PRIMARY RESEARCH PAPER

A comparison of lake classifications based on aquatic macrophytes and physical and chemical water body descriptors

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Received: 28 February 2008/Revised: 5 January 2009/Accepted: 12 January 2009/Published online: 10 February 2009 © Springer Science+Business Media B.V. 2009

Abstract The Water Framework Directive (WFD) requires that surface water bodies within a river basin be differentiated according to type, defined according to prescribed geographical or physico-chemical water body descriptors. Type-specific biological reference conditions, representing high ecological status, must be established for each derived water body type. A reference network of 20 lakes in Northern Ireland. representative of a WFD environmental typology, was sampled for physical and chemical variables and the lakes were classified on the basis of their aquatic macrophytes. A comparison was made between the efficacy of a WFD based multimetric approach and a multivariate approach at partitioning variation in lacustrine macrophyte communities. It was demonstrated by canonical correspondence analysis that a multivariate model explained more biological variation than a WFD multimetric classification. The predictive power of a set of environmental variables was tested using multiple discriminant analysis and canonical analysis of principal coordinates. These statistical

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methods were used to test how well the variables discriminated between groups in both models. The continuous variables effectively discriminated the a priori macrophyte classification groups; poor classification cross-validation rates were obtained using the WFD-based environmental classification lake groups. It was concluded that the WFD-based environmental classification did not adequately describe the ecological continuum that was evident from a classification based on aquatic macrophytes. It is implied from the findings that type specific reference conditions derived from large scale environmental classifications are inadequate as they do not sufficiently describe the ecological variation in lake macrophyte communities.

Keywords Lake macrophytes · Classification · Water framework directive · Reference conditions

Introduction

The general relationships between macrophyte species and the environmental characteristics of freshwaters, particularly water chemistry, are well established (e.g. Seddon, 1972; Hutchinson, 1975; Palmer et al., 1992; Jeppesen et al., 2000; Heegaard et al., 2001; Murphy, 2002; Duigan et al., 2007). Species–environment relationships are central to new bioassessment techniques that are being developed to help manage freshwater resources. Adopted in 2000, the EU Water

Handling editor: L. M. Bini

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Framework Directive (WFD) has established an ecological-based water management system in Europe. The overriding objective of the WFD is to achieve 'good surface water status', defined as when ecological status and chemical status are at least good (European Parliament, Directive 2000/60/CE).

Species-environment relationships in the WFD are described by a water body typology. Reference conditions correspond to a natural or near natural state and are of 'high ecological status'. According to WFD guidance, reference conditions do not equate to water bodies in a pristine state. They describe waters experiencing minimal disturbance, with human pressure resulting in very minor effects on biological, hydromorphological, and physico-chemical elements. The directive provides normative definitions of high ecological status in lakes for each quality element. Quality elements listed for aquatic macrophytes are taxonomic composition and abundance. The directive states that for 'high ecological status', these characteristics must correspond 'totally or nearly totally to undisturbed conditions'. As deterioration in biological status at a site is assessed by comparing what is found at the site with the biological reference conditions, it is imperative that type specific biological reference conditions are accurately derived. In regions where anthropogenic disturbances are not widespread, survey data may be used (a spatial approach), either alone or in conjunction with modelling to ascertain reference conditions. In areas with severe anthropogenic disturbances, derivation of reference conditions may involve methods such as paleoecology and modelling (Wallin et al., 2003). In Ireland, where many water bodies exist in relatively unimpacted catchments, there may be lakes that demonstrate no more than the required minimal deviation from a reference state, therefore facilitating a spatial approach to help determine biological reference conditions (Leira et al., 2006).

Reference conditions for European lakes have been developed on the basis of phytoplankton chlorophyll concentration (Carvalho et al., 2008), the majority of the proposed reference lakes having total phosphorus (TP) concentrations of $<50 \ \mu g \ l^{-1}$. Chlorophyll reference concentrations in the European study varied according to humic content, depth and alkalinity types, being lowest in deep, clear water, low alkalinity sites and highest for shallower, high alkalinity, humic lake types. A set of aquatic macrophyte species indicative of reference communities in European lakes using species data across a TP gradient has been derived (G-Tóth et al., 2008). Species indicative of reference conditions were found at lower TP concentrations ($<35-40 \ \mu g \ l^{-1}$), whereas tolerant species were found at higher concentration values ($>50-60 \ \mu g \ l^{-1}$).

The WFD states that water body types may be differentiated according to 'System A' or 'System B'. These two systems are comprised of core mandatory descriptors (altitude, depth, size and geology). System A provides descriptor categories for lake characterisation and divides Europe into 25 Ecoregions for surface waters, the island of Ireland being Ecoregion 17. The alternative, System B, outlines two additional obligatory descriptors (latitude and longitude) that determine lake characteristics and therefore the biological community structure and its composition. System B also provides 10 optional physico-chemical descriptors for typing lakes, which are mean water depth, lake shape, residence time, mean air temperature, air temperature range, mixing characteristics, acid neutralising capacity (ANC), background nutrient status, mean substratum composition and water level fluctuation (European Parliament, Directive 2000/60/CE). System B is therefore more flexible than System A and facilitates the use of statistical clustering methods, spatial classifications and expert judgement to characterise water bodies (Wallin et al., 2003).

The System A and B approaches of the WFD assume that on the basis of a set of similar physical or chemical descriptors, lakes should support similar, predictable biological communities. This type of landscape-based classification system has become popular despite there being a paucity of studies that demonstrate good agreement between the landscape classifications and biological variation (Heino & Mykrä, 2006). This study investigated the concordance between a biological lake classification based on aquatic macrophytes and a classification based on the System B approach. Successful partitioning of biological variation is essential if a multimetric approach is to be effective at fulfilling the needs of the WFD. The investigation was achieved using a spatial network of small (<50 ha), minimally disturbed lakes across Northern Ireland and an existing System B-based water body typology for lakes in Northern Ireland (Rippey et al., 2001).

Methods

Environmental classification

An environmental water body classification comprised of seven types derived using the WFD System B method was available (Rippey et al., 2001). This environmental typology placed lakes in classes, based on categories, for altitude (<175 m and 175–375 m), area (<2 ha and \geq 2 ha), geology (siliceous/calcareous) and ANC (<0.05 meq l⁻¹ and \geq 0.05 meq l⁻¹). All lakes were included in one depth and one mixing category as calculated using an empirical relationship (Ruiz, 1999).

Study sites

Twenty lakes (Table 1), with at least two from each of the System B seven water body types, were selected and the floating and submerged aquatic macrophyte abundance was estimated. The lakes chosen in each type were minimally disturbed and likely to be closest to the biological reference condition for aquatic macrophytes. Total phosphorus was used as the major determinant of reference state due to the relationship between TP and anthropogenic enrichment (Vollenweider, 1968; Wetzel, 2001). The TP concentration recorded during a previous survey of these lakes was less than 30 µg l⁻¹ (Wolfe-Murphy et al., 1992); the major ion composition was typical of undisturbed lakes (Rippey & Gibson, 1984) and a site visit revealed minimal disturbance.

Macrophyte abundance and distribution

Macrophyte sampling was carried out during spring, summer and autumn to fully represent the species composition of each lake. Lakes were circumnavigated and representative bays and headlands were chosen, recorded on a map and sampled for aquatic macrophytes. Macrophyte sampling was carried out from an inflatable boat, collecting data along six belt transects using a two-headed rake-grapnel and an underwater viewer. A transect was sampled in each of three bay areas and three exposed headlands (Department of Environment, 1987). The transect belts measured at least 100 m in length and were parallel to the shore; the width of each transect was variable as it extended to include the entire zone of macrophyte colonisation. The total circumference of smaller lakes

Table 1 Lakes sampled including classification group mem-
bership for a biological classification based on aquatic
macrophytes and a water framework directive-based System B
classification (Rippey et al., 2001)

Lake	Irish grid reference	System B class	Biological class
Lough Craigfad A	D263 168	А	1
Loughnacrackin	H568 786	А	1
Mill Lough	H742 886	А	1
Annachullion Lough	H519 303	В	4
Cloghcor Lough	H530 487	В	4
Coolyermer Lough	H181 424	С	3
Lough NaCranagh	D179 427	С	2
Lough Formal	H047 474	С	2
Cashel Lough Upper	H968 196	D	3
Lattone Lough	H001 455	D	3
Legane Lough	H737 538	D	3
Lough A Waddy	H041 644	D	2
Forkhill (unnamed lake)	J006 169	Е	3
Oak Lough	H498 841	Е	1
Sheetrim Lough	H907 194	Е	4
Lough Shannagh	J295 262	F	1
Lough Skale	H309 441	F	2
Black Lough	H652 755	G	1
Loughnabrick	D258 199	G	2
Lough Nabrickboy B	H036 502	G	2

was sampled if six transects of 100-m length were in excess of the lake circumference. A five-point semiquantitative scale (Plant Importance Value) was used to record the abundance and distribution of the macrophytes (Wolfe-Murphy et al., 1992).

Water analyses

Total phosphorus, ANC, conductivity and pH were recorded for each lake in each season and the mean value was taken. Methods to determine ANC, pH and conductivity followed The Standing Committee of Analysts (1979, 1982). Total phosphorus was determined according to Murphy & Riley (1962) and Eisenreich et al. (1975).

Lake classifications

A biological lake classification, based on aquatic macrophyte vegetation, was created using TWIN-SPAN (Hill, 1979a). Aquatic macrophyte taxa used

were as for the Northern Ireland Lake Survey (Wolfe-Murphy et al., 1992). The resultant macrophyte lake groups were confirmed by complementary analysis using Detrended Correspondence Analysis (DCA) (Hill, 1979b).

The relationship between the biological and abiotic data was explored using BIOtic/ENVironmental matching (BIOENV) (Clarke & Warwick, 1994) and Canonical Correspondence Analysis (CCA) (ter Braak, 1986). The robustness of the System B-type lake classification was assessed using ANalysis of SIMilarities (ANOSIM). ANOSIM compares statistics of within class biotic similarity to between class similarity. The 'R statistic' indicates the amount of similarity between lake groups, with 1 being the highest value obtainable in this type of analysis (maximal separation) (Clarke & Gorley, 2001).

Multiple Discriminant Analysis (MDA) (Tatsuoka, 1971) and Canonical Analysis of Principal coordinates (CAP) (Anderson & Willis, 2003) were used to test whether the continuous variables could predict the a priori lake groups that resulted from the macrophyte and System B classifications. Multiple discriminant analysis is a form of discriminant function analysis that uses more than one environmental variable to distinguish between groups. This method is used to determine whether groups differ with regard to the mean of a variable and then to use that variable to predict group membership (Field, 2001). Canonical analysis of principal coordinates is a method of generalised canonical variates analysis. This ordination uncovers the axes in principal coordinate space that best discriminate between a priori groups. The functions obtained from the analyses were used to allocate lakes to the a priori classification groups; MDA also determined the relative contribution of the chemical variables to the distinction among the lake groups. Leave-one-out cross validation was employed in both methods to provide a measure of classification success.

Results

Biological classification based on aquatic macrophytes

The TWINSPAN classification separated the lakes into four groups (Fig. 1), and physico-chemical characteristics of the groups are outlined in Table 2. The DCA ordination confirmed that the TWINSPAN endgroups appear as distinct clusters of lakes (Fig. 2). The DCA revealed an altitude gradient with lakes found at a higher altitude (Group 1) occurring towards positive values on the first axis and lower altitude lakes occurring closer to the origin.

The components of Group 1 (n = 6) are generally small, low diversity lakes (Table 3), occurring >150 m above sea level. Dominant macrophytes (frequency >60%) of Group 1 lakes include *Juncus bulbosus* L., bryophytes such as *Fontinalis antipyretica* Hedw. and the acidophilous *Sphagnum cuspidatum* Hoffm. This Group has the lowest median values for conductivity, ANC and pH.

Group 2 (n = 6) is relatively species rich, with 23 macrophyte taxa, and all lakes occurring >100 m



Fig. 1 TWINSPAN classification ontogeny of 20 minimally disturbed Northern Irish lakes based on aquatic macrophyte abundance

Table 2 The physico-chemical characteristics of the macrophyte lake groups

	Altitude (m)	Size (ha)	Conductivity $(\mu S \text{ cm}^{-1})$	ANC (meq 1 ⁻¹)	pН	$\begin{array}{c} TP \\ (\mu g \ l^{-1}) \end{array}$
Group 1 $(n =$	6)					
Mean	262	4.00	44	0.21	5.7	19
Median	228	1.50	38	0.13	5.8	15
Range	165-415	1.00 - 17.00	34–72	0-0.71	4.0-7.0	13–39
Group 2 ($n =$	6)					
Mean	210	5.04	81	0.45	6.8	19
Median	203	5.75	68	0.37	6.9	24
Range	125-350	1.25-8.25	48-142	0.18-0.95	6.0-7.2	7–27
Group 3 $(n =$	5)					
Mean	99	11.20	136	1.24	6.9	19
Median	105	6.00	116	0.68	6.8	13
Range	55-140	1.00-30.00	89–222	0.52-2.46	6.2–7.8	8–37
Group 4 ($n =$	3)					
Median	105	1.25	177	1.38	7.1	26
Mean	103	1.42	220	2.19	7.1	27
Range	65–140	1.00-2.00	146–336	1.32–3.86	6.7–7.4	26–32

Lakes were classified by TWINSPAN using aquatic macrophyte abundance. ANC Acid neutralising capacity, TP total phosphorus



Fig. 2 Detrended correspondence analysis ordination of 20 Northern Irish lakes. Groups created by a TWINSPAN classification appear as symbols: *filled square* Group 1 lakes, *cross* Group 2 lakes, *filled triangle* Group 3 lakes, *filled circle* Group 4 lakes

above sea level. Frequently occurring species include the isoetids, *Isoetes lacustris* L. and *Littorella uniflora* (L.) Asch. in association with *Myriophyllum alterniflorum* DC. The group's water chemistry is characterised by circum-neutral pH, low ANC and conductivity.

Group 3 (n = 5) is the most diverse in the macrophyte classification (24 taxa), all lakes occurring >50 m above sea level. Macrophytes occurring with a high frequency are *Elodea canadensis* Michx.,

Nymphaea alba L., Potamogeton natans L. and Sparganium angustifolium Michx.

Group 4 (n = 3) supports 13 taxa. This group of low altitude lakes has the highest mean and median values for ANC, conductivity and pH. The median TP concentration (26 µg l⁻¹) places this group in the meso-eutrophic range. The commonly occurring species of the group are *Nuphar lutea* (L.) Sm., *N. alba, Sparganium emersum* Rehmann and *P. natans*, which inhabit mesotrophic to eutrophic waters (Haslam et al., 1982; Preston, 1995; Preston & Croft, 1997).

Ordination of the reference lakes

The first DCA axis (Fig. 2) has a high eigenvalue (0.560), demonstrating that the community structure is dominated by a single gradient. Species distribution across this gradient is evident (Fig. 3) with macrophytes characteristic of oligotrophic conditions generally occurring on the positive side of the first axis; species tolerant of more eutrophic or alkaline conditions occur near the origin or have negative values on the first axis.

Canonical correspondence analysis (CCA) showed that TP explained little variance in the data. This is

Table 3 Frequency of aquatic macrophyte taxa occurrence across lake groups formed by a TWINSPAN macrophyte classification

Taxonomic group	Taxa code	Group 1 N = 6	Group 2 N = 6	Group 3 N = 5	Group 4 N = 3 S = 13	
		S = 12	S = 23	S = 24		
Algae (filamentous)	Alga fil	III	II	_	_	
Algae (other)	Alga oth	IV	II	Ι	_	
Batrachospermum spp.	Batr chs	II	_	_	_	
Callitriche hamulata	Call ham	_	_	Ι	_	
Characeae	Characeae	_	II	III	Ι	
Eleogiton fluitans	Eleo flu	_	_	Ι	_	
Elodea canadensis	Elod can	_	Ι	IV	_	
Fontinalis antipyretica	Font ant	III	III	Ι	_	
Isoetes lacustris	Isoe lac	II	IV	_	_	
Juncus bulbosus	Junc bul	IV	Ι	III	_	
Lemna minor	Lemn min	_	_	II	_	
Lemna trisulca	Lemn tri	_	_	II	Ι	
Littorella uniflora	Litt uni	_	V	III	_	
Lobelia dortmanna	Lobe dor	II	III	Ι	_	
Moss (other)	Moss oth	IV	II	Ι	II	
Myriophyllum alterniflorum	Myri alt	_	V	II	_	
Myriophyllum spicatum	Myri spi	_	_	_	Ι	
Nuphar lutea	Nuph lut	_	Ι	III	v	
Nymphaea alba	Nymp alb	_	_	V	v	
Persicaria amphibia	Pers amp	Ι	_	_	_	
Potamogeton alpinus	Pota alp	_	IV	Ι	Ι	
Potamogeton berchtoldii	Pota ber	_	_	III	_	
Potamogeton lucens	Pota luc	_	II	III	Ι	
Potamogeton natans	Pota nat	_	IV	IV	IV	
Potamogeton obtusifolius	Pota obt	_	Ι	Ι	_	
Potamogeton pectinatus	Pota pec	_	Ι	_	Ι	
Potamogeton perfoliatus	Pota per	_	Ι	III	_	
Potamogeton polygonifolius	Pota pol	Ι	II	II	_	
Potamogeton praelongus	Pota pra	_	Ι	_	_	
Potamogeton pusillus	Pota pus	_	_	Ι	_	
Sparganium angustifolium	Spar ang	Ι	IV	V	_	
Sparganium emersum	Spar eme	_	Ι	_	IV	
Sphagnum spp.	Spha spe	III	III	_	II	
Utricularia australis	Utric au	_	_	_	Ι	

I: >0 to 20%, II: >20 to 40%, III: >40 to 60%, IV: >60 to 80%, V: >80 to 100%. N number of lakes in each lake group, S number of taxa in each lake group

most likely due to the lakes being chosen on the basis of their relatively low TP concentration. Therefore, TP was excluded from subsequent analyses. A CCA of macrophyte abundance, conductivity, ANC, pH, size and altitude values yielded eigenvalues of 0.491 and 0.236 for axis 1 and 2, respectively. These axes explained 23% of the variation in the macrophyte species data. A Monte Carlo permutation test showed that both axes were significant at the 5% significance level. Forward selection of environmental variables showed that conductivity and ANC were the most important parameters in explaining the macrophyte

Fig. 3 Detrended correspondence analysis ordination of 20 Northern Irish lakes showing aquatic macrophyte taxa. Taxa codes are shown in Table 3



community variation; since they are highly and negatively correlated to altitude, these parameters dominate the first CCA axis. Lakes with relatively high values for conductivity and ANC tended to be lowland lakes. There is a progression from Group 1 lakes (higher altitude, lower conductivity and ANC) to Group 4 (lower altitude with higher values for conductivity and ANC) (Fig. 4). BIOENV analysis confirmed the findings of the CCA with conductivity and ANC the most important variables at explaining variation in the macrophyte data. A CCA using only the categorical variables of the System B classification produced eigenvalues of 0.373 and 0.204 for the first two axes, both significant axes explaining 18% of biological variance.

Strength of the classifications

ANOSIM was used to investigate within-group and between-group similarities of the System B classification. A global R statistic of 0.29 (significant at the 5% probability level) was obtained, demonstrating the weakness of the category-based multimetric classification at capturing the variance in the lake macrophyte communities. MDA and CAP were used to investigate the predictive power of both classifications. The discriminant functions obtained from the analyses were used to allocate the lakes to a priori macrophyte and System B groups; MDA determined the relative contribution of the environmental variables to the distinction among the groups. Using the continuous variables and the a priori macrophyte lake groups, the MDA eigenvalues for the first three discriminant functions were obtained as 4.64, 0.47 and 0.38, respectively. The first function explained a high percentage of the variance (85%) in the lake groups, which were found to be significantly different (Wilks' lamba = 0.088) at the 1% level. The main descriptors for the first function were conductivity, pH and altitude (standardised canonical discriminant function coefficients of 0.916, 0.653 and -0.519, respectively). Using the functions based on these



Fig. 4 Canonical correspondence analysis ordination of 20 Northern Irish lakes. Groups created by a TWINSPAN classification appear as symbols. *ANC* Acid neutralising capacity, *filled square* Group 1 lakes, *filled circle* Group 2 lakes, *filled triangle* Group 3 lakes, *cross* Group 4 lakes. Conductivity and ANC have been transformed (log x + 1)

variables to predict the macrophyte lake group, with leave-one-out cross validation led to 50% of the lakes being assigned to the correct macrophyte classification group (Table 4). Using the same predictor variables, the a priori groups from the System B classification were used in the analysis; the main descriptors of the first function were conductivity and altitude (standardised canonical discriminant function coefficients 0.723 and -0.464, respectively) explained 48% of the variance. A leave-one-out cross validation test gave a value of 15% of cases being allocated to the correct System B lake group (Table 5); the System B groups

 Table 4 Cross validated classification success of the macrophyte classification resulting from a multiple discriminant analysis; 50% of lakes are correctly classified

	Lake macro group	Pre me	dicte mber	Total		
		1	2	3	4	-
Cross validated count	1	3	3	0	0	6
	2	2	3	1	0	6
	3	0	3	1	1	5
	4	0	0	0	3	3

Table 5 Cross validated classification success of a water framework directive-based environmental classification (Rippey et al., 2001) resulting from a multiple discriminant analysis; 15% of lakes are correctly classified

	System B lake group	Pr me	Predicted group membership						Total
		A	В	С	D	Е	F	G	
Cross validated count	А	1	0	0	0	1	0	1	3
	В	0	1	1	0	0	0	0	2
	С	0	1	0	1	0	0	1	3
	D	0	1	1	0	2	0	0	4
	Е	1	1	0	0	1	0	0	3
	F	1	0	0	0	0	0	1	2
	G	2	0	0	0	1	0	0	3

were not found to be significantly different. CAP was also used to generate generalised discriminant functions and test for differences between lake groups by permutation. The CAP method also provides a measure of classification success. Using the macrophyte classification, a priori lake groups yielded a first axis explaining 81% of the variation. The first canonical axis was highly correlated with altitude and conductivity (-0.89 and 0.72, respectively). The cross validation also gave a success rate of 50%, the groups being significantly different at the 1% level. When the ordination was performed using the System B a priori lake groups, the first canonical axis was highly correlated with altitude (-0.83); however, only 20% of lakes were classified into the correct group when the classification was cross validated.

Discussion

Successful implementation of the WFD requires the incorporation of a broad range of biological, physical and chemical variables. The legislation provides aquatic biologists and water managers with only cursory guidelines as to how to achieve working classifications and subsequent monitoring systems for the sustainable use of waters and conservation of important habitats and features. There have been attempts to adapt current classification and monitoring systems to meet the needs of the Directive (Clarke et al., 2003; Davy-Bowker et al., 2006); however, there are still many questions regarding the effectiveness of the various approaches within the framework

provided by the Directive (Moss et al., 2003). The WFD advocated approach assumes that physicochemical characteristics account for most of the biological variation and that these parameters measured at large scales will influence factors that control local biological attributes (Pyne et al., 2007). These types of landscape-based classifications have been widely applied to lotic systems (Hawkins et al., 2000) with many studies using invertebrates. However, ecoregion and geology models have been consistently found to account for a poor amount of variation in biological communities in rivers, local effects being stronger than landscape characteristics (Van Sickle & Hughes, 2000; Verdonschot, 2006; Pyne et al., 2007).

The System B classification performed relatively poorly in explaining biological variance (as shown by CCA and ANOSIM). The classification based on aquatic macrophytes was more effectively explained by a set of quantitative variables than the System B typology (MDA cross classification validation rates of 50% and 15%, respectively). G-Tóth et al. (2008) found that lake classes based on three WFD intercalibration types derived using physico-chemical and geological parameters had little concordance with a classification based on aquatic macrophytes. This lack of congruence demonstrated between the biological and physico-chemical classifications has serious implications for implementing the WFD, which advocates the use of a multimetric based typology of water bodies to define reference conditions.

Although some similarity was evident between the category-based System B lake classification and the macrophyte lake classification (Table 1), it would not be possible to use the physico-chemical-based classification of lakes (Rippey et al., 2001) to predict the macrophyte flora of lakes. This implies that typologybased methods are not as successful as those employing continuous variables at estimating reference values for a metric. Carvalho et al. (2008) also suggest that European type specific chlorophyll reference conditions may not be ideal as they do not adequately represent the continuous nature of water colour and depth. They propose the use of site specific reference-based assessments developed by empirical regression models. Similarly, the System-B type classification did not adequately describe the ecological continuum evident in the study lakes. Similar continua have been described by Heuff (1984), Wolfe-Murphy et al. (1992), Palmer et al. (1992) and Duigan et al. (2007). Therefore, site specific reference conditions are also advised for lake macrophytes. Good correspondence was found between the macrophyte classification groups in this study and those of Duigan et al. (2007), with the exception of Group 3, which has some correspondence to Group E. Similarities are not strong for these two groups, especially regarding the lower Northern Irish values observed for conductivity and ANC. This lack of agreement may be due to the inclusion of impacted water bodies by Duigan et al. (2007); as lowland lakes are the most vulnerable to eutrophication, it would not be expected to find close agreement between the two classifications for lowland lake groups.

A recent model for predicting site specific reference phosphorus concentration in European lakes (Cardoso et al., 2007) found TP concentration to be negatively correlated with altitude. Altitude was found to be an important surrogate for unmeasured variables, which together make up a complex gradient (Whittaker, 1967). In Northern Ireland, a close relationship exists between the solid geology (which is closely related to altitude) and the water chemistry of the lakes (Gibson et al., 1995). Altitude was correlated with water chemistry in this study with upland lakes being of lower conductivity compared to lowland lakes. Altitude is a strong predictor of aquatic plant richness (negatively correlated), a fact attributed to the indirect effects of temperature and length of growing season (Jones et al., 2003). Heegaard et al. (2001) established that the chemical and nutrient composition of lakes in Northern Ireland was strongly correlated with altitude and that the occurrence of certain macrophyte species was dependent on catchment and local scale land use. It was concluded that the major influence on lakes was anthropogenic eutrophication, correlated with altitude and derived mainly from agricultural activities. Intensive agricultural activities occur mainly in the lowland areas of the country. Catchments of the lakes in this study were the least impacted lakes available with the majority of lake catchments containing habitats of national or international conservation importance. Countries such as the Republic of Ireland (Leira et al., 2006) and Scotland (Bennion et al., 2004) have used paleolimnological methods to confirm their reference lake suite, and similar validation of Northern Irish reference lakes would be useful, especially in relation to lowland lake reference sites.

Instead of predicting the macrophyte lake groups using predefined physico-chemical categories, it has been found in this study that it is more effectual to first study the biota and then subsequently investigate its relationship with physico-chemical variables. The superiority of this approach is supported by the results of the ANOSIM, MDA and CAP analyses. Various modelling methods are available for subsequent macrophyte prediction at reference sites. The RIVPACS technique, developed for invertebrates (Wright et al., 1997; Wright, 2000), has inspired many similar methods globally (Smith et al., 1999; Carlisle & Meador, 2007; Feio et al., 2007) and a similar paradigm for macrophytes PLANTPACS, (Maberly et al., 2000) and combinations of biological groups (Williams et al., 1998). It is common for dystrophic peat or acid moorland lakes to naturally support very low numbers of aquatic macrophyte taxa (Duigan et al., 2007). Therefore, assessment methods based solely on taxon occurrence, which have been highly successful for organisms such as river invertebrates, may be more challenging to use for lake macrophytes. Approaches based on biological metrics or a combination of indices (Williams et al., 1998; Schaumburg et al., 2004; Johnson et al., 2006; Penning et al., 2008) may be useful complements.

This study signifies the preliminary stages of the development of a predictive model for macrophyte communities in lakes. The work must be built upon using a variety of modelling methods to meet the needs of the WFD. Descriptors for System A and System B classifications have been described as too rigid with temperature, current and size being more successful in partitioning invertebrate variation in rivers (Verdonschot, 2006). Area, pH and altitude have been found to explain most variation in macroinvertebrate lake communities (Neale & Rippey, 2007). It has been shown in this study that a multimetric System B method for water body classification was not as successful as using a multivariate approach in explaining biological variation in lentic habitats. Ecoregion classifications remain useful for initial stratification of sites (Hawkins et al., 2000) and may be improved, for example, by the use of tree-based models (Lamon et al., 2008). These models may help derive more sensitive and numerous descriptor categories for the parameters identified as the main drivers of biological variation, which in the case of macrophytes in this study were altitude and conductivity/ANC.

Acknowledgements The manuscript has benefited from comments by three anonymous reviewers and the editor. The work was made possible by funding from the University of Ulster.

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