers, 1999). Several biological control agents have already been found in South America. The most promising is a leaf-feeding beetle from Argentina that turned out to be an undescribed species of the genus *Lysathia*. *Lysathia* n.sp. (previously known as *L. flavipes* (Boh.) was introduced in South Africa in 1994 (Cilliers, *loc.-cit.*). The results are encouraging and at one site the water surface that was covered by *M. aquaticum* declined by more than 50% over 3 years (Cilliers, *loc.cit.*). However, in general, *M. aquaticum* plants are able to recover after suffering severe levels of herbivore damage. This ability, together with the tendency of the beetles to migrate from heavily damaged plants to areas where healthy plants occur, allows the weed population to recover. A stem-boring weevil, *Listronotus marginicollis* (Hustache) from Argentina is a promising candidate to supplement the damage caused by *Lysathia* sp.

The case of *Hydrocotyle ranunculoides*, which is spreading very rapidly despite concerted control efforts, is fairly promising. Cordo et al. (1982) refer to a South American weevil (recently renamed *Listronotus elongatus* Hustache) with an apparently restricted host range damaging this floating aquatic. Nonetheless little is known about the complex of arthropod or fungal natural enemies associated with other invasive species in Europe in their native range such as *Crassula helmsii*, *Elodea* spp., *Lagarosiphon major* (Ridley) Moss and *Egeria densa* Planch.

Conclusion

Whilst there are no reliable methods for predicting the impact or success of biological control agents, a retrospective analysis shows that free-floating and emergent aquatic plants with genus-specific species of chrysomelid and curculionid beetles should be the first choice for biological control programmes in Europe. This fact should encourage European countries to embark on biological control of invasive aquatic species with priority given to *Ludwigia* spp., and *Azolla filiculoides* and other species, such as *Crassula helmsii* or *Lemna minuta*, for which very little is known in their respective native range. Species in the genus *Myriophyllum* appear to be relatively difficult targets, although the results obtained with *M. spicatum* and *M. aquaticum* in North America and in South Africa, respectively, are encouraging. Submerged species such as *Elodea canadensis* Michx, *E. nuttallii* (Planch.) St. John, *Lagarosiphon major*, *Egeria densa* could be more difficult targets but, as long as their complex of natural enemies remains unknown, it would be premature to discount any of them as potential targets for classical biological control.

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Factors influencing the distribution of *Hydrocharis morsus-ranae* L. and *Rumex hydrolapathum* Huds. in a mowed low-lying marshland, Réserve de Cheyres, lac de Neuchâtel, Switzerland

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Key words: reed beds, modelling, mowing, ruts, species distribution

Abstract

Using environmental parameters we studied the distribution of two endangered species, *Hydrocharis morsus-ranae* L. and *Rumex hydrolapathum* Huds., in a low-lying marshland of the Swiss Plateau, a region in which aquatic vegetation is particularly threatened. A large part of the study site is regularly mown by a machine for site management purposes. The caterpillar mower digs ruts, which are especially pronounced along the tracks used to reach the mown compartment. To assess the effects of site management on these species, we have tested six environmental parameters (vegetation unit, water conductivity, water supply indicated by altitude, time elapsed since the last cut, distance from major ruts and disturbance of major ruts) that can potentially influence plant distribution. All the plots of these two species have been found in mowed compartment, which seems to indicate a correlation between site management operations and occurrences. The other factors driving the distribution of these plants are vegetation unit, distance from major ruts and water supply.

Introduction

Many wetland plant and animal species are threatened with extinction at the regional, national or even European level. A total of 66% of the extinctions of continental species involve wetlands (Denny, 1994). On the Swiss Plateau the situation for aquatic vegetation is very grave, with more than 60% of the species on the Red List of threatened ferns and flowering plants of Switzerland (Moser et al., 2002). The principal reason for this is the destruction of habitats. In this context, remaining wetlands have to be protected. Maintenance operations are carried out to control invasion by shrubs and to prevent the degradation of these valuable natural environments. The open marshes of the study area are mown using a machine with caterpillar tracks. These tracks mark the ground with more or less major ruts, dependent on the frequency of passage and on the nature of the substrate. With a view to maintenance of the wetlands and conservation of rare species, our principal objective is to assess the impact of management on the presence and abundance of two endangered species: *Hydrocharis morsus-ranae* and *Rumex hydrolapathum*. This depends upon detailed prior knowledge on the occurrence sites of the two plants in the study area, and their status at each station. Many environmental parameters can potentially influence the distribution of the two species. We have tried to identify which factors are involved by using a geographical information system (GIS) in conjunction with modelling tools, on a data set covering six environmental parameters: type of vegetation, type of water supply indicated by altitude, electric conductivity of water, time elapsed since the last cut, distance from major mowing ruts and disturbance frequency of the major ruts.

Description of the study site and of the management operations

The 0.98 km^2 study area is at an average altitude of 430 m, in a protected part of the Grande Cariçaie wetland, on the southern shore of Neuchâtel Lake (Switzerland), known as the Cheyres nature reserve. It includes a strip of 0.59 km^2 of open marshland approximately 2 km long and 300–500 m broad, which skirts the lake edge. This zone is above the level exposed to wave action and is out of its direct influence (Buttler et al., 1995). Water supply is primarily runoff from cliffs dominating the marsh and also surface streams and rainfall (Cuccudoro, 1990 in Buttler et al., 1995). The substrate is sand and silts, replaced locally by the underlying molassic bedrock.

Mowing of compartment of open marshes by the crawler-mounted machine Elbotel constitutes the principal management operation. The open marsh is cut in 15 adjacent compartments disposed along the banks, each of 0.02–0.05 km^2. They are mown in rotation, such that each is cut every third year, with 4–5 of them cut each year. One compartment is used as an uncut control. To access to compartment distant of one of the two service roads, the machine must pass trough adjacent compartment. This repeated passage digs major ruts with a higher disturbance frequency and often deeper than the lateral minor ruts let by a single moving passage per 3 year.

Materials and methods

The nomenclature employed here follows that of Aeschimann & Burdet (1994). The sites at which the two plants occurred were plotted and charted along parallel transect evenly space out each 5 m throughout the whole study area, between August 15 and October 25, 2000. The boundaries of the plot correspond to the limit of colonisation of the species. Inside each plot, the mean cover was estimated according to the Braun–Blanquet scale. The individual isolated were recorded in the class 1 (see Table 1).

To describe the vegetation, we used the vegetation map of the southern shore of Neuchâtel Lake (Clerc, 2003), merging categories as necessary, according to the standardised typology of Delarze et al. (1998). Water conductivity characterises water bodies by their content of dissolved salts and gives indirect information on nutrient content, conductivity being positively correlated with concentration of phosphorus (Wang & Yin, 1997). We measured conductivity two time in 3 days at 120 randomly chosen points, following Jenness (2001), but with 50% of the sample points located at sites where species occurred (30 points each). Variations in altitude, which indicates the discharge direction and persistence of water in the marsh, were given using a series of 608 points determined by photogrammetry and distributed in a zone projecting the study area. Series of measurement points have been interpolated in the GIS to generate a continuous layer of information from the point measurements. Results derived from interpolation were assembled into four classes. The mowing cycle indicates the number of years since the last mowing operation. The distance to major ruts allows quantification of the proportion of plots located in these ruts and their range of influence in the lateral minor ruts. The disturbance of major ruts gives the frequency of passage of the mower during a complete management cycle (3 years). This value increases with the proximity of a service road because the rut is more often use to deserve remote compartment. An information field has been created in the GIS

Table 1. Braun–Blanquet cover scale

Braun–Blanquet cover scale	Interval of covering (%)	Class
+	<1	1
1	1–5	2
2	6–25	3
3	26–50	4
4	51–75	5
5	<75	6

for each of the six environmental parameter, the classifications are given in Table 2.

We model the distribution of plots where the target species occurred with generalised additive models (GAM). Characteristics of GAM are explained in Hastie & Tibshirani (1990), Bio et al. (1998) and Lehmann (1998). Regression is mainly data-driven and explanatory variables are smoothed by a spline function instead of depending on a with-priori model. Three parameter diagnoses are used to evaluate quality of models (Hosmer & Lemeshow, 1989): proportion of deviance explained by the model (D^2), coefficient of correlation (val) and coefficient of correlation between the values predicted and observed, obtained by cross validation (cross-val), the model being calculated on 5/6th of the data and tested on the remaining 1/6th. This modelling was carried out with the software GRASP (Generalised Regression Analysis and Spatial Prediction) developed by Lehmann et al. (2002). The table of explanatory variables used for modelling was extracted from the GIS. It is composed of 197 lines, each corresponding to either the centroid of a plot of occurrence of one of the species or to a point from which the species were absent and at which water conductivity has been measured. Its composition is shown in Table 3.

Results and discussion

The complete cartography of the occurrences of the two species for the whole study site allow us to enumerate 68 plots of *Hydrocharis morsus-ranae* (sized from 2 to 680 m^2) and 65 plots of *Rumex hydrolapathum* (sized from 1 to 328 m^2). The two species only occur in the mowed area.

The incidence of *Hydrocharis morsus-ranae* is associated with mainly two vegetation unit (Fig. 1): Phragmition (class 1, Table 2) in particular its variant with *Typha* sp., and Magnocaricion (class 3, Table 2), more specifically meadows with *Carex elata* and *C. panicea*, mixed with *Cladium mariscus*. This preferential localisation is related to the persistence of water throughout the growing season in this particular environment. For the same reason, occurrences of *Hydrocharis* are more frequent than absences in the lowest part of the marsh, which is most frequently inundated (altitude classes 1 and 2, Table 2). This plant is preferentially localised in water with a high nutrient content, indicated here by a high conductivity (classes 3 and 4, Table 2). Because of the digging action caused by the repeated passage of the mower, the major ruts are depressed and accumulate water. For this reason occurrences in major ruts are more frequent than absences. The major ruts provide the larger surfaces colonised by *H. morsus-ranae*, 65.5% of the colonised surface (determined from the complete cartography) representing 38% of the plots are located in major ruts and their direct neighbourhood (classes 1 and 2 in Table 2). Interpretation of the influence of the mowing cycle is more difficult. It would require data from the complete rotation cycle of 3 years, rather than from 1 year as available to this study. For interpreting the effects of the disturbance of major ruts, the situation is different,

Table 2. Unit, interval and frequency classes of the six environmental parameters

Vegetation unit		Conductivity		Altitude		Mowing cycle		Distance to major ruts		Disturbance of major ruts	
Unit	Class	Interval (µS/cm)	Class	Interval (m)	Class	Years elapsed	Class	Interval (m)	Class	Passage/3 years	Class
Phragmition	1	≤300	1	428–429.7	1	Pilot zone	0	0	1	0 (minor ruts)	1
Phalaridion	2	301–500	2	>429.7–429.9	2	2 years	1	0–5	2	2	2
Magnocaricion	3	501–600	3	>429.9–430.4	3	1 year	2	>5–15	3	4	3
Caricion davallianae	4	>600	4	>430.4	4	0 year	3	>15–30	4	6	4
Glycerio-Sparganion	5							>30–50	5	8	5
								>50	6	10	6

Table 3. Description of the table of explanatory parameters (legend of classes see Table 2)

Vegetation unit			Conductivity			Altitude			Mowing cycle			Distance to major ruts			Disturbance of major ruts		
Class	*n*	%	Class	*n*	%	Class	*n*	%	Class	*n*	%	Class	*n*	%	Class	*n*	%
1	43	21.8	1	8	4	1	37	18.8	0	3	1.5	1	37	18.8	1	160	81.2
2	49	24.9	2	45	23	2	69	35.0	1	58	29.4	2	26	13.2	2	5	2.5
3	77	39.1	3	86	44	3	71	36.0	2	73	37.1	3	26	13.2	3	11	5.6
4	17	8.6	4	58	29	4	20	10.2	3	63	32.0	4	23	11.7	4	5	2.5
5	11	5.6										5	23	11.7	5	12	6.1
												6	62	31.5	6	4	2.0
n =	197			197			197			197			197			197	

because frequency of disturbance is constant through the 3-year management cycle. Nevertheless, the real effect of this parameter is difficult to assess, because it must be dissociated from under-representation of particular frequency of disturbance. It shows essentially that occurrence of the plant is possible at every level of disturbance. The Generalised Additive Models (GAM) of presence/absence as well as GAM of abundance are driven by three explanatory parameters which explain more than 60% of the distribution: the distance to the major ruts, the vegetation unit and the altitude. The contribution of each variable is described in Table 4 and response curves are given in Figure 2. The two models predict a positive correlation between occurrences and a buffer area of 30 m

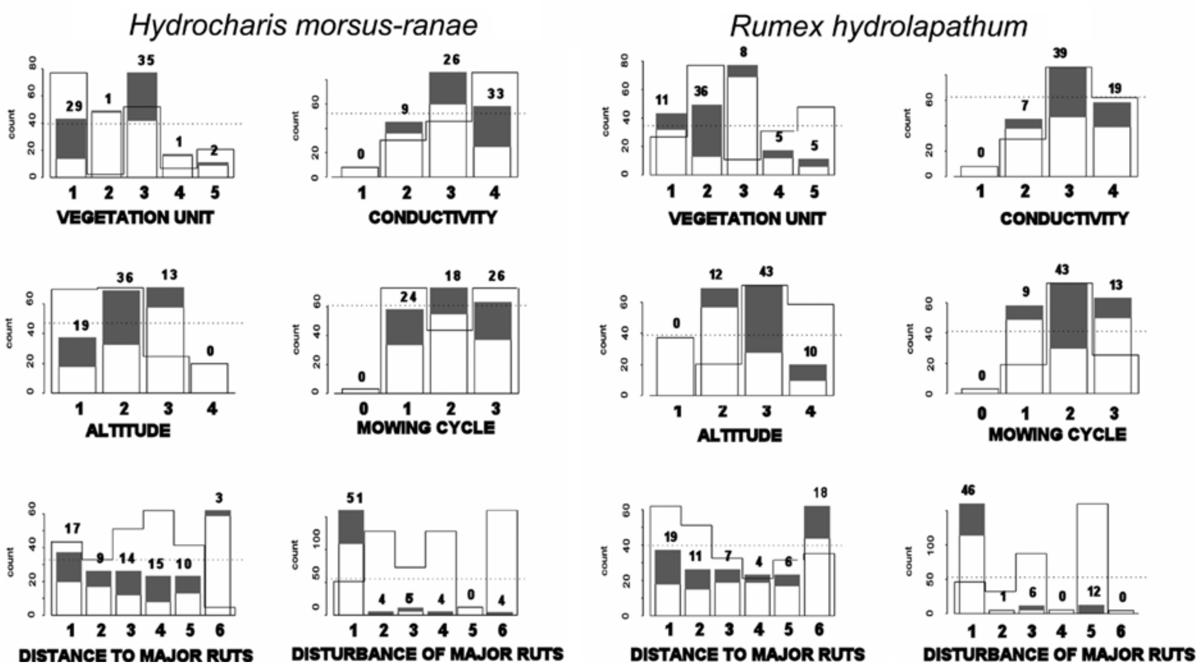

Figure 1. Histogram of the presences/absences (n = 197). (On the left *Hydrocharis morsus-ranae*; on the right *Rumex hydrolapathum*.) The legends of the classes are given in Table 1. White portion of histograms indicates absences. The black portion, as well as the number printed above the column, indicates the number of plots where the species is present. The continuous line indicates proportion of presence, compared to the whole of the plots: when it passes above the dotted line, the ratio of this attribute for the particular species is higher than in the whole sample.

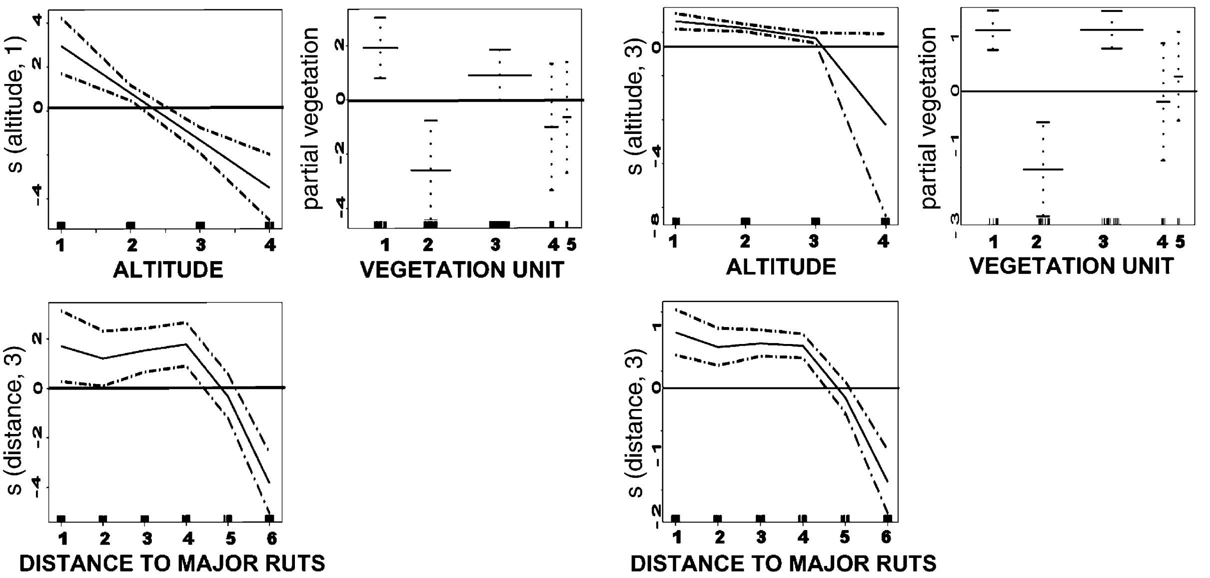

Figure 2. Response curves for the variables incorporated in presence/absence GAM (left) and in abundance GAM (right) for *Hydrocharis morsus-ranae*. On *X*-axes the classes and on *Y*-axes the response of the model. Unities of *Y*-axis are smoothed function of order gave in the parenthesis. Response curves are given by the black lines and intervals of confidence by the dot lines. The horizontal continuous black lines indicate the limit between negative effect (below) and positive effect (above) on the species occurrence.

around the major ruts. This relation can be partly explained by the colonisation of the minor lateral ruts from the large plots located in major ruts. Colonisation of the minor ruts is limited by the persistence of water, which increase with the proximity of the major ruts. There is also a positive correlation between occurrence and the two preferential vegetation unit cited above. On the other hand there is a negative correlation between occurrences and altitude, which is related to the water supply.

Our results relate the occurrence of *Rumex hydrolapathum* to the presence of two plant associations (Fig. 1): principally the Phalaridion (class 2, Table 2) and in less importance the Glycerio Sparganion (class 5, Table 2). *R. hydrolapathum* can colonise ground subjected to various hydrological regimes (Landolt, 1977), and for this reason, it is present in the upper, less regularly flooded part of the study area (altitude classes 3 and 4, Table 2). Its plots are recorded primarily in water with a conductivity higher than 500 μS/cm (Fig. 1). As in the case of *H. morsus-ranae*, assessment of the influence of the mowing cycle requires field information for a complete 3-year cycle. Concerning distance from ruts, the species is more frequently recorded within a radius of 5 m from the ruts (Fig. 1). Interpretation of the disturbance frequency data suffers from the same limitation as identified in considering *H. morsus-ranae*, but the rut of class disturbance 5 (containing only plots where the plant was present) nevertheless support a particular type of Phalaridion, pioneer vegetation with *Alisma plantago-aquatica* favoured by a high disturbance frequency. This class of disturbance is correlated positively with the abundance of *R. hydrolapathum* at the sites. The general additive model of presence/absence is driven by five explanatory parameters: vegetation unit, mowing cycle, water conductivity, altitude and distance to major ruts. Approximately 40% of the distribution is explained by this model. Individual contributions are given in Table 4 and response curves are plotted in Figure 3. The model predicts a positive correlation of the occurrences of *R. hydrolapathum* with the two vegetation unit cited above and also with conductivity and altitude. Because of its large confidence interval, the model predicts possible occurrence at every distance from the major ruts. The abundance model also shows a poor level of explained deviation. It retains altitude and the same vegetation unit as the presence/absence GAM, as positively correlated with abundance

Table 4. Characteristics of the GAM models (PA – presence/absence; REC – covering; hydroch – *Hydrocharis morsus-ranae*; rumex – *Rumex hydrolapathum*) $n = 197$. Values for each parameter quantify the drop contribution (correspond to the loss of significance of the model without this parameter). D^2, validation and cross-validation are the diagnostic parameters of model. Variables without drop contribution were not retained in the model

Model	PA hydroch	REC hydroch	PA rumex	REC rumex
p	< 0.004	< 0.004	< 0.004	< 0.004
Vegetation	36	69.56	25.91	26.89
Conductivity			10.36	
Altitude	38.45	41.32	9.29	52.33
Mowing cycle			24.05	
Distance to major ruts	79.45	110.88	6.28	
Disturbance of major ruts				11.54
D^2	0.63	0.65	0.45	0.42
val	0.96	0.8	0.92	0.61
cross-val	0.94	0.77	0.86	0.5

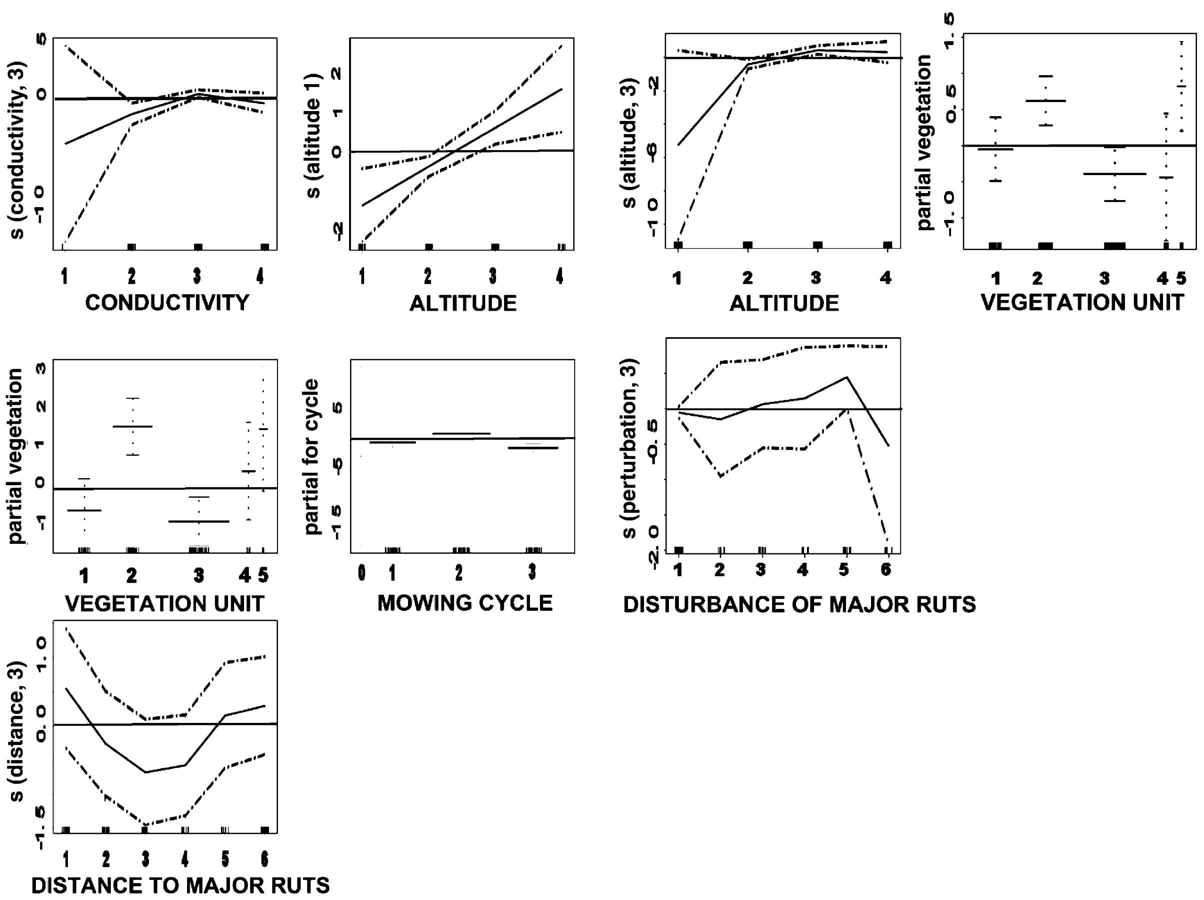

Figure 3. Response curves for the variables incorporated in presence/absence GAM (left) and in abundance GAM (right) for *Rumex hydrolapathum*. On *X*-axes the classes and on *Y*-axes the response of the model. Unities of *Y*-axis are smoothed function of order gave in the parenthesis. Response curves are given by the black lines and intervals of confidence by the dot lines. The horizontal continuous black lines indicate the limit between negative effect (below) and positive effect (above) on the species occurrence.

(Fig. 3). For modelling the distribution of *Rumex hydrolapathum* more accurately explanatory parameters, like soil chemistry or sediment structure, probably require to be brought into play.

The fact that neither of the occurrence plots of one of the two species were observed in the unmowed pilot zone suggest that the survival of the two species at the site may be dependent upon maintenance of open areas of marsh.

Conclusions

The results of this work show up that the mowing of the marshes does not seem to have a negative impact on *Hydrocharis morsus-ranae* and *Rumex hydrolapathum*, the two species are able to colonise the ruts and occur even at the most recently mowed plots. The repeated rutting of the major track servicing the compartments has even a positive impact on the target species. With the management programme currently operated, it appears that the populations of *Hydrocharis morsus-ranae* and *Rumex hydrolapathum* are not threatened with disappearance in the short-term.

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Evaluating the necessity of additional aquatic plant testing by comparing the sensitivities of different species

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Key words: sensitivity, aquatic plants, herbicides, risk assessment

Abstract

At present, at least three and up to five plant species are required to assess the potential risks of herbicides to non-target aquatic plants. Several regulatory authorities are considering whether there should be further requirements based on concerns about the possible selectivity of herbicides (e.g., specific modes of action against dicotyledonous plants). The relative sensitivity of a range of aquatic plants is assessed in our work in order to evaluate the implications of differences in species sensitivity for aquatic risk assessment of herbicides. We therefore present results from ecotoxicological tests performed at Syngenta Crop Protection AG on various aquatic plants and compare them to available studies and results in literature. The criterion used for sensitivity ranking is the EC50 (median effect concentration) value, which allows a better comparison of values from different testing methods and conditions. The overall results obtained in the present work show that the aquatic risk assessment procedure for herbicides based on *Lemna* sp. and algae is sufficiently protective while identifying potential toxicity to non-target plants. Only few exceptions concerning herbicides with selective modes of action (e.g., auxin simulators) may require additional species testing for proper risk assessment.

Introduction

The preliminary risk assessment procedure for assessing the effects of herbicides on non-target aquatic plants requires data from a whole series of test organisms. For the EU, two species of freshwater algae (a green algae such as *Selenastrum capricornutum* Printz and a species from another taxonomic group e.g., the blue-green alga *Anabaena flos-aquae* (Lyngb) Bréb.) are tested. In addition, for US registration, data are required for a freshwater diatom (e.g., *Naviculla pelliculosa* (Bréb.) Hilse) and a marine alga (e.g., the diatom *Skeletonema costatum* Grev.). For both the EU and the US, data on the aquatic macrophyte *Lemna* sp. L. are also required.

A number of regulatory authorities are currently considering whether such a database is sufficient to adequately assess potential risks to aquatic plants. For example, the US EPA (2001) published a 'Proposal to Update Non-Target Plant Toxicity Testing under NAFTA' and concluded that aquatic macrophytes are under-represented. They propose that the number of vascular plants should be increased.

While evaluating the necessity for further aquatic plant testing, one should consider applying an appropriate uncertainty factor which could lead to a risk assessment that is sufficient to protect from harmful effects under field conditions.

In order to assess the potential impact of differences in species sensitivity on aquatic risk assessment for non-target aquatic plants, available data from our laboratory and from the literature were reviewed.

Materials and methods

Algal and macrophyte species used in the sensitivity ranking at Table 2 are presented by full name in Table 1.

A description of the various methods investigating the toxicity of herbicides on non-target aquatic plants can be found in Table 2. The complete methodology of the experiments taken from literature can be found in Fairchild et al. (1998), Green & Westerdahl (1990) and Netherland & Getsinger (1992). The toxicity values referring to algae and *Lemna* sp. for the auxin simulating compounds are taken from Brock et al. (2000) and are based on the geometric mean of the available results in literature for each species. Toxicity values from required test species of the preliminary risk assessment (algae and *Lemna* sp.) obtained at Syngenta Crop Protection AG are performed according to existing guidelines (ASTM, 1995; FIFRA, 1989a, b; OECD, 1984) or draft documents (OECD, 2001) already in discussion.

Table 1. Algal and macrophyte species compared in the sensitivity ranking

Algae tested	Macrophytes tested
Anabaena flos-aquae (Lyngb) Bréb.	*Ceratophyllum demersum* L.
Chlamydomonas reinhardii Dangeard	*Elodea canadensis* Michx.
Chlorella pyrenoidosa Chick	*Egeria densa* Planch.
Chlorella vulgaris Beijer.	*Glyceria maxima* (Hartm.) Holmb.
Microcystis sp. Kütz.	*Hydrilla verticillata* (L.f.) Royle
Navicula pelliculosa (Bréb.) Hilse	*Lemna gibba* L.
Scenedesmus quadricauda (Turp.) Bréb.	*Lemna minor* L.
Scenedesmus subspicatus Chodat	*Myriophyllum heterophyllum* Michx.
Selenastrum capricornutum Printz	*Myriophyllum spicatum* L.
Skeletonema costatum Grev.	*Myriophyllum verticillatum* L.
	Najas sp. L.
	Potamogeton densus L.

Results

Syngenta test results presented in Table 3 were performed in-house during the last months. A wider classification of the compounds than the one recommended by HRAC was performed based on their mode of action. The most sensitive species in the selected studies was identified and highlighted. The source of the data and the mode of action of specific compounds as well as a sensitivity ranking for the tested species based on median effect concentrations (EC50) were reported for each compound. The most sensitive species tested was identified, and taking this as a reference, the test species were grouped accordingly. Groupings were made according to EC50 values that differed by less than five times, less than 10 times and more than 10 times from the most sensitive species tested.

Discussion

Comparing plant species across the different experiments, the range of sensitivities identified show that there is no one plant species that is always the most sensitive, even for compounds with the same mode of action. It should also be noted that differences in the testing method (e.g., emerged, submersed or rooted form of the plant in the test, temperature, test medium, pH, light intensity) or changes in the application method can lead to substantial differences in the values recorded as test endpoints. In the sensitivity ranking shown here, this problem is partly avoided because the comparison among species is made in the same study with the same testing method (see Fairchild et al., 1998 and Syngenta results). The difficulties related to data comparison between tests can be exemplified by the study of Fairchild et al. (1998), in which every alga was tested for 96 h with chlorophyll fluorescence as an endpoint while the duckweed *Lemna* sp. was tested over 96 h with the frond count as test endpoint.

Table 2. Experimental conditions for algae and vascular plants testing used in different studies

Species tested	Duration (in hours)	Photoperiod (light/dark)	Temp. (in °C)	Medium	Endpoints	Remarks	Reference
Algae	96-h	16:8	25	1× ASTM[a]	Chlorophyll fluorescence		
Duckweed (*L. minor*)	96-h	16:8	25	10× ASTM[a]	Frond counts		Fairchild et al. (1998)
Macrophytes	14 d	16:8	25	1× ASTM[a]	Wet weight increase	With sediment	
C. pyrenoidosa	120-h	n.d.	n.d.	n.d.	Growth		
C. reinhardii	192-h	n.d.	n.d.	n.d.	Growth		After Brock et al. (2000)
S. capricornutum	96/120-h	n.d.	n.d.	n.d.	Growth		
L. minor	4 d	n.d.	n.d.	n.d.	Frond counts		
M. spicatum	72-h	13:11	21	Simulated hard water	Biomass (dry weight)	With sediment	Green & Westerdahl (1990)
M. spicatum	84-h	14:10	22	Simulated hard water	Biomass (dry weight)	With sediment	Netherland & Getsinger (1992)
Blue-green algae/diatoms[a]	96-h	Continuous	22–24/18–22	1× ASTM[a]	Growth		Syngenta
Green algae[b]	72-h	Continuous	20–25	1× OECD[b]	Growth		Syngenta
L. gibba G3[c]	7 d/14 d	Continuous	24	20X-AAP[c]	Frond counts/dry frond weight		Syngenta
L. minor	7 d/14 d[d]	Continuous/12:12[d]	24/20[d]	20X-AAP[c]/tap water[d]	Frond counts/biomass[d]		Syngenta
E. canadensis	21 d	14:10	23	M4-medium[e]	Length, biomass	with sediment	Syngenta
E. densa	14 d	12:12	20	Tap water	Length, wet/dry weight	small scale microcosm	Syngenta
G. maxima	14 d	16:8	15	Tap water	Growth, wet/dry weight		Syngenta
P. densus, *E. canadensis*, *H. verticillata*, *M. spicatum*, *M. verticillatum*	4 d	12:12	20	Mod. Gerloff-medium[f]	Conductivity, oxygen saturation, pH		Syngenta

[a] After ASTM-guideline E1218–90.
[b] After OECD-guideline No. 201.
[c] After FIFRA-guidelines No. 122-2 and 123-2, and OECD guideline draft.
[d] Small scale microcosm study (see *Egeria densa*).
[e] See Elendt (1990). [f] See Selim et al. (1989). n.d. not described.

Table 3. Species sensitivity ranking for different herbicides with relation to their database and mode of action (vascular plants, *algae*); (required test species for preliminary risk assessment procedure (grey); most sensitive species (underlined))

Mode of action (Inhibition of)	Species sensitivity ranking					Reference
	Mean effective concentrations (EC50) differing:					
	< five times		> five times		> > 10 times	
Photosynthetic electron transport (compound 1)	C. demersum *C. vulgaris* E. canadensis L. minor *Microcystis* sp. Najas sp.	>	*C. reinhardii* M. heterophyllum *S. quadricauda* *S. capricornutum*	> >	*A. flos-aquae*	Fairchild et al. 1998
Photosynthetic electron transport (compound 2)	C. demersum *C. reinhardii* *C. vulgaris* E. canadensis L. minor M. heterophyllum Najas sp. *S. capricornutum*	>	*Microcystis* sp. *S. quadricauda*	> >	*A. flos-aquae*	Fairchild et al. 1998
Cell division	C. demersum *C. vulgaris* L. minor Najas sp. *S. capricornutum*			> >	*A. flos-aquae* *C. reinhardii* E. canadensis *Microcystis* sp. M. heterophyllum *S. quadricauda*	Fairchild et al. 1998
Auxin simulators (compound 1)	M. spicatum			> >	*C. pyrenoidosa* *C. reinhardii* L. minor *S. capricornutum*	Green & Westerdahl 1990 / Brock *et al.* 2000
Auxin simulators (compound 2)	M. spicatum			> >	*S. capricornutum*	Netherland & Getsinger 1992 / Brock et al. 2000
Amino acid synthesis (compound 1)	L. gibba G3			> >	*A. flos-aquae* G. maxima L. minor *N. pelliculosa* *S. capricornutum* *S. costatum*	Syngenta
Amino acid synthesis (compound 2)	E. canadensis L. gibba G3	>	L. minor	> >	*A. flos-aquae* *N. pelliculosa* *S. capricornutum* *S. costatum*	Syngenta

Continued on p. 235

Table 3. (Continued)

Mode of action (Inhibition of)	Species sensitivity ranking					Reference
	Mean effective concentrations (EC50) differing:					
	< five times		> five times		> > 10 times	
Amino acid synthesis (compound 3)	E. densa L. gibba G3 L. minor			> >	*A. flos-aquae* *N. pelliculosa* *S. capricornutum* *S. costatum*	Syngenta
Lipid-biosynthesis (grass-killer)	G. maxima			> >	L. gibba G3 *S. capricornutum*	Syngenta
Photosynthesis	L. gibba G3 *Microcystis* sp. *N. pelliculosa* *S. subspicatum*	>	E. canadensis H. verticillata M. verticillatum M. spicatum P. densus			Syngenta

The other vascular plants were tested over 14 days with a measurement of wet weight increase. Consequently, robust comparisons of inherent sensitivity are difficult. For most of the existing studies, this particular issue is also a problem when comparing the results obtained in tests with the same plant species. For vascular plants in particular, this is an issue because there are no harmonised testing guidelines, test methods and endpoints are different. Even for species like the duckweed *Lemna* sp. where a guideline draft is available, there are still differences in methods. Only the testing of algal species is mostly performed according to the existing guidelines, which leads to comparable results due to standardisation of conditions and methodology. Brock et al. (2000) tried to solve the problem of comparing results from tests performed under different conditions by taking the geometric mean for the existing EC50 values of one species. In their report they compared the database available for algae, *Lemna* sp. and for a series of vascular plant species with respect to a list of nearly 20 compounds. They concluded that for over 80% of the compounds, the existing testing scheme with a green algae and *Lemna* sp. was sufficient to detect potential toxicity against non-target aquatic plants. What was apparent though, was that algae and *Lemna* sp. were inadequate for auxin simulating herbicides because in these tests the threshold value was underestimated up to a factor of 100. Auxin simulators generally appear to be more applicable to dicotyledonous macrophytes other than the monocot *Lemna* sp.

In our studies we always included *Lemna* sp. and green algae, which are test species required for the standard database in the preliminary risk assessment scheme. The results showed that the required test species produced EC50 values differing by less than five times from the most sensitive species. The only discernible exceptions were for two auxin simulating and a grass killing herbicide, where differences in the EC50 values between green algae and *Lemna* sp. were more than 10 times as compared to other vascular plants. For an acceptable risk assessment procedure, the existing testing scheme could be considered most protective under field conditions. However, this has to be proven by comparable results obtained under laboratory and field conditions using environmentally relevant concentrations.

At the moment, no international harmonised guidelines for aquatic plant testing exist and required testing protocols of different countries vary. Prior to the definition of further testing requirements by the US EPA we recommend to perform further investigations to define adequate test species and experimental designs (FIFRA SAP, 2001).

Conclusions

The results presented by Brock et al. (2000) and our present comparison of literature and in-house data indicate that further test species need to be identified for testing the impact of auxin simulating herbicides or grass specific compounds to see if toxicity to non-target aquatic plants is underestimated.

Further studies will have to characterise adequate test species and to develop standardised experimental protocols before decisions on new regulations may be taken.

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Plasticity of *Lythrum salicaria* and *Phragmites australis* growth characteristics across a European geographical gradient

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Abstract

Plants of *Lythrum salicaria* and *Phragmites australis* originating from localities across the European north–south geographical gradient were cultivated in parallel in an outdoor tub experiment. A strong correlation was found between growth and morphometric characteristics related to plant size (plant height, basal diameter, aboveground- and belowground plant biomass, etc.) and the position of the respective populations along the north–south gradient. Plants of both *L. salicaria* and *P. australis* from the southern localities grew taller and more vigorously and flowered later than plants from relatively more northern localities. From this point of view, the plants originating from south European populations were comparable to invasive North American plants. Our study indicates that explanation of the competitive success of populations invading new geographical areas may involve the role of geographic gradients within the species native range.

Introduction

Lythrum salicaria L. and *Phragmites australis* Cav. (Trin. ex Steud.) both represent species that are common components of wetland communities in Europe (Hejný, 1960), which behave in an invasive manner in North American wetland habitats. *L. salicaria*, a Eurasian native species, is a broad-leaved dicotyledonous plant, which spreads mostly by sexual reproduction. Introduced accidentally to North America at the end of the 18th century, *L. salicaria* spread rapidly from the 1930s along rivers and into marshes in the northeastern USA and neighbouring areas of Canada (Stuckey, 1980). It is now found in all states and provinces between 35° and 51° N latitudes. In both, native and invasive ranges of occurrence, *L. salicaria* grows in a many plant communities and habitats (Dubyna et al., 1993; Bastlová-Hanzélyová, 2001). While in native areas *L. salicaria* became a dominant species of plant communities only exceptionally, it was documented as important plant species of invaded wetland habitats in North America (Bastlová-Hanzélyová, 2001).

Phragmites australis is a cosmopolitan perennial grass that relies on both generative reproduction and vegetative spreading of its clones. *P. australis* is an important dominant species in Eurasian wetlands, especially in littoral zones of lakes and ponds (Haslam, 1972, 1973; Rodewald-Rudescu, 1974). The species is apparently native not only to Eurasia, but also to North America, where many populations are non-invasive and do not form monospecific stands (Marks et al., 1994; Havens et al., 1997; Saltonstall, 2002). At many localities in the United States and Canada, however, populations of *P. australis* behave invasively, forming very dense, monospecific stands. The aggressively invasive *P. australis* populations in North America are widely believed to have been introduced from Eurasia (Marks et al., 1994).

The general purpose of our investigation is to elucidate some of the biological mechanisms behind the competitive ability of selected European populations of *L. salicaria* and *P. australis* in relation to their potential invasiveness in North America. This particular study is focused on the variability in life history characteristics of native populations of the two species along the north–south geographical gradient in Europe.

Materials and methods

Lythrum salicaria and *P. australis* plants were cultivated in a parallel outdoor tub experiment in Třeboň, Czech Republic. Four populations (represented by four parental plants per each population) of *L. salicaria* originating from four countries (Sweden, Poland, Slovenia and Israel) and six populations of *P. australis* (each represented by six clones) originating from six countries (Sweden, The Netherlands, Czech Republic, Hungary, Romania and Spain) were used for the experiment. Offspring of each parental plant was planted in four replicates in 2.5 l pots in *L. salicaria*. Rhizome cuttings of each clone were planted in six replicates in 5 l buckets in *P. australis*. Full nutrition was added to the pots or buckets in the form of a slowly diluting granulate fertilizer, Osmocote Plus, in a dose of 6 g l^{-1} of substrate, mixed with the sand medium at the time of plantation. The containers were placed in tubs situated outdoors and kept flooded throughout cultivation to one half of their height and to the substrate surface for plants of *L. salicaria* and *P. australis*, respectively. The cultivation started in May and ended in September 2000, when all the plant material was harvested. At the time of harvest the shoot height, total number of primary lateral branches (growing directly from the main stem) and basal stem diameter were recorded for each *L. salicaria* plant sampled. The specific leaf area (SLA), dry weight of roots, stems and lateral branches (without leaves and inflorescences), leaves and reproductive parts (inflorescences on main shoot and on lateral shoots + flower-bearing parts of the stems) were then determined. In *P. australis* the total number of shoots and that of flowering shoots, the length, basal stem diameter and number of nodes in the longest shoot, SLA and the ratio of aboveground/belowground total dry weight per bucket, were determined at the time of harvest.

Statistical treatment

The dependence of plant growth characteristics on latitude of original geographical location was fitted to generalised linear models (GLM) (McCullagh & Nelder, 1989) using S-plus software package (Statistical Sciences, 1995a, b). GLM was also used for hierarchical analyses of data to explain partitioning of total variability between the variability at geographical location level, between populations and within population levels. In *L. salicaria* three levels of variability were distinguished: (1) within population, i.e. between offspring of different mother plants from the population, (2) between populations within geographical location, and (3) between geographical locations. In *P. australis* two levels of variability were tested: (1) between clones within geographical location, and (2) between geographical locations. Significance of the GLM was tested using the *F*-test (Zar, 1984).

Results

Plants of both *L. salicaria* and *P. australis* originating from localities across the geographical gradient differed in their morphological traits, with the following growth characteristics being negatively correlated with latitude in both plant species (Fig. 1): plant height, aboveground and belowground dry weight, stem diameter and dry weight. In contrast, the SLA was positively correlated with latitude in both plant species.

Plants of *L. salicaria* originating from Sweden were semi-prostrate with small leaves and their lateral branches were almost as thick as the main stem. On the other hand, plants from Israel were tall, with vigorous, erect main stem and lateral branches shorter and thinner than the main stem.

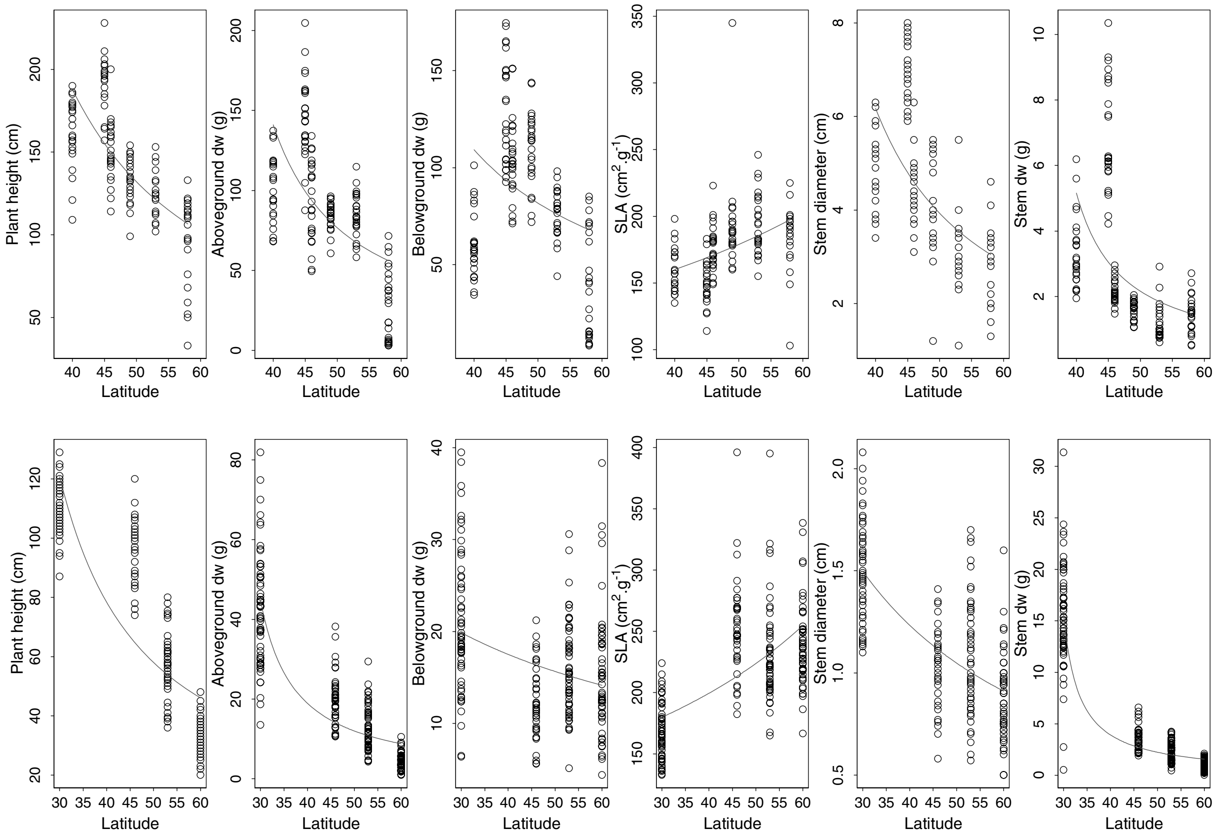

Figure 1. Dependence of *P. australis* (above) and *L. salicaria* (below) plant growth characteristics on the latitude of the plant populations original geographical location. The regression curves were fitted using GLM. (dw = dry weight, SLA = specific leaf area).

A similar trend was observed for *P. australis*. Plants of northern (Swedish and Dutch) populations had fairly thin semi-prostrate shoots with small leaves while plants of southern (Romanian and Spanish) populations formed tall erect shoots. In *L. salicaria* plants from Sweden and Israel did not flower, or flowered only rarely (Sweden). Flowering plants from the southern locality (Slovenia), flowered later and had a lower dry weight of inflorescences than plants from northern localities (Sweden, Poland). In *P. australis*, only Czech and Hungarian populations flowered by the end of experiment.

Based on the hierarchical analysis of variance, a highly significant proportion of total variability was ascribed to the effect of geographical location on the life history characteristics related to plant size (Table 1). A much smaller proportion of variability in these growth characteristics can be attributed to either within or between population differences in both species.

Discussion

The observed significant dependence of most of the plant morphological and growth characteristics on the latitude of the plants' geographical location suggests that a natural gradient exists in the variability of both studied plant species. The variability in phenology and life history characteristics across the north–south gradient may result from long-term adaptation to prevailing geographical conditions (Peacock & McMillan, 1968; Kudoh et al., 1995; Li et al., 1998; Pollard et al., 200l). Photoperiod is one factor of great importance that is highly variable with latitude and influenced plant life history characteristics. For example, populations of two *Solidago* species originating from northern locations flowered earlier and reached a smaller size at maturity than plants from southern locations (Weber & Schmid, 1998). The same applies to plants of *P. australis* from different

Table 1. Partitioning of variation among geographical locations, between populations within one geographical location (between population – in *L. salicaria* only) and between parental plants (in *L. salicaria*) or clones (in *P. australis*) originating from one population (within population)

	L. salicaria			*P. australis*		
	Explained variability			Explained variability		
	F	%	*p*	*F*	%	*p*
Plant height						
Geographical location	169.70	91	***	107.80	64	***
Between populations	3.61	2	***			
Within population	1.48	2	*	10.09	21	***
Shoot basal diameter						
Geographical location	18.65	49	***	71.79	63	***
Between population	3.86	10	***			
Within population	1.14	10	NS	3.97	13	***
Number of internodes						
Geographical location	0.09	0	NS	8.68	20	*
Between population	3.69	24	**			
Within population	1.07	25	NS	2.81	24	***
Single shoot dry weight						
Geographical location	147.51	83	***	257.88	82	***
Between population	2.28	2	***			
Within population	1.50	4	*	8.96	10	***
Aboveground dry weight						
Geographical location	64.06	79	***	110.71	53	**
Between populations	3.14	4	**			
Within population	1.81	6	**	19.76	34	***
Belowground dry weight						
Geographical location	5.59	21	***	89.51	54	**
Between population	2.32	14	***			
Within population	2.14	24	***	14.84	32	***
SLA						
Geographical location	49.80	55	***	18.67	39	***
Between population	1.10	4	*			
Within population	1.70	15	*	2.11	16	**

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, *F*-test of GLM.

latitudes in Europe (Véber, 1978; Clevering et al., 2001). The differences between *L. salicaria* plants from northern and southern populations found in the present experiment corresponded with those distinguishing short-day from long-day plants according to Shamsi & Whitehead (1974). Similarly as in the above studies, the variability in phenology and growth characteristics found in our study for both plant species may be strongly correlated with physiological requirements for floral initiation and is probably genetically based (Shamsi & Whitehead, 1974; Jouve et al., 1998).

Implications for invasiveness of Lythrum salicaria *and* Phragmites australis

In comparative studies on native and invasive populations of *L. salicaria* from areas with similar climatic conditions, the invasive populations showed more vigorous growth (Edwards et al., 1998, 1999; Bastlová & Květ, 2002). This is in accordance with the view that invasive populations of *L. salicaria* grow generally taller and show higher competitive ability in comparison with native European populations (Blossey & Nötzold, 1995; Willis & Blossey, 1999). However, when comparing native and invasive populations of *L. salicaria* from wider geographical areas, some of the native populations studied were more similar to the invasive ones than the others, e.g. native plant from more eutrophic sites (Edwards et al., 1998, 1999) or southern locations (Bastlová et al., 2004) were similar to invasive populations. In our present study, plants of both *L. salicaria* and *P. australis* from the southern localities grew taller and more vigorously and flowered later than plants from relatively more northern localities. From this point of view, the plants of both species originating from south European populations were comparable to invasive North American plants in terms of their vigorous vegetative growth and reduced proportion of dry-mass allocated to generative reproduction.

It has been suggested that species that successfully invade new geographic areas tend to allocate more biomass to vegetative growth, and less to reproduction or herbivore defence in a enemy-free space of secondary area (EICA hypothesis) (Blossey & Nötzold, 1995), or come from more competitive genotypes of the species in its native area (Mooney & Drake, 1986; di Castri et al., 1990). Our study indicates that explanation of the competitive success of populations invading new geographical areas may involve the role of geographic gradients within the species native range.

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Use of Geographic Information Systems to monitor and predict non-native aquatic plant dispersal through north-eastern North America

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Key words: macrophytes, GIS, *Myriophyllum spicatum*, *Trapa natans*, *Potamogeton crispus*

Abstract

North America has a growing problem with invasive aquatic plants. At every level of the aquatic food web, long-standing checks and balances have become dramatically eroded by the introduction of non-native species. The northeastern United States contains thousands of diverse freshwater habitats, highly heterogeneous in geology and locale, where nuisance aquatic plant growth results in decreased lake water quality, interference with recreational access, degraded flood control structures, and impacts to their aesthetic quality. Early infestation and spread of exotic species is often poorly documented at local and state levels. Consequently, successful management of these species depends on continuous monitoring and definitive identification by both public officials and waterside homeowners. With new mapping capabilities employing software such as Geographic Information Systems (GIS) coupled with the growing computerization of taxonomic records and online availability of regional herbarium records, we have been able to illustrate the temporal and geographic spread of these species, thus enabling aquatic ecologists and managers to make predictions of future infestations. In this paper we evaluate these techniques focusing on three of the most pervasive of exotic aquatic plant species in New York State: *Myriophyllum spicatum*, *Trapa natans* and *Potamogeton crispus*.

Introduction

Exotic species are one of the major causes of loss of biodiversity in aquatic ecosystems in North America (Reed, 1977; Madsen, 1994). This is especially true in the Northeastern United States where thousands of lakes and ponded waters exist with considerable heterogeneity in chemical, physical and geographic characteristics. Although multimillion-dollar control programs are currently underway in the US to manage these species (Cooke et al., 1993), early detection is often difficult and control measures come into play only after a species has spread to nuisance proportions. Lake associations made up of residents who live around the shore privately own many lakes. Therefore stakeholder education and observations, as well as predictive dispersal capabilities are both important in early detection and management.

New generation mapping software such as Geographic Information Systems (GIS), coupled with a growing availability of computerized taxonomic and online herbarium databases, may provide aquatic plant ecologists and managers with a new set of tools to observe, via computer simulation, dispersal across time and space. The objective of this paper is to evaluate the usefulness of these software programs in mapping and projecting a historical framework of geographic movement based upon herbarium records and documented dates of discovery. Three exotic species were chosen to illustrate the power of the software to track their ecological movements geographically: *Myriophyllum spicatum* (Eurasian watermilfoil), *Trapa natans* (Waterchestnut) and *Potamogeton crispus* (Curly-leaf Pondweed). These species represent different growth and dispersal patterns. New York State was chosen as our geographic unit because of our familiarity with state and university herbarium collections. Data "mining" of information is difficult because it is often relegated to "grey" literature if published at all.

Myriophyllum spicatum is a highly aggressive perennial. In northern latitudes growth begins in spring earlier than most native plants, with stem elongation in excess 1 cm per day. Once stems reach the water surface they grow laterally to form a canopy, reducing light penetration and eliminating native plants growing as an understory (Madsen et al., 1991). The plant spreads through auto and allo-fragmentation. Fragments remain viable for weeks drifting throughout a lake system before taking root (Madsen et al., 1988). Recreational boaters, fishermen, and waterfowl disperse fragments. First observed in New York State in 1949, *M. spicatum* has been reported in 47 of the 62 counties of the state and is currently found in over 190 lakes statewide (Eichler, 2002).

Trapa natans is an annual that prefers calm, shallow, nutrient-rich lakes and river systems. Inflated stems offer buoyancy to a canopy of surface leaf clusters. Shallow waters can become completely covered. Annual production of nutlets is the principal means of dispersal of this species. *T. natans* was imported to New York State as an ornamental in 1884. It escaped from cultivation into the Mohawk River/Erie Canal around 1900 (Muenscher, 1934, 1936). From there it has rapidly moved throughout the state barge canal system (NYS DEC, 2001).

Potamogeton crispus proliferates in the spring and early summer when native plants are yet to germinate. Seed production and die-back occur in mid-July. Vegetative growth from turions or overwintering buds begins under the ice during the late winter. One of earliest aquatic plants to invade New York State, *P. crispus* was first reported in the Finger Lakes region in 1878 (Gilman, 1983, 1985). Since that time it has spread throughout most of the western portion of the state and along the eastern tier where more alkaline waters are found (Harman et al., 2001).

Materials and methods

Historical data for distribution maps were compiled by searching herbarium records of universities and state archives located within New York. The NYS Department of Environmental Conservation Citizens Statewide Lake Assessment Program (Kishbaugh, 2001), the Aquatic Plant ID Programs of Darrin Fresh Water Institute (Taggett, 1989; Eichler, 2002), and databases at Cornell University (Robert Johnson, per. comm.), Syracuse University (Miller, 1978; Oswego County Planning Department, 1990), College of the Finger Lakes (Gilman, 1983), and others (LCI, 1998; Harman et al., 2001) provided recent colonization data. A database for each species was constructed containing lake name, location and approximate year of first identification (date of discovery). Date of discovery was defined as the year when the species in question was identified by a knowledgeable taxonomist. Date of actual invasion and date of discovery can vary, depending on location and accessibility of a given lake. Lakes and rivers geographically closer to active university herbarium collections or with better recreational access are expected to have dates of discovery that more accurately reflect dates of invasion.

Latitude and longitude coordinates were determined at the outlet for each lake or its respective county border. MapInfo Professional™ version 6.5 GIS software (MapInfo Corp., Troy, NY) was used to create thematic maps. Inverse distance weighting (IDW) of the discovery date was used for visual representation of date and location for each species. IDW interpolates a data point by using a neighborhood (search radius) about the point that is identified and a weighted average is taken of the values within this neighborhood. The weights are a decreasing function of distance. A search radius of 15 miles was applied to limit influence of geographically distant points. The software program uses color to correlate discovery dates with geographic location because it provides visual clarity over time, and helps to distinguish patterns of species movement across distance.

Results

Myriophyllum spicatum is clearly the most expansive of invasive species utilizing both asexual and sexual propagation. Figure 1 demonstrates wide-range spread from multi-foci over a short period of time (since 1949) throughout most of the state. Numerous points of introduction and non-uniform spread patterns suggest that the primary vector of dispersal involves human interaction. Both primary (Interstate) and secondary road systems are included on each figure to illustrate the proximity of affected lakes to vehicular access and movement across the state. The spread of milfoil closely parallels New York's major river systems and highways, suggesting transport via plant fragments attached to boats or trailers. Although this plant appears to cover nearly all of New York State, there is a significant gap in the north central region (Adirondack Park). Approximately 3000 lakes and ponds exist in the Adirondacks, yet most are either inaccessible or are possibly chemically restrictive to *M. spicatum* due to their low pH.

Trapa natans was first identified in New York State during the late 19th century at Collins Lake near Schenectady. By plotting all the known locations of this species throughout the state, and referencing discovery dates by color, we have a clear representation of its spread over time (Fig. 2). From a single point of introduction, this species has expanded through the Hudson and Mohawk River systems, and more recently has been introduced to peripheral lakes and ponds. As an annual, *T. natans* reproduces only by seeds. Because these seeds are encased in large thorny

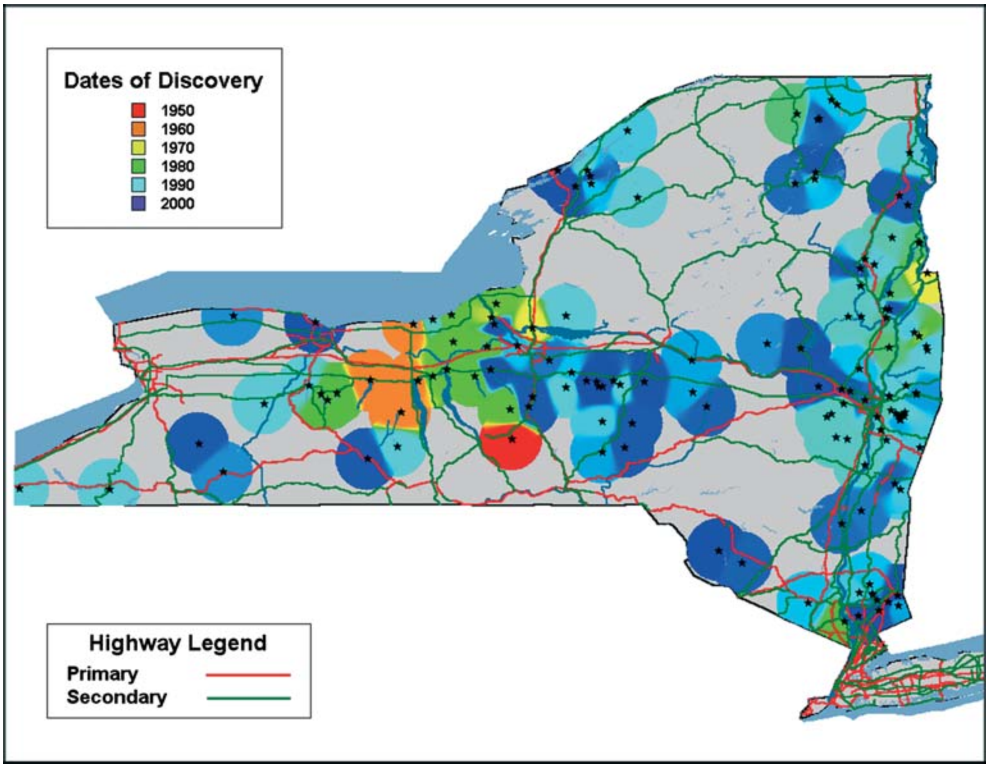

Figure 1. Thematic map of New York State, USA, correlating location and date of discovery of the exotic aquatic plant species *Myriophyllum spicatum*. Mapping software uses inverse distance weighting of the discovery date to represent species location with time. A black star indicates the specific location of each water body or its respective county border.

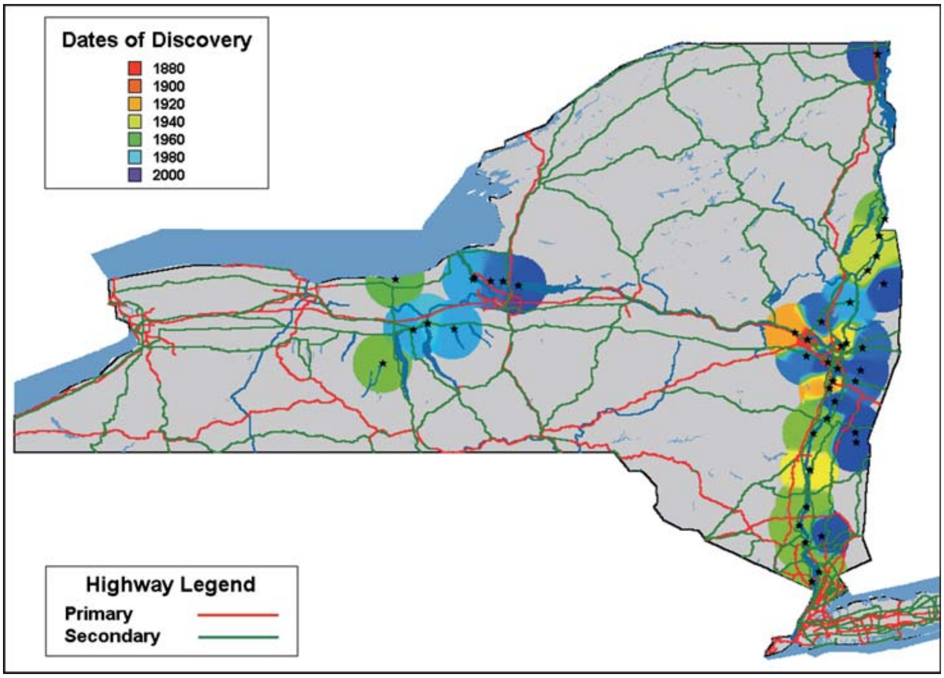

Figure 2. Thematic map of New York, USA, correlating location and date of discovery of the exotic aquatic plant species *Trapa natans*. Mapping software uses inverse distance weighting of the discovery date to represent species location with time. A black star indicates the specific location of each water body or its respective county border.

nutlets, they are not a common animal food source, limiting the likelihood of wildlife as a vector of dispersal. Rather, the seeds drop to the river bottom traveling with flow, thus its spread has been more restrictive than with milfoil and, as far as New York State is concerned, is mostly contained within the state river and canal systems.

Potamogeton crispus does not demonstrate a single point of introduction into New York State (Fig. 3). The earliest discoveries of this species appear in the western part of the state. From these locations, there are numerous clusters of dispersal expanding over the last century. *P. crispus* has natural preferences to more alkaline waters, which may explain its abundance in the western portion of the state where limestone sediments are common. Also demonstrated in Figure 3, is its tendency to inhabit ponds and lakes rather than major river systems. It reproduces both by seeds and overwintering turions. The most likely dispersal mechanism is waterfowl with both the Finger Lakes and the eastern perimeter of New York State on major migratory routes for waterfowl (e.g., Canadian geese, Snow geese, ducks, etc.)

Discussion and conclusions

New York State and New England contain thousands of lakes and ponds ranging in size from only a few to thousands of hectares of surface area. Typically lakes of 100 ha or greater are used for recreational boating. Public ramps provide water access for power craft between lakes. Public lakes within New York in wilderness designated areas are restricted from powered watercraft. Consequently, areas of the state such as the 6 million acre Adirondack region preclude vehicular access. Such regulations have minimized the movement of exotic species to these lakes. Lakes in the lowlands and valleys of the state have historically seen more human activity and perturbation. Such bodies of water are usually mesotrophic where lake nutrient availability favors rapid proliferation of exotic

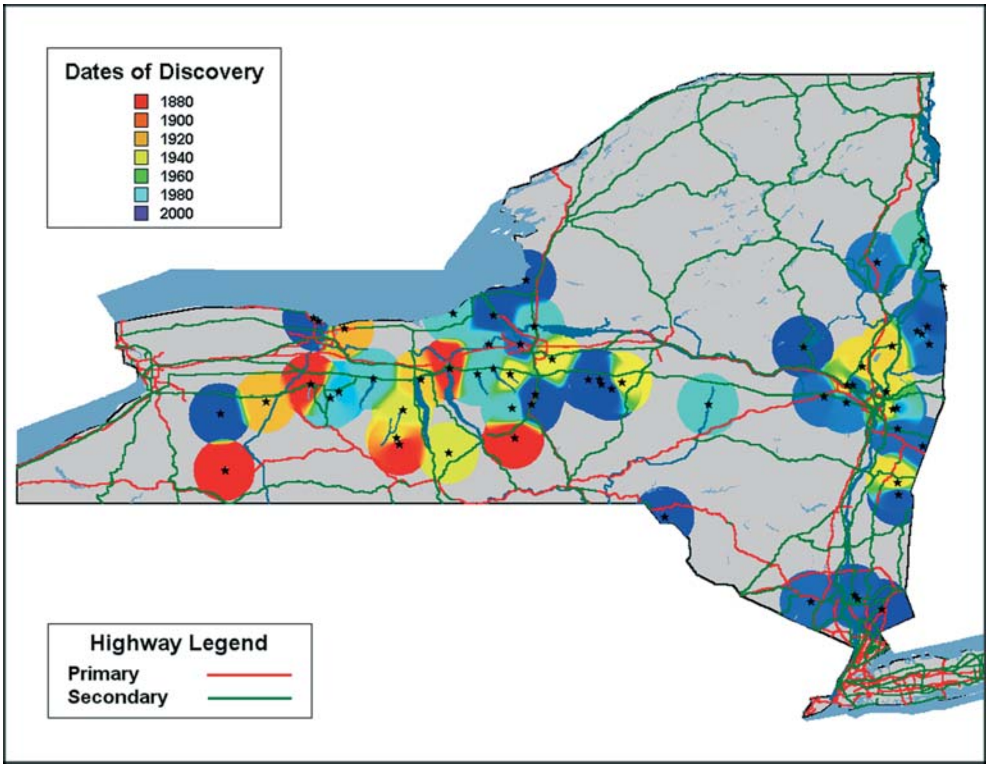

Figure 3. Thematic map of New York, USA, correlating location and date of discovery of the exotic aquatic plant species *Potamogeton crispus*. Mapping software uses inverse distance weighting of the discovery date to represent species location with time. A black star indicates the specific location of each water body or its respective county border.

species once they have been introduced. Higher elevation lakes in the Adirondack and Catskill mountain regions have limited vehicular access and have maintained their more natural oligotrophic state.

The geographical distribution of each exotic plant species chosen for this study is dictated by its mode of dispersal. Fragmentation and human transport appear to be the most effective methods for rapid dispersal. Within 50 years, *M. spicatum* has spread throughout much of the state. The post WWII expansion of a burgeoning middle class has spurred a boom in recreational boating. Numerous boat launch ramps have been built to provide lake access throughout the state. Researchers cite public boat launching areas as the focus of introduction of *M. spicatum* (Madsen, 1994).

Dependent exclusively on dispersal of large seeds, *T. natans* is largely limited to the principal waterways in New York State. Its distribution is mainly in the Hudson and Mohawk River systems and their connecting canals, including connections to Lake Champlain and the Saint Lawrence River. Intensive management efforts prior to annual seed production as practiced in the 1950s and 1960s offers the possibility of eradication of this species from New York State.

Seed and turion production with transport by waterfowl has proven an effective dispersal mechanism for *P. crispus*. The expansion of this species has slowed in the last two decades. Water chemistry may be the primary limiting factor in the current distribution of this species, with low ionic strength waters in the Adirondack region excluding the expansion of this species (Crow & Hellquist, 2000).

With each species the connection of aquatic plant dispersal with major interstate highways and/or water-based transportation corridors is apparent. A concerted effort to restrict transport of propagules by recreational and commercial boating traffic is imperative. Public education to assure rapid identification and remedial management of exotic species will greatly simplify management efforts.

The current project shows the value of "mining" data from historical sources and using software mapping capabilities to better utilize existing datasets. In order to fully recognize the abilities of these methods, one must also incorporate herbarium data from neighboring states and countries. By doing this, a more complete understanding of spatial and temporal movement of a species can be established without being limited to theoretical government borders. Overlays of bedrock, soil and alkalinity maps can also be incorporated, allowing for more accurate predictions on future distribution where water chemistry could be a limiting factor. Survey efforts can then focus on areas not currently infested, but at risk of invasion, resulting in reduced management efforts and costs.

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Present distribution of the genus *Elodea* in the Alsatian Upper Rhine floodplain (France) with a special focus on the expansion of *Elodea nuttallii* St. John during recent decades

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Key words: *Elodea canadensis*, *Elodea nuttallii*, *Elodea ernstiae*, invasive species, habitat parameters, impacts on species richness

Abstract

Three species of *Elodea* (*Elodea canadensis* Michaux, *E. nuttallii* St John and *E. ernstiae* St John) have colonized Europe from the American continent. All three arrived in the Alsatian Rhine floodplain (north-eastern France) soon after their arrival in Europe, i.e. in the mid-19th century for *E. canadensis*, and in the mid-20th century for *E. nuttallii* and *E. ernstiae*. The paper investigates the present distribution of *Elodea* spp. in the floodplain by quantifying the species' respective occurrences and by describing their habitats. The study further focuses on *E. nuttallii* which is presently colonizing other parts of Europe. It analyses whether it has continued to expand in the Alsatian Rhine floodplain during recent decades, and it checks whether changes in the abundance of *E. nuttallii* have had an impact on species richness of water plant communities. *E. nuttallii* has been found to be at present one of the most dominant and most frequent aquatic plant species in the study sector, while *E. canadensis* and *E. ernstiae* are less abundant. The species' distributions differ with regard to water chemistry and water temperature: *E. canadensis* occurs in oligo-mesotrophic, rather stenothermic habitats, whereas *E. nuttallii* and *E. ernstiae* can be encountered in meso- to eutrophic sites with little or no arrival of stenothermic ground water. By comparing successive vegetation relevés from the same sites the study revealed further that the distribution of *E. nuttallii* has been stable in recent decades, despite local fluctuations in abundance. No relationship could be established between those fluctuations and changes in species richness or type of local plant communities. The sum of the results suggests that the expansion of *E. nuttallii* in the Alsatian Rhine floodplain had been completed prior to the study period. The species' present distribution in the study sector as well as its position in local plant communities might therefore be considered a model for what can be expected to happen in areas where *E. nuttallii* has only recently arrived.

Introduction

The impact of species which expand their geographic range is a strong concern to scientists, nature conservation managers and, increasingly, the public living in the areas were those species are arriving. It is generally admitted that new species can only become established in existing communities when they are successful in competition with local flora or fauna, or when they present advantages such as a high growth rate that allows them to occupy rapidly new space after disturbance. Because of their supposed high competitive ability, range-expanding species are generally considered a threat to local biodiversity, since they may alter local communities. Possible impacts include the competitive exclusion of local species from sites where the invader becomes established.

Within aquatic macrophyte communities in western Europe, *Elodea canadensis*, a Hydrocharitacea of North American origin was introduced in the mid 19th century (Cook & Urmi-König, 1985). Its common name of a "pest" in several European languages (e.g., "Kanadische Wasserpest" in German) is a reflection of the fact that it has been considered a nuisance since its arrival in Europe because of its rapid spread. After 100 years, it has become a common member of many aquatic plant communities. Its detailed impact on local plant diversity is, however, difficult to document today. Within recent decades, two other representatives of the North American *Elodea* genus, *Elodea nuttallii* and *E. ernstiae*, have been observed in Europe. While *E. ernstiae* (synonym: *E. callitrichoides*; Cook & Urmi-König, 1985), occurs only locally, *E. nuttallii* is expanding in Europe. It was first observed in Belgium in 1939 (Wolff, 1980), its first appearance in France is mentioned in the 1950's in the Alsace region (north-eastern France) (Geissert et al., 1985). It still continues to spread, since its first appearance in south-eastern France for example occurred in the 1990's (Barrat-Segretain, 2001). Several authors have observed that the arrival of *E. nuttallii* in a new geographic sector resulted in local replacements of *E. canadensis* by *E. nuttallii* (Mériaux & Géhu, 1980; Simpson, 1990; Barrat-Segretain, 2001), suggesting a higher competitive ability of the latter. The arrival of *E. nuttallii* may therefore also have a negative impact on other aquatic macrophytes and could represent a potential threat to the species richness and/or diversity of its new habitats.

In the Alsatian Rhine floodplain in north-eastern France, all three *Elodea* species were observed soon after their first arrival in Europe: The oldest known record of *E. canadensis* in Alsace dates from 1875 (Rencker, 1882, database "Brunfels" of the *Société Botanique d'Alsace*), the oldest record of *E. ernstiae* from 1958 (Sell, 1959). *E. nuttallii* was also first observed in the 1950's (Geissert et al., 1985). The present paper investigates the occurrences of *Elodea* spp. in the Alsatian Rhine floodplain in recent decades. The aims of the study are:

(i) to quantify the respective abundances of the three *Elodea* in the present macrophyte communities and to characterize their habitats
(ii) to investigate whether and to what extent *E. nuttallii* has expanded during recent decades
(iii) to check for impacts of *E. nuttallii* on the species richness of macrophyte communities.

Methods

Present distribution of Elodea *spp.*

The present frequency distribution of the *Elodea* was derived from vegetation relevés in rivers and groundwater streams that were recorded in 2000 and 2001. Vegetation sampling followed the Braun–Blanquet method and was carried out in four eographical sectors. From North to South: (1) the river Sauer (13 relevés), (2) the groundwater stream Rossmoerder near Offendorf (7 relevés), (3) the sector «Illwald», i.e. the river Ill and nearby groundwater streams near Sélestat (60 relevés, centre of Alsace), (4) two former Rhine arms at Biesheim (23 relevés). The sampling covers the whole trophic range encountered in the Rhine floodplain.

Habitat characteristics

Habitats were described by water chemistry (temperature, conductivity, pH, $N–NO_3^-$, $N–NH_4^+$, $P–PO_4^{3-}$ and Cl^-) and by the degree of shade (5 classes), flow velocity (5 classes), sediment texture (7 classes: from 1: very fine grained to 7: very coarse) and total plant cover (% of surface covered). These parameters were extracted from vegetation relevés, most of them realized in 2000/2001. Since the dataset was small in the case of the less frequent species, data were completed with information from previous years, gathered in two additional sectors (river Zembs and sector of Daubensand). Water chemistry data were the mean values of 3 or 4 (one monthly sampling during the spring months). Standard deviation of water temperature over the sampling period was used as an indicator for stenothermy. The parameter values encountered in the habitats of each species were compared by Kruskal–Wallis tests (Sokal & Rohlf, 1995). Sample sizes were in general at least 7 and as large as 33.

Expansion of E. nuttallii *within recent decades*

46 aquatic plant habitats have been visited at least twice during recent decades. Most of the second visits occurred in the second half of the 1990's, while most (85%) of the first visits took place in late 1980's or early 1990's. A few visits reached back to the early 1970's. The studied plant habitats span the whole trophic range encountered in the floodplain. In order to evaluate a possible expansion of *E. nuttallii*, the species' abundances in successive relevés from the same sites were compared. The time interval between both relevés was about a decade in most cases, but extended up to three decades in a few cases (Fig. 3). When *E. nuttallii* was present in both relevés, we analyzed whether it had modified its position within the community through time. This was done by comparing *E. nuttallii's* cover abundance with the median cover abundance in the community. Since the Braun–Blanquet scale is not based on regularly spaced additive steps, cover classes were transformed into cover percentage by taking, for each Braun–Blanquet class, its average cover percentage: class +: 0.5%; class 1: 2.5%; class 2: 15%; class 3: 37.5%; class 4: 62.5%; class 5: 87.5%.

Results

Present distribution of Elodea *spp.*

In 2000/2001, 63 aquatic plant taxa, including hydrophytes, helophytes, algae and bryophytes were recorded in the study sector. *E. nuttalli* occurred in 42%, *E. canadensis* in 10%, and *E. ernstiae* in 3.8% of the relevés. *E. nuttallii* was one of the most frequent species overall after *Callitriche obtusangula* (63%), *Sparganium emersum* (48%) and *Berula erecta* (45%) and before *Lemna minor* (30%). *E. ernstiae* and *E. nuttallii* were locally abundant with maximum cover indices of 4, and mean cover indices of, respectively, 2 and 1.85. *E. canadensis* developed less extensive cover in sites where it was present (maximum cover: 2, average cover: 0.85). Because of both its high frequency and high local abundance, *E. nuttallii* was the third most dominant species (dominance = % occurrence x mean cover) in the study sector after *B. erecta* and *C. obtusangula*. *E. canadensis* ranked 22nd and *E. ernstae* 27th. All three species can be encountered in the phytosociological communities *Callitrichetum obtusangulae*, *Ceratophylletum demersii* and *Ranunculetum fluviantis* according to Oberdorfer (1992), however, with different frequencies (Fig. 1): in *E. canadensis* 42% of all occurrences belong to the Calletrichetum, 5% to the Ceratophylletum and 10.5% to the Ranunculetum. *E. nuttallii* is in 15% of its occurrences part of the Calltrichetum, in 9%

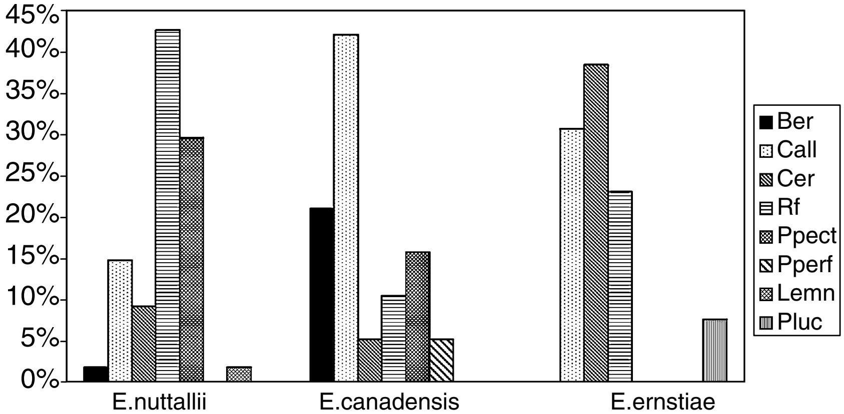

Figure 1. Frequency of the three species in the phytosociological communities determined according to Oberdorfer (1992). Call: *Callitrichetum*; Ber: *Sietum erecti submersi*; Cer: *Ceratophylletum*; Rf: *Ranunculetum fluitantis*; Ppect: *Potametum pectinati*; Pperf: *Potametum perfoliati*; Lem: *Lemnetum minoris*; Pluc: *Potametum lucentis*. Sample sizes: *E. nuttallii* $N = 54$; *E. canadensis* $N = 19$; *E. ernstiae* $N = 13$.

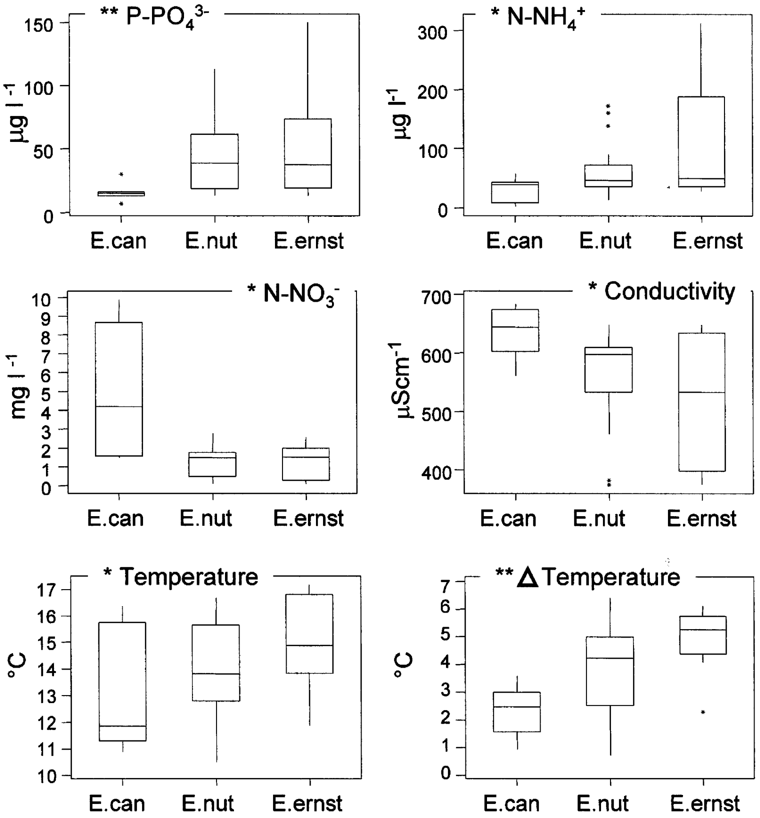

Figure 2. Habitat characteristics of *Elodea spp.* in the Alsatian Rhine floodplain. Represented are median values, the central 50% (box around median) and total range of parameter values (vertical lines) that were encountered for each species. *: outliers (values at least 1.5 times superior to standard deviation of the central 50% of values). Represented are only habitat parameters in which median values were significantly different between species. Level of significance (Kruskal–Wallis test): * $p < 0.1$, ** $p < 0.05$. Δ Temperature: standard deviation of water temperature during monthly samples. Sample sizes: P–PO$_4$: *E. canadensis*: $N = 8$, *E. nuttallii*: $N = 23$; *E. ernstiae*: $N = 8$. N–NH$_4$: *E. canadensis*: $N = 8$, *E. nuttallii*: $N = 24$; *E. ernstae*: $N = 8$. N–NO$_3$: *E. canadensis*: $N = 8$, *E. nuttallii*: $N = 24$; *E. ernstiae*: $N = 7$. Conductivity: *E. canadensis*: $N = 8$, *E. nuttallii*: $N = 20$; *E. ernstiae*: $N = 8$. Temperature: *E. canadensis*: $N = 8$, *E. nuttallii*: $N = 24$; *E. ernstiae*: $N = 10$.

of the Ceratophylletum and in 43% of the Ranunculetum phytosociological communities. *E. ernstiae* is relatively evenly distributed among the communities (Callitrichetum 31%, Ceratophylletum 38.5%, Ranunculetum 23%).

Habitat characteristics

The three *Elodea* showed a relatively segregated distribution: All three species coexisted in only one of 45 *Elodea* sites, *E. nuttallii* and *E. ernstiae* coexisted in 10, *E. canadensis* and *E. nuttallii* in three, and *E. canadensis* and *E. ernstiae* in one of those sites. The habitats of the three species differed only in parameters related to water chemistry and temperature. Habitats of *E. canadensis* had low water phosphate and ammonium levels, a wide range of nitrate levels and high conductivity (Fig. 2). Temperature was stable and, in most sites, relatively low. *E. nuttallii* and *E. ernsitae* showed the opposite pattern. *E. nuttallii* is singled out by its wide range with regard to temperature, *E. ernstiae* by its wide range with regard to ammonium and conductivity.

Expansion of E. nuttallii *in recent decades*

In most of the visited sites, the situation was stable through time with regard to the occurrence of *E. nuttallii*: either the species was absent from both successive relevés (44%), or it was present in both (37%). In 12% of the cases, *E. nuttallii* appeared

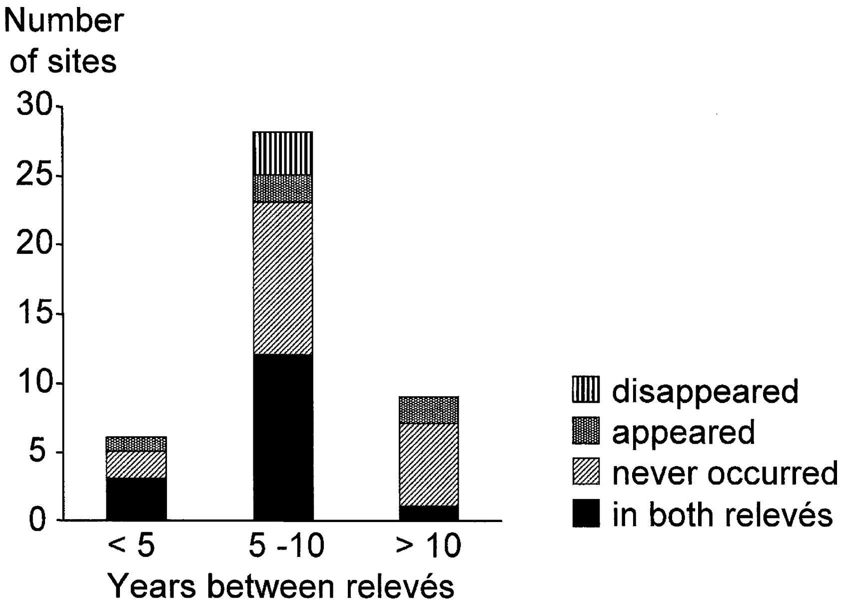

Figure 3. Changes in the distribution of *E. nuttallii* within recent decades. Represented are sites that were visited twice during recent decades and the change with regard to the presence or absence of *E. nuttallii.*

between the first and the second relevé, in 7% of the cases it disappeared. Even when more then a decade lay between the first and the second relevé, most of the sites (Fig. 3) had remained *E. nuttallii*-free.

In sites where *E. nuttallii* occurred, its cover corresponded in most cases either to the median cover developed by plant species in that community, or it was superior to the communities median cover (Table 1). The position of *E. nuttallii* with regard to median cover, i.e. *E. nuttallii's* relative abundance, varied largely through time (Table 1) since the position of *E. nuttallii* with regard to median cover was stable in only 4 out of 16 cases. Increases in relative abundance did not exceed decreases.

Table 1. Relative abundance of *E. nuttallii* within plant communities of the Alsatian Rhine floodplain, expressed as the number of sites in which the cover of *E. nuttallii* is inferior, equal or superior to the median cover of plant species in those sites

	< median	= median	> median
Date 1	3	6	7
Date 2	4	5	5

Date 1: situation at the first visit, date 2: situation at the second visit. Change of position of *E. nuttallii* with regard to median cover: increase: 5 sites; decrease: 7 sites; unchanged: 4 sites.

Changes in species richness between date 1 and date 2 occurred in many of the studied sites, with the number of losses exceeding that of gains (Fig. 4). These changes can, however, not be related to changes in the abundance of *E. nuttallii*: losses of 5 or more species occurred both with increasing, decreasing or stable *E. nuttallii* cover, and in none of the sites did a decrease of *E. nuttallii* result in an increase in species richness. The most frequently encountered plant community during both visits was the eutrophic *Potametum pectinati* (43 and 56%, respectively, during visits one and two). *E. nuttallii* was dominant in none of the few sites in which community changed between the two visits.

Discussion

This study shows that *Elodea nuttallii* has become, some 50 years after its arrival in the Alsatian Rhine floodplain, the most abundant of the three *Elodea* species, and one of the most common aquatic macrophytes in the sector. Its pattern of distribution is characterized by its occurrence in a

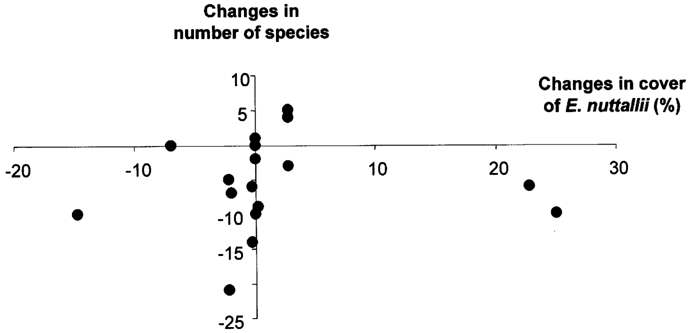

Figure 4. Variation in species richness *vs*. variation in *E. nuttallii* abundance (cover percentages) during the same time period in a series of aquatic habitats in the Alsatian Rhine floodplain.

large number of sites, combined with an often high local abundance. This pattern contrasts with that of *E. canadensis* (intermediate frequency, intermediate local abundance) and *E. ernstiae* (low frequency, high local abundance). Its distinctive habitat characteristics, i.e. phosphate- and ammonium levels belonging to the meso- to eutrophic range and a wide range of temperature, indicate that *E. nuttallii* grows in sites that may have some input of stenothermic, oligotrophic groundwater. *E. ernstiae* sites have similar characteristics but go further into high trophic levels. Habitats of *E. canadensis*, on the other hand, are limited to the oligo-mesotrophic range. The temporal stability of water temperature and the high conductivity indicate that those habitats have relatively high groundwater input. Thus, the spatial distribution of the *Elodea* has been found to be close to allopatric. Similar patterns have been observed for example on the German side of the Rhine floodplain (Tremp, 2001) and in the nearby Northern Vosges (Thiébaut et al., 1997).

The plant communities in which the different *Elodea* occur show partly their differences with regard to physical and chemical habitat characteristics. Thus, *E. canadensis* occurs mainly in communities typical of mesotrophic conditions (Sietum erecti submersi and Callitrichetum), while *E. nuttallii* occurs more often in communities typical of eutrophic conditions (Ranunculetum and Potametum pectinati). In a significant number of cases, however, the three *Elodea* can be encountered in the same types of communities. This seems to be in contradiction with their rather segregated distribution as regards their habitat characteristics. The contradiction can be explained as follows: due to engineered structures, the watercourses of the Rhine floodplain have lost their connection to the Rhine River. This has caused an oligotrophization of watercourses in several sectors during the second half of the 20th century. Water chemistry has changed, but the plant communties as a whole often reflect a more ancient situation.

This study did not reveal a trend with respect to the further expansion of *E. nuttallii* in the Alsatian Rhine floodplain. The situation was especially stable in terms of the number of colonized sites, whereas relative local abundance of *E. nuttallii* fluctuated considerably, but without showing a trend towards a general increase. Those elements let us conclude that the distribution of *E. nuttallii* in the Alsatian Rhine floodplain is at present stable, with all potential and attainable habitats being occupied by the species. Several authors have observed competitive replacements of *E. canadensis* by. *E. nuttallii* in nutrient-richer sites in several geographic sectors (Simpson, 1990; Barrat-Segretain, 2001). The allopatric distribution of *E. nuttallii* and *E. canadensis* in the Alsatian Rhine floodplain suggests that this kind of replacement might have taken place before the period of observation of the study and has by now come to an end.

The findings of this study do not confirm that *E. nuttallii* has a strong negative impact on the plant species richness of the sites in which it grows. One might argue that the major impact might have occurred during or shortly after the expansion phase of *E. nuttallii* and could therefore no longer be documented by the present study. This view cannot be refuted, nevertheless, *E. nuttallii* generally coexists with a rather high number of species (13 species in average), a finding confirmed on the German side of the Upper Rhine floodplain (Tremp, 2001). This suggests that *E. nuttallii* does, at worst, represent a limited threat to the species richness of the sites it is colonizing. The Alsatian Rhine floodplain, because of its relatively ancient colonization by *Elodea* spp. and the present stable distribution of *E. nuttallii* can serve as a projection of what might be expected in geographic sectors where *E. nuttallii* has arrived only recently. Nevertheless, the current invasions should be carefully monitored. Systematic, fine-grained monitoring should especially check whether the species' impact on plant communities differs according to the type of habitat (e.g., mesotrophic vs. eutrophic). This kind of fine-scale analysis needs proper experimental design and can rarely be reconstituted from historic data.

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Invasibility of four plant communities in the Llobregat delta (Catalonia, NE of Spain) in relation to their historical stability

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Key words: landscape history, alien species, coastal habitats, Llobregat delta

Abstract

Presence and cover of alien plants were analysed in relation to recent naturalness changes (1956–1999) in the Llobregat delta by means of GIS techniques and field surveys. Two land cover maps of 1956 and 1999 were generated by photo-interpretation of orthoimages and they were then reclassified into naturalness classes, defined as the degree of preservation of the pristine state. The resulting naturalness maps were combined in order to obtain a naturalness change map, which was used to design field sampling in four pristine communities: reedbeds, rushbeds, halophilous scrubs and fixed dune communities. Two study areas were selected for each community and three stability regimes (stable, semi-stable and non-stable) obtained from the naturalness change map. Five vegetation inventories were performed on average in each of these areas using the classical sigmatist method. Results showed a negative relationship between stability and invasibility, with several variations between communities. No alien species were found in stable areas of all communities. Alien species number, species percentage and relative cover increased from semi-stable to non-stable regimes in reedbeds and dune communities, indicating that reversion towards the climax reduces opportunities for alien establishment in these communities. In contrast, halophilous habitats such as rushbeds and scrubs did not exhibit significant differences between semi-stable and non-stable plots, probably because saline stress makes their invasion by alien plants difficult, even under disturbance.

Introduction

Invasion by alien species is a worldwide phenomenon with recognised negative effects on the conservation of native biodiversity (Lodge, 1993). The close relationship between habitat disturbance and invasibility is widely accepted (di Castri, 1990; Vitousek et al., 1997; Hobbs, 2000). Disturbance reduces competition and increases the availability of safe sites, providing more opportunities for alien colonization and spreading (Hobbs & Huenneke, 1992; Olenin & Leppänoski, 1999). Aquatic ecosystems are particularly susceptible to invasion because of their intermediate to high disturbance regimes (di Castri, 1990; Rauchich & Reader, 1999), with a number of studies indicating the concentration of alien species in ponds and rivers (Pyšek & Prach, 1993; Alpert et al., 2000).

Land use changes driven by man are a main source of habitat disturbance, and consequently of alien introduction, in terrestrial ecosystems (Hobbs, 2000) but also in wetlands (Ewel, 1986).

Despite the major role of land use changes in habitat invasibility, there are few studies aimed at examining the association between stability and the risk of invasion of natural habitats from an historical perspective. The present study analyses long-term (1956–1999) changes (including persistence, recuperation and degradation) and their possible association with invasibility in several communities in the Llobregat delta (central coast of Catalonia, NE of Spain), in the metropolitan area of Barcelona. Because of intense land use change and trading, the Llobregat delta has become one of the regions most invaded by alien plants in Catalonia (Casasayas, 1990). In our study, GIS techniques have been used to find areas of contrasting habitat stability comparing 1956 and 1999. A field survey of main wetland and dune communities has been conducted in these areas in order to assess the relationship between their stability and invasibility.

Description of site

The study was done in the southern hemidelta of the Llobregat, adjacent to the city of Barcelona and still dominated by natural and agricultural habitats (Fig. 1). At present, marshlands and fixed dunes correspond to 13% of the total land cover, with reedbeds (*Phragmition australis* W. Koch), rushbeds (*Juncion maritimi* Br. Bl.), and secondary pine (*Pinus pinea* L.) forests as dominant plant communities. Fragments of halophilous scrublands (*Arthrocnemion fruticosi* Br. Bl.) and fixed dune communities (*Crucianellion maritimae* Rivas Goday and Rivas Mart.) with variable conservation status still persist. Despite some of the remaining natural areas having been declared Natura 2000 sites, their conservation is threatened by intense land-use change, water pollution and degradation. About 15% of the areas occupied by natural habitats exhibit moderate to intense degradation.

Material and methods

Two land cover maps of 1956 and 1999 of the study area were generated by photo-interpretation of orthophotomaps at 1:5 000 scale. The map of 1956 was photo-interpreted on an orthophotomap generated *ad hoc*, by geo-correction and mosaic of black and white aerial photographs. In contrast, the 1999 map was directly photo-interpreted on colour orthophotomaps produced by the Cartographic Institute of Catalonia. Contrasting colour and quality of images, and the availability of ancillary information on vegetation categories for 1999 but not for 1956 determined a contrasting thematic resolution for both land cover maps. In order to homogenise the results of photo-interpretation, to facilitate map comparison and to reduce the number of land cover classes to be analysed, the land cover classes were reclassified into four naturalness classes. We defined naturalness as the degree of preservation of the pristine state (Figs. 1a,b): High (natural marshland habitats), medium (disturbed natural habitats and formerly abandoned fields), low (crops and recently mowed areas), and null (urban areas, roads and railways). A naturalness change map describing land stability was then obtained by combining the naturalness maps of 1956 and 1999 (Fig. 1c). Only three stability regimes were considered for the present study: stable (high naturalness in 1956 and 1999), semi-stable (medium or low naturalness in 1956, and high in 1999), and non-stable (medium naturalness in 1999).

The relationship between invasibility and stability was assessed in four plant communities representing several of the pristine habitats in the Llobregat delta (Bolòs & Bolòs, 1950) and responding to contrasting conditions of water availability and conductivity: reedbeds, rushbeds, halophilous scrublands, and fixed dune communities. Two study areas for each plant community and stability regime were selected using the naturalness change map and additional information on current vegetation. In each area, a mean of 5 10×10 m plots were marked out and vegetation inventories were performed therein following the classical sigmatist method. The number and percentage of alien species and their relative cover were compared among communities, stability regimes and areas, by means of ANOVA tests after normalisation by arcsin transformation. Pairwise comparisons between semi stable and non-stable regimes were carried out a posteriori for each community using Tukey's test.

Results

A total of 17 alien species were found in the study (Table 1). Fixed dune communities were invaded by most species (11), followed by reedbeds (10), rushbeds (4) and halophilous scrubs (2). *Aster squamatus* (Spreng.) Hieron. was the most frequent alien, growing in 25% of samples and colonizing all the communities studied. *Cortaderia selloana* (Schultes ex Schultes) Asch. et Graetbn. was found in reedbeds and secondarily in fixed dune communities, whereas *Cuscuta campestris* Yuncker was mainly located in fixed dunes and occasionally in rushbeds. *Oenothera glazioviana* Minchx. and *Carpobrotus edulis* (L.) N.E. Br.were exclussively found in fixed dune communities. Seven species were found only once.

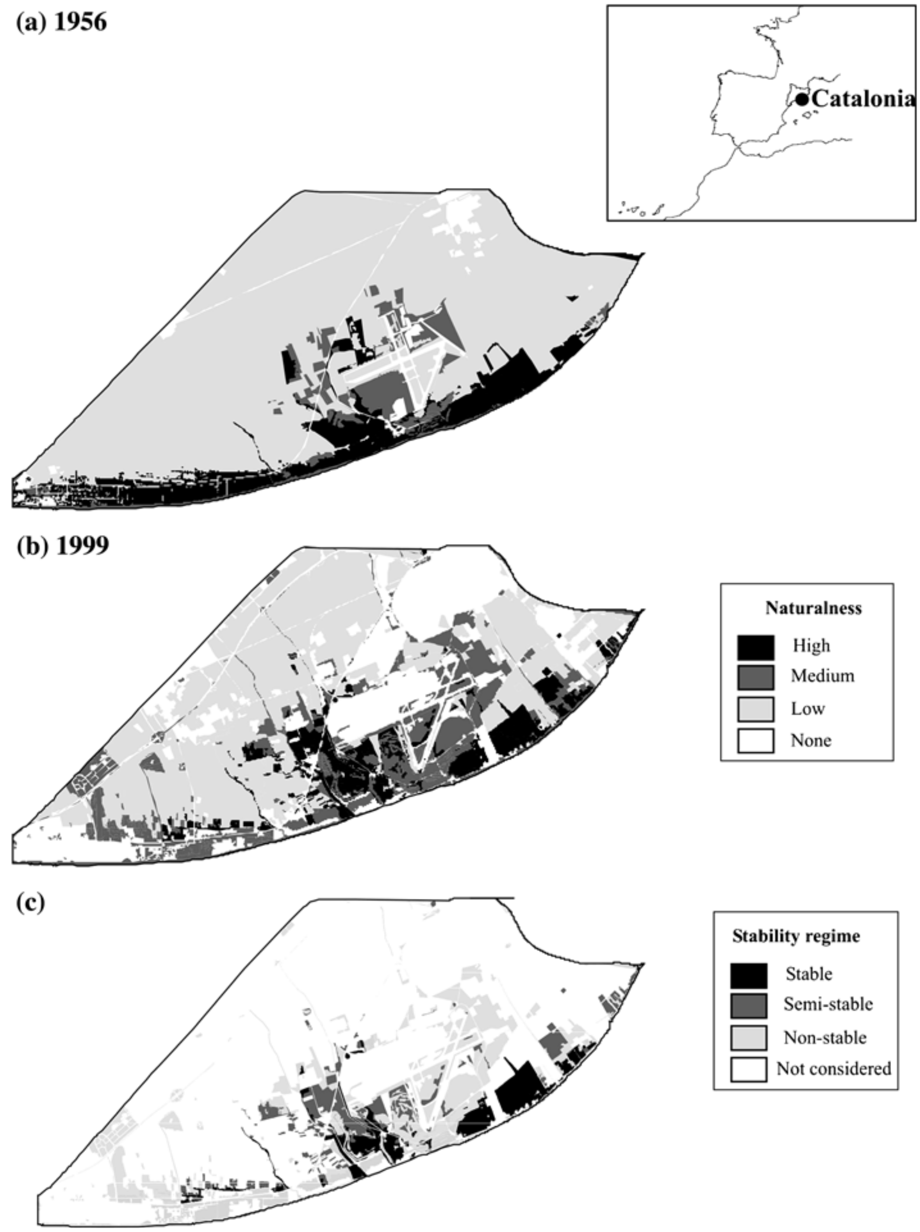

Figure 1. Habitat naturalness in (a) 1956, and (b) 1999 in the southern hemidelta of Llobregat, and (c) the associated naturalness change map showing the stability regimes selected for the study (see text for more details).

Table 1. Percentage of inventories of each community and stability regime with presence of each alien species. Stability regimes: SS, semi-stable; NS, non-stable. There were no alien species in the inventories of the stable regime

	Reedbeds		Fixed dunes		Rushbeds		Halophilous scrubs	
	SS	NS	SS	NS	SS	NS	SS	NS
Amaranthus retroflexus L.		7.7						
Ambrosia coronopifolia Torrey et A. Gray		7.7						
Arundo donax L.		23.1		7.7				
Asparagus officinalis L.				7.7				
Aster squamatus (Spreng.) Hieron.	18.2	23.1	9.1	38.5	75.0	83.3	45.5	50.0
Carpobrotus edulis (L.) N.E. Br.			9.1	30.8				
Chenopodium ambrosioides L.		7.7		7.7				
Conyza bonariensis (L.) Cronq.				7.7				
Conyza sumatrensis (Retz.) E. Walter		30.8	18.2	30.8	8.3			
Cortaderia selloana (Schultes ex Schultes) Asch. et Graetbn.		30.8		7.7				
Cuscuta campestris Yuncker			36.4	30.8		8.3		
Ipomoea indica (Burm.) Merr.		7.7						
Lonicera japonica Thunb. in Murray		7.7						
Oenothera glazioviana Minchx.			45.5	23.1				
Phoenix canariensis Chabaud			9.1					
Rumex palustris Sm		23.1						40.0
Xanthium echinatum Murray				38.5		33.3		

Non-stable plots concentrated the majority of alien citations in reedbeds and dune communities. *A.squamatus, C. edulis, Conyza sumatrensis* (Retz.) E. Walker, and *Xanthium echinatum* Murray were found in more than 30% of non-stable plots, but in less than 20% of semi stable ones in fixed dune communities. *C. sumatrensis* and *C. selloana* were found in more than 30% of non-stable plots in reedbeds, whereas *Arundo donax* L., *A. squamatus*, and *Rumex palustris* Sm colonised more than 20% of these plots. Semi stable plots were only colonised by *A. squamatus*, which was also commonly found in rushbeds and in halophilous scrubs, either in semi-stable or non-stable plots. *R. palustris* and *X. echinatum* were, respectively, frequent in non-stable plots in halophilous scrubs and rushbeds, but absent in semi-stable plots. There were no records of alien species in stable plots, despite *A. squamatus* being observed at times in areas nearby these plots.

Stability was significantly related to the number and the proportion of alien species, and also to their relative cover (Table 2). Differences between communities were significant for the number and the relative cover of alien species, and marginally significant ($p = 0.057$) for the relative number of aliens. This was the only significantly different factor between areas. The interactions between stability and community, and between area and community were significant for both the number and the percentage of aliens, but not for the relative cover of aliens. The interactions between stability and area were never significant, whereas third-order interactions between all the parameters studied were always significant. Semi-stable and non-stable regimes exhibited contrasting invasibility patterns among communities (Fig. 2). Non-stable reedbeds and fixed dune communities showed significantly higher number, percentage and relative cover of alien plants than semi-stable ones. In contrast, there were no significant differences between non-stable and semi-stable plots in rushbeds and halophilous scrubs.

Discussion

Most of the Catalan alien species occur in heavily human-disturbed habitats, with few of them able to succeed in natural communities (Casasayas,

Table 2. Summary of ANOVA aimed at comparing the effects of stability regime, community, and area on the number and the percentage of alien species, and on the percentage of species cover corresponding to aliens

	df	MS	*F*	*p*
Number of alien species				
Stability	2	169.216	25.567	< 0.001
Community	3	481.798	72.795	< 0.001
Area	1	4.796	0.725	0.396
Stability × community	6	107.464	16.237	< 0.001
Stability × area	2	3.512	0.531	0.590
Community × area	3	86.410	13.056	< 0.001
Stability × community × area	6	34.821	5.261	0.001
Percentage of alien species				
Stability	2	8.383	63.063	< 0.001
Community	3	0.343	2.584	0.057
Area	1	1.010	7.597	0.007
Stability × community	6	0.461	3.471	0.003
Stability × area	2	0.323	2.429	0.093
Community × area	3	0.584	4.397	0.006
Stability × community × area	6	1.288	9.688	< 0.001
Percentage of alien species cover				
Stability	2	3.695	36.210	< 0.001
Community	3	0.290	2.840	0.041
Area	1	0.251	2.462	0.119
Stability × community	6	0.196	1.923	0.083
Stability × area	2	0.063	0.619	0.540
Community × area	3	0.186	1.825	0.146
Stability × community × area	6	0.745	7.296	< 0.001

1990). We have found that natural, coastal plant communities in the Llobregat delta are potentially invaded by a number of alien plants, which appear to be relatively non-specific with the exception of several characteristic aliens of dune communities (*C. edulis, O. erythrosepala*, and to a minor extent *C. campestris*). In addition, habitat invasibility was related negatively to stability. Communities enclosed in areas that have persisted unaltered since 1956 exhibited a very low invasibility, with only a single species, *A. squamatus*, growing at extremely low densities. It is well established that disturbed habitats are regarded to be more vulnerable to invasion than unaltered ones (di Castri, 1990; Vitousek et al., 1997; Hobbs, 2000). This is particularly true in perennial communities dominated by one or a few species, especially in reedbeds, but also in several rushbeds and even dune communities, whose vegetative regeneration mechanisms are responsible for a dense canopy that prevents invasion. Disturbance might provide gaps that would enhance the establishment of invaders, as Jones & Doren (1997) reported for the exotic tree *Schinus terebinthifolius* in the Everglades.

Invasibility of reedbeds and fixed dune communities is related to historical stability, since alien species number and proportion and relative alien cover increased significantly from semi-stable to non-stable regimes. Assuming that historical change indicates how long ago main disturbances occurred, the decrease in invasibility from non-stable to semi-stable regimes would indicate a reversion towards the climax. During this process, the establishment of non-natives may be less opportune, basically as a result of decreasing resources or increasing colonization of clonal, dominant species that would reduce safe sites for alien germination and establishment (Hobbs & Huenneke, 1992). However, this pattern was not valid for rushbeds nor for

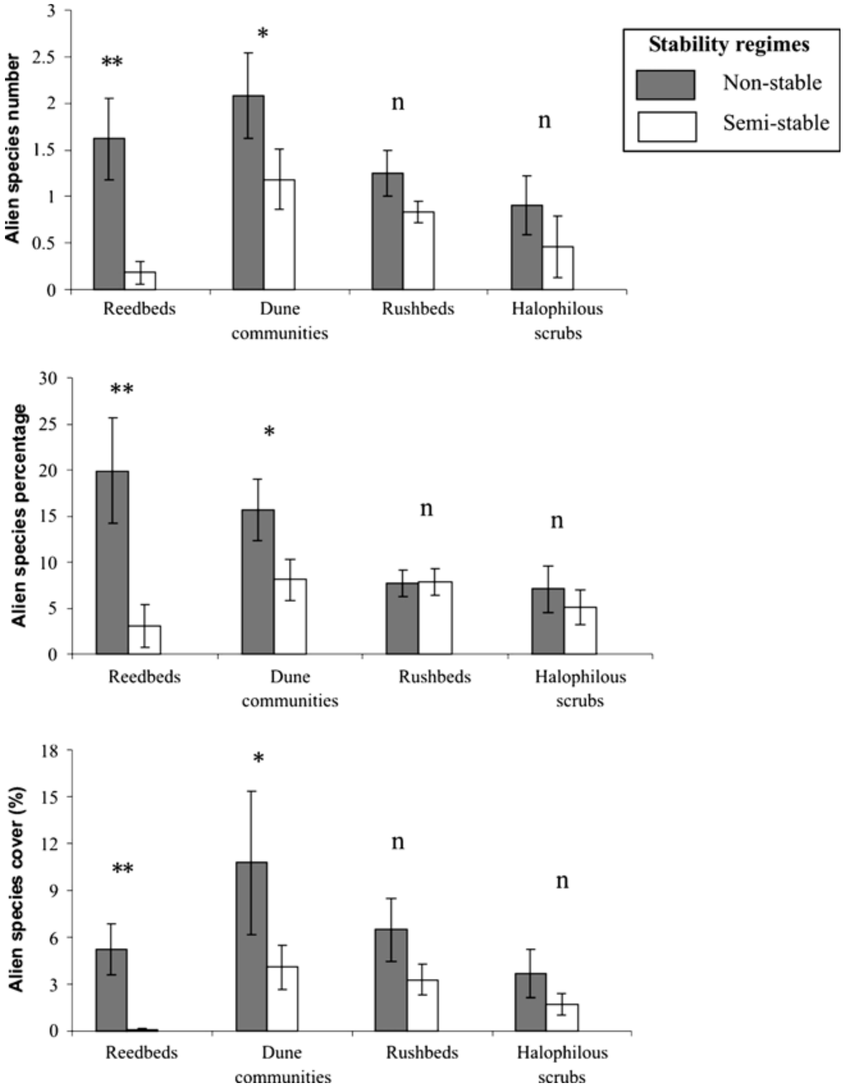

Figure 2. Number and percentage of alien species, and relative alien cover in four coastal communities in the Llobregat delta, in relation to the stability regimes selected for the study (see text for more details). Means and standard errors are shown. Significance of pairwise comparisons between semi-stable and non-stable regimes is shown (*: $p < 0.05$; **: $p < 0.01$; n: no significant).

Arthrocnemum scrubs in the Llobregat delta. Invasibility of a given habitat also depends on traits related to their adequacy for plant establishment, such as resource supply, non-biotic and biotic conditions (Alpert et al., 2000; Heger, 2001). Saline stress probably prevents these halophilous communities from invasion, as Alpert et al. (2000) reported for other harsh habitats such as xeric grasslands and desert vegetation in relation to drought and nutrient stress. Although bare soil might be abundant in halophilous communities, its colonisation by alien or even native plants is extremely difficult, being restricted to vegetation patches and nearby areas with lower conductivity and higher water and nutrient contents (Rubio-Casal et al., 2001).

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