Science of the Total Environment 624 (2018) 366–376

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Characterisation of a major phytoplankton bloom in the River Thames (UK) using flow cytometry and high performance liquid chromatography

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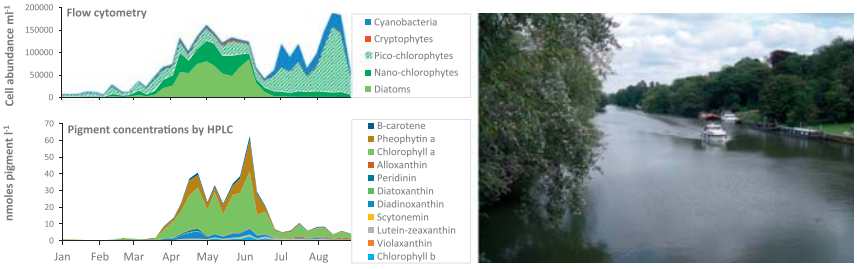
HIGHLIGHTS

- Controls on river phytoplankton succession and biomass poorly understood.
- Weekly HPLC and flow cytometry used to characterise major river phytoplankton bloom.
- Community shifts from diatoms (spring) to picochlorophytes and cyanobacteria (summer).
- Combined analytical approaches provide knowledge of wider range of planktonic groups.
- HPLC/flow cytometry provide key data to improve understanding of bloom dynamics.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 11 October 2017
Received in revised form 11 December 2017
Accepted 11 December 2017
Available online xxxx

Editor: Jay Gan

Keywords:
Algae
Cyanobacteria
Eutrophication
Water quality
High-frequency monitoring
Photosynthetic pigments

ABSTRACT

Recent river studies have observed rapid phytoplankton dynamics, driven by diurnal cycling and short-term responses to storm events, highlighting the need to adopt new high-frequency characterisation methods to understand these complex ecological systems. This study utilised two such analytical methods; pigment analysis by high performance liquid chromatography (HPLC) and cell counting by flow cytometry (FCM), alongside traditional chlorophyll spectrophotometry and light microscopy screening, to characterise the major phytoplankton bloom of 2015 in the River Thames, UK. All analytical techniques observed a rapid increase in chlorophyll *a* concentration and cell abundances from March to early June, caused primarily by a diatom bloom. Light microscopy identified a shift from pennate to centric diatoms during this period. The initial diatom bloom coincided with increased HPLC peridinin concentrations, indicating the presence of dinoflagellates which were likely to be consuming the diatom population. The diatom bloom declined rapidly in early June, coinciding with a storm event. There were low chlorophyll *a* concentrations (by both HPLC and spectrophotometric methods) throughout July and August, implying low biomass and phytoplankton activity. However, FCM revealed high abundances of pico-chlorophytes and cyanobacteria through July and August, showing that phytoplankton communities remain active and abundant throughout the summer period. In combination, these techniques are able to simultaneously characterise a wider range of phytoplankton groups, with greater certainty, and provide improved understanding of phytoplankton functioning (e.g. production of UV inhibiting pigments by cyanobacteria in response to high light levels) and ecological status (through examination of pigment degradation products). Combined HPLC and FCM analyses offer rapid and cost-effective characterisation of phytoplankton communities at

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https://doi.org/10.1016/j.scitotenv.2017.12.128
0048-9697/

appropriate timescales. This will allow a more-targeted use of light microscopy to capture phytoplankton peaks or to investigate periods of rapid community succession. This will lead to greater system understanding of phytoplankton succession in response to biogeochemical drivers.

1. Introduction

Phytoplankton plays a vital role within the aquatic ecosystem. In large rivers, they are often the principal autotrophs, supplying organic carbon to the system, oxygenating the water column and providing the base of aquatic food webs (Thorp and Delong, 1994; Wehr and Descy, 1998). However, under certain conditions (exacerbated by anthropogenic nutrient enrichment, river flow regulation, land-use change and food-web disturbance), excessive phytoplankton growth can occur (Bowes et al., 2012). These phytoplankton blooms can pose serious threats to ecosystem structure and function, water quality, and threaten drinking water supplies (Dodds et al., 2009). They also reduce sunlight penetration within the water column, resulting in the loss of macrophytes and the vital habitat that they provide for zooplankton, macroinvertebrates and fish. Blooms can be aesthetically unappealing, resulting in significant financial losses for the tourism industry and reduce the value of riverside properties (Pretty et al., 2003; Dodds et al., 2009). High densities of phytoplankton can also increase operating costs for water companies due to blocking of filters and toxin/taint production by cyanobacteria. When blooms terminate, there is often depletion in dissolved oxygen concentration as the algae are consumed by heterotrophs, and these oxygen sags can ultimately result in fish-kills (Hilton et al., 2006).

The plankton communities of marine, estuarine and lake environments have been extensively monitored, principally by chlorophyll analysis and algal identification/quantification by light microscopy. This has meant that the characteristics and drivers of phytoplankton succession are relatively well understood in these systems (Sommer et al., 2012). Over the last few decades, high performance liquid chromatography (HPLC) has been widely used to quantify pigments associated with phytoplankton groups (Barlow et al., 1993; Pinckney et al., 1998), which provide further information on phytoplankton biomass and community structure in estuarine and marine environments. In recent years, a new high-throughput technique, flow cytometry (FCM), has been employed to rapidly characterise and quantify marine and lake planktonic communities (Veldhuis and Kraay, 2004; Sarmento et al., 2006; Pomati et al., 2011). When used in combination, these techniques can provide a much more complete description of plankton dynamics, and are able to produce data at a much higher temporal resolution than traditional light microscopy alone.

In contrast, river phytoplankton succession has been relatively understudied in comparison to marine and lake environments (Reynolds and Descy, 1996). As a consequence, our knowledge of the causes of, and controls on river phytoplankton bloom development are not well understood (Reynolds, 1993). Previous studies of temperate rivers have shown how phytoplankton biomass can be affected by a combination of factors including hydrology (Reynolds and Descy, 1996; Hardenbicker et al., 2014), nutrient limitation (Tavernini et al., 2011; Wu et al., 2011), grazing (Basu and Pick, 1997; Twiss et al., 2010), light (Dokulil, 2014) and temperature (Desortova and Puncochar, 2011). Most river phytoplankton studies are based on infrequent (fortnightly to seasonal) chlorophyll analysis (to give an estimate of phytoplankton biomass), and/or identification and quantification by light microscopy to give some indication of phytoplankton succession.

Recent riverine studies have highlighted the rapid sub-daily changes in chlorophyll *a* concentration that can occur (Dubelaar et al., 2004; Bowes et al., 2016), highlighting the need for future river research to embrace new, high-throughput monitoring techniques such as HPLC and FCM to capture these short term phytoplankton dynamics and enable greater process understanding. However, to date, the number of riverine HPLC pigment studies are limited, and have been used to capture general changes in phytoplankton communities through the annual cycle (Descy and Metens, 1996; Dorigo et al., 2004) or along the river continuum (Dagg et al., 2005; Descy et al., 2017). Application of FCM to rivers is extremely limited. Dubelaar et al. (2004) used FCM to characterise daily changes in the phytoplankton community over a two week period in the Oude Rijn River, Netherlands, highlighting the rapid dynamics that occur in riverine environments. FCM has also been used to quantify river bacterioplankton abundances (Paulse et al., 2007; Besmer et al., 2014; Read et al., 2015). Read et al. (2014) used FCM to enumerate ten phytoplankton groups (including diatoms, chlorophytes, cryptophytes and cyanobacteria) at 23 sites across the River Thames basin at weekly intervals, and these data have been utilised to model the algal dynamics and potential impact of climate change on phytoplankton community composition (Whitehead et al., 2015; Bussi et al., 2016).

This study aimed to characterise the phytoplankton community succession of the River Thames, southern England, at weekly interval through the spring to autumn period of 2015, using both traditional techniques (spectrophotometric chlorophyll *a* determination and light microscopy screening) alongside HPLC and FCM. To our knowledge, this is the first time these two instrumental techniques have been applied simultaneously to monitor a river phytoplankton bloom. The monitoring data was then assessed to identify strengths and potential weaknesses of these techniques, and to determine if greater understanding of phytoplankton dynamics can be achieved by combining these multiple approaches.

2. Methods

2.1. Study site

The River Thames is the longest river wholly in England (354 km), and the second longest in the UK, with a freshwater basin of 9948 km^2. It rises at Thames Head in Gloucestershire, and flows in an easterly direction through the relatively rural Cotswold Hills, then passing through a number of large towns and cities, such as Swindon, Oxford, Reading and Maidenhead, before flowing through the centre of London and into the North Sea (Fig. 1). Approximately one fifth of the UK population (with a population density of 960 people km^2) reside in the catchment (Merrett, 2007). This study was conducted on the River Thames at the town of Wallingford, 134 km from the source. Previous studies along the River Thames have shown that chlorophyll maximum concentrations occur followed by depletions of soluble reactive phosphorus and silicon within the reach from Oxford to Wallingford (Lazar et al., 2012; Bowes et al., 2016) (Fig. 1), making this productive site ideal to study phytoplankton dynamics, hence its choice for this study. Mean river flow at Wallingford is approximately 32 $m^3\ s^{-1}$ with a base flow index of 0.64 (due to significant groundwater input from the Cretaceous Chalk and Oolitic Limestone geology) and mean annual rainfall of 715 mm (Marsh and Hannaford, 2008). The catchment area upstream of Wallingford is 4213 km^2, with land use consisting of 45.1% arable, 35.6% grassland, 10.3% woodland, and 7.3% urban and semi-urban development (Fuller et al., 2002). The sewage treatment works (STW) population estimate upstream of Wallingford is approximately 1.03 million people (Bowes et al., 2014). The large number of STW, high population density and areas of relatively intensive agriculture result in the River Thames at Wallingford having elevated nutrient