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Comparative summer dynamics of surface cyanobacterial communities in two connected lakes from the west of Ireland

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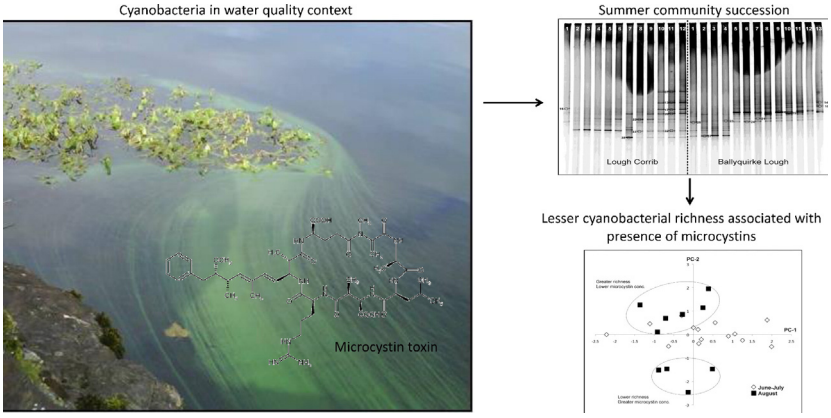
HIGHLIGHTS

- DGGE highlighted cyanobacterial community differences in two connected lakes.
- Microcystin-coding genes and microcystin-like activity were detected in both lakes.
- Low cyanobacterial richness was associated with increased microcystin-like activity.

GRAPHICAL ABSTRACT



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ABSTRACT

The eutrophication of lakes is typically associated with high biomass proliferations of potentially toxic cyanobacteria. At a regional level, the sustainable management of water resources necessitates an approach that recognises the interconnectivity of multiple water systems within river catchments. This study examined the dynamics in summer diversity of planktonic cyanobacterial communities and microcystin toxin concentrations in two inter-connected lakes from the west of Ireland prone to nutrient enrichment. DGGE analysis of 16S rRNA gene amplicons of genotype-I cyanobacteria (typically spherical) showed changes in the communities of both Lough Corrib and Ballyquirke Lough throughout the summer, and identified cyanobacterial genotypes both unique and shared to both lakes. Microcystin concentrations, estimated via the protein phosphatase 2A inhibition assay, were greater in August than in July and June in both lakes. This was concomitant to the increased occurrence of *Microcystis* as evidenced by DGGE band excision and subsequent sequencing and BLAST analysis. RFLP analysis of PCR amplified mcy-A/E genes clustered together the August samples of both lakes, highlighting a potential change in microcystin producers across the two lakes. Finally, the multiple factor analysis of the combined environmental data set for the two lakes highlighted the expected pattern opposing greater water temperature and chlorophyll concentration against macronutrient concentrations, but also indicated a negative relationship between microcystin concentration and cyanobacterial diversity, possibly underlining allelopathic interactions. Despite some element of connectivity, the dissimilarity in the composition of the cyanobacterial

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assemblages and the timing of community change in the two lakes likely were a reflexion of niche differences determined by meteorologically-forced variation in physico-chemical parameters in the two water bodies.

1. Introduction

The management of water quality is an important endeavour worldwide relevant to biodiversity preservation, ecosystem balance and public health safety (Papastergiadou et al., 2010). Increasing anthropogenic pressures on aquatic environments have been met with the development of stringent policies, such as the EU Water Framework Directive in Europe, implemented to preserve the sustainability of aquatic resources (European Communities, 2000). Their efficient management requires a better understanding of the interconnections between the factors that determine quantitative and qualitative patterns in the biota components of aquatic ecosystems.

The eutrophication of continental waters is a significant environmental problem, which has usually been associated with high biomass algal 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 toward animal and human health (Codd et al., 2005). Cyanobacteria are ubiquitous components of phytoplankton assemblages in aquatic environments and although proliferations are typically seasonal, perennial blooms have been documented in several eutrophic lakes (Mur et al., 1999).

The dynamics of cyanobacterial populations in lakes have been extensively studied due to the potential noxious effects some species can elicit. Animal death and human illness have been associated with the production by some species of a diverse array of biotoxins (Codd et al., 2005). The most common are the hepatotoxic cyclic heptapeptides microcystins, which have been non-exhaustively found in the *Microcystis*, *Anabaena*, *Nostoc* or *Planktothrix* genera (Vezie et al., 1998; Marsalek et al., 2000). Many microcystin structural forms of varying toxicity have been characterised with a mode of action associated with the inhibition of protein phosphatases.

Cyanotoxins constitute a serious problem for the management of drinking water as toxin concentrations in lakes do not necessarily reflect the size of cyanobacterial communities. Succession in toxigenic cyanobacteria may also impact upon the overall amount of cyanotoxins present in water (Kardinnal and Visser, 2005). Moreover, variation in cellular toxin quotas may result from changes in environmental conditions (Dokulil and Teubner, 2000). It has hence been recommended to base the management of cyanobacterial blooms on both toxin concentration and biomass. For example, guideline values for microcystin concentration and cyanobacterial biomass in water, issued by the World Health Organization, have been set to 1 $\mu g \cdot l^{-1}$ and 10 $\mu g \cdot l^{-1}$, respectively (WHO, 2003).

The determination of the taxonomic diversity of cyanobacterial communities has traditionally been carried out by light microscopy. Difficulties in the management of toxic blooms have lied in the common co-occurrence of multiple morphologically similar species, which may or not synthesise toxins. Difficulties have been further compounded by the fact that there exist in individual species both toxic and nontoxic forms (Kurmayer et al., 2004).

Molecular techniques have permitted to better characterise microbial genetic diversity in various ecosystems. In particular for cyanobacteria, phylogenetic studies based on 16S rDNA polymorphism have demonstrated the existence of both polyphyletic and monophyletic groups interspersed in various clades (Willmotte and Herdman, 2001; Abed et al., 2002; Litvaitis, 2002). Other studies have led to the design of taxa-specific molecular markers suitable for the discrimination of morphologically similar species (Zehr et al., 1997; Runnegar et al., 1995; Beard et al., 1999; Iteman et al., 2000; Tillett et al., 2001; Zeidner et al., 2003). The molecular fingerprinting of microbial communities using typing methods such as denaturant gradient gel electrophoresis (DGGE) of PCR-amplified genes have notably proved successful to determine patterns in microbial species succession and community structure, including cyanobacteria (Geiss et al., 2004; Song et al., 2005; Briand et al., 2009). The elucidation of the genetic basis of non-ribosomal microcystin biosynthesis has also resulted with the characterisation of the microcystin toxin gene cluster (*mcy*) in several cyanobacterial genera (Neilan et al., 1999; Christiansen et al., 2003; Rouhiainen et al., 2004). Primers have since been tested using both culture and field samples, for the specific amplification of different domains of the *mcy* gene cluster (Pan et al., 2002; Hisbergues et al., 2003).

These methodological developments offer opportunities to better manage the risks associated with cyanobacterial blooms. As aquatic resources are increasingly being managed at the water catchment or regional level, there is a need to better understand the dynamics in space and time of biota communities in relation to the degree of connectivity of water bodies. In this context this study was carried out to compare the dynamics in summer diversity of planktonic cyanobacterial communities in two inter-connected lakes prone to nutrient enrichment. The main hypothesis focused on ascertaining whether or not there was a difference in cyanobacterial community composition in Ballyquirke Lough and Lough Corrib. To this end, cyanobacterial diversity was estimated by genotypic analysis via DGGE resolution of 16S rDNA amplicons. Along with the measurement of limnological variables, the potential existence of a relationship between variation in cyanobacterial community composition and the onset of microcystin production in the two lakes was also examined.

2. Material and methods

2.1. Study area

Sampling was carried during summer 2010 in two connected lakes in the west of Ireland (Fig. 1). Western Irish lakes are managed as recreational fisheries resources and constitute important tourist attractions for the region. Changes in farming practices near some lakes have however generated concerns about their vulnerability to eutrophication (McCarthy et al., 2001). Lough Corrib is the largest of the western Irish lakes with a surface extending over 180 km^2 and a catchment area of ~3000 km^2. The lake spans two geological basins mainly constituted of granite and limestone and contains multiple islands and bays. Lough Corrib Lower has an average depth of 2.1 m (max. 5.5 m) while that of Lough Corrib Upper is 8.4 m (max. 42.0 m). Ballyquirke Lough lies southeast of Lough Corrib Lower and an ~3 km long river connects the two water bodies. The underlying limestone bedrock also provides ample subterranean drainage routes. Ballyquirke Lough is much smaller with a surface and catchment area of 79.2 ha and 7319 ha, respectively.

2.2. Sampling and data acquisition

Sampling was carried out on a near weekly basis in the two lakes along transects of stations between June 6th and September 2nd 2010. Surface water temperature and depth profiles were recorded using a YSI MultiQuatroPro probe. Geographical coordinates were taken with a GPS and water transparency was determined with a Secchi disk. Solar irradiance, wind speed and daily rainfall data were retrieved from the meteorological station at the National University of Ireland Galway but were only available from mid-July for seven weeks. Surface water samples were immediately filtered onto Whatmann GF/F filters for subsequent analysis of chlorophyll-*a* and microcystin-like activity.