Environmental RTDI Programme 2000-2006

WATER FRAMEWORK DIRECTIVE

A Reference Based Typology and Ecological Assessment System for Irish Lakes

Preliminary Investigations

(2002-W-FS-1-M1)

Synthesis Report

Main Report available for download on
www.epa.ie/EnvironmentalResearch/ReportsOutputs

Prepared for the Environmental Protection Agency
by
University College Dublin; National University of Ireland, Galway;
Trinity College Dublin.

Authors:

Gary Free, Ruth Little, Deirdre Tierney,
Karol Donnelly and Rossana Caroni

ENVIRONMENTAL PROTECTION AGENCY
An Ghníomhaireacht um Chaomhnú Comhshaoil
PO Box 3000, Johnstown Castle, Co. Wexford, Ireland

Telephone: +353 53 916 0600 Fax: +353 53 916 0699
Email: info@epa.ie Website: www.epa.ie

DISCLAIMER

Although every effort has been made to ensure the accuracy of the material contained in this publication, complete accuracy cannot be guaranteed. Neither the Environmental Protection Agency nor the authors accept any responsibility whatsoever for loss or damage occasioned or claimed to have been occasioned, in part or in full, as a consequence of any person acting, or refraining from acting, as a result of a matter contained in this publication. All or part of this publication may be reproduced without further permission, provided the source is acknowledged.

WATER QUALITY

The Water Quality Section of the Environmental Research Technological Development and Innovation (ERTDI) Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in this area. The reports in this series are intended as contributions to the necessary debate on water quality and the environment.

Environmental RTDI Programme 2000-2006

Published by the Environmental Protection Agency, Ireland

Printed on recycled paper

ISBN:1-84095-215-6 04/07/300
Price: €10

ACKNOWLEDGEMENTS

This report has been prepared as part of the Environmental Research Technological Development and Innovation Programme under the Productive Sector Operational Programme 2000-2006. The programme is financed by the Irish Government under the National Development Plan 2000-2006. It is administered on behalf of the Department of the Environment and Local Government by the Environmental Protection Agency which has the statutory function of co-ordinating and promoting environmental research.

The authors wish to acknowledge the considerable amount of assistance received throughout the course of this study. We particularly thank the laboratory staff at EPA Regional Inspectorates in Dublin and Castlebar for processing chemical samples. We would like to thank the staff of the Environment and Heritage Service (EHS) of Northern Ireland for their help, in particular Peter Hale and Imelda O'Neill. James King and Joe Caffrey of the Central Fisheries Board provided training in the identification of macrophytes, as did the EHS and Nigel Holmes. Norman Allott, Jonathan White and Pascal Sweeney conducted training courses in phytoplankton, littoral invertebrates and profundal invertebrates respectively. Wayne Trodd helped in processing the profundal invertebrate samples and Noriana Kennedy provided invaluable assistance with fieldwork in 2003.

We would like to thank the Western Regional Fisheries Board, Duchás, Ian Donohue and staff in the zoology department National University of Ireland, Galway (NUIG) for aid in sampling particular lakes. The authors would also like to acknowledge the Rosses, Lough Anure and Lough Rea Anglers associations, the Newport, Ballinahinch, Glenicmurren and Lough Inagh Fisheries, Delphi Lodge, Donegal Co. Council, the ESB (Ballyshannon) and all land owners who allowed access across their property. We thank Ken Irvine (Trinity College Dublin (TCD)), Declan Murray (National University of Ireland, University College Dublin (UCD)), Mary Kelly-Quinn (UCD) and T.K. McCarthy (NUIG) for administrative support. Many thanks also to the EPA staff of the Environmental Research Technological Development and Innovation Unit (ERTDI), in particular Loraine Fegan, Kevin Woods and Claire Fahy. We are particularly grateful for helpful comments and guidance provided by Jim Bowman and Martin McGarrigle throughout the course of this project.

Details of Project Partners

Gary Free,
European Commission
- Joint Research Centre,
Building 30 (TP 300),
Via Enrico Fermi,
Ispra,
I-21020 ITALY
E-mail: gnfree@hotmail.com

Rossana Caroni,
Department of Zoology,
Trinity College,
Dublin 2
IRELAND

Ruth Little,
EPA Regional Inspectorate,
John Moore Road,
Castlebar, Co. Mayo,
IRELAND
Tel: +353 (0)94 904 8432
E-mail: r.little@epa.ie

Karol Donnelly,
104 Manor Village,
Westport Road,
Castlebar,
Co. Mayo,
IRELAND
Tel: +353 (0)87 243 1560
E-mail: kdonnelly2@mayococo.ie

Deirdre Tierney,
Environmental Protection Agency,
Dublin Regional Inspectorate,
McCumiskey House,
Richview,
Clonskeagh Road,
Dublin 14
IRELAND
Tel: +353 (0)1 268 0176
E-mail: d.tierney@epa.ie

Table of Contents

1. Introduction

The European Union (EU) Water Framework Directive (WFD) which came into force in December 2000 (Council of European Communities (CEC), 2000) provided the impetus for this research project through its requirement for the ecological assessment of lakes. The WFD has shifted emphasis from chemical measures of water quality to those based on ecology. Chemical and physical components of water quality are still an integral part of assessment but are regarded as 'supporting elements' for the biology.

The main objectives of this project were as follows.

Objective 1: Collection of ecological information

The biological elements investigated were phytoplankton, macrophytes and macroinvertebrates (from both littoral and profundal zones). The remaining biological elements required to be monitored by the WFD (phytobenthos and fish) were outside the scope of this study. When selecting the lakes to be sampled, one of the objectives was to ensure that there was sufficient representation of lakes that were close to their natural state or in reference condition.

Objectives 2 and 3: Development of a lake typology and the description of reference conditions

In developing a system to ecologically assess lakes it must be recognised that there are different types of lakes, which naturally support different flora and fauna in their reference condition. The WFD requires that ecological quality is measured as deviation away from undisturbed or reference condition for each lake type. The purpose of a typology is to allow ecological change, caused by anthropogenic pressure, to be detected with greater reliance (REFCOND, 2003).

The WFD allows member States to define their lake typology using one of two systems: System A or System B (Table 1.1). The proviso is that use of System B must result in at least the same degree of differentiation as would be achieved if System A had been used.

The initial primary objective of this project was to develop a lake typology which is biologically relevant, *i.e.* a typology defined by environmental boundaries that is demonstratively successful in partitioning natural variation in the biology. The first step was to select a set of lakes thought to be in reference condition that contain representatives of the types of lakes in Ireland. The second step was to examine each of the biological elements to see if 'biological' types were evident using multivariate grouping techniques. Such ‘biological’ types were then examined in terms of potential typology factors (*e.g.* Table 1.1) to see if they were also distinct in terms of environmental variables. This led to a working definition of what a lake type is:

A lake type describes a group of lakes that, in reference condition, has a unique composition or abundance of flora or fauna which is related to a distinct combination of environmental factors for that group.

Table 1.1 Parameters that may be used to define types using System A or B (CEC, 2000). Obligatory factors are in bold.

WFD typology factors	System	
Ecoregion	**A**	
Latitude		**B**
Longitude		**B**
Altitude	**A**	**B**
Depth	**A***	B
Lake area	**A**	**B**
Geology	**A**	**B**
Mean water depth		**B**
Mean air temperature		B
Air temperature range		B
Acid neutralising capacity		B
Residence time		B
Mixing characteristics/Stratification		B
Background nutrient status		B
Lake shape		B
Mean substratum composition		B
Water level fluctuation		B

* based on mean depth

The next objective step was to define environmental boundaries for each of the types that are effective in partitioning the biological variation in reference condition. The typology was initially developed at biological element level (*e.g.* macrophytes or phytoplankton) as this is the scale at which assessment systems will work, acknowledging that many typological factors may be of greater relevance to one group over another.

Following the development of a typology, the third objective was to describe type-specific reference conditions. Future assessment systems will be based on a deviation from these defined reference conditions. The description may be achieved in two ways. The first is a general description of the composition and abundance of taxa for each of the biological elements and the second is by using a summary statistic such as the mean value assigned to reference lakes for the assessment metric used. One underlying premise in the above approach is that lakes are available that are in reference condition.

Objective 4: Development of WFD compliant classification tools

The WFD requires that classification tools or metrics are developed which measure ecological quality for each biological element. Ecological quality is to be expressed as deviation away from reference conditions in the form of an Ecological Quality Ratio (EQR). Thus, the fourth objective of this project was to carry out developmental work on ecological assessment tools for the biological elements phytoplankton, macrophytes, littoral macroinvertebrates and profundal macroinvertebrates. Metrics may be developed to work along one or more pressure gradients. This project focused on nutrients, represented by the parameter total phosphorus, as the main pressure affecting Irish lakes.

The project examined a number of ecological assessment approaches depending on their suitability to the biological element under consideration. These included multimetric indices, published indices, simple empirically-based indices and multivariate classification. The different methods are described in the relevant chapters of the final report (Free *et al.,* 2006).

The aim of this work is to position Ireland better so as to meet its commitments to assess lakes ecologically as required by the WFD. The successful ecological assessment of lakes was recognised at an early stage by the Irish Environmental Protection Agency (EPA) to be crucially dependent on research. This is the second EPA commissioned research project on the ecological assessment of lakes: the first by Irvine *et al*. (2001) was commissioned in 1995.

2. Overview of Lakes Sampled

201 lakes, principally located in the midlands and along the western seaboard and representative of the physico-chemical and trophic gradients typically found in Ireland were selected Figure 2.1. These include a large proportion of the lakes in the country over 50 hectares (ha) surface area (50-100 ha being the smallest area type in the System A typology of the WFD) as well as a selection of smaller lakes. The vast majority of the lakes was sampled in both spring and summer for a minimum of one year between 2001 and 2003. Full details of sampling frequency, the physical and chemical characteristics of the lakes and catchment land use data are listed in the final report (Free *et al*., 2006).

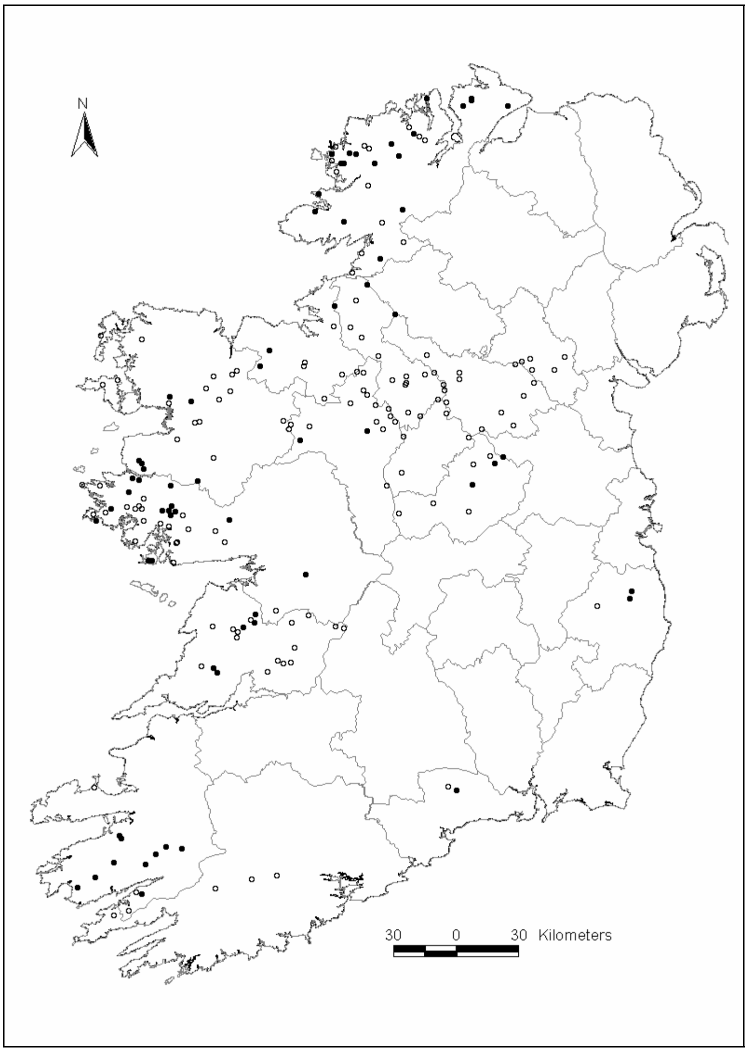

Figure 2.1 Location of the lakes sampled. Candidate reference lakes are indicated by solid circles.

3. General Methodology

A full description of the sampling, analytical and taxonomic methodologies employed are in Free *et al*. (2006).

3.1 Water Chemistry

All lakes were surveyed by boat. The following measurements were taken in the field: Secchi depth, temperature (^{o}C) and oxygen (mg l^{-1}) profiles, conductivity (µS cm^{-1}) and pH. Alkalinity was determined *in situ* by the appropriate titration method. Subsamples of a composite water sample were used for the determination of total phosphorus, total nitrogen, dissolved nutrients (filtrate) and colour. Chlorophyll *a* was also measured. Laboratory analysis was carried out by the chemistry laboratories at the EPA regional inspectorates in Castlebar and Dublin.

3.2 Biological Elements

3.2.1 Phytoplankton

Two phytoplankton samples, a quantitative (from the composite water sample) and a qualitative sample (net haul), were collected from each site on each date sampled.

3.2.2 Macrophytes

Macrophyte sampling was undertaken during the summer sampling season. At each lake, between four and six transects, perpendicular to the shoreline and relatively evenly spaced around the lake, were surveyed. A transect was 100 m long and consisted of a series of sites at intervals of 0, 2.5, 5, 7.5, 10, 25, 50, 75, and 100 m. Samples were obtained using four throws of a double-headed rake. At each sampling point on the transect, the macrophytes collected from the first throw of the rake were weighed collectively on a digital balance, identified and the percentage species composition was recorded. A further three rakes were thrown and the relative macrophyte abundance was recorded using a 5 point scale (5 = dominant to 1 = rare) on the basis of the occurrence of the collected species on all four rakes. Macrophytes were primarily identified in the field. Difficult specimens were returned to the laboratory for closer examination. All specimens were identified using the available taxonomic keys.

3.2.3 Phytobenthos - Epilithic Algae

Epilithic algae samples were collected from the littoral zone from two stones. Three small stones from the lake littoral zone were collected for the estimation of epilithic chlorophyll *a*.

3.2.4 Benthic Invertebrates

Profundal invertebrates

Profundal invertebrates were predominantly collected during the spring sampling period in either 2001 or 2002. At each site, five replicate samples were collected using a 225 cm^2 Ekman grab, filtered *in situ* and preserved in 70% alcohol. Samples were subsequently sorted and identified in the laboratory to lowest taxonomic resolution practicable (typically genus) using available taxonomic keys.

Littoral macroinvertebrates

Lakes were sampled for littoral macroinvertebrates in spring 2001 and both spring and summer 2002. Two minute kick/sweep samples were collected using a rectangular framed hand net (260 mm wide, 200 mm high, 670 µm mesh). All samples were preserved *in situ* in 70% alcohol and subsequently sorted and identified to the lowest taxonomic resolution practicable (typically species) using the available taxonomic keys.

3.3 Statistical Analysis

A number of univariate and multivariate statistical techniques were used to assist in the development of a biologically validated typology on an element by element basis.

Data were classified using Two-Way-Indicator-Species-Analysis (TWINSPAN) and Cluster Analysis to identify lake groups on the basis of their biological communities.

Indirect ordination methods (Multi-dimensional Scaling (MDS) / Non-Metric Multidimensional Scaling (NMS) and Detrended Correspondence Analysis (DCA)) were used to map the community data and provide visual support to the groups obtained by the classification. Multiple Random Permutation Procedures (MRPP) or Analysis of Similarities (ANOSIM) were used to test for differences between lake groups based upon their biological communities. Indicator Species Analysis was used to identify statistically significant indicator species for each group. Direct ordination methods (Canonical Correspondence Analysis (CCA) and Canonical Variates Analysis (CVA)) were used to identify and describe underlying environmental gradients.

TWINSPAN, MRPP, NMS and Indicator Species Analysis were performed using the PC-ORD v4.25 computer programme (McCune and Mefford, 1999). Cluster analysis, ANOSIM and MDS ordinations were performed using PRIMER v5.2.9 (Clarke and Warwick, 1994). Additional Cluster Analysis was done using STATISTICA™ 5.1 (Statsoft Inc, 1998). CCA and CVA ordinations were performed in CANOCO v4.52 (ter Braak and Šmilauer, 2002).

4. Phytoplankton

4.1 Development of a Typology Using Phytoplankton Composition and Abundance

Cluster analysis, carried out on transformed ($x^{0.5}$) abundance data, (cells or colonies ml^{-1}) resulted in the formation of nine clusters. Non-metric multidimensional scaling (NMS) generally supported the findings of the cluster analysis and canonical variates analysis identified alkalinity, altitude, catchment to lake area ratio (an indicator of water residence time) and colour as the more important discriminant factors between the clusters.

Alkalinity was found to be the most important variable distinguishing between clusters. Given the limited range of altitude in the reference lakes sampled, the influence of altitude is likely to be related to a surrogate variable such as alkalinity, rather than having a direct influence. The catchment to lake area ratio was not included in the typology at this time as it is only an approximate indicator of residence time. Colour was selected as a factor to form a typology, as it is likely to affect phytoplankton through its influence on light quantity and quality. With increasing nutrient input, deeper lakes are likely to show a smaller increase in phytoplankton abundance than shallow lakes owing to less light exposure for these algae when mixed to deeper depths. Mean depth was therefore considered essential to form a typology. Alkalinity, colour and depth in that order were considered to be the principal factors necessary to form a typology.

The proposed typology consists of three alkalinity categories. The lower alkalinity boundary of 10 mg l^{-1} $CaCO_3$ (calcium carbonate) for the typology, was defined using the relative abundance of Desmids in net samples as the majority of Desmids are well known to have a preference for soft-water (Hutchinson, 1967). An upper alkalinity boundary was set at 100 mg l^{-1} $CaCO_3$ as this was close to the lower value of 94 mg l^{-1} $CaCO_3$ for cluster 6 (excluding 2 misclassified sites). Two categories were determined for colour, the boundary being set at 40 mg l^{-1} PtCo (platinum cobalt), as this was the value that marked the lower end of the distribution of cluster 3, the cluster which appeared most influenced by colour. Using a colour of 40 mg l^{-1} PtCo the reference compensation depth (depth where 1% of surface light remains) was predicted using equations in Free *et al*., (2005) to be 5 m. This was lowered to 4 m to coincide with the mean depth boundary chosen for the macrophyte typology (chapter 5).

In order to determine if the proposed typology resulted in types which were biologically distinct, pair-wise multi-response permutation procedure (MRPP) tests were carried out on phytoplankton abundance. The A values resulting from the procedure (chance-corrected within-group agreement) indicate the homogeneity within a group to that expected by chance: 1 equals complete within group homogeneity whereas an A of 0 equals within group heterogeneity equal to that expected by chance (McCune *et al*., 2002).

Comparison of the overall A values from MRPP analysis showed that the proposed typology (overall A value = 0.12, $p < 0.001$) was slightly less effective at partitioning natural variation than the WFD default System A typology (overall A = 0.14, $p < 0.001$). However, if depth is removed as a typing factor from the proposed typology, the overall A value improves (0.16, $p < 0.01$). Such a typology may be overly simple, having just 3 alkalinity bands (< 10, 10-100, > 100 mg l^{-1} $CaCO_3$) and two colour bands (< or > 40, mg l^{-1} PtCo). Depth is likely to be important in governing the response to nutrient input and therefore was retained in the phytoplankton typology.

A description of the reference community for the proposed typology was achieved by calculating indicator values of the phytoplankton across the types (see Free *et al*., 2006 for details). A high (> 50%) indicator value (IV) typically indicates that a taxon is both frequent and abundant in a type. As expected, low alkalinity types (< 10 mg l^{-1} $CaCO_3$) had more Desmids than the other types. *Merismopedia* had its highest IV recorded in low alkalinity coloured lakes. Of the dinoflagellates (Pyrrophyta), *Gymnodinium* had a higher IV in low alkalinity lakes whereas *Ceratium* had a higher IV at higher alkalinities. Higher alkalinity bands tended to have more diatoms (Bacillariophyta). The highest alkalinity band (> 100 mg l^{-1} $CaCO_3$) was typically comprised of marl lakes and was characterised by higher amounts of *Peridinium* spp. and *Dinobryon* spp.

4.2 Development of a Phytoplankton Index for Ecological Assessment

A phytoplankton index that met the requirements of the Water Framework Directive *i.e.* incorporating information on the composition, abundance and biomass of the phytoplankton community was developed (see Free *et al*., 2006) using quantitative summer samples. The relationship between the phytoplankton index and log (TP + 1) ($r^2 = 0.67$, $p \le 0.0001$, $n = 129$) is shown in Figure 4.1. Figure 4.2 illustrates that the phytoplankton index had reasonable success in detecting eutrophication in a set of independent lakes ($r^2 = 0.62$, $p \le 0.0001$, $n = 30$). The phytoplankton index appeared to work across the typologies although the importance of typology factors may be more apparent in a larger dataset.

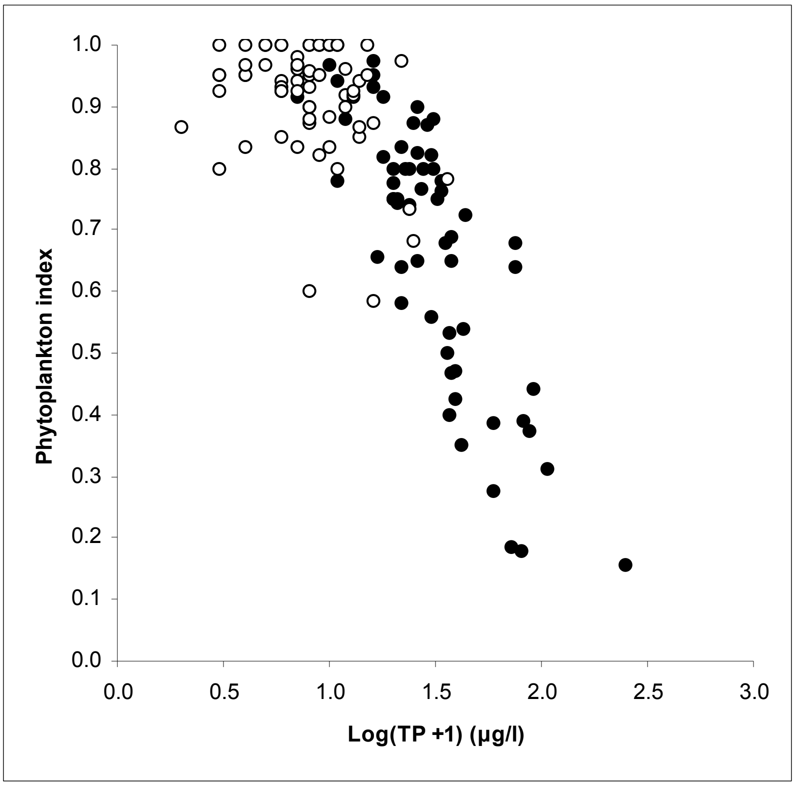

Figure 4.1 **Relationship between the phytoplankton index and sample TP (µg l^{-1}) (n = 129). ○ = reference lakes, ● = non-reference lakes.**

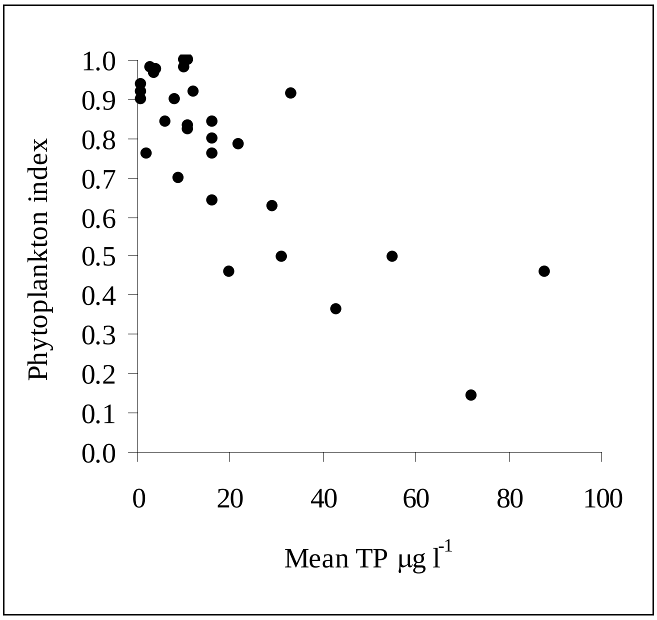

Figure 4.2 **Relationship between the phytoplankton index and mean (1996-7) TP (µg l^{-1}). Phytoplankton data are from 30 lakes sampled in June or July 1996 (Irvine *et al.,* 2001).**

5. Macrophytes

5.1 Development of a Typology Using Macrophyte Composition and Abundance

Cluster analysis was carried out on transformed ($x^{0.5}$) macrophyte abundance (g) data from 58 reference lakes resulting in six clusters. Four clusters were reasonably well separated along the most important axes (2 and 3) of a non-metric multidimensional scaling (NMS) ordination and two clusters were more distinct along axis 1.

No significant differences were found in the selected environmental variables among clusters 1, 3, 5, and 6 using pair-wise *post-hoc* tests with Bonferroni adjustment. These groups could all be classified as low alkalinity lakes with a medium to deep transect depth. In contrast, clusters 2 and 4 had a number of significant differences with the other clusters in terms of environmental variables as well as indicator species.

Because environmental factors can have compounding effects, Canonical Variates Analysis (CVA) was used to determine which linear combination of environmental factors best discriminated between clusters.

In the resulting ordination, the group centroids for clusters 1, 3, 5, and 6 are located close together indicating that they are not distinct environmentally (Figure 5.1). In contrast, the centroids for clusters 4 and 2 are more distinct and are more closely surrounded by each clusters lakes. Alkalinity, lake surface area and mean transect depth were the most important discriminant factors along axes 1, 2 and 3 respectively. In contrast, colour and altitude were not found to be significant in separating clusters in the model.

In summary, the analysis of 58 reference lakes indicated that there were three groups distinct in terms of both macrophytes and environmental characteristics. The first is a high alkalinity group characterised by *Chara sp*. (cluster 2), the second is a low alkalinity shallow type characterised by rich growth of several Isoëtid growth forms (cluster 4). The third group are low alkalinity lakes of medium to deep depth, comprising clusters 1, 3, 5 and 6. Alkalinity, area and mean transect depth were the most important factors separating clusters and will be used to form type boundaries.

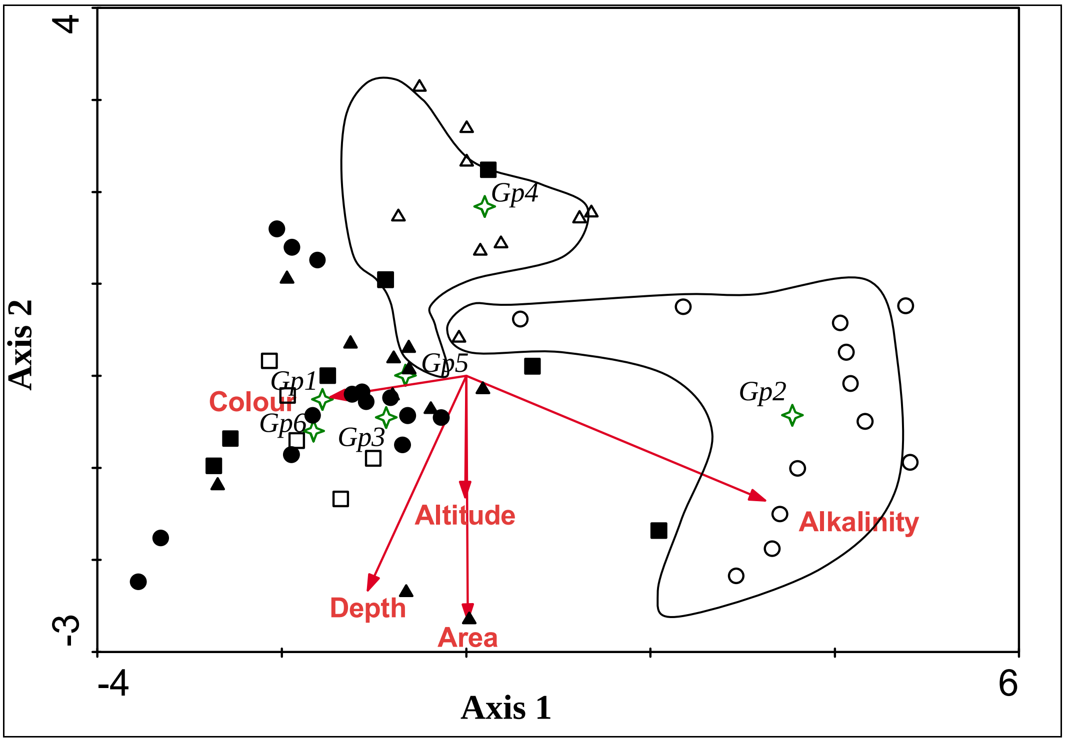

Figure 5.1 **Axes 1 and 2 of CVA plot of six clusters identified by cluster analysis and Indicator Species Analysis. Group centroids (✧) are labelled. Groups 2 and 4 are outlined. Legend: Group 1 (Gp1) = ●, Group 2 (Gp2) = ○, Group 3 (Gp3) = ▲, Group 4 (Gp4) = Δ, Group 5 (Gp5) = ■, Group 6 (Gp6) = □ . Arrows indicate direction and strength of parameters.**

The distribution of *Isoetes lacustris* and *Chara* species was used to set the alkalinity boundaries at 20 and 100 mg l^{-1} $CaCO_3$. Plant communities of low nutrient status lakes may be characterised by *Chara* species in hard-water lakes and *Isoetes* species in soft-water lakes (Rørslett and Brettum, 1989; John *et al*., 1982). *Isoetes lacustris* was virtually absent at alkalinity > 20 mg l^{-1} $CaCO_3$ and *Chara* species increased markedly between 85 and 100 mg l^{-1} $CaCO_3$. These levels also broadly corresponded to the upper 25th percentile alkalinity found in siliceous catchments (26 mg l^{-1} $CaCO_3$) and the lower 25th percentile (108 mg l^{-1} $CaCO_3$) found in catchments with 100% limestone (Free *et al*., 2005). A third type (20-100 mg l^{-1} $CaCO_3$) was present by default. There may be some biological support for this default type. Lakes in this alkalinity band have previously shown some separation in an NMS ordination of macrophyte abundance (Free *et al*., 2005).

The depth boundary was determined from the mean transect depth of 2.2 m for cluster 4. Cluster 4 appeared to be strongly influenced by the shallow depths of the constituent lakes resulting in a distinctly higher amount of littoral rosette type species The mean transect depth of 2.2 m was converted into a mean lake depth of 4 m (see Free *et al*., 2006).

The type boundary for lake area was selected as 50 ha based on the Water Framework Directive System A (CEC, 2000). An additional reason was that cluster 4, whose macrophytes may have been influenced by lake area (Figure 5.1), group centroid 4 at opposite end of area vector) were mostly smaller than 50 ha (upper 25th percentile = 37 ha).

In order to determine if the proposed typology resulted in types that were biologically distinct, pair-wise multi-response permutation procedure (MRPP) tests were carried out on transformed ($x^{0.5}$) macrophyte abundance (g). The A values from MRPP indicate the homogeneity within a group to that expected by chance: 1 equals complete within group homogeneity whereas an A of 0 equals within group heterogeneity equal to that expected by chance (McCune *et al*., 2002). The majority of types were biologically distinct (see Free *et al.,* 2006). Of the twenty-one tests which were possible, sixteen were significant with A values ranging from 0.11 to 0.45. Insignificant test results were largely due to low sample numbers *i.e.* < 5 lakes. Lack of reference lakes precluded the testing of some types.

The proposed typology must be as effective as the default System A typology in partitioning biological variation. This requirement was evaluated using MRPP. The proposed typology for macrophytes had an overall A value of 0.35 ($p < 0.001$) whereas the default System A typology had an overall A of 0.21 ($p < 0.001$). This provides some evidence that the biologically derived typology was better at partitioning natural variation than the default System A typology.

A description of the proposed typology for macrophytes was achieved by calculating indicator values (IV) of the macrophytes across the types (see Free *et al*., 2006). There was a clear distinction between low and high alkalinity bands in terms of species composition, *viz.* the higher IV for *Chara* at high alkalinity. In the low alkalinity, small shallow lake type, littoral rosette species were both frequent and abundant but as area and depth increase across the low alkalinity band their IV decreases.

5.2 Metrics and Multimetric Indices

Two approaches were adopted in developing a multimetric index:

(a) based on the draft European Committee for Standardisation (CEN) (2004) guidance and

(b) stepwise multiple regression.

5.2.1 Development of a Multimetric Index for Ecological Assessment Based on Linear Metrics

A macrophyte index composed of the following metrics: maximum depth of colonisation (Zc); relative frequency of Elodeids (functional group); mean depth of macrophyte presence; relative frequency of tolerant taxa; relative frequency of *Chara* (for lakes > 100 mg l^{-1} $CaCO_3$ alkalinity only) and a plant trophic score based on TP concentration was developed (see Free *et al*., 2006) for lakes with an alkalinity greater than 20 mg l^{-1} $CaCO_3$ alkalinity.

The relationship between the macrophyte index and TP is shown in Figure 5.2. Reference lakes clearly have higher values of the index. A linear regression between the macrophyte index and Log (TP+1) for lakes with alkalinity > 20 mg l^{-1} $CaCO_3$ resulted in an r^2 of 0.59 ($p = 0.0001$, $n = 93$, and outliers Salt and Cloonagh removed). The index values for low alkalinity lakes are also plotted in Figure 5.2. Independent data should be used to test the index when it becomes available.

5.2.2 Development of an Assessment Tool for Aquatic Macrophytes Based on Multiple Linear Regression

The multimetric index in the preceding section was developed by averaging several metrics that had a linear response to anthropogenic pressure (TP). Stepwise multiple regression models were developed for all the lakes and lake types (based on alkalinity and depth typology bands) (Table 5.1). The models explained between 57 and 80% of the variation in TP (Table 5.1). Relative metrics such as relative frequency of (RF) tolerant taxa, RF filamentous algae and taxa found once or twice as a percentage of all taxa found were selected more frequently in the models produced for the high alkalinity lakes (> 100 mg l^{-1} $CaCO_3$). In contrast, the plant trophic score and depth of angiosperm colonisation were more important in the lakes of intermediate alkalinity (20 – 100 mg l^{-1} $CaCO_3$).

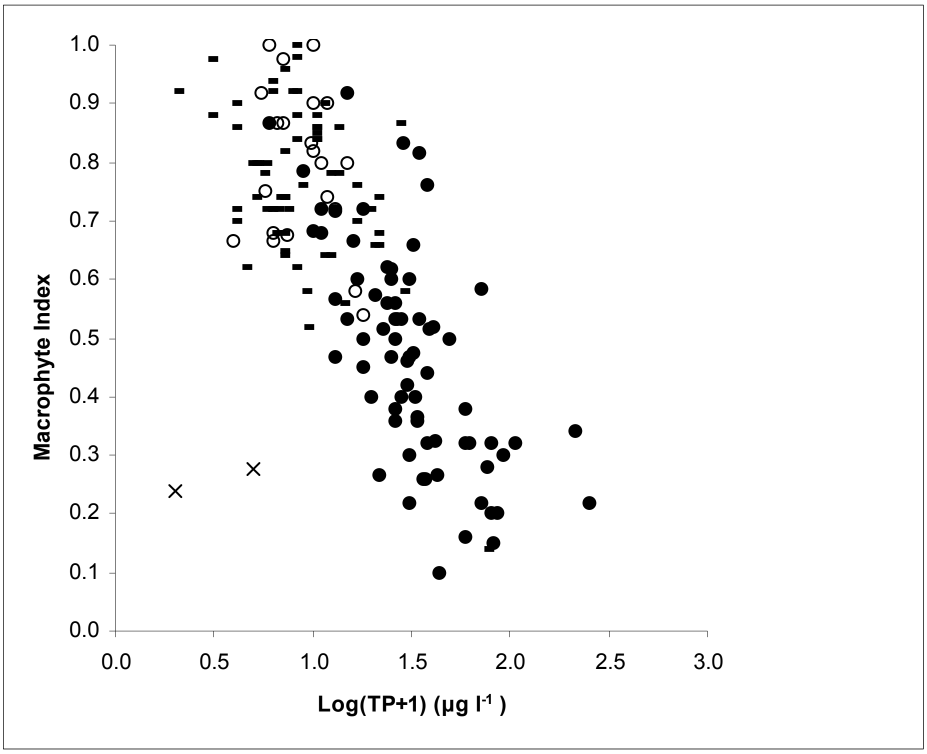

Figure 5.2 **Relationship between the macrophyte index and Log (TP + 1) (µg l^{-1})**

○ **= reference lakes > 20 mg l^{-1} $CaCO_3$ (n = 22),**
● **= non reference lakes > 20 mg l^{-1} $CaCO_3$ (n = 71) and**
- **= lakes with alkalinity < 20 mg l^{-1} $CaCO_3$ (n = 64).**
- **Outliers (×) are Loughs Cloonagh and Salt.**

Table 5.1 **Multiple regression models of Log (TP + 1) (µg l^{-1}) against macrophyte metrics for all lakes and lake sub-types.**

Typology	Model	n	r^2	p	Outliers
All lakes	- 0.558 + 1.525 * log(1 + Plant trophic score) - 0.673 * log(1 + Zc) - 0.003 * RF Isoetids	153	**0.67**	< 0.001	Cloonagh, Salt
> 20 Alkalinity	- 0.576 + 0.006 * RF tolerant taxa - 0.525 * log(1 + Zc Angiosperms) + 1.182 * log(1+Plant trophic score)	92	**0.64**	< 0.001	Cloonagh, Salt
20-100 Alkalinity	- 0.611 + 1.539 * log(1 + Plant trophic score) - 0.651 * log(1 + Zc Angiosperms)	46	**0.73**	< 0.0001	Egish, Salt
> 4 m	1.111 + 0.018 * Plant trophic score - 0.609 * log(1 + Zc Angiosperms)	25	**0.77**	< 0.0001	Egish, Salt
< 4 m	1.886 - 0.023 * Species richness - 0.523 * log(1 + Zc Angiosperms)	21	**0.64**	< 0.05	
> 100 Alkalinity	1.056 + 0.007 * RF tolerant taxa -0.005 * once or twice as % of taxa found	44	**0.64**	< 0.01	Cross, Cloonagh
> 4 m	1.982 - 0.671 * Mean taxa max depth in lake * taxa max depth recorded in survey	22	**0.57**	< 0.0001	
< 4 m	1.016 + 0.006 * RF tolerant taxa - 0.005 * once or twice as % of taxa found + 0.010 * RF filamentous algae	22	**0.80**	< 0.05	Cross, Cloonagh

5.3 Epilithic Chlorophyll *a*

Phytobenthos are required to be monitored by the Water Framework Directive. This project carried out an initial examination of the abundance of epilithic algae, as indicated by chlorophyll *a*, in the littoral zone of 99 lakes. Epilithic chlorophyll *a* was most highly related to alkalinity ($r^2 = 0.43$, $p < 0.001$) (Figure 5.3). Total phosphorus was not significant in explaining additional variation in chlorophyll *a* in multiple regression. This indicates that the abundance of epilithic algae may not be related in a straightforward way to mid-lake nutrient concentrations. The higher chlorophyll *a* found at higher alkalinities might be related to the habit of particular species. For example, high chlorophyll *a* was recorded from some marl-encrusted stones with which colonies of *Schizothrix* spp. can be naturally associated (John *et al*., 1982). This strong influence of alkalinity on abundance underlines the need for a typology to be developed for epilithic algae in Irish lakes.

Figure 5.3 also compares values recorded for epilithic chlorophyll a in 1997 (Irvine *et al*., 2001) with those recorded from this study. Many of the lakes deviate from a 1:1 relationship indicating that there is a high degree of inter-annual variation in abundance or that several sites within a lake should be sampled to better account for natural variation. The high variation in abundance between years and the lack of a relationship between abundance and pressure (TP) indicate the need to focus attention on the use of species composition in ecological assessment.

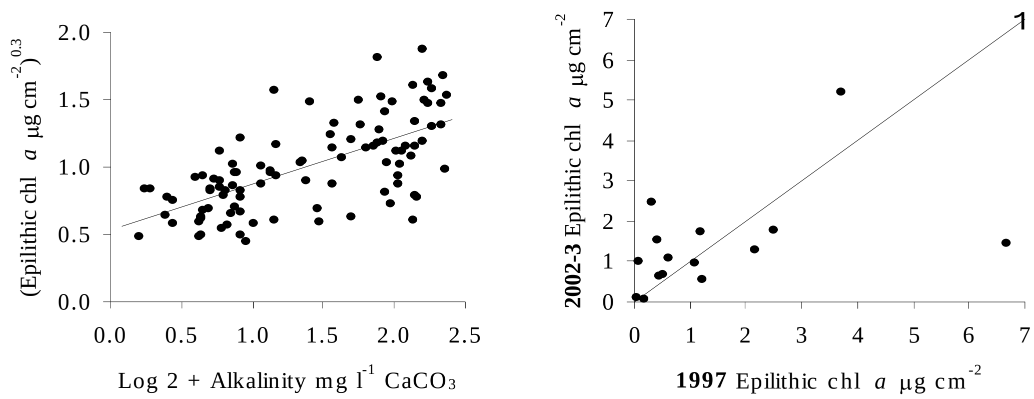

Figure 5.3 Relationship between transformed epilithic chlorophyll *a* and alkalinity (n = 99) and comparison between levels found in 1997 (Irvine *et al*., 2001) and this survey (n = 15).

6. Littoral Macroinvertebrates

6.1 Development of a Typology Using Littoral Invertebrates

The macroinvertebrate community data used to develop a lake typology were collected predominantly from stony shorelines in potential reference lakes sampled in spring 2001 and 2002, and summer 2002. The spring data (n = 69) comprised almost 43,000 macroinvertebrates representing 149 taxa, identified predominantly to species level, encompassing 105 genera. The combined season data set (it was not always possible to get summer samples from all of the lakes therefore for lakes with both spring and summer samples, n = 58) comprised over 67,000 individuals representing 197 taxa encompassing 126 genera.

Two different methods of classification, TWINSPAN and cluster analysis, were applied to 12 different treatments – 3 taxonomic resolutions x 2 transformations x 2 seasons - to group reference lakes on the basis of their biological communities.

ANOSIM was used to identify the most effective analysis and treatment. The strength of the ANOSIM test lies in the fact that values of the test statistic, R, close to unity are indicative of complete separation of the groups, and small values close to zero imply little or no segregation (Clarke and Warwick, 1994). The highest global R value, R = 0.573, was obtained using TWINSPAN classification (5 groups) on spring presence-absence data, excluding rare species. The next highest global R value, R = 0.556, was generated using cluster analysis (4 clusters) on the combined season square root transformed species data. Subsequent analysis concentrated on these two treatments.

Pair-wise *post-hoc* tests with Bonferroni adjustment indicated that there were few significant differences in environmental variables among the TWINSPAN groups. Group 5 was significantly different ($p < 0.05$) from groups 1, 2 and 4 in terms of pH and percentage peat and from group 4 in terms of alkalinity. None of the other groups were significantly different from each other for any of the other environmental variables.

Among the cluster groups, the *post-hoc* tests indicated that Group 2 (high alkalinity group) was significantly different from all other groups in terms of pH, conductivity, alkalinity and percentage peat ($p < 0.001$). Groups 1 and 4 were also different in terms of pH, alkalinity ($p < 0.008$) and area ($p < 0.035$). Group 4 were primarily small, acid lakes and the ANOSIM R between Group 1 and 4 was the highest found (R = 0.68).

The groups generated by the biological classifications were generally supported by the MDS ordinations although there was some overlap among the groups. The stress values for the MDS ordinations were 0.26 and 0.23 respectively. Stress values > 0.2 are likely to yield plots which may be difficult to interpret (Clarke 1993) and values > 0.25 indicate that the points are close to being arbitrarily placed in ordination space; any subsequent interpretation should be treated sceptically. Stress values < 0.2 would be potentially useful (Clarke and Warwick, 1994).

Canonical variates analysis (CVA) was used to determine which linear combination of seven environmental factors best discriminates between groups. For the TWINSPAN groups, percentage peat was identified by CVA as the only significant environmental variable. For the cluster end groups, alkalinity, conductivity, and percentage peat were the selected significant variables. Canonical correspondence analysis (CCA) was performed on the data sets analysed earlier. pH, conductivity and alkalinity were the most important underlying variables along axes 1, 2 and 3, respectively, for both data sets. Although the CCA axes explain approximately half the variation in biotic data, compatibility between the location of the sites in the ordinations and the groupings developed using biological classifications based upon species assemblages were poor.

The results indicated a clear tendency for littoral invertebrate communities to be dependent on an underlying alkalinity gradient, but apart from this distinction into hard and soft water lakes, the littoral invertebrate community in Ireland is quite homogenous. It was not possible to define typing boundaries using the invertebrate community and three alkalinity bands were chosen (< 20, 20 – 100, > 100 mg l^{-1} $CaCO_3$) using expert judgement and taking into account boundaries suggested by the phytoplankton and macrophyte communities (chapters 4 and 5, respectively).

Indicator Species Analysis (Dufrene and Legendre, 1997) identified 11 statistically significant indicator species from the three lake types. Visual inspection of the reference littoral community data did not reveal any large shifts in the major invertebrate orders in terms of relative abundance across an alkalinity gradient. Spearman rank correlation coefficients revealed that 27 of the 197 taxa in the combined log (x+1) transformed abundance data set were significantly ($p < 0.05$) correlated with alkalinity. Four of these were negatively correlated and the remaining 23 were positively correlated. When presence/absence data were examined an additional four taxa were correlated with alkalinity, one negatively so and the other three positively.

6.2 Assessment of Ecological Quality Using Littoral Invertebrates

Thirty-five candidate invertebrate metrics were originally selected from the literature for investigation and a macroinvertebrate trophic score was constructed. Spearman rank correlation coefficients (r_S) were calculated to identify metrics which had a linear or log linear response to TP in different alkalinity bands. The metric that consistently performed the best was ASPT and this recorded the highest coefficient value (r_S = -0.50) using the full data set, followed by the number of intolerant taxa (r_S = -0.48).

The macroinvertebrate trophic score was developed using data from 190 samples collected in spring 2001 and 2002. The lakes were divided into six TP bands (< 10, 10 – 20, 20 – 30, 30 – 40, 40 – 60, > 60 µg l^{-1} TP) and for each invertebrate taxon the average TP value was calculated within each of those bands. To allow for the different sampling intensity among the various TP bands, a weighted average TP concentration for each taxon was calculated based on the percentage of sites where the taxon was present in each TP band. Weighted averages were calculated only for taxa found in five or more samples. For each lake the weighted average taxon scores were averaged to give a macroinvertebrate trophic score. The relationship between this macroinvertebrate trophic score and Log (TP+1) can be seen in Figure 6.1.

Using all lakes, the invertebrate trophic score was significantly correlated with Log (TP+1) (r^2 = 0.43, $p < 0.05$). In lakes with TP values less than 15 µg l^{-1}, there was no significant correlation between the invertebrate trophic score and TP (r^2 = 0.03). Above a TP level of 15 µg l^{-1}, there is a significant correlation between the score and the TP levels in the lake (r^2 = 0.50, $p < 0.05$), suggesting that the invertebrate communities are not influenced by relatively low TP levels. However, once a certain threshold is reached, the community starts to respond and taxa that are more tolerant of the higher nutrient levels begin to increase (Figure 6.1).

A macroinvertebrate index was developed composed of the following metrics: invertebrate trophic score, ASPT (Average Score per Taxon), no. intolerant taxa, % EPT (Ephemeroptera, Plecoptera, Trichoptera), % Trichoptera, BMWP (Biological Monitoring Working Party), HBI (Hilsenhoff's Biotic Index),MBI (Modified Biotic Index), % Oligochaetes and % Shredder taxa (see Free *et al*., 2006).

The relationship between the invertebrate index and TP (Figure 6.2) was poor with a r^2 value = 0.22, though the reference lakes did tend to have a higher value of the index. Combining and averaging the metrics did not improve the relationship with total phosphorus.

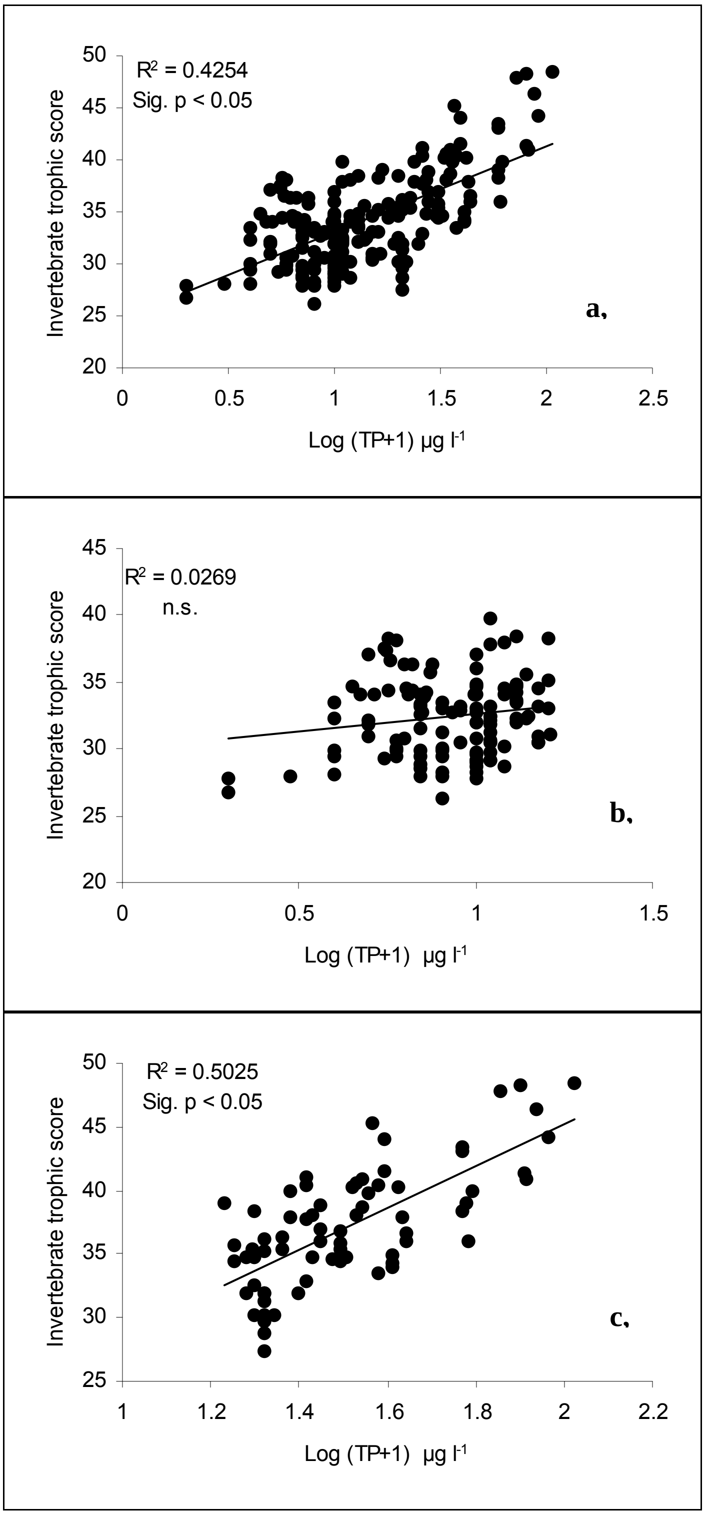

Figure 6.1 **Relationship between invertebrate trophic score and Log (TP + 1) for: (a) all lakes (n = 191), (b) lakes with TP ≤ 15 µg l^{-1} (n = 119) and (c) lakes with TP > 15 µg l^{-1} (n = 72).**

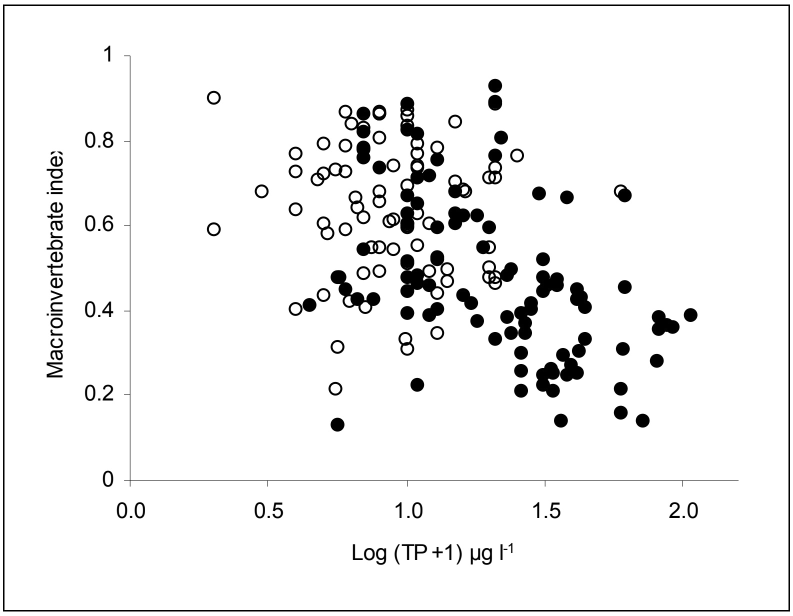

Figure 6.2 Relationship between the macroinvertebrate index and Log (TP+1) (µg l^{-1}). ○ = reference samples (n = 80). ● = non reference samples (n = 100)

7. Profundal Macroinvertebrates

7.1 Development of a Typology Based on Profundal Macro-invertebrates

TWINSPAN classification of profundal macroinvertebrate data from candidate reference lakes (n = 63) resulted in the production of three end-groups. Multi-response permutation procedure (MRPP) showed that the three groups: Groups 1, 2 and 3 were significantly different. Forward selection in CCA chose nine of the nineteen environmental variables that comprised dataset 1- alkalinity, maximum depth, TP, peat, colour, catchment area, catchment perimeter, other catchment use (*i.e.* a combination of sparsely vegetated, natural woodland /scrub to bare ground, see Appendix 1 in Free *et al*., 2006) and chlorophyll *a* - which explained most of the variance in the biological community from dataset 1 (see Free *et al.,* 2006 for components). The resulting ordination showed a reasonable separation of the TWINSPAN groups without a serious loss of information as indicated by comparable eigenvalues. The variables TP and chlorophyll *a* had short gradients indicating that they had little influence on the faunal data. This supported the theory that the majority of the lakes were in reference condition. Alkalinity, maximum depth, colour and altitude were selected from dataset 2 (a subset of dataset 1 consisting of 10 variables) as explaining most of the variance.

The variables: % peat, maximum depth, % forest, lake area, TP and lake perimeter were identified by CVA as the variables important in discriminating between TWINSPAN end-groups. The three groups were clearly separated on the CVA ordination diagram. Five variables: conductivity, maximum depth, lake perimeter, altitude and lake area were identified by CVA from dataset 2 as being significant. The findings of the CCA and CVA were also supported by Mann-Whitney tests.

The preceding analyses of the profundal data suggested a typology based on alkalinity and maximum depth, with possibly some influence from colour. The proposed boundaries for alkalinity are < 20 mg l^{-1} $CaCO_3$ (Group 2 and 3) and > 100 mg l^{-1} $CaCO_3$ (Group 1). This was also suggested from the classification of the oligochaetes and chironomids (section 7.2). A tentative third category was 20 to 100 mg l^{-1} $CaCO_3$ alkalinity. However, few reference lakes were in this category and they did not form a separate TWINSPAN group; furthermore, such a category was not suggested by the classification of the oligochaetes and chironomids (see Section 7.3.7, Final report). The suggested type categories for depth were < 12 m (Group 3) and > 12m (Group 1 and 2), closely approximating the lower 25th percentile of Groups 1 and 2. The distribution of the three littoral chironomid taxa, *Polypedilum* spp., *Pagastiella* spp. and *Pentaneurini* also indicated a depth boundary between 10 and 15 m.

Distinct biological communities were verified for each type by MRPP. Six significant indicator taxa were identified: three for the type alkalinity > 100 mg l^{-1} depth < 12 m - *Chironomus* spp., *Cladotanytarsus* spp. and Hydracarina, one for type alkalinity 20-100 mg l^{-1} type depth < 12 m - *Pagastiella* sp.-, one for the alkalinity < 20 mg l^{-1} depth < 12 m - *Heterotanytarsus* spp.- and one for alkalinity > 100 mg l^{-1} depth > 12 m – *Asellus* sp. However, identification of indicator taxa for some lake types in reference condition was difficult for many reasons such as insufficient lake numbers.

Testing of the WFD System A typology was undertaken using MRPP. Comparison of the overall A value of the proposed typology (A = 0.14) and System A typology (A = 0.11) showed an improvement in the A value using the proposed typology.

7.2 Oligochaete and Chironomid Community Structure and Lake Trophic Status

7.2.1 Oligochaete Community Structure and Lake Trophic Status

TWINSPAN classification of oligochaete data from 166 lakes across the trophic range resulted in 16 communities validated by ANOSIM. Divisions were based on changes in abundance and often dominance of the five taxa; *Potamothrix-tubifex* group, *Limnodrilus* group, *Lumbriculus variegatus*, *Aulodrilus pluriseta* and *Spirosperma ferox*. It was evident from Mann-Whitney tests, CCA and CVA that oligochaete community structure was primarily influenced by alkalinity or a correlated variable (pH, conductivity), trophic status (TP or chlorophyll *a*) and to a lesser extent depth.

In summary, 16 oligochaete communities were recognised for the 166 lakes which could be divided into two alkalinity types, > 20 mg l^{-1} $CaCO_3$ – Groups 2 to 8 – and < 20 mg l^{-1} $CaCO_3$ – Groups 1, 9 to 16 excluding Group 13. Group 13 was an exception having a higher alkalinity than the other groups (Groups 9 to 16). Lakes with alkalinities between 20 and 100 mg l^{-1} $CaCO_3$ were not distinct from those with alkalinities greater than 100 mg l^{-1} $CaCO_3$. This led to the conclusion that for oligochaetes, there were only 2 alkalinity types. It was evident, from the median TP values for Groups 2 to 8, that these communities represented a range in trophic status. In terms of community structure this was expressed as increased abundance in the *Limnodrilus* and *Potamothrix-Tubifex* groups. The low alkalinity lakes had no clear trophic gradient and were mostly oligotrophic. *Spirosperma ferox* and *L. variegatus* typified these groups by their presence but not necessarily dominance. *Limnodrilus* group and *Potamothrix-Tubifex* group were usually present. There was no suggestion that stratification influenced the oligochaete community but stratified lakes were concentrated among the moderate to high alkalinity lakes with increasing prevalence in Groups 5 to 8.

7.2.2. Chironomid Community Structure and Trophic Status

Chironomid community structure was examined using TWINSPAN for 93 lakes with alkalinities > 20 mg l^{-1} $CaCO_3$. Lakes with a lower alkalinity were excluded because they lacked a TP pressure gradient. The TWINSPAN classification resulted in nine communities. Divisions were predominantly based on the presence/absence and changes in abundance of three taxa (*Procladius* spp., *Tanytarsus* spp. and *Chironomus* spp.) and also on the presence/absence of *Polypedilum* spp. and the oligotrophic species *Protanypus* spp.. ANOSIM validated all end-groups for division levels I to V except where the group size was small. Indicator species analyses confirmed TWINSPAN indicator species as significant indicators and identified additional indicator taxa. The latter were usually rare in abundance and infrequent in occurrence.

DCA showed that the End-groups 8 and 9 were distinct from the remaining groups. End-groups 1 to 7 were not distinct because these groups were predominantly distinguished by the presence / absence of only three taxa (*Procladius* spp., *Tanytarsus* spp. and *Chironomus* spp.) and their abundance. Lake morphology related variables (depth, ILBS (index of lake basin shape)) conductivity and trophic status (TP, chlorophyll *a*) were identified by CCA as the primary variables influencing community structure. This was supported by Mann-Whitney tests. Chlorophyll a and other agriculture (*i.e.* not pasture see Appendix 1 in Free *et al.,* 2006) were identified by CVA as the variables that best explained the 9 end-groups.

In summary, the factors considered responsible for the structure and composition of the chironomid communities of moderate to high alkalinity lakes were trophic status (TP) and lake morphology related characteristics, *viz.* tendency to stratification, either separately or in tandem. Both factors impact on oxygen availability.

7.3 Assessment of Ecological Quality Using Profundal Invertebrates

The following literature based oligochaete indices were selected for investigation: the Environmental Index (EI) by Howmiller and Scott (1977); a modified version of the Environmental Index (mEI) (Milbrink, 1980) and the Benthic Quality Index (BQI) using oligochaetes (Wiederholm, 1980). Three indices, termed Index 3 to 5, were devised based on the mEI. Taxa trophic preferences were reassigned and the coefficient values altered. The coefficient values were determined by the total abundance of oligochaetes. Saether's key to chironomid associations of the profundal zone (Saether, 1979) and the BQI for chironomids (Wiederholm, 1980) were also applied. The BQI for chironomids and modifications thereof were also calculated and termed Der BQI 1, 2, 3 and 4. Two versions of the Quirke Index - Quirke 1 and Quirke 2- (Irvine *et al.*, 2001) were also calculated.

Of the mixed (chironomid and oligochaete) literature-based indices examined, Quirke 1 had the best correlation with TP (Table 7.1). The index Der BQI 2 had the highest correlation with TP of the chironomid-based indices. However, based on visual inspection, Der BQI 4 was considered a better discriminator of chironomid TWINSPAN groups. The index IV 5 had the highest correlation with TP of the oligochaete based indices. Both these indices were developed as generalised systems across all types, the effect of which was to generate different reference values for each type. The relationship between log TP and the Quirke 1 index, the IV 5 oligochaete index and the Der BQI 4 for chironomids is presented in Figure 7.1.

Of the metrics explored using the linear multiple regression method, depth adjusted lp/sv, had the highest correlation (r^2 (adjusted) = 45.4%) with TP. The inclusion of other metrics did not explain a requisite additional 5% of the variation.

A profundal index was developed based on linear metrics and concentrating on shallow lakes across all alkalinities. This was done to minimise any stratification related effects. There was the possibility that the trends detected were due to alkalinity and not TP because there was no segregation by alkalinity type. However, the profundal index was no better than one of its component metrics (Dlp) alone in terms of TP correlation (Table 7.2). Multimetric indices following the approach of Karr and Chu (1999) which included non-linear metrics were also constructed. The metric combination (Karr 4) with the highest correlation (r = -0.647) to log TP was selected for presentation. The relationship between Log TP and the profundal index along with the Karr based index: Karr 4 and the metric with the best regression for all shallow lakes: Dlp/sv is presented in Figure 7.2.

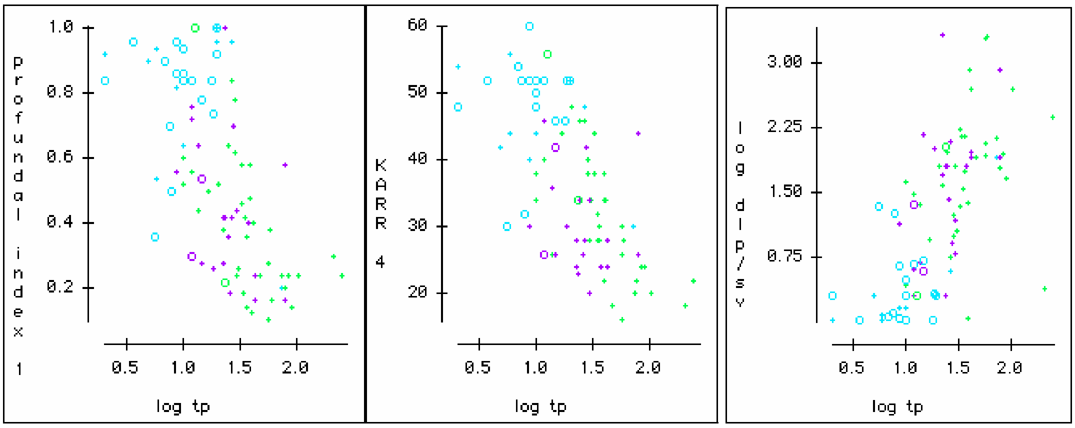

Figure 7.1 **The relationship between Log TP (µg l^{-1}) and the profundal index along with the Karr based index: Karr 4 and the metric with the best regression for all shallow lakes: Dlp/sv. Reference lakes are highlighted by o. The three alkalinity bands: low = blue, moderate = green and high = purple are highlighted.**

Table 7.1 **Pearson product moment correlations (r^2) of literature-based ecological quality indices with log TP (µg l^{-1})**

Oligochaete based			**Chironomid based**			**Mixed**		
	n	**r^2**		**n**	**r^2**		**n**	**r^2**
IV 5	174	0.556	Chir BQI	168	-0.139	Quirke 1	174	0.528
IV 4	174	0.514	Der BQI 1	168	-0.279	Quirke 2	174	0.509
IV 3	174	0.519	Der BQI 2	168	-0.299			
BQI	148	-0.427	Der BQI 3	168	-0.128			
EI - HS	139	0.444	Der BQI 4	168	-0.28			
Mod EI M	148	0.477						

Table 7.2 Pearson Product-Moment Correlation for the Profundal Index and its components (transformed) for shallow lakes.

	Profundal Index	O/C	log DIp	ch-cPT	% Tubificidae	% eutrophic sp	log TP
Profundal	1						
O/C	-0.91	1					
log DIp	-0.90	0.77	1				
ch-cPT	0.84	-0.68	-0.66	1			
%Tubificida	-0.94	0.87	0.83	-0.66	1		
%eutrophic	-0.95	0.81	0.82	-0.78	0.91	1	
log TP	-0.63	0.58	0.64	-0.52	0.58	0.58	1

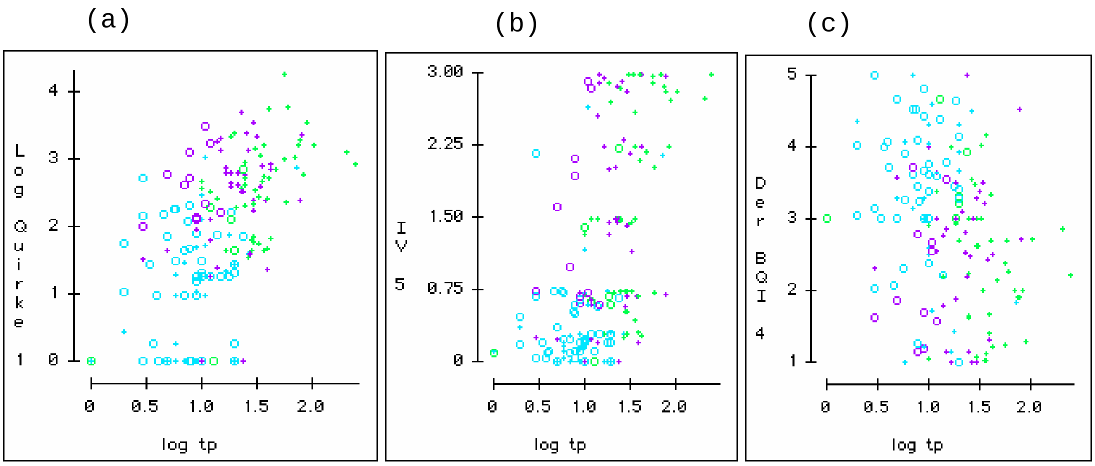

Figure 7.2 The relationship between log TP (µg l^{-1}) and the (a) Quirke 1 index (n = 174), (b) IV 5 oligochaete index (n = 174) and (c) the Der BQI 4 for chironomids (n = 158). The three alkalinity bands: low = blue, moderate = green and high = purple and reference lakes (o) are also highlighted.

8. An Overall Lake Typology for Ireland

It was apparent from the preceding chapters that the typologies based on the analysis of the individual biological elements had defining parameters in common, with similar bands (Figure 8.1). This facilitates the construction of an overall lake typology for the island of Ireland (Ecoregion 17).

For each of the System B parameters, altitude, latitude and longitude, one category only is proposed. Most lakes in Ireland lie below 200 m. There were insufficient data on lakes located above 200 m to allow a statistical analysis to be carried out and there was little evidence from the classification of the existing data for an altitude influence (Figure 8.1). Both latitude and longitude have narrow bands due to the island's size and therefore would have little influence.

The additional parameters selected for the overall typology were: alkalinity, depth and lake area. Their selection and order of importance were supported by statistical analysis (Table 8.1). Alkalinity or correlated variables: conductivity and % peat, were identified by CVA of the biological elements as the main parameters responsible for discriminating between biological end-groups. Depth was frequently selected as the second most important factor and lake area was found to significantly influence macrophytes (Figure 8.1).

At least two alkalinity categories < 20 mg l^{-1} $CaCO_3$ and > 100 mg l^{-1} $CaCO_3$ were evident for each biological element, with particularly strong evidence for the form*er* (Table 8.2). There were few reference lakes in the moderate alkalinity category, (20-100 mg l^{-1} $CaCO_3$), consequently this is to be considered a tentative category. Further classification of profundal data (including non-reference lakes) suggested that the types: 20-100 mg l^{-1} $CaCO_3$ and > 100 mg l^{-1} $CaCO_3$ may be amalgamated. Depth was clearly a typing parameter for three of the elements with particularly good agreement on boundaries at a mean depth of 4 m (Table 8.2). In the case of the profundal invertebrates, maximum (station) depth (12 m) was equated to mean depth (4 m) using an equation derived from data in Irvine *et al.* (2001). The boundaries for lake area were based on the macrophyte end-groups.

However, Figure 8.1 suggests that area may also have influenced the littoral and profundal macroinvertebrates, although the development of a typology for each of these elements did not indicate area as a strong typing parameter. Three alkalinity types, two depth types and two area types were recognised. These gave rise to 12 lake types (Table 8.3).

It is suggested that further work be carried out to investigate the possible inclusion of other typology parameters such as stratification, residence time and colour, to better define marl lakes and to incorporate turloughs and high altitude (mountain) lakes (which were not included in the selection of candidate reference lakes) in the typology.

Table 8.1 For each biological element, the significant parameters identified by CVA as discriminating between end-groups are presented. Parameters are numbered in order of importance *i.e.* variation explained. Shaded cells indicate that a parameter was not used in the original analysis.

Biological Element		CVA- forward selection	Conductivity	Alkalinity	Colour	TP	Altitude	Lake Area	Lake Perimeter	Depth	Catchment/lake are	% Forestry	% Peat
Phytoplankton				1	2		2				3		
Macrophytes				1				3		2			
Invertebrates	Littoral	cluster endgroups	2	1									3
		TWINSPAN endgroups											1
	Profundal	dataset 1				5		4	6	2		3	1
		dateset 2	1				4		3	2			

Table 8.2 Boundary settings for the typology parameters (indicated by colour changes) based on the classification of candidate reference lakes for the biological elements.

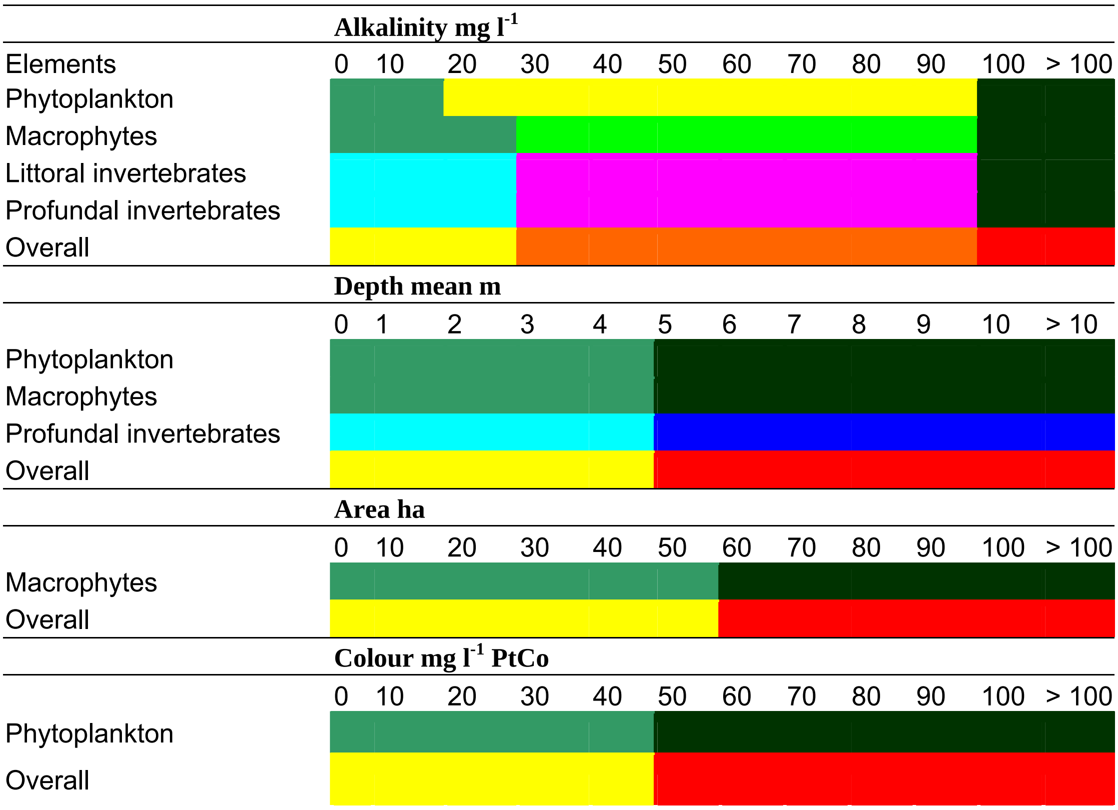

Table 8.3 The overall typology for Irish lakes.

Parameters	**Boundaries**											
Alkalinity (mg l^{-1} $CaCO_3$)	< 20				20 - 100				> 100			
Depth (m)	< 4		> 4		< 4		> 4		< 4		> 4	
Area (ha)	< 50	> 50	< 50	> 50	< 50	> 50	< 50	> 50	< 50	> 50	< 50	> 50
Type	1	2	3	4	5	6	7	8	9	10	11	12

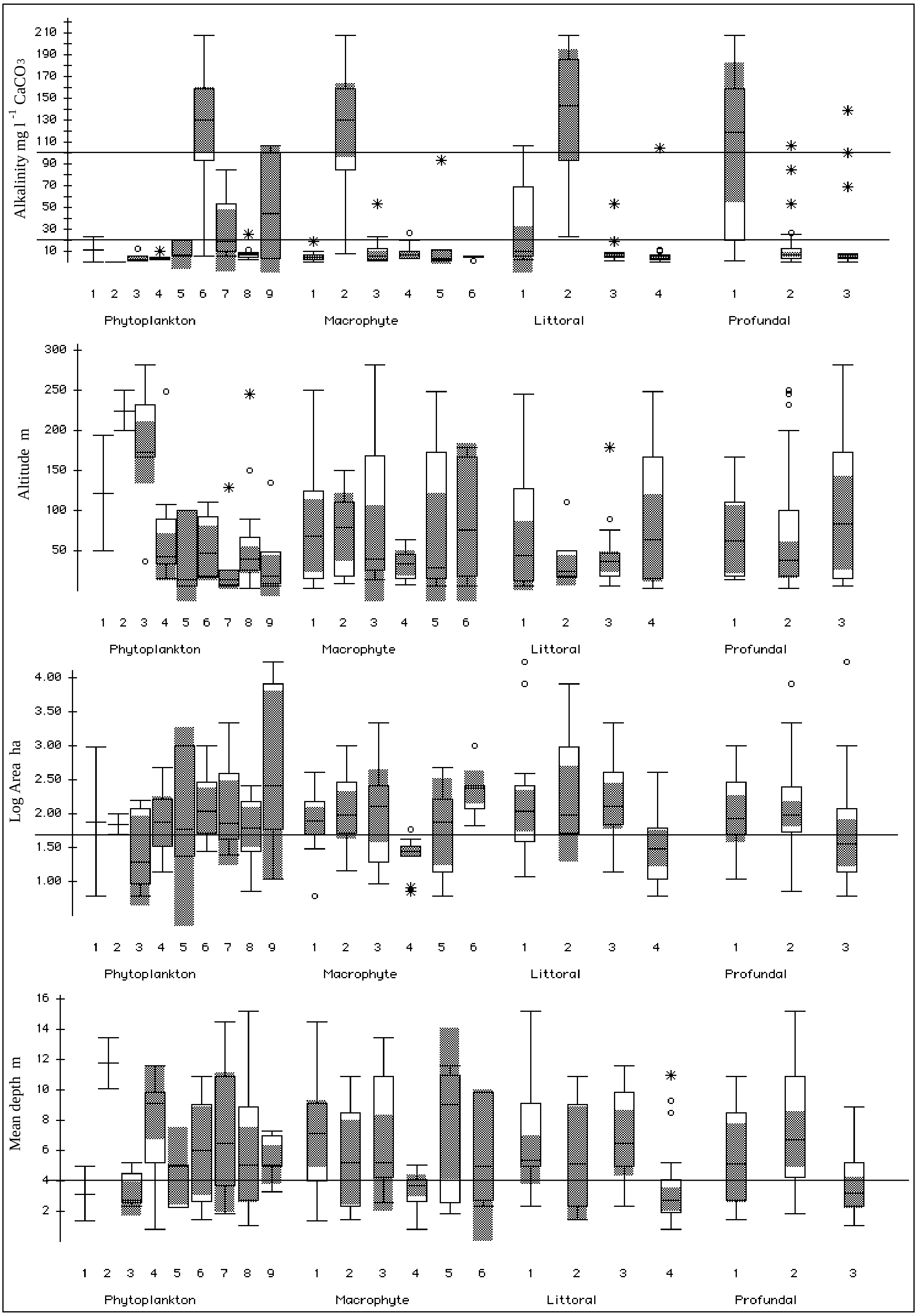

Figure 8.1 **Boxplots of typology parameters by classification end-groups of phytoplankton, macrophytes, littoral macroinvertebrates (cluster analysis) and profundal macroinvertebrates. The parameter boundaries for the overall typology are highlighted by horizontal lines.**

9. Defining Ecological Quality Classes

9.1 Introduction

The purpose of this chapter is to examine the response of certain metrics to pressure to identify levels of pressure that result in ecological change which can clearly be related to the status classes as defined by the WFD. Ultimately, such boundaries will be adjusted through an intercalibration exercise with EU countries in order to ensure comparability between member States (CEC, 2000).

9.2 Results

Increased input of total phosphorus was considered to be the main pressure affecting lakes in the Republic of Ireland. Ecologically relevant boundaries for TP concentrations were initially chosen based on the response of macrophyte diversity to TP. Figure 9.1 shows a unimodal relationship between Simpson's diversity index and log(TP + 1) for lakes with an alkalinity greater than 20 mg l^{-1} $CaCO_3$. Four bands of < 10, 10-25, 25-70 and > 70 $\mu g\ l^{-1}$ TP were selected to correspond to points of change in diversity with TP. The good/moderate boundary was taken to be 25 $\mu g\ l^{-1}$ TP on the basis that it corresponds with normative definitions *i.e.* it is the point where diversity starts to decrease therefore resulting in an 'undesirable disturbance to the balance of organisms' (WFD Annex V section 1.2.2). The increase in diversity between 10 and 25 $\mu g\ l^{-1}$ TP may correspond to normative definitions of good status in that the change is not an 'undesirable' one.

The TP bands above were examined for further relevance in two sub-sets of lakes in alkalinity bands of the proposed typology that are most subject to pressure: 20 – 100 and > 100 mg l^{-1} $CaCO_3$. In the alkalinity band 20 – 100 mg l^{-1} $CaCO_3$, the 25 $\mu g\ l^{-1}$ TP boundary was marked by an increased loss of littoral rosette species including *Littorella uniflora*, an increase in the relative frequency (RF) of Nymphaeids (rooted macrophytes with floating leaves) and a marked reduction in the RF of Charophytes. Phytoplankton showed a more log linear response to TP for example *Pediastrum* spp., although it only occurred in significant numbers at > 25 $\mu g\ l^{-1}$ TP indicating that it may be a more useful indicator over that part of the TP gradient.

In lakes with an alkalinity > 100 mg l^{-1} $CaCO_3$ (typically marl-precipitating lakes), the relative frequency (RF) of *Chara* spp. was typically > 40% at TP concentrations below 10 $\mu g\ l^{-1}$. The RF of *Chara* spp. declined rapidly at higher TP concentrations from 10 – 25 $\mu g\ l^{-1}$. The RF of Lemnids and the percentage of taxa occurring with filamentous algae also increased after 10 $\mu g\ l^{-1}$ TP. As found in the intermediate alkalinity band, *Pediastrum* spp. typically only occurred in significant numbers at > 25 $\mu g\ l^{-1}$ TP. *Cryptomonas* and *Scenedesmus* spp.

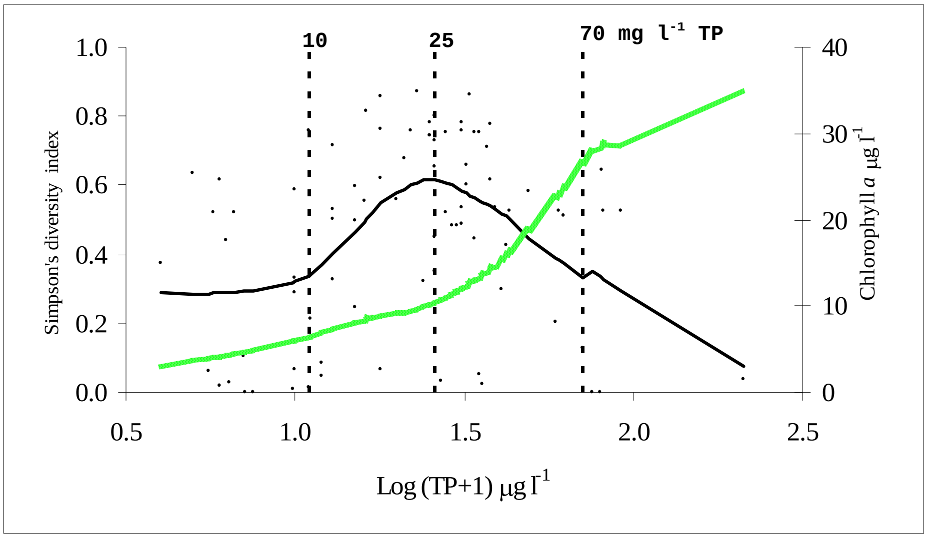

Figure 9.1 **Selection of TP bands (- - -) based on the lowest smoothed relationship (──) between Simpson's diversity index for the macrophytes samples and Log (TP+1) (µg l^{-1}). Smoothed relationship of chlorophyll *a* with Log (TP+1) is overlain (══). Graph refers to lakes > 20 mg l^{-1} $CaCO_3$ only, TP values were mostly measured in spring.**

displayed an unclear relationship with TP in this alkalinity band.

An alternative approach may be to examine literature based relationships that might lend support to boundary setting. A series of regression models (Table 9.1), initially based on spring TP, were used to successively predict summer chlorophyll *a* (Dillon & Rigler, 1974), Secchi depth (Free, 2002), depth of colonisation of charophytes (Blindow, 1992) and depth of colonisation of angiosperms (Chambers and Kalff, 1985). The prediction of Secchi depth used multiple regression based on predicted chlorophyll *a* and a colour of 30 mg l^{-1} PtCo.

Figure 9.2 shows the predicted relationships between TP (as a pressure gradient) and chlorophyll *a*, Secchi depth, depth of colonisation of charophytes and the depth of colonisation of angiosperms. The predictions provide a literature-based example of the interactions between a pressure gradient and ecological quality. As chlorophyll *a* increases with TP, it leads to a rapid decrease in Secchi depth (transparency) which reduces the depth of colonisation of charophytes and angiosperms. As the rate of extinction of light is exponential with depth, the initial change from an oligotrophic state to a mesotrophic state is where the most change takes place.

The high/good boundary was placed at 10 μg l^{-1} TP and this appears to be where there is a significant change in slope/response of the depth of macrophyte colonisation to TP concentration for a colour of 30 mg l^{-1} PtCo (Figure 9.2).

The good/moderate boundary was placed at 25 μg l^{-1} TP which corresponded to a point where the depth of colonisation of charophytes is reduced by 24% from reference condition (assumed to be 10 μg l^{-1} TP). This appears to fit normative definitions where phytoplankton biomass is such as to produce a significant, undesirable disturbance in the condition of another biological quality element. The depth of colonisation of angiosperms is less useful in this regard as a reduction in transparency may be accompanied by a shift to taller growing species such as *Potamogeton lucens*.

Table 9.1 Models used to generate Figure 9.2. Sources: 1: Equation 2 Dillon and Rigler (1974), 2: Free (2002), 3 Equation 4 Chambers and Kalff (1985), 4: Blindow 1992. TP and chlorophyll *a* are expressed in μg l^{-1}, Secchi depth in m and colour in mg l^{-1} PtCo. A colour value of 30 mg l^{-1} PtCo was used.

Source	Dependent variable	r^2	Model
1	Log (chlorophyll *a*)	0.92	1.45 * log TP - 1.14
2	Log (1 + Secchi depth)	0.82	1.35 - 0.41 * log (colour + 1) - 0.21 * log (chlorophyll *a* + 1)
3	$(Zc\ Angiosperms)^{0.5}$		1.33 * log (Secchi depth + 1.4)
4	Log (Zc Charophyta)	0.83	1.03 * log (Secchi depth + 0.18)

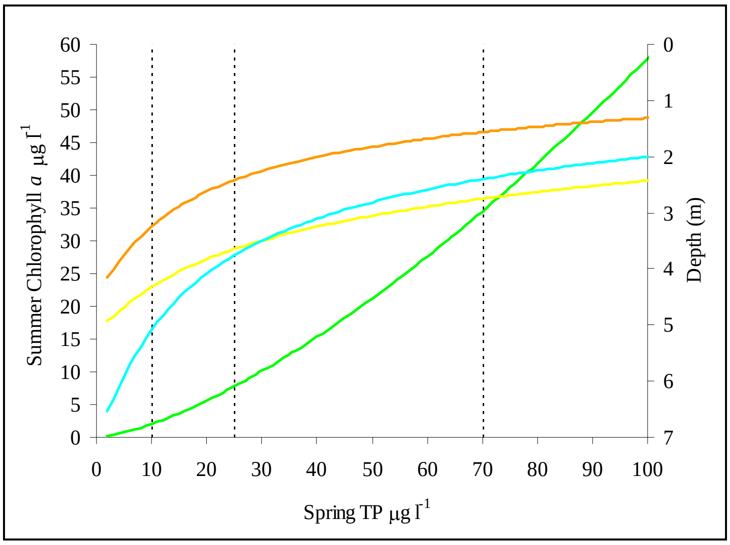

Figure 9.2 Relationship between spring TP (µg l^{-1}) and summer chlorophyll *a* (µg l^{-1}) (━), and predicted Secchi depth (━), predicted depth of colonisation of Charophytes (━) and Angiosperms (━) (m). Sources and models are listed in Table 9.1. Dashed lines represent boundaries of 10, 25 and 70 µg l^{-1} TP.

9.3 Discussion

The good/moderate boundary is perhaps more important than other boundaries in that member States will have to restore all lakes found to be in moderate status, or below, to good status by the end of 2015. However, it is also a requirement of the directive that any further deterioration of water quality be prevented. For the moderate alkalinity lakes (20 – 100 mg l^{-1} $CaCO_3$), the setting of boundaries at 10 $\mu g\ l^{-1}$ TP for the high/good boundary and at 25 $\mu g\ l^{-1}$ for the good/moderate boundary seemed reasonable based on the changes in macrophyte diversity, the increase in Nymphaeids and the tendency of littoral rosette macrophyte taxa to be absent.

For the high alkalinity lakes (> 100 mg l^{-1} $CaCO_3$) which are predominantly marl-precipitating in Ireland, the boundaries suggested by the overall relationship between macrophyte diversity and TP appeared too high when individual metrics were examined. In particular, there appeared to be a sharp reduction in the relative frequency of *Chara* spp. at TP concentrations above 10 $\mu g\ l^{-1}$. Charophytes typically dominate marl lakes in reference condition and, owing to their deep depth of colonisation, are sensitive to even small increases in phytoplankton due to the exponential decrease of light with depth. In order to conform with normative definitions for moderate status, the TP boundary for good/moderate status in these lakes could be placed at 15 $\mu g\ l^{-1}$, which, for *Chara* spp., would correspond to a significant decline from good quality. A TP concentration of 7 $\mu g\ l^{-1}$ may represent the boundary between high and good status, based on the TP distribution of lakes with a high relative frequency of *Chara* spp.

There was some disagreement between the model run using published equations and the observed response of charophytes. A more rapid decline in the relative frequency of charophytes with TP was observed than indicated by the published models. However, the outcome of the model is largely dependent on input values; if they are changed to a colour of 20 mg l^{-1} PtCo (more typical of marl lakes) and a initial reference TP of 5 $\mu g\ l^{-1}$, then a 15 $\mu g\ l^{-1}$ TP good/moderate boundary would correspond to a 24% reduction in the depth of charophytes.

The lower boundaries of TP suggested for the high alkalinity marl lakes take cognisance of sensitivity of charophytes to eutrophication pressure but are also appropriate because of the conservation importance of these lakes (John *et al*., 1982). In addition, lower boundaries of TP are appropriate because natural background nutrient concentrations are likely to be lower in these lakes due to the co-precipitation of phosphorus (Otsuki and Wetzel, 1972) and the lower nutrient inputs associated with groundwater fed rather than surface water fed lakes.

The suggested boundaries must be regarded as preliminary owing to the limited availability of nutrient data for this study.

10. General Discussion

10.1 Typology

The approach taken to develop the typology examined differences in composition and abundance of flora and fauna in potential reference lakes using multivariate techniques. An alternative approach would be to develop a typology based on ecological assessment metrics rather than the composition and abundance of the flora and fauna. This would entail the examination of both metric variation in reference condition and metric response to pressure for each of the biological elements. Focusing on metrics may be more appropriate as the purpose of a typology is to help detect ecological change by accounting for factors that naturally effect ecology.

Other elements that could be considered useful to refine the typology at a later date are information on residence time, more detailed information on stratification and mixing depth, a description of the substrate for macrophytes and invertebrates and a measure of exposure for macrophyte and littoral invertebrate sites. In addition, the biological elements - fish and phytobenthos - were not considered in this study but should be incorporated at the earliest opportunity.

The upper alkalinity band to the typology (> 100 mg l^{-1} $CaCO_3$) was generally taken to correspond to marl-precipitating lakes. These lakes are typically situated on limestone and are characterised by their abundance of charophytes in reference condition. It may be worthwhile to confirm which lakes with > 100 mg l^{-1} $CaCO_3$ are marl-precipitating on a site-by-site basis at a later stage. Some lakes, located in the northeast may have high alkalinity but may not be considered marl lakes, as they are not located on limestone. It may be appropriate to consider two subtypes – marl and non-marl lakes – within the > 100 mg l^{-1} $CaCO_3$ lake type. This is important as it was argued in chapter 9 that marl lakes should have more stringent environmental criteria as they respond to pressure at an earlier stage.

10.2 Ecological Assessment

The success of the ecological assessment metrics developed was found to vary with the biological elements. Some elements such as phytoplankton and macrophytes were clearly more useful in detecting the eutrophication pressure. The phytoplankton index showed a good relationship with total phosphorus (r^2 = 0.67) as would be expected, given the well-established relationship between indicators of phytoplankton biomass (chlorophyll a) and TP (Sakamoto, 1966; Dillon and Rigler, 1974). The macrophyte multimetric index also had a good relationship with TP (r^2 = 0.59).

Macrophytes may show a close relationship with the pressure gradient because they reflect the influence of eutrophication on the light climate over the growing season. The performance of the trophic score for littoral macroinvertebrates had limited success (r^2 = 0.43) and needs further development. The response of littoral macroinvertebrates to eutrophication pressure may be complex, perhaps owing to the non-uniform substrate, diverse communities or a complex food web. The relationship between profundal invertebrates and pressure was also concluded to require further work. The complicating influence of depth and stratification on the effects of eutrophication may make it difficult to develop an assessment system that can be used in all situations. Thus, some degree of expert judgement may be necessary in the application of ecological assessment metrics for profundal invertebrates.

It is useful to make some remarks about the level of taxonomy required for an effective ecological assessment system. While some of the metrics developed suggest that focusing on a very reduced set of taxa, such as 'eutrophic' taxa for phytoplankton, is justified, it may be premature to adopt such an approach. The development of ecological assessment tools is ongoing in Europe and it is recommended that future work on ecological assessment be carried out at the lowest taxonomic resolution possible so that future assessment tools may be availed of. It is possible to make observations (Table 10.1) on the level of taxonomy which was considered feasible during the operation of this project. This was largely a function of the biological group considered, as some groups are more difficult taxonomically than others.

Table 10.1 Recommended level of taxonomy

Biological group	Level of taxonomy practical
Phytoplankton	Largely Genus with selected taxa to species
Macrophytes	Species where possible
Littoral invertebrates	Species where possible
Profundal invertebrates	Largely Genus with selected taxa to species

11. Conclusions and Recommendations

The conclusions and recommendations which that follow from the investigations described and the discussion in Chapter 10 are:

- Information on the ecology of 201 lakes was successfully collected. The resulting dataset is the most extensive on lake ecology in Ireland.

- The typology described in chapter 8 is to be used as the reporting typology for Ireland. Comparison of the typologies developed at the individual element level indicated that they were at least as successful as the default typology system of the WFD – system A. The typology should prove effective in partitioning natural variation and allow ecological change to be detected more easily.

- Further work may help the application of the typology. The relative benefits of using the typologies developed at individual element level versus the overall typology level should be further considered.

- Further work may be conducted for typology refinement such as the development of typologies based on the variation of the values recorded for ecological assessment metrics in reference condition for the biological elements.

- Research into the background nutrient status of lakes is needed. Owing to the uncertainty associated with background (reference) nutrient concentration estimates, it may be preferable to apply a correction factor to an ecological assessment metric after application, based on background nutrient status.

- Other elements that may be useful to refine the typology at a later date are information on residence time, more detailed information on stratification and mixing depth, a description of the substrate for macrophytes and invertebrates and a measure of exposure for macrophyte and littoral invertebrate sites. In addition the biological elements: fish and phytobenthos were not considered in this study but should be incorporated at the earliest opportunity via research developments.

- The success of the ecological assessment metrics developed was found to vary with the biological elements. Some elements such as phytoplankton and macrophytes were clearly more useful in detecting pressure.

- The multimetric index developed for the macrophytes appears to have achieved similar success in detecting the effects of pressure on ecological quality in the lakes as the long established Q-value system has for river quality in Ireland and should provide a useful tool in ecological assessment.

- The performance of the invertebrate trophic score for littoral macroinvertebrates had limited success ($r^2 = 0.43$) and needs further development. The response of littoral macroinvertebrates to eutrophication pressure may be complex, perhaps owing to the non-uniform substrate, diverse communities or complex food web.

- The relationship between profundal invertebrates and eutrophication pressure was also concluded to require further work. The complicating influence of depth and stratification on the effects of eutrophication may make it difficult to develop an assessment system that can be used in all situations.

- Focusing on a limited depth range such as the sub-littoral as well as the profundal zone may help extract the influence of stratification and depth from that of eutrophication. Most ecological change was found to take place at an early stage of nutrient enrichment - generally between 10 and 20 $\mu g\ l^{-1}$ TP. Ecological status boundaries need to reflect this.

- Ecological assessment metrics were developed for eutrophication only. It is recommended to complement current data with a separate study whose site selection is stratified across a range of known acidification and hydromorphological pressures in Ireland.

- Further chemical and physical characterisation of lakes is necessary and should be carried out by the River Basin Districts.

- Reference conditions were unavailable or poorly represented for certain lake types – mainly in the 20 – 100 mg l^{-1} $CaCO_3$ alkalinity band. A palaeolimnological project focusing on biological elements such as phytoplankton, chironomids and macrophytes would help to characterise reference conditions for these types. This would complement ongoing work by the EPA funded project INSIGHT (*2002-W-MS-17-M1*) which aims to test the validity of many of the reference lakes selected by this project using palaeolimnological techniques.

- Fundamental research into the functioning of aquatic ecosystems at third level should be supported to provide a basis for continued refinement of ecological assessment techniques.

- Work should be immediately carried out to refine metrics, develop estimates of uncertainty in assessment and an exercise on integrating assessment, based on all biological elements, should be performed.

12. References

Blindow, I. 1992. Declines of charophytes during eutrophication: comparison with angiosperms. *Freshwater Biology* **28:** 9-14

CEC (Council of the European Communities), 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. *Official Journal of the European Communities*, 72p.

CEN (European Committee for Standardization), 2004. *Water Quality – Guidance standard on the design on multimetric indices*. (Draft). CEN 2004.

Chambers, P.A. and J. Kalff 1985. Depth distribution and biomass of submersed macrophyte communities in relation to Secchi depth. *Can. J. Fish. Aquat. Sci*. **42:** 701-709

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology **18**: 117-143.

Clarke, K.R. and Warwick, R.M., 1994. Change in Marine communities: an approach to statistical analysis and interpretation. 2nd Edition. PRIMER-E, Plymouth.

Dillon, P.J. and Rigler, F.H., 1974. The phosphorus - chlorophyll relationship in lakes. *Limnology and Oceanography* **19**: 767-773.

Dufrêne, M. and Legendre, P., 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs* **67:** 345-366.

Free, G., 2002. The relationship between catchment characteristics and lake chemistry in the Republic of Ireland. Unpublished PhD Thesis, Trinity College Dublin.

Free, G., Bowman, J., Caroni, R., Donnelly, K., Little, R., McGarrigle, M. L., Tierney, D., Kennedy, N., Allott, N. and Irvine, K., 2005. The identification of lake types using macrophyte community composition in Ireland. *Verhandlungen Internationale Vereinigung für theoretische und angewandte Limnologie*. **29:** 296-299.

Free, G., Little, R., Tierney, D., Donnelly, K., and Caroni, R., 2006. *A reference based typology and ecological assessment system for Irish Lakes. Preliminary Investigations*. Final Report. Environmental Protection Agency, Ireland. IN PRESS

Howmiller, R. P and Scott, M. A., 1977. An environmental index based on relative abundance of oligochaete species. *Journal of the Water Pollution Control Federation* **49**: 809-815.

Hutchinson, G.E., 1967. *A treatise on limnology, Volume 2*. John Wiley and Sons, New York.

Irvine, K., Allott, N., de Eyto, E., Free, G., White, J., Caroni, R., Kennelly, C., Keaney, J., Lennon, C., Kemp, A., Barry, E., Day, S., Mills, P., O'Riain, G., Quirke, B., Twomey, H. and Sweeney, P., 2001. *Ecological assessment of Irish lakes*. Environmental Protection Agency, Wexford, 503p.

John, D. M., Champ, W. S. T., Moore, J. A., 1982. The changing status of Characeae in four marl lakes in the Irish midlands. *Journal of Life Sciences, Royal Dublin Society* **4:** 47-71.

Karr, J. R. and Chu, E. W., 1999. *Restoring Life in Running Waters. Better Biological Monitoring*. Island Press, Washington, D.C.

McCune, B. and Mefford, M. J., 1999. PC-ORD. *Multivariate Analysis Ecological Data*. Version 4. MjM Software

Design, Gleneden Beach, Oregon, USA.

Milbrink, G., 1980. Oligochaete communities in pollution biology. The European situation with special reference to lakes in Scandinavia. *In*. Brinkhurst, R.O. and Cook, D.G. (*eds*.) *Aquatic Oligochaete Biology*. Plenum. *pp*. 433-455.

Otsuki, A. and Wetzel, R.G., 1972. Coprecipitation of phosphate with carbonates in a marl lake. *Limnology and Oceanography* **17:** 763-767.

REFCOND, 2003. Common implementation strategy for the Water Framework Directive (2000/60/EC), guidance document 10, river and lakes – typology, reference conditions and classification systems. Office for Official Publications of the European Communities, Luxembourg, 87p.

Rørslett, B. and Brettum, P., 1989. The genus *Isoëtes* in Scandinavia: an ecological review and perspectives. *Aquatic Botany* **35:** 223-261.

Saether, O.A., 1979. Chironomid communities as water quality indicators. *Holarctic Ecology* **2:** 65-74.

Sakamoto, M., 1966. Primary production by phytoplankton community in some Japanese lakes and its dependence on lake depth. *Archiv für Hydrobiologie* **62:** 1-28.

STATSOFT, Inc. 1998. *STATISTICA for Windows*. StatSoft Inc. 23000 East 14th Street, Tulsa, OK 74104.

ter Braak, C.J.F., and Šmilauer, P., 2002. CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Micro-computer Power, New York, 500p.

Wiederholm, T., 1980. Use of benthos in lake monitoring. *Journal of the Water Pollution Control Federation* **52**: 537-547.

13. Acronyms and Notation

ANOSIM	**Analysis of Similarities**
ASPT	**Average Score per Taxon**
BMWP	**Biological Monitoring Working Party**
BQI	**Benthic Quality Index**
$CaCO_3$	**Calcium Carbonate**
CCA	**Canonical Correspondence Analysis**
CEC	**Council of European Communities**
CVA	**Canonical Variates Analysis**
DCA	**Detrended Correspondence Analysis**
EHS	**Environment and Heritage Service (of Northern Ireland)**
EI	**Environmental Index (by Howmiller and Scott)**
EPA	**Environmental Protection Agency (of Ireland)**
EPT	**Ephemeroptera, Plecoptera, Trichoptera**
EQR	**Ecological Quality Ratio .**
ERDTI	**Environmental Research Technological Development and Innovation**
EU	**European Union**
HBI	**Hilsenhoff's Biotic Index**
ILBS	**Index of Lake Basin Shape**
ISBN	**International Standard Book Number**
MBI	**Modified Biotic Index**
MDS	**Multidimensional Scaling**
mEI	**Modified version of the Environmental Index**
MRPP	**Multiple Random Permutation Procedures**
NMS	**Non-Metric Multidimensional Scaling**
NUIG	**National University of Ireland – Galway**
PtCo	**Potassium Cobalt**
RF	**Relative Frequency**
RTDI	**Research Technological Development and Innovation**
spp	**Species**
TCD	**Trinity College Dublin**
TP	**Total Phosphorus**
TWINSPAN	**Two-Way-Indicator-Species-Analysis**
UCD	**University College Dublin**
WFD	**Water Framework Directive**