

# STRIVE

## Report Series No.88

# Molecular Diversity and Dynamics of Toxigenic Blue-green Algae in Irish Lakes

## STRIVE

Environmental Protection  
Agency Programme

2007-2013

# Environmental Protection Agency

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**EPA STRIVE Programme 2007-2013**

# **Molecular Diversity and Dynamics of Toxigenic Blue-green Algae in Irish Lakes**

Molecular Diversity and Dynamics of Toxigenic Cyanobacteria  
in Irish Lakes

**(2008-FS-EH-3-S5)**

## **STRIVE Report**

Prepared for the Environmental Protection Agency

by

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# Executive Summary

Cyanobacteria are cosmopolitan micro-organisms that occasionally reach high population densities in aquatic environments, events usually referred to as blooms. These cyanobacteria, or blue-green algae, are particularly prominent in freshwater ecosystems and can be of major concern for water managers. Microalgal proliferations can have negative consequences on ecosystem dynamics and affect human health due to the production by several species of biotoxins, which have also been implicated in poisoning incidents of livestock, wildlife and domestic animals. In Ireland, the management of anthropogenic pressures on water quality and the assessment of biodiversity in aquatic environments have been identified as priorities to comply with the EU Water Framework Directive. Seasonal blooms of toxic cyanobacteria, largely promoted by anthropogenic inputs of nutrients, can render the water unsafe locally for recreational activities or the production of drinking water. Hence, the risks of human exposure to cyanotoxin-containing water and the possibility of spreading to other water reservoirs make characterising the dynamics of toxic populations a necessity.

In this context, this study investigated (1) the distribution, diversity and abundance of planktonic cyanobacteria in lakes from the west and northwest of Ireland, and (2) the summer dynamics in the composition and toxic potential of cyanobacterial assemblages in a selection of water bodies. Of particular interest was to characterise potential trends between land cover typology and the capacity for microalgal biomass development, and to identify lakes at risk of harbouring future blooms of toxigenic cyanobacteria. To this end, given the range of anthropogenic pressures the study area is subjected to and the high number of water bodies within, a regionalisation framework was applied, based on a stratified sampling strategy of lakes designed following GIS analysis of river catchment attributes.

Results highlighted the linkage between land topography and water quality across the study area. In particular, the midland zone, subjected to substantial agricultural

activity, harboured a greater proportion of lakes of lesser quality compared to the more natural habitats found in coastal and mountainous areas. The summer diversity of cyanobacterial communities in the lakes was investigated using a genetic fingerprinting methodology and showed complex distribution patterns both in space and time. In particular, the analysis showed the presence of the toxigenic genus *Anabaena*, which was common in the lakes sampled. The potential of cyanobacteria to produce hepatotoxic microcystins, the only cyanotoxin for which the World Health Organisation has issued recommended safety levels, was ascertained using molecular, biochemical and analytical techniques. The toxin signature activity, the gene coding their synthesis or the toxin themselves were detected in several of the lakes sampled, showing that most water bodies are at risk of harbouring toxic blooms of cyanobacteria should the favourable environmental conditions allowing their development be met. The dynamics of planktonic cyanobacteria were monitored during summer 2010 in Lough Corrib and Ballyquirke Lough (Co. Galway) and showed variation in time, with the occurrence at the end of the summer of microcystin-producing populations. Finally, fine resolution time-lapse sampling was carried out with an Aquamonitor (Envirotech) to trace the surface dynamics of phytoplankton in Lough Graney (Co. Clare) in late August 2010. Results showed a change in the phytoplankton community concomitant to variation in water temperature. The use of passive adsorption devices for sequestering natural toxins also showed the presence of microcystin-like activity in the corresponding extracts.

Overall, the regional assessment of cyanobacterial diversity shows great potential for incorporation into a battery of methods for the management of aquatic resources. In the future, the inclusion of molecular analysis to microbial monitoring programmes will facilitate the evaluation of the efficiency of the environmental policies introduced to improve water quality.



# 1 Introduction

Occurrences of harmful algal blooms (HABs) have increased since the 1970s in marine and freshwater environments worldwide (Hallegraeff 2004). In continental waters, eutrophication has usually been associated with the development of high biomass cyanobacterial blooms. Those dominated by toxic species have caused a variety of ecological disruptions and present serious threats towards animal and human health (Mur *et al.* 1999). Poisoning incidents of livestock, wildlife, domestic animals and humans have been documented after exposure to or consumption of water contaminated by toxins produced by cyanobacteria species – compounds also referred to as ‘cyanotoxins’. The range of symptoms in humans includes gastroenteritis, fevers, vomiting, rashes, liver and kidney damage, respiratory irritation but also primary liver cancer and death (Codd *et al.* 2005). Intoxications usually derive from consumption of contaminated drinking water or from contact during recreational activities. Cyanotoxins show a wide variety of chemical forms and have been grouped into several classes according to their structure and biological activity. The most commonly encountered cyanobacterial toxins are the hepatotoxic microcystins, which are produced by several genera, including, non-exhaustively, planktonic forms of *Anabaena*, *Microcystis*, *Oscillatoria*, *Nostoc* or *Anabaenopsis*. Structural variability among toxin variants for each class confer cyanotoxins with a wide range of toxicity, the most acute forms constituting potential problems for water body and health managers worldwide.

Cyanobacteria are ubiquitous components of phytoplankton assemblages in aquatic environments. They exhibit a variety of sizes and shapes and have traditionally been classified into five groups according to morphological criteria (Mur *et al.* 1999). The introduction and application of molecular biology methodologies have affected the perception of relationships amongst cyanobacterial taxonomic groups. Phylogenetic studies based on rDNA analysis have permitted assessing the evolution of different morphotypes and determining their lineages, showing that both polyphyletic and monophyletic groups,

interspersed in various clades, existed among cyanobacteria (Moore *et al.* 1998, Wilmotte & Herdman 2001, Abed *et al.* 2002). The genetic characterisation of cultured cyanobacteria strains has also provided the basis for designing taxa-specific molecular markers for the discrimination of morphologically similar species in both culture and environmental samples (Zehr *et al.* 1997, Rudi *et al.* 1998, Beard *et al.* 1999, Itean *et al.* 2000, Tillet *et al.* 2001, Zeidner *et al.* 2003).

The dynamics of natural cyanobacteria populations have been studied extensively in many countries due to the potential noxious effects they can elicit. However, only few studies have investigated their spatial and temporal distributions at mesoscale levels, over extensive geographic areas including several water bodies. Typical limitations to such studies include sampling, methodological, time and budgetary constraints. However, the application of regional approaches for lake water quality assessment, monitoring and management is increasing. Regionalisation frameworks applied to sampling surveys can combine geographical, geological and hydromorphological data to define contiguous spatial regions that share similar features, the underlying assumption being that lake characteristics within regions are more similar than those of waterbodies across regions (Gerritsen *et al.* 2000). Advances in the field of geographic information systems (GIS) have permitted water bodies to be placed in better context with their geomorphologic surroundings and to include changes in land cover and usage, which can impact upon water quality (Aspinal & Pearson 2000). Risk assessments based on such approaches and new technologies can enable regulatory and managing bodies to take informed decisions for the development of guidelines for watershed management. The design and application of sampling plans have seldom been considered for aquatic microbial ecology studies. This limitation in mind, Catherine *et al.* (2008) applied a sampling strategy based on regional attributes to study the distribution of cyanobacteria in lakes within a geographic area subjected to various land use around Paris, France.

The development of risk management strategies and the definition of tolerable levels of cyanobacterial toxins in water have been addressed in recent years in several countries (CYANONET 2007). Guideline values of cyanotoxin concentration and cyanobacterial biomass have also been decided upon (World Health Organisation [WHO] 2003). Developing prediction and mitigation capacities requires a sound understanding of the dynamics of toxinogenic cyanobacterial populations, in particular in identifying the environmental cues that affect their distribution, abundance and toxicity both in space and time. Assessing the impact of anthropogenic pressures on aquatic environments is part of policies that are enforced in many countries. In Europe, the implementation of the European Water Framework Directive (WFD) by member states partly aims at preventing the eutrophication of water bodies and achieving good water quality status (EC Parliament and Council 2000). Excluding some regions subject to intensive agriculture, the general quality status of lakes and rivers is satisfactory in Ireland (Environmental Protection Agency [EPA] Ireland 2009). Water quality in lakes is of great importance from a human health aspect but also in terms of region attraction and commercial activities. However, mortalities of wild and domestic animals have on occasions been associated with blooms of toxic cyanobacteria in several Irish lakes (James *et al.* 1997). Investigations on harmful cyanobacteria previously focused on relating the presence of cyanobacterial biomass with lake trophic state or detecting cyanotoxins in selected water bodies (Reynolds & Petersen 2000, Furey *et al.* 2003, Allis *et al.* 2007). Still, little is known about the distribution, diversity and toxigenic potential of planktonic cyanobacteria at a regional scale encompassing multiple river catchments and water bodies. Likewise, the understanding of the summer dynamics of cyanobacterial communities in specific lakes, and of the toxins they synthesise, is largely limited and fragmentary.

## **1.1 Objectives**

European member states are compelled to address the various elements comprised in the WFD. With respect to water quality, phytoplankton is one of the recognised elements used to assess the ecological state of water bodies (European Communities 2000). Planktonic cyanobacteria can dominate phytoplankton assemblages during the summer months and form

high-biomass blooms in eutrophied water bodies, with the associated risk of production of a range of biotoxins at levels unsafe for wildlife and human health.

In this context, the specific objectives of this study were to investigate (1) the distribution, genetic diversity and abundance of planktonic cyanobacteria in representative inland water bodies from the west and north-west of Ireland, and (2) the summer dynamics in the composition and toxinogenicity of planktonic cyanobacterial assemblages in a selection of water bodies. Of particular interest was the attempt to characterise potential trends between land cover typology and the capacity for microalgal biomass development, and to identify lakes at risk of harbouring future blooms of toxigenic cyanobacteria. To this end, given the range of anthropogenic pressures the study area is subjected to and the high number of water bodies within, a regionalisation sampling framework was applied, based on a stratified sampling strategy of water bodies designed following GIS analysis of river catchment attributes. The detection, distribution and diversity of planktonic cyanobacteria in selected water bodies sampled were then assessed using a range of molecular biology and microscopy methods. This project was undertaken with the anticipation that the generated results could contribute in assisting monitoring authorities with the elaboration of future sampling and risk assessment schemes in support of the management strategy of aquatic resources in regions of Ireland.

## **1.2 Classification, Morphology and Ultrastructure of Cyanobacteria**

Cyanobacteria, often referred to as blue-green algae, are one of the largest and most important lineages of bacteria. They constitute a group of aquatic and photosynthetic microorganisms that have colonised many habitats (Mur *et al.* 1999). Their roles in shaping an oxygenated atmosphere through photosynthesis and in the evolution of many eukaryotic organisms have been essential. With about 2,000 species and 150 genera, cyanobacteria are recognised in both the International Code of Botanical Nomenclature and the International Code of Nomenclature of Bacteria (Mur *et al.* 1999). The botanical approach relies mainly on the examination of morphological and physiological criteria such as pigment composition, cell wall structure and the presence of

differentiated cells. The bacteriological classification is based on biochemical and genetic analyses of cultured reference strains. Some differences exist between the two systems but they remain generally congruent for hierarchical taxa above genera.

Analysis of metabolic markers and ribosomal gene sequences suggests that cyanobacteria form a genetically homogeneous group (monophyletic) within the Eubacteria (Wilmotte 2001). They have traditionally been classified according to morphological and reproductive criteria into five orders referred to as (1) Chroococcales, (2) Pleurocapsales, (3) Oscillatoriales, (4) Nostocales and (5) Stigonematales. Chroococcales are unicellular and usually aggregate in colonies whereas Pleurocapsales have the ability to form multiple internal spores called baeocytes. The three other groups comprise filamentous species; the Oscillatoriales form chains of cells that do not differentiate into specialised cell types whereas Nostocales and Stigonematales do so. Some single-celled cyanobacterial morphotypes show spherical, ovoid or cylindrical cellular shapes and reproduce by binary fission. Multicellular structures organised in straight or coiled filaments are called trichomes, which may vary in size and shape. Species in the Oscillatoriales are composed of identical cells whereas species from the Nostocales and Stigonematales, which may show branched or multiseriated trichomes, are differentiated into several cell types. In particular, photosynthetic cells are distinguished from akinetes, climate-resistant spores that may form during adverse environmental conditions, and heterocysts, thick-walled cells containing the enzyme nitrogenase that enables the fixation of atmospheric dinitrogen.

Cyanobacteria possess a Gram-negative type cell envelope composed of a plasmic membrane, a peptidoglycan and an outer membrane. They lack a nuclear membrane, membrane-bound organelles and the histone proteins associated with eukaryotic chromosomes. They are equipped with an organised system of internal membranes folded into several lamellae referred to as thylakoids, which contain the chlorophyll-a and accessory pigments involved in photosynthesis. The thylakoid membranes are studded with phycobilisomes, multimolecular complexes of hemidisoidal shape composed of pigments (phycobiliproteins) that function as

harvesting antennae for the photosystems (Douglas 1994). The relative composition and abundance of those hydrosoluble pigments (mostly phycoerythrin, phycocyanin, allophycocyanin) are responsible for the specific colouration of cyanobacteria. The cytoplasm of cyanobacteria is filled with various inclusions, which act as reserve products. Those include granules of lipid, glycogen or polyphosphates, or inclusions with specialised functions such as carboxysomes, which contain the rubisco enzymatic complex (Fay & Van Baalen 1987). Several planktonic species are also equipped with gas vesicles that permit buoyancy regulation and enable the adjustment of vertical position in the water column (Walsby 1987).

### 1.3 Characteristics of Cyanotoxins and Methods of Analysis

Several cyanobacteria are able to produce a suite of harmful compounds referred to as cyanotoxins, which are grouped into several categories according to their biological activity (hepatotoxic, neurotoxic, dermatotoxic, cytotoxic) or chemical structure (peptide, alkaloid, lipopolysaccharide).

#### 1.3.1 Hepatotoxic Cyclic Peptides – Microcystins and Nodularins

The most commonly encountered cyanobacterial toxins in aquatic environments are heptacyclic peptides called microcystins. They are hepatotoxins produced by several cyanobacteria species, in particular *Microcystis* spp., from which the toxin name derives. About 60 structural variants of microcystins have been characterised from environmental samples and isolated cyanobacterial strains. The pentacyclic nodularins have a chemical structure similar to that of microcystins as both types of toxins share the uncommon amino acid Adda((2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid). This amino acid is responsible for the characteristic wavelength absorbance of both microcystins and nodularins (238 nm), which enables their detection and quantification after separation by reverse phase HPLC (Harada *et al.* 1999). Microcystins have at least been characterised from planktonic *Anabaena*, *Microcystis*, *Oscillatoria* (*Planktothrix*), *Nostoc*, and *Anabaenopsis* species while nodularins have been found from the brackish water cyanobacterium *Nodularia spumigena* and the

marine sponge *Theonella* (Sivonen *et al.* 1992, Vezie *et al.* 1998, Marsalek *et al.* 2000).

The relative toxicity of structural variants of microcystin and nodularin can differ greatly and is mediated by binding to protein phosphatase enzymes (Runnegar *et al.* 1995). Those hepatotoxins can cause death in mice by liver haemorrhage within a few hours after intra-peritoneal (i.p.) injection, with toxicities ranging from 50 to 300  $\mu\text{g.kg}^{-1}$  (body weight). Structural modifications to the Adda and glutamate moieties usually reduce the toxicity of microcystins and nodularins (Rinehart *et al.* 1988, Harada *et al.* 1991). Only a few non-toxic variants have been identified.

### 1.3.2 Neurotoxic Alkaloids – Saxitoxins and Anatoxins

Mass occurrences of neurotoxic cyanobacteria have caused wild and domestic animal poisonings in North America, Europe and Australia. Two families of cyanobacterial neurotoxins can be distinguished: saxitoxin derivatives and anatoxins. Saxitoxins (STX) are highly toxic molecules with lethal doses 50 ( $\text{LD}_{50}$ ) as low as 10  $\mu\text{g.kg}^{-1}$  (i.p.) in mice whereas the toxicity of anatoxins varies between 20 and 250  $\mu\text{g.kg}^{-1}$  (Oshima 1995). Both groups are alkaloid molecules that contain at least a nitrogen atom combined in a heterocycle. They are polar and soluble in water, with a molecular weight generally inferior to 1000 Da. Saxitoxins are carbamates categorised according to their chemical structure: non-sulfated (STX), mono-sulfated (GTXs) and bi-sulfated (C-toxins) forms (Shimizu 2000). Saxitoxins (~20 forms) elicit their toxicity by inhibition of voltage-dependent sodium channels in neuromuscular synapses, leading to death by respiratory failure in most severe cases. Toxin profiles can vary greatly according to the producing organism and its geographic origin (Lagos *et al.* 1999, Pomati *et al.* 2000, Sivonen & Jones 1999).

Anatoxin-a and homoanatoxin-a are low molecular weight secondary amines (MW 156 and 179, respectively) produced by a number of cyanobacterial genera including *Anabaena*, *Aphanizomenon*, *Oscillatoria*, *Phormidium* and *Cylindrospermum* (Carmichael *et al.* 1975, Bumke-Vogt *et al.* 1999). They are potent neurotoxins that block neuromuscular depolarisation by binding to post-synaptic acetylcholine receptors (Swanson *et al.* 1991). Anatoxin-a(S) is a structurally

different phosphate ester of a cyclic N-hydroxyguanine (MW 252) synthesised by some *Anabaena* spp. (Onodera *et al.* 1997b). It is a very potent inhibitor of acetylcholinesterase acting similarly to the organophosphate pesticides malathion and parathion.

### 1.3.3 Cytotoxic and Dermatotoxic Alkaloids

The hepatotoxic cylindrospermopsin is a cyclic guanidine alkaloid (MW 415) produced by *Cylindrospermopsis raciborskii*, *Umezakia natans* and *Aphanizomenon ovalisporum* (Harada *et al.* 1994, Banker *et al.* 1997, Hawkins *et al.* 1997). Pure cylindrospermopsin is a potent inhibitor of protein synthesis with an  $\text{LD}_{50}$  in mice (i.p.) of 2.1  $\text{mg.kg}^{-1}$  after 24 h (Ohtani *et al.* 1992). Like microcystins, it primarily affects the liver, although provokes in mice considerable damage to kidneys, spleen, thymus and heart. It also exhibits carcinogenic activity by causing aneugenic and clastogenic effects, which initiate dysfunctions in chromosome migration (Humpage *et al.* 2005).

Benthic marine cyanobacteria from the genera *Lyngbya*, *Oscillatoria* and *Schizothrix* produce inflammatory alkaloid toxins causing severe dermatitis and/or severe oral and gastrointestinal inflammation among swimmers in contact with the filaments. The molecules involved, known as lyngbyatoxin-a, aplysiatoxins and debromoaplysiatoxin, are potent tumour promoters and protein kinase C activators (Mynderse *et al.* 1977, Fujiki *et al.* 1990).

### 1.3.4 Irritant Toxins and other Bioactive Compounds

The lipopolysaccharide (LPS) component of the outer cell wall of Gram-negative bacteria, including cyanobacteria, can trigger irritant and allergenic responses in humans. Lipopolysaccharides are conjugated products of sugars and lipids. Further evaluation of the chemical structures and health risks of cyanobacterial LPS are needed as the few results available indicate that cyanobacterial LPS is less toxic than that of other bacteria, such as *Salmonella* (Keleti & Sykora 1982, Raziuddin *et al.* 1983). The endotoxin component of the LPS of the cyanobacterial cell wall may still contribute to human health problems associated with exposure to mass occurrences of cyanobacteria.

Beta-methylamino alanine (BMAA) is a recently discovered cyanotoxin, but it is uncertain whether or

not cyanobacteria are the only source (Cox *et al.* 2005). BMAA was identified in 1967 from extracts of cycads seeds in Guam and described as a possible cause of the neurodegenerative disorders occurring on the island (Vega & Bell 1967). It is a potent glutaminergic agonist causing excitotoxicity at high concentrations (Weiss *et al.* 1989). Potential risks for the provision of safe drinking water have been identified since it has been demonstrated that many cyanobacteria could produce BMAA.

### 1.3.5 Detection and Quantification of Cyanotoxins

#### *Biological methods*

The mouse bioassay has long been the reference method for screening blooms of microalgae and cultured strains. Its use has been contested in recent years for ethical reasons and low sensitivity (Fernandez *et al.* 2003). Various invertebrate and vertebrate models have alternatively been used to assess the presence of biotoxins in samples, including copepods, prawns, locusts, flies, batracians or fish, after injection, ingestion or cutaneous application (McElhiney *et al.* 1998, Wiegand *et al.* 1999, Smith 1996, Saker & Eaglesham 1999). The response of biological tests is mostly qualitative, and integrates the toxic effects of a suite of molecules, including possible synergistic or antagonistic effects. As a result, the interpretation of results must be cautious and a battery of tests may be used to orientate diagnostics. Assays on cultured mammal cell lines are based on the mode of action of toxins. For example, the cytotoxic effects of cylindrospermopsin and microcystins have been characterised using rat primary hepatocytes, eliciting lethal effect and a significant reduction of protein synthesis (Chong *et al.* 2002, Gácsi *et al.* 2009). An assay using rat brain synaptosomes also exists for the detection and semi-quantification of neurotoxins such as STX that act on voltage-gated sodium channels (Dechraoui *et al.* 1999).

#### *Biochemical methods*

Once biological or cytotoxic effects have been observed using bioassays, identification and quantification of the responsible compounds are required. Sensitive biochemical and immunological methods based on the chemical structures and modes of action of toxins are available. Commercially available Enzyme Linked Immuno-Sorben Assay (ELISA) kits for microcystins using antibodies targeting the Adda moiety have shown

good repeatability and reproducibility with a detection limit as low as 0.05 µg.l<sup>-1</sup> (Rivasseau *et al.* 1999a). Such assays exist for STX but the variability in their chemical structures has caused difficulties in selecting antibodies with a suitable recognition spectrum (Robillot & Hennion 2001).

Enzyme-inhibition based methods have also been developed as screening tests to evaluate the toxic potential of samples through the mode of action of toxins. The enzyme activity is revealed by colourimetric reaction linked to the transformation of a substrate into a coloured product. For example, microcystins are detected using the protein phosphatase inhibition assay, whereby the global inhibition reflects the action of all possible variants present in the sample. It has shown a detection limit of less than 0.1 µg.l<sup>-1</sup> (Rivasseau *et al.* 1999b). The inhibitory activity of anatoxin-a(S) can also be characterised using the acetylcholinesterase enzyme, but the absence of commercial standards prevents its quantification.

#### *Physico-chemical methods*

A range of analytical liquid chromatography methods with a variety of stationary and mobile phases is available for the detection, identification and quantification of cyanotoxins. High performance liquid chromatography (HPLC) methods coupled to UV or fluorescence detection are generally used. For example, microcystins are often separated by reverse-phase polarity HPLC and detected using a UV detector with diode (DAD) that allows the visualisation of the characteristic maximum absorbance at 238 nm of microcystins (Lawton *et al.* 1994). Sometimes pre- or post-column derivatisation, in particular for STX, is required for fluorescence detection (Oshima 1995, Lawrence *et al.* 2004). Mass spectrometry has become popular in recent times due to the possibility of identifying multiple toxin variants in environmental samples. However, the lack of toxin standards and the need for toxin pre-concentration constitute limits to the precise characterisation of compounds in natural samples (Lawton & Edwards 2008).

## 1.4 Molecular Characterisation of Harmful Cyanobacteria

The significant advances in molecular ecology in the last decades have affected the classification of living

organisms profoundly. Woese and Fox (1977) pioneered the exploitation of the information retained in the universally shared ribosomal RNA (rRNA) for inferring the phylogenies of all life forms. Molecular techniques have improved the acquisition of DNA sequence information, mainly thanks to the introduction of the polymerase chain reaction (PCR) for the amplification of gene fragments (Saiki *et al.* 1988). The additional use of gene probe technology has been essential to facilitate the detection, identification and enumeration of species in complex plankton assemblages.

#### **1.4.1 Inferring the Phylogeny of Cyanobacteria using Molecular Markers**

Analysis of rDNA genes has become very popular in ecological genetics for understanding the evolutionary history of organisms owing to the high degree of functional and structural consistency of rRNA transcripts. Some regions are universally conserved while other domains are highly variable, providing adequate resolution at a variety of taxonomic levels for determining evolutionary relationships.

Studies on rDNA genes have permitted estimating the evolution of different cyanobacterial morphotypes and the determination of their lineages (Moore *et al.* 1998, Abed *et al.* 2002). Phylogenetic studies have hence shown that both polyphyletic and monophyletic groups, interspersed in various clades, existed among cyanobacteria, contradicting in part the previous classifications based on the examination of morphological criteria alone (Wilmotte & Herdman 2001, Litvaitis 2002).

Assessing the diversity and relationships among taxa at the genus and species levels has required using more variable markers since the small sub-unit (SSU) rDNA gene did not offer sufficient resolution. Additional markers have included the interspacer rDNA regions but also coding and non-coding sequences of genes such as the nitrogenase (*nifH*), the units of the rubisco operon (*rbcL/X*), the spacer located between the A and B genes of the photosystem-a (*psaA/B*) and that of phycocyanin (PC-IGS), or gas vesicle proteins (*gvpA/C*) (Zehr *et al.* 1997, Rudi *et al.* 1998, Beard *et al.* 1999, Iteanu *et al.* 2000, Tillet *et al.* 2001, Zeidner *et al.* 2003). In recent times, attention was placed upon the characterisation of the genes involved in toxin synthesis. Significant advances have been made for the peptidic toxin microcystin whose gene is organised into an

~75 kb operon (Tillet *et al.* 2000, Kurmayer *et al.* 2002). The presence or absence of the microcystin genes in cultured strains of various cyanobacterial genera has permitted discriminating between potentially toxic and non-toxic stains (Kurmayer *et al.* 2004).

#### **1.4.2 Assessing the Molecular Diversity of Cyanobacterial Communities**

Molecular techniques based on gene variability among taxa have been used to assess the composition of cyanobacterial assemblages in various environments. Those techniques, which rely on PCR to specifically amplify genes commonly shared by the target taxa, are known as fingerprinting methods, and have been used extensively to assess species succession in microbial populations.

A widely used fingerprinting method for monitoring microbial communities is restriction fragment length polymorphism (RFLP) analysis. For instance, the genetic diversity of toxic cyanobacteria from various origins has been assessed using RFLP and the phycocyanin locus marker (Neilan *et al.* 1995). The method requires PCR amplification followed by digestion of the amplicon by restriction enzymes. Diversity estimation is then carried out after electrophoretic separation and examination of the resulting band migration pattern, which is used for the constitution of operational taxonomic units.

A more sophisticated high resolution fingerprinting technique that has also been applied to various environments is denaturant gradient gel electrophoresis (DGGE) analysis of PCR-amplified genes. In particular, the method has proved successful for analysing cyanobacterial communities in soil crusts, alpine rocks, paddy fields or estuaries using cyanobacteria-specific 16S rDNA-targeting PCR primers (Nübel *et al.* 1997, Garcia-Pichel *et al.* 2001, Sigler *et al.* 2003, Gei *et al.* 2004, Song *et al.* 2005). PCR amplification of the target gene generates a mixture of alleles of near-equal length but heterogeneous sequences, which are individually resolved during electrophoresis using a polyacrylamide gel containing a linear denaturant gradient of urea and formamide. The separation is based on the nucleotide composition of the alleles, which cease their migration at different positions along the gradient according to their specific melting characteristics. Analysis of the fingerprints using statistical methods such as non-metric multidimensional scaling or similarity analysis followed by appropriate



clustering methods can provide an estimate of the diversity and reveal community structures (Casamayor *et al.* 2002, Rothrock & Garcia-Pichel 2005).

#### 1.4.3 *Molecular Detection and Quantification of Species and Toxin Genes*

The monitoring of toxic species mostly relies on the detection and quantification of target species in complex phytoplankton assemblages. Traditional enumeration methods by light microscopy have proved time consuming and require high levels of taxonomic expertise. Moreover, the discrimination between toxic and non-toxic forms is sometimes not possible using morphological traits. A suite of methods based on molecular probes has hence been adapted to facilitate the differentiation between morphologically similar species and to provide an indication about their toxic potential. Molecular probes typically include a variety of compounds (lectins, antibodies, nucleic acids) that selectively bind to specific target receptors usually found within or on the surface of targeted taxa, enabling their selective detection and quantification (Scholin *et al.* 2003). The detection and identification of cyanobacteria with gene probes involve the use of specific oligonucleotide sequences conjugated to fluorescent labels. Such probes are originally designed by selecting DNA sequence fragments that are unique to the target taxa. Three major types of molecular biology applications have been adapted for the detection and quantification of cyanobacteria and cyanotoxin genes using nucleic acid probes: (1) fluorescent *in situ* hybridisation (FISH), (2) PCR and quantitative real-time PCR, and (3) microarray chip technology (Schonhuber *et al.* 1999, Castiglioni *et al.* 2004, Briand *et al.* 2008).

A current trend is to target the genes involved in toxin synthesis directly. Because of their peptidic nature, microcystins are the most investigated cyanotoxins: this simplifies the study of their genomics as their gene organisation is structured into a single operon. The genes encoding for the microcystin synthetase have been identified and characterised for several cyanobacteria species (Neilan *et al.* 1999, Christiansen *et al.* 2003, Rouhiainen *et al.* 2004). Specific genetic primers have been developed for the amplification of different domains, such as the *mcy*-A and -B regions, and been validated using both culture and field samples (Pan *et al.* 2002, Hisbergues *et al.* 2003).

## 1.5 **Dynamics of Cyanobacterial Populations and Cyanotoxins**

Cyanobacterial growth to noxious levels can occur very quickly under favourable environmental conditions. Variations in the composition of phytoplankton communities result from physico-chemical interactions and from the biological characteristics and responses of individual species. Population dynamics of phytoplankton are therefore very complex and can be driven by mixing processes, light regimes, nutrient limitation or competition and predation.

### 1.5.1 *Physical Control*

Physical mixing processes can influence the dynamics of phytoplankton populations. Vertical mixing, in particular, is a major determinant of the development of surface blooms of cyanobacteria (Hutchinson 1967, Spigel & Imberger 1987). In weak mixing conditions, the vertical positions of species are governed by their growth characteristics, sinking and floating velocity, and random motion caused by turbulent diffusion. Changes in turbulent mixing can hence affect the competitive balance between buoyant cyanobacteria and other sinking phytoplankton. For example, shift of dominance from diatoms during high regimes of vertical mixing to buoyant cyanobacteria during weak vertical mixing periods have been observed in many aquatic environments (Harris & Baxter 1996, Bormans & Condie 1998, Sherman *et al.* 1998).

Cyanobacteria require access to light as a resource for their photosynthesis activity. Since most species tend to sink in the water column, where the amount of penetrating light decreases exponentially with depth, there is a species-specific critical light intensity below which populations will cease to grow. Models predict that species with the lowest critical light intensity (with low light level requirement) are better competitors for light in well-mixed waters (Huisman & Weissing 1994). Continuous culture experiments carried out using a number of phytoplankton species have validated such predictions (Huisman *et al.* 1999).

### 1.5.2 *Chemical Control*

The potential role of nutrient limitation on the growth of cyanobacterial populations has been long recognised. Competition for limiting nutrients has a major impact on the composition of phytoplankton assemblages, as

species that compete for the same resource reduce its availability for both themselves and other competing species. Since the beginning of eutrophication management in the 1970s, the idea that phytoplankton biomass production is determined by the availability of phosphorus in lakes is commonly shared worldwide (Shapiro 1988, Correll 1999). Investigations on the competitive abilities of cyanobacteria for sequestering phosphorus have shown that they are relatively poor competitors compared to diatoms, prochlorophytes and green algae – confirming the many field observations that indicate that cyanobacteria rarely dominate in lakes where phosphorus is a major limiting factor (Tilman *et al.* 1986, Fujimoto *et al.* 1997, Downing *et al.* 2001). Nitrogen is an essential element that can also limit phytoplankton growth. Several cyanobacterial species are able to fix nitrogenous gas, which confer them with advantages when dissolved nitrate and ammonium concentrations are low. Phytoplankton community composition also depends on the ratio of nitrogen and phosphorus supplies. Smith (1983) compiled data from 17 lakes and observed that cyanobacteria were rare for N:P ratios greater than 29 and could become dominant at lower values. These findings were of major practical importance from a management perspective, because N:P ratios can be manipulated in many lakes, but a potential flaw resides in the underlying assumption that nitrogen and phosphorus are the only limiting factors.

### **1.5.3 Biological Control**

Cyanobacteria may achieve dominance in aquatic environments owing to superior competitive abilities for accessing resources such as light, but also by releasing inhibiting compounds against their competitors (Gross 2003). The cyanobacterium *Microcystis*, for example, is able to inhibit carbon fixation of competing phytoplankton species (Sukenik *et al.* 2002). Such interactions are known as allelopathic interactions. Theoretical competition models have shown that the outcome of allopathy between ‘toxic’ and ‘sensitive’ species largely depends on mixing intensity (Kerr *et al.* 2002, Hulot & Huisman 2004).

Like many phytoplankters, cyanobacteria constitute a potential alimentary resource for grazers and filter-feeding organisms. However, a number of structural and chemical properties confer them with a relatively low edibility. The size and morphology of cyanobacteria generally protect them from grazing pressure as they

reach the upper size limit of food particles that can be handled by zooplankton (DeMott *et al.* 2001, Gliwicz 2003). The nutritional quality of cyanobacteria is also relatively low due to their limited levels of poly-unsaturated fatty acids (Muller-Navarra *et al.* 2004). Finally, some cyanobacteria species can utilise a chemical arsenal targeted towards potential predators. Laboratory experiments have demonstrated that cyanobacterial compounds could inhibit various zooplankton taxa, including cladocerans and copepods (DeMott 1999, Reinikainen *et al.* 2002).

### **1.5.4 Cyanotoxin Dynamics in the Environment: Example of Microcystins**

The most encountered cyanotoxins in freshwater ecosystems are the hepatotoxic microcystins, which are mostly synthesised by species of the widely distributed genera *Microcystis*, *Anabaena* and *Planktothrix*. Monitoring in various lakes has revealed a high degree of spatial and temporal variability in microcystin concentrations. This may be attributed to a variation of cyanobacterial biomass, different physiological responses to environmental variations and changes in cyanobacterial species/genotype composition (Kardinaal & Visser 2005). The factors that affect cyanobacterial growth and dominance in lakes are multiple and non-exhaustively include temperature, irradiance, water-column stability, grazing pressure, pH and N:P ratios (Dokulil & Teubner 2000). Cyanobacteria concentrations usually increase during the summer months and can often be sustained until early autumn. As a result, microcystin concentrations in lakes can be related to changes in cyanobacterial biomass, whereby higher concentrations of microcystin-producing cyanobacteria will typically yield higher amounts of microcystins. Numerous studies have demonstrated that microcystin dynamics correlate well with many of the environmental factors that influence cyanobacterial growth, in particular light and nutrients (Kardinaal & Visser 2005).

The variability in microcystin concentration observed in lakes can greatly exceed the two- to three-fold variability which is typically obtained among strains maintained in the laboratory (Fromme *et al.* 2000, Ame *et al.* 2003). It has been suggested that changes in species and genotype composition in cyanobacterial communities may explain the dynamics of toxin concentrations in lakes (Kardinaal & Visser 2005). There is a growing

body of evidence indicating that microcystin dynamics in freshwater lakes may result from successive replacements of closely related genotypes that differ in toxin production (Briand *et al.* 2002, Carillo *et al.* 2003, Welker *et al.* 2003). Field studies carried out in 31 globally distributed lakes showed for *Microcystis* sp. colonies that their microcystin content declined as the population increased. It was suggested that the seasonal succession might commence with toxic genotypes that are gradually displaced by genotypes of lesser toxicity during the course of the summer (Kardinaal & Visser 2005).

## 1.6 Emerging Technologies for Automated Sampling and Analysis of Phytoplankton

Research efforts toward the development of new tools and methodologies for the rapid detection, identification and quantification of both phytoplankton and biotoxins have been attempted in recent years to improve monitoring capacity in aquatic environments. The maximum cell concentrations of many microalgal species are often encountered in subsurface thin layers that are usually associated with water column stratification (Gentien *et al.* 2005). Recent advances in *in situ* instrumentation technologies for studying phytoplankton dynamics at spatio-temporal scales of high resolution have enabled the observation of phytoplankton thin layer structures in the water column (Babin *et al.* 2005). Thin layer formation is believed to derive from the interaction of vertical shear created by near-inertial waves with horizontal patchiness of phytoplankton. Those thin layers may only measure several decimetres in vertical thickness, yet they can accumulate high cell densities and locally alter the physico-chemical environment (Donaghay & Osborn 1997). The growing need for *in situ* analysis has seen the emergence of several submersible

instruments. The Ocean Response Coastal Analysis System (ORCAS) is an autonomous bottom-up profiler used to collect time series of vertical profiles and specifically designed to resolve thin layer structures of phytoplankton (Sullivan *et al.* 2010). Of particular promise, the FlowCytobot has been successfully deployed at coastal-cabled observatory sites on the United States east coast (Olson *et al.* 2003). The instrument is an automated underwater flow cytometre that measures the presence of particular microalgal taxa by recognising the specific pigment signature of some algal groups. The environmental sample processor (ESP) is also a promising fully independent instrument that samples the water column and measures in a near real-time fashion the levels of a selected number of HAB species and their biotoxins using immunoassay- and hybridisation-based techniques (Doucette *et al.* 2009).

From a laboratory-based aspect, methods enabling a reliable and rapid high-throughput analysis of samples have been developed to support the time-consuming and expertise-demanding monitoring of phytoplankton levels in aquatic systems. In particular, the FlowCAM instrument is an imaging flow cytometer combined with conventional microscopy attributes, which has been designed to characterise and analyse microparticles in a fluid stream (Alvarez *et al.* 2011). FlowCAM can measure light scattering and fluorescence in individual phytoplankton cells larger than 5 µm in size. The instrument automatically recognises, images and enumerates cells in a continuous flow, providing over 20 measurements for each individual particle. One of the main advantages of FlowCAM against traditional microscopic methods is the rapid establishment of digitally and automatically catalogued records of the multiple phytoplankton taxa present in samples, which may degrade over time and often require rapid analysis following water sample collection and preservation.

## 2 Methodology

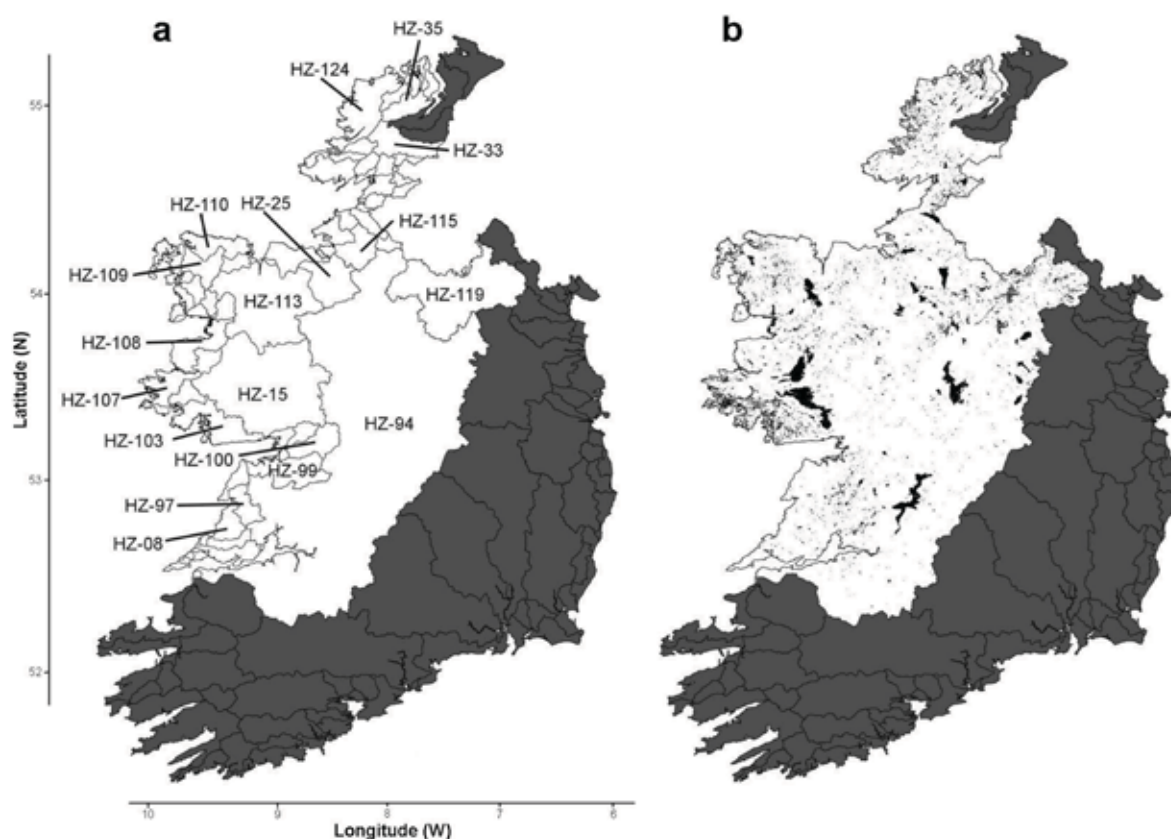
### 2.1 Sampling Strategy Design for the 2009 Summer Survey

Database management tools in ArcGIS were used to design a sampling strategy of water bodies based on attributes of river catchments from the west and northwest of Ireland.

#### 2.1.1 Study Area, Target Population and Sample Size

Sampling was carried out over a period of 15 weeks during the summer of 2009 (26 May to 3 September) extending in latitude from 52°20' south to 55°10' north, with a surface area of ~40,000 km<sup>2</sup> and a population of ~1,230,000 (Fig. 2.1). The study area crossed over three river basin districts (North-Western, Western and Shannon) and was constituted of soils predominantly composed of carboniferous limestone and blanket peats in the central region and the western-northern areas,

respectively. Modifications in land cover in the region for the period 2000–2006 have mostly been restricted to changes to transitional woodland scrubs, coniferous forests and some degree of discontinuous urban fabric (EPA Ireland, 2008a). Interrogation of a GIS database maintained by the EPA showed the study area to contain 9,019 water bodies, accounting for ~75% of the national amount. Some lakes within the study area are subject to a range of human uses, in particular water abstraction and recreational activities. Two major lakes, Lough Corrib and Lough Ree, have been attributed the status of Nutrient Sensitive waters under the WFD and are listed in Ireland's Urban Waste Water Treatment Regulations (S.I. 254/2001). Only water bodies greater than 5 ha surface area were considered for sampling, which amounted to a total of 1,230 lakes. The sampling effort was set to 50 lakes (~4% of population) across an area containing 59 river catchments.



**Figure 2.1.** Maps of Ireland showing the delineation of the river catchments (a) and the water bodies contained within the study area (b).

### 2.1.2 Index Definition and Typology of the River Catchments in the Study Area

A stratified semi-random sampling design based on an analysis of river catchment attributes was used for the selection of water bodies to sample. The typology of the catchments was described based on topographical and environmental parameters following ArcGIS analysis of data sets obtained from the EPA. Catchments within the study area that did not contain water bodies of superficies greater than 5 ha were excluded from the analysis (catchments 24, 93, 123). River catchment attributes were described through seven indices based on surface area, drainage, altitude and land cover type as detailed in Catherine *et al.* (2008) (Fig. 2.2). Definition of the indices is provided in Table 2.1, except for river quality. The composite river quality index was derived from the river ecological status data sets provided by the EPA and based on the Quality Rating System (Q-value) (EPA Ireland, 2009). River quality was selected here as a criterion for river catchment typology owing to the large amount of data available compared to that compiled for lakes. The frequency of high- to poor-quality attributes of the rivers contained within each

catchment were weighted and the river scores then combined for each catchment. Data was absent for four catchments with respect to the river quality index (27, 31, 102, and 114). All seven indices were attributed an identical weight, ranging from 0 to 1 to avoid the introduction of quantitative biases for the subsequent similarity analysis.

Similarity analysis based on the indices was applied to build clusters of catchments. This was carried out using Gower's method, which allows for the presence of missing data during the generation of the distance matrix (Legendre & Legendre, 1998). Similarity analysis and hierarchical clustering (Ward's method) were performed using the R package (Casgrain & Legendre, 2004). The sampling effort was set to 50 lakes, which were allocated to each cluster of catchments proportionally according to the total number of water bodies in each. The lakes sampled were mainly selected at random, but a small proportion (~15%) was also chosen according to previous knowledge about cyanobacteria bloom occurrences and usage for recreation or water abstraction (Table 2.2).

**Table 2.1. GIS-derived indices used to characterise the typology of the river catchments of the west and north-west of Ireland.**

Index	Abbreviation	Formulation
<i>Morphology</i>		
Relative surface index	$I_{rs}$	$(S_{catch}/\Sigma S_{catch})/[(S_{catch}/\Sigma S_{catch})_{max}]$
Relative altitude index	$I_{alt}$	$(Alt_{av})/[(Alt_{av})_{max}]$
<i>Land cover<sup>1</sup></i>		
Forest surface index	$LC_f$	$(S_f/S_{catch})/[(S_f/S_{catch})_{max}]$
Agriculture surface index	$LC_a$	$(S_{ua}/S_{catch})/[(S_a/S_{catch})_{max}]$
Wetlands and water bodies surface index	$LC_w$	$(S_w/S_{catch})/[(S_w/S_{catch})_{max}]$
<i>Hydrology and water quality</i>		
Drainage index	$H_{dr}$	$(L_{riv}/S_{catch})/[(L_{riv}/S_{catch})_{max}]$
Composite river quality index	$H_{riv}$	***

$S_{catch}$ : surface area of the river catchment;  $Alt_{av}$ : average altitude of the river catchment;  $S_f$ : forest cover surface (%);  $S_a$ : agricultural surface area (%);  $S_w$ : wetland and aquatic surface area (%);  $L_{riv}$ : length of river stream within a river catchment.

<sup>1</sup> Indices obtained through analysis of CORINE land cover Ireland 2000 data set. \*\*\* See text for details.



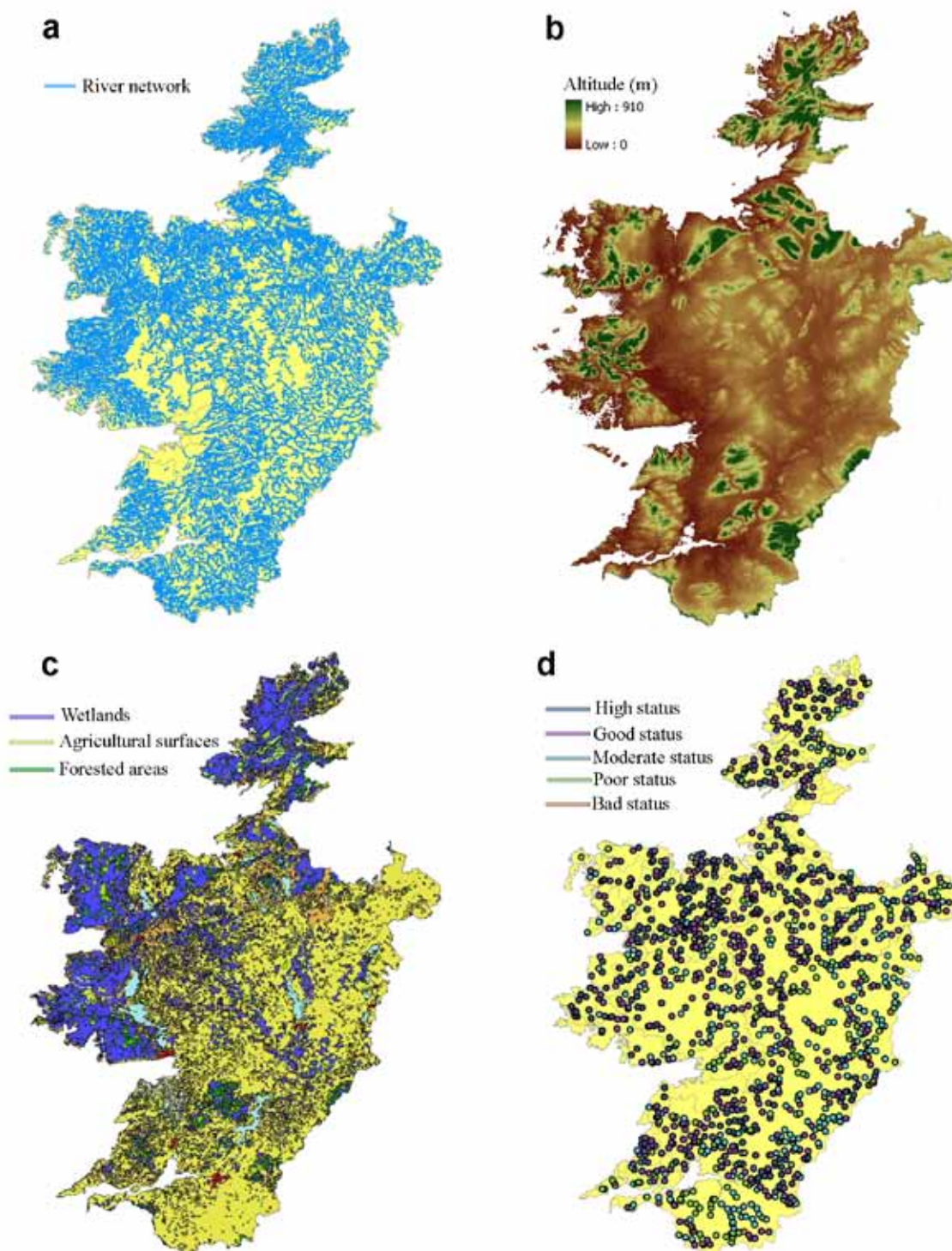


Figure 2.2. Distributions in the study area of the river network (a), land elevation (b), land cover type (c) and river quality (d). The data was extracted from GIS layers provided by the EPA and available online at: <http://maps.epa.ie/internetmapviewer>.

## 2.2 Field Sampling

### 2.2.1 Summer 2009 and 2010 Surveys

In 2009, one station was sampled in each water body, directly from the shore or within 300 m using a floating platform. An effort was also made to spatially segregate sampling occasions within the clusters so as to encompass potential seasonal succession changes (Fig. 2.3, Table 2.2). The station location was selected according to road access so as to limit the time requirement on the lake and minimise sampling costs. All samples were taken just beneath the water surface.

In 2010, sampling was carried out on a near weekly basis in Lough Corrib (Co. Galway) and Lough Ballyquirke (Co. Galway) where surface water samples were collected along a transect of stations (Fig. 2.4). Samples from Lough Graney (Co. Clare) were also periodically collected from the shoreline throughout the summer and using an autonomous water column sampler (Aquamonitor, EnviroTech) for 30 days during August–September (Fig. 2.4). The instrument collects independently up to 50 water samples according to a predefined sampling sequence through a syringe pumping mechanism and a 50-way multi-port valve. The instrument was programmed to collect 200 ml water sample every two days during the deployment period. Samples were stored in gas-impermeable bags containing 3 ml of Lugol's Iodine for preserving the planktonic material. In addition, in-line cartridges containing the polymer resin SP700 (Sepabeads®) were used as passive samplers for the adsorption of microcystins. Every two days and for one week, 400 ml were passed through one cartridge, four cartridges being connected to different collecting ports of the

instrument. *In situ* temperature was also recorded during the sampling period using a StowAway TidbiT Temperature Logger, which was attached to the frame of the Aquamonitor. After the instrument was retrieved, the content of each bag was transferred in the laboratory into a tissue culture bottle supplemented with ~0.5 ml of Lugol's Iodine for ulterior phytoplankton analysis, and the cartridges were stored at -20°C until further processing.

### 2.2.2 Data Acquisition

Samples and data were acquired for each lake/station as follows. Surface water temperature and depth were measured using a handheld echo sounder, geographical coordinates were taken with a GPS and water transparency was determined with a Secchi disk. Water samples were collected and preserved with Lugol's Iodine in 50 ml bottles for microscopy analysis. Water samples were also filtered onto Whatmann GF/F filters (47 mm diameter) for subsequent analysis of chlorophyll-a, used as a phytoplankton biomass proxy, and the cyanobacterial toxins microcystins. Additional water samples were filtered (150 ml) onto cellulose nitrate filters (Whatmann, 25 mm diameter, 1.0 µm pore size) and placed into 2 ml tubes for ulterior nucleic acid extraction. All filters were stored in the dark at -20°C until further processing. The meteorological conditions at the time of sampling were logged under the following designations: sunny spells, overcast and rain. Additionally in 2010, water column profiling up to 15 m depth was carried out in both Lough Corrib and Ballyquirke Lough using an YSI MultiQuatroPro probe, which was slowly lowered from the side of the sampling platform to measure temperature, conductivity, pH and dissolved oxygen levels.

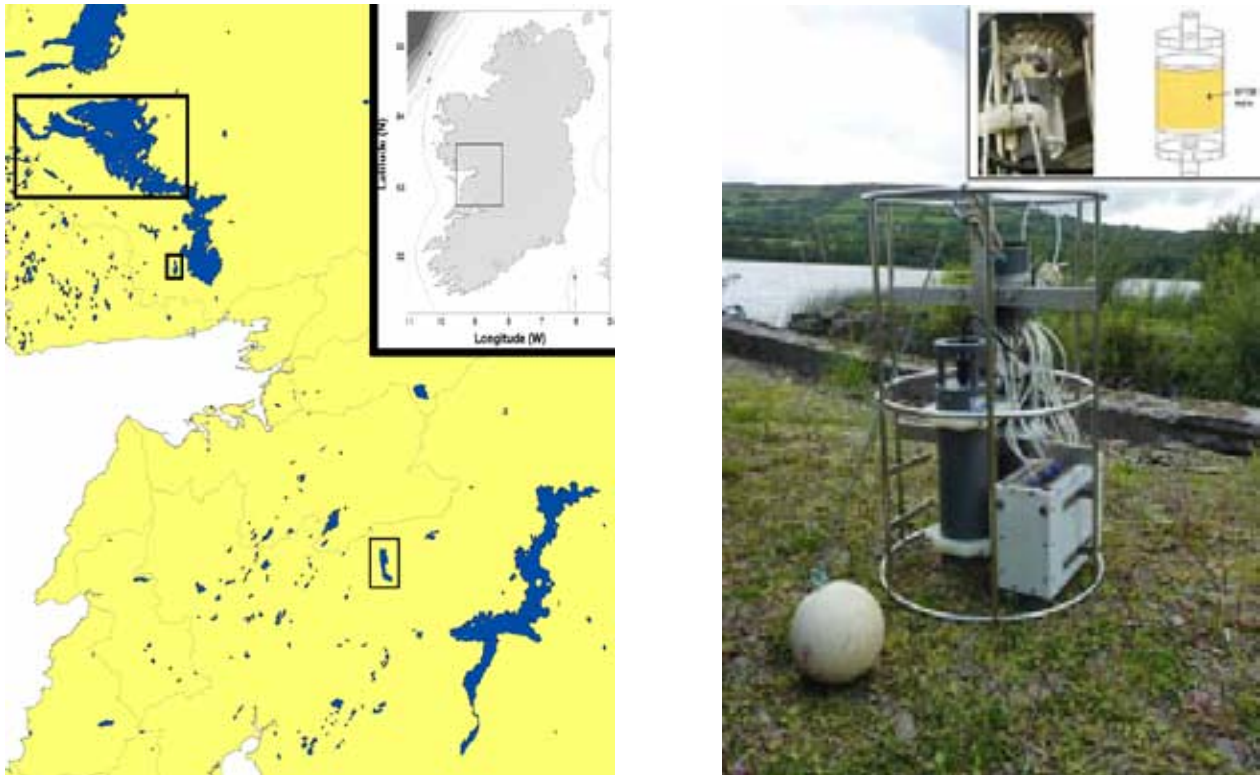
	cores 1	cores 2	cores 3	cores 4	cores 5	cores 6	cores 7	cores 8	cores 9	cores 10	cores 11	cores 12	cores 13	cores 14	cores 15
Cluster A	201	204	205	206	209	211	212	214	215	216	217	218	219	220	221
Cluster B	202	203	207	208	210	213	214	215	216	217	218	219	220	221	222
Cluster C	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215
Cluster D	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215
Cluster E	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215
Cluster F	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215

**Figure 2.3. Designations and sampling timeline of the 50 lakes selected in 2009 from the clusters of river catchments defined by similarity analysis and hierarchical clustering.**

**Table 2.2. Sampling and geographical information pertaining to the west and northwest Irish lakes investigated during summer 2009.**

Date	Station	Location	Cluster	Latitude (N)	Longitude (W)	Area (ha)	Hydro. zone
26/05	201	Lough Inagh	D	53.30.31	09.44.34	309	13
28/05	202	Rosslara Lough	B	52.53.47	08.42.06	15	94
28/05	203	Tully Lough	E	52.53.51	08.41.89	10	103
02/06	204	Lough Arrow	A	54.02.88	08.31.57	1247	25
04/06	205	Lough Glinn	B	53.83.88	08.56.57	55	94
09/06	206	Dahybaun Lake	D	54.06.90	09.32.08	56	109
09/06	207	Derrywaking lough	E	53.28.64	09.59.61	6	107
11/06	208	Lough Derg	B	53.00.88	08.16.56	12005	94
16/06	209	Lough Derg	E	54.36.57	07.51.45	862	37
16/06	210	Glencar Lough	A	54.20.33	08.24.11	115	117
18/06	211	Lough Ardderry	E	53.27.22	09.32.34	81	103
23/06	212	Cross Lake	E	54.12.04	10.04.47	111	110
23/06	213	Ard lough	D	09.52.64	53.52.76	14	106
23/06	214	Doo Lough	C	53.38.78	09.41.91	155	108
25/06	215	Lough Corrib	A	53.24.90	09.08.60	16631	15
30/06	216	New Lake	E	55.10.41	07.58.82	126	124
30/06	217	Durnesh Lake	A	54.33.92	08.10.99	70	122
30/06	218	Lake Unsheen	B	54.31.14	08.05.07	27	119
07/07	219	Carrowmore Lough	D	54.10.52	09.47.11	915	109
09/07	220	Lough Graney	B	52.58.12	08.39.01	372	94
09/07	221	Knocka Lough	A	52.42.84	09.16.34	33	5
14/07	222	Lough Gall	E	53.56.17	09.49.24	5	111
14/07	223	Lough Fee	E	53.35.54	09.49.87	174	107
16/07	224	Lough Gill	C	54.14.36	08.26.23	1381	115
16/07	225	Lough Talt	A	54.04.67	08.55.06	97	113
21/07	226	Dungloe Lake	E	54.56.91	08.20.42	65	124
21/07	227	Lough Adderry	D	54.41.11	08.26.34	18	120
23/07	228	Cavetown Lake	B	53.55.72	08.15.29	64	94
23/07	229	Ballyquirke Lake	A	53.20.26	09.09.38	74	15
28/07	230	Lough Ree	B	53.29.74	08.00.33	10353	94
28/07	231	Shliabh an Aonaigh	E	53.19.09	09.15.73	16	103
30/07	232	Loughrea	F	53.11.21	08.33.94	301	100
30/07	233	Lough Lickeen	A	52.57.76	09.14.76	84	97
04/08	234	Lough Eske	D	54.41.57	08.03.57	387	32
04/08	235	Garadice Lake	B	54.03.40	07.42.20	389	119
06/08	236	Lough Carra	A	53.40.81	09.13.23	1564	15
06/08	237	Nambrackmore lake	E	53.26.72	09.55.97	10	107
11/08	238	Lough Conn	A	54.00.87	09.11.52	5728	113
11/08	239	Aconeera Lough	E	53.23.46	09.38.25	24	103
13/08	240	Polleagh Lough	B	53.39.68	08.38.86	12	94
13/08	241	Maumeen Lake	E	53.23.83	10.01.69	56	104
18/08	242	Lough Sheelin	B	53.47.17	07.22.16	1816	94
20/08	243	Lough Feeagh	D	53.56.99	09.34.04	395	20
25/08	244	Lough Gara	B	53.55.82	08.25.96	1197	94
25/08	245	Lough Easky	A	54.09.28	08.50.71	119	116
27/08	246	Lough Cutra	C	53.02.09	08.46.24	384	99
27/08	247	Uggabeg Lake	E	53.15.92	09.25.82	24	10
01/09	248	Inchiquin Lake	B	52.57.07	09.04.74	108	94
01/09	249	Lough Anillaun	E	53.24.52	09.44.13	57	103
03/09	250	Beltra Lough	D	53.56.16	09.23.75	403	105





**Figure 2.4. Summer 2010 sampling. Left: Upper Lough Corrib, Ballyquirke Lough and Lough Graney. Right: the Aquamonitor deployed in Lough Graney with, in the encased panel, details of the 50-valve system and the drawing of an inline SP700-containing cartridge.**

### 2.3 Phytoplankton Biomass Determination, Light Microscopy and Nutrient Analyses

Analysis of chlorophyll-*a*, used as an indicator of phytoplankton biomass, was performed on filtered surface samples collected from each lake and station. This was carried out by spectrophotometry after overnight extraction in 90% acetone (Lorenzen 1967). Briefly, 7.5 ml of 90% acetone was added into 15 ml tubes containing the filters. Samples were homogenised using a vortex mixer and kept in darkness overnight at 5°C. Samples were centrifuged (4000 rpm, 15 min) prior to carrying out the measurements using a spectrophotometer (Hitachi U-1100) at 665 nm and 750 nm wavelengths. Measurements were also taken after adding a drop of 0.1N chloridric acid to each extract to compensate for the presence of phaeopigments.

Water samples preserved in Lugol's Iodine were examined by light microscopy using an inverted Olympus CKX-41 microscope. Samples were settled overnight in 50 ml flat-bottomed bottles and

genus-level identifications of phytoplankton were carried out at  $\times 100$ -400 magnifications based on the morphological descriptions provided in the literature (Cox 1996, John *et al.* 2002). Dissolved inorganic nutrients, specifically nitrate+nitrite and phosphate, were measured by flow injection using a LaChat QC 8000 automated analyser with computer-controlled sample selection and peak processing with the methods recommended by the manufacturer.

### 2.4 Nucleic Acid Extraction from Filtered Water Column Samples

For each sampling site, 150 ml volume of water was filtered (25 mm diameter membrane, 1.0  $\mu$ m pore size) and kept in a 2 ml tube at -24°C until extraction. Total genomic DNA extraction was carried out on field samples using the Qiagen DNeasy® Blood & Tissue Kit according to the manufacturer's instructions for Gram-negative bacteria. Minor modifications to the protocol were carried out as follows. First, the membranes containing the filtered material were disrupted by bead-beating in a Hybaid ribolyser (full

power, three cycles of 20 sec each) using 180 µm diameter glass beads. Cell lysis was then carried out with proteinase-K in a Thermomixer (56°C, 180 min). An additional extraction step in the Thermomixer was performed after the addition of buffer AL (70°C, 10 min). Nucleic acids were eluted in a final volume of 100 µl with double distilled water and the concentration and quality of the extracts determined using a Nanodrop 1000 spectrophotometer (Thermo scientific). Samples were stored at -20°C until use.

## **2.5 Cyanobacteria Community Fingerprinting by 16S rDNA Denaturant Gradient Gel Electrophoresis Analysis**

The cyanobacterial community structure was assessed in the lakes sampled during summer 2009 and 2010 by DGGE fingerprinting. A semi-nested PCR approach was carried out with an initial partial amplification of the 16S rDNA gene performed on nucleic acid extracts of field samples using the cyanobacteria-specific primer combination CYA359f and 23S30r (Boutte *et al.* 2006). The PCR cocktail contained 1X buffer with loading dye, 2 mM MgCl<sub>2</sub>, 200 µM dNTPs, 0.2 µM of both primers, 1 unit of *Taq* polymerase (Promega, GoTaq) and 1 µl of template DNA. The thermocycling conditions were as follows: initial denaturation step (94°C, 5 min), 15 cycles of amplification [denaturation (94°C, 0.5 min), annealing (58°C, 1 min) and extension (72°C, 2 min)], and a final extension step (72°C, 30 min). The second master mix had a similar composition and was supplemented with 1 µl of the first PCR reaction prior to subjecting the samples to the second amplification carried out with the primers CYA359f and CYA781r(a) or (b) with the GC clamp on the forward primer. The cycling conditions were as follows: initial denaturation step (94°C, 5 min), 40 cycles of amplification [denaturation (94°C, 1 min), annealing (62°C, 1 min) and extension (72°C, 1 min)], and a final extension step (72°C, 30 min). Visualisation of the PCR products was carried out by 1%-agarose gel electrophoresis using the nucleic acid stain Sybr<sup>®</sup>Safe (Invitrogen). Separation of individual amplicons was then performed on vertical denaturing acrylamide gels using the DCode Mutation

Detection Systems (Bio-Rad Laboratories, USA). Gels were constituted of 8% acrylamide/bis 40/1 (v/v) with a 45–55% denaturant gradient prepared from a 100% denaturation solution of 7 M urea and 40% formamide (v/v). Amplicons were loaded into the wells (35 µl) and separation allowed for 16.5 h at 60°C at a constant voltage of 60 V. Prior to visualisation under UV illumination, gels were stained in 300 ml of 10 mg ml<sup>-1</sup> Ethidium Bromide in TAE buffer for 40 min and destained in 300 ml of deionised water for 12 min. All gels were imaged by UV illumination using the G:Box and the GeneSnap<sup>®</sup> programme (Syngene). Additional DGGE profiles were run to verify the consistency of band separation for selected lake samples. Selected bands on each gel were excised with sterile blades and the DNA eluted overnight at 5°C in 100 µl of PCR grade water. Re-amplification was then performed using 1 µl of template with the internal primer combination CYAseq-f and CYAseq-r using the second round PCR. Amplicons were purified using the QIAquick PCR purification kit as described in the manufacturer's instructions. The samples were eluted in 20 µl of double distilled water and stored at 5°C prior to external sequencing (MWG Biotech, Germany). Analysis of the DGGE amplicon migration patterns representing the cyanobacterial community composition was carried out using the Total-Lab TL120 software (Nonlinear Dynamics, UK). A densitometric scan of the gels was created and background noise was subtracted using a rolling disc algorithm. Operational taxonomic units were defined as the total number of defined bands per sample. A band-matching matrix was constructed using peak height values for each gel. The matrices of band intensities (peak height) for both spherical and filamentous cyanobacteria were then analysed through the PASWStatistic17.0 module in SPSS for Mac by hierarchical clustering using Ward's method construction and Chebichev distance.

## **2.6 Phylogenetic Inference**

Upon reception, the rDNA sequences of amplified cyanobacterial DGGE bands were initially screened using BLAST (<http://www.ncbi.nlm.nih.gov>) (Basic, Local, Alignment, Search, Tool, Altschul *et al.* 1990) to orientate the identification of the isolates. The

sequences were then compiled with other partial 16S rDNA sequences of cyanobacteria imported from GenBank, and aligned using the pairwise alignment function of the GeneDoc and Clustal-X programs. The software package PAUP version 4.0b10 (Swofford 2002) was used to infer the phylogeny of cyanobacteria and determine the relative position of the sequenced Irish isolates. Modeltest3.7 (Posada & Crandal 1998) was used to determine the optimal base substitution models for the alignments relative to the partial 16S rDNA sequences. Maximum likelihood (ML) analyses were carried out using PAUP\* with the parameters derived from the Akaike Information Criterion (AIC) in Modeltest. Phylogenies were then reconstructed by performing heuristic searches with random addition of sequences (10 replicates) and a tri-bisection-reconnection branch swapping algorithm. Bootstrap analyses were finally performed on the tree topologies to evaluate the robustness of the sequence arrangements. Due to computation constraints, bootstrap values were derived using the 'fast' stepwise addition with 10000 replicates under the distance criterion. The out-group selected was the Gram-positive bacterium *Clostridium lavalense* (strain CCRI-9929, GenBank entry: EF564278).

## 2.7 Polymerase Chain Reaction Amplification of Cyanotoxin Biosynthesis Genes in Field Sample Extracts

The *mcy-A* gene of the microcystin operon has proved useful for identifying toxigenic cyanobacterial genera in culture and field samples (Christiansen *et al.* 2003, Hisbergues *et al.* 2003). The *mcy* operon comprises ~10 genes coding for the enzymes involved in microcystin biosynthesis, the amplified *mcy-A* region encoding part of the condensation domain of three non-ribosomal peptide synthetases (Borner & Dittmann 2005). The nucleic acid extracts obtained from lake samples were amplified by PCR in a 50 µl reaction

volume with the forward and reverse primers MCY-A-cd1-f (5'-AAAAGTGTTTTATTAGCGGCT CAT- 3') and MCY-A-cd1-r (5'-AAAATTTAAAGCCGTATCAAA-3') (Hisbergues *et al.* 2003). The PCR was carried out using a cocktail consisting of 1X buffer, 3 mM MgCl<sub>2</sub>, 200 µM dNTPs, 0.5 µM of both primers, 1 unit of *Taq* polymerase (Promega, GoTaq) and 1 µl of template. The thermocycling programme was as follows: an initial denaturation step (94°C, 5 min, 40 cycles of amplification [denaturation (94°C, 5 min), annealing (50°C, 1 min) and extension (72°C, 2 min)], and a final extension step (72°C, 5 min). Visualisation of the PCR products was carried out as in Section 2.5. Prior to RFLP, PCR purification was carried out on the amplicons using a QIAquick PCR purification kit and the products were eluted in 20 µl of double distilled water. The restriction enzymes *Rsa.I* and *Hha.I* (Fermentas) were used according to the manufacturer's instructions to conduct the dual digestion (15 min, 37°C) of the purified partial *mcy-A* gene amplified from the field sample extracts. The digests were resolved on a 2% agarose-1XTAE buffer gel. The interpretation of the restriction patterns was carried out by recording the presence/absence of bands to create a binary matrix. The similarity matrix was then converted into a dendrogram using the UPGMA method (un-weighted pair group method with arithmetic averages) in PAUP version 4.0b10.

Additionally, analysis for the presence of the biosynthetic genes for the cyanobacterial toxins cylindrospermopsin (CYN), anatoxin-a (ANA) and STXs was carried by PCR. The designations and sequences of the primers are indicated in [Table 2.3](#). The thermocycling conditions applied were the same for the three types of PCR and were as follows: an initial denaturation step (95°C, 5 min, 35 cycles of amplification [denaturation (95°C, 1 min), annealing (58°C, 1 min) and extension (72°C, 1 min)], and a final extension step (72°C, 10 min). Visualisation of the PCR products was carried out as in Section 2.5.

**Table 2.3. Sets of primers used for the polymerase chain reaction (PCR) analysis of the 16S rDNA gene and cyanotoxin genes (microcystins, anatoxin-a, saxitoxins and cylindrospermopsin) in the water column sample extracts collected during summers 2009 and 2010.**

Designation	Direction	Sequence (5' → 3')	Target	T <sub>m</sub>	Reference
CYA359	Forward	ggggaattttccgcaatggg	16S	59.4	Nübel <i>et al.</i> 1997
23S30	Reverse	cttcgcctctgtgtgcctaggt	16S	64.0	Nübel <i>et al.</i> 1997
CYA781-ra	Reverse	gactactggggtatctaattccatt	16S	61.3	Nübel <i>et al.</i> 1997
CYA781-rb	Reverse	gactacaggggtatctaattcccttt	16S	61.3	Nübel <i>et al.</i> 1997
CYAseq-f	Forward	gcgaaagcctgacggagc	16S	60.5	this study
CYAseq-r	Reverse	ggggtatctaattccattcgct	16S	60.3	this study
MCY-A- cd1-f	Forward	aaaagtgtttattagcggtcat	MCY	55.9	Hisbergues <i>et al.</i> 2003
MCY-A- cd1-r	Reverse	aaaataaaagccgatcaaa	MCY	48.1	Hisbergues <i>et al.</i> 2003
M14	Forward	gatgaacatcgctcactggtg	CYN	62.1	Schembri <i>et al.</i> 2001
M13	Reverse	ggcaaattgtgatagccacgagc	CYN	62.4	Schembri <i>et al.</i> 2001
Sxt-A-f	Forward	aggtcttgacttgcatcaa	STXs	55.2	Ledreux <i>et al.</i> 2010
Sxt-A-r	Reverse	aaccggcgacatagatgata	STXs	55.2	Ledreux <i>et al.</i> 2010
Antx-f	Forward	cgaaatcgatgctcactta	ANA	55.2	Cadel-Six <i>et al.</i> 2009
Antx-r	Reverse	ccactggctccatcttgatt	ANA	57.3	Cadel-Six <i>et al.</i> 2009

## 2.8 Analysis of Microcystin by Protein Phosphatase 2A Assay and Analytical Chemistry

Initially developed for detecting okadaic acid in contaminated marine shellfish, the protein phosphatase 2A assay (PP2A) has been adapted for the semi-quantification of cyanobacterial hepatotoxins (microcystins and nodularins) (Simon & Vernoux 1994, Tubaro *et al.* 1996, Rivasseau *et al.* 1999b). The reaction relies on the inhibition by microcystins of the dephosphorylation by the enzyme phosphatase 2A of the substrate para-nitrophenyl-phosphate (pNPP) into the product paranitrophenol (pNP), which is detectable by spectrophotometry. Microcystins were extracted from GF/F filters containing the biological material concentrated on station during sampling. The addition of 4 ml of 100% methanol to the filters was followed by further disruption by bead-beating, sonication and multiple freezing-thawing cycles. The homogenates were passed through a 5 ml syringe fitted with an in-line 0.45 µm pore size filter. The eluates were then evaporated to dryness, resuspended with 400 µl of 100% methanol and stored at -20°C until analysis. A similar process was applied to extract the microcystins from the SP700 resin contained in the cartridges used during the Aquamonitor deployment in Lough Graney during summer 2010. The PP2A assay was carried out in a 96-well plate format.

Each well was inoculated with 100 µl of buffered sample (field extract, microcystin ((MC)-LR standard or buffer blanks, in 40 mM Tris/HCl, 34 mM MgCl<sub>2</sub>, 4 mM EDTA and 4 mM DL-dithiothreitol, pH 8.0) and 50 µl of the enzyme (0.05 U.ml<sup>-1</sup> final concentration). Each determination was done in duplicate and a set of three dilutions for each extract was tested. The reaction was started by the addition of 50 µl of the substrate p-NPP (28.2 mM final concentration) and carried out for 1 h at 37°C. The hydrolysis of p-NPP to p-NP was recorded at 405 nm using a Microplate Autoreader (Biotek Instruments, USA). Microcystin concentration in samples, expressed in µg.l<sup>-1</sup> of MC-LR eq., was determined by interpolation of the absorbance values of the samples from the linear portion of the standard curve.

Selected field sample extracts were further analysed to determine the composition of the microcystin variants. Analysis was carried out at the Museum National d'Histoire Naturelle (MNHN) in Paris, France, through collaborative work with Prof. C. Bernard and Dr. A. Marie. A time-of-flight hybrid tandem mass spectrometer (Q-Star Applied Biosystems) equipped with an electrospray ionization source was used. All runs were carried out in positive-ion mode. The capillary voltage was set to 2500 V, and the declustering potential was 20 V for the mass spectrometry mode.

Data were acquired and processed using Analyst Qs software (Applied Biosystems, Framingham, MA, USA). The mass scan range was from  $m/z$  800 to 1200, and the scan cycle was 1 sec. For MS/MS experiments, the collision energy was set to 30 eV with a declustering potential of 60 V. The mass scan ranged from  $m/z$  30 to 170 so as to select the adda group-containing ion at  $m/z$  135.

## 2.9 Data Treatment and Analysis

For the summer 2009 survey, contingency tables were used to determine the presence of significant relationships between variables. Those included meteorological conditions, sampling periods, the trophic state of the lakes sampled, land-cover typology, lake groups based on phytoplankton community composition, lake groups based on *mcy-A* restriction profile, cumulated *Oscillatoria* and *Anabaena* concentrations, and PP2A-based microcystin concentration estimates. Non-parametric Kruskal-Wallis and Mann-Whitney analyses were carried out to identify significant relationships in the distribution of quantitative variables in space and time (Kruskal & Wallis 1952). The post-hoc analysis consisted of carrying out Mann-Whitney tests on each pairing and applying a Bonferroni correction onto the  $p$  values to identify the treatments that significantly differed from each other (Mann & Whitney 1947). A hierarchical classification of the 50 lakes sampled, based on phytoplankton community structure, was performed using TWINSpan (Two-Way Indicator Species Analysis) and DECORANA (DEtrended CORrespondence ANALysis) programs in VESpan III (Malloch 1988, Hill 1994). For this analysis, only the protist taxa present in more than three sites were considered. Focusing specifically on cyanobacteria, hierarchical clustering was also carried out on the DGGE band migration profiles obtained for both filamentous and spherical types. Cyanobacterial diversity estimates were determined based on the number and peak intensity of DGGE bands retrieved for each lake after nested PCR amplification. Species richness was defined as the number of bands detected in each lane. Principal component analysis (PCA), which simplifies data sets by reducing the dimensionality of multivariate data, was also applied to assess the relationships between different variables, including surface temperature, chlorophyll-*a* concentration, lake

size, land cover typology, spherical and filamentous cyanobacterial richness and DGGE band intensity based estimated abundance. The PCA was run for a correlation matrix in a Varimax rotation mode. The data sets were normalised by column standardisation prior to analysis. The relationships between the ordination axes and individual environmental variables were assessed using Spearman rank correlations.

For the summer 2010 surveys, parametric statistics were applied to the data. Analyses of variance (ANOVA) and Student *t*-tests were carried out to identify significant differences in the distribution of quantitative variables in Lough Corrib and Ballyquirke Lough. Those included temperature, Secchi depth, chlorophyll-*a* concentration, dissolved inorganic nitrogen and phosphorus, phytoplankton abundances, PP2A-based microcystin concentrations and DGGE-based estimate of spherical cyanobacteria abundance. Hierarchical clustering was also performed on both the RFLP and DGGE band migration profiles obtained with the samples collected throughout the summer. Fisher's exact test was applied to selected indicator bands to determine the presence of relationships between the presence/absence of some taxa with environmental descriptors. Finally, multiple factor analysis (MFA) (Escofier & Pages 1990) was applied to the Lough Corrib and Ballyquirke Lough data sets to assess the relationships between different variables. MFA explores the common structures present in several data sets in two steps. Principal component analyses (PCA) are first performed on each individual data set, which is then weighted by dividing all its elements by the square root of the first eigenvalue obtained from its PCA. The normalised tables are juxtaposed into a single matrix and subjected to a PCA again. The analyses were run for the following variables: temperature, Secchi depth, chlorophyll-*a* concentration, dissolved inorganic nitrogen and phosphorus, and DGGE-based estimate of spherical cyanobacteria richness and abundance. The final PCA was run for a correlation matrix in a Varimax rotation mode. The data sets were autoscaled by column standardisation prior to analysis. Pearson correlation was used to determine the significance of the relationships between the MFA components and selected quantitative variables.

All statistical treatments were performed using PASWStatistic17.0 module in SPSS for Mac.



### 3 Results

#### 3.1 River Catchments Characteristics

The parameters used to describe the typology of the river catchments studied in the west and northwest regions of Ireland during the summer 2009 survey showed some variability, both in terms of hydrogeology and land cover (Table 3.1).

A gradient of land cover types was apparent in the study area (Fig. 3.1). A high proportion of wetlands were contained in ten catchments (13, 14, 23, 36, 103, 106, 107, 111, 124, 128), corresponding to coastal areas in west Connemara, west Mayo and west Donegal. Five catchments (37, 98, 99, 127, 130) in areas of Counties Clare and Donegal showed a high forestry cover. High proportions of artificial/agricultural cover were contained in 18 catchments (8, 10, 15, 25, 27, 30, 31, 94, 95, 96, 97, 100, 101, 102, 114, 117, 118, 119), which corresponded mainly to pastures, highlighting farming activities in the area extending from Limerick to North Sligo. The remaining 23 river catchments showed intermediate characteristics and were mostly located in a geographical band extending from Cos Mayo to Donegal.

#### 3.2 Typology of River Catchments and Site Selection

The apparent gradient in land cover suggested possible assemblages of river catchments, delineating regions with a contrasting range of geomorphologic environments. Representations of the seven indices used to characterise the river catchments within the study area are compiled in Fig. 3.2. In general, the patterns reflected a zoning of catchments with three north-south bands orientated in a near parallel manner along the longitudinal axis. These 'ecoregions' identified the midland areas (zone I), the intermediate Galway, Mayo, Sligo, South Donegal area (zone II) and the west and northwest coastal band of catchments (zone III). Zone I corresponds to low altitude and domesticated lands, with human activity dominated by agriculture, while the other regions relate to wilder natural habitats, with the west band in particular corresponding to well-drained and mountainous coastal areas.

**Table 3.1. Summary statistics of geomorphology, hydrological characteristics and land cover in the river catchments selected within the study area (n=56).**

		Average	Median	s.d.	Min.	Max.
Hydro-geology	Surface (km <sup>2</sup> )	577.8	146.8	1981.1	6.2	14919.4
	Altitude (m)	97.3	91.1	50.5	15.1	206.0
	Drainage (m.km <sup>2</sup> )	1275.3	1325.8	593.6	0.0	2804.8
Land cover	Urban (%)	1.4	0.6	2.6	0.0	12.7
	Agriculture (%)	47.2	49.3	28.2	3.8	95.8
	Forests (%)	17.9	15.8	10.8	0.0	45.1
	Wetlands (%)	31.4	29.8	23.7	0.0	88.4
	Water (%)	2.1	1.1	2.5	0.0	11.6

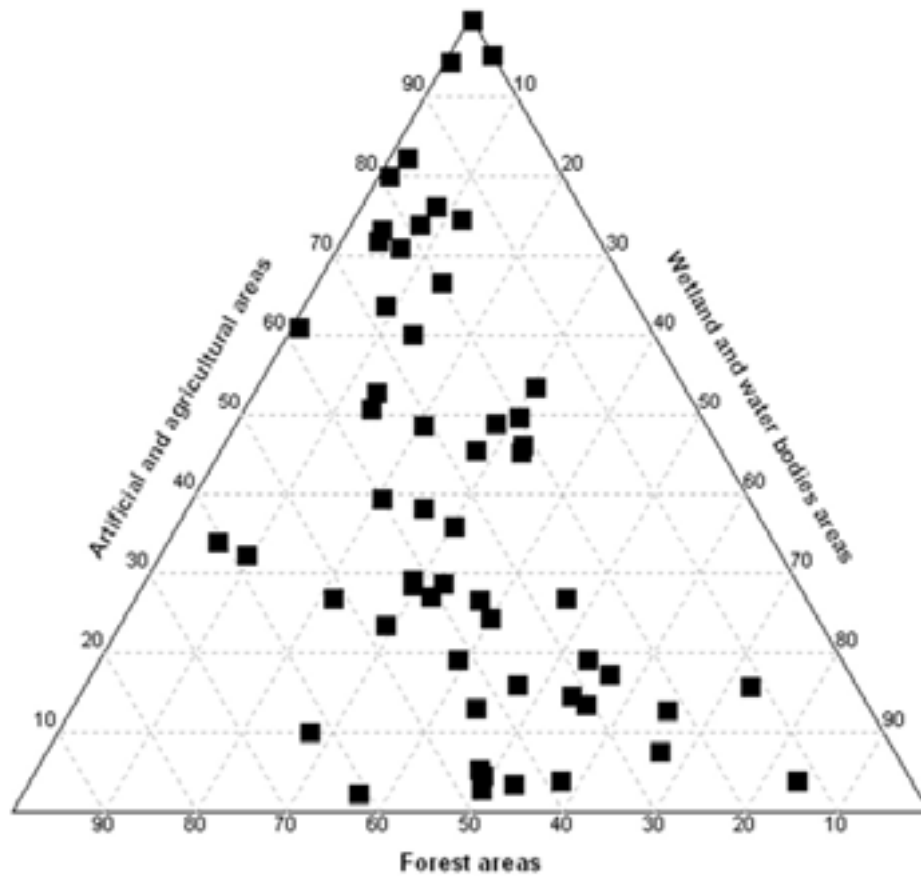


Figure 3.1 Ternary diagram showing the proportions of wetland, agricultural and forestry surfaces within the study area.

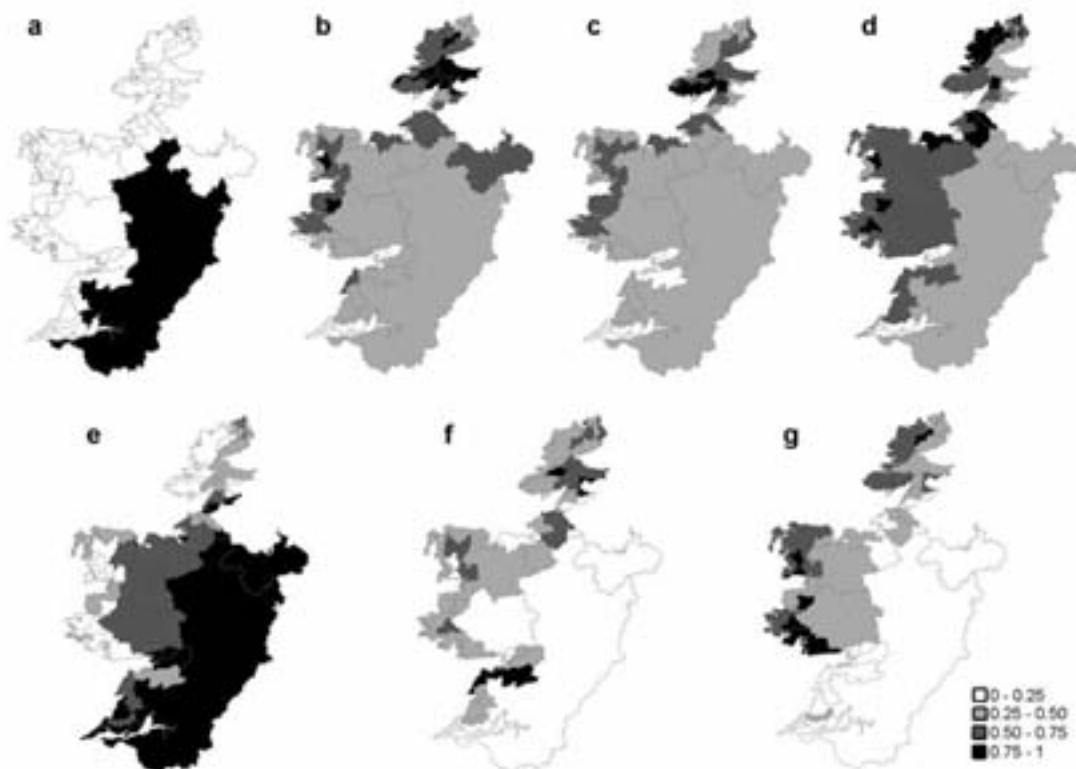


Figure 3.2. Index score distribution for the river catchments sampled during summer 2009: surface (a), altitude (b), drainage (c), river quality (d), agricultural cover (e), forest cover (f) and wetland cover (g).

The grouping of river catchments into assemblages sharing resemblances was carried out following similarity analysis and hierarchical clustering of catchment attributes based on seven indices derived for each hydrological zone. A similarity level forming six river catchment assemblages was retained (Fig. 3.2). All indices were relatively homogenous amongst the six clusters, except the values pertaining to the relative surface index (mean 0.11, s.d. 0.22), which were very low across all groups except cluster B, which contained the largest Irish river catchment (94, 'Shannon') (Table 3.2).

The six defined clusters contained from 2 to 13 river catchments and 124 to 2804 water bodies (Table 3.3). Clusters A, B and E accounted for 77% of the total amount of water bodies within the study area with cluster

B containing the highest number of lakes greater than 500 ha in surface. Within each cluster a number of lakes was selected proportionally to the number of potential sampling sites per cluster. The range of water bodies to sample varied from 1 to 15 across the six clusters of river catchments, clusters F and E representing 2 and 30% of the sampling effort, respectively.

The hierarchical clustering reflected to some extent the zonation groups identified in Fig. 3.3, with cluster B as zone I, clusters A, C and F as zone II and clusters D and E as zone III. The lakes selected for the investigation were sampled during a 15-week period in a total of 29 river catchments from the 56 initially selected within the study area. The most sampled catchment was from cluster B ('Shannon', n. 94), in which 10 lakes were sampled during the course of the summer.

**Table 3.2. Average index values for the clusters of river catchments sampled during summer 2009 and defined by similarity analysis and hierarchical clustering.**

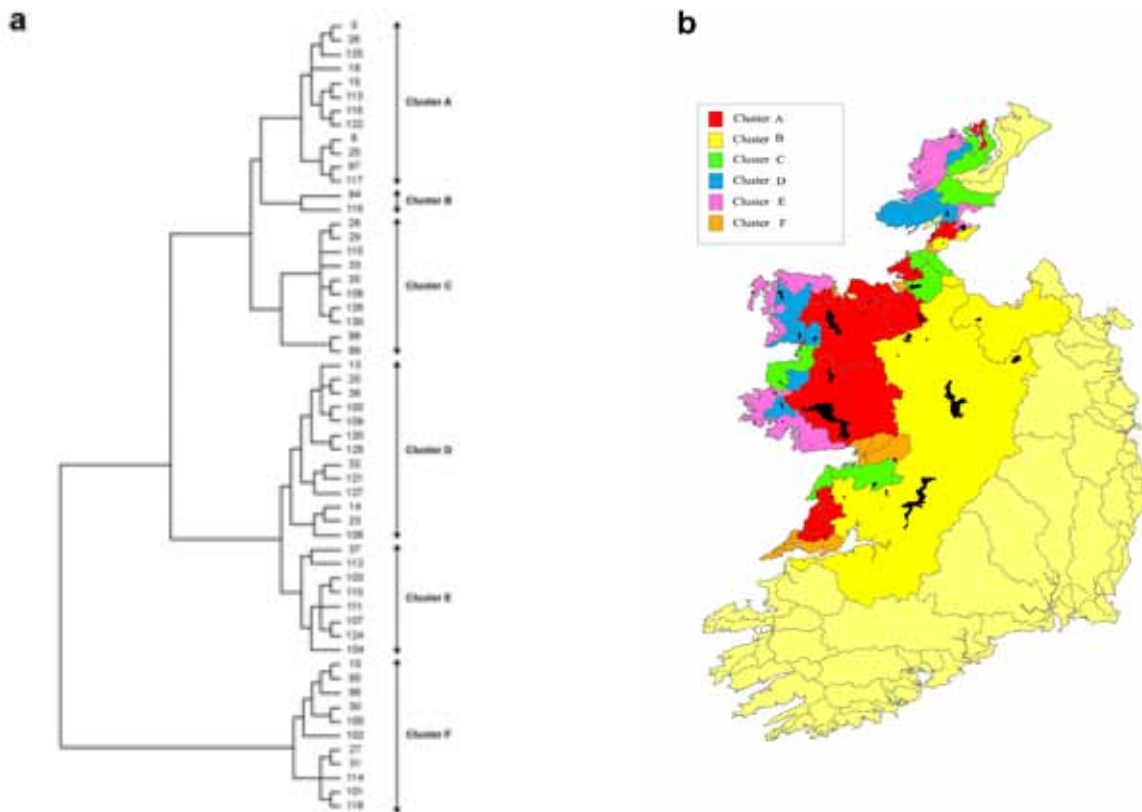
Cluster	I <sub>rs</sub>	H <sub>dr</sub>	LC <sub>ua</sub>	LC <sub>f</sub>	LC <sub>w</sub>	I <sub>alt</sub>	H <sub>riv</sub>
A	0.04	0.47	0.61	0.32	0.28	0.39	0.66
B	0.58	0.33	0.79	0.19	0.14	0.48	0.39
C	0.02	0.51	0.43	0.62	0.34	0.61	0.67
D	0.01	0.69	0.15	0.53	0.69	0.76	0.73
E	0.02	0.41	0.19	0.48	0.67	0.43	0.57
F	0.01	0.16	0.88	0.19	0.04	0.17	0.24

See Table 1.1 for abbreviations.

**Table 3.3. Water body and river catchment attributes of the six clusters defined by similarity analysis and hierarchical clustering.**

Cluster	Total no. of lakes	% of lakes in study area	no. of lakes [5-50 ha]	no. of lakes [50-500 ha]	no. of lakes >500 ha	no. of river catchments	no. of lakes to sample
A	1983	22	157	29	5	12	11
B	2199	24.5	441	62	12	2	12
C	469	5	71	10	2	10	3
D	1455	16	75	15	1	13	8
E	2804	31	306	31	1	8	15
F	124	1.5	12	1	0	11	1





**Figure 3.3.** Hierarchical clustering based on the attributes of the river catchments contained within the study area (a) and map of the Republic of Ireland indicating the 6 defined clusters and the 50 water bodies that were sampled during summer 2009 (b).

### 3.3 Distribution of Physical and Biochemical Variables (2009)

#### 3.3.1 Temperature and Light Penetration

The average surface temperature of the lakes sampled over the duration of the summer was 16.7°C (s.d. = 1.9) (Fig. 3.4). Considering temperature on a weekly basis, and irrespective of lake membership to the different clusters, the average surface water temperature increased from week 1 to 6, reaching a maximum value of 21.0°C. The temperature progressively dropped thereafter until week 15 to 14.0°C. There was no significant difference in the average temperatures obtained between weeks 1 and 7 and weeks 8 and 15 (Mann-Whitney,  $p > 0.05$ ). The local maximum temperature of 22.0°C was recorded in Lough Gall (cluster E) on 30 June. The minimum temperature of 11.5°C was recorded in Lough Inagh (cluster D) on 26

May. No significant difference was detected between the clusters (excluding cluster F) when considering the temperatures recorded within each assemblage (averages comprised between 16.0 and 17.4°C) over the length of the summer (Kruskal-Wallis,  $p > 0.10$ ,  $H = 3.29$ ,  $df = 4$ ,  $n = 49$ ).

Light penetration was measured in the lakes using a Secchi disk when feasible. The Secchi depth was very variable between lakes, with an average of 2.15 m (s.d. = 1.37,  $n = 31$ ). The Secchi depths recorded during June, July and August were all significantly different from each other (Kruskal-Wallis,  $p < 0.01$ ,  $H = 11.07$ ,  $df = 2$ ,  $n = 31$ ), but not so spatially between clusters (Kruskal-Wallis,  $p > 0.10$ ,  $H = 2.56$ ,  $df = 4$ ,  $n = 31$ ). The local minimum and maximum Secchi depths were 0.50 m and 5.60 m and were recorded in Carrowmore Lough (07 July) and Doo Lough (23 June), respectively.

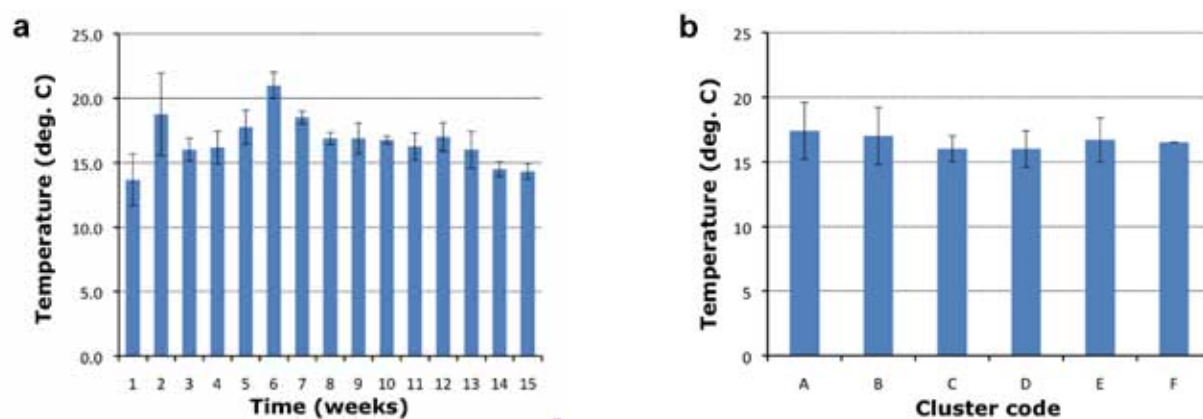


Figure 3.4. Average surface water temperatures determined in the lakes sampled on a weekly basis (a) and in the lakes grouped in clusters (b) during summer 2009. Error bars denote standard deviation.

### 3.3.2 Chlorophyll-a Concentrations

Chlorophyll-a was used as a phytoplankton biomass indicator and was on average  $4.03 \mu\text{g.l}^{-1}$  in the lakes sampled (s.d. = 4.73) (Fig. 3.5). Most lakes (26) showed chlorophyll-a levels below  $2.5 \mu\text{g.l}^{-1}$ , 14 lakes showed levels between  $2.5$  and  $5 \mu\text{g.l}^{-1}$ , and levels greater than  $5 \mu\text{g.l}^{-1}$  were recorded in 10 lakes. No significant pattern in the distribution of chlorophyll-a levels was found in the three monthly time periods (Kruskal-Wallis,  $p >$

$0.10$ ,  $H = 3.53$ ,  $df = 2$ ). On the other hand, moderately significant relationships were found in the distribution of chlorophyll-a concentrations in space, between zones I and III (Kruskal-Wallis,  $p < 0.10$ ,  $H = 5.77$ ,  $df = 2$ ) and between clusters B and D (Kruskal-Wallis,  $p < 0.10$ ,  $H = 9.11$ ,  $df = 4$ ,  $n = 49$ ). Chlorophyll-a concentrations greater than  $15 \mu\text{g.l}^{-1}$  were determined in only two lakes, with the maximum being recorded in Ballyquirke Lough (cluster A,  $29.2 \mu\text{g.l}^{-1}$ ) on 23 June.

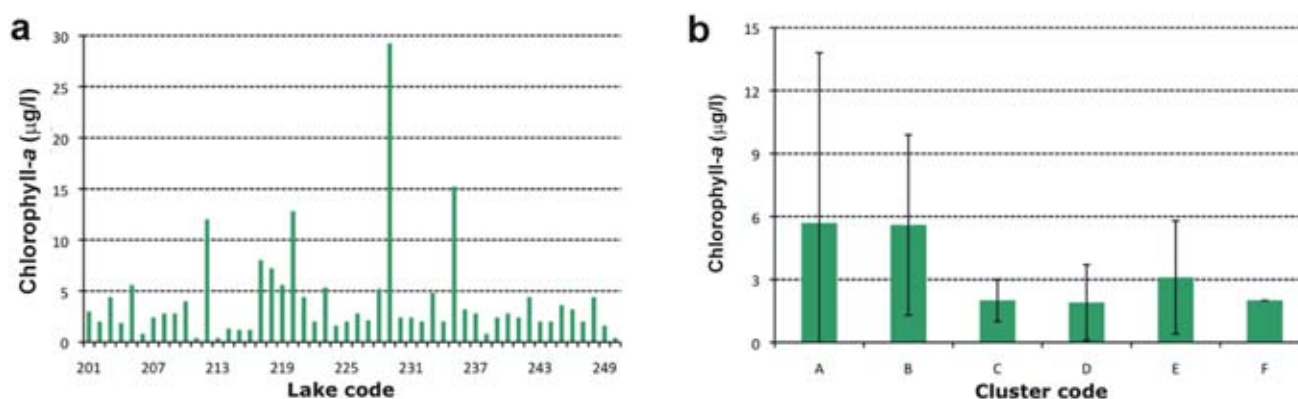
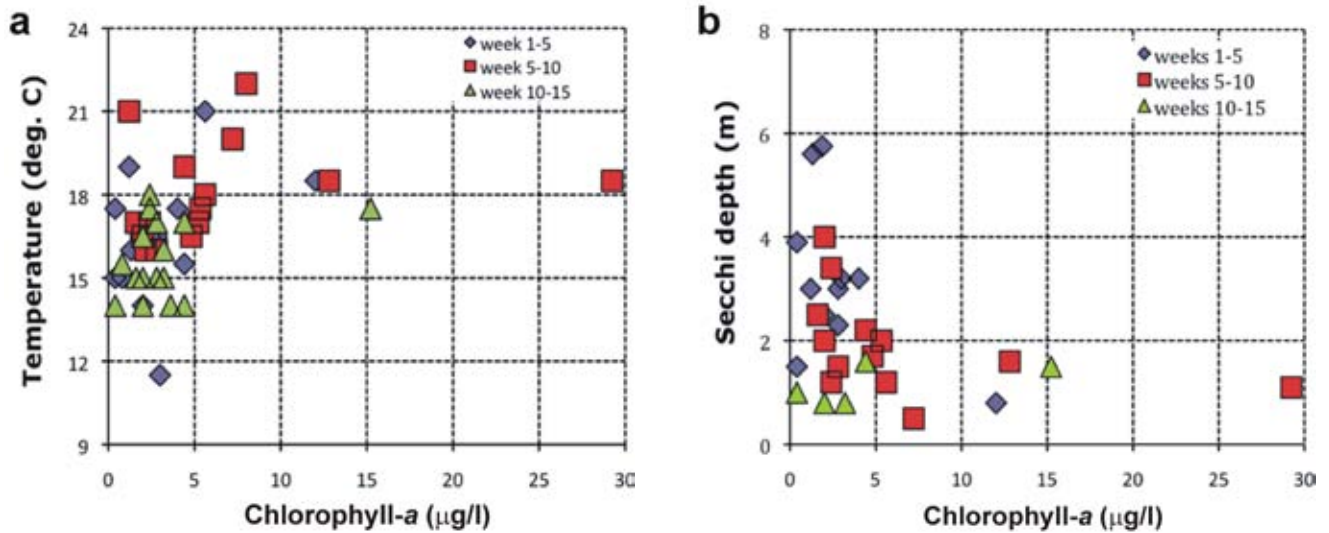


Figure 3.5. Surface chlorophyll-a concentrations determined in the 50 lakes sampled (a) and in the lakes grouped per cluster (b) during summer 2009. Error bars denote standard deviation.



**Figure 3.6. Scatter plots between chlorophyll-a and temperature (a), and between chlorophyll-a and Secchi depth (b) in the lakes sampled during summer 2009.**

### 3.3.3 Relationships between Chlorophyll-a, Temperature and Secchi Depth

Chlorophyll-a concentrations greater than  $5 \mu\text{g.l}^{-1}$  were generally reached when temperatures were near  $18^\circ\text{C}$  (Fig. 3.6). Higher chlorophyll-a levels were associated with low Secchi depths, in particular between weeks 5 and 10. The low chlorophyll-a concentrations observed at the beginning of the study coincided with high water clarity, whereas the lakes sampled near the end of the summer showed both low phytoplankton biomass and weak light penetration, suggesting the presence of humic substances or perhaps heavy loads of suspended matter in the water column concomitant to bad weather and rainfall.

### 3.3.4 Trophic Status of Lakes and Associated Land Cover Type

Contingency analysis was carried out to determine the presence of significant relationships between the nature of land cover and the trophic state of the

waterbodies sampled in the study area (Fig. 3.7). The analysis was performed using four trophic state classes (ultra-oligotrophic, oligotrophic, mesotrophic and eutrophic) and the three geographical zonation groups defined following examination of the indices data. When feasible, publicly available water body categorisation, previously carried out by the Irish EPA, was used for the lakes sampled (EPA Ireland, 2008b). The trophic state of the remaining lakes was estimated using the chlorophyll-a concentrations and Secchi depth values determined in this study according to the eutrophication classification levels defined in OECD (1982). The contingency analysis showed a significant relationship between trophic status and the zonation groups ( $X = 17.35$ ,  $p < 0.05$ ,  $\text{df} = 6$ ). A further significant relationship was found in relation to spatial distribution between trophic status and the clusters of river catchment (excluding lakes from clusters C and F) ( $X = 20.22$ ,  $p < 0.05$ ,  $\text{df} = 9$ ,  $n = 46$ ).

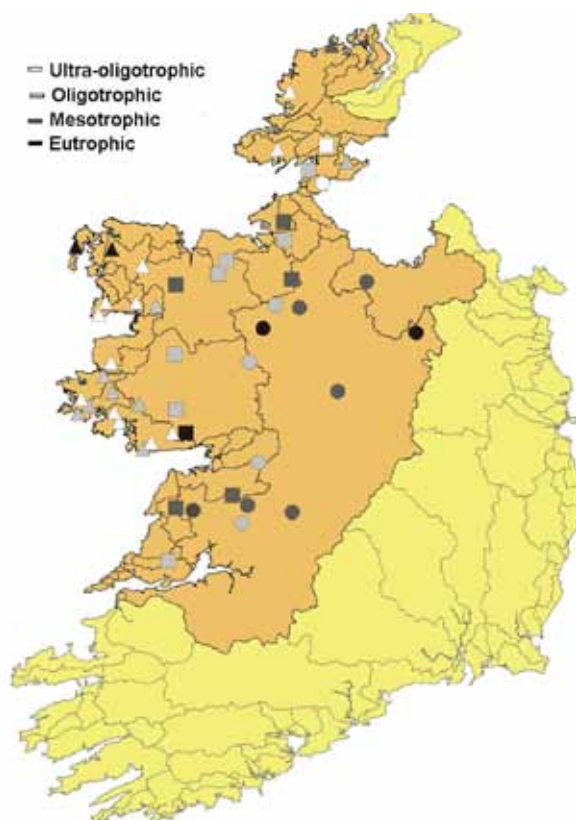
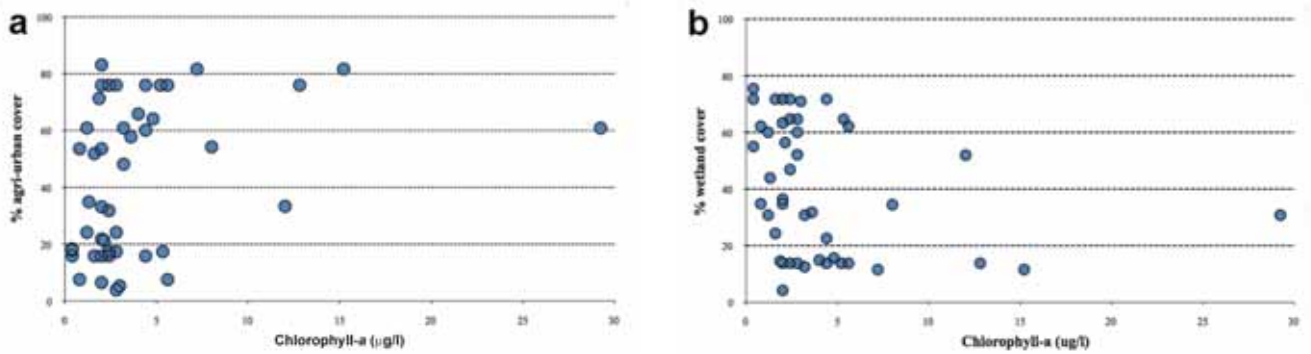


Figure 3.7. Trophic status and associated land cover type of the lakes sampled during summer 2009: ■, ●, ▲ represent zones III, II and I, respectively.

Table 3.4. Significance<sup>1</sup> in space and time of the non-parametric Spearman correlation tests carried out between land cover type (percentage cover) and chlorophyll-a for the 50 lakes sampled during summer 2009.

	Agricultural type cover	Forestry type cover	Wetland type cover
Zone I	$p = 0.339$ $r = 0.134$	$p = 0.007^{**}$ $r = -0.690$	$p = 0.329$ $r = -0.143$
Zone II	$p = 0.246$ $r = 0.193$	$p = 0.424$ $r = -0.054$	$p = 0.334$ $r = -0.121$
Zone III	$p = 0.287$ $r = -0.124$	$p = 0.245$ $r = 0.152$	$p = 0.310$ $r = -0.109$
Weeks 1–5	$p = 0.188$ $r = 0.238$	$p = 0.369$ $r = 0.091$	$p = 0.086$ $r = -0.36$
Weeks 5–10	$p = 0.186$ $r = 0.224$	$p = 0.049^{*}$ $r = -0.401$	$p = 0.23$ $r = -0.186$
Weeks 10–15	$p = 0.011^{*}$ $r = 0.565$	$p = 0.041^{*}$ $r = -0.448$	$p = 0.008^{**}$ $r = -0.594$
Whole period	$p = 0.010^{**}$ $r = 0.329$	$p = 0.058$ $r = -0.225$	$p = 0.005^{**}$ $r = -0.364$

<sup>1</sup> \* and \*\* indicate 2-tail significance at the 0.05 and 0.01 levels, respectively.



**Figure 3.8. Relationships between chlorophyll-a concentration and the percentage of agriculture (a) and wetland (b) cover types of the river catchments harbouring the water bodies sampled.**

Non-parametric Spearman correlations were also performed to investigate the relationships between chlorophyll-a levels and the proportion of river catchment covered by the three land cover types retained for this study (Table 3.4). Considering the whole data set, there were significant correlations between the chlorophyll-a levels determined in the lakes sampled and the percentage of river catchment area covered with wetland ( $r_s = -0.364$ ,  $p = 0.005$ ) and agriculture ( $r_s = 0.329$ ,  $p = 0.010$ ) cover types, respectively (Fig. 3.8). Specifically in time, a negative correlation was found between chlorophyll-a and forest cover in the river catchment containing the lakes sampled during July, when the highest chlorophyll-a concentrations were found ( $r_s = -0.401$ ,  $p = 0.041$ ,  $n = 18$ ). Considering chlorophyll-a concentrations in space, the only significant relationship was negative and obtained for forest cover in zone I ( $r_s = -0.690$ ,  $p = 0.007$ ,  $n = 12$ ).

### 3.4 Phytoplankton Community Structure Analysis by Light Microscopy

#### 3.4.1 General Regional Distribution of Broad Taxonomic Units

The phytoplankton community was analysed at the genus level in the 50 water samples collected during summer 2009. Analysis was carried out by light microscopy and the identification of broad taxonomic units was performed according to morphological descriptions found in the literature. Figure 3.9 illustrates the abundances of the main classes of protistean plankton – namely microflagellates, diatoms, chlorophytes, cyanobacteria, flagellates and ciliates. Microflagellates dominated the phytoplankton assemblages numerically in all samples. Of interest also are the great concentrations of diatoms

(near or above  $1 \times 10^6$  cells  $l^{-1}$ ) reached in loughs Cross, Unsheen and Gall, sampled in late June and mid-July. The frequency of miscellaneous flagellates was also greater in the first half of the sampling period than from mid-July to August.

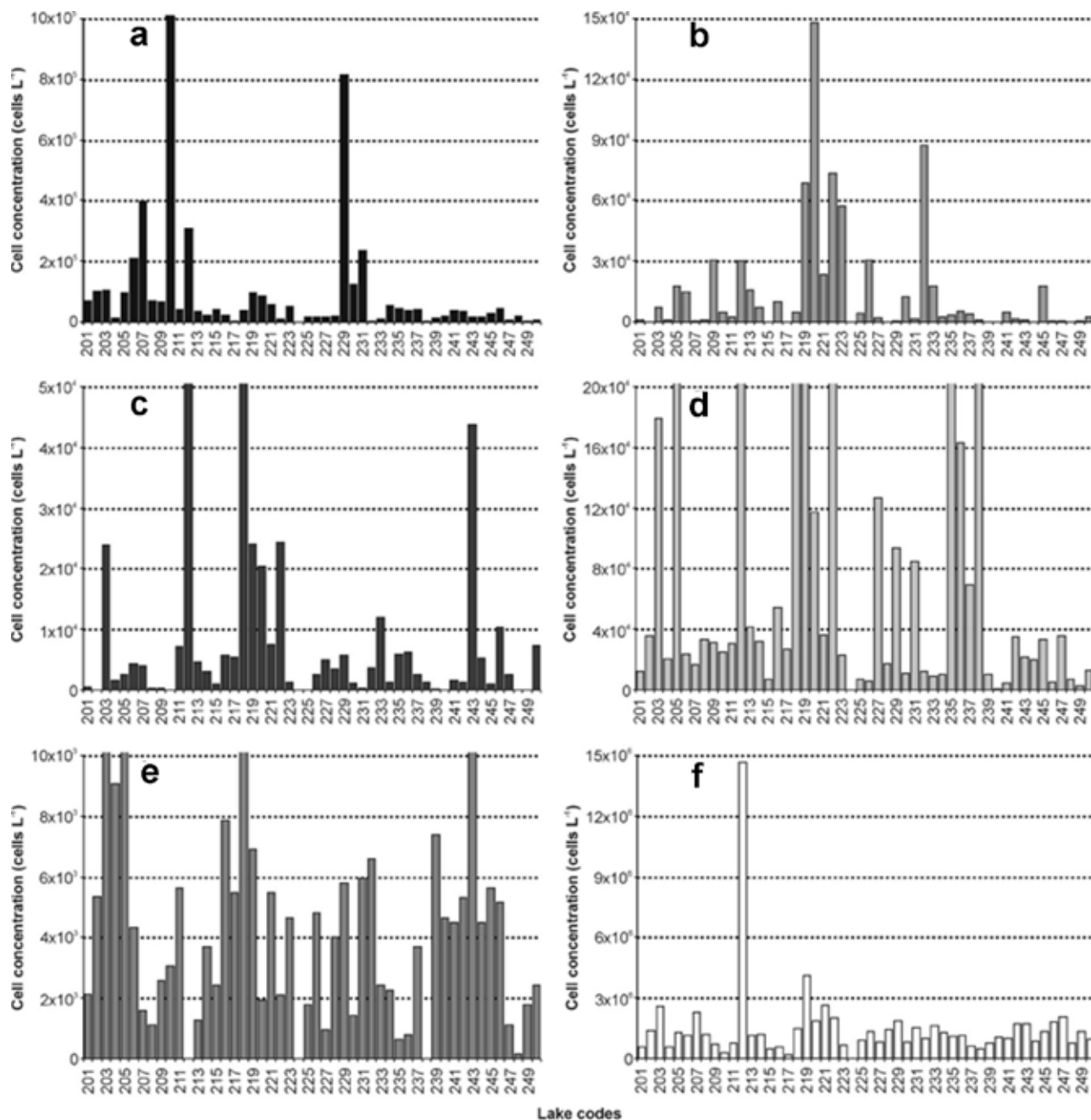
The temporal and spatial distribution of those taxa was investigated (Table 3.5). In time, the abundances of microflagellates and cyanobacteria were significantly greater in June and July, respectively (Kruskal-Wallis,  $p < 0.05$ ,  $H > 8.20$ ,  $df = 2$ ). In space, no significant relationship was obtained between the taxa abundances and their distribution in the three catchment zones (Kruskal-Wallis,  $p > 0.10$ ,  $H < 4.60$ ,  $df = 2$ ). Neither was a significant relationship found between the three sampling periods and phytoplankton richness (Kruskal-Wallis,  $p > 0.10$ ,  $H = 1.37$ ,  $df = 2$ ), but there was significantly greater richness in zone III than in zones I and II (Kruskal-Wallis,  $p < 0.05$ ,  $H = 7.83$ ,  $df = 2$ ). The greater phytoplankton richness recorded in cluster D compared to cluster B was moderately significant (Kruskal-Wallis,  $p < 0.10$ ,  $H = 9.45$ ,  $df = 4$ ,  $n = 49$ , lake from cluster F excluded).

**Table 3.5. Pearson's chi-square values obtained from the contingency tables drawn to determine patterns in the distribution of the phytoplankton groups in space and time (df = 2, n = 50).**

	Time period	Zonation group
Flagellates	7.38*	0.18
Green algae	4.40	0.52
Ciliates	1.17	0.18
Cyanobacteria	7.70*	1.14
Diatoms	0.63	0.79
Microflagellates	1.11	0.18

\* indicates a significant relationship at the 0.05 level.

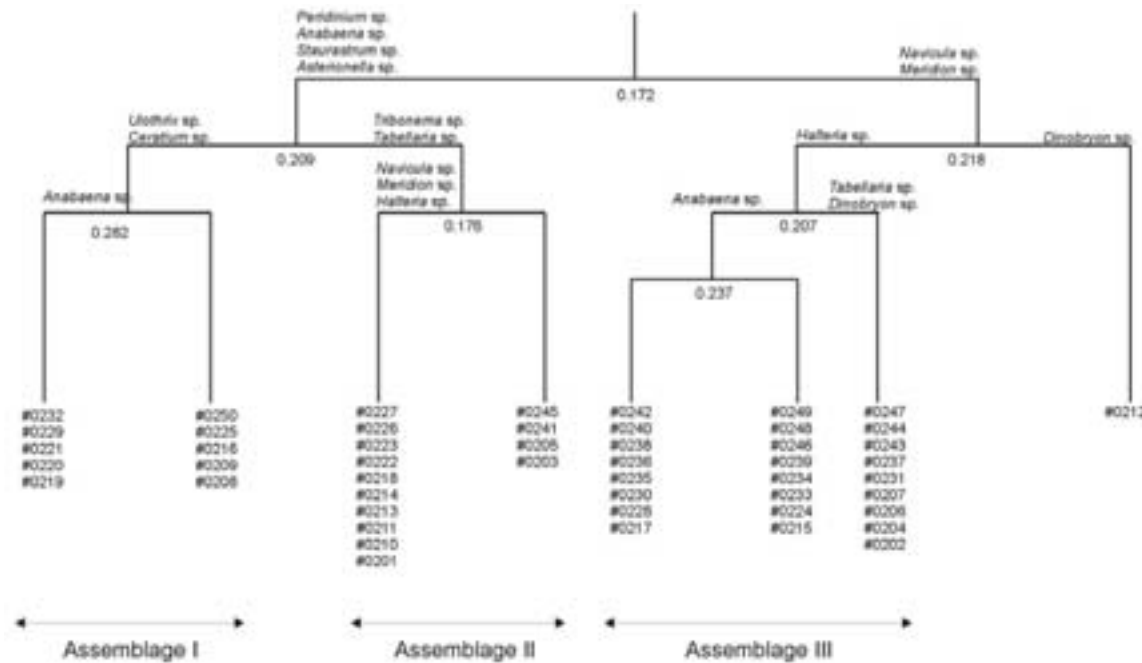




**Figure 3.9. Abundances of the main phytoplankton taxonomic groups determined in the 50 surface water samples collected in the lakes sampled during summer 2009: flagellates (a), cyanobacteria (b), chlorophytes (c), diatoms (d), ciliates (e), microflagellates (f).**

The data set was subjected to a multivariate analysis to identify structural patterns and similarities in the phytoplankton communities of the lakes sampled. The hierarchical classification of the sites performed by TWINSpan clustered the lakes into three main assemblages based on phytoplankton community composition (Fig. 3.10). The initial TWINSpan division separated lakes containing the indicator taxa *Peridinium*, *Anabaena*, *Staurastrum* and *Asterionella* on

the left side of the dendrogram from those containing the indicators *Navicula* and *Meridion* to the right. The second division on the left side of the dendrogram separated the lakes into two main assemblages (I and II), containing the indicator taxa *Ulothrix* and *Ceratium* on the one hand, and *Tribonema* and *Tabellaria* on the other. Assemblage II was subdivided into two groups based on the presence/absence of the indicators *Navicula*, *Meridion* and *Halteria*. The second division on

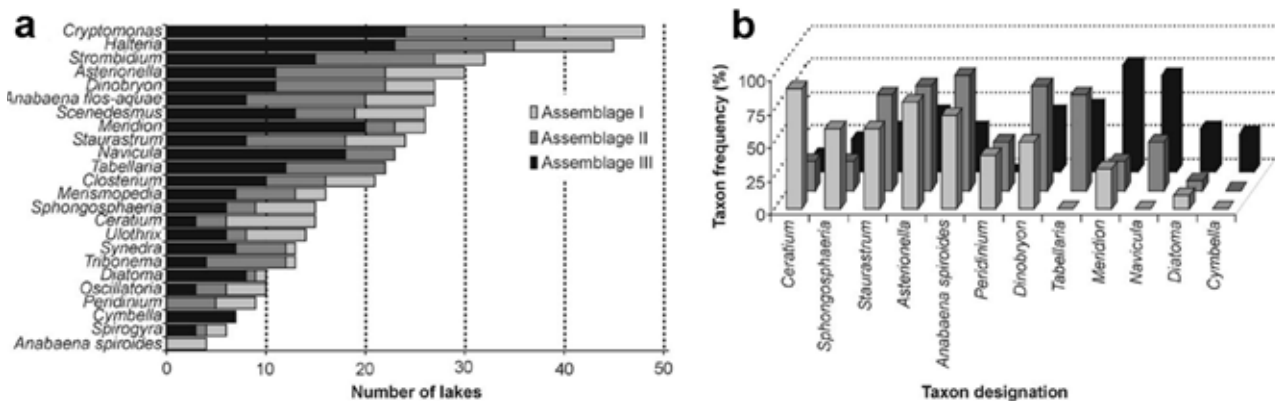


**Figure 3.10.** Groups of lakes identified by Two-Way Indicator Species Analysis (TWINSpan). Eigenvalues are shown at each level together with the indicator taxa.

the right side of the dendrogram-isolated Cross Lake, with the indicator *Dinobryon*, from the rest of the lakes constituting assemblage III and containing the indicator taxon *Halteria*. Subdivision in assemblage III was based on the presence of *Anabaena* in the 16 lakes of the first group, as opposite to the presence of the indicator taxa *Tabellaria* and *Dinobryon* in the 9 lakes from the second group.

Figure 3.11 shows a range of taxa that occurred with varying frequencies across the three assemblages. The dinoflagellate *Ceratium* and cyanobacterium *Sphongosphaerium* occurred more frequently in

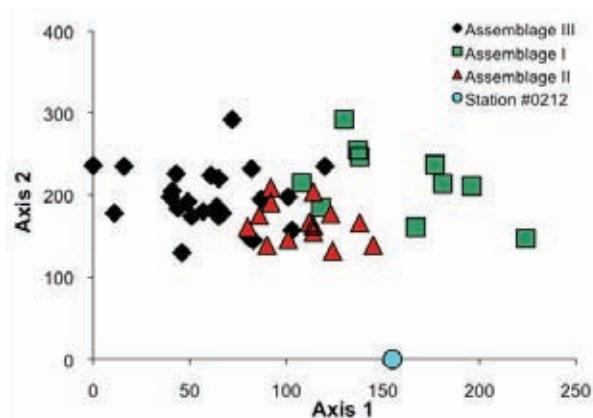
assemblage I. The lakes grouped in assemblage II contained, along with *Dinobryon*, less cyanobacteria and more diatoms (mostly *Tabellaria* and *Tribonema*). The taxa that occurred the most in assemblage III were essentially diatoms (*Cymbella*, *Diatoma*, *Meridion* and *Navicula*), whose frequency of occurrence was greater in assemblage III (Kruskal-Wallis,  $p < 0.05$ ,  $H = 6.34$ ,  $df = 2$ ,  $n = 49$ , lake 0212 excluded). About ~75% of the individual taxa in the lakes grouped in Assemblage III were less abundant than in Assemblages I and II. An assessment of variations in the distribution of the broad phytoplankton taxonomic units among the three assemblages showed that a significant difference was



**Figure 3.11.** Occurrence frequency of selected phytoplankton taxa in the 2009 sampled lakes (a). Occurrence frequency of selected taxa in lakes from the three assemblages defined by Two-Way Indicator Species Analysis (TWINSpan) analysis (b).

found only for cyanobacteria, with a lesser abundance in assemblage III (Kruskal-Wallis,  $p < 0.05$ ,  $H = 16.6$ ,  $df = 2$ ,  $n = 49$ , lake 0212 excluded).

The determination of the presence of relationships between lake groups, based upon membership of the three assemblages defined through the phytoplankton community, trophic state, meteorological, temporal and spatial patterns was carried out using contingency tables. A significant relationship was found only for time ( $X = 13.98$ ,  $p < 0.05$ ,  $df = 4$ ,  $n = 49$ , Cross Lake excluded). Relationships were investigated further through data ordination of the lakes and phytoplankton community composition data via detrended correspondence analysis (DCA). The eigenvalues obtained were 0.216, 0.140, 0.110 and 0.084 for axes 1–4, respectively. The first two axes represented the greatest amount of variation with the clustering of stations along axis 1, in particular, reflecting the associations made in TWINSpan (Fig. 3.12). Non-parametric Spearman correlations were performed between the sample scores on the first axis and several variables, including temperature, chlorophyll-*a*, Secchi depth and percentage of agriculture, wetland and forestry cover of the river catchments harbouring the lakes sampled (Table 3.6). Significant positive correlations were observed when plotting axis 1 values against temperature and river catchment surface covered with forest landscape (Fig. 3.13).

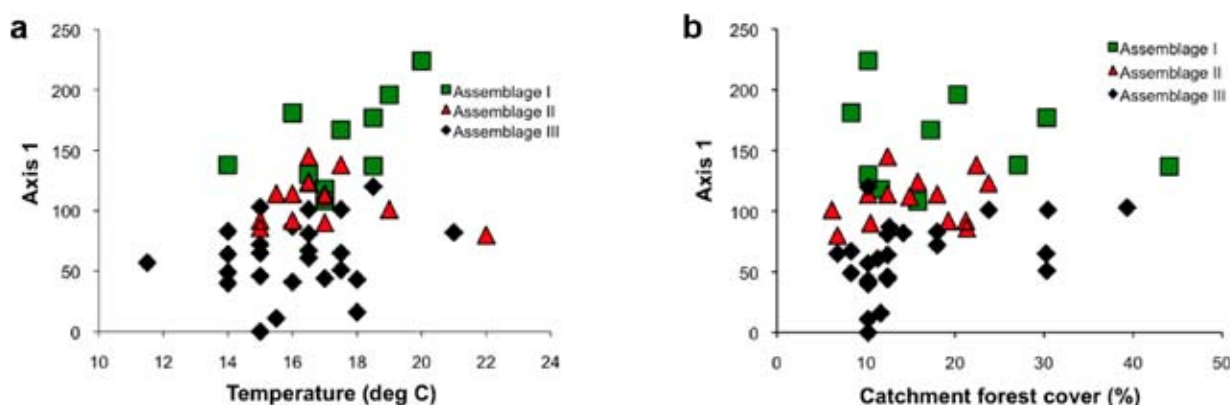


**Figure 3.12.** Detrended correspondence analysis (DCA) scatterplot of axes 1 and 2 reflecting the groups of lakes identified by Two-Way Indicator Species Analysis (TWINSpan).

**Table 3.6.** Spearman correlation coefficient and 2-tail significance of the relationships between the detrended correspondence analysis (DCA) axis 1 and selected variables.

Variable	<i>r</i>	<i>p</i>
Temperature	0.368	0.004**
Chlorophyll- <i>a</i>	0.141	0.165
Agricultural type cover	-0.193	0.090
Forestry type cover	0.330	0.010**
Wetland type	0.131	0.183
Secchi depth	-0.094	0.307

\* and \*\* indicate significant relationships at the 0.05 and 0.01 levels, respectively.

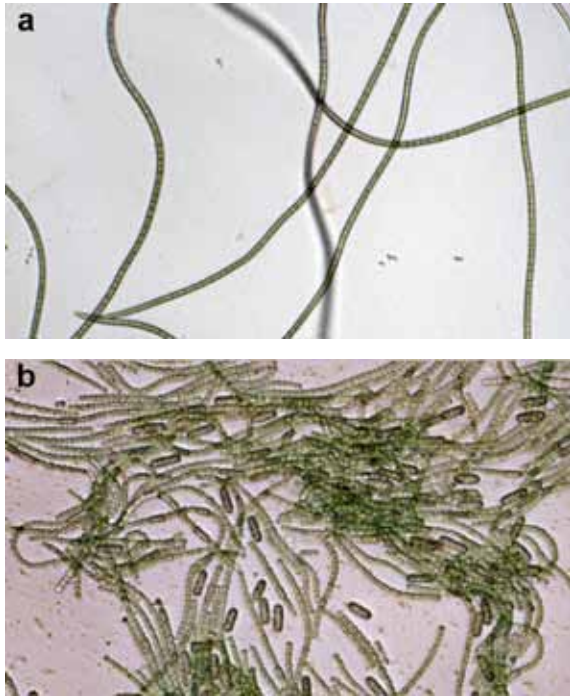


**Figure 3.13.** Scatterplots showing the distribution of the lake assemblages identified by Two-Way Indicator Species Analysis (TWINSpan) (Cross Lake excluded) along the detrended correspondence analysis (DCA) axis 1 and temperature (a), and the forest cover percentage of the river catchments containing the lakes sampled (b).



### 3.4.2 Distribution of Toxinogenic Cyanobacteria

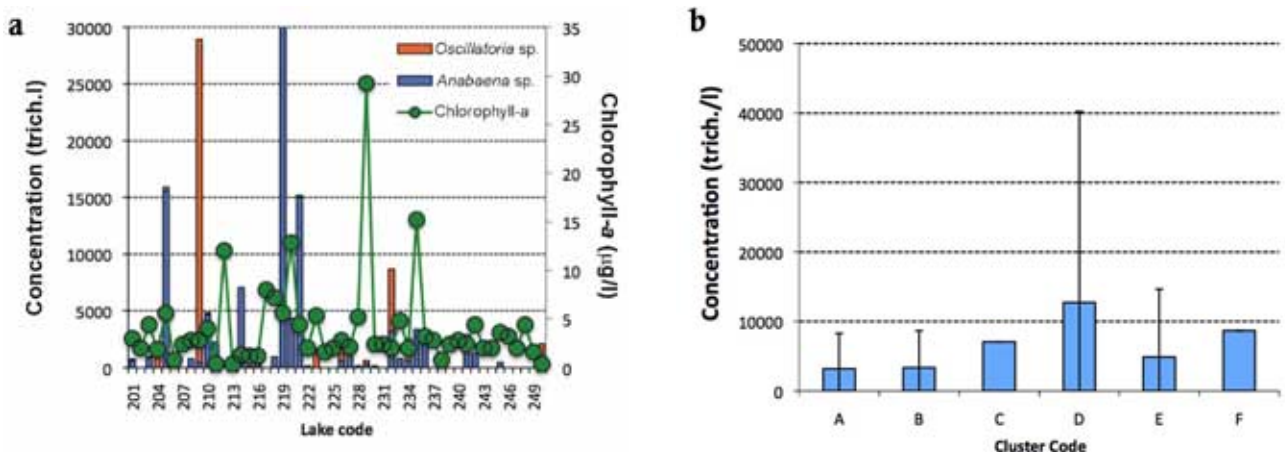
The toxigenic cyanobacterial genera *Oscillatoria* sp. and *Anabaena* sp. were identified and enumerated by light microscopy in preserved water samples collected from each sampled lake (Fig. 3.14). About 65% of the lakes contained either *Oscillatoria* sp. or



**Figure 3.14.** Micrographs of live *Oscillatoria* sp. (a) and *Anabaena* sp. (b) trichomes isolated from Irish lakes ( $\times 200$  magnification).

*Anabaena* sp. trichomes, and  $\sim 25\%$  contained both types of organism. The frequencies of occurrence of *Oscillatoria* sp. and *Anabaena* sp. in the lakes sampled were 35% and 55%, respectively. The maximum trichome concentration was found in Lough Carrowmore with  $\sim 40 \times 10^3$  trichomes. $\text{l}^{-1}$  of *Anabaena* sp. Cumulated concentrations of *Oscillatoria* sp. and *Anabaena* sp. greater than 4000 trichomes/ $\text{l}$  were found in eight lakes: Lough Derg (cluster E), Carrowmore Lough (cluster D), Lough Lickeen (cluster A), Knocka Lough (cluster A), Lough Graney (cluster B), Doo Lough (cluster C), Glencar Lough (cluster A) and Lough Glinn (cluster B) (Fig. 3.15).

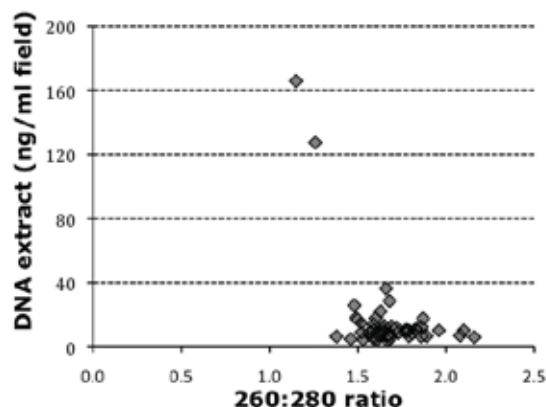
Even at high concentrations, the concentration of *Anabaena* sp. and *Oscillatoria* sp. contributed in minor proportions to the phytoplankton biomass, as suggested by the corresponding chlorophyll-*a* profile. Given the high variability in cell abundances, there was no significant difference in trichome concentrations in the lakes sampled in both time and space (Kruskal-Wallis,  $p > 0.10$ ,  $H < 4.60$ ,  $df = 2$ ). This was also apparent when comparing the average cumulated *Anabaena* sp. and *Oscillatoria* sp. trichome concentrations amongst clusters, with the high level found in Lough Carrowmore, in particular, contributing to the high standard deviation observed in cluster D.



**Figure 3.15.** *Oscillatoria/Anabaena* trichome concentrations and chlorophyll-*a* levels recorded in the sampled lakes over time (a), and average trichome concentrations in clusters of river catchments (b). Error bars denote standard deviation.

### 3.5 Molecular Analysis of Cyanobacteria: Regional Diversity

#### 3.5.1 Quality and Quantification of Nucleic Acids Extracted from Field Samples



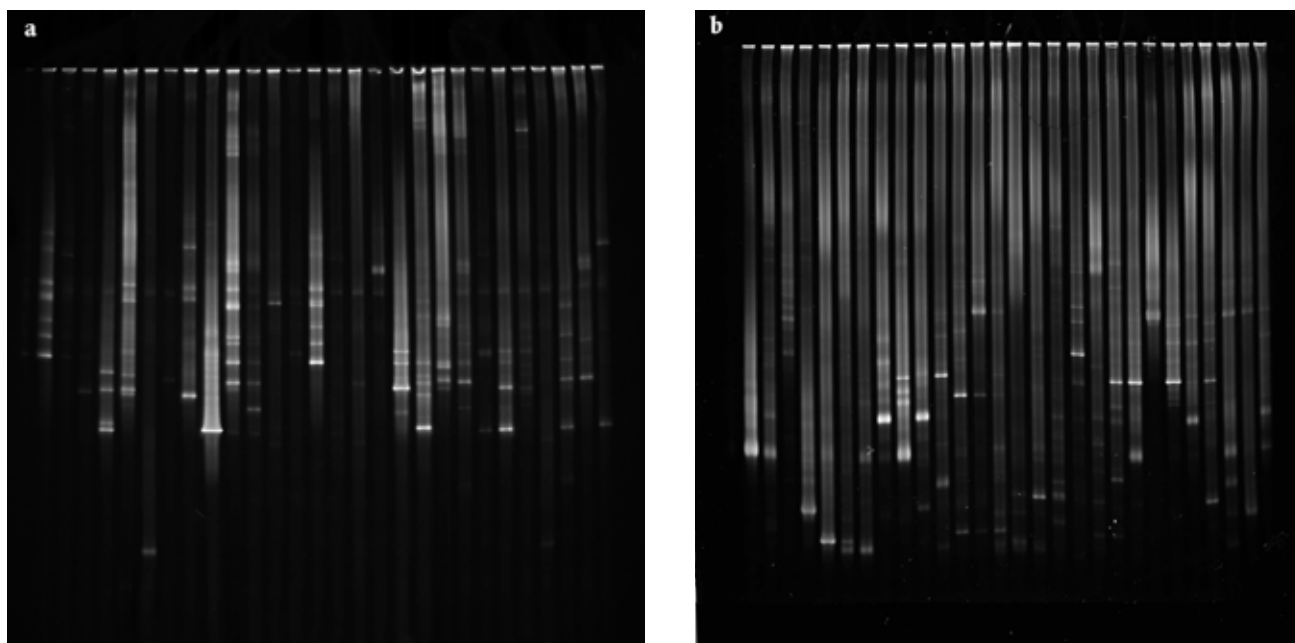
**Figure 3.16.** Scatter plot showing the relationship between DNA extract quality and concentration obtained in the 50 field samples collected during summer 2009.

Total genomic DNA was extracted from each sample and quality and concentration assessed using a Nanodrop. The quality of the nucleic acid extracts was ascertained using the 260:280 absorbance ratio. The average ratio value was 1.68 (s.d. = 0.19,

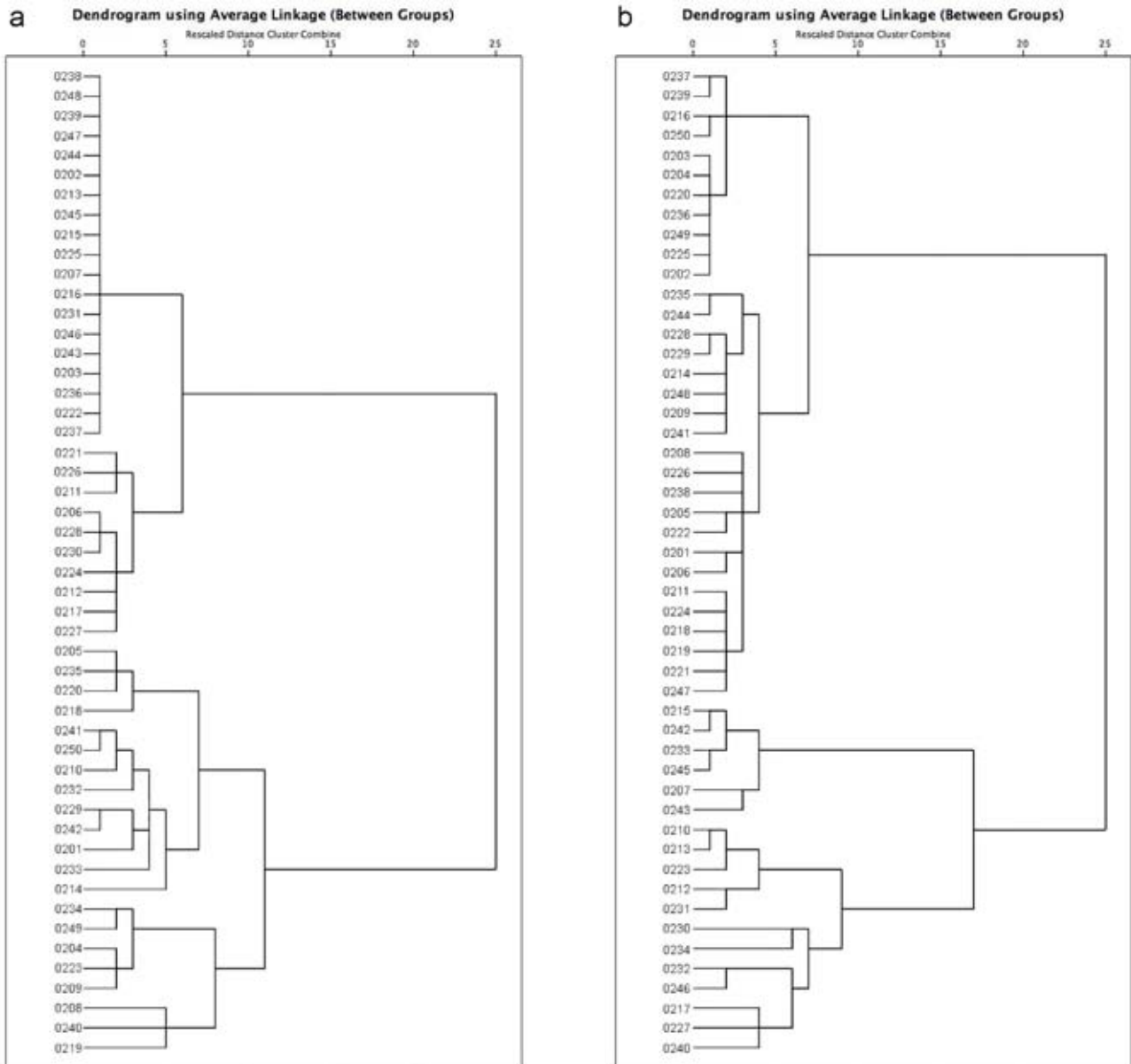
$n = 50$ ). About 30% of the extracts were of relatively good quality ( $> 1.75$ ), whereas 55% and 15% were of intermediate (between 1.50 and 1.75) and low quality ( $< 1.5$ ), respectively. The average amount of DNA retrieved from the filtered water samples was  $1.89 \mu\text{g}$  (s.d. = 0.64,  $n = 50$ ). Two samples (Cross Lake and New Lake), showing high concentration and poor extract quality were clearly discernible ([Fig. 3.20](#)).

#### 3.5.2 Analysis of Molecular Diversity and Community Structure of Cyanobacterial by DGGE

A segment of the cyanobacterial 16S rRNA gene was amplified from field extracts using a nested PCR approach and specific primers. Two reactions were carried out following the primary PCR to preferentially amplify filamentous and spherical cyanobacteria, respectively. Electrophoretic separation of amplicons was carried out by DGGE and showed great variation in profiles, both in pattern complexity and band intensity ([Fig. 3.17](#)). Grouping of lakes was carried out based on hierarchical clustering of the similarity matrices reflecting the band migration profiles. Samples were segregated into two main constitutive groups both for filamentous and spherical cyanobacterial communities ([Fig. 3.18](#)).



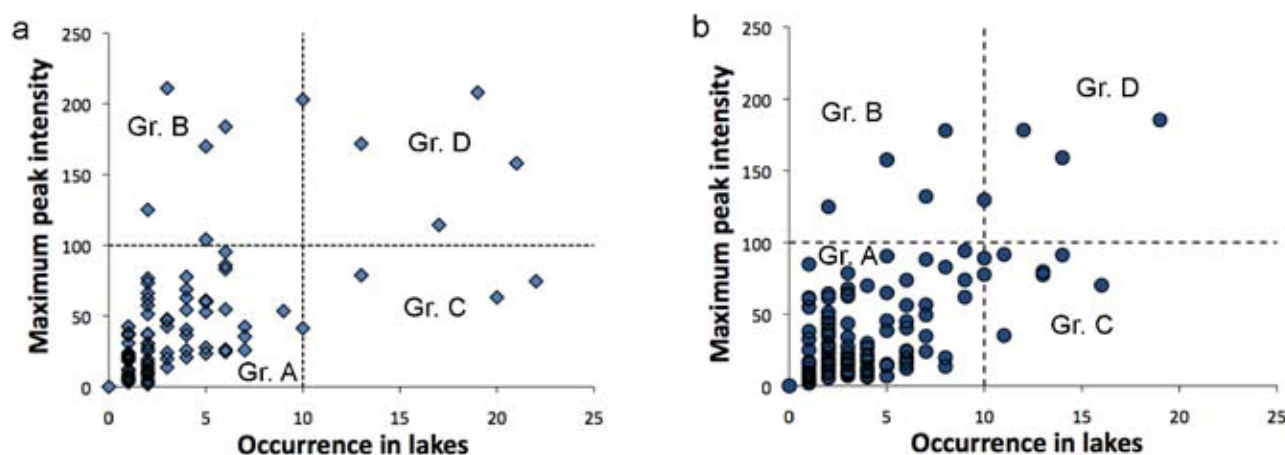
**Figure 3.17.** Examples of partial 16S rDNA DGGE migration patterns obtained for filamentous (a) and spherical (b) cyanobacteria. Each lane displays the community recorded for a particular lake sampled during summer 2009.



**Figure 3.18. Dendrogram showing the clustering of the lakes sampled during summer 2009 according to their similarity in filamentous (a) and spherical (b) cyanobacteria community composition.**

The number of DGGE bands visible provided an estimate of species richness, which varied for filamentous and spherical cyanobacteria from 1 to 23 and 4 to 24 bands, respectively. The Shannon-Weaver diversity index was also determined based on the relative intensity of the bands for each sample and was on average significantly greater for spherical cyanobacteria (av. = 1.84, s.d. = 0.40) than for filamentous types (av. = 1.42, s.d. = 0.71) (Mann-Whitney,  $p < 0.01$ ). Band-based species richness and Shannon-Weaver index were positively correlated for both cyanobacterial morphotype categories (Spearman,  $p < 0.001$ ,  $r_{\text{filamentous}} = 0.94$ ,  $r_{\text{spherical}} = 0.72$ ). The presence

of significant patterns in the distributions of richness and total cyanobacterial abundance, estimated through cumulated band intensity for each sample, was investigated in time, space and according to the weather conditions at the time of sampling. No significant relationship was found for filamentous cyanobacteria (Kruskal-Wallis,  $p > 0.10$ ,  $H < 4.60$ ,  $df = 2$ ). However, the richness of spherical cyanobacteria was significantly lesser in August than in June and July (Kruskal-Wallis,  $p < 0.10$ ,  $H < 4.60$ ,  $df = 2$ ). Even though the cumulated band intensities obtained for the lakes sampled in August were in general also lower, this was not supported statistically. However, cumulated band intensities were



**Figure 3.19.** Segregation of the denaturant gradient gel electrophoresis (DGGE) bands for both filamentous (a) and spherical (b) cyanobacteria according to their maximum intensity and frequency of occurrence in the lakes sampled during summer 2009.

significantly greater when sampling was carried out under fair weather conditions (Kruskal-Wallis,  $p < 0.05$ ,  $H = 6.28$ ,  $df = 2$ ).

The occurrence frequency of individual cyanobacterial bands in the DGGE gels and their peak intensities were also considered. [Figure 3.19](#) shows the segregation of bands for both filamentous and spherical cyanobacteria into four groups: rare bands of low intensity (Gr.A), rare bands of high intensity (Gr.B), common bands of low intensity (Gr.C) and frequent bands of high intensity (Gr.D). No significant pattern was apparent in the distribution of those groups in space and time for both filamentous and spherical cyanobacteria (Kruskal-Wallis,  $p > 0.10$ ,  $H < 4.60$ ,  $df = 2$ ). However, for spherical cyanobacteria only, the peak heights of bands from

Gr.D were significantly lower when rainfall events coincided with the time of sampling (Kruskal-Wallis,  $p < 0.05$ ,  $H = 7.39$ ,  $df = 2$ ).

Individual bands from groups B, C and D were successfully excised from the gels and re-amplified prior to purification and sequencing. The occurrence of these indicator bands was assessed in relation to various environmental descriptors using Fisher's exact test ([Table 3.7](#)). In particular, bands 64, 78, 80, 83 and bands 55, 66, 72, 78, 79, for spherical and filamentous cyanobacteria, respectively, showed some significant correlations with the descriptors pertaining to cyanobacterial richness, the time of the summer, temperature, weather conditions and catchment forest cover.

**Table 3.7.** Significant correlation (2-tail Fisher's exact test) between the presence of a selected band within a gel pattern and a qualitative descriptor.

Band code	Descriptors					
	Cyanobacteria richness	Summer period	Lake temperature	Weather conditions	Catchment forest cover	16S rDNA identification (% similarity)
Bd64	-	-	-	0.000	-	Chroococcales
Bd78	-	-	0.001	-	-	Chroococcales
Bd80	-	-	0.040	-	0.010	Chroococcales
Bd83	-	0.020	-	0.050	0.050	Oscillatoriales*
Bd55	-	0.004	-	-	0.050	Nostocales
Bd72	0.000	-	-	-	-	Nostocales
Bd78	0.001	-	-	-	0.009	Nostocales
Bd79	0.020	-	-	-	-	Nostocales
Bd66	0.018	-	-	-	-	Nostocales

\* refers to a filamentous cyanobacterial morphotype which is closely related, in the 16S rDNA phylogeny found in [Fig. 3.22](#), to spherical genotypes.

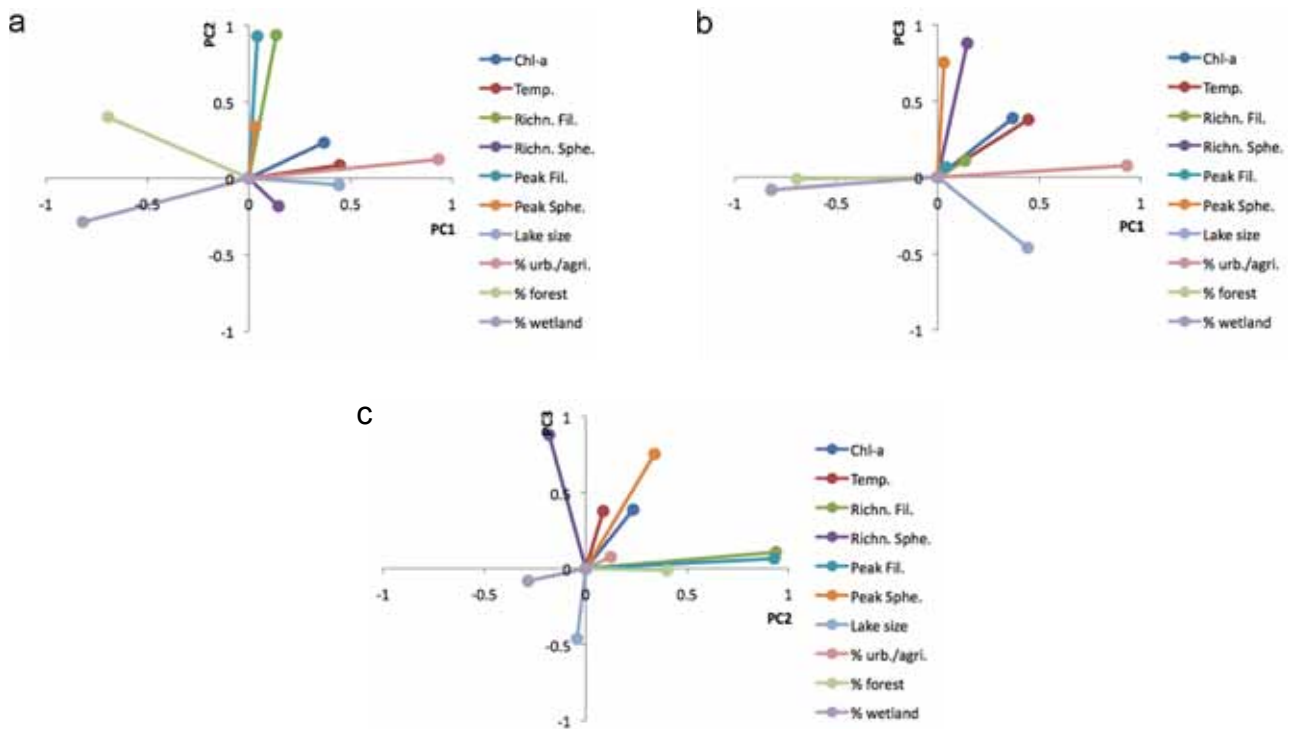
### 3.5.3 Assessment of Relationships between Cyanobacterial and Environmental Variables

Principal component analysis (PCA) was applied on the autoscaled summer 2009 data set, which resulted in the identification of three components that accounted for 67% of the total variance. The projections of the variable loadings on the principal components and the relationships between the ordination axes and individual environmental variables are shown in [Fig. 3.20](#).

Component 1 (PC1) was positively related to the proportion of river catchment covered by agricultural type surface, lake surface temperature, lake size and chlorophyll-a concentration while the proportions of river catchment covered by forested areas and wetland type surfaces scored negatively on the axis. Cumulated filamentous cyanobacteria DGGE band intensity, filamentous cyanobacteria richness and to a lesser extent proportions of river catchment covered by forested areas scored positively on component 2 (PC2). Only the cumulated DGGE band intensity of

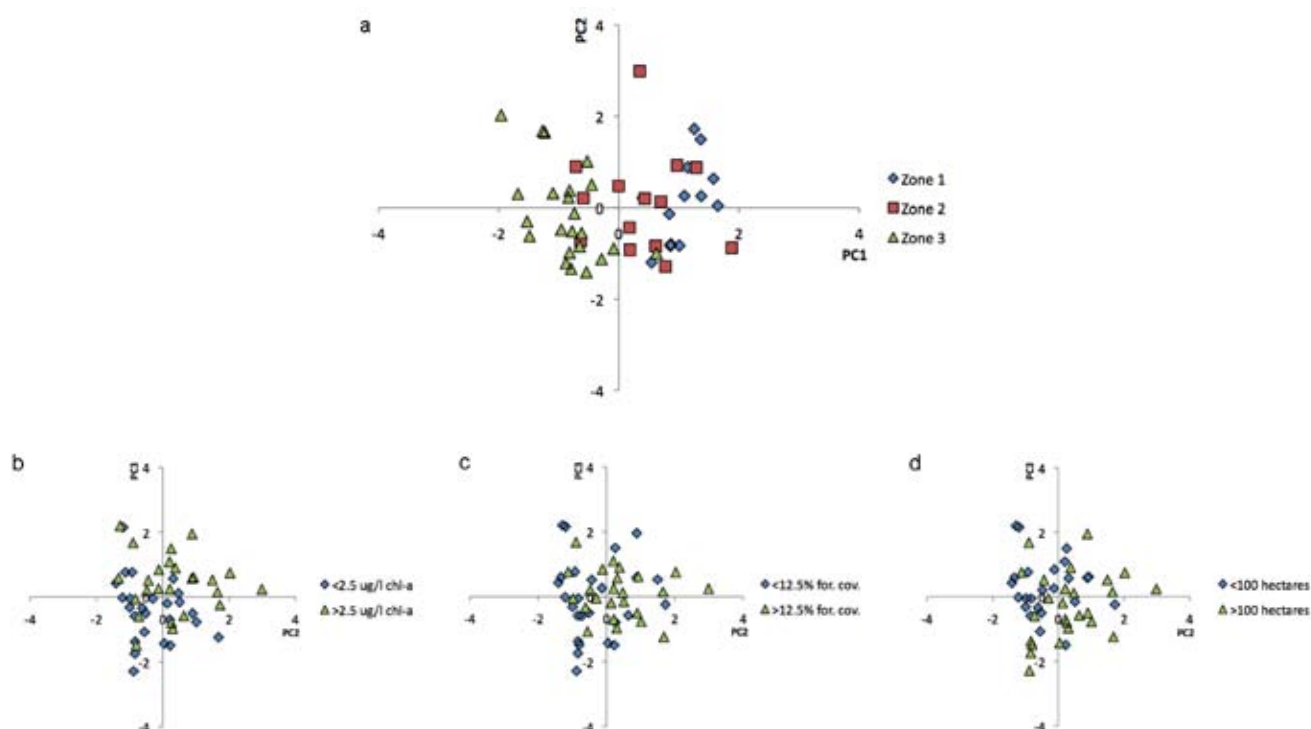
spherical cyanobacteria and spherical cyanobacteria richness contributed substantially to the positive domain of component 3 (PC3), while the size of the sampled lakes scored negatively.

The corresponding projected score plots for the individual water bodies along the component axes did reveal some clustering tendencies ([Fig. 3.21](#)). A land cover gradient reflecting the geographic delineation of zones I, II and III was clearly defined along PC1. In the plane defined by PC2 and PC3, representing spherical and filamentous cyanobacteria, respectively, partial segregation of the lakes was visible with respect to the trophic status of individual lakes, based on the distribution of chlorophyll-a concentrations. In addition, 16 out 25 lakes contained within a river catchment with a forest cover of at least 25% scored positively along PC2. Likewise, for this particular data set, it appeared that filamentous cyanobacteria occurred preferentially in sizeable water bodies as 17 lakes with a surface greater to 100 ha scored positively on PC2.



**Figure 3.20.** Projections along the principal components showing the distributions of the variables pertaining to the summer 2009 data set in the planes defined by PC1-PC2 (a), PC1-PC3 (b) and PC2-PC3 (c).





**Figure 3.21.** Sample projections along the principal components showing the distributions of data pertaining to chlorophyll-based trophic state, lake size, river catchment forest cover and the three ecoregions within the study area. (a) lake zonation in PC1-PC2 plane (b, c, d) chlorophyll-a distribution, forest cover and lake size in PC2-PC3 plane, respectively.

#### 3.5.4 Planktonic Cyanobacteria from Irish Lakes: Partial 16S rDNA Phylogenetic Inferences

Selected 16S rDNA fragment bands excised from the DGGE gels run with the summer 2009 field extracts, and successfully re-amplified by PCR, were used for phylogenetic analysis. Following PCR amplification, the products were purified and sequenced using a commercial service provider (MWG Biotech, Germany). The returned sequences of putative spherical and filamentous cyanobacteria were then subjected to BLAST analysis for an initial diagnosis of identity. The positions of the isolates in the 16S rDNA phylogeny of cyanobacteria were then ascertained. Maximum likelihood (ML) analysis was carried out using PAUP\* version 4.b with the parameters derived from the AIC in Modeltest to construct the phylogenetic tree (Fig. 3.22). The data set relative to the 16S rDNA alignment contained 88 taxa. Of the 382 characters, 186 were constant sites, 45 were variable parsimony-

uninformative and 151 parsimony-informative. One most likely tree was returned from the ML analysis with a score of  $-\ln 6750.32$ . The unresolved 'Oscillatoriales-V' clade diverged first and contained genera such as *Phormidium* and *Tychonema*. Thereafter, the topology of the tree displayed two main lineages. The first lineage supported the 'Oscillatoriales-IV' clade and the two sister assemblages 'Stigonematales' and 'Nostocales' clades, containing non-exhaustively the genera *Fischerella* and *Hapalosiphon* on the one hand, and *Anabaena*, *Nodularia*, *Nostoc* on the other. The second lineage supported the 'Pleurocapsales' clade and six groups of 'Oscillatoriales' and 'Chroococcales' interspersed into several clusters. In general, bootstrap values reinforced the intra-clade relationships between taxa, but groupings between the main assemblages were not well resolved. As anticipated, the sequences retrieved from Irish lake samples clustered within all the lineages except the 'Pleurocapsales' clade.



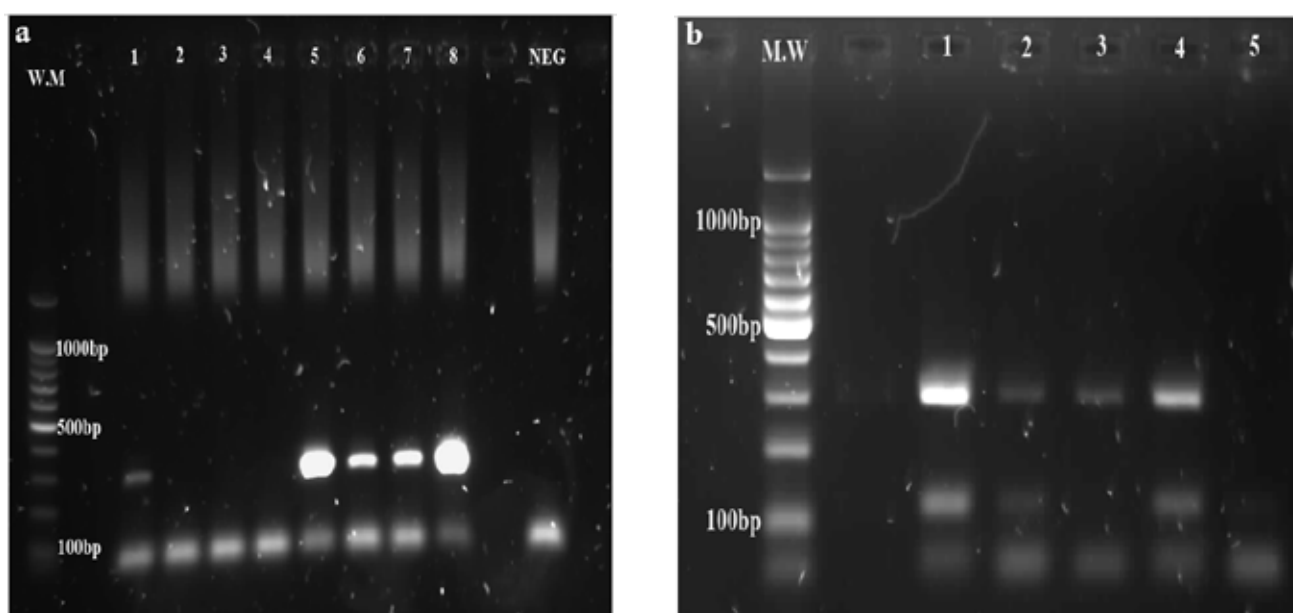
### 3.6 Cyanotoxins Detection in West and Northwest Irish Lakes

#### 3.6.1 Detection of Cyanotoxin Genes in Field Samples

The nucleic acid extracts obtained from the lakes sampled were subject to PCR analysis to determine the presence of toxigenic cyanobacteria potentially synthesising cyanotoxins. PCR products were successfully amplified for microcystin genes only. Bands of the expected size (~300 bp) were observed for 18 out of 50 samples, and varied in intensity (Fig. 3.23). Double digestion was carried out on the amplicons using the restriction enzymes *Rsa.I* and *Hha.I*, producing a number of RFLP patterns.

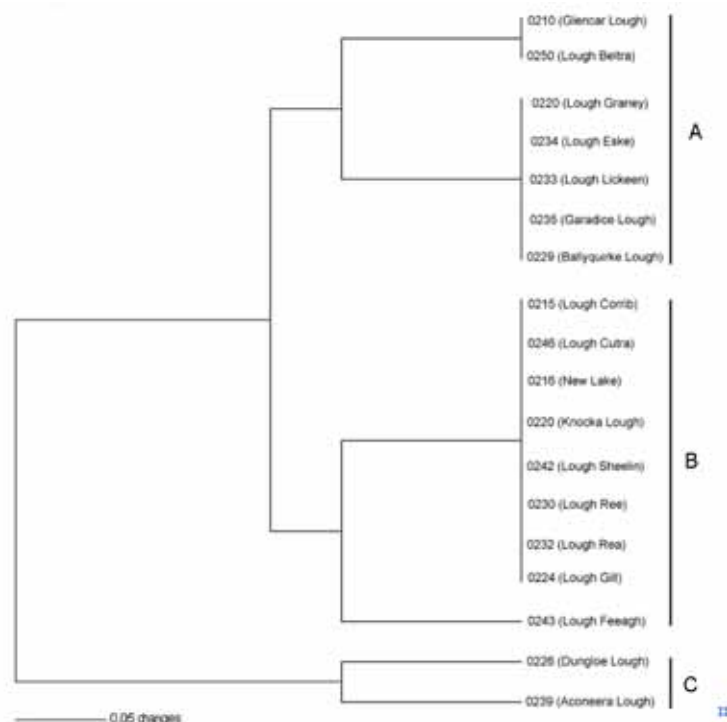
The restriction profiles were converted into a binary matrix and subsequent cluster analysis defined three

groups A, B and C of water bodies containing 7, 9 and 2 lakes, respectively (Fig. 3.24). Contingency tables showed there were no significant relationships between the clustering of lakes based on their PCR restriction profiles and the time period during which they were sampled, or their distribution in the three identified longitudinal ecoregions ( $X < 6.11$ ,  $p = 0.05$ ,  $df = 4$ ,  $n = 18$ ). However, focusing on the samples of clusters A and B only, the cumulated abundances of *Anabaena* and *Oscillatoria* were significantly lesser in cluster B than in cluster A (Mann-Whitney,  $p = 0.04$ ,  $n = 16$ ). This was further corroborated using the DGGE-derived data pertaining to the diversity and abundance of filamentous cyanobacteria (Mann-Whitney,  $p = 0.001$ ,  $n = 16$ ). A reverse pattern was found for spherical cyanobacteria but with no statistical support (Mann-Whitney,  $p = 0.462$ ,  $n = 16$ ).



**Figure 3.23.** *Mcy-A* PCR and RFLP analysis of field sample extracts. 1% agarose gel showing the *mcy-A* amplicons with lanes 1-8 corresponding to samples 0210-0213-0218-0219-0220-0221-0229-0233, respectively (a). 2% agarose gel showing the restriction profiles obtained for samples 0233 (1), 0221 (2), 0229 (3), 0220 (4) and 0210 (5), respectively (b).





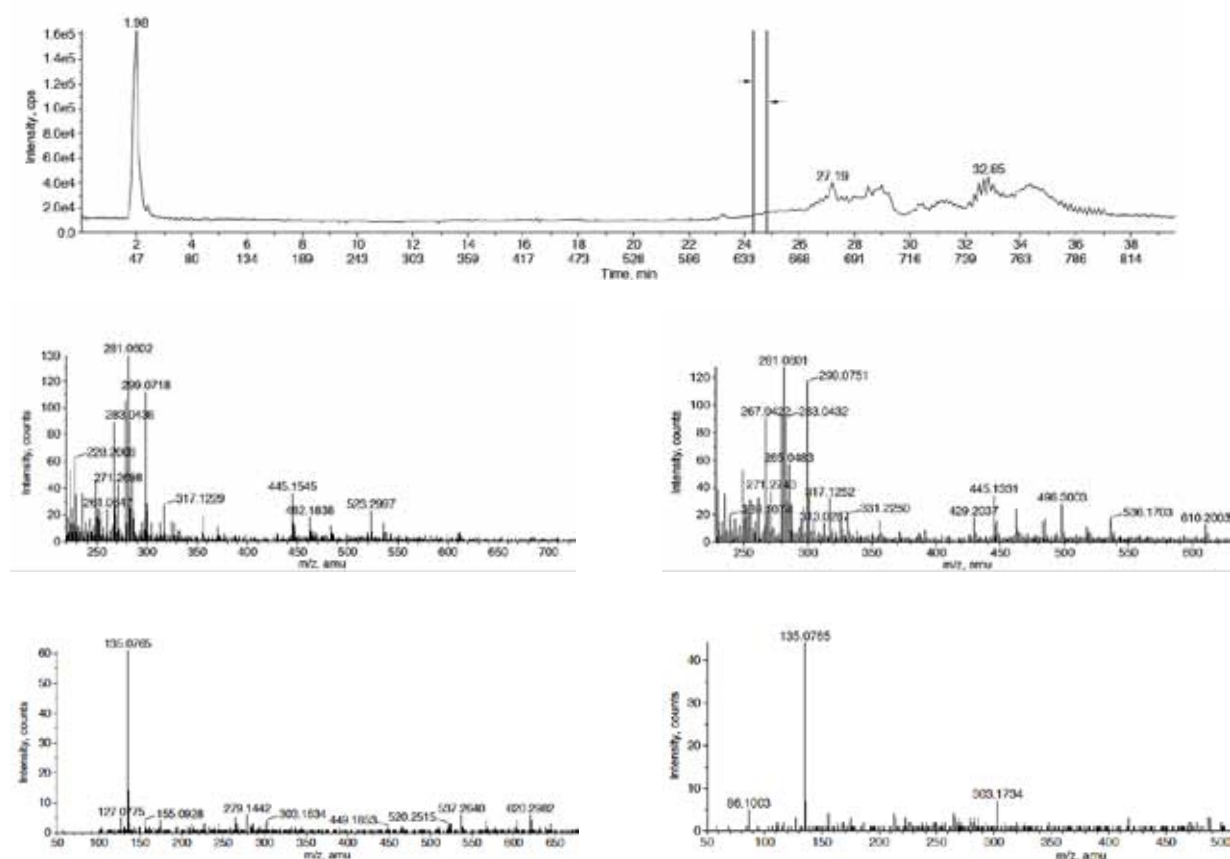
**Figure 3.24. Un-weighted pair group method with arithmetic averages (UPGMA) dendrogram generated in PAUP based on the restriction profile analysis of the polymerase chain reaction (PCR) amplified *mcy-A* gene fragments from the water extracts obtained during summer 2009.**

### 3.6.2 Detection of Microcystins in Field Samples by PP2A and Analytical Chemistry

All the methanolic extracts obtained from the filtered surface water samples were tested using PP2A. Inhibition was quantifiable in 12 extracts only, with a range of estimated concentration from 0.006 to 0.020  $\mu\text{g.l}^{-1}$  MC-LR eq. in the corresponding lakes, values well below the recommended WHO limit of 1  $\mu\text{g.l}^{-1}$ . The highest concentrations were determined in the methanolic extracts from loughs Graney, Sheelin and Lickeen. Contingency tables showed there were no significant patterns in space and time for the detection of microcystins in the lakes sampled ( $X < 5.06$ ,  $p = 0.05$ ,  $df = 2$ ). There was also no significant correlation between the MC-LR equivalent concentration estimates found from the water extracts with the trichome concentrations of *Anabaena* sp. and

*Oscillatoria* sp. determined in the corresponding water column samples ( $p = 0.32$ ,  $r = -0.15$ ,  $n = 12$ ). Only in 7 out of the 12 lakes where MC-LR activity was detected by PP2A was the *mcy-A* gene detected by PCR. Those were both from cluster A (Lough Graney, Lickeen, Garadice) and from cluster B (New Lake, Knocka Lough, Lough Ree, Lough Sheelin).

Confirmatory HPLC and MS/MS analysis was carried out on the methanolic extracts that proved positive by either *mcy-A* or PP2A analysis. The presence of microcystins was positively confirmed in only three extracts from Lough Lickeen, Garadice Lough and Lough Sheelin, all three both positive for PP2A and *mcy-A*. The variant microcystin LR (MC-LR) was identified in all the three lakes while MC-YR was additionally present in the Lough Sheelin extract (Fig. 3.25).



**Figure 3.25.** Chromatogram and mass spectra from the LC-MS/MS analysis of the Lough Sheelin (0246) methanolic surface water extract collected during August 2009 indicating the presence of both microcystin-LR (MC-LR) and microcystin-YR (MC-YR). The ion masses of the two toxins ( $m/z$  498.30 and 523.29, respectively) and of the characteristic -adda moiety ( $m/z$  135.07) are circled in the MS and MS/MS spectra, respectively.

### 3.7 Summer Dynamics of Cyanobacteria in Lakes from the West of Ireland (2010)

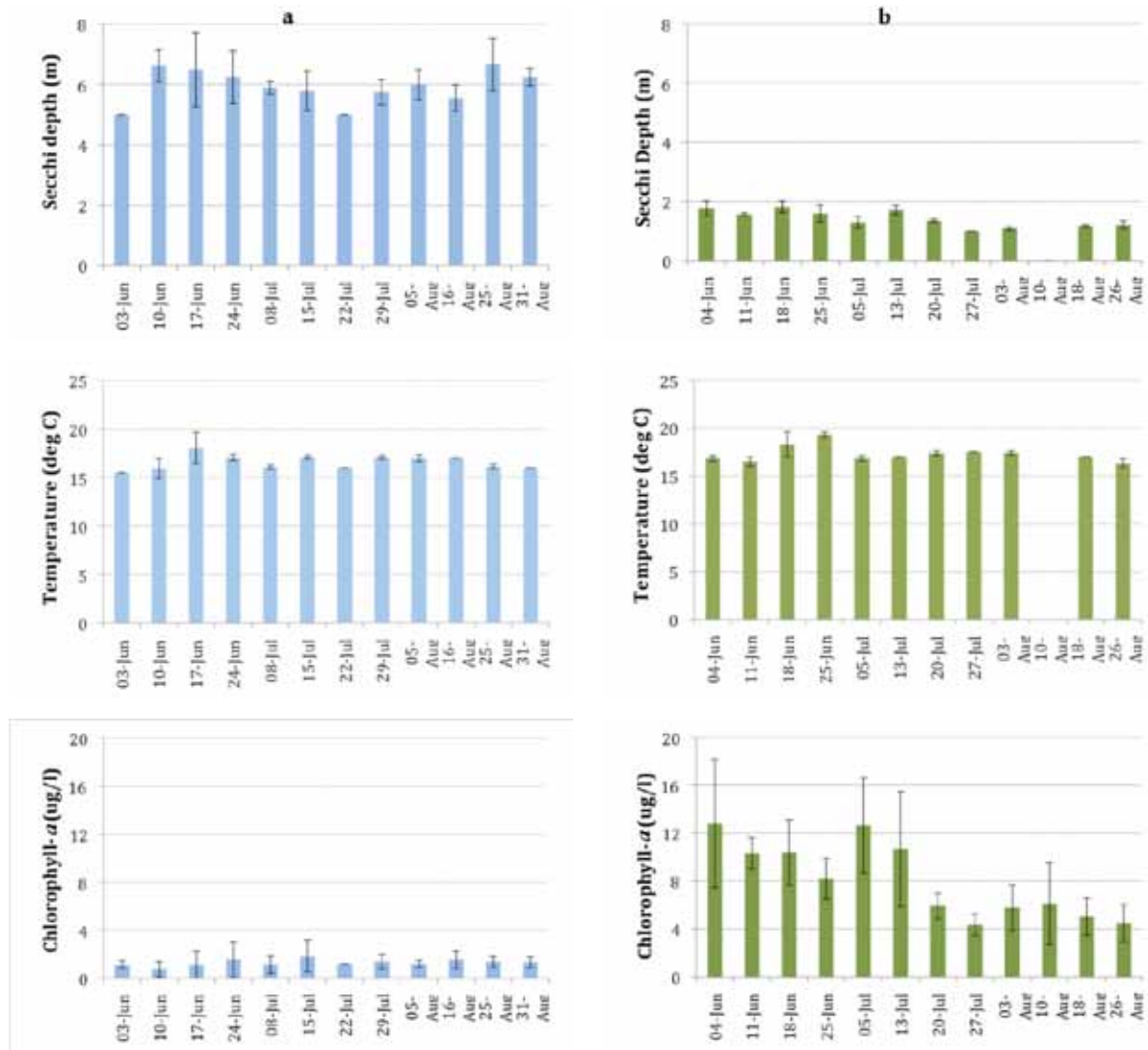
#### 3.7.1 Variation of Selected Environmental Variables

The physical parameters, surface temperature and light penetration in the water column were measured throughout summer 2010 in both Lough Corrib and Lough Ballyquirke, along with phytoplankton biomass estimated through chlorophyll-*a* level determination (Fig. 3.26). Information on the metrics relative to these parameters are presented in Table 3.8. The dynamics of the three variables were compared on a monthly basis for the two lakes. There was no significant difference in the average temperature and

chlorophyll-*a* concentration recorded in June, July and August in Lough Corrib (ANOVA,  $F < 1.01$ ,  $p > 0.37$   $n > 64$ ), whereas the Secchi depth was significantly lower in July than in June and August (ANOVA,  $F = 3.66$ ,  $p = 0.03$ ,  $n = 62$ ). In Lough Ballyquirke, the average surface temperature, Secchi depth and chlorophyll-*a* concentration were all significantly lower in August than in June and July (ANOVA,  $F > 5.82$ ,  $p < 0.01$ ,  $n > 43$ ). On a comparative basis, average temperatures and chlorophyll-*a* concentrations over the course of the summer were significantly greater in Lough Ballyquirke than in Lough Corrib (Student *t*-test,  $p < 0.04$ ,  $n = 23$ ). Average Secchi depth was also significantly lower in Lough Ballyquirke than in Lough Corrib (Student *t*-test,  $p < 0.01$ ,  $n = 23$ ).

**Table 3.8. Metrics pertaining to the surface environmental variables recorded in Lough Corrib and Ballyquirke Lough throughout summer 2010.**

	Temperature (°C)		Secchi depth (m)		Chlorophyll-a ( $\mu\text{g.l}^{-1}$ )	
	L. Corrib	L. Ballyquirke	L. Corrib	L. Ballyquirke	L. Corrib	L. Ballyquirke
Minimum	15.0	16.0	5.0	1.0	0.0	1.6
Maximum	20.0	20.0	8.0	2.0	4.9	18.4
Average	16.7	17.4	6.1	1.4	1.3	7.7
Std deviation	0.9	1.0	0.8	0.3	0.8	3.8



**Figure 3.26. Average weekly surface temperature, Secchi depth and chlorophyll-a levels determined during summer 2010 from sites in Lough Corrib (a) and Ballyquirke Lough (b). Error bars denote standard deviation.**

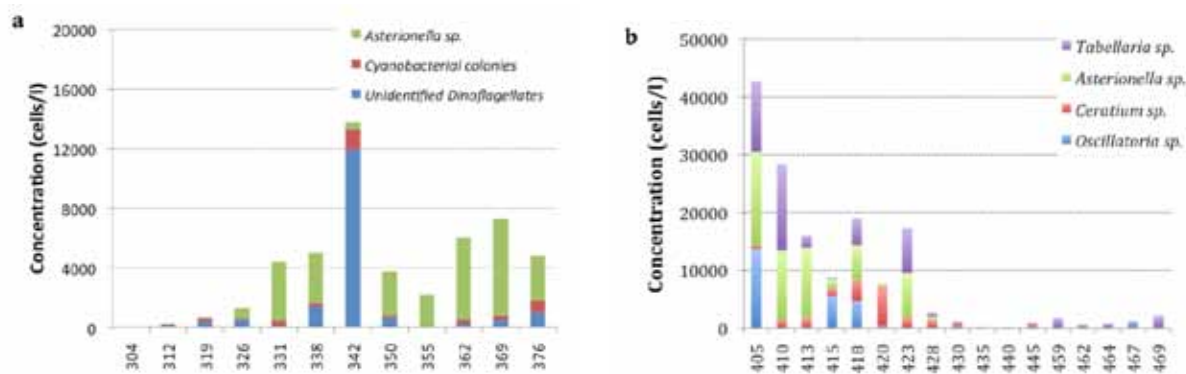


Figure 3.27. Microphytoplankton concentrations determined from surface samples in Lough Corrib (a) and Ballyquirke Lough (b) collected during summer 2010.

### 3.7.2 Phytoplankton Analysis and Relationships with Environmental Variables

Surface phytoplankton community dynamics were investigated in preserved water samples for both Lough Corrib and Ballyquirke Lough by light microscopy. Only the most abundant taxa that were recognisable at  $\times 100$  magnification were considered (Fig. 3.27). There was little microphytoplankton observed in the samples collected from Lough Corrib and only the following taxonomic units were enumerated: unidentified dinoflagellates, spherical cyanobacterial colonies and *Asterionella* sp. In general, *Asterionella* sp. was the most abundant and frequent taxon, with a maximum concentration of  $\sim 6,500$  cells  $l^{-1}$  achieved on 25 August (sample 369). However, unidentified dinoflagellates were numerically more abundant on 22 July (sample 342) with a maximum concentration of  $\sim 12,000$  cells  $l^{-1}$ . Phytoplankton concentrations reached greater levels in Lough Ballyquirke, where the taxa *Oscillatoria* sp., *Tabellaria* sp., *Ceratium* sp. and *Asterionella* sp. were

enumerated. The four taxa were relatively abundant with a cumulated average concentration of 20,000 cells  $l^{-1}$  until 5 July, after which the concentrations remained below 3,000 cells  $l^{-1}$ .

The relationships between phytoplankton concentrations and other environmental variables were assessed using Pearson's parametric correlation analysis for both lakes. Significant correlations were found between temperature and chlorophyll-*a* in Lough Corrib ( $p = 0.04$ ,  $r = 0.59$ ,  $n = 12$ ), and between chlorophyll-*a* and cumulated phytoplankton abundance for Ballyquirke Lough ( $p = 0.05$ ,  $r = 0.395$ ,  $n = 17$ ).

### 3.7.3 Water Column Profiling and Surface Nutrient Concentrations

Water column profiling was carried out at each station during summer 2010 using a YSI Pro Quatro instrument which was lowered overboard from the side of the sampling platform (Fig. 3.28).

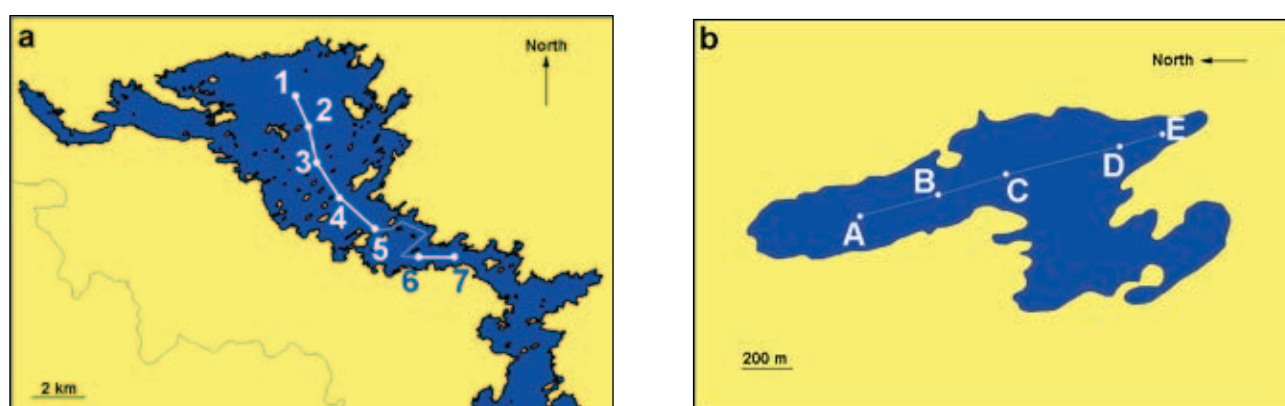
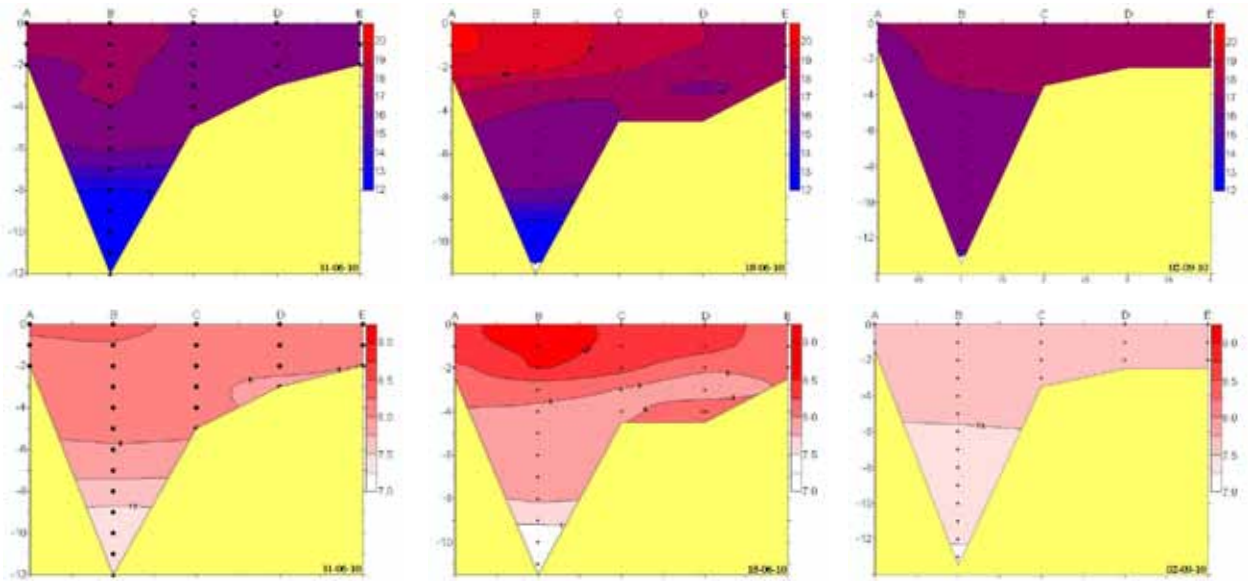


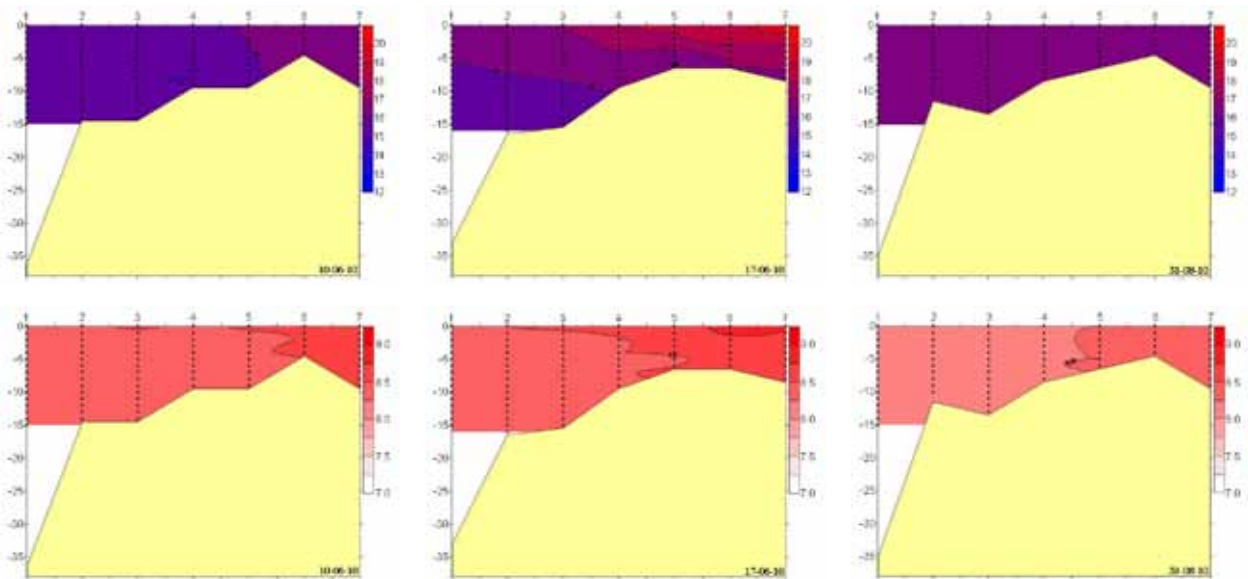
Figure 3.28. Maps of Lough Corrib (a) and Ballyquirke Lough (b) showing in each lake the locations sampled on a near-weekly basis during summer 2010.

The variables measured were dissolved oxygen, temperature, conductivity and pH. Thermal stratification was observed in both lakes on occasions depending on the meteorological conditions (Figs 3.29 and 3.30). Notably, the temperature levels in the upper water column of Ballyquirke Lough were always greater than those recorded for the same period in Lough Corrib. The temperature in Ballyquirke Lough near 10 m depth

was also cooler than that measured for Lough Corrib at similar depths. The conductivity values in Lough Corrib were significantly higher than those measured in Lough Ballyquirke (Student *t*-test,  $p < 0.001$ ) and in general greater in the domain extending from stations 1 to 4 (data not shown). The values recorded for pH and dissolved oxygen concentrations were positively correlated for both lakes (Pearson's,  $p = 0.001$ ,

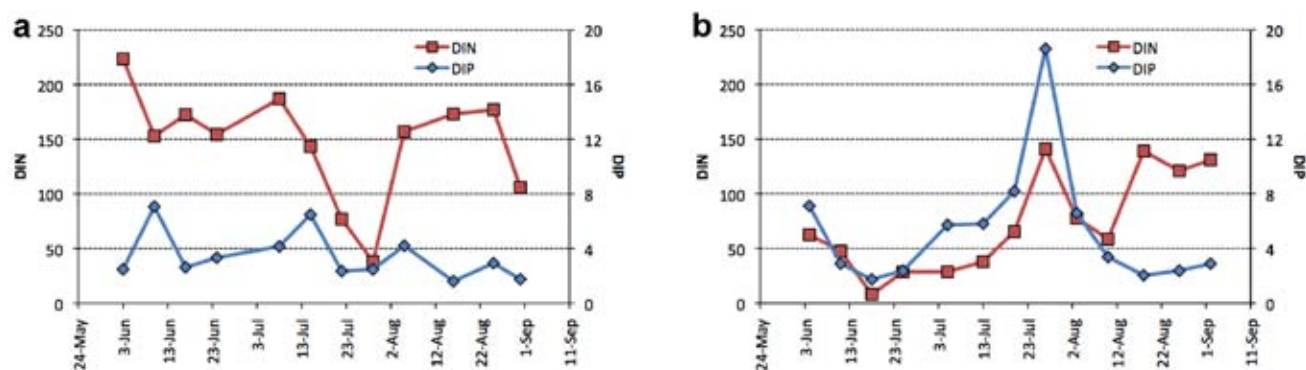


**Figure 3.29.** Vertical profiles of temperature and pH across a transect of stations in Ballyquirke Lough on three sampling surveys during summer 2010. Axes: horizontal = transect of stations as in Fig. 3.28; vertical = depth (m); colour gradient = temperature in degree Celsius and pH.



**Figure 3.30.** Vertical profiles of temperature and pH across a transect of stations in Lough Corrib on three sampling surveys during summer 2010. Axes: horizontal = transect of stations as in Fig. 3.28; vertical = depth (m); colour gradient = temperature in degree Celsius and pH.





**Figure 3.31. Surface dynamics of dissolved inorganic nitrogen and phosphorus (DIN, DIP, respectively) during summer 2010 in Lough Corrib station 4 (a) and Ballyquirke Lough station E (b).**

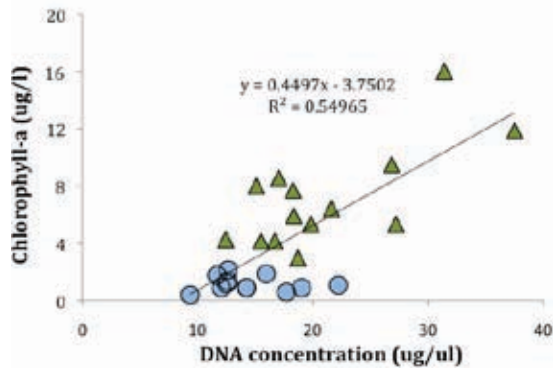
$r = 0.832$ ) (data not shown). For clarity, pH values, and not oxygen, are presented in [Figs 3.29](#) and [3.30](#) since the dramatic divergence of oxygen profiles between Lough Corrib and Ballyquirke Lough, when displayed on a common scale, would have made the visualisation and interpretation of the distributions difficult. Because the ordination of pH values are inherently based on a logarithmic scale, the display of units makes the profiles easily comparable between the two lakes.

Surface water samples were also collected in both lakes to determine the concentrations of major inorganic nutrients. The weekly dynamics of phosphate and nitrite+nitrate over the duration of the summer are shown in [Fig. 3.31](#). In Lough Corrib, the concentrations of nitrate and nitrite were in general superior to  $150 \mu\text{g.l}^{-1}$  at station 4, except from mid July to early August where the concentrations dropped below  $50 \mu\text{g.l}^{-1}$ . On average, the concentration of nitrate and nitrite at station 4 was  $146.8 \mu\text{g.l}^{-1}$  (s.d. =  $50.7$ ,  $n = 12$ ), whereas that of phosphate was  $3.5 \mu\text{g.l}^{-1}$  (s.d. =  $1.7$ ,  $n = 12$ ). At the southern end of Ballyquirke Lough, the general trend of nitrate and nitrite concentrations was toward an increase during the summer, from  $\sim 50 \mu\text{g.l}^{-1}$  in early June to  $125 \mu\text{g.l}^{-1}$  at the end of August. The dynamics of phosphate concentration were different, with a progressive increase from early June ( $\sim 4 \mu\text{g.l}^{-1}$ ) to the end of July ( $\sim 18 \mu\text{g.l}^{-1}$ ), after which a drop occurred reaching levels of  $\sim 4 \mu\text{g.l}^{-1}$  at the end of August. On average, the concentration of nitrate and

nitrite at station E was  $72.9 \mu\text{g.l}^{-1}$  (s.d. =  $45.7$ ,  $n = 13$ ), whereas that of phosphate was  $5.4 \mu\text{g.l}^{-1}$  (s.d. =  $4.5$ ,  $n = 13$ ). The concentration of nitrate and nitrite was on average significantly greater in Lough Corrib than in Lough Ballyquirke during summer 2010 at the stations sampled (Student  $t$ -test,  $p = 0.001$ ). However, there was no significant difference when comparing the concentrations of phosphate in the two lakes (Student  $t$ -test,  $p = 0.19$ ). Furthermore, there were no significant correlations between the weekly evolution of inorganic nitrogen and phosphorus concentrations for the two lakes (Pearson,  $p > 0.100$ ) (data not shown).

#### 3.7.4 *Cyanobacteria-specific 16S rDNA PCR Amplification and RFLP Analysis*

Total genomic DNA was extracted from the surface water sample filtrates collected in Lough Corrib and Lough Ballyquirke throughout the summer. The quality of the extracts, determined as per 260:280 absorbance ratio, was satisfactory. The average ratio value was  $1.77$  (s.d. =  $0.13$ ,  $n = 27$ ). About 50% of the extracts were of relatively good quality ( $>1.75$ ), whereas 45% and 5% were of intermediate ( $1.75$  to  $1.50$ ) and low quality ( $<1.50$ ), respectively. The average concentration of DNA determined in the environmental extracts was  $17.9 \text{ ng.}\mu\text{l}^{-1}$  (s.d. =  $6.5$ ,  $n = 27$ ). On a comparative basis, there was no significant difference in the quality of the nucleic acid extracts obtained from Lough Corrib and Lough Ballyquirke (Student  $t$ -test,  $p = 0.320$ ).



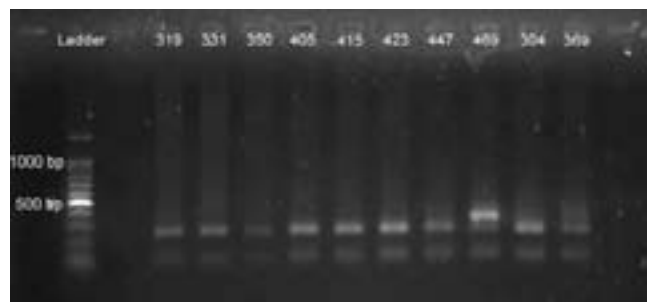
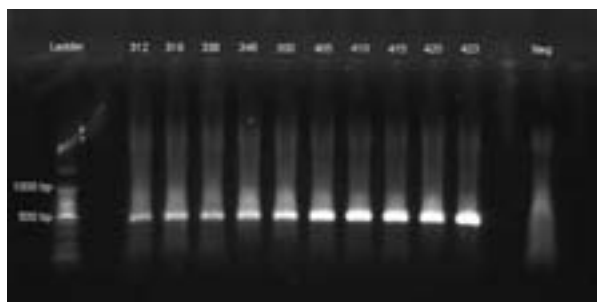
**Figure 3.32. Scatter plot showing the correlation between DNA extract and chlorophyll-a concentrations for samples collected in Lough Corrib (circle) and Ballyquirke Lough (triangle).**

However, the nucleic acid extract concentrations were on average significantly greater in Lough Ballyquirke than in Lough Corrib (Student *t*-test,  $p < 0.010$ ). This reflected the chlorophyll-a concentrations determined for the corresponding samples in both lakes as there was an overall significant positive correlation between chlorophyll-a and nucleic acid concentrations (Pearson correlation coefficient = 0.741,  $p < 0.001$ ,  $n = 26$ ) (Fig. 3.32).

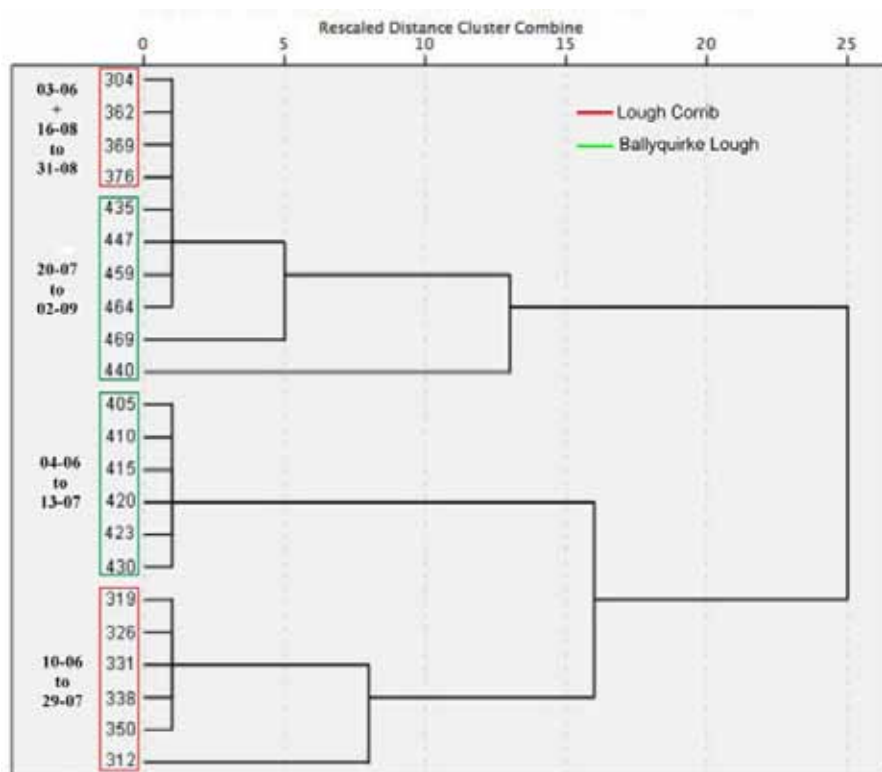
A region of the 16S rDNA gene was amplified using a nested PCR approach and two reverse primers

specific for filamentous and spherical cyanobacteria, respectively. The samples used were collected near Inchiquin in the upper Corrib and at the southern end of Lough Ballyquirke. Most extracts were amplified successfully, returning amplicons of various intensities and suggesting the common presence of cyanobacteria in the two lakes during the sampling period (Fig. 3.33). In a preliminary attempt to compare the cyanobacterial communities in both lakes and possibly detect seasonal changes, RFLP analysis was carried out using a dual digestion protocol on the pooled (filamentous and spherical) cyanobacterial nested PCR products. The restriction profiles were analysed visually by recording the presence and absence of bands for each sample.

A cluster diagram showing the relationships between the samples was then constructed from the binary matrix (Fig. 3.34). Two major clades were apparent, separating first samples from Lough Corrib and Lough Ballyquirke, clustered into two separate groups, and corresponding to all samples collected during the June–July period of the survey. A mixed group of samples from both lakes, mainly collected during August (except for Lough Corrib, sample 304), was identified. This suggests that a seasonal change in the cyanobacterial community of the two lakes might have occurred at the end of July.



**Figure 3.33. 16S rDNA polymerase chain reaction (PCR) (a) and restriction fragment length polymorphous (RFLP) electrophoresis gels (b) of field sample extracts collected from Lough Corrib and Ballyquirke Lough during summer 2010. The 300s and 400s number series correspond to samples from Lough Corrib and Ballyquirke Lough, respectively.**



**Figure 3.34. Restriction fragment length polymorphous (RFLP)-based dendrogram illustrating the relationships between cyanobacterial communities for Lough Corrib and Lough Ballyquirke during summer 2010.**

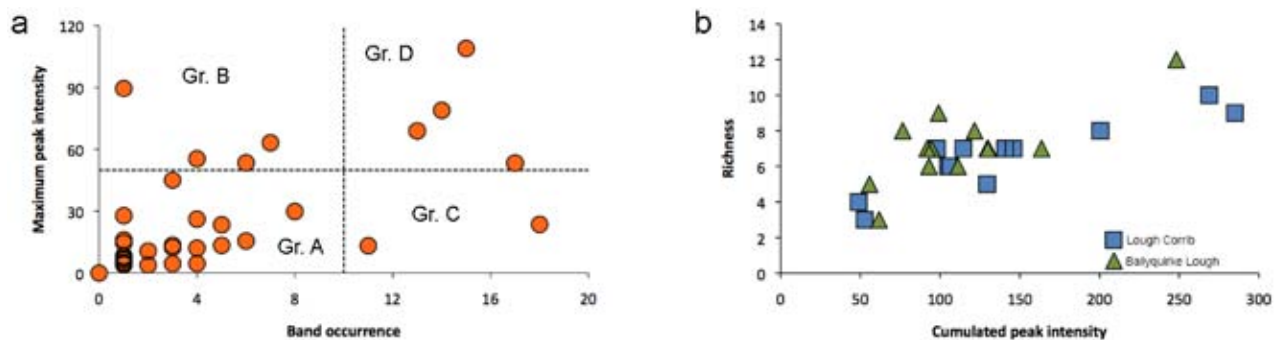
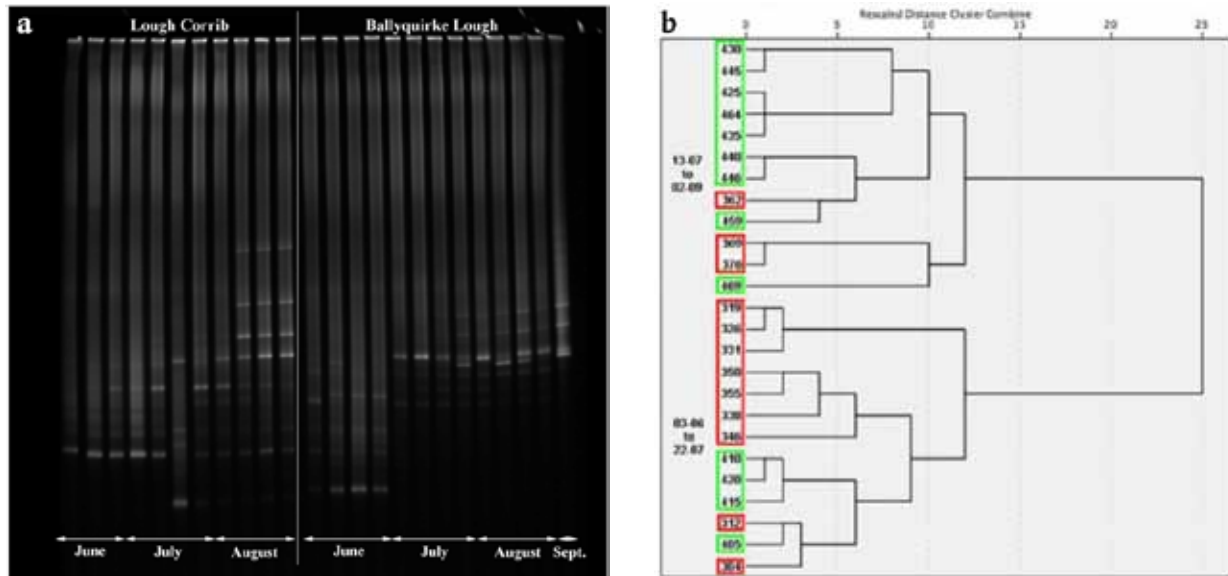
### 3.7.5 *Cyanobacteria Community Fingerprinting by 16S rDNA DGGE Analysis*

The seasonality pattern observed in both Lough Corrib and Ballyquirke Lough through RFLP analysis was investigated further using DGGE analysis. The interpretation of the DGGE migration patterns showed clear switches in the spherical cyanobacteria communities for both lakes during the length of the summer. This was most noticeable for Lough Corrib after 5 August, while in Ballyquirke Lough the first apparent switch was observed after 5 July, followed by a gradual change between 20 July and 10 August (Fig. 3.35). Filamentous cyanobacteria were only detected in some of the Ballyquirke samples during August, showing low richness and band intensity (data not shown).

For Lough Corrib, samples collected at two different locations (station 7 close to KnockFerry Pier and station 4 near Inchiquin in the Upper Corrib) were used for the analysis during August. Results showed dissimilarities

in the spherical cyanobacteria communities with variation in the intensity of specific bands for both locations. In particular, bands 22 and 52 were of greater intensity at station 7 than at station 4, with a reverse pattern for band 15 (Student *t*-test,  $p < 0.04$ ). Additionally, bands 28 and 39 were found only at station 4 and 7, respectively. The frequency of occurrence of individual cyanobacterial bands in the DGGE gels and their peak intensities were considered for both lakes. Figure 3.36 shows the segregation of bands for spherical cyanobacteria into four groups: rare bands of low intensity (Gr.A), rare bands of high intensity (Gr.B), common bands of low intensity (Gr.C) and frequent bands of high intensity (Gr.D). The existence of patterns in the distribution of those groups in space and time was ascertained. The cumulated peak intensities of DGGE bands from groups B, C and D were significantly lesser in Lough Corrib than Ballyquirke Lough (Student *t*-test,  $p < 0.05$ ). A difference in the cumulated peak intensities during the summer was found only for the bands from Gr.D,

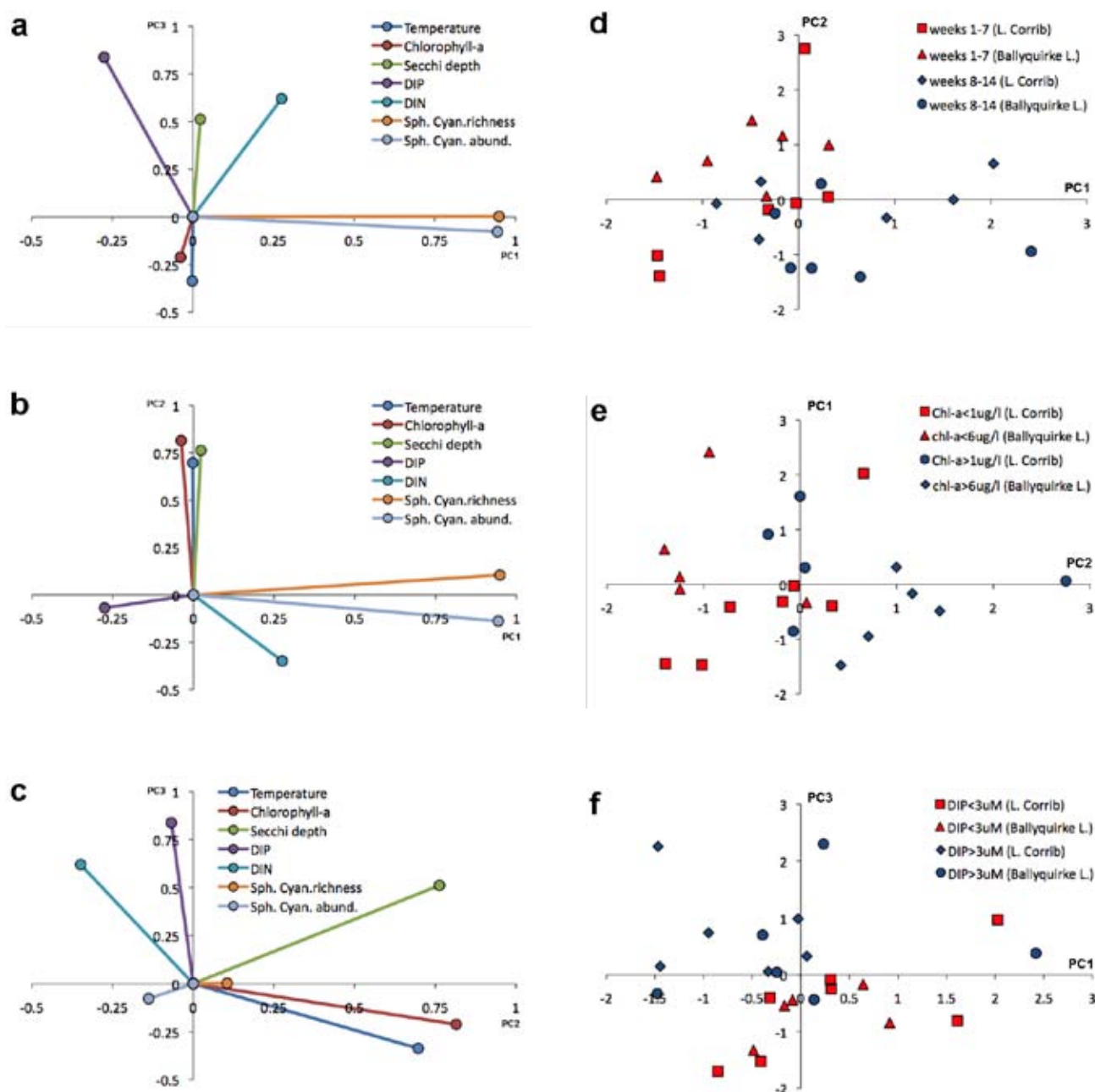




with those from August significantly greater than those from June and July (ANOVA,  $F = 13.02$ ,  $p < 0.01$ ).

Individual bands from groups B, C and D were successfully excised from the gels and re-amplified prior to purification and sequencing. The occurrence of the indicator bands was assessed in relation to

environmental descriptors using Fisher's exact test ([Table 3.9](#)). In particular, bands 15, 22, 28, 46, 49 and 52 for spherical cyanobacteria showed, for each lake, significant correlations with the descriptors pertaining to the time of the summer, surface chlorophyll-*a* levels, light penetration and inorganic nitrogen levels.



**Figure 3.37.** Sample projections along the principal components showing the distributions of variables (a, b, c). The projections of the lakes sampled are also shown (d, e, f) with respect to seasonality, chlorophyll-a concentrations and dissolved phosphate.

The MF analysis applied on the Lough Corrib and Ballyquirke Lough autoscaled data sets resulted in three components that accounted for 76.4% of the total variance. The projections of the variable loadings on the principal components and sample score plots are shown in [Fig. 3.37](#). Component 1 (PC1) was positively related to cyanobacterial richness and abundance.

Component 2 (PC2) was positively related to surface water temperature, Secchi depth and chlorophyll-a concentration, while Component 3 (PC3) was positively related to both nitrate and phosphate concentrations, and to a lesser extent Secchi depth. The sample score plot did reveal some clustering tendencies when considering the distribution for selected variables

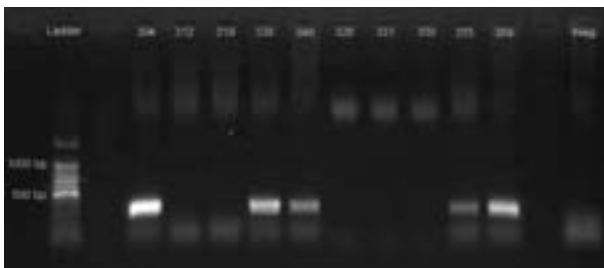
**Table 3.9. Significant correlation ( $P < 0.10$ , 2-tail Fisher's exact test) between the presence of a selected band within the gel and a qualitative descriptor in Lough Corrib and Ballyquirke Lough.**

Lake	Band Code	Descriptors				
		Summer period	Light penetration	Chl-a concentration	Inorganic nitrogen	16S rDNA fragment identification (% identity)
Lough Corrib	Bd15	0.015	-	-	-	<i>Synechococcus</i> sp.
	Bd22	0.002	-	-	-	<i>Synechococcus</i> sp.
	Bd28	-	-	-	-	<i>Synechococcus</i> sp.
	Bd46	-	-	-	-	<i>Synechococcus</i> sp.
	Bd49	-	-	-	-	Unsequenced band
	Bd52	0.015	-	-	-	<i>Synechococcus</i> sp.
Ballyquirke Lough	Bd15	0.027	0.027	0.015	0.027	<i>Synechococcus</i> sp.
	Bd22	0.060	0.080	-	-	<i>Synechococcus</i> sp.
	Bd28	0.015	0.028	0.015	0.027	<i>Synechococcus</i> sp.
	Bd46	0.060	-	-	-	<i>Synechococcus</i> sp.
	Bd49	0.060	-	-	-	Unsequenced band
	Bd52	-	-	-	-	<i>Synechococcus</i> sp.

– in particular species richness and phosphate concentrations. Notably, the separation along PC1 of stations from Lough Corrib and Ballyquirke Lough that were sampled after mid-July, when dissolved inorganic phosphate was low, was clearly discernible.

### 3.7.6 Determination of the Presence of Microcystins in Natural Samples

PCR amplification of a portion of the *mcy-A* gene was carried out on selected field sample extracts from Lough Corrib and Lough Ballyquirke to ascertain the presence of toxigenic cyanobacteria potentially synthesising hepatotoxic microcystins (Fig. 3.38).



**Figure 3.38. Partial polymerase chain reaction (PCR) amplification of the *mcy-A* gene in field samples from Lough Corrib collected during the summer of 2010.**

Amplicons of the expected size (~ 400 bp) were detected in 10 out of 15 and 16 out of 16 samples collected from Lough Corrib and Lough Ballyquirke, respectively.

Analysis of methanolic extracts using the PP2A assay was carried out on weekly samples collected from the two lakes: this showed the presence of microcystin-like activity in all the samples tested (Fig. 3.39). The microcystin concentration estimates ranged from 0.005 to 1.150 µg eq. MC-LR per litre and were on average 0.033 (s.d. = 0.031,  $n = 11$ ) and 0.104 (s.d. = 0.315,  $n = 13$ ) for Lough Corrib and Lough Ballyquirke, respectively. Microcystin concentrations in Lough Corrib were significantly higher in August than in June and July (ANOVA,  $F = 13.64$ ,  $p < 0.01$ ,  $n = 11$ ). No significant difference was found in time for the microcystin levels determined in Lough Ballyquirke (ANOVA,  $F = 0.92$ ,  $p = 0.43$ ,  $n = 13$ ). However, this is largely due to the high standard deviation of the August data set, attributable to sample 469, for which the microcystin concentration estimate was 1.150 µg eq. MC-LR per litre. On a comparative basis, and even when excluding sample 469, there was no significant difference in the average microcystin concentrations determined in Lough Corrib and Lough Ballyquirke during summer 2010 (Student  $t$ -test,  $p = 0.08$ ,  $n = 23$ ).

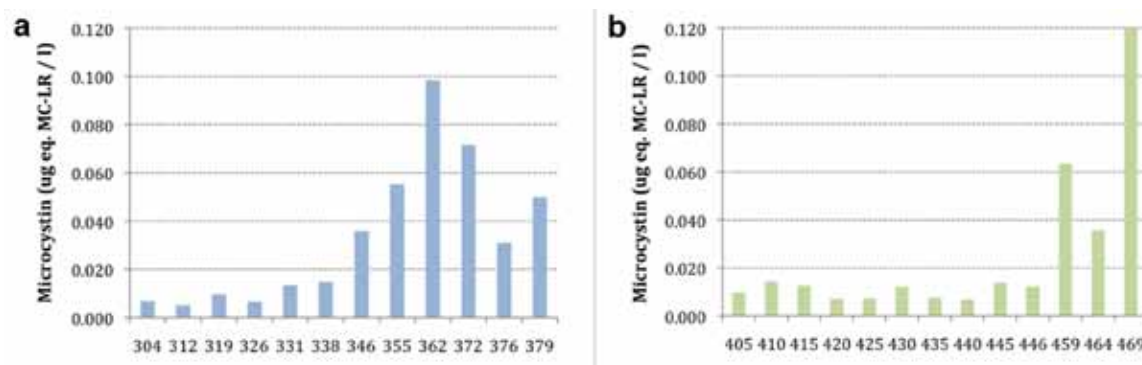


Figure 3.39. Evolution of microcystin surface concentration estimates in near-weekly samples collected in Lough Corrib (station 4, a) and Lough Ballyquicke (station E, b) during summer 2010.

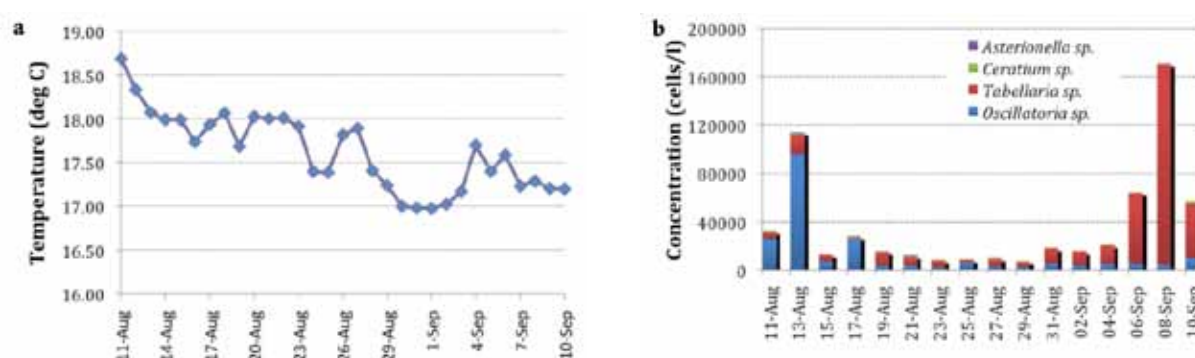


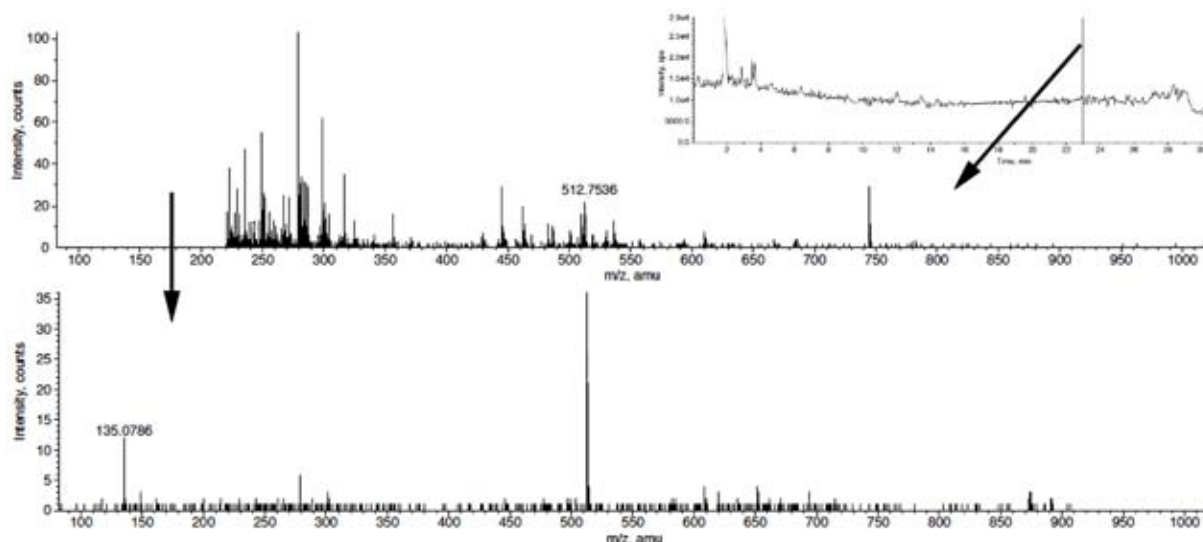
Figure 3.40. Evolution of subsurface temperature (a) and most abundant phytoplankton taxa (b) in Lough Graney, Co. Clare, during August–September 2010.

Four methanolic extracts from each lake were analysed by HPLC/MS/MS to confirm the presence of microcystins in the samples. The presence of MC-LR was confirmed in samples 362 and 379 from Lough Corrib while, surprisingly, none of the four Ballyquicke Lough samples analysed, including sample 469, contained the –adda fragmented group ( $m/z$  135) characteristic of microcystins.

### 3.7.7 Aquamonitor Deployment in Lough Graney

High frequency sampling of Lough Graney was carried out for 30 days throughout August and September 2010. The Aquamonitor instrument was complemented with a temperature logger and was deployed to collect water samples every two days for both phytoplankton and microcystin analyses. The temperature profile showed an overall decreasing

trend with maximal and minimal values of 18.7°C and 17.1°C, recorded on the days of deployment and retrieval, respectively (Fig. 3.40). Examination of the water samples by light microscopy showed that phytoplankton abundance varied during the sampling period. The most abundant taxa were enumerated at  $\times 100$  magnification and comprised the filamentous cyanobacteria *Oscillatoria* sp., dinoflagellate *Ceratium* sp. and the diatoms *Asterionella* sp. and *Tabellaria* sp. The cyanobacterium *Oscillatoria* sp. dominated the phytoplankton assemblage during the first week of deployment, after which the phytoplankton levels dropped down to  $\sim 10,000$  cells  $l^{-1}$ . Abundances rose again during the first week of September and the community was dominated at that stage by *Tabellaria* sp., with a maximum cumulated numerical phytoplankton concentration of 170,000 cells  $l^{-1}$ .



**Figure 3.41.** LC-MS/MS analysis of the Lough Graney extract collected in September 2010. The ion mass of Asp(3) MC-RR ( $m/z$  512.75) and of the characteristic -adda moiety ( $m/z$  135.07) are indicated.

An attempt was made to assess the suitability of the SP700 polymer resin to detect the presence of microcystins in the water column. Following methanolic extraction of the resin entrapped in the cartridges, analysis was carried out using the PP2A assay. Results all proved positive, showing the presence of microcystin activity in the extracts tested, with concentrations

ranging from 5.5 to 9.2  $\mu\text{g}$  eq. MC-LR per gram of resin. The SP700 extracts were also analysed by HPLC/MS/MS. No confirmation of the presence of microcystins was obtained from this analysis. However, a concentrated water column sample collected at the end of the autosampler deployment period (16 September) showed positive (Fig. 3.41).

## 4 Discussion

### 4.1 General Context of the Study

There is increasing global recognition of the need to strike a better balance between environmental sustainability requirements and human developmental activities. Inland water bodies are considered as integrated parts of the landscape and the monitoring of water quality is of great importance from a human health aspect but also in terms of regional attractiveness for securing economic benefits from leisure activities. In Ireland, excluding regions subject to intensive agriculture, the general quality status of lakes and rivers is satisfactory (EPA Ireland, 2009). Eutrophication of continental waters is a significant environmental problem, which has usually been associated with high biomass phytoplankton proliferations, in particular cyanobacteria (Carpenter *et al.* 1998, Schindler 2006, Istvánovics 2009). Those dominated by toxic species have caused a variety of ecological disruptions and present serious threats towards animal and human health (Codd *et al.* 2005). Although improvements in water quality have been achieved following diversion of effluent from nutrient point sources, nonpoint sources are now the main cause of aquatic pollution in regions of Europe and North America (Smith 2003, Jeppesen *et al.* 2005, Smith *et al.* 2006). The intermittent nature of nutrient diffusion fluxes linked to seasonal agricultural activity or to precipitation regimes makes their assessment more difficult (Carpenter *et al.* 1998). In Europe, a number of biological variables, including phytoplankton, have been defined as suitable descriptors of water quality through the enactment of the WFD (European Communities 2000). Increases in nutrient inputs usually translate directly into enhanced primary productivity. Phytoplankton biomass, by estimating chlorophyll-*a* levels, can therefore serve as an indicator of the trophic status of water bodies (OECD 1982).

Responses of lacustrine species richness to environmental gradients vary over a range of spatial and temporal scales, depending on niche differentiation, competition for resources, noncyclic disturbances, food web structure or colonisation processes (Dodson *et al.* 2000, Chase & Leibold 2002, Morin & Fox 2004,

Stomp *et al.* 2004, Reynolds 2006, Clegg *et al.* 2007). Hence, the sustainable management of aquatic resources requires a better understanding of the interconnections between the biotic and abiotic factors that determine quantitative and qualitative patterns in the basic components of the food chain, in particular the phytoplankton assemblage. Conservation and restoration efforts of water bodies with decreased water quality can be carried out on a case-by-case basis but an efficient strategy for the sustainable management of multiple watersheds and river catchments must be done at a regional level.

In this context, this study analysed the topographical attributes of river catchments with the view to delineating potential ecoregions for future regionalised watershed management. A regional survey based on such an analysis was carried out in western and northwestern Irish lakes during the summer of 2009 to relate the defined spatial structures to features of the water bodies sampled. In particular, distribution patterns in phytoplankton biomass were investigated so as to identify potential associations of lake trophic state with river catchment land cover attributes. Multivariate analysis of phytoplankton assemblages, and cyanobacteria in particular, was also carried out to determine whether taxa associations reflected meteorology-linked estival succession or specific spatial distributions. Given the high number of water bodies within the study area, a regional sampling strategy approach was applied, based on the semi-random stratified sampling of lakes following ArcGIS analysis of river catchment attributes. In a second phase, the summer dynamics of cyanobacteria were investigated in Lough Corrib, Ballyquirke Lough and Lough Graney in 2010. Analysis was based on the molecular fingerprinting of cyanobacterial communities by DGGE separation of 16S rDNA amplicons and was linked to the determination of the microcystin synthesis potential through gene detection and the use of both enzymatic and chemical detection methods. Additionally, an autonomous water column sampler was deployed in Lough Graney to monitor short-term phytoplankton dynamics and investigate the suitability

of the SP700 resin (Sepabed) for the passive adsorption of microcystins.

## 4.2 Regionalisation Frameworks and Sampling Schemes

Regionalisation frameworks enable the delineation of adjacent geographic regions, which can be used as spatial units for the design of sampling programmes and monitoring strategies (Sandin & Johnson 2000, Jenerette *et al.* 2002, Santoul *et al.* 2004, Robertson *et al.* 2006). Regionalisation schemes do not specifically resolve variability among water bodies but rather facilitate the capture of broad-scale patterns, enabling the identification of relationships between natural attributes of watersheds and anthropogenic pressures (Gerritsen *et al.* 2000, Cheruvilil *et al.* 2008). Some degree of uncertainty lies in the geographic extent across which lake groups can be formed as different regionalisation frameworks can return assemblages of substantially differing sizes (Cheruvilil *et al.* 2008). The hierarchical clustering of river catchments applied to the study area was based on seven indices given identical weights. This allowed for a compensation of the biases introduced by highly divergent features among contiguous zones, such as the size of the 'Shannon' river catchment in cluster B, which accounts for ~50% of the total surface of the study area. An additional layer of heterogeneity was added by spreading the sampling effort over a period of 15 weeks, a temporal scale that can complicate the interpretations of inferred relationships. However, unlike temporally focused surveys that return environmental snapshots, sampling in extended time periods incorporates punctual heterogeneities into a broader frame.

Regionalisation frameworks use large spatial units containing several water bodies as the delineation of elementary units and the building of strata at a resolution based on the watershed level is still fragmentary over extensive geographic areas. The use of river catchments as referential units in this study enabled the stratification of the sample population and ensured that the lakes sampled were distributed across heterogeneous land cover and hydrogeomorphology. Future mesoscale surveys may be refined by incorporating additional environmental variables into the distance matrix, and for which data are available at the spatial unit required. These could

include interconnecting networks between lakes but also encompass the geologic nature of soils and subsoils, information on human population density, precipitation metrics or the typology of the agricultural activity carried out in the river catchments within the sampling population. Construction of time-series and further exploration of phytoplankton dynamics and water quality inside representative hydrological zones, and of their relationships with watershed attributes, could be carried out based on the typology of substrata or application of sample grids. Finally, the selection of lakes to sample could be based on documented data relative to residence time, stratification regime, alkalinity, mean depth or other environmental descriptors.

## 4.3 Relationships between Lake Trophic State and Adjacent Land Cover

The results obtained from sampling western and northwestern Irish lakes through the summer of 2009 confirmed the overall good-quality status of the lakes in the region. Despite the large temporal and spatial scales considered in this study, significant relationships were identified between some environmental features and water-quality variables. In general, the highest chlorophyll-*a* levels coincided with the warmest period from mid-June to mid-July. The lowest temperatures were mostly associated with low chlorophyll-*a* levels in August, which also coincided with low light penetration, suggesting the presence of heavy loads of suspended matter in the water column concomitant to rainfalls. However, this turbidity could have been associated to a certain degree with water colour, which is a strong determinant of Secchi depth in some Irish lakes (Free *et al.* 2000).

Relationships were also assessed in space and a range of trophic status was observed among the lakes sampled in the study area, according to a longitudinal geographic gradient. About 65% of the lakes sampled could be classified as ultra-oligotrophic and oligotrophic habitats, in particular in western coastal and remote areas, reflecting the relatively low local anthropogenic pressures. The 17 lakes classified as mesotrophic to eutrophic, several of which have been the focus of attention for improvement (EPA Ireland, 2008b), were mostly located inland within zone I, where agricultural activities are predominant. In addition, considering land cover typology, chlorophyll-*a* levels



in the water bodies sampled across the whole study area were negatively and positively correlated with the percentage of river catchment area covered by wetland surfaces and by agricultural surfaces, respectively. Chlorophyll-*a* levels were negatively correlated with the percentage of the hydrographical zone covered by forestry surface, and this for zone I only, where there was also no apparent correlation between chlorophyll-*a* and agricultural surfaces. These findings are to some extent similar to the observations made for watersheds in France and the USA (Jones *et al.* 2004; Catherine *et al.* 2008). A recent study demonstrated the feasibility of predicting the eutrophication status of water bodies based on river catchment attributes and land use (Catherine *et al.* 2010). A similar approach could probably be used to define and test a eutrophication risk index. Paleolimnological records indicate that lake enrichment in nutrients, through non point source mechanisms at the landscape level, is linked to the conversion of native land cover to agricultural and urban uses (Reavie & Smol 2001). An inherent linkage between urban and agricultural land cover categories may explain why eutrophic water bodies can be found within hydrographical systems showing intermediate urban and cropland cover (Catherine *et al.* 2008). Comparative analysis of the dynamics of nutrients and chlorophyll-*a* in areas with mixed landscapes may return new insights on the relationships between water quality and the environmental attributes of the zones contiguous to water bodies of interest. Trans-regional approaches may also orientate choices for improving the management of constrained resources, and help in assessing the future regional condition of aquatic resources based on the status of representative watersheds. To this end, monitoring based on the use of technologies enabling real-time and *in situ* water column profiling may assist management bodies substantially by rapidly informing upon the physical, chemical and biological status of water bodies across extensive geographic areas. For example, minor adaptations to the Environmental Sample Processor (ESP), currently used for investigating natural dynamics of marine-harmful algal bloom species, could be carried out for the autonomous measurement of key environmental variables pertaining to inland water quality (Scholin *et al.* 2009).

#### 4.4 Structuring in Time and Space of Phytoplankton Communities

Information on phytoplankton dynamics and diversity in Irish lakes is scarce, and knowledge mostly concentrates on a selection of water bodies that are important for water abstraction and other aquatic resources (King & Champ 2000, Reynolds & Petersen 2000, EPA Ireland 2008b). Heterogeneity was observed in the distributions and abundances of major phytoplankton groups during the 2009 survey, which was carried out after the typical diatom-dominated spring period. Considering broad taxonomic units, significant temporal relationships were found only for microflagellates and cyanobacteria, which predominantly occurred in the lakes sampled in June and July, respectively, periods during which the meteorological conditions were fair. The fact that no vertical profiling was carried out in the lakes in this study also needs to be re-emphasised as phytoplankton biomass is often maximal a few meters under the water surface and some species can also develop under spatial structures described as thin layers.

Patterns in lacustrine phytoplankton diversity, and their relationships with environmental gradients, have traditionally been studied based on morphological examinations (Chalar 2009, Jaanus *et al.* 2009). Other approaches to lake eutrophication assessment have included the use of diatom assemblages as indicators of lake trophic status to predict phosphorus levels, or the application of a phytoplankton functional diversity index showing strong responses to environmental drivers (Yang *et al.* 2008, Longhi & Beisner 2010). In this study the community composition of phytoplankton was analysed by multivariate analysis in 2009, an approach commonly used for vegetation cover studies and previously applied to phytoplankton biogeography and ecology (Okolodkov & Dodge 1996, McDonald 2001). Three main clusters were characterised, grouping lakes according to no clear geographical pattern. Some taxa were ubiquitous in the samples whereas others were associated with specific lake assemblages. For example, the cyanobacterium *Anabaena* sp. was mostly observed in lakes of assemblage I, whereas the dinoflagellate *Peridinium*, usually found in stratified environments, was mostly found in assemblage II. The diatoms *Cymbella* and *Diatoma*, which are typical components of benthic periphyton, were present



in lakes from assemblage III, and their presence in surface water samples probably reflects water column mixing concomitant to climatic conditions characterised by regimes of wind and precipitations. It is also possible that those taxa reflect the near-shore sampling strategy which was applied to the survey. Data ordination in a planar projection showed the first component to be positively correlated with lake surface temperature, enabling the discrimination of the three lake assemblages across the temperature gradient. The first component was therefore interpreted in this study as a general indicator of seasonality and meteorological conditions.

Shifts in climatic conditions, in particular those that result in physical mixing processes, can impact significantly upon the structuring of phytoplankton communities (Reynolds *et al.* 1983, Spigel & Imberger 1987, Huissman *et al.* 2004). It has been predicted that the outcome of competition for light in a well-mixed water column depends on the individual critical light intensities of the organisms, which can differ considerably between species (Huissman & Weissing 1994, Huisman & Hulot 2005). In particular, studies in Lake Nieuwe Meer, The Netherlands, confirmed that turbulent mixing could shift the competitive balance – hence, phytoplankton community composition – between buoyant cyanobacteria and sinking diatoms (Huisman *et al.* 2004). Despite the lack of measurements of wind stress and vertical turbulent diffusivities in the present study, it can be hypothesised, based on the evolution of surface water temperature, Secchi depth and meteorological conditions that a similar shift occurred at the end of July, which lasted throughout August. The phytoplankton community structure in Ireland changed toward lower chlorophyll-*a* levels and the dominance of diatoms over this period.

The relationship between community complexity and primary productivity is important for trophic interactions within the aquatic food chain, and greater phytoplankton diversity has been observed in lakes of low productivity (Leibold 1999, Dodson *et al.* 2000). In this study, phytoplankton richness was significantly lower in zone I (and cluster B), which contained the greatest proportions of mesotrophic and eutrophic lakes in the sampled population. Niche differentiation can be reduced in well-mixed environments with low spatial heterogeneity, and

is accompanied by lower diversity in the water column (Jäger *et al.* 2008). Since sampling within each cluster of river catchment was spread over a 15-week period encompassing a range of weather conditions, it is likely that the lower richness observed in the lakes sampled from zone I originates from the interactions of multiple factors rather than meteorological effects only.

#### **4.5 Environmental Fingerprinting: Molecular Assessment of Communities**

Advances in molecular biology have enabled the study of microbial communities in multiple environments based on the variability between species in the nucleotide sequences of a number of genes. Molecular ecology has strongly benefited from the adaptation of genetic fingerprinting techniques to unravel the wide diversity in microbial communities. The generalisation of the use of PCR and sequencing to genetically characterise isolates of globally distributed taxa has also permitted the resolution of some taxonomic ambiguities.

The summer variation in lacustrine cyanobacterial diversity was investigated using 16S rDNA DGGE fingerprinting across a region of Ireland containing multiple river catchments during 2009. Even though nested PCR-DGGE is a powerful molecular fingerprinting method for detecting low copy number genes or specific hierarchical taxonomic groups in microbial communities, it has usually been described as unsuitable for quantitative assessments, as the degree of proportionality between initial template amounts and resulting amplicon concentration can be lost through the amplifications cycles (Farrelly *et al.* 1995, Muylaert *et al.* 2002, Zwart *et al.* 2005). The abundances of amplicons may also not reflect those of all target genes due to differences in primer binding, elongation efficiency or initial proportions of target DNA in template samples (Raeymaekers 1995, Suzuki & Giovannoni 1996). Those aspects have been examined while coupling the nested approach with quantitative PCR, in which the number of amplifications cycles in the secondary PCR did affect the reliability of the quantification. Park and Crowley (2010) also demonstrated that the number of first-round PCR cycles was essential to limit the bias associated with nested PCR-DGGE, recommending less than 20 cycles for amplifying low copy number sequences and

maintaining the reliability of quantifications.

Cyanobacterial diversity in surface water samples was estimated based on DGGE analysis of a ~400 bp 16S rDNA gene fragment. Results showed the presence of both spherical and filamentous cyanobacterial ribotypes in the great majority of the samples, with relatively complex DGGE banding patterns for both types. Light microscopy analysis of microphytoplankton was also effectuated at the genus level to ascertain the presence of potentially toxic taxa and assess the correspondence between DGGE data and numerical abundance. As anticipated, the molecular method was more sensitive in that it provided greater resolution for cyanobacterial community composition assessment. Overall, more cyanobacteria were numerically found in the lakes sampled in June and July than in August, a pattern which was significantly related to the weather conditions observed at the time of sampling. Although weakly supported statistically, this was also apparent after cumulating the individual intensities of each DGGE band for each station. However, given the potential distortion associated with PCR biases, this aspect warrants caution. The general discrepancy obtained between the two methods was still largely anticipated considering that the constitutive units measured during numerical abundance and DGGE analyses are inherently different. Still, considering all samples, a positive correlation was observed between cumulated DGGE band intensities for filamentous cyanobacteria and joint numerical abundances of filamentous genera determined by microscopy.

The large number of available 16S rDNA gene sequences in databases facilitates the identification and phylogenetic classification of cyanobacterial taxa in complex environmental samples. Most of the bands excised from the DGGE gels were successfully re-amplified and sequenced, returning cyanobacterial sequences only and no heteroduplex products, suggesting that amplification of nontarget sequences was limited. However, identification of the taxa at the species level was not optimal given the short size of the amplicons and high nucleotide homology between sequences, even though the 16S rDNA gene domain amplified is described as a variable region (Boutte *et al.* 2006). Still, the phylogenetic inference including

the sequences retrieved from the sampled lakes indicated the presence of members from the main cyanobacterial lineages in the study area. Nostocales, in particular *Anabaena*, were the dominant taxa that the sequencing analysis of selected bands returned, with 27% of the sequences returned clustering with *Anabaena* and *Aphanizomenon* sequences.

Even though the presence of genera such as *Merismopedia* sp., *Sphongosphaerium* sp. or *Microcystis* sp. was observed in some samples by microscopy, no particular focus was placed on spherical cyanobacteria due to the general small size of individual cells, the relatively low occurrence of colonies and the poor structural integrity of preserved samples over time. Substantial trichome concentrations of the potentially toxic genera *Oscillatoria* and *Anabaena* were found in eight lakes mostly sampled in zones I and II during June and July, when the meteorological conditions were fair, suggesting that those morphotypes might develop in lakes of lesser trophic state under mild environmental conditions. Detection of cyanobacteria in surface water samples implies the presence of positively buoyant species, whose rate of photosynthesis can be enhanced by higher irradiance (Visser *et al.* 2005). Alternatively, it could suggest uniform vertical distribution in the epilimnion following wind-driven turbulence (Huisman and Hulot 2005). The competitor, stress tolerator and ruderal (CSR) model defining the three primary ecological and evolutionary strategies identified among terrestrial plants, based on resource stress and habitat disturbance, has to some extent been applied satisfactorily to phytoplankton organisms (Grime 2001, Reynolds 2006). Although probably variable for individual species, the S-strategy nature of buoyant *Anabaena* and *Microcystis*, both K-selected microorganisms viewed as resource gleaners, can hence be contrasted to the R-strategist *Oscillatoria*, often associated with density gradients and viewed as acclimating organisms (Reynolds 2006).

Sampling was carried out in a range of water bodies of differing trophic states with the expectation that cyanobacterial diversity and abundance would be greater in the lakes of lesser quality, given they frequently support toxic cyanobacterial blooms. Results showed that lakes classified as mesotrophic or

eutrophic generally supported greater cyanobacterial diversity. The meteorological conditions in August, dominated by precipitations and high-wind regimes, were typically not supportive of cyanobacterial growth, which was reflected in the low levels recorded during this period. Hierarchical classification of the DGGE profiles segregated the lakes with low and high diversity of spherical and filamentous cyanobacteria. Those associations were also underpinned by significant relationships, in particular between chlorophyll-*a* levels and spherical cyanobacteria and meteorological conditions with filamentous cyanobacteria. This was further evidenced from the multivariate analysis. Surface filamentous cyanobacteria also tended to occur in larger water bodies contained in river catchments covered substantially with forestry surface. However, cyanobacterial biomass has in previous studies been observed to be lesser in water bodies contained within river catchments substantially covered by forested areas, which might limit diffusion fluxes of nutrients and therefore phytoplankton growth (Catherine *et al.* 2008). Nostocales cyanobacteria such as *Anabaena* sp. can fix dinitrogen; this attribute can provide them with a competitive advantage over other co-occurring phytoplankton species in more oligotrophic environments and could be part of the reason for the pattern observed in the distribution of filamentous cyanobacteria in this study.

Genotypes and competitor species are continuously replaced in planktonic microbial assemblages by fitter ecotypes (Giovannoni 2004). It has been suggested for bacteria that lineages with higher diversity make a higher contribution to ecosystem functioning than those with comparable abundance but lower diversity (Peter *et al.* 2011). In addition, as noted by Youssef and Elshahed (2009), for soil bacterial communities in clone libraries, a positive correlation between diversity and abundance within a specific lineage suggests the recruitment of new species to the ecosystem through immigration, or the elevation of rare, previously undetected members of this specific lineage. A similar trend was observed in this study for the lacustrine planktonic cyanobacteria sampled throughout the summer. However, filamentous and spherical cyanobacterial ribotypes can hardly be viewed as separate lineages since rDNA-based phylogenies have shown the interspersing of cyanobacterial

morphotypes into various clades of paraphyletic origins (Litvaitis 2002). Still, results showed on average greater diversity of spherical cyanobacteria compared to filamentous cyanobacteria. The two groups contain both *r*- and *K*-selected species, so attempts to generalise should be cautious (Reynolds 2006).

#### 4.6 Dynamics of Cyanobacterial Communities: Environmental Determinism

The diversity and community dynamics of surface cyanobacteria were investigated in Lough Corrib and Ballyquirke Lough during summer 2010 using DGGE-based molecular fingerprinting. Cyanobacteria were detected on each sampling occasion throughout the summer but well-resolved profiles were obtained only for spherical cyanobacteria. Cyanobacterial species composition differed to some extent between the two lakes and showed variability with multiple successions of genotypes. Analysis of bands excised from the gel returned highly similar Chroococcales sequences closely related to *Merismopedia*, *Aphanothece* and *Microcystis*. The latter is known as one of the most important toxin-producing genus in lakes (Sivonen & Jones 1999) and this cyanobacterial genotype appeared in Lough Corrib during August only, concomitantly with an increase in microcystin levels.

A substantial amount of work has previously been carried out in both lakes. One study focused on determining the eutrophication potential of a number of western Irish lakes, including Lough Corrib (McCarthy *et al.* 1999). The Secchi depth and chlorophyll-*a* concentrations recorded at the time were very similar to those obtained 15 years later in this study. Seven stations were sampled on a near weekly basis in the Upper Corrib during summer 2010 and analysis showed spatial variability in the distribution of some environmental variables. In particular, the domain delineated by Knocferry Pier and the western end of Rabbit Island (stations 7 to 5, respectively) proved on several occasions subject to increased temperatures, Secchi depths, chlorophyll-*a* and nutrient levels compared to the domain extending from Inchiquin to the north of Inchagoill Island (stations 4 to 1, respectively). This is not surprising given the topography of the basin

in those areas; the latter domain is much larger and deeper and more susceptible to wind exposure and associated water column mixing. On the other hand, the lower end of the Upper Corrib is a very shallow and narrow strait. Hence, this body of water can warm up more quickly and is often protected from wind-driven turbulence, two aspects that can contribute to phytoplankton biomass development when sustained by the necessary nutrient inputs. A comprehensive study of Ballyquirke Lough, where cyanobacterial proliferations have occurred in the past, was previously carried out with respect to water quality determination and analysis of a number of physical, chemical and biological variables (Irvine *et al.* 1998). Interestingly, the phosphate and chlorophyll-*a* concentrations obtained in 1997 were largely comparable to those determined in the present study, suggesting that the lake status has probably remained stable in the past 15 years. A major difference between Lough Corrib and Ballyquirke Lough during summer 2010 was the observation in the latter of an anoxic layer in the deepest part of the lake (station D) on several occasions. Even though thermal stratification was also observed in Lough Corrib (station 4), the dissolved oxygen levels measured were always greater than 7.0 mg.l<sup>-1</sup>. The likely difference in microbiological activity in the anoxic and oxic compartments of Ballyquirke Lough needs however to be considered in relation with the rapid flushing rate of the lake and the frequency of wind-driven water column homogenisation, as near-uniform distribution of the physical and chemical variables across the water column was also observed on occasions.

Even though the morphological and hydrological features of Lough Corrib and Ballyquirke Lough differ markedly, a shift in spherical cyanobacteria community occurred in July in both lakes. Temporal changes in microbial community may occur in freshwater over a short period of time (Jaspers *et al.* 2001, Van der Gucht *et al.* 2001). It is likely that the changes in community structure could be related to changes in water column structure. Unfortunately, vertical profiling was not carried out during July due to instrument malfunction. Together with the interactions with phytoplankton, bacteria, viruses, protozoa and zooplankton, nutrient availability, temperature and

meteorological conditions influence the composition and dynamics of cyanobacterial communities in aquatic environments (van Hannen *et al.* 1999, Riemann *et al.* 2000, Pernthaler *et al.* 2001, Muylaert *et al.* 2002). It has been demonstrated that abundance variations caused by competition for a few limiting abiotic resources can account for the coexistence of multiple species (Armstrong & McGehee 1980, Hastings 1980, Huisman & Weissing 1999). Wind re-suspension and rainfall cause pulsed availability of phosphorus and nitrogen forms; different strategies in response to nutrient limitation, such as storage or high affinity uptake, may hence support the coexistence of multiple species and affect the outcome of competitive interactions (Gons *et al.* 1991).

#### **4.7 Monitoring Cyanobacteria: Molecular Biology, Toxins and Autonomous Sampling**

Microcystins are the most common cyanotoxins and the only type for which the World Health Organisation has issued recommended levels (Sivonen and Jones 1999). In 2009, the presence of the *mcy-A* gene was detected in 18 nucleic acid extracts obtained from surface water samples of western and north-western Irish lakes. Subsequent RFLP analysis showed the clustering of the lakes into three assemblages. After examining microscopy-derived abundances, lakes in cluster B had significantly less amounts of the filamentous cyanobacterial genera *Anabaena* and *Oscillatoria*, suggesting the existence of polymorphism in *mcy-A* gene sequences between the two filamentous taxa and spherical cyanobacteria such as *Microcystis* and *Gomphosphaeria*, which were more abundant in cluster B. Nevertheless, interpretation about cyanobacterial toxicity based solely on *mcy-A* gene analysis warrants caution given that the many existing variants of microcystins display a wide range of toxicities and that toxin profiles can be complex and vary in time according to the physiological state of the cells (Kardinaal & Visser, 2005). It has been hypothesised that there might be a succession of genotypes of varying toxicity according to light competition during cyanobacterial bloom development. In particular, the competitive replacement of toxic genotypes by nontoxic strains offers a plausible explanation for the

gradual decrease in average toxicity per cell, which has been observed during the development of dense *Microcystis* blooms (Kaardinal *et al.* 2007).

The phytoplankton material collected in each lake was processed to solubilise microcystin toxins and the corresponding extracts were analysed using the PP2A assay, which has often been used for the analysis of cyanobacterial hepatotoxins and diarrhetic shellfish poisoning toxins in seafood products (Tubaro *et al.* 1996, Rivasseau *et al.* 1999b). Only 12 samples from the 2009 surveys were positive with the assay whereas microcystin-like activity was detected in virtually all the samples collected during summer 2010 in Lough Corrib and Ballyquirke Lough. The great majority of the extracts tested with the PP2A assay were well below the recommended WHO safety level of 1 µg.l<sup>-1</sup> for the 2009 samples. In 2010, the maximum microcystin concentration estimate in Lough Corrib was 0.1 µg.l<sup>-1</sup> while that found in Ballyquirke Lough was 1.1 µg.l<sup>-1</sup>, both found during August when spherical cyanobacterial colonies were visible at the surface of the lakes. Substantial PP2A inhibition coincided with the detection of the *mcy-A* gene on only six occasions in 2009, whereas this correspondence was much greater during summer 2010 while measuring cyanobacterial and microcystin dynamics in Lough Corrib and Ballyquirke Lough. Relating PP2A inhibition to the detection of *mcy-A* genes is speculative, as gene presence does not necessarily mean toxin expression. PP2A inhibition could also be caused by unknown compounds co-extracted from the biological matrix and associated with *mcy-A* negative results. Likewise, weakly toxic variants could be synthesised in small quantities, returning negative PP2A response but *mcy-A* positive results. It is likely however for a toxic population that a relationship exists between cyanobacterial biomass and the response of the *mcy-A* PCR assay, which should reflect the total number of *mcy-A* gene copies. Quantitative real-time PCRs have been designed in recent years, targeting individual species and microcystin genes, and tested successfully with natural samples (Kurmayer & Kutzenberger 2003). Their use in ecological surveys could prove very valuable for assessing the potential risk of water body contamination with microcystins produced by particular cyanobacteria such as *Microcystis* sp. and *Anabaena* sp.

A number of samples which returned positive results with the PP2A assay were analysed by HPLC/MS/MS to confirm the presence of microcystins. The comparison of PP2A and analytical chemistry results is often difficult given the inherently different nature of the measurements for each method. The PP2A test is a functional assay based on the inhibition of enzymatic activity by microcystins or other compounds with similar biological properties. Analysis by HPLC and mass spectrometry informs about the identity and amounts of compounds based on their chemical structure. The presence of microcystins was only confirmed in six samples collected during both summers 2009 and 2010, including the variants MC-LR, -RR and -YR. It is noteworthy that microcystins were detected in three extracts from the 2009 survey that were also positive after PP2A and *mcy-A* analyses. Given the overall low levels of measured phytoplankton biomass and microcystins, it is likely that insufficient material was collected to satisfy the requirements of LC/MS/MS analysis and confirm the presence of microcystins in the other PP2A positive samples. Recent work carried out in Northern Irish lakes has led to the determination of microcystin profiles in natural cyanobacteria populations (Mooney *et al.* 2011). Notably a range of microcystin variants was identified and it is likely that similar toxin forms could be found in lakes across the island of Ireland. Interestingly, the authors did not find a relationship between Zebra mussel lake contamination and selection of toxic phytoplankton, such as microcystin-producing cyanobacteria.

Lough Graney (Co. Clare) was also sampled in 2010 in response to the observation on site of microcystin-producing cyanobacteria during the preceding summer survey. Given the constraining sampling regime already in place in Lough Corrib and Ballyquirke Lough, an autonomous water sampler was deployed in Lough Graney for four weeks at the end of August. An attempt at monitoring the dynamics of microcystins during this period was effectuated using passive adsorption, a technique successfully employed in coastal embayments to monitor the phytoplankton toxins that render shellfish unsafe for human consumption (McKenzie 2010). Microcystin-like activity was detected for each week of deployment using the PP2A assay. Unfortunately, the corresponding extract concentrations were too low for confirmation with analytical chemistry.

However, a large water sample collected independently from the shore and analysed by liquid chromatography and mass spectrometry did confirm the presence of MC-LR in the lake during the instrument deployment period. This result warrants more frequent measurements of water quality variables to ascertain the potential risk toward recreational activities and drinking water safety. Previous research has shown that microcystins can have negative effects on reproduction and developmental processes in fish larvae and juveniles (Malbrouck & Kestemont 2006). It is hence important to estimate

the extent and frequency to which fish populations, in lakes prone to cyanobacterial biomass development, are exposed to cyanotoxins. A new generation of instruments and buoys has been developed in recent years and the autonomous sampling and remote sensing of both marine and lacustrine environments is now feasible (Babin *et al.* 2005). The installation of a battery of autonomous instruments in sentinel lakes across Ireland would be beneficial to watershed management bodies and enable the continuous monitoring of water quality variables in the near future.

## **5 Future Work and Recommendations**

The management of toxic cyanobacteria in water bodies should encompass the interactive nature of the physical, chemical and biotic parameters that govern the determinism of bloom occurrences. A better understanding of the genetic mechanisms underlying cyanotoxin synthesis has been achieved in the last decade. There is now a need to better appreciate the ecological and physiological pressures that drive the regulation of cyanotoxins in various toxin-producing species. This will require the gathering of information at the cellular level to predict periods of enhanced toxicity given a particular physiological state in a developing population. Additional aspects that should be addressed in the future for improving the monitoring and management of water quality and aquatic resources are provided below.

- 1 The sustainable management of aquatic resources requires the adjunction of new standards and methods to the conventional monitoring procedures already in place for measuring the diversity and abundance of the biota. The molecular genotyping of aquatic communities offers a resolution power greater than that of morphology-based sample examination for species identification and enumeration. The molecular analysis of a fragment of the 16S rDNA gene by DGGE fingerprinting proved useful for ascertaining cyanobacterial diversity in the present study. On a larger-scaled programme, a similar approach could be used for comparing and contrasting the seasonal community dynamics of cyanobacteria in multiple representative sentinel lakes so as to identify potential patterns and responses to human-assisted environmental forcing.
- 2 Recognition of cyanobacterial toxic genotypes is essential to forecast the dynamics of toxins. There is, hence, an urgent need for the development of high throughput sample analysis capacities for both species and toxins. The use of real-time PCR with both toxin- and species-specific primers and probes, or some flow-cytometry type apparatus with fluorescently labelled toxin-targeting antibodies and species-specific probes, would provide reliable quantitative data on the distribution of toxic and non-toxic genotypes in natural populations.
- 3 The cost of environmental monitoring prevents high-frequency sampling in multiple lakes over extensive time periods. Modern technologies based on optical detection and remote sensing could enable the mapping of biomass distribution and, following interpretation, the design of targeted sampling strategies in sentinel water bodies or across multiple watersheds. Valuable data could be generated through the use of shipboard, airborne or satellite spectrometry, which can already differentiate between various groups of algae on the basis of their photosynthetic pigments. The coupling of sentinel type monitoring systems (fixed platforms or floating arrays) with satellite connection for real-time data acquisition will improve research capacities and provide a basal platform for improving current monitoring and management strategies of the environment dramatically.
- 4 The management of toxic cyanobacterial blooms requires extensive knowledge about the environmental factors that control phytoplankton biomass and toxin production. Some blooms dominated by potential toxin-producing species are not toxic at all, whereas others, dominated by the same species, can be highly toxic. The acquisition and analysis of relevant biological and physico-chemical data are therefore needed for the successful parameterisation of future predictive models of cyanobacteria bloom development in lakes. The easy to monitor development of such models, linked with meteorological data and environmental variables will facilitate decision-making in management plans aimed at preventing and controlling harmful phytoplankton blooms.



## 6 Conclusions

The enactment of environmental policies has made the identification and mediation of the factors that degrade surface waters essential. Evidence indicates that the incidence, duration and intensity of harmful algal blooms are increasing because of largely human-assisted alterations of the quality of aquatic environments. Blooms of noxious cyanobacteria occur worldwide and the risks they pose toward human and animal health have been demonstrated clearly. While significant efforts have been made to define the toxicological properties of cyanotoxins, additional efforts from researchers and environmental agencies to determine their toxicity potential and monitor and map their occurrences are required.

Cyanobacteria form a very diverse group of microorganisms whose population dynamics are governed by the interplay of multiple ecological variables. The idea of a single-factor explanation elucidating the determinism of cyanobacteria blooms is not realistic. Instead, the development of adapted measures for particular species in specific aquatic ecosystems is more appropriate for preventing and mitigating noxious effects. Successful water-management strategies pertaining to occurrences of toxic cyanobacteria blooms will necessitate accurate identification of cyanobacteria species, together with a sound understanding of the environmental processes that govern their dynamics at the watershed level. In this context, regionalisation frameworks can enable the identification of groups of water bodies in spatially contiguous hydrological zones at risk of ecological status degradation. In complement, molecular methods constitute promising approaches for monitoring populations of both toxic and non-toxic cyanobacterial genotypes, and for elucidating the factors that drive their dynamics within phytoplankton assemblages.

In Ireland, the management of anthropogenic pressures on water quality and biodiversity assessments in aquatic environments are of strategic relevance. Mortalities of wild and domestic animals have been associated with blooms of toxic cyanobacteria in Irish lakes. Ensuring that recreational and drinking waters are

free of toxins and pathogens is one of the most important challenges for regulatory authorities in the future. Due to the risks of human exposure to water contaminated with toxic cyanobacteria, it is necessary that the population dynamics of these organisms be investigated in Irish lakes for the future development of monitoring strategies and management tools, such as predictive models.

This study investigated the summer variations in the molecular diversity of lacustrine cyanobacteria at a regional level in the west of Ireland. DGGE fingerprinting of 16S rDNA enabled an assessment of the variation in planktonic cyanobacteria community composition among lakes interspersed within various river catchments, highlighting seasonality and the range of land cover typology in the study area. As anticipated, there was wide variation in the structure of the communities sampled. PCR-based analysis of a microcystin-coding gene and analysis of natural extracts by PP2A and analytical chemistry also confirmed the presence of potentially toxic species in multiple lakes within the study area. It is important to consider both spatial and temporal scales to understand the dynamics of primary productivity in relation to biophysical interactions and nutrient loadings in aquatic systems. This study suggested that the application of regionalisation schemes for watershed sampling could be complemented effectively by spreading out the sampling effort in time, returning a dynamic environmental assessment. The assessment of regional diversity patterns in cyanobacterial assemblages constitutes an innovative approach to microbial ecology and shows great potential for incorporation into a battery of methods for the management of aquatic resources. In the future, coupling molecular analysis to routine microbial monitoring programmes could allow the rapid generation of cyanobacterial assemblage fingerprints. The subsequent interpretation of their diversity and dynamics, at varying scales of space and time, would contribute then to the evaluation of the efficiency of the environmental policies introduced to improve lake trophic state and water quality in general.

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## Acronyms and Annotations

AIC	Akaike Information Criterion
ANA	Anatoxin-a
ANOVA	Analyses of variance
BLAST	Basic Local Alignment Search Tool
BMAA	Beta-methylamino alanine
C-toxins	Bi-sulfated
CYN	Cyanobacterial toxins cylindrospermosin
DAD	Diode array detector
DCA	Detrended correspondence analysis
DGGE	Denaturant gradient gel electrophoresis
ELISA	Enzyme-Linked ImmunoSorbent Assay
FISH	Fluorescent in situ hybridisation
GIS	Geographic information system
GTX	Mono-sulfated
HAB	Harmful algal blooms
HPLC	High performance liquid chromatography
LD <sub>50</sub>	Lethal Dose 50%
LPS	Lipopolysaccharide
MFA	Multiple factor analysis
ML	Maxium likelihood
PCA	Principal component analysis
PCR	Polymerase chain reaction
PP2A	Protein Phosphatase 2A
RFLP	Restriction fragment length polymorphism
STX	Non-sulfated
TWINSpan	Two-Way Indicator Species Analysis
WFD	Water Framework Directive

# Appendix 1

**Summary Results Table for Summer 2009**

Station	Location	mcya <sup>1</sup>	PP2A	HPLC-MS/MS	Anabaena sp. trich. <sup>2</sup>	Chroococcales colonies <sup>2</sup>	Oscillatoriales trich. <sup>2</sup>
201	Lough Inagh	-	<LOD				
202	Rosslara Lough	-	<LOD				
203	Tully Lough	-	<LOD				3200
204	Lough Arrow	-	<LOD				
205	Lough Glinn	*	+	<LOD	15600		
206	Dahybaun Lake	-	<LOD				14800
207	Derrywaking lough	-	<LOD				
208	Lough Derg	-	<LOD				
209	Lough Derg	*	+	<LOD		28400	1600
210	Glencar Lough	+	<LOD	<LOD	4600		
211	Lough Ardderry	-	<LOD		2250		
212	Cross Lake	-	<LOD				
213	Ard lough	-	<LOD				
214	Doo Lough	-	<LOD		7100		
215	Lough Corrib	+	<LOD	<LOD			
216	New Lake	+	+	<LOD			3700
217	Durnesh Lake	-	<LOD				
218	Lake Unsheen	-	<LOD				
219	Carrowmore Lough	*	+	<LOD	43500	25200	
220	Lough Graney	+	+	<LOD	4300		
221	Knocka Lough	+	+	<LOD	15000		5000
222	Lough Gall	-	<LOD				
223	Lough Fee	-	<LOD			1500	59800
224	Lough Gill	+	<LOD	<LOD			
225	Lough Talt	-	<LOD				4500
226	Dungloe Lake	+	<LOD	<LOD			
227	Lough Adderry	-	<LOD		2100		
228	Cavetown Lake	*	+	<LOD			
229	Ballyquirke Lake	+	<LOD	<LOD			
230	Lough Ree	+	+	<LOD			
231	Shliabh an Aonaigh	-	<LOD				
232	Loughrea	+	<LOD	<LOD			
233	Lough Lickeen	+	+	+	3000	5500	
234	Lough Eske	+	<LOD	<LOD		2000	
235	Garadice Lake	+	+	+	3300		
236	Lough Carra	*	+	<LOD	2700		
237	Nambrackmore lake	-	<LOD				2600
238	Lough Conn	-	<LOD				
239	Aconeera Lough	+	<LOD	<LOD			
240	Polleagh Lough	-	<LOD				
241	Maumeen Lake	-	<LOD		2900		2100
242	Lough Sheelin	+	+	+	1300		

Continued overleaf

**Summary Results Table for Summer 2009 (continued)**

Station	Location	mcvA <sup>1</sup>	PP2A	HPLC-MS/MS	<i>Anabaena</i> sp. trich. <sup>2</sup>	Chroococcales colonies <sup>2</sup>	Oscillatoriales trich. <sup>2</sup>
243	Lough Feeagh	+	<LOD	<LOD			
244	Lough Gara	-	<LOD				
245	Lough Easky	-	<LOD				
246	Lough Cutra	+	<LOD	<LOD			
247	Uggabeg Lake	-	<LOD				
248	Inchiquin Lake	-	<LOD				
249	Lough Anillaun	-	<LOD				
250	Beltra Lough	+	<LOD	<LOD		1600	

<sup>1</sup> \*\* indicates the lakes where PP2A positive results were not unambiguously corroborated by the mcvA PCR analysis.

<sup>2</sup> Figures are shown only for the lakes where concentrations were >1000 trichomes or colonies per litre at the time of sampling.



# An Ghníomhaireacht um Chaomhnú Comhshaoil

Is í an Ghníomhaireacht um Chaomhnú Comhshaoil (EPA) comhlachta reachtúil a chosnaíonn an comhshaol do mhuintir na tíre go léir. Rialaímid agus déanaimid maoirsiú ar ghníomhaíochtaí a d'fhéadfadh truailliú a chruthú murach sin. Cinntímid go bhfuil eolas cruinn ann ar threochtaí comhshaoil ionas go nglactar aon chéim is gá. Is iad na príomhnithe a bhfuilimid gníomhach leo ná comhshaol na hÉireann a chosaint agus cinntiú go bhfuil forbairt inbhuanaithe.

Is comhlacht poiblí neamhspleách í an Ghníomhaireacht um Chaomhnú Comhshaoil (EPA) a bunaíodh i mí Iúil 1993 faoin Acht fán nGníomhaireacht um Chaomhnú Comhshaoil 1992. Ó thaobh an Rialtais, is í an Roinn Comhshaoil, Pobal agus Rialtais Áitiúil.

## ÁR bhFREAGRACHTAÍ

### CEADÚNÚ

Bíonn ceadúnais á n-eisiúint againn i gcomhair na nithe seo a leanas chun a chinntiú nach mbíonn astuithe uathu ag cur sláinte an phobail ná an comhshaol i mbaol:

- áiseanna dramhaíola (m.sh., líonadh talún, loisceoirí, stáisiúin aistrithe dramhaíola);
- gníomhaíochtaí tionsclaíocha ar scála mór (m.sh., déantúsaíocht cógaisíochta, déantúsaíocht stroighne, stáisiúin chumhachta);
- diantalmhaíocht;
- úsáid faoi shrian agus scaoileadh smachtaithe Orgánach Géinathraithe (GMO);
- mór-áiseanna stórais peitreal;
- scardadh dramhuisce.

### FEIDHMIÚ COMHSHAOIL NÁISIÚNTA

- Stiúradh os cionn 2,000 iniúchadh agus cigireacht de áiseanna a fuair ceadúnas ón nGníomhaireacht gach bliain.
- Maoirsiú freagrachtaí cosanta comhshaoil údarás áitiúla thar sé earnáil - aer, fuaim, dramhaíl, dramhuisce agus caighdeán uisce.
- Obair le húdaráis áitiúla agus leis na Gardaí chun stop a chur le gníomhaíocht mhídhleathach dramhaíola trí chomhordú a dhéanamh ar líonra forfheidhmithe náisiúnta, díriú isteach ar chiontóirí, stiúradh fiosrúcháin agus maoirsiú leigheas na bhfadhbanna.
- An dlí a chur orthu siúd a bhriseann dlí comhshaoil agus a dhéanann dochar don chomhshaol mar thoradh ar a ngníomhaíochtaí.

### MONATÓIREACHT, ANAILÍS AGUS TUAIRISCIÚ AR AN GCOMHSHAOIL

- Monatóireacht ar chaighdeán aer agus caighdeáin aibhneacha, locha, uiscí taoide agus uiscí talaimh; leibhéil agus sruth aibhneacha a thomhas.
- Tuairisciú neamhspleách chun cabhrú le rialtais náisiúnta agus áitiúla cinntiú a dhéanamh.

### RIALÚ ASTUITHE GÁIS CEAPTHA TEASA NA HÉIREANN

- Caimníochtú astuithe gáis ceaptha teasa na hÉireann i gcomhthéacs ár dtiomantas Kyoto.
- Cur i bhfeidhm na Treorach um Thrádáil Astuithe, a bhfuil baint aige le hos cionn 100 cuideachta atá ina mór-ghineadóirí dé-ocsaíd charbóin in Éirinn.

### TAIGHDE AGUS FORBAIRT COMHSHAOIL

- Taighde ar shaincheisteanna comhshaoil a chomhordú (cosúil le caighdeán aer agus uisce, athrú aeráide, bithéagsúlacht, teicneolaíochtaí comhshaoil).

### MEASÚNÚ STRAITÉISEACH COMHSHAOIL

- Ag déanamh measúnú ar thionchar phleananna agus chláracha ar chomhshaol na hÉireann (cosúil le pleananna bainistíochta dramhaíola agus forbartha).

### PLEANÁIL, OIDEACHAS AGUS TREOIR CHOMHSHAOIL

- Treoir a thabhairt don phobal agus do thionscal ar cheisteanna comhshaoil éagsúla (m.sh., iarratais ar cheadúnais, seachaint dramhaíola agus rialacháin chomhshaoil).
- Eolas níos fearr ar an gcomhshaol a scaipeadh (trí cláracha teilifíse comhshaoil agus pacáistí acmhainne do bhunscoileanna agus do mheánscoileanna).

### BAINISTÍOCHT DRAMHAÍOLA FHORGHNÍOMHACH

- Cur chun cinn seachaint agus laghdú dramhaíola trí chomhordú An Chláir Náisiúnta um Chosc Dramhaíola, lena n-áirítear cur i bhfeidhm na dTionscnamh Freagrachta Táirgeoirí.
- Cur i bhfeidhm Rialachán ar nós na treoracha maidir le Trealamh Leictreach agus Leictreonach Caite agus le Srianadh Substaintí Ghuaiseacha agus substaintí a dhéanann ídiú ar an gcrios ózóin.
- Plean Náisiúnta Bainistíochta um Dramhaíl Ghuaiseach a fhorbairt chun dramhaíl ghuaiseach a sheachaint agus a bhainistiú.

### STRUCHTÚR NA GNÍOMHAIREACHTA

Bunaíodh an Ghníomhaireacht i 1993 chun comhshaol na hÉireann a chosaint. Tá an eagraíocht á bhainistiú ag Bord lánaimseartha, ar a bhfuil Príomhstíúrthóir agus ceithre Stíúrthóir.

Tá obair na Ghníomhaireachta ar siúl trí ceithre Oifig:

- An Oifig Aeráide, Ceadúnaithe agus Úsáide Acmhainní
- An Oifig um Fhorfheidhmiúchán Comhshaoil
- An Oifig um Measúnacht Comhshaoil
- An Oifig Cumarsáide agus Seirbhísí Corparáide

Tá Coiste Comhairleach ag an nGníomhaireacht le cabhrú léi. Tá dáréag ball air agus tagann siad le chéile cúpla uair in aghaidh na bliana le plé a dhéanamh ar cheisteanna ar ábhar imní iad agus le comhairle a thabhairt don Bhord.

### **Science, Technology, Research and Innovation for the Environment (STRIVE) 2007-2013**

The Science, Technology, Research and Innovation for the Environment (STRIVE) programme covers the period 2007 to 2013.

The programme comprises three key measures: Sustainable Development, Cleaner Production and Environmental Technologies, and A Healthy Environment; together with two supporting measures: EPA Environmental Research Centre (ERC) and Capacity & Capability Building. The seven principal thematic areas for the programme are Climate Change; Waste, Resource Management and Chemicals; Water Quality and the Aquatic Environment; Air Quality, Atmospheric Deposition and Noise; Impacts on Biodiversity; Soils and Land-use; and Socio-economic Considerations. In addition, other emerging issues will be addressed as the need arises.

The funding for the programme (approximately €100 million) comes from the Environmental Research Sub-Programme of the National Development Plan (NDP), the Inter-Departmental Committee for the Strategy for Science, Technology and Innovation (IDC-SSTI); and EPA core funding and co-funding by economic sectors.

The EPA has a statutory role to co-ordinate environmental research in Ireland and is organising and administering the STRIVE programme on behalf of the Department of the Environment, Heritage and Local Government.



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Environment, Community and Local Government