

## **Towards the modeling of benthic cyanobacteria abundance and toxicity in a regulated river**

Vers une modélisation de l'abondance et de la toxicité des cyanobactéries benthiques en rivière régulée

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### **RÉSUMÉ**

Le changement climatique impacte les écosystèmes aquatiques dans le monde entier, entraînant une augmentation des températures et des événements hydrologiques extrêmes comme les étiages sévères. Ces changements favorisent le développement de producteurs primaires incluant les cyanobactéries potentiellement toxiques. A l'inverse des milieux lenticques, peu de connaissances sont disponibles sur les causes de leur développement en eaux courantes. Pourtant la toxicité des cyanobactéries de rivière a été fréquemment mise en cause dans de nombreux cas de mortalité de mammifères. A l'aide d'un modèle hydraulique-2D permettant de quantifier les valeurs hydrauliques passées (vitesse de courant, hauteur d'eau) à une échelle de 4 m<sup>2</sup> pour différents débits, nous avons défini trois zones hydrauliques dans lesquelles les biofilms de cyanobactéries et les paramètres abiotiques ont été échantillonnés sur 247 placettes de 4 m<sup>2</sup> durant 6 campagnes estivales (de début juin à fin août 2020) sur la basse rivière d'Ain. La présence de genres potentiellement toxiques de cyanobactéries est significative avec 60% des placettes colonisées. Par ailleurs, nos résultats suggèrent un rôle majeur des variables hydrauliques et de l'abondance des familles d'algues concurrentes (Chlorophycées, Cyanobactéries non toxiques, Diatomées) sur le développement des biofilms de cyanobactéries en rivière, la physico-chimie ayant un rôle ponctuel en fin d'été (Nitrates).

### **ABSTRACT**

Climate change impacts freshwater ecosystems worldwide, causing a rise in water temperatures and an increase in extreme flow events including droughts. These changes promote the development of primary producers including toxic cyanobacteria. Though these organisms are well studied in lakes, little is known on which biotic or abiotic components cause their occurrence in running waters whereas recently, the toxicity of riverine cyanobacteria has been pointed out through an increased occurrence of mammal deaths. We defined three zones using a two-dimensional hydraulic model enabling to depict past hydraulic values (velocity and water depth) at a 4 m<sup>2</sup> scale and we sampled cyanobacterial biofilm and abiotic parameters in 247 plots at six occasions during the summer period in the Lower Ain River. Potentially toxic genera of cyanobacteria occurred in 60% of the plots. Our results suggest that the hydraulic variables and the abundance of other algae families (Chlorophyta, non-toxic cyanobacteria, diatoms) mainly influence the development of cyanobacterial biofilms in rivers, whereas physico-chemistry has a minor effect, essentially in the late summer (nitrates).

### **MOTS CLES**

Anatonin-a, benthic biofilm, cyanobacteria, modelling, river.

## 1 INTRODUCTION

Cyanobacterial biofilms development has been rising worldwide in the last two decades. Benthic cyanobacteria are often found in running waters and have been proven to produce cyanotoxins that can be harmful to aquatic and terrestrial organisms (Quiblier et al. 2013). Even though the main environmental factors allowing their developments have been studied (Quiblier et al. 2013), no predictive model exist to date. Here, we analyzed the abiotic and biotic parameters that can potentially drive toxic cyanobacteria occurrence in river biofilms, with the aim of improving the assessment of toxic risk in rivers. We mainly focus on potentially toxic cyanobacteria genera (producing anatoxin-a and other toxic genera). Given the current knowledge, we hypothesized that the hydraulic constraints would be a major factor structuring their development. In contrast, as some cyanobacteria can proliferate under low nutrient conditions (Wood et al. 2020), nutrient concentrations were not expected to limit their development whereas higher concentrations could enhance it.

## 2 METHODS

### 2.1 Field sampling of biofilm

Four locations of the Lower Ain river (France) were selected from known past occurrence of benthic cyanobacteria. Six sampling campaigns (2 days) were performed during summer 2020: 2-3, June (noted C1), 29-30, June (C2), 15-16 July (C3), 23-24 July (C4), 27-28 July (C5) and 24-25 August (C6). Three zones having different hydraulic patterns (depth and water velocity) were determined on the river prior to sampling (2-D hydraulic model from INRAE/EDF): a zone experiencing low velocity magnitude and variability (class 1: velocity  $\leq 0.8$  m/s and velocity variability  $< 0.1$  m/s between 15 and 40 m<sup>3</sup>/s), a zone experiencing low velocity and high variability (class 2: velocity  $< 0.8$  m/s and variability  $< 0.1$  m/s under 20 m<sup>3</sup>/s and variability  $> 0.1$  m/s between 20 and 40 m<sup>3</sup>/s) and a high velocity zone (class 3:  $v > 0.8$  m/s even at low flows under 20 m<sup>3</sup>/s). Plots of 2m x 2m were used as sampling units. Three pebbles were selected randomly within 4m<sup>2</sup>-plots for biofilm collection. The samples were fixed with Lugol's iodine solution and stored in the dark until identification.

### 2.2 Field measurements

Water depth and velocity were measured at each pebble. Water temperature, pH, dissolved oxygen concentration and conductivity were measured at each plot. We analyzed the concentrations of ammonium, nitrates and orthophosphates following standard colorimetric methods. We estimated the percentage cover of substrate types (sand, gravel, small cobbles and pebbles) and derived mean grain size. We considered past 15-days mean discharge (Dis15).

### 2.3 Identification and enumeration of taxa

Biofilm samples were homogenized before identification and enumeration. Several subsampling allowed identifying and counting of all algal cells using a light microscope and a Malassez chamber. Photosynthetic communities were identified at the genus level. The cyanobacteria phylum was divided into three categories: potentially anatoxic cyanobacteria (PAC), other potentially toxic cyanobacteria (PTC) and non-toxic cyanobacteria. The other phylums included diatoms (Bacillariophyta) and Chlorophyta.

### 2.4 Statistical analysis

We performed ANOVA II models to assess the effect of hydraulic zones and time on the occurrence of PAC and PTC. In addition, we performed RDA to select the best model explaining the PAC and the PTC assemblage composition. These models were performed separately at each time (C1-C6). Models were selected by permutation tests using a forward selection on adjusted R<sup>2</sup> and P-value. Finally, we used variance partitioning (VP) to assess the amount of variance (adjusted R<sup>2</sup> in RDA) explained in PAC and PTC assemblages by the physical and chemical variables, the other biofilm algae, and the hydraulic variables.

## 3 RESULTS

### 3.1 Occurrence of potentially toxic cyanobacteria in biofilms

The total number of PAC and PTC in biofilms could reach  $7.34E+06$  cells/cm<sup>2</sup> and  $1.51E+07$  cells/cm<sup>2</sup>, respectively. Four PAC genera (especially *Phormidium* sp. and *Oscillatoria* sp.) and 6 PTC genera (especially *Lyngbya* sp. and *Planktolyngbya* sp.) occurred throughout the sampling period. The overall frequency of PAC and PTC was highest in the hydraulic class 2 (Anova II model,  $F_{(2,54)}=38.8$ ,  $p=3.53e-11$  and  $F_{(2,105)}=9$ ,  $p=0.00025$  respectively). In addition, the interaction between time and hydraulic classes was apparent for PAC ( $F_{(10,54)}=5.058$ ,  $p=3.58e-05$ ) but not for PTC (Fig.1).

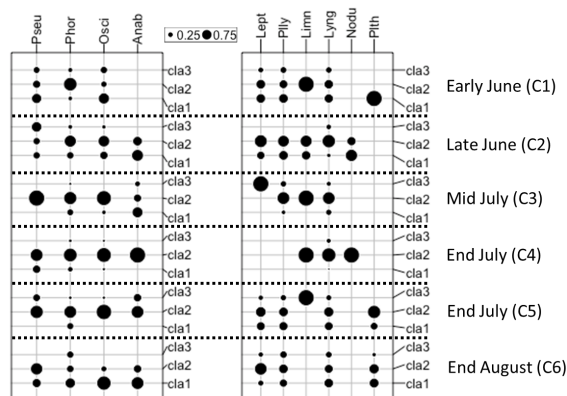


Figure 1: Frequency of occurrence of potentially toxic cyanobacteria (left) PAC; (right) PTC, at 6 sampling dates.

### 3.2 Drivers of potentially toxic cyanobacteria abundance in biofilms

The variance of PAC explained ranged between 9.8% and 25.5%. NO<sub>3</sub> were an evident driver in late summer ( $p<0.05$ ). Non-toxic cyanobacteria were prominent in mid-summer ( $p=0.046$ ), chlorophytes in and diatoms in early summer ( $p=0.044$  and  $p=0.03$ , respectively). According to season, hydraulic variables accounted for in RDA models included depth, velocity, grain size, hydraulic zone, and Dis15 ( $p$  [0.002-0.004]). According to VP, hydraulics was a major source of variation at any campaign ( $R^2$  [0.101-0.282];  $p$  [0.001-0.041]). Other algae represented a secondary source of variation ( $R^2$  [0.098-0.268];  $p$  [0.001-0.043]) in mid and late summer. Finally, physical and chemical variables were influential only in late summer ( $R^2$  [0.171-0.157];  $p$  [0.004-0.027]). The variance of PTC explained ranged between 15.1% and 58.4%. Temperature ( $p=0.008$  and  $0.002$  respectively), pH ( $p=0.010$ ) and NO<sub>3</sub> ( $p=0.018$ ) had a main influence in the early summer. Non-toxic cyanobacteria and chlorophytes had a prominent influence in late summer ( $p=0.002$  and  $p=0.002$  respectively) and diatoms in early summer ( $p=0.002$ ). Hydraulic variables accounted for included depth, velocity, grain size, hydraulic zone, and Dis15 ( $p$  [0.002-0.008]). VP demonstrated that hydraulics were a major source of variation at nearly all campaign ( $R^2$  range [0.169-0.580],  $p$  [0.001-0.008]). Other algae represented the second important source of variation at all the campaigns ( $R^2$  [0.104-0.541];  $p$  [0.001-0.034]). Finally, physical and chemical variables were influential in early and late summer ( $R^2$  [0.116-0.286];  $p$  [0.009-0.001]).

## 4 DISCUSSION

Our results show that PAC and PTC in biofilms mainly depends on past and local hydraulic parameters and time conforming previous studies (Echenique et al. 2018). We add to this knowledge that intermediate hydraulic variability favors them. In addition, the influence of algae suggests potential competition or association. However, each of the potentially toxic genus showed a different spatio-temporal pattern of occurrence, implying different responses to environmental parameters. This is consistent with the assumption of Wood et al. (2020) that different cyanobacterial genus may have different ecological preferences and development strategies.

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