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Assessment of *in situ* fluorometry to measure cyanobacterial presence in water bodies with diverse cyanobacterial populations

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ABSTRACT

A YSI EXO2 water quality sonde fitted with fluorometric sensors for chlorophyll-*a* (Chl-*a*) and phycocyanin (CPC) was used to determine its applicability in cyanobacterial quantification in three small urban ponds in Sydney, Australia displaying considerable variations in cyanobacterial community composition and abundance, as well as eukaryotic algae, turbidity and chromophoric dissolved organic matter. CPC and Chl-*a* measured *in situ* with the instrument was compared against laboratory measures of cyanobacterial biovolume over two summer sampling periods. A good correlation was found between CPC and total cyanobacterial biovolume in two of the three ponds. The poor correlation in the third was due to the frequent dominance of picoplanktonic sized cyanobacteria. CPC did not correlate well with cell counts, and Chl-*a* was a poor measure of cyanobacterial presence. The relationship between CPC measured by fluorometry varied according to the dominant cyanobacterial taxa present in the ponds at any one time. Fluorometry has good potential for use in environmental monitoring of cyanobacterial biovolume, but may need to be based on predetermined relations applicable to local water bodies. Management guidelines based on CPC concentrations would also enhance the usefulness of *in situ* CPC measurements.

1. Introduction

Cyanobacterial blooms in freshwater bodies are of considerable concern worldwide, because they pose a major hazard to human health, livestock, wildlife and the aquatic environment (Ibelings et al., 2008; Pilotto, 2008; Merel et al., 2013). A number of commonly occurring species have the ability to produce potent toxins that require removal prior to the water being safe for potable use (Zamyadi et al., 2012a), and which can poison domestic and wild animals drinking from impacted waters (Stewart et al., 2008). Cyanobacteria have also been hypothesised to be a possible cause of neurodegenerative illness (Holtcamp, 2012; Bradley et al., 2013), and their cell walls contain compounds (lipopolysaccharides) that can act as contact irritants (Pilotto, 2008). Because of the public health risk, the management of cyanobacteria in freshwater systems, especially those used as a source of drinking water and for recreation, is a major activity in many parts of the world (Chorus, 2012; Ibelings et al., 2014).

Monitoring techniques for cyanobacteria are many and varied, as reviewed by Srivastava et al. (2013). The traditional method most widely used is the collection of water samples for microscopic analysis which provides identification and enumeration of the major taxa present, and is also used as the basis for biomass or biovolume estimations which are used in some countries for management purposes (Chorus, 2012; Ibelings et al., 2014). Other methods include pigment analysis, remote sensing and chemical, biochemical and genetic analysis to determine toxin presence or the toxigenicity of blooms (Srivastava et al., 2013). Major drawbacks of most of these methods are that they are costly, require considerable laboratory expertise and are time consuming, and therefore they are seldom able to deliver information for management use in a timely manner (Zamyadi et al., 2012a).

The measurement of freshwater cyanobacterial presence utilising the *in vivo* fluorescence of the photosynthetic and ancillary

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pigments they contain, notably chlorophyll-*a* (Chl-*a*) and phycocyanin (CPC), is one potential means of obtaining near real-time assessments for management purposes. A range of instruments manufactured by several different companies are available for this purpose. The performance of these instruments has been studied over a variety of lakes, reservoirs and rivers (e.g. Gregor et al., 2005; Brient et al., 2008; McQuaid et al., 2011; Bastien et al., 2011; Catherine et al., 2012; Bowling et al., 2013; Song et al., 2013; Kong et al., 2014), as has their use in on-line monitoring of raw and treated water in water treatment plants (e.g. Izydorczyk et al., 2005, 2009; Gregor et al., 2007; Zamyadi et al., 2012b, 2016a). Although these studies have generally found good relationships ($0.6 < r^2 < 0.9$) between cyanobacterial presence measured *in situ* using fluorometric instruments and laboratory measures, chromophoric dissolved organic matter (CDOM), total suspended material (TSM), eukaryotic algal presence and variations in cyanobacterial community composition and bloom condition may cause limitations in their accuracies (Chang et al., 2012; Zamyadi et al., 2012c, 2016a; Kring et al., 2014). Recently Zamyadi et al. (2016a) concluded that the applicability of *in situ* fluorescence measurements to implement a reliable monitoring strategy and trigger action requires further investigation following a robust field experimental plan.

The main objective of this study was to examine the performance of an *in situ* fluorometry instrument to monitor cyanobacterial presence in mixed phytoplankton communities. The specific objectives of the study were (1) to compare the data collected *in situ* in three urban ponds with relevant laboratory measured data for water samples collected at the same time as the field data and (2) to identify water quality/conditions and phytoplankton community factors that influence the accuracy of *in situ* measurements. To the best of the authors knowledge this study presents novel information on the application of fluorometric probes for cyanobacterial monitoring in water bodies with variable phytoplankton communities, which allows us to test the hypothesis that fluorometric measurements will differ in response to what taxa are present in the water body.

2. Material and methods

2.1. The YSI EXO2 instrument

A newly purchased YSI EXO2 water quality sonde fitted with a fluorometric probe (EXO Total Algae PC Smart sensor) that measures both chlorophyll-*a* (Chl-*a*) and phycocyanin (CPC) was used for the study. The latter is an ancillary pigment found predominantly in cyanobacteria in freshwater systems (but also in cryptophytes), and which is therefore a potentially useful indicator of cyanobacterial biovolume in these systems. This multiprobe system was selected because: (1) It provides access to raw fluorescence readings, as relative fluorescent units (RFU); (2) Suitable lightweight probe design for long term continuous monitoring; (3) Features incorporated into its design, e.g. wipers, to minimise maintenance requirements; (4) User friendly hardware and software; and (5) Reported linear ($0.6 < r^2 < 0.9$) and sensitive measurements. The probe was calibrated according to the manufacturer's instructions using rhodamine, providing readout as RFU, as recommended by Zamyadi et al. (2012c). Although it also provides data as $\mu g\ l^{-1}$ of pigment derived via an onboard default conversion factor, laboratory tests (not reported here) have indicated that these data may considerably underestimate the actual pigment concentration. The sonde was also fitted with sensors for temperature, electrical conductivity, dissolved oxygen, pH, turbidity and Fluorescent Dissolved Organic Matter (fDOM), but for this study, we were principally interested in the pigment measurements.

2.2. The ponds

Three small urban ponds in Sydney, New South Wales, Australia were chosen for this study, namely at Sir Joseph Banks Park in Botany (henceforth termed SJBP), and Kensington and Duck Ponds in Centennial Park, Randwick (Fig. 1). Details of their morphology and water quality are provided in Supplementary Table S1. The ponds are eutrophic and usually have cyanobacterial blooms each summer, being subjected to urban stormwater runoff during wet periods, and water loss by evaporation and possibly to groundwater during dry periods. Water levels can fluctuate up to 0.5 m. Because of their shallow depth and exposure they are assumed to be well mixed both vertically and horizontally, and additionally SJBP has artificial mixing devices operating. Cyanobacterial surface scums were present at both SJBP and Kensington Pond on occasions during the sampling program.

2.3. Field sampling and laboratory analysis

Field sampling at the ponds took place over two austral spring, summer and autumn periods, from September 2013 to May 2014, and from October 2014 to May 2015, on an approximately weekly basis. The YSI EXO2 sonde was placed in the water from off the shoreline at SJBP, from a bridge midway over Kensington Pond, and from an overwater platform at Duck Pond, with the sensors 25 cm below the water surface. The same location in each pond was used on each sampling occasion. The sonde was set to log data at 10 s intervals, with logging conducted over a period of approximately 10 min.

Water samples were collected for laboratory analysis from 25 cm below the water surface at the same location as the YSI EXO2 sonde in each pond using a 3 m long extension pole in new 250 ml

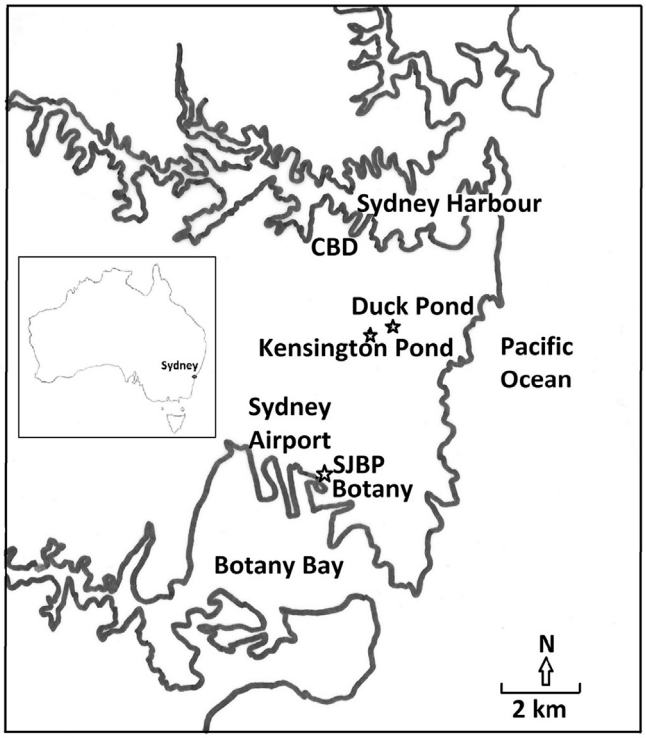

Fig. 1. Map showing the location of the urban ponds sampled for this study.