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► **To cite this version:**

Brigitte Vinçon Leite, Nicolas Clercin, Lenora Ludolf Gomes, Francesco Piccioni, P Dubois, et al.. High-frequency monitoring for in situ cyanobacterial risk assessment. IWA World Water Congress, International Water Association (IWA), Oct 2020, Copenhagen, Denmark. hal-04216165

HAL Id: hal-04216165

<https://enpc.hal.science/hal-04216165v1>

Submitted on 23 Sep 2023

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High-frequency monitoring for *in situ* cyanobacterial risk assessment

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Abstract: Worldwide, water resources for drinking water supply are severely impacted by multiple anthropogenic pressures. Cyanobacteria blooms can generate serious health risks. Current methods for determining toxic species are based on microscope identification. These require specialized skills and are time-consuming. Thus, easy to implement methods must be developed such as those based on fluorescence probes of chlorophyll and cyanobacteria-specific pigments. Collected data are very variable depending on the hardware characteristics and may be affected by large errors. In order to investigate their reliability, a field survey has been performed for 2 years, in a small experimental lake (Great Paris, France). This included high-frequency measurements of both Chl-*a* and phycocyanin fluorescence. Lab analysis and Fluorescent Excitation-Emission Matrices (F-EEM) were also realised. The dataset was analysed in order to define sensor reliability conditions for assessing the cyanobacteria biomass.

Keywords: Cyanobacteria, Fluorescence probes, monitoring

Introduction. Lakes and reservoirs are strategic water resources for drinking water supply in metropolitan areas. They are severely impacted by multiple anthropogenic pressures, including water abstraction, water pollution, eutrophication, erosion, micropollutants, which raise major problems, for both water quantity and quality. Crucial water quality issues are related to eutrophication, leading to harmful algal blooms (*e.g.* Cyanobacteria). Due to global warming the risk of toxic cyanobacteria blooms increases as well as public health problems (Lüring et al., 2017; O'Neil et al., 2012). Threshold levels of cyanobacteria biomasses and toxin concentrations are provided by the World Health Organization (WHO) guidelines and, in many countries, by national regulations which require that cyanobacteria biomass is monitored to avoid health risks. Generally, these are based on monitoring schemes comprising cyanobacteria cell numbers. The delay between water sampling and the availability of the laboratory results can take a few days. This can lead to wrong decisions regarding the adaptation of the raw water treatment process. In this context, low cost, adaptive and efficient monitoring system of cyanobacteria blooms are increasingly required. During the last decade, specialized sensors enabling the possibility to measure *in situ* proxies of cyanobacterial biomass have been developed. Paradoxically, operational measuring systems of cyanobacterial specific pigment, phycocyanin, are not widely used and seldom *in situ* validated with reference methods. In this paper we present (i) the design of a high-frequency (HF) monitoring of cyanobacteria biomass in a small experimental lake; (ii) the field survey

implemented for validating the real-time monitoring system; (iii) the analysis of the dataset obtained.

Material and methods. The study site, Lake Champs-sur-Marne, located in the eastern Great Paris area (France) is a sand-pit lake fed by the water table from the nearby Marne River (Fig. 1). The lake is small (12 ha) and very shallow (mean depth 2.4 m).

High-frequency measurements (every 10 min) of physical-chemical variables (water temperature, conductivity, pH, O₂), chlorophyll-*a* fluorescence (Chla), as a proxy of total phytoplankton biomass, and phycocyanin fluorescence, as a proxy of cyanobacterial biomass, were performed at three different sites (P, A and B, Fig. 1). At each site, the continuous monitoring was set at three fixed depths for sites A and B and two depths for site P using a set of different underwater sensors. At sites A and B, two thermal sensors were installed at depths of 0.5 and 2.5 m; at site P only at 0.5 m depth. At all sites, a multi-parameter sensor was placed at 1.5 m depth for measuring water temperature, pH, conductivity, dissolved oxygen, Chla and additionally PC at site B. At this latter, continuous data were measured at 1.5 m with a multiparameter probe (NKE Sambat) and then transmitted by a GPRS system to an onshore server once per day. Fortnightly (in summer and autumn) to monthly (in spring and winter) field profiles have been measured for 2 years, from May 2018 to October 2019. Four phytoplankton groups (green algae, diatoms, cryptophytes and cyanobacteria) were measured with a spectrofluorometric probe (BBE Fluoroprobe), temperature, conductivity, dissolved oxygen and pH with a multi-parameter probe (Seabird SBE19). Laboratory quantification of Chla and PC were also done.

Water sample splits were preserved in Lugol's iodine solution before phytoplankton identification and enumeration in the lab using the Ütermohl method (1958). Organisms were identified to the lowest taxonomic levels. Biovolume conversions were calculated according to Hillebrand and co-authors (1999).

Fluorescence Excitation-Emission Matrices (F-EEM) from collected raw water samples were obtained using a Cary Eclipse Spectrofluorometer (Agilent Technologies). Sample splits were placed in a 1-cm path length quartz cuvette and fluorescence intensities were measured at excitation wavelengths between 400 and 660 nm and, at emission wavelengths between 550 and 750 nm. Excitation and emission slit widths were 5 nm. Scan speed was 9,000 nm min⁻¹.

Results. Our data highlighted a good correlation of Chla concentration measured by lab spectrophotometry and by the BBE fluoroprobe ($r^2=0.70$, $p<0.001$). A good correlation was also obtained between the cyanobacterial concentration assessed by the PC sensor and the BBE fluoroprobe ($r^2=0.86$, $p<0.001$). Reversely, no correlation was found between Chla and PC data measured by the HF sensors (Fig.2). Deeper investigations into time series indicated that major discrepancies appeared when a high biomass of cyanobacteria was present. No correlation was found between HF sensor's Chla concentrations and lab's concentrations.

Data analysis of F-EEM illustrated the detection of main photosynthetic pigments, *e.g.* Chla and PC, in the visible range during seasonal occurrences of cyanobacteria (Fig. 3). Fluorescence peaks were identified according their Excitation wavelength (λ_{EX}) and Emission wavelength (λ_{EM}). Maximum peak intensities were recorded at $\lambda_{EX} = 410-425$ nm and $\lambda_{EM} = 665-675$ nm for Chla and, at $\lambda_{EX} = 615-625$ nm and $\lambda_{EM} = 645-660$ nm for PC. A fluorescent peak corresponding to phycoerythrin was detected at $\lambda_{EX/EM} = 560/651$ nm. Belonging to other algal groups, photosynthetic

pigments such as peridinine and fucoxanthin were missing. Other fluorescent compounds were also detected by the F-EEM technique but have not yet been identified.

We then performed a Principal Component Analysis (PCA) using as variables Chla and cyanobacterial concentrations as measured by the BBE fluoroprobe, the HF sensors, lab analysis, and both phytoplankton and cyanobacteria cell densities. The PCA results evidenced that, in presence of elevated biomasses of cyanobacteria, the Chla sensor was giving very low chlorophyll concentration values.

Discussion and conclusion. Our results showed that the high-frequency Chla sensor of the type implemented in our survey cannot be used alone for cyanobacterial bloom monitoring. The set excitation/emission wavelengths were only reliable for estimating biomasses of non-cyanobacterial species when cyanobacteria were not dominant. During a bloom of *Aphanizomenon flos-aquae* in Lake Champs-sur-Marne during the summer 2019, the F-EEM provided evidences that the Chla sensor went totally blind and missed the chlorophyll peak which fluoresced strongly at $\lambda_{EX/EM} = 420/670$ nm. Meanwhile, PC was peaking intensively at $\lambda_{EX/EM} = 615/655$ nm. The phycocyanin sensor did not catch precisely the maximum intensity of the PC signal but was not so far off. The captured signal corresponded to the shoulder of the peak as recorded in higher emission wavelengths (Fig. 3). In our study, as the PC sensor provided reliable measurements for cyanobacterial biomass estimations, it can be fairly adapted to an early warning assessment of cyanobacterial risks in lakes and reservoirs. F-EEM fingerprints may vary seasonally with algal community changes but can be used conjointly with online sensors to address accurate statements of cyanobacterial risks in surface waters.

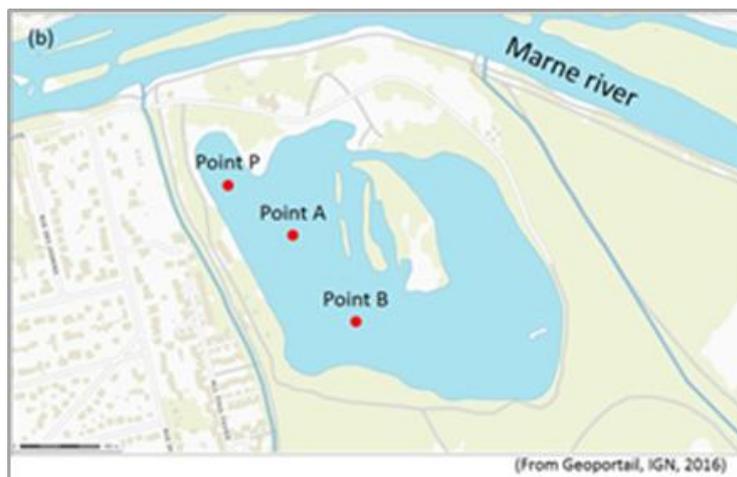


Figure 1 Location of the monitoring sites

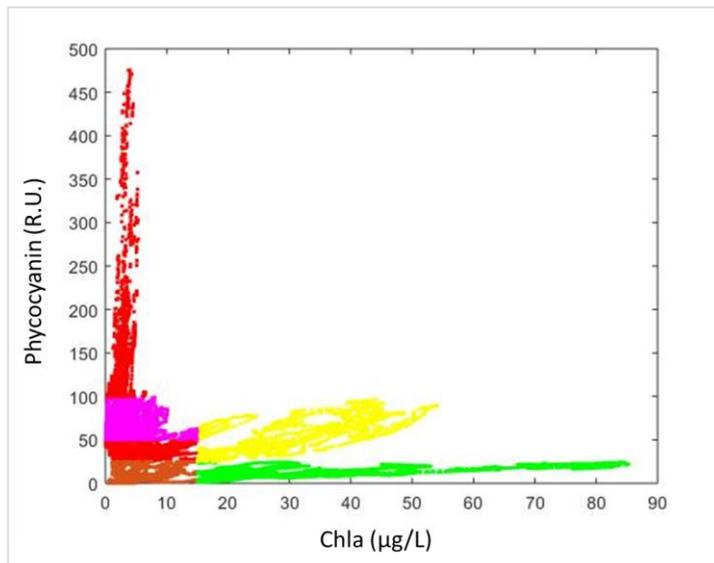


Figure 2 Chla sensor data and phycocyanin sensor data

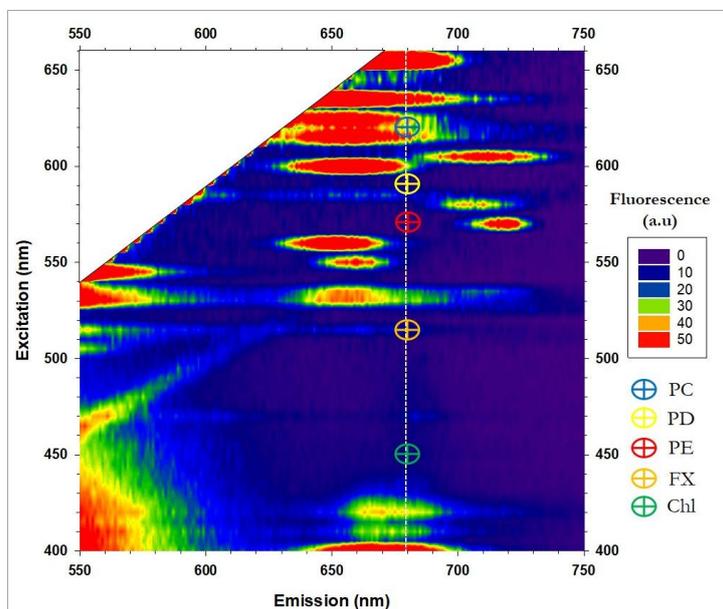


Figure 3 Lake Champs raw water fluorescence EEM and BBE Fluoroprobe sensor emission wavelength (680 nm) for pigment fluorescence detection; with Phycocyanin (PC), Peridinine (PD), Phycoerythrin (PE), Fucoxanthin (FX) and Chlorophylls (Chl).

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