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An ecological assessment system for sub-alpine lakes using macroinvertebrates –

The development of a parsimonious tool for assessing ecological health of European lakes

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1. Introduction

The Water Framework Directive (WFD; DIRECTIVE 2000/60/EC) has initiated a change in both the concept of water quality and its assessment throughout Europe. It has started a shift from the mindset of Europe's water resource being a product which may be monitored chemically to ensure its suitability for human use to one that regards water as a heritage. A more holistic assessment of functioning and structure of aquatic ecosystems is now legally required to be preformed by member states and must include the biological groups: fish, phytoplankton, macrophytes and phytobenthos and macro-invertebrates (Irvine *et al.* 2002; Heiskanen *et al.* 2004). For member-states this represents a highly complex task.

Benthic macroinvertebrates play an essential role in key processes of lakes (food chains, productivity, nutrient cycling and decomposition: Reice & Wohlenberg 1993). Profundal macroinvertebrates form an important link between detrital deposits and higher trophic levels in aquatic food webs (Brinkhurst, 1974). In principle, any environmental changes in lakes, for example in nutrient concentrations, would be reflected by changes in the structure of the benthic invertebrate community (Carvalho *et al.* 2002).

Recent extensive reviews of the current state-of-the-art of ecological water quality assessment systems in Europe have revealed that, while practical (and WFD-compliant) assessment tools using macroinvertebrate parameters are already in use to assess the ecological quality of rivers, in many European countries there are currently no working macroinvertebrate assessment systems for lakes (Cardoso *et al.* 2005; Nõges *et al.* 2005). Indeed, this has been recently identified as one of the major ecological 'knowledge gaps' impeding the full assessment of ecological quality of lakes as required by the WFD in a literature review carried out within the EU project REBECCA[1] (Heiskanen and Solimini 2005). The current lack of knowledge is also limiting the fulfilment of the EU-wide intercalibration of the lake ecological quality assessment systems in Europe, and thus compromising the basis for setting the environmental objectives as required by the WFD, particularly concerning quantification of the ecosystem impacts of nutrient loading pressures (i.e. eutrophication), which is one of the most wide-spread pressures on surface water ecological quality in Europe (EEA, 2003).

Despite their key role in aquatic ecosystems, benthic macroinvertebrates are perhaps one of the most difficult biological groups to develop an assessment system for in lakes. Three factors may be largely responsible for this: their complex biotic structure, high temporal variability and the high spatial variability associated with substrate heterogeneity found in lakes (Brose *et al.*, 2004). A solution needs to be found to experimentally control natural variability so that anthropogenic impact may be identified and extracted from other sources of variation.

Generally, the benthic zone of lakes can be divided along the depth profile into the littoral, sub-littoral and profundal zones. The littoral zone is defined as the nearshore lake bottom areas where emergent macrophytes grow. The sub-littoral zone is defined

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[1] Specific Targeted Research Project under the EC 6th Framework programme (SSPI-CT-2003-502158) http://www.environment.fi/syke/rebecca.

as the bottom area covered by submerged macrophyte or algal vegetation. Often, empty shells of molluscs are accumulated at its lower end (littoriprofundal) and thus form a specific sediment type. The lake bottom area extending deeper is called the profundal zone, which consists of exposed fine sediment free of vegetation.

Previous work by Free *et al*. (2006) examined benthic macroinvertebrates in 200 lakes from both the littoral (~0.5 m deep) and profundal zones (>12 m deep). The development of assessment systems in the profundal region of lakes was obstructed by the effect of natural deoxygenation in stratified lakes – which has the same effect as anthropogenic mediated deoxygenation and therefore was impossible to differentiate. In contrast, the development of an assessment system based on littoral invertebrates was largely obstructed by the complex biotic structure and the high substrate heterogeneity typical of the littoral zone.

This project aimed to develop an ecological assessment system sensitive to eutrophication pressure using benthic macroinvertebrates. There are many factors that affect macroinvertebrate communities in the natural environment. This work controlled as many environmental factors as possible by sampling design so that the influence of eutrophication on macroinvertebrates could be more clearly understood and an assessment system produced. Table 1 lists the main factors controlled and the rational.

Table 1 Environmental factors controlled by sampling design and rational.

Factor controlled	Rational
Season	Benthic macroinvertebrates communities display substantial seasonal variation. This project focused on Spring.
Typology	Different types of lakes may respond to eutrophication differently. Overall the lakes selected fit the typology: of medium sized lakes (0.5 - 4 km^2), high alkalinity (> 2 meq l^{-1}), mid altitude (mostly 200 - 800 m) and medium depth (maximum depth typically 12-35 m).
Depth, oxygen and lake zone	Lakes were sampled in the sub-littoral zone between 4-5 m depth. This should reduce the influence of oxygen on benthic macroinvertebrate communities as it is typically above the depth of thermocline development (Irvine *et al.*, 2001).
Substrate	Benthic macroinvertebrate communities vary depending on substrate. Only soft sediment was sampled using an Ekman grab.

The key aspect of the research approach taken here that should mark an improvement over previous attempts to develop an assessment system, is the focus on the sub-littoral zone (sampling at 4-5 m depth). This has the benefit over sampling in the profundal zone (>12 m) where the effects of natural deoxygenation complicate interpretation of community changes with eutrophication. The sub-littoral also has the benefit over sampling in the littoral in that it suffers less from the complex heterogeneity of substrate and exposure. In addition the focus on a narrow typology should reduce the complicating influence of other environmental variables at the broader scale.

Key tasks of the work were:
1. The identification of key environmental factors to control in order to elucidate the response of benthic macroinvertebrate communities to eutrophication.
2. Selection of 45 lakes in Austria, Germany and Italy of similar typology.
3. To carry out an extensive sampling programme targeting the sub-littoral habitat.
4. The laboratory identification and enumeration of benthic macroinvertebrate species.
5. The measurement of key supporting chemical and physical parameters.
6. Integration of data into a database
7. Analysis of data
8. Development of an ecological assessment tool for benthic macroinvertebrates for sub-alpine lakes.

2. Materials and Methods

Fieldwork

Biological samples were taken from soft sediment in the sub-littoral (4-5 m) using an Ekman grab. Three sites around the lake were sampled with two replicates per site (6 samples per lake in total). A previous sampling campaign in 2005 had indicated that 6 samples from the sub-littoral may be enough to distinguish differences in taxa richness between lakes (Figure 1). Samples were sieved in a mesh size not exceeding 250 µm. Samples were preserved in either 70% ethanol or 5% formalin. From a mid-lake station, supporting environmental parameters were measured: temperature and oxygen profiles, alkalinity, pH, Secchi depth and conductivity (reported at 25°C), total phosphorus and chlorophyll *a*. Total phosphorus was also measured at all sub-littoral stations. A 100g sediment sample was taken from each of the 3 macroinvertebrate sub-littoral stations and dried at 40°C for determination of loss on ignition and other sediment characterisation parameters.

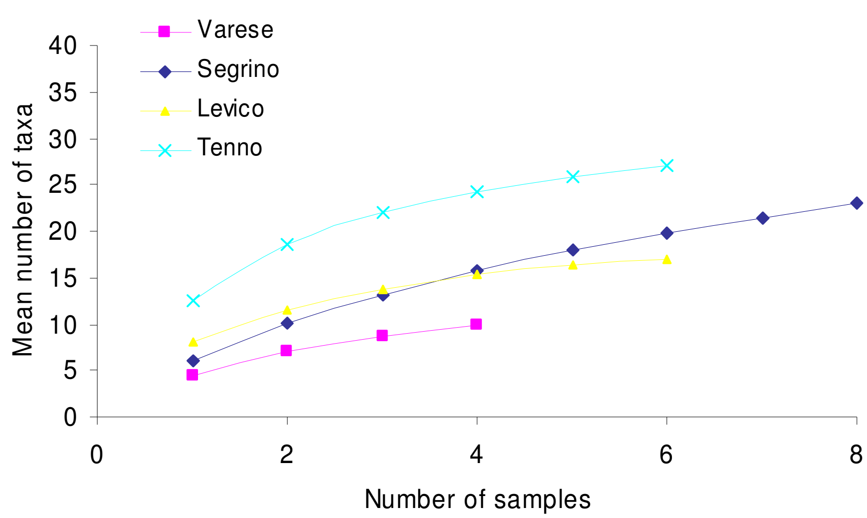

Figure 1 Species area curves for the sub-littoral of four lakes sampled in 2005 using a Ponar grab (from Free *et al*. 2005). Lakes have different trophic status (Tenno<Levico<Segrino<Varese).

Laboratory methods

Physicochemical measurements

Total phosphorus was measured following Eisenreich *et al.* (1975), Loss on ignition was measured following Heiri *et al.* (2001). Alkalinity was measured by titration with H_2SO_4 following Mackereth *et al* (1989) or HACH (1997).

Lake altitude, area and maximum depth were obtained from national authorities. The index of lake basin shape (ILBS) was calculated as maximum depth (m) divided by the square root of lake area (km^2) following Nürnberg (1995).

Biological analysis (Sorting, elutriation and identification)

Sorting

1.1. Samples were washed trough nested sieves (from 5 mm to 0.250 mm) to help separation of animals and associated material.
1.2. Samples separated by sieve were preserved in alcohol 75° or formalin 5%.
1.3. All the material to be sorted was divided in small portions and each portion was placed in a white tray, large animals were sorted by eye (elutriation was needed, see 2 below).
1.4. The remaining material was concentrated in Petri dishes and all the remaining animals were sorted under 25x magnification.
1.5. Sub-sampling was adopted in very few cases when the number of animals of a given taxon was more than 200 individuals. In almost all cases the entire sample was examined.

Elutriation

Samples were elutriated in the laboratory to remove inorganic sample debris (e.g. sand and gravel) before sorting. The purpose of this step was to minimize the effects that inorganic debris has on spreading organisms in a sorting tray.

Procedure for elutriation

2.1. The entire sample was carefully swirled in a tub of water to suspend the organic debris and organisms.
2.2. Once suspended, the organic debris was poured into another wash basin, leaving behind the heavier, inorganic debris (cased caddisfly were very rare and when present were collected).

Identification

Taxa were identified using the following references:

To identify chironomids:

Wiederholm, T. (ed.) 1983. Chironomidae of the Holartic region. Keys and Diagnoses. Part I: Larvae. Entomologica Scandinavica Supplement 19: 1-457.

Wiederholm, T. (ed.) 1986. Chironomidae of the Holartic region. Keys and Diagnoses. Part II: Pupae. Entomologica Scandinavica Supplement 28: 1-482.

Wiederholm, T. (ed.) 1989. Chironomidae of the Holartic region. Keys and Diagnoses. Part III: Adult males. Entomologica Scandinavica Supplement 34: 1-532.

Langton, P. H. 1991 A Key to Pupal Exuviae of West Palaearctic Chironomidae. Privately published by the author. 386 pp.

To identify Oligochaetes:

Brinkhurst, R.O. & BGM. Jamieson, 1971. Aquatic Oligochaeta of the world. – Oliver and Boyd. pp. 860.

Tarmo Timm 1999 A Guide to Estonian Annelida. Estonian Academy Publishers, 207 pp.

For all the other taxa:

Campaioli S., Ghetti P.F, Minelli A., Ruffo S. (1994) “Manuale per il riconoscimento dei macroinvertebrati delle acque dolci italiane” Vol. I – II Prov. Autonoma di Trento.

Quality control

Total phosphorus (TP) concentration in water was one of the key parameters focused on as the primary indicator of the pressure of interest in this study. It was therefore important to subject this parameter to both quality control and quality assurance procedures. Figure 2 shows that the internal quality control prepared for the innovation competition project had a mean TP of 30.5 μg l^{-1}. Most of the analysis fell within one standard deviation from this and all fell within two standard deviations. An external PO_4 quality assured sample (BCR CRM 617) was analysed to provide a degree of quality assurance. The quality assurance material was taken from groundwater and therefore most of the P fraction would be expected to be present as PO_4 thereby being suitable to indicate TP values. The mean of the CRM 617 measured was 262.5 μg l^{-1} TP, which was within one standard deviation (10 μg l^{-1}) of the indicative value of 272 μg l^{-1} PO_4.

For Loss on ignition (LOI) at 550 and 950^{o}C a QC sample was run alongside samples. The QC was always within ±1% of mean values for both 550 and 950^{o}C.

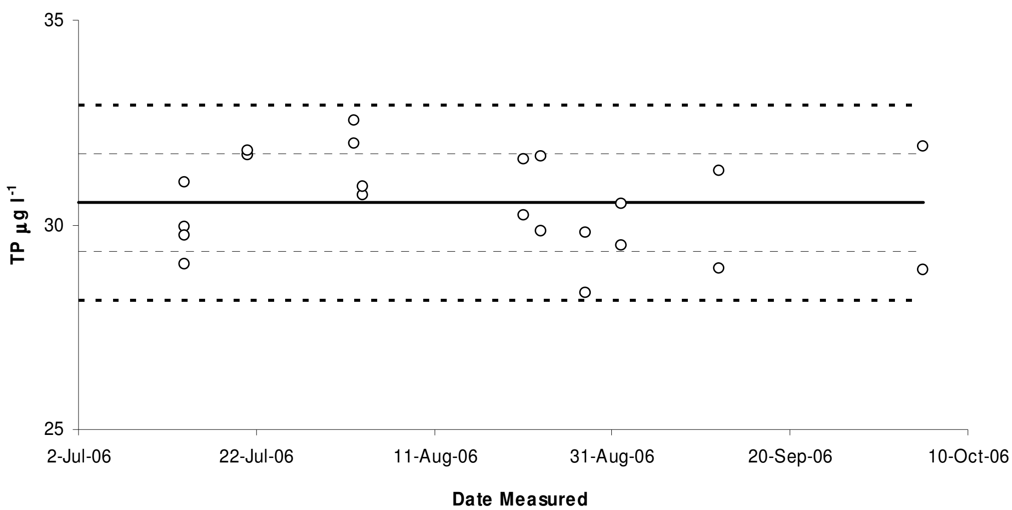

Figure 2 Quality control chart for total phosphorus. Solid line represents mean QC, dashed lines represent one and two standard deviations respectively.

Statistical methodology

For ordinations and weighted average approaches rare species, defined as those occurring in less than five of the study lakes were excluded from the analysis. Statistical analysis was performed in Microsoft Excel and PC-ORD (version 4).

Weighted average approach (1)

For each species the mean (weighted by species abundance) total phosphorus of the lakes where it occurred was calculated. These weighted averages were then used as TP scores to assess each lake using the formula (McCune & Mefford, 1999):

$$v_i = \frac{\sum_{j=1}^{p} a_{ij} w_j}{\sum_{j=1}^{p} a_{ij}} \qquad \textbf{Equation 1}$$

Where v_i = lake score for lake i, a_{ij} = abundance of species j in lake i and w_j is the TP score of taxa j.

Weighted average approach (2)

A non-metric multidimensional scaling ordination (NMDS) was carried out on transformed ($x^{0.5}$) macroinvertebrate abundance. The ordination was rotated so that axis one had maximum correspondence with the TP vector. The axis one values were then used as species scores to assess each lake using equation 1.

Biological indices

Fourteen biological indices were calculated to investigate their potential use in assessing the lakes (Table 2).

Table 2 Biological indices calculated and source.

Index	**Source**
Mean abundance	
Sum abundance	
Species richness	
Evenness	Pielou (1969)
Shannon index	Shannon & Weaver (1949)
Simpson's index	Modified version of McCune & Mefford (1999)
Sum sensitive	Sum abundance of taxa with a weighted average TP < 30 µg l^{-1} (Table 4)
RA sensitive	Relative abundance of taxa with a weighted average TP < 30 µg l^{-1} (Table 4)
Chironomus plumosus / Chironomidae	Solimini *et al.* (submitted)
Orthocladiinae / Chironomidae	Solimini *et al.* (submitted)
Tanypodinae / Chironomidae	Solimini *et al.* (submitted)
Oligochaeta / Chironomidae	Solimini *et al.* (submitted)
Quirke index	Irvine *et al.* (2001)
(Limnodrilus Group+ Potamothrix) / Sample depth	Free *et al.* (2006)

3. Results

Lake selection

Owing to the limited resources available and to maximise the chance of developing a successful assessment system it was decided to focus on a limited typology. The aim of this was to control as many factors as possible, other than eutrophication, so as to more clearly allow the influence of eutrophication on macroinvertebrates to be understood. Initial lake selection took place based on alkalinity, maximum depth, area and altitude. A total of 45 lakes were selected in Austria, Germany and Italy. Figure 3 shows boxplots of key environmental parameters by country. Overall the lakes selected for the innovation project may be said to fit the typology of medium sized lakes (0.5 – 11 km^2), high alkalinity (> 2 meq l^{-1}), mid altitude (mostly 200 - 800 m) and medium depth (maximum depth typically 12 – 35 m).

Although the lakes were selected to fit an overall broad typology there were some notable differences, most notably in the Italian lakes which are at a significantly lower altitude, tended to have a shallower depth and had higher total phosphorus concentrations. Such geographic differences need to be taken into account in an assessment system as they cannot be overcome by sampling design.

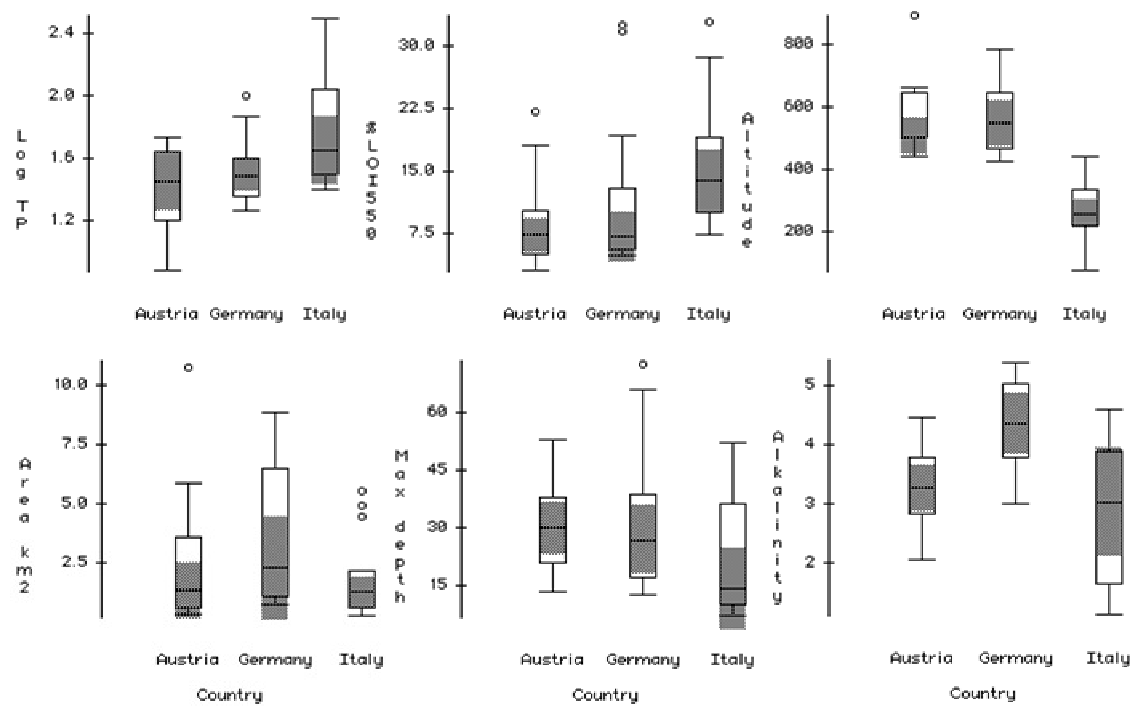

Figure 3 Box plots of lakes in Austria, Germany and Italy by log TP (µg l^{-1}), %LOI at 550^{o}C, Altitude (m), Area (km^2), Maximum depth (m) and Alkalinity (meq l^{-1}). Shaded areas represent 95% confidence intervals.

General description of macroinvertebrate dataset

A total of 270 biological samples were analysed from the 45 lakes. 10,996 individuals were counted and identified to species or a higher taxonomic level. Ninety-seven taxa were found, the group containing the most taxa were the Chironominae with a total of 32 taxa identified. The Tubificidae were the most abundant group accounting for 45% of the individuals found (Figure 4). On average 16 taxa were found per lake with species richness ranging from 7 to 38 taxa (the maximum being found in Tegernsee in Germany) (Figure 5). Fifty-four percent of the taxa found occurred rarely, being found in three or less of the 45 lakes.

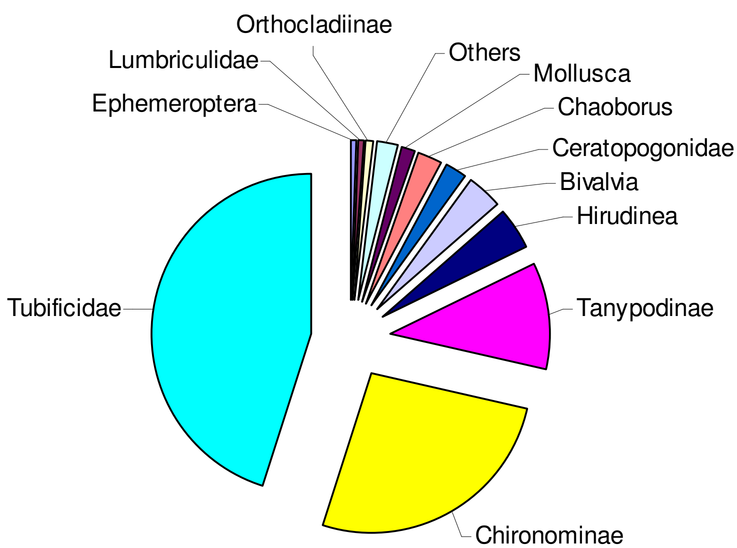

Figure 4 The relative abundance of taxa found in the 45 lakes.

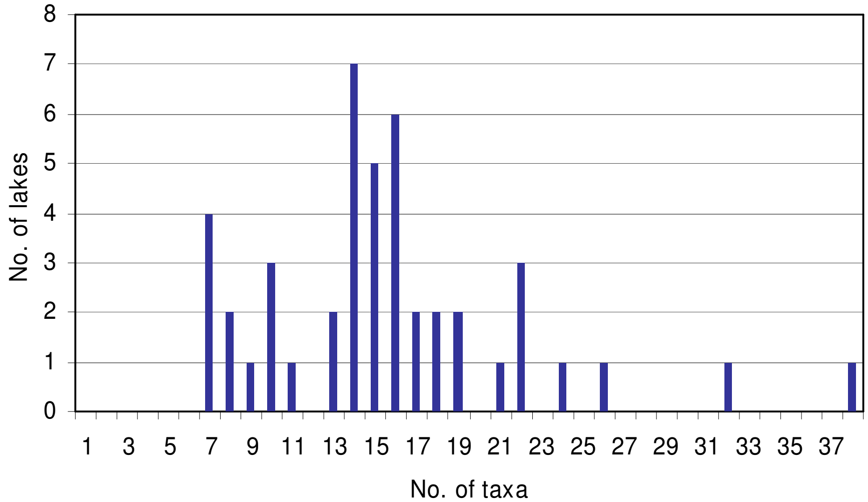

Figure 5 Taxa richness in the study lakes

One of the obstacles to be overcome in developing assessment systems is the natural distribution pattern of organisms between regions (biogeography). Table 3 shows that 23 taxa were found to be a significant indicator of either a country or side of the Alps. However, several taxa such as *Potamothrix hammoniensis* and *Limnodrilus hoffmeisteri* that were found to be significant indicators of the Italian lakes were only so because of the higher abundance in these lakes owing to their higher trophic state as these species were also common in the Austrian and German lakes. One species, *Ilyodrilus templetoni* was highly indicative (IV = 73) of the northern side of the Alps and found to be absent from the Italian lakes sampled. The approach taken to develop an index in this study that overcomes biogeographic differences is the development of weighted averages which translate the trophic preferences of the species into a common scale to allow comparison across countries.

Table 3 Taxa that were found to be significant ($p \leq 0.05$) indicators of a country or side (North or South) of the Alps. The p value was determined by Monte Carlo test - proportion of 1000 randomised trials where the observed indicator value (IV) was equalled or exceeded (McCune & Mefford, 1999).

Taxa	**Country indicative of**	**IV**	**Indicative of north or south of Alps**	**IV**
Ablabesmyia sp.			N	58.8
Micropsectra atrofasciata	Italy	26.7	S	26.7
Pisidium casertanum	Italy	52.7	S	56.1
Procladius choreus	Germany	52.4		
Cladotanytarsus Sp.	Germany	62.9	N	86.4
Ephemera danica	Germany	33.5	N	30.0
Spirosperma ferox	Germany	30.8		
Chaoborus flavicans	Italy	62.6	S	64.6
Tanytarsus gregarius			N	74.3
Potamothrix hammoniensis	Italy	81.6	S	84.1
Limnodrilus hoffmeisteri	Italy	86.5	S	92.8
Lumbricidae			S	20.0
Nematoda			S	20.0
Parachironomus sp.			S	20.0
Microtendipes pedellus	Germany	38.7		
Physa sp.	Italy	26.7	S	26.7
Planorbidae			S	25.9
Branchiura sowerbyi			S	41.1
Ilyodrilus templetoni	Germany	52.3	N	73.3
Bithynia tentaculata			S	45.7
Valvata sp.	Italy	33.3	S	33.3
Ceratopogonidae vermiformes	Italy	64.2	S	71.2
Cladopelma viridula	Italy	61.9	S	67.2

Development of an ecological assessment system

Weighted averaging

Three weighted averaging approaches were followed in order to develop an ecological assessment system for sub-alpine lakes. The approach which produced the best result was that which calculated the weighted average TP concentration (TP score) for each taxa for which sufficient data was available ($n \geq 5$). These scores were derived from measurements of the total phosphorus concentration in the sub-littoral at the time of sampling and were weighted by the abundance of the species. These values may be interpreted as TP 'optima' for each of the species. Figure 6 show the results of the application of the TP scores to the 45 lakes studied. The metric score had a highly significant relationship ($r^2 = 0.48$, $p \leq 0.001$) with total phosphorus concentration. However, the performance of the metric fell short of expectations; it was hoped that the metric would have an r^2 of at least between 0.7 and 0.8 before it could be recommended for use. It is still possible however for member states to apply the metric in an exploratory way using a simple 4 step procedure:

1) Assign the TP scores in Table 4 to each of the taxa collected from the sub-littoral during monitoring
2) Enter the abundance estimates for the species and TP scores from Table 4 into equation 1 to produce an initial metric value for each lake
3) Obtain the upper 10th percentile (or alternatively median or mean as appropriate) metric value for a set of reference lakes.
4) Enter the metric result, reference value and worst value of the metric into equation 2. This produces the EQR that measures deviation away from reference condition.

Equation 2 modified from CEN (2004)

$$EQR = \frac{Metric\ result\ (i.e.\ \text{model}\ prediction) - worst\ value\ of\ metric}{Upper\ 10^{th}\ percentile\ of\ metric\ for\ reference\ sites - worst\ value\ of\ metric}$$

The scores are only valid for the lakes in the sub-alpine region that have a similar typology as the lakes examined in this study (Figure 3). Further guidance on reference conditions and EQR development may be found in REFCOND (2003).

Another approach followed was the calculation of species scores using a non-metric multidimensional scaling ordination (NMS). Figure 7 and Figure 8 show the ordination of the lakes coded by country with environmental variables overlain as vectors. Lakes that have a similar abundance and composition of species are positioned closer together in the ordination. Most of the Italian lakes are positioned on the right of axis 1 at the end of the total phosphorous vector reflecting their higher trophic status. A multiple response permutation procedure (MMRP) test was carried out to see if there was a significant difference between countries. Evidence for a significant difference between all countries was found ($A = 0.27$, $p < 0.001$) with the difference being stronger, indicated by the higher A value, for the Italian lakes (Table 5). Species scores were obtained by rotating the ordination so that axis 1 had a maximum correlation with the total phosphorus vector and then recording the species scores along axis one. These scores were then used to develop a metric score, the same as the previously, using equation 1. Figure 9 shows the relationship between the application of the metric score and TP. The relationship was highly significant ($p \leq 0.001$) but performed more poorly than the TP scores developed for the previous approach.

A third approach applied published species scores that were derived using a similar weighted averaging approach but with an integrated pressure / secondary effects gradient that incorporated TP, oxygen and Secchi depth (Rossaro *et al*. 2006). Although a significant relationship was found ($p = 0.036$), the relationship was poor having an r^2 of only 0.098 (Figure 10).

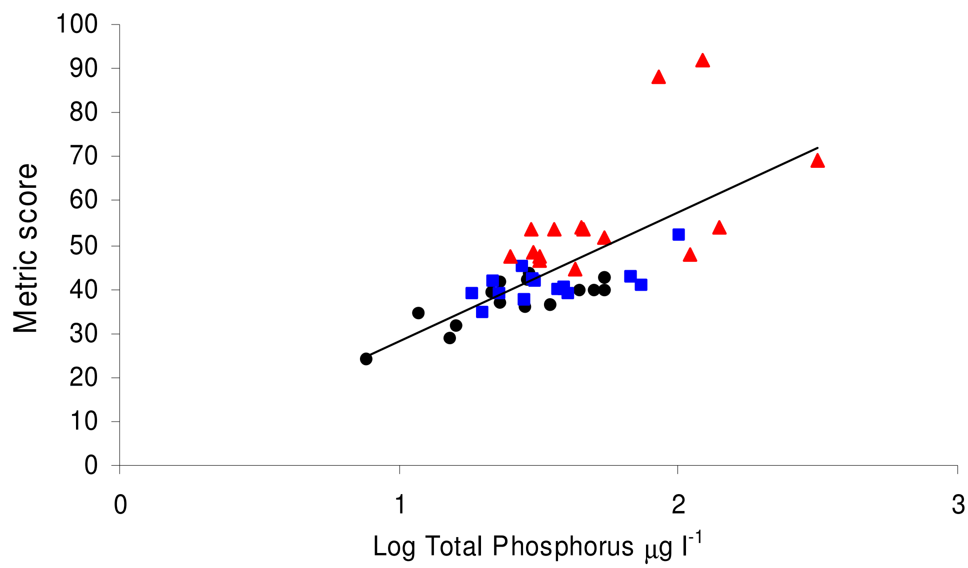

Figure 6 Log total phosphorus and metric score (weighted averages using equation 1) for 45 lakes in Italy (▲), Austria (●) and Germany (■). $r^2 = 0.48$, $p < 0.001$ ($y = 29.376x - 1.1185$).

Table 4 TP scores calculated from average TP concentration (weighted by taxa abundance) used to generate Figure 6. Scores were only calculated for taxa that were present in ≥5 lakes.

Taxa	TP Score	n
Paracladius conversus	13.0	7
Tanytarsus gregarius	19.3	30
Harnischia curtilamellata	22.3	7
Zavrelimyia sp.	24.9	5
Microtendipes pedellus	25.7	10
Spirosperma ferox	25.8	6
Ephemera danica	26.1	9
Phryganea sp.	27.0	8
Stylodrilus heringianus	28.0	7
Parakiefferiella sp.	28.7	5
Paralauterborniella nigrohalteralis	30.1	8
Sialis sp.	31.7	10
Pseudochironomus prasinatus	31.7	14
Asellus aquaticus	32.3	7
Dicrotendipes nervosus	32.3	20
Rhyacophila sp.	32.5	5
Psectrocladius sp	34.0	9
Einfeldia dissidens	34.7	8
Planorbidae sp.	35.2	5
Caenis macrura	35.6	5
Ablabesmyia sp.	36.2	21
Procladius choreus	38.6	40
Cladotanytarsus sp.	39.5	27
Dreissena polymorpha	40.2	11
Ceratopogonidae vermiformes	40.8	29
Valvata sp.	41.4	5
Ilyodrilus templetoni	41.4	22
Erpobdella octoculata	41.7	5
Bithynia tentaculata	41.7	17
Polypedilum nubeculosum	44.6	23
Glyptotendipes pallens	45.0	11
Cryptochironomus defectus	45.2	35
Limnodrilus hoffmeisteri	45.7	40
Chironomus anthracinus	50.2	10
Branchiura sowerbyi	51.8	16
Pisidium casertanum	52.5	15
Conchapelopia pallidula	55.3	11
Cladopelma viridula	55.9	24
Chironomus plumosus	61.2	15
Chaoborus flavicans	89.6	21
Potamothrix hammoniensis	98.9	21

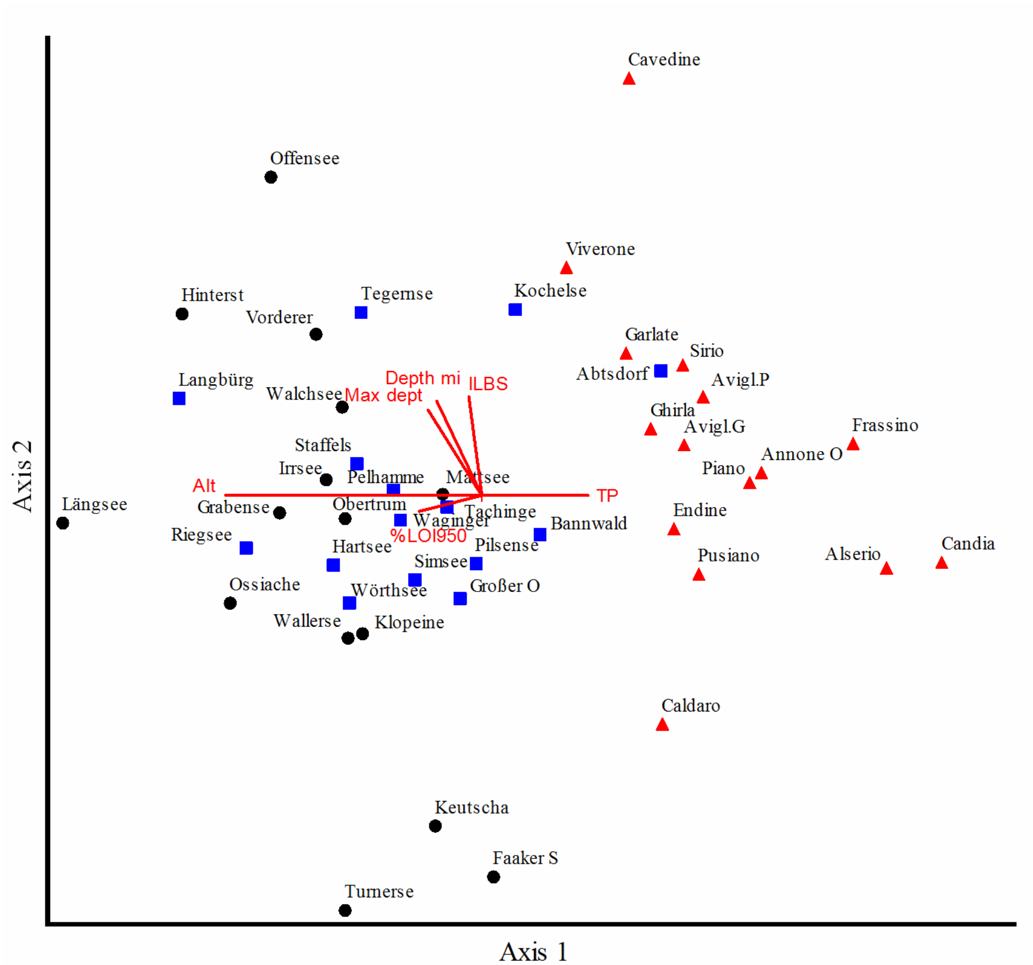

Figure 7 NMS ordination (Axis 1 and 2) of 45 lakes using transformed ($x^{0.5}$) benthic macroinvertebrate abundance. Sorensen (Bray-Curtis) distance measure used; stress: 15.7. The proportion of variance explained by axis 1 was 35% and axis 2: 31%. Italy (▲), Austria (●) and Germany (■).

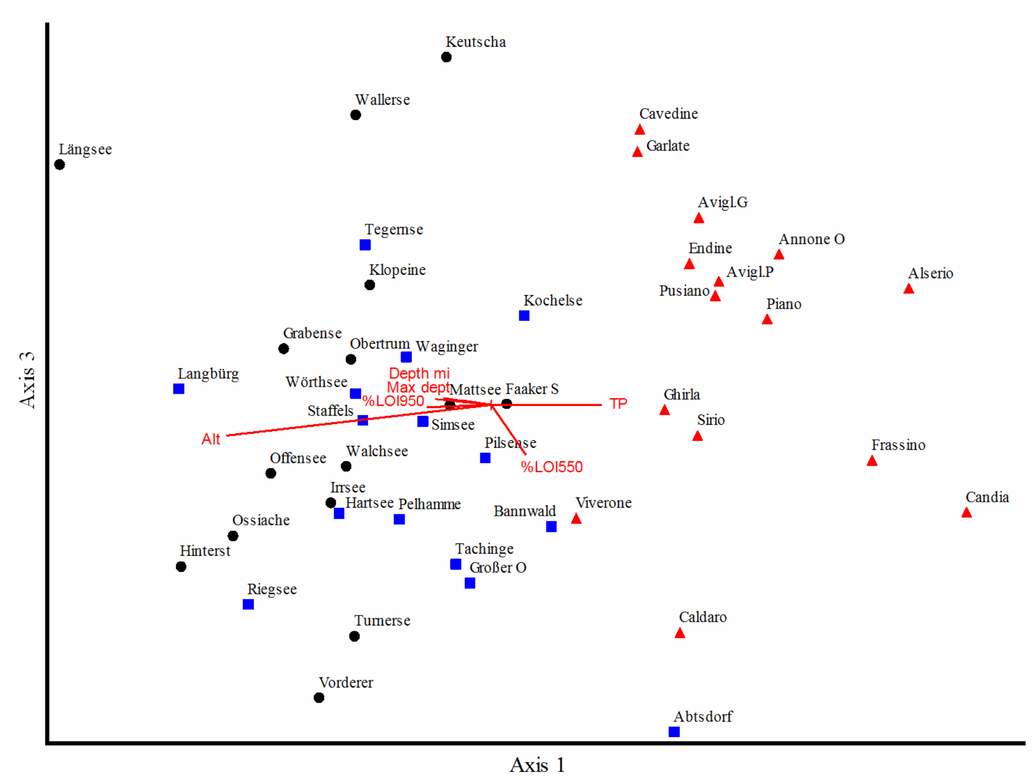

Figure 8 NMS ordination (Axis 1 and 3) of 45 lakes using transformed ($x^{0.5}$) benthic macroinvertebrate abundance. Sorensen (Bray-Curtis) distance measure used; stress: 15.7. The proportion of variance explained by axis 1 was 35% and axis 3: 16%. Italy (▲), Austria (●) and Germany (■).

Table 5 Results (A values and p) of pair-wise MRPP tests for the countries using transformed ($x^{0.5}$) benthic macroinvertebrate abundance and the Sorensen (Bray-Curtis) distance measure (rank transformed matrix). An A of 1 = complete within group homogeneity, an A of 0 = within group heterogeneity equal to that expected by chance.

	Austria	Germany	Italy
Austria	•		
Germany	0.047 ($p = 0.006$)	•	
Italy	0.303 ($p < 0.001$)	0.283 ($p < 0.001$)	•

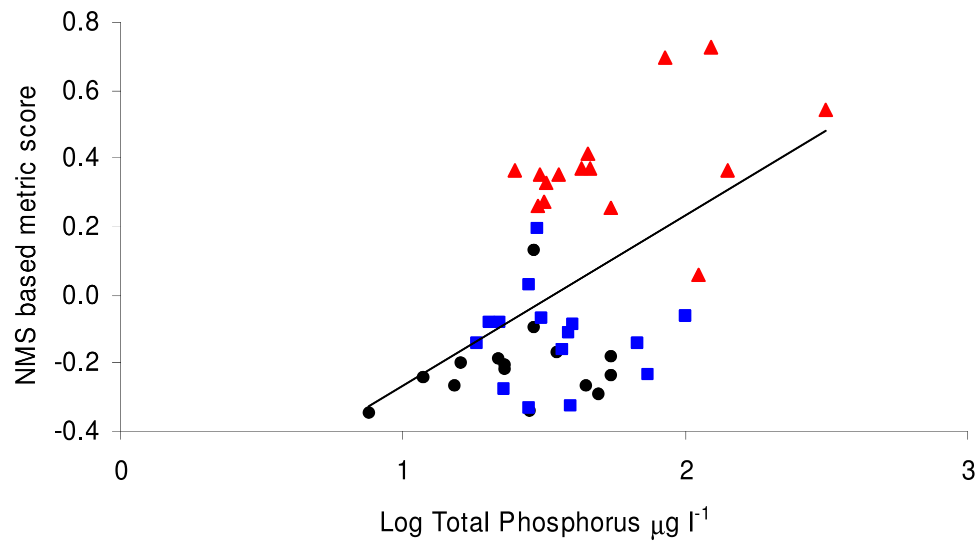

Figure 9 Log total phosphorus and weighted average score (2) derived from an NMS ordination for 45 lakes in Italy (▲), Austria (●) and Germany (■). $r^2 = 0.25$, $p < 0.001$ ($y = 0.5036x - 0.7736$).

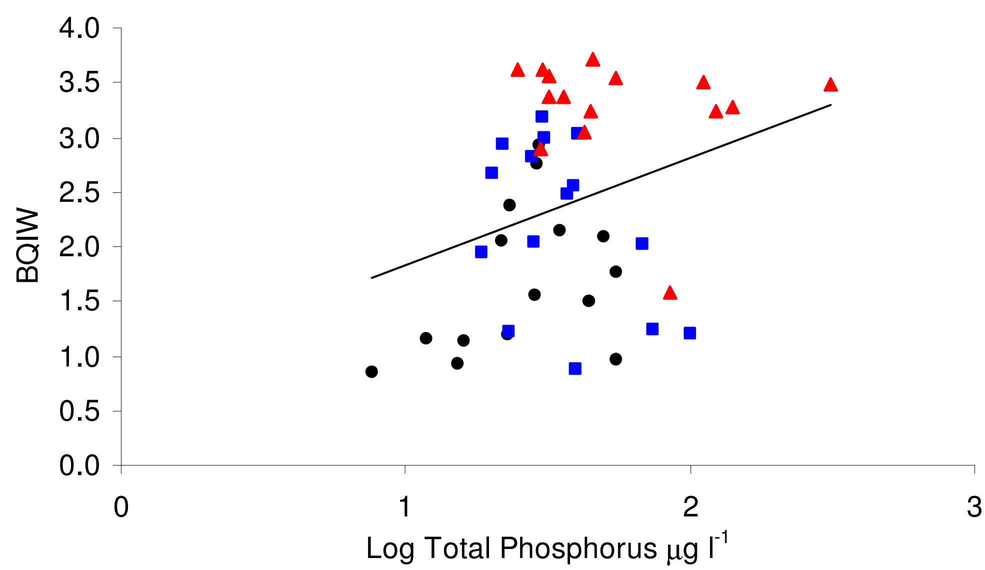

Figure 10 Log total phosphorus and applied BQIW scores (Rossaro *et al*. 2006) for 45 lakes in Italy (▲), Austria (●) and Germany (■). $r^2 = 0.0981$, $p = 0.036$ ($y = 0.9813x + 0.8539$).

Biological indices

Fourteen biological indices were calculated and examined using Spearman rank correlation coefficients. Seven of the metrics had a significant correlation with log total phosphorus (Table 6). The most highly correlated metric was the proportion of sensitive taxa which had an r_S of -0.68 (Figure 11). This index was however too variable to be used for predictive or assessment purposes alone but is worthwhile considering for future incorporation alongside other indices into a multimetric assessment system.

Table 6 Spearman Rank correlation coefficients between log total phosphorus (TP) and calculated biological indices. Significant coefficients ($p \leq 0.05$) are in bold.

	Log TP	Mean abundance	Sum abundance	Species richness	Evenness	Shannon index	Simpson's index	Sum sensitive taxa	Pr sensitive taxa	Chironomus plumosus / Chironomidae	Orthocladiinae / Chironomidae	Tanypodinae / Chironomidae	Oligochaeta / Chironomidae	Quirke index	(Limnodrilus Group+ Potamothrix) / Sample depth
Log TP	•														
Mean abundance	0.14	•													
Sum abundance	0.14	**1.00**	•												
Species richness	-0.13	**0.50**	**0.50**	•											
Evenness	-0.10	**-0.54**	**-0.54**	-0.09	•										
Shannon index	-0.19	-0.07	-0.07	**0.55**	**0.72**	•									
Simpson's index	-0.13	-0.17	-0.17	**0.35**	**0.86**	**0.95**	•								
Sum sensitive	**-0.64**	0.21	0.21	**0.56**	-0.06	**0.33**	0.17	•							
Pr sensitive	**-0.68**	-0.13	-0.13	**0.32**	0.18	**0.39**	0.29	**0.88**	•						
Chironomus plumosus / Chironomidae	**0.30**	0.15	0.15	-0.07	**-0.40**	**-0.32**	**-0.42**	-0.21	-0.29	•					
Orthocladiinae / Chironomidae	**-0.33**	0.21	0.21	**0.36**	-0.15	0.09	-0.01	**0.48**	**0.41**	0.08	•				
Tanypodinae / Chironomidae	-0.01	-0.02	-0.02	0.02	0.14	0.11	0.13	-0.02	0.08	**-0.41**	-0.24	•			
Oligochaeta / Chironomidae	**0.43**	**0.33**	**0.33**	-0.14	-0.28	-0.27	-0.26	**-0.40**	**-0.50**	**0.40**	-0.08	-0.04	•		
Quirke index	**0.32**	**0.68**	**0.68**	0.21	**-0.41**	-0.13	-0.20	-0.14	**-0.37**	**0.47**	-0.02	0.00	**0.78**	•	
(Limnodrilus Group+ Potamothrix) / Sample depth	**0.35**	**0.68**	**0.68**	0.18	**-0.37**	-0.13	-0.17	-0.17	**-0.41**	**0.35**	-0.03	0.04	**0.81**	**0.98**	•

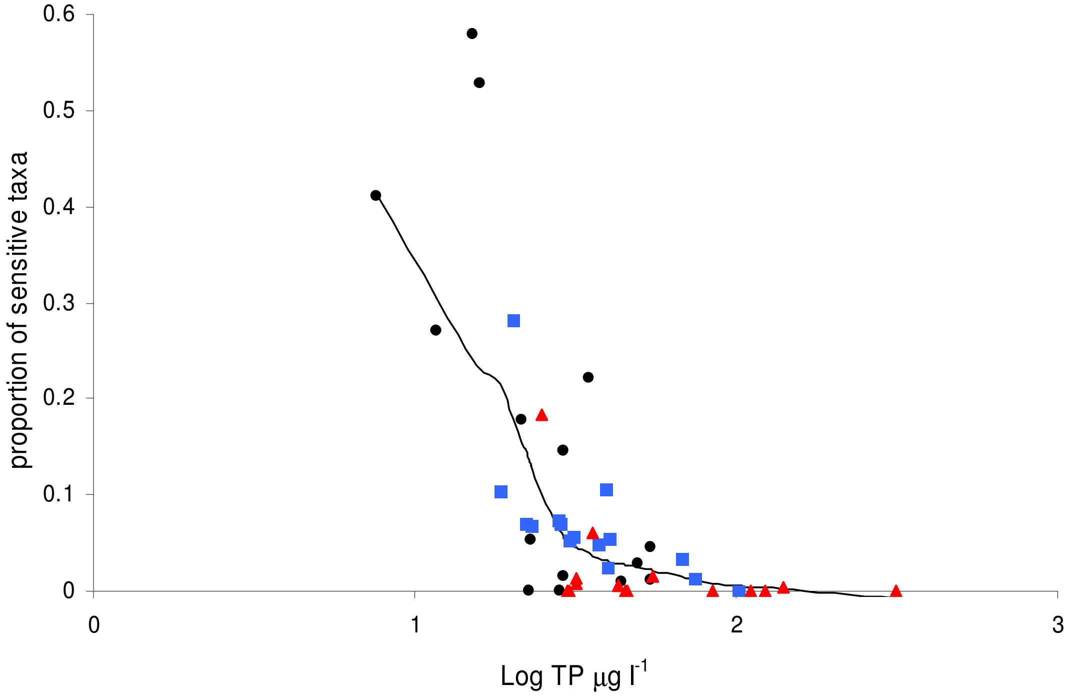

Figure 11 Log total phosphorus and the proportion of sensitive taxa for 45 lakes in Italy (▲), Austria (●) and Germany (■). A Lowess smoothed line was fitted to the data.

4. Discussion

A total of 45 lakes in Austria, Germany and Italy were successfully sampled in the Spring of 2006. The extensive laboratory work that followed involved the examination of eleven thousand biological specimens and detailed chemical and physical characterisation of the water and sediments collected from the lakes. The work has been carried out using biologists with international expertise in the area and following a quality control procedure for key environmental parameters.

The new approach taken in this study to improve the understanding of benthic macroinvertebrate response to eutrophication pressure had 2 key elements:

1) A focus on a reduced typology of the most relevant environmental parameters: season, alkalinity, depth, lake size and altitude.
2) A concentration of work in the sub-littoral lake zone and only on soft substrata.

The control of environmental factors and focus on one lake zone by sampling design was to allow the influence of eutrophication on macroinvertebrates to be more clearly understood and an assessment system produced.

Evidence was found that several species, either through their differential presence or abundance were indicative of either a country or side of the Alps (Table 3). The approach taken to develop an index in this study that overcomes such biogeographic differences is the development of weighted averages which translate the trophic preferences of the species into a common scale to allow comparison across countries.

The approach which produced the best result was that which calculated the weighted average TP concentration (TP score) for each taxa for which sufficient data was available ($n \geq 5$). These scores were derived from measurements of the total phosphorus concentration in the sub-littoral at the time of sampling and were weighted by the abundance of the species. These values may be interpreted as TP 'optima' for each of the species. The metric score had a highly significant relationship ($r^2 = 0.48$, $p \leq 0.001$) with total phosphorus concentration. However, the performance of the metric fell short of expectations; it was hoped that the metric would have an r^2 of at least between 0.7 and 0.8 before it could be recommended for use. It is still possible however for member states to apply the metric in an exploratory way using a simple 4 step procedure outlined in the results section. However, one of the limitations of the weighted average approach is that the true optima for many of the species may lie outside that covered by the sampling strategy. Therefore the averages must to some extent be considered specific to the typology considered in this study, although the approach has a wide applicability.

The performance of the metric ($r^2 = 0.48$) was comparable to previous work by Free *et al*. (2006) who found that attempts to develop assessment indices for benthic macroinvertebrates in the littoral zone ($r^2 = 0.42$) and profundal zone ($r^2 = 0.40$) were less successful than for other biological elements such as phytoplankton ($r^2 = 0.67$) and macrophytes ($r^2 = 0.59$). As pointed out above, the reasons for the difficulty in understanding and developing assessment systems to assess anthropogenic pressure using benthic macroinvertebrates include their complex biotic structure and high temporal and spatial variability. This project attempted to control many environmental factors by sampling design (Table 1) but only a slight improvement (~6-8%) on previous models was achieved.

Despite the attempts to focus on a limited typology a complex biotic structure was still encountered. For example, a large proportion of the taxa (54%) occurred rarely, being found in approximately less than 5% of the lakes sampled. This seems to be a feature of benthic macroinvertebrates in lakes. A similar figure was found by Free *et al*. (2006) in the littoral zone (42 - 54%). This fact is likely one of the reasons why there are current difficulties in developing assessment systems for lake benthic macroinvertebrates. It is difficult to develop an understanding of community structure and response to anthropogenic pressure when over 50% of the taxa are rarely found, thereby leading to inadequate knowledge about the environmental preferences of many species. Such problems may only be overcome by very extensive sampling campaigns across the range of typologies.

As part of WFD implementation the intercalibration exercises taking place between different EU countries has involved the integration of datasets that when analysed, for example using ordination techniques, often remain strongly indicative of their country. There is often a high degree of uncertainty whether such differences are due to differences in field and laboratory methodology or real ecological differences. One of the useful outcomes of this study is a dataset that focused on three countries and employed standard methodology and the same taxonomic contractors for the study. Therefore the differences in fauna between the countries represent real differences rather than those introduced by differences in methodology and skill or level of taxonomic determination.

Key stakeholders were informed of the results of the project during an EU Lakes Intercalibration Expert Workshop held in Ispra, Italy between the 26 and 27th of October 2006. It is intended to communicate further developments through this network.

Conclusions

The key conclusions of the research were:

1) Data on benthic macroinvertebrates and supporting environmental parameters was successfully collected from 45 sub-alpine lakes.

2) Several approaches were followed to develop an ecological assessment system for benthic macroinvertebrates. The best results were achieved using the weighted average TP concentration (TP score) for each taxa to develop a metric score using equation 1.

3) The model had a highly significant relationship ($r^2 = 0.48$, $p \leq 0.001$) with total phosphorus concentration. However, the variation explained by the model was too low for it to be recommended to national authorities in the sub-alpine ecoregion for implementation of the WFD.

4) Although, the variance explained by the model was lower than desirable it is still possible for member states to apply the metric in an exploratory way as outlined in the results section.

5) The approach used in this research has a wide applicability and can be followed by member states in developmental work for the Water Framework Directive.

6) The standardised methodology used in the collection and analysis of the benthic macroinvertebrates should provide highly comparable data for use by member states in the ecoregion for future intercalibration exercises.

7) The metric using the proportion of sensitive taxa is promising and is worthwhile to consider for future incorporation alongside other indices into a multimetric assessment system.

8) There is still a need to further understand the lake benthic macroinvertebrate community structure and response to anthropogenic pressure in order to reduce uncertainty of developed indicators. Such problems may only be overcome by very extensive sampling campaigns across the range of typologies.

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