Ann. Limnol. - Int. J. Lim. 2021, 57, 19
© M. Moiron *et al.*, Published by EDP Sciences, 2021
https://doi.org/10.1051/limn/2021014

Available online at:
www.limnology-journal.org

RESEARCH ARTICLE OPEN ACCESS

Die hard in Lake Bourget! The case of *Planktothrix rubescens reborn*

Marthe Moiron[1,2], Frédéric Rimet[2], Cyrille Girel[1,3] and Stéphan Jacquet[2,*]

[1] CISALB, 42 rue du Pré Demaison 73000, Chambéry cx, France
[2] Université Savoie Mont Blanc, INRAE, UMR CARRTEL, 75 bis avenue de Corzent 74200, Thonon les Bains cx, France
[3] Grand Chambéry, 106 allée des Blachères 73026, Chambéry cx, France

Received: 10 June 2021; Accepted: 24 August 2021

Abstract – Blooms of *Planktothrix rubescens* have been recorded for 15 years in Lake Bourget (France), from 1995 to 2009. Then, the presence of this filamentous and toxic cyanobacterium became anecdotic between 2010 and 2015 and it was thought that its proliferation was over. However, blooms occurred again in 2016 and 2017 despite apparent low phosphorus concentrations in surface waters of the lake. We have attempted to explain the reasons for this come back in order to develop scenarios helpful to stakeholders who are concerned such proliferations may occur in the future. We show that phosphorus input, both from the main tributaries to the lake and possibly from the sediments, were likely the triggers of the new development of the cyanobacterium provided a minimum autumn/winter inoculum of *P. rubescens* was detected the year before. The subsequent bloom was observed deeper than previous years and associated with a conjunction of factors known to favour the development of this species (*i.e.,* mild winter temperature, water column stability, available light at depth, surface water transparency, low predation, etc.). Although many factors and processes could account for the occurrence and bloom of the cyanobacterium, a plausible scenario is proposed. One thing remains unclear: where does this cyanobacterium "hide" when it is not observed during the routine monitoring surveys and from which place it could initiate its development (nearshore, the pelagic zone, or from the sediment?).

Keywords: Lake / cyanobacteria / *Planktothrix rubescens* / bloom / phosphorus / river input / scenario / prediction

1 Introduction

Lake Bourget, the largest deep natural lake in France, has undergone eutrophication during the second part of the 20th century, as with many other ecosystems worldwide (Jenny *et al.*, 2020). While the phosphorus concentration was lower than 10 µg/L before 1940, it reached up to 100 µg/L during the 1980s' because of massive discharges from industrial effluents and insufficiently treated domestic wastewaters (Jacquet *et al.*, 2005). With eutrophication, biological changes within the eukaryotic microbial communities were noted; initially *ca.* 1940 and again between 1960 and 1980 (Capo *et al.*, 2016, 2017). Management actions were set up to reduce nutrient concentrations in the lake, leading to a lower phosphorus concentration which dropped under 20 µg/L after 2000 (Capo *et al.*, 2016, 2017; Jacquet *et al.*, 2017). Significant ecological changes occurred in the pelagic compartments during the lake's reoligotrophication, notably regarding the toxic filamentous cyanobacterium *Planktothrix rubescens* (Jacquet *et al.*, 2014; Frossard *et al.*, submitted).

Planktonic cyanobacterial species are characterized by adaptations and tolerances to various environments and can produce important amounts of various toxins. Among these species, *P. rubescens* (Anagnostidis and Komarek, 1988) is a red-coloured filamentous and gas-vacuolated cyanobacterium, with photo-heterotrophic capabilities, likely to develop in meso- to moderately eutrophic conditions (Zotina *et al.*, 2003; Jacquet *et al.*, 2005; Ernst *et al.*, 2009; Dokulill and Teubner, 2012). It can self-adjust its position in the water column *via* buoyancy to its preferred low light environment, and maybe in response to other resources, where it often forms deep water maxima in stratified lakes of temperate latitudes (Feuillade, 1994; Micheletti *et al.*, 1998; Bright and Walsby, 2000; Vinçon-Leite *et al.*, 2002; Zotina *et al.*, 2003; Jacquet *et al.*, 2005; Posch *et al.*, 2012). This taxon can be considered an R-strategist according to Reynolds *et al.* (2002), and is known as an important producer of hepatotoxic microcystins (MCs) (Fastner *et al.*, 1999; Briand *et al.*, 2005) that are harmful to a variety of animals, including humans (Sotton *et al.*, 2011; Sotton *et al.*, 2012; Kurmayer *et al.*, 2015). Indeed, *P. rubescens* produces a variety of microcystins, especially MC-LR and MC-RR, and these toxins have been shown to contaminate different fish tissues. Both filaments and toxins

---

*Corresponding author: `stephan.jacquet@inrae.fr`

have been observed in intestinal tracts of whitefish and the presence of MC-LR has been detected in their intestine and liver. MCs were also detected in the muscles and liver of young perch of the year through dietary routes, particularly *via* the consumption of MC-containing *Daphnia* (Sotton *et al.*, 2011, 2012). Consequently, such toxins can be incorporated into different organisms by ingestion of *P. rubescens* filaments, leading to potential adverse effects on the animals' health. The issue is particularly important since Lake Bourget is a place for an important professional and recreational fishing activity and is a source of drinking water for thousands of inhabitants.

While paleolimnological data first revealed the importance of this species in Lake Bourget during the early years of eutrophication (*i.e.,* prior to 1930; Savichtcheva *et al.*, 2015), *P. rubescens* was mainly observed and counted as a dominant species in the lake between the mid-1990s and 2009. This was explained as a response to the reoligotrophication process of this lake, intermediate phosphorus levels, important water column stability, increasing transparency, etc. (Jacquet *et al.*, 2005, 2014). The success of reoligotrophication was noticed in that, from 2010, the species declined and "disappeared", and, in the same time, phytoplankton biomass was considerably reduced and taxa composition changed to more preferably species (Frossard *et al.*, submitted). However, with increasing phosphorus concentrations in surface waters of the lake, *P. rubescens* "reappeared" and biomasses were recorded in 2016 and 2017.

This study aims at explaining this paradoxical situation (apparent phosphorus depletion and *P. rubescens* reappearance) and proposes scenarios to predict future possible developments of this cyanobacterium in Lake Bourget. Long-term ecological monitoring survey in this lake offers the possibility to analyze factors responsible for the development of cyanobacteria/algal species, therefore we used the dataset available for the lake and of its main tributaries to explain what could have been the reasons of the 2016–2017 bloom episodes of *P. rubescens*. Our hypotheses were that phosphorus input from rivers and/or sediment could be important factors triggering a new development of the cyanobacterium, whose growth and development could be sustained thereafter thanks to a conjunction of favorable conditions. Our results tend to confirm these hypotheses and highlight also the importance of a minimal autumn/winter concentration of the cyanobacterium (referred latter to as an inoculum) to develop further during the year.

2 Methods

2.1 Description of the site

Lake Bourget (45°44'N, 231 m altitude) is the largest natural deep lake in France and is located on the edge of the Alps. It is a warm, meromictic and elongated (18 km in length and 3 km for maximal width). It is north-south orientated with an area of 42 km^2, a total volume of 3.5×10^9 m^3, maximum and average depths of 145 m and 80 m respectively, and a water residence time of approximately 10 years. Mixing from winter turnover reaches the bottom of the lake only during very cold winters. It has a catchment area of about 560 km^2, with maximum and average altitudes of 1845 and 700 m respectively. There are two important cities beside the lake; Chambéry to the south and Aix-les-Bains to the east, with a combined population of 180,000 inhabitants, plus a large influx of tourists (>50,000) each summer. The lake has suffered from eutrophication since 1950 and water quality restoration programs started in the 1970s. These programs involved the development and improvement of wastewater treatment plants, and in 1981, the diversion of the treated sewage of the two main cities from the lake, directly downstream the lake into the Rhône River. Other improvements of the functioning of the sewer system –especially the control of the combined sewer overflows – lead in the recent years to an additional reduction of the nutrient loading to the lake. Two rivers, the Leysse and the Sierroz, are the main inputs to the lake, with average flow rates of 6.5 and 2.5 m^3/s, respectively. The flow rates of these two rivers can occasionally (during floods for instance) reach more than 120 and 30 m^3/s, respectively. The outflow from the lake, located on its northern shore, is known as the Savière channel (length: 4.5 km, mean flow rate: 10–30 m^3/s, annual output: 0.5 km^3) and it flows into the Rhone River.

2.2 Data

All lake data used in this paper correspond to sampling performed at the reference sampling site located in the middle and deepest part of the lake, referred to as point B and are part of the lake observatory and its information system (Rimet *et al.*, 2020; © *OLA-IS, AnaEE-France, IRLife, INRAE of Thonon-les-Bains, CISALB).* The site is more than 1.5 km from each bank and more than 5 and 10 km from the Sierroz and Leysse rivers, respectively. A conductivity-temperature-depth (CTD) measuring device (CTD SEABIRD SBE 19 Seacat profiler) was used to obtain vertical profiles of temperature, dissolved oxygen, pH, and conductivity.

Temperature data were used to determine the onset of water column stratification. We assumed that stratification had occurred when there was a temperature differential of more than 1 °C between the 2- and 50-m depths on two consecutive sampling dates. The Brunt-Väisälä frequency (which measures the natural frequency of oscillation of a vertical column of water, and can be viewed as an index of the water column stability, Lemmin, 1978) was also calculated from the temperature values, according to the following equation:

$$N^2 = \frac{g}{\rho}\left(\frac{d\rho}{dz}\right)$$

where N^2 is the stability coefficient (in s^{-2}); g is the acceleration parameter (in m/s^2); ρ is the water density (no unit); z is the depth (in m); with: $\rho(\mathrm{T}) = 1000–7 \times 10^{-3}\ (\mathrm{T}-4)^2$ according to Lemmin (1978) where T is the temperature (in °C).

Nutrient concentrations (such as total phosphorus and P-PO$_4$, but also total nitrogen, N-NO$_3$, N-NH$_4$) were measured monthly to bi-monthly at ten different depths (from surface to bottom) according to normalised procedures and protocols (AFNOR, 2015). Transparency data were obtained using a Secchi disk. The concentration of chlorophyll *a* was determined using the method of Strickland and Parsons (1972). Phytoplankton species and biovolumes were analysed according to the European standardised method (AFNOR, 2006) of Uthermöhl (1958). For both chlorophyll *a* and phytoplankton determination, samples consisted in an integrated water sample collected between 0 and 20 m using a dedicated sampler (Pelletier and Orand, 1978). Species biovolumes used for this monitoring are available in Rimet and Druart (2018). For *P. rubescens*, the cell concentrations were estimated by counting 200-µm length filaments and by assuming a mean cell length of 5 µm. Several vertical profiles of the main phytoplankton groups, including *P. rubescens,* and of the temperature were also obtained using a submersible spectrofluorimeter (BBE-Fluoroprobe, Germany). This *in situ* measuring/recording device, which can be used to perform chlorophyll *a* analysis and integrated algal class determination, has been shown to provide a realistic estimation of the abundance and dynamics of the cyanobacterial population after specific calibration (Leboulanger *et al.*, 2002). Zooplankton was obtained using a 212-µm mesh vacuum net, during vertical sampling made from 50 m to the surface. The micro-crustaceans were counted from formalin fixed samples using a standard microscope (Olympus BX40) and abundances were given in number of individuals per m^2.

River data come from two automatic sampling stations located in the two main tributaries, *i.e.*, the Leysse and Sierroz, responsible for >75% of the water flowing into the lake. Samplers (ASP station 2000 Hendress + Hauser) are located at 1.3 km and 0.75 km from the lake, for the Leysse and the Sierroz, respectively. Waters for nutrient concentrations (such as total phosphorus, P-PO_4, N-NO_3 and N-NH_4) are collected using a daily time scale and have been measured since 2003. This sampling allows obtaining an accurate estimation of the quantity of nutrient discharge into the lake as well as the key periods of such inputs.

Data such as air temperature, wind force and direction, irradiance, cloudiness, and precipitation were obtained at a 3-hour time step from the meteorological station Voglans at Chambéry airport, located less than 1 km from the southern shore of the lake.

3 Results

3.1 *Dynamics and distribution of* planktothrix rubescens

P. rubescens began to bloom in Lake Bourget in 1995/1996. Each year until 2009 (except 2004), the cyanobacterium developed significantly reaching regularly >50% of the total phytoplankton biomass (Vinçon-Leite *et al.*, 2002; Jacquet *et al.*, 2005; Jacquet *et al.*, 2014). In 2009, its cells concentration was still high, but much lower than in 2008 (a record year with 185,600 cells/mL recorded in July around 15 m). However, following a conjunction of factors, as explained in Jacquet *et al.* (2014), the cyanobacterium "disappeared" during the winter 2009/2010 (Fig. 1A). However, at the end of 2015 (*i.e.,* October-November) and during the 2015–2016 autumn/winter period, *P. rubescens* reappeared (Fig. 1B, Tab. 1) and proliferated latter in the year 2016 reaching >50,000 cells/mL in September in the metalimnion, at a depth greater than observed by past (*i.e.*, between 20 and 25 m *vs.* between 15 and 20 m for the period before 2009). In details, we observed at the end of October 2015 ~500 cells/mL that were counted at 20 m and this concentration increased until mid-November to reach ~2000 cells/mL. Subsequently in early 2016, the cyanobacterium spread over the surface water column and remained observed along the year, reaching >15,000 cells/mL in early summer and ~50,000 cells/mL at the end of summer/early autumn. Then, a progressive decrease of the cyanobacterial biomass and a shift of *P. rubescens* cells towards the surface was recorded. Cells maintained however at a relatively high level (up to 8000 cells/mL) at all depths between surface and 50 m during the winter 2016/2017 (Fig. 1B). Thereafter, in 2017, *P. rubescens* developed massively at depth (reaching >19,000 cells/mL by the end of May at 19 m, >22,000 cells/mL at 22.5 m on June 13, >31,000 cells/mL at 25 m on June 27 and >45,000 cells/mL at 21.5 m on July 10). High concentrations (>25,000 cells /mL) were measured until the end of September at various depths (Fig. 1B). During the winter 2017/2018 the cyanobacterium disappeared, and no new development of *P. rubescens* has been observed until now, *i.e.,* 2021 (Supplementary Fig. S1a).

3.2 Meteorological data

Over the period 2010–2019, the time of sunshine followed approximately the same annual pattern, with an increase during summer and a decrease in winter. While the summer sunshine level was almost similar each year, some differences were, however, recorded in winter. During winters 2012/13, 2017/18 and 2018/19, the daily time of sunshine stayed relatively low (<4.0 h) from November to March. This phenomenon was even more important for the winter 2017–18 with a very low sunshine time (maximum and average sunshine time of 2.84 h and 2.17 h, respectively) and no significant fluctuation was recorded (Fig. 2A). By contrast, during winters 2015/16 and 2016/17 the sunshine decreased to low values but oscillated with higher values (Fig. 2B).

3.3 Transparency

The transparency of the water column ranged from 1.9 m to 14.4 m between 2010 and 2019. It was higher from 2010 to 2015 compared to subsequent years. Indeed, winter peaks were recorded with a maximum depth ranging from 13.0 m to 14.4 m, unlike the 2016–2019 period, where depths varied between 7.3 m and 10.6 m (Fig. 3).

3.4 Phosphorus input from rivers

From 2010 to 2019, the phosphorus input from the two main lake tributaries (*i.e.,* the Leysse and Sierroz) were distributed differently. While such input was rather low from 2010 to 2012, it increased significantly at the end of 2012 until the end of 2016. Nutrient loads were also measured to be particularly high when important river floods occurred, such as for example in 2016, June 16th (Fig. 4). During the year 2017, low inflows from the tributaries were observed, whereas the beginning of 2018 was characterised again by high inputs (>8.6 tons of total-phosphorus and >0.2 tons of P-PO_4). In 2019, nutrient loads from the tributaries were still relatively important, especially for PO_4.

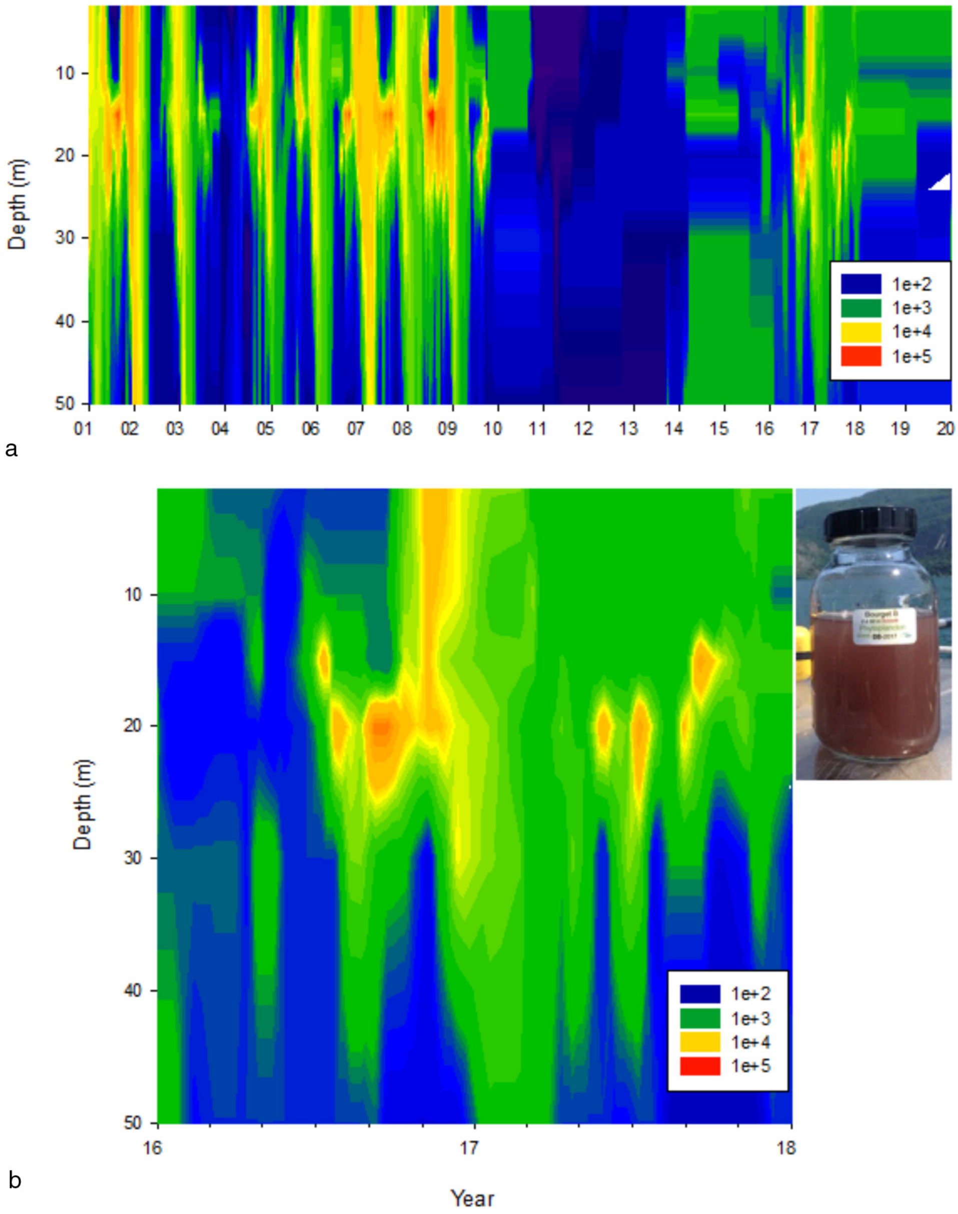

Fig. 1. (A) *P. rubescens* dynamics and distribution from 2001 to 2019 at point B in Lake Bourget. (B) Zoom of the 2016–2017 bloom episode associated to a picture of a <62 μm plankton net sample obtained in spring 2017.

3.5 Phosphorus concentration

From 2010 to 2019, both total phosphorus and orthophosphates concentrations (*i.e.*, the mean values along the water column or in surface waters) decreased regularly. It was observed, however, an increase for the resource for the two winter periods of 2015 and 2016 (Fig. 5).

3.6 Water column mixing and stratification

During winter 2016, the water column was poorly homogenised, and the partial mixing occurred only from surface to 65 m deep. At mid-April, the maximal dissolved oxygen concentration only reached 6.3 mgO_2/L. It is noteworthy that the reoxygenation at 140 m was the worst recorded for the last 10 years (not shown). During autumn 2016, the wind could blow at high speed (with gusts exceeding regularly 30 to 40 km/h, some reaching more than 100 km/h) and this favoured destabilization of the water column. The mixing was also partial in 2017 (despite a colder winter than in 2016) and reached at least 110 m deep. The maximal dissolved oxygen concentration was recorded on February 22nd with 9.2 mgO_2/L. In 2017, the reoxygenation at 140 m was better than for 2015 and 2016 but stayed significantly lower than for the years 2010 to 2013 (Fig. 6A). At the same time, the stability of the water column, from spring to autumn, was generally important (especially during the summer time). It is noteworthy, however, that mixing and thus the destratification

Table 1. Average values (from all sampled depths) calculated for each season of the cell abundance (cells/mL) of *P. rubescens.*

	Winter (DJF)	Winter (JFM)	Spring (AMJ)	Summer (JAS)	Autumn (OND)
2001	7910	6196	8716	9640	18521
2002	12600	6597	719	552	5028
2003	5429	4162	1444	742	45
2004	113	169	335	3662	7182
2005	5058	1473	646	5259	4328
2006	5796	4569	1433	6216	6624
2007	13604	11520	8187	7327	5710
2008	5040	4107	8846	18193	15958
2009	7504	3212	1111	3659	478
2010	32	0	0	4	15
2011	22	14	7	2	4
2012	12	21	0	0	19
2013	0	0	0	0	75
2014	71	386	72	0	10
2015	0	0	21	75	215
2016	193	130	442	4465	5775
2017	3827	2181	1491	2739	1541
2018	129	10	17	0	0
2019	66	66	66	37	25

of the water column was relatively important at the end of 2017 (Fig. 6B) while the stability was globally higher for 2016 and 2017 compared to 2015 and 2018 (Fig. 7).

3.7 Phyto- and zooplankton

For the last decade, the phytoplankton biomass was higher when *P. rubescens* was recorded during the two years 2016 and 2017, more particularly during the spring season. For the zooplankton, it was usually found a more important density during the summer months (July and August). For the two years 2016 and 2017, the zooplanktonic biomass was the highest (with >22,000 ind/mL), especially the proportion of the herbivores (Supplementary Fig. S1b).

4 Discussion

Amongst aquatic microorganisms, cyanobacteria occupy an important place on Earth because of their historical and ongoing importance in ecosystem function. Moreover, many cyanobacteria are threats since that can generate toxins, especially in inland water bodies. Today, it remains very important to address key issues and highlight key items and new insights regarding these unique organisms. Typically, we still need pieces of information dealing with diversity and functional roles of cyanobacteria, harmful blooms (*i.e.*, determinism, toxin risk, predictive models, management), molecular pathways (including toxin production), abiotic and biotic interactions with cyanobacteria, role of toxin production, as well as about the variety of applications (food supply, socio-economic models). Our study deals with the issue of harmful blooms, more particularly about the attempt to propose some scenarios (even imperfect) about the occurrence of harmful cyanobacterial blooms in lakes (*e.g.,* Anneville *et al.*, 2015; Gallina *et al.*, 2017; Derot *et al.*, 2020). This work makes sense when one knows that future climate scenarios project an increase of such proliferations both in terms of frequency and duration (Paerl and Huisman, 2009).

Blooms of *P. rubescens* have been important in Lake Bourget at different periods of the last 3 decades (Jacquet *et al.*, 2005, 2014) and the 2016–2017 episode reminded us that such event may still occur and could impact ecosystem functioning and services (*e.g.,* drinkable water). This study brings new insights on a variety of factors, processes and mechanisms likely to intervene and regulate the blooms of *P. rubescens* in Lake Bourget, which can be viewed and used as a successful model case of ecosystem restoration and reoligotrophication but possibly threatened by cyanobacteria. Indeed, our study highlights that toxic filamentous cyanobacteria such as *P. rubescens* can still proliferate in oligotrophic conditions, and thus not only, as most other nuisance cyanobacteria, in meso- to eutrophic conditions. We believe that this information is very important for both the scientific community and environmental mangers.

In Lake Bourget, *P. rubescens* declined after 2010 after a relatively long blooming period (from 1996 to 2009). Jacquet *et al.* (2014) explained that this collapse was likely due to a conjunction of factors and processes. It was suggested, among other things, that an important factor could be the existence during the autumn and/or winter period of a minimal biomass for the cyanobacterium to be able to develop during the following seasons. We tested this last information and found indeed that this autumn/winter inoculum was very important. By simply making average calculations with concentration values at each season, we found indeed that it was necessary for *P. rubescens,* to develop and bloom during the year, to reach a precedent autumn (OND) or winter (either DJF or JFM)

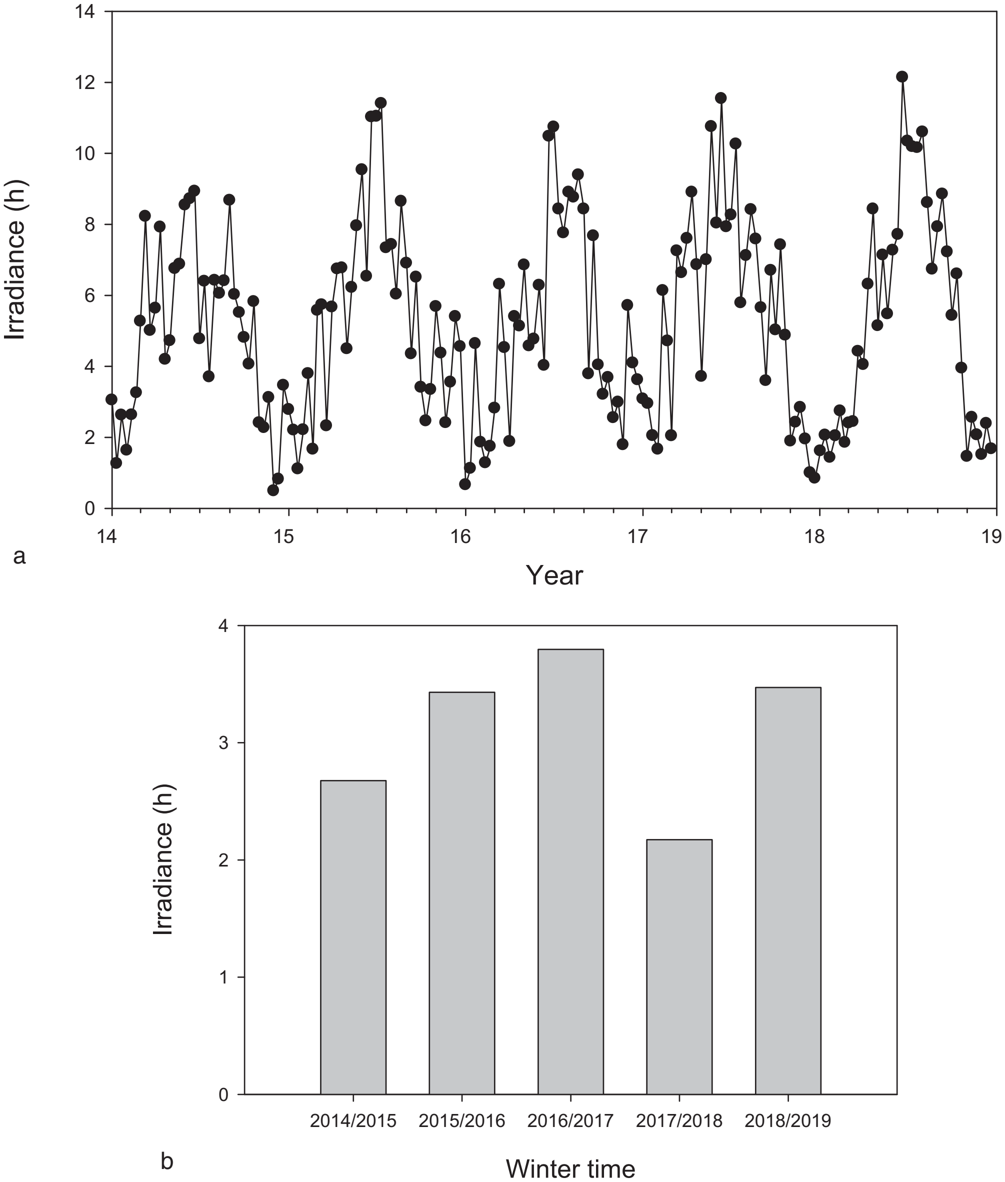

Fig. 2. (A) Chronicle of sunshine time averages from January 2014 to December 2018, and (B) average of sunshine time for winters 2014-15 to 2018-19.

Table 2. Relationships (*r* value) between each season for cell abundance (cells/mL) of *P. rubescens* from 2001 to 2020 ($n = 20$).

	DJF	JFM	AMJ	JAS	OND
DJF	1	–	0.61	0.44	0.44
JFM	–	1	0.75	0.51	0.51
AMJ	0.61	0.75	1	0.85	0.82
JAS	0.44	0.51	0.85	1	0.87
OND	0.44	0.51	0.82	0.87	1

threshold above 180 cells/mL (Tab. 1). Significant positive correlations were clearly found between each season suggesting the importance of the previous months or seasons to explain subsequent blooms later during the year of *P. rubescens* (Tab. 2). Thus, by sequencing the years into seasonal periods, and examining the variability in the ecological response of *P. rubescens* to environmental forcing, we show that the successions of events have considerable importance for its

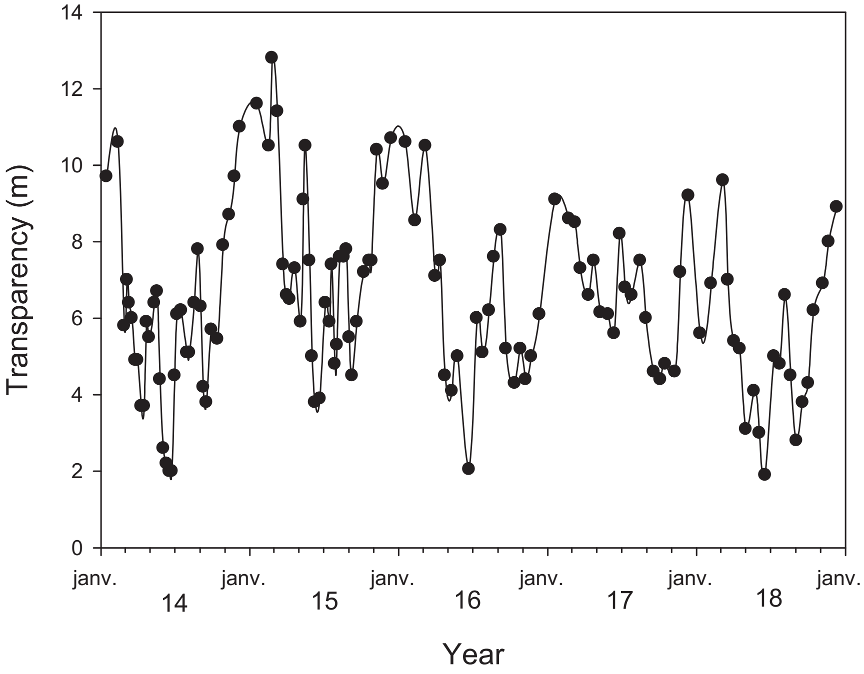

Fig. 3. Transparency evolution January 2014 to December 2018.

development or decline, provided that a minimal initial cell concentration has a determining role for *P. rubescens* dynamics in the following growth season.

After its disappearance during the 2009/2010 winter period, *P. rubescens* reappeared in the autumn of 2015. During spring 2014, partial water turnover was observed while complete winter mixing occurred annually between 2010 and 2014. In spite of adequate daylight during 2014, these conditions were not sufficient to allow the appearance of this cyanobacterium. It was only at the end of 2015 (November and December), that an inoculum was recorded, linked to favourable conditions such as significant summer inputs of nutrients from tributaries (mainly phosphorus) and a relatively high time of subsequent sunshine and mild winter. Such "warm" conditions and the absence of complete overturn may have reduced the dilution of the cyanobacterium and its growth inhibition or mortality at depth (because of light absence and gas vesicle [that intervene in its buoyancy] collapse under high pressure), not to mention the direct effect of the temperature likely to enhance cell metabolism. Incomplete winter mixing has already been reported to be favourable to the development of the cyanobacterium (*e.g.,* Walsby *et al.*, 1988; Jacquet *et al.*, 2005; Posch *et al.*, 2012). Here, we can assume that vertical and lateral transport mechanisms, induced by internal waves or upwelling events, occurred, as already demonstrated by past in Lake Bourget (Cuypers *et al.*, 2011).

Strong inputs of total-phosphorus and P-PO_4 in June 2016, coupled with significant sunshine during summer and a high stability of the water column, were likely the main factors leading to the important development of *P. rubescens* at depth where it is known to be very competitive over most eukaryotic microalgae (Dokulil and Teubner, 2012). The population ended up being the dominant species starting from summer by forming a dense layer at the metaliminion (due to its low light tolerance and stability requirement) and prevented the growth of other phytoplankters through the reduction of nutrient availability in the upper lit layers. It was in July that the bloom phase was really observed at 15 m depth. With an above-average presence of phosphorus at this period, a high transparency, as well as a well-established stability of the water column and possibly low predation pressure until September, conditions were clearly favourable to allow this new development of *P. rubescens*.

The autumn of 2016 was characterised by a decrease in sunshine and the arrival of autumn gust of wind. Despite a general and progressive decrease in cell density, the search for light by migration was probably the reason of the rise of *P. rubescens* cells to the surface (Bright and Walsby, 2000). But the destabilization of the water column due to the autumn wind may have a negative impact on the presence of the cyanobacterium on the surface. Indeed, numerous wind gusts exceeding 30 to 40 km/h, some reaching more than 100 km/h, were recorded during this period.

The decrease in sunshine accompanied by a reduction in transparency, and low nutrient inputs from tributaries during the autumn/winter period of 2016/2017, contributed to the decrease of the cyanobacterium, but without total disappearance. We observed that P could come from the bottom of the lake, because the mild winter of 2016/2017 did not allow a complete water column turnover, and hypoxic conditions at depth were clearly observed, a phenomenon likely to allow the release of phosphorus into the water column. Additionally, the sunshine stayed sufficiently high to allow *P. rubescens* to maintain and grow during the winter, since concentrations were about 2,600 cell/mL on average.

Then, from spring 2017, low phosphorus and P-PO_4 inputs from tributaries were recorded, but the partial reversal of the water column confirmed at the end of March, and phosphorus

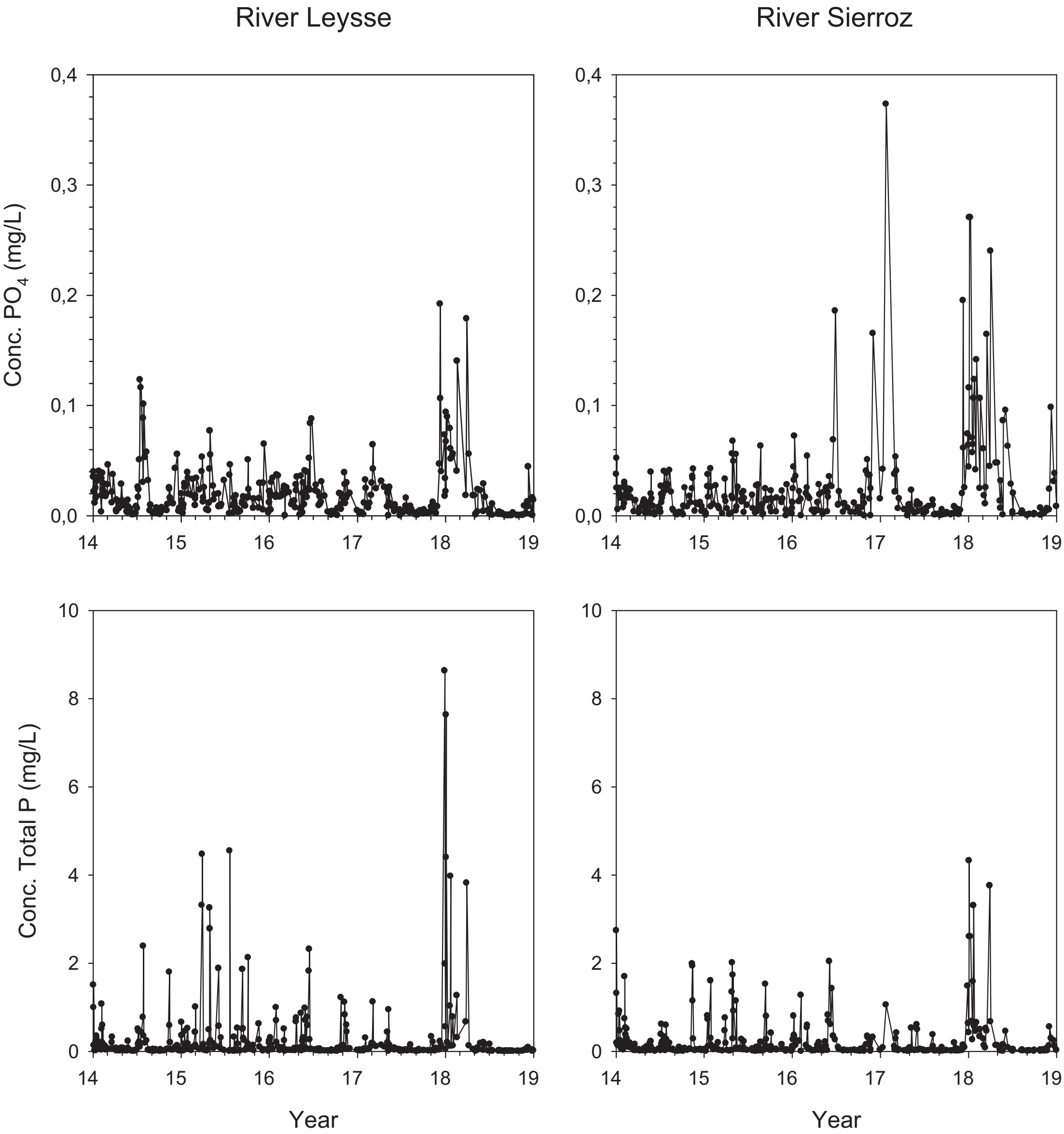

Fig. 4. Evolution of total phosphorus and PO_4 input to the lake from the rivers Leysse and Sierroz from January 2014 to December 2018.

gradually moved up from deep sediments, what have compensated this lack. These favourable nutrient conditions, the return of sunshine and a transparency that was maintained at good levels during the spring/summer, allowed the winter inoculum to maintain and be the cause of a summer bloom at depth (above 20 m in spring and early summer) with a well-marked presence between 15 and 30 m. P. *rubescens* beginning from a relatively high concentration, the population could end up being the dominant species starting from summer by forming a dense layer in the metalimnion (due to its low light tolerance and stability requirement) and prevent the growth of other phytoplankters through the reduction of nutrient availability in the upper lit layers. Such competitive exclusion facilitated by the priority effect in which the species with higher initial concentrations outcompete the competitors (characterised by lower initial concentrations) by making the abiotic environment inhabitable has been shown elsewhere and for other species (*e.g.*, Tapolczai *et al.*, 2014).

From September 2017, *P. rubescens* was present in surface, and it was correlated with a decrease in transparency and an important decrease in the sunshine. A reduction in resources with an important water column destratification (favouring dilution of the population and collapse of intracellular gas vesicles below a critical depth) probably contributed to the "disappearance" of *P. rubescens* from January 2018. Despite "record" inputs from the tributaries from January to May, no reappearance occurred, likely due the absence of the inoculum. The decrease in transparency and low spring sunshine probably also contributed to the end of the cycle initiated in winter 2015. While we did not measure it directly here, predation by zooplankton could have also been important as shown by Jacquet *et al.* (2014) since metazoan feeders can, in

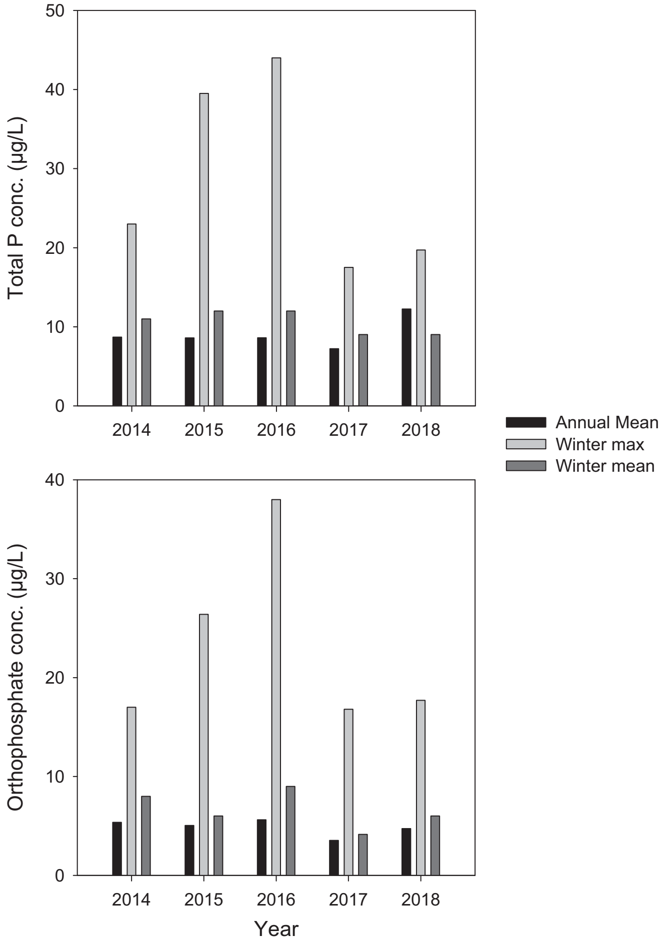

Fig. 5. Evolution of winter (max and mean values) and annual (mean value) of total phosphorus and PO_4 concentrations over the water from 2014 to 2018.

some situations (*i.e.*, low growth, size reduction, toxin absence or weak concentrations), impact *P. rubescens* significantly (Oberhaus *et al.*, 2007; Perga *et al.*, 2013; Jacquet *et al.*, 2014). An increased vulnerability to parasites such as viruses or chytrids (Tao *et al.*, 2020) could also be proposed although it remains to be tested.

After 2018, the lack of an inoculum, and a sunshine average particularly low prevented *P. rubescens* to develop again. The absence of the cyanobacterium was also confirmed in 2019 and until now (end of 2020). This agrees with previous results (Jacquet *et al.*, 2014), highlighting again that the decline of filamentous and/or colonial cyanobacteria blooms are firstly attributed to phosphate limitation (Walve and Larsson, 2007) despite the capacity of *Planktothrix* to excrete alkaline phosphatases, allowing for the use of dissolved organic phosphorus when phosphate is depleted (Feuillade *et al.*, 1990).

We are aware that when blooms reemerge, it is important to look at additional factors beyond those that previously drove the prior blooms. In multiple systems where the blooms were traditionally P-limited, anthropogenic loading of nutrients has led to eutrophication and N-limitation and N and P co-limitation arising (*e.g.,* Barnard *et al.*, 2021; Paerl *et al.*, 2011, 2016; Jansson *et al.*, 2001). However, nitrogen concentrations in the pelagic zone and river inputs did not change dramatically and did not seem to have a significant impact on the resurgence of *P. rubescens.* As already observed and discussed elsewhere (*e.g.,* Jacquet *et al.*, 2005; Posch *et al.*, 2012), *P. rubescens* blooms went in parallel to increasing NO_3/PO_4 ratio in the surface lit layers of the lake and was probably favoured as it requires inorganic nitrogen such as NO_3 and cannot fix atmospheric N2.

Finally, it is noteworthy that filamentous cyanobacteria can also produce bio-active compounds with antibacterial properties for instance so that such chemical interactions in microbial communities could also play an important role in facilitating the development of the cyanobacterium while preventing the others (Mazur-Marzec *et al.*, 2013; Legrand *et al.*, 2003). We conducted experiments and found indeed that allelopathic effects could be induced by extracts from *P. rubescens* on other phytoplankters (Oberhaus *et al.*, 2008; Chiapusio *et al.*, unpublished).

5 Conclusion

Beyond its role as an indicator of environmental degradation and/or major ecological changes occurring in Lake Bourget, bloom forming *P. rubescens* may constitute a serious threat for the lake's functioning (by inhibiting a part of the matter and energy transfer through the food webs,

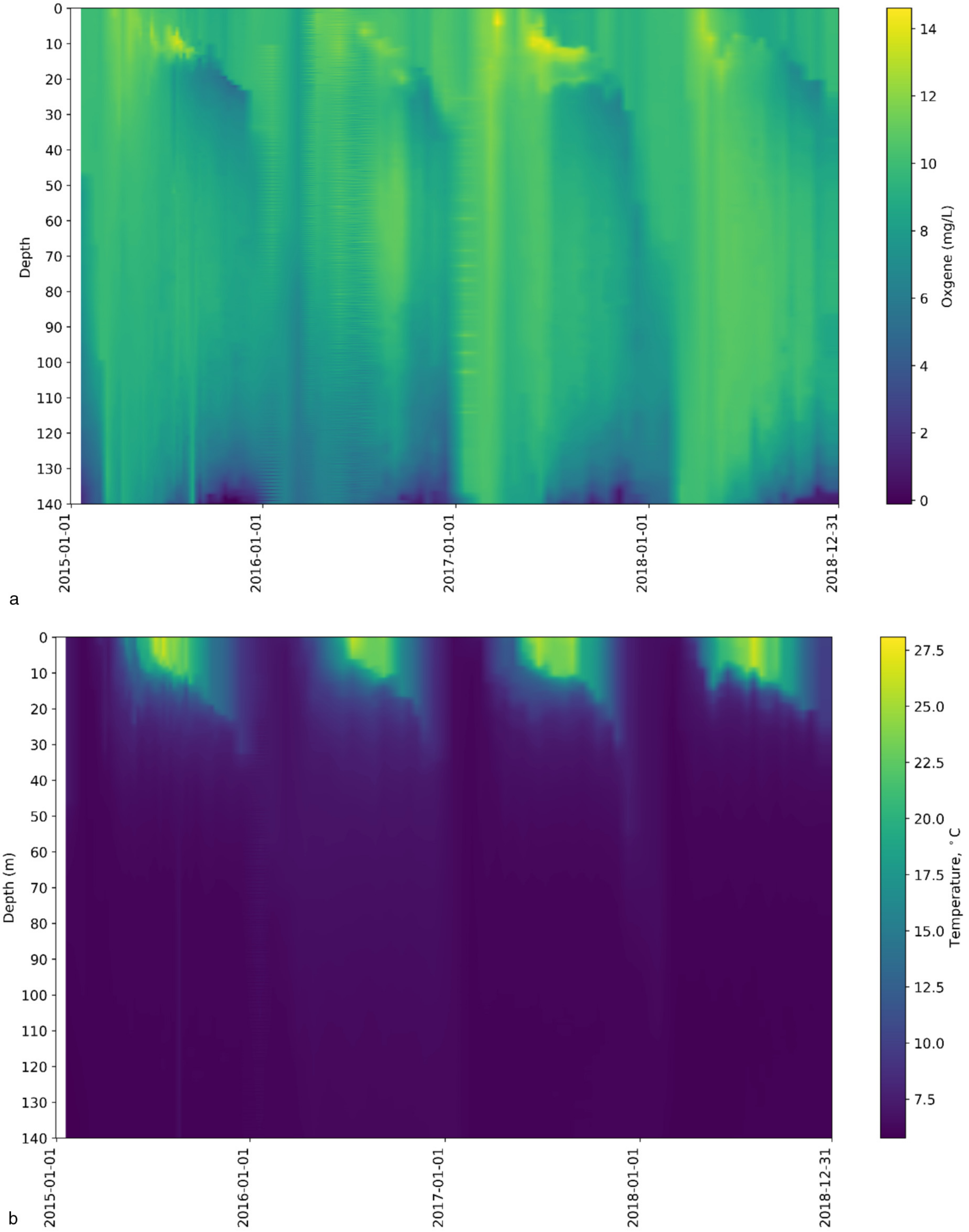

Fig. 6. Evolution of dissolved oxygen concentrations (A) and temperature (B) over the water column from 2015 to 2018.

because of chemical alterations of the water) and ecosystem services (*e.g.,* animal kills, health hazards for humans via drinking water, consumption of fish, recreational use). *P. rubescens* is very opportunistic and can still develop and bloom following environmental shifts inducing favourable conditions for its growth and development. Our results suggest that (i) when meso- to moderately eutrophic conditions are encountered, the proliferation of *P. rubescens* remains possible (Jacquet *et al.*, 2005; Dokulill and Teubner, 2012) and (ii) confirm that the success of *P. rubescens* can be attributed to

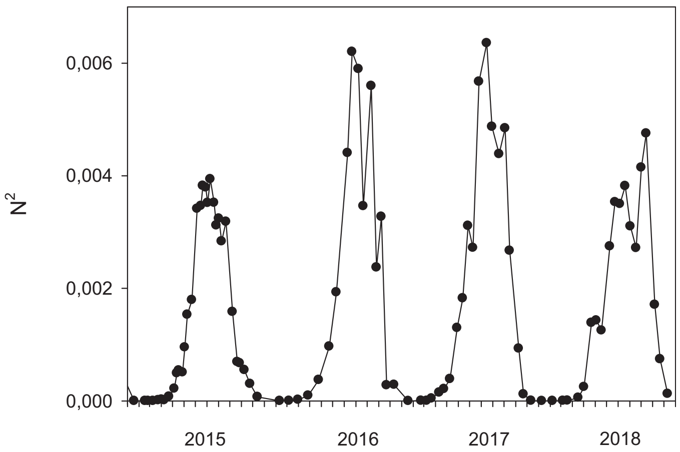

Fig. 7. Evolution of the Brunt-Väisälä frequency N^2 from 2015 to 2018.

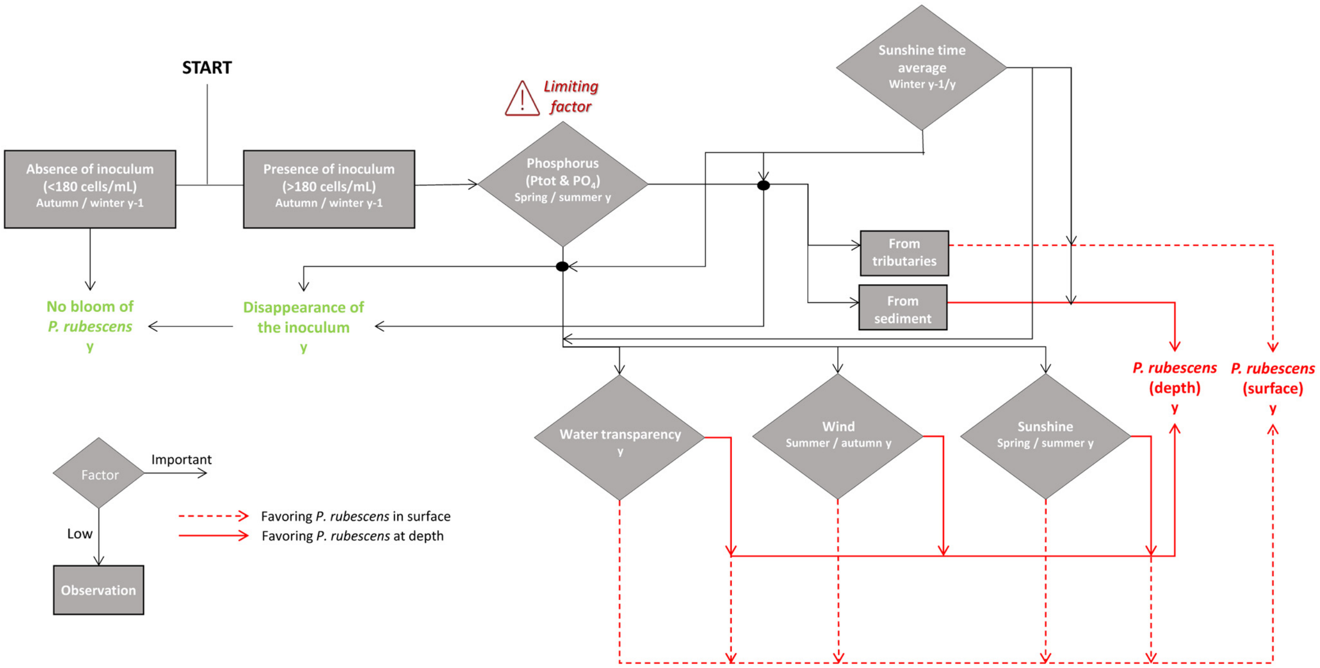

Fig. 8. *P. rubescens* is likely to bloom in Lake Bourget following a conjunction of factors and processes. Firstly, the presence of an autumn/winter innoculum of the cyanobacterium the year before is significant. Secondly, the phosphorus resource remains a key issue and severe limitation in winter/spring of the year can prevent the bloom whereas presence or input (*i.e.*, P already present in surface waters of the lake or coming from tributaries or from the sediment) during the winter/spring of the year can be crucial. Moreover, a relatively high level of winter irradiance and/or water transparency may clearly favor the initial development of *P. rubescens* merely at depth (*i.e.*, between 15 and 25 m).

a combination of physico-chemical factors and ecological processes (*e.g.*, Jacquet *et al.*, 2005, 2014; Posch *et al.*, 2012). This study merely suggests that future environmental conditions (extreme events and runoff, temperature increase, deoxygenation and P release from the bottom) may potentially provide conditions for the development and bloom of the cyanobacterium in Lake Bourget, while the latter is oligotrophic. Associated to the counting of a threshold of filaments during the autumn/winter period the year before the bloom (*e.g.*, >180 cells/mL) and relatively warm winter conditions known to be an important trigger to serve at maintaining the population at a level where it will be possible to proliferate (*e.g.*, Jacquet *et al.*, 2014; Anneville *et al.*, 2015; Gallina *et al.*, 2017; Kerimoglu *et al.*, 2017), a simple alert can be imagined and is proposed in Figure 8. This study may allow for predictions in a variety of lakes on how P. *rubescens* dynamics could develop in times of climate change and respond to inter-annual weather conditions. However, important questions remain: Where did the inoculum come from? Are lateral transport mechanisms induced for instance by internal waves and upwelling events, which occur frequently in Lake Bourget (Cuypers *et al.*, 2011), important drivers for population development? Were filaments present but not detected because of methodical bias due to sampling, sample preparation and/or counting? Or were these filaments located somewhere else in the lake? Etc. In the future, to be able to predict a potential new development of *P. rubescens*, it could be useful to propose a 3D model of the dynamics and distribution of this cyanobacterium, in relation to different factors, themselves linked to the growth of this such particular species.

Conflict of interest

Authors declare no conflict of interest.

Acknowledgements. We are grateful to the technicians who make possible to obtain a large amount of data of high quality (© OLA-IS, AnaEE-France, INRAE of Thonon-les-Bains, CISALB [Rimet *et al.* https://doi.org/10.4081/jlimnol.2020.1944]) and Orlane Anneville for her critical reading of a former version of this article. Professor Steven Wilhelm is greatly acknowledged for improving a former version of this manuscript and English editing.

Supplementary Material

Supplementary Fig. S1. Evolution of the phytoplanktonic biomass (a) using the main classes and the (b) zooplankton using the proportion of the total microcrustacean zooplankton, herbivores and herbivore cladocerans and between 2004 and 2019. A zoom is also proposed for years 2015 to 2018 for the herbivores at the different season.

The Supplementary Material is available at https://doi.org/10.1051/limn/2021014.

References

AFNOR EN 15204. 2006. Water quality – Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique). *Afnor* 1–39.

AFNOR. 2015. Qualité de l'eau. Recueil des normes. ISBN: 978-2-12-3179311-5

Anagnostidis K, Komarek J. 1988. Modern approach to the classification system of cyanophytes. 3-Oscillatoriales. *Arch Hydrobiol* 80: 327–472.

Anneville A, Domaizon I, Kerimoglu O, Rimet F, Jacquet S. 2015. Blue-Green Algae in a "Greenhouse Century"? New insights from field data on climate change impacts on Cyanobacteria abundance. *Ecosystems* 18: 441–458.

Barnard MA, Chaffin JD, Plaas HE, *et al.* 2021. Roles of nutrient limitation on western Lake Erie CyanoHAB toxin production. *Toxins* 13: 47.

Bright DI, Walsby AE. 2000. The daily integral of growth by *Planktothrix rubescens* calculated from growth rate in culture and irradiance in Lake Zurich. *New Phytolog* 146: 301–316.

Briand J-F, Jacquet S, Flinois C, *et al.* 2005. Variations in the microcystins production of *Planktothrix rubescens* (cyanobacteria) assessed by a four-years in situ survey of Lac du Bourget (France) and by laboratory experiments. *Microb Ecol* 50: 418–428.

Capo E, Debroas D, Arnaud F, *et al.* 2016. Long-term dynamics in microbial eukaryotes communities: a palaeolimnological view based on sedimentary DNA. *Molec Ecol* 25: 5925–5943.

Capo E, Debroas D, Arnaud F, Perga ME, Chardon C, Domaizon I. 2017. Tracking a century of changes in microbial eukaryotic diversity in lakes driven by nutrient enrichment and climate warming. *Environ Microbiol* 19: 2873–2892.

Cuypers Y, Vinçon-Leite B, Groleau A., Tassin B, Humbert J-F. 2011. Impact of internal waves on the spatial distribution of *Planktothrix rubescens* (cyanobacteria) in an alpine lake. *ISME J* 5: 580–589.

Derot J, Yajima H, Jacquet S. 2020. Advances in forecasting harmful algal blooms using machine learning models: A case study with *Planktothrix rubescens* in Lake Geneva. *Harmful Algae* 99: 101906.

Dokulil MT, Teubner K. 2012. Deep *living Planktothrix* rubescens modulated by environmental constraints and climate forcing. *Hydrobiologia* 698: 29–46.

Ernst B, Hoeger SJ, O'Brien E, Dietrich DR. 2009. Abundance and toxicicty of *Planktothrix rubescens* in the pre-alpine Lake Ammersee, Germany. *Harmful Algae* 82: 329–342.

Fastner J, Erhard M, Carmichael WW, *et al.* 1999. Characterization and diversity of microcystins in natural blooms and strains of the genera *Microcystis* and *Planktothrix* from German freshwaters. *Arch Hydrobiol* 145: 147–163.

Feuillade J. 1994. The cyanobacterium (blue-green algae) *Oscillatoria rubescens* D.C. *Arch Hydrobiol Beih Eregbn Limnol* 41: 77–93.

Feuillade M, Feuillade J, Blanc P. 1990. Alkaline phosphatase activity fluctuations and associated factors in a eutrophic lake dominated by Oscillatoria rubescens. *Hydrobiology* 207: 233–240.

Frossard V, Grandrémy N, Arthaud F, Guillard J, Jacquet S. Evidence of ecological shifts in fresh water ecosystems: A case study with Lake Bourget (France).

Gallina N, Beniston M, Jacquet S. 2017. Estimating future cyanobacterial occurrence and importance in lakes: a case study with *Planktothrix rubescens* in Lake Geneva. *Aquat Sci* 79: 249–263.

Jacquet S, Briand J-F, Leboulanger C, *et al.* 2005. The proliferation of the toxic cyanobacterium *Planktothrix rubescens* following restoration of the largest natural French lake (Lac du Bourget). *Harmful Algae* 4: 651–672.

Jacquet S, Kerimoglu O, Rimet F, Paolini G, Anneville O. 2014. Cyanobacterial bloom termination: the disappearance of *Planktothrix rubescens* from Lake Bourget after restoration. *Freshw Biol* 59: 2472–2489.

Jacquet S, Arthaud F, Barbet D, *et al.* 2017. Suivi environnemental des eaux du lac du Bourget pour l'année 2016. Rapport INRA-CISALB-CALB, 211 pages.

Jansson M, Bergstrom AK, Drakare S, Blomqvist P. 2001. Nutrient limitation of bacterioplankton and phytoplankton in humic lakes in northern Sweden. *Freshw Biol* 46: 653–666.

Jenny JP, *et al.* 2020. Scientists' Warning to Humanity: Rapid degradation of the world's large lakes. *J Great Lakes Res* 46: 686–702.

Kerimoglu O, Jacquet S, Vinçon-Leite B, *et al.* 2017. Modelling the plankton groups of the deep, peri-alpine Lake Bourget. *Ecol Model* 359: 415–433.

Kurmayer R, Blom JF, Deng L, Pernthaler J. 2015. Integrating phylogeny, geographic niche partitioning and secondary metabolite synthesis in bloom-forming *Planktothrix*. *Isme J* 9: 909–921.

Leboulanger C, Dorigo U, Jacquet S, LeBerre B, Paolini G, Humbert J-F. 2002. Application of a submersible spectrofluorometer for rapid monitoring of freshwater cyanobacterial blooms: a case study. *Aquat Microb Ecol* 30: 83–89.

Legrand C, Rengefors K, Fistarol GO, Granéli E. 2003. Allelopathy in phytoplankton - biochemical, ecological and evolutionary aspects. *Phycologia* 42: 406–419.

Mazur-Marzec H, *et al.* 2013. Occurrence of cyanobacteria and cyanotoxins in the Southern Baltic Proper. Filamentous cyanobacteria vs. single-celled picocyanobacteria. *Hydrobiologia* 701: 235–252.

Micheletti S, Schanz F, Walsby AE. 1998. The daily integral of photosynthesis by *Planktothrix rubescens* during summer stratification and autumnal mixing in Lake Zürich. *New Phytolog* 138: 233–249.

Oberhaus L, Gelinas M, Pinel-Alloul B, Ghadouani A, Humbert J-F. 2007. Grazing of tow toxic *Planktothrix* species by *Daphnia pulicaria*: potential for bloom control and toxin transfer of microcystins. *J Plankton Res* 29: 827–838.

Oberhaus L, Briand J-F, Humbert J-F. 2008. Allelopathic growth inhibition by the toxic, bloom-forming cyanobacterium *Planktothrix rubescens*. *FEMS Microbiol Ecol* 66: 243–249.

Paerl HW, Huisman J. 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environ Microbiol Rep* 1: 27–37.

Paerl HW, Xu H, McCarthy MJ, *et al.* 2011. Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): the need for a dual nutrient (N & P) management strategy. *Water Res* 45: 1973–1983.

Paerl HW, Scott JT, McCarthy MJ, *et al.* 2016. It takes two to tango: When and where dual nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems. *Environ Sci Technol* 50: 10805–10813.

Pelletier JP, Orand A. 1978. Appareil de prélèvement d'un échantillon dans un fluide. Brevet d'invention 76.08579.

Perga ME, Domaizon I, Guillard J, Hamelet V, Anneville O. 2013. Are cyanobacterial blooms trophic dead ends? *Oecologia* 172: 551–562.

Posch T, Koster O, Salcher MM, Pernthaler J. 2012. Harmful filamentous cyanobacteria favoured by reduced water turnover with lake warming. *Nature Clim Change* 2: 809–813.

Reynolds C, Huszar V, Kruk C, Naselli-Flores L, Melo S. 2002. Towards a functional classification of the freshwater phytoplankton. *J Plankton Res* 24: 417–428.

Rimet F, *et al.* 2020. The Observatory on LAkes (OLA) database: Sixty years of environmental data accessible to the public. *J Limnol* DOI: 10.4081/jlimnol.2020.1944.

Rimet F, Druart J-C. 2018. A trait database for phytoplankton of temperate lakes. *Ann Limnolog* 54: 18.

Savichtcheva O, Debroas D, Perga ME, *et al.* 2015. Effects of nutrients and warming on *Planktothrix* dynamics and diversity: a palaeolimnological view based on sedimentary DNA and RNA. *Freshw Biol* 60: 31–49.

Sotton B, Anneville O, Cadel-Six S, Domaizon I, Krys S, Guillard J. 2011. Spatial match between *Planktothrix rubescens* and whitefish in a mesotrophic peri-alpine lake: evidence of toxins accumulation. *Harmful Algae* 10: 749–758.

Sotton B, Guillard J, Bony S, *et al.* 2012. Impact of toxic cyanobacterial blooms on Eurasian perch (*Perca fluviatilis*): experimental approaches and in situ observations in a peri-alpine lake. *PLoS ONE* 7: e52243.

Strickland JDH, Parsons TR. 1972. A practical handbook of seawater analysis. 2nd Ed. Bull. Fish. Res. Bd. Canada 167: 311.

Tao Y, Wolinska J, Hölker F, Agha R. 2020. Light intensity and spectral distribution affect chytrid infection of cyanobacteria via modulation of host fitness. *Parasitology* 147: 1206–1215.

Tapolczai K, Anneville O, Padisak J, *et al.* 2014. Occurrence and mass development of Mougeotia spp. (Zygnemataceae) in large, deep lakes. *Hydrobiologia* 745: 17–29.

Uthermöhl H. 1958. Zur Vervollkommung der quantitativen phytoplankton-methodik. *Mitt Int Ver Limnol* 9: 38.

Vinçon-Leite B, Tassin B, Druart J-C. 2002. Phytoplankton variability in Lake Bourget: Phytoplankton dynamics and meteorology. *Lakes & Reservoirs: Research and Management* 7: 93–102.

Walsby AE, Avery A, Schanz F. 1988. The critical pressure of gas vesicles in *Plantktothrix rubescens* in relation to the depth of winter mixing in lake Zurich, Switzerland. *Journal of Plankton Research* 20: 1357–1375.

Walve J, Larsson U. 2007. Blooms of Baltic Sea *Aphanizomenon* sp. (Cyanobacteria) collapse after internal phosphorus depletion. *Aquatic Microbial Ecology* 49: 57–69.

Zotina T, Koster O, Juttner F. 2003. Photoheterotrophy and light-dependent uptake of inorganic and organic nitrogenous compounds by *Planktothrix rubescens* under low irradiance. *Freshwater Biology* 48: 1859–1872.

Cite this article as: Moiron M, Rimet F, Girel C, Jacquet S. 2021. Die hard in Lake Bourget! The case of *Planktothrix rubescens reborn*. *Ann. Limnol. - Int. J. Lim.* 57: 19