


## Article

# Increases in Picocyanobacteria Abundance in Agriculturally Eutrophic Pampean Lakes Inferred from Historical Records of Secchi Depth and *Chlorophyll-a*

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**Abstract:** Phytoplankton size structure has profound consequences on food-web organization and energy transfer. Presently, picocyanobacteria (size < 2 µm) represent a major fraction of the autotrophic plankton of Pampean lakes. Glyphosate is known to stimulate the development of picocyanobacteria capable of degrading the herbicide. Due to the worldwide adoption of glyphosate-resistant crops, herbicide usage has increased sharply since the mid-1990s. Unfortunately, there are very few studies (none for the Pampa region) reporting picocyanobacteria abundance before 2000. The proliferation of µm sized particles should decrease Secchi disc depth (ZSD). Therefore ZSD, conditional to *chlorophyll-a*, may serve as an indicator of picocyanobacteria abundance. We use generalized additive models (GAMs) to analyze a “validation” dataset consisting of 82 records of ZSD, *chlorophyll-a*, and picocyanobacteria abundance from two Pampean lakes surveys (2009 and 2015). In support of the hypothesis, ZSD was negatively related to picocyanobacteria after accounting for the effect of *chlorophyll-a*. We then fitted a “historical” dataset using hierarchical GAMs to compare ZSD conditional to *chlorophyll-a*, before and after 2000. We estimated that ZSD levels during 2000–2021 were, on average, only about half as deep as those during 1980–1999. We conclude that the adoption of glyphosate-resistant crops has stimulated outbreaks of picocyanobacteria populations, resulting in lower water transparency.

**Keywords:** underwater light climate; eutrophication; glyphosate; cyanobacteria

## 1. Introduction

The size structure of phytoplankton has profound consequences on food-web organization and energy transfer within plankton communities [1]. The dominance of small-sized algae (picophytoplankton < 2 µm in diameter) would tend to increase the abundance of smaller grazers (i.e., heterotrophic flagellates and ciliates), promoting the heterotrophic pathway (picoplankton → heterotrophic protists → zooplankton) over the classical trophic chain (phytoplankton → zooplankton), therefore decreasing the energy transfer efficiency toward upper trophic levels [2–4]. Changes in land use might affect aquatic community composition and processes, altering food-web interaction and ultimately impacting carbon fluxes in lakes [5].

The fertile Pampa region of Argentina (33–39° S, 57–64° W) harbors large numbers of shallow, eutrophic lakes [6]. Over the last two centuries, the Pampean landscape changed progressively from natural grasslands into cultivated grasslands and croplands due to animal production and intensive crops (wheat, corn, sunflower, and, since the mid-1990s, soybean) [7]. The process of agricultural intensification aggravated the eutrophication of shallow Pampean lakes through increased nutrients (N, P) and agrochemical inputs into the aquatic environment [8,9]. Since the last decade of the 20th century, Pampean lakes have experienced remarkable increases in nutrients (N and P) and *chlorophyll-a* concentration (Chla) and decreases in water transparency, estimated as Secchi disk depth (ZSD) [10]. Within a historical framework, the eutrophication increase in shallow Pampean lakes was concurrent with the massive conversion to glyphosate-resistant crops (GRC) technologies, adopted by major grain producer countries [11] including Argentina. Glyphosate (N-(phosphonomethyl) glycine) is a broad-spectrum, post-emergent herbicide that is presently among the most widely used agricultural chemicals globally. Usage trends of this herbicide in Argentina have increased steadily, fueled by the widespread adoption of transgenic soybean monoculture and no-tillage practices since around the mid-1990s [12].

In aquatic environments, glyphosate tolerance is widespread among cyanobacteria [13,14], particularly within small-sized species [15]. Studies performed in mesocosms [16–18] demonstrated that additions of glyphosate to experimental vessels stimulated the development of picocyanobacteria (Pcy), which reached densities much higher than in control treatments. More recently, a survey of shallow, eutrophic lakes from Argentina [19] provided additional, non-manipulative evidence on the stimulatory effect of glyphosate on Pcy communities in their natural habitats (i.e., Pcy abundance was positively correlated with glyphosate indicators within the Pampa region). Bullerjahn and coworkers [20,21] investigated the mechanism of glyphosate tolerance in picocyanobacteria. They reported that Pcy, isolated from the Laurentian Great Lakes, were able to metabolize glyphosate and its main degradation product, aminomethylphosphonic acid (AMPA), which allowed them to grow in cultures with glyphosate or AMPA as the only sources of phosphorus. Glyphosate tolerance and its utilization by various freshwater *Synechococcus* strains is mediated by the phosphonate transporter gene *phnD*, suggesting that detection of *phnD* gene in plankton samples is an indication of the capacity of picoplankton to metabolize phosphonates, either natural or synthetic (e.g., glyphosate) [20]. We assessed the occurrence of the *phnD* gene using primers specific for *Synechococcus* in lake plankton from the Pampa (highly impacted by agricultural practices) and Patagonia (not impacted by agriculture activities) regions of Argentina. Detection of the *phnD* gene was more frequent and Pcy abundance much higher in lakes from the Pampean region (29% of lakes,  $1.8 \times 10^6$  cells mL<sup>-1</sup>) than in similarly eutrophic lakes from Patagonia (5% of lakes,  $2.0 \times 10^5$  cells mL<sup>-1</sup>) [19]. Along the same lines, Saxton and coworkers [14] concluded that glyphosate influences the phytoplankton community structure in Lake Erie, serving as a nutrient source to microbes (i.e., heterotrophic bacteria and Pcy) able to tolerate the herbicide effects of the compound, while killing those less tolerant algal species.

Presently, Pcy represent a major fraction of the autotrophic plankton of Pampean lakes. Current estimates often exceed  $10^7$  cells mL<sup>-1</sup> and rank among the highest abundance reported for lakes worldwide [19,22,23]. Considering such values within the context of the previous arguments, we suspect that the continued usage of glyphosate-based herbicides during the last decades may have impacted natural phytoplankton assemblages of shallow Pampean lakes. More specifically, we hypothesize that by favoring glyphosate-resistant picocyanobacteria, modern agricultural practices have inadvertently contributed to the increase in Pcy abundance up to the very high values typical of present-day Pampean phytoplankton. Unfortunately, epifluorescence microscopy [24] and flow cytometry [25] techniques, which would later allow routine quantification of Pcy concentrations, were not readily implemented until around the 2000s. However, rather conveniently for our purposes, large numbers of small-sized particles, such as Pcy, should produce optical signals that, at least in principle, can be retrieved from historical records of ZSD measurements.

Although regression models of ZSD vs. Chla (often transformed as reciprocal-log or log-log) tend to be highly significant within the mesotrophic–eutrophic range [26,27], Chla usually accounts for only a modest fraction of the total variability in ZSD. The analysis of the residual variability has been used to explore the effect of additional variables, such as inorganic turbidity or water color [28]. ZSD is particularly affected by light scattering due to suspended particles, with small particles having a disproportionately large effect [29]. Stramski and Kiefer [30] estimated that in waters where picophytoplankton concentration approaches  $10^6$  cells  $\text{mL}^{-1}$ , the microorganisms may dominate light scattering, accounting for >50% of its total magnitude. Gallegos et al. [31] reported long-term decreasing trends in ZSD in Chesapeake Bay associated with changes in phytoplankton size structure. In their study, increases in picoplankton abundance resulted in greater light scattering and shallower ZSD. Similarly, analysis of different phytoplankton assemblages differing in size structure showed that the dominance of small-sized algae resulted in remarkably shallower ZSD [32,33]. In other words, ZSD, conditional to Chla (i.e., after statistically removing the effect of Chla), can be expected to bear information on the size structure of the phytoplankton assemblage in general, and particularly, on the abundance of the small-sized plankton fraction.

Summarizing, the accumulated evidence consistently suggests that glyphosate-based herbicides stimulate Pcy populations, resulting in higher abundance of small-sized phytoplankton cells. On the other hand, based on previous theoretical and empirical studies, it is expected that larger numbers of small-sized ( $\sim 1 \mu\text{m}$ ) particles would decrease ZSD. The question that lingers is whether, under the complex optical climate of Pampean lakes [34], the decrease in ZSD would be sufficiently pronounced, i.e., not masked by confounding factors. Our first objective was to assess this hypothesis. For this purpose, we assembled a “validation” dataset combining two relatively recent (2009 and 2015) surveys of Pampean lakes, reporting data on ZSD, Chla, and Pcy abundance, among other variables. Using statistical tools, we asked whether, at comparable Chla, ZSD was negatively related to Pcy abundance—the practical implication being that ZSD, conditional to Chla, could then be used as an indicator of Pcy abundance. Our second objective was to compare ZSD measurements before and after the massive adoption of glyphosate-based herbicides in Argentina. If confirmed, this would provide a lacking piece of evidence supporting our main hypothesis that the continued use of glyphosate has impacted the phytoplankton size spectrum of shallow Pampean lakes by favoring pico-sized cyanobacteria populations, as previously observed in mesocosms experiments [16,17]. For achieving the second objective, we compiled a “historical” dataset (combining a bibliographic search with our own data).

## 2. Materials and Methods

The Argentine Pampa region is a large fertile plain in the center-east part of the country. The climate is temperate, with warm summers and mild winters. Precipitation ranges from  $1000 \text{ mm year}^{-1}$  in the northeast to  $400 \text{ mm year}^{-1}$  in the southwest. Shallow lakes are the most important freshwater ecosystems in the region. Only in Buenos Aires Province, there are roughly 14,000 large ( $>10 \text{ ha}$ ) and 146,000 small ( $0.05\text{--}10 \text{ ha}$ ) shallow lakes ([6], and references therein).

Our first objective was to evaluate whether, at comparable Chla, ZSD was negatively related to Pcy abundance. For testing this hypothesis, we assembled a “validation” dataset combining two relatively recent (2009 and 2015) surveys of Pampean lakes that report data on ZSD, Chla, and Pcy abundance, among other variables [19,23]. The combined dataset totaled 82 observations from 60 Pampean shallow lakes (22 lakes visited twice and 38 lakes visited once), ranging from mesotrophic to hypertrophic (see Table 1). For assessing whether Pcy abundance and Chla (i.e., the predictor variables) significantly contributed to explain the variability in ZSD (i.e., response variable), we used generalized additive models (GAMs) [35]. GAMs allow one to model flexible functions among the response and predictor variables without imposing an a priori specified functional form (e.g., linear, quadratic, etc.).

Rather conversely, the shape of the relationship between the response and the predictors arises from the data analysis itself. GAMs were fitted to log<sub>10</sub> transformed data to comply with the assumption of normality. We used REML (restricted maximum likelihood) to estimate model coefficients and smoothing parameters and thin plate regression splines (TPRS) as the smoother. The initial basis dimension was checked for adequacy and increased whenever necessary. The model was then refitted according to [36]. We evaluated the uncertainty of the fitted trends under a Bayesian formulation, using 10,000 simulations of the trend from the posterior distribution of the fitted GAM. Model selection was based on Akaike's Information Criterion (AIC). We estimated GAMs using the mgcv package (version 1.8–36) [35] and uncertainty in the adjusted trends using the gratia package (version 0.6.0) [37]. Graphical outputs were obtained using the ggplot2 package [38] for R [39].

**Table 1.** Median and range of limnological variables corresponding to the “validation” dataset (i.e., 82 shallow Pampean lakes sampled during spring and summer surveys performed in 2009 and 2015).

Limnological Parameter	Median	Range
ZSD (cm)	14	2–191
Chla ( $\mu\text{g L}^{-1}$ )	64.28	1.58–1549.79
Pcy (cells $\text{mL}^{-1}$ )	$9.60 \times 10^5$	$3.3 \times 10^3$ – $7.72 \times 10^7$
Total phosphorus ( $\mu\text{g L}^{-1}$ )	620	124.2–4538
Total nitrogen ( $\mu\text{g L}^{-1}$ )	5051	1702–12,846
pH	8.9	8–10.2

ZSD: Secchi disk depth; Chla: *chlorophyll-a* concentration; Pcy: picocyanobacteria abundance.

For our second objective (i.e., using ZSD, conditional to Chla, to infer changes in Pcy abundance after the adoption of glyphosate resistant crops in Argentina), we analyzed a “historical” data set consisting of 534 observations of shallow Pampean lakes, covering the period from 1984 to 2021. The database included published records, unpublished data collected by us, and unpublished data provided by colleagues. The full database, including its sources, has been published elsewhere [10]. For the present analyses, only data corresponding to the spring and summer seasons were included. In recent times, a few individual lakes were sampled on many occasions and they were therefore overrepresented. To avoid this potential bias, we randomly subsampled the set of data, leaving 5 observations per lake at most after the year 2000. The final working subset totaled 207 observations and included information from 1984 to 1999 (referred to as 20th century) and from 2000 to 2021 (21st century). We assessed the significance of the relationship between ZSD and Chla and evaluated whether such relationship differed between the two above mentioned periods. For these analyses, we used hierarchical GAM (HGAM) [40], which allows modeling nonlinear functional relationships between the predictor and response variables. Moreover, the model can handle different functional shapes for each grouping level (different centuries, in our case). Model selection involves the analysis of different degrees of intergroup (i.e., between centuries) variability in functional response. The model structure must be specified according to the hypothesis to be tested. Briefly, one must decide (i) whether each group should have its own smoother, or whether a common smoother would suffice; (ii) whether the group-specific smoothers should have the same wiggleness, or whether each group should have its own smoothing; and (iii) whether the smoothers for each group should have a similar shape to one another (i.e., a shared global smoother). Depending on the answers to the above questions, there are five possible model structures: a single common smoother for all observations (model G), a global smoother plus group-level smoothers with the same wiggleness (model GS), a global smoother plus group-level smoothers with different wiggleness (model GI), group-specific smoothers, but all of them having the same wiggleness (model S), and group-specific smoothers with different wiggleness (model I) [40]. Given that our interest was to assess the significance of inter-group (i.e., between centuries) variability, rather than investigating the functional form of the relationship between predictor and response variables, the suitable model structures are either S or I. The performance of these two models was compared based on

AIC, which is a robust approach for comparing different model structures [40]. According to this criterion, the model with the lowest AIC should be preferred, and as a rule of thumb, the performance of models differing by less than 2 units are equivalent [41]. We used mgcv package to fit HGMs and estimate the confidence interval of the fitted trends, and ggplot2 to illustrate plots.

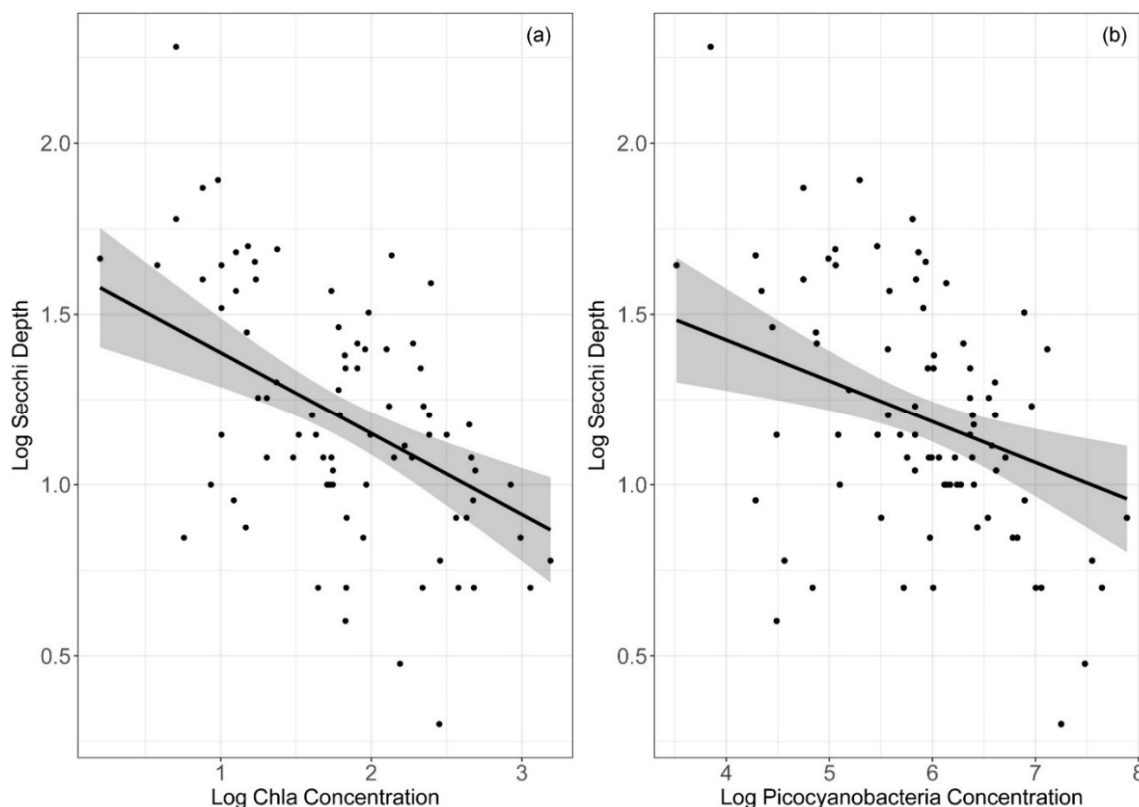
### 3. Results

The validation dataset included lakes in eutrophic–hypereutrophic status, which is the dominant condition in shallow Pampean lakes. In general, they are characterized by high nutrient and Chla concentrations, high Pcy abundance, and alkaline waters (Table 1). Despite the wide range in ZSD, most of them had shallow ZSD (median = 14 cm, Table 1). In order to assess whether Pcy abundance negatively affects ZSD at comparable Chla (objective 1), we fitted two models relating ZSD to either Chla or Pcy abundance separately and a model evaluating the combined effect of Chla and Pcy abundance (Table 2). The first two models significantly explained a percentage of the deviance (Pcy: 24.70%, Chla: 31.30%). However, the best fitting model included both predictor variables (explained deviance: Pcy + Chla: 38.70%). Moreover, the combined model had the lowest AIC, and the difference in AIC to the next best model (i.e., delta AIC) was greater than 4 units. The combined model indicates additive negative effects of Pcy and Chla on ZSD, i.e., increases in either Chla concentration and/or Pcy abundance result in decreased ZSD (Figure 1).

**Table 2.** Statistics from fitted GAMs to the “validation” dataset; grey shadow represents the best fitted model based on AIC and the percentage of deviance explained; edf, estimated degrees of freedom; *p*-value < 0.05 is statistically significant; AIC, Akaike Information Criterion; deltaAIC is the difference with respect to the lowest AIC value.

Model	edf	<i>p</i> -Value	Deviance	AIC	DeltaAIC
Secchi ~ Pcy	1.4	$2.75 \times 10^{-5}$	24.70%	47.68	16.18
Secchi ~ Chla	1	$<2 \times 10^{-16}$	31.30%	38.8	7.3
Secchi ~ Pcy + Chla	1	0.00288	38.70%	31.5	0
	1	$3.82 \times 10^{-5}$			

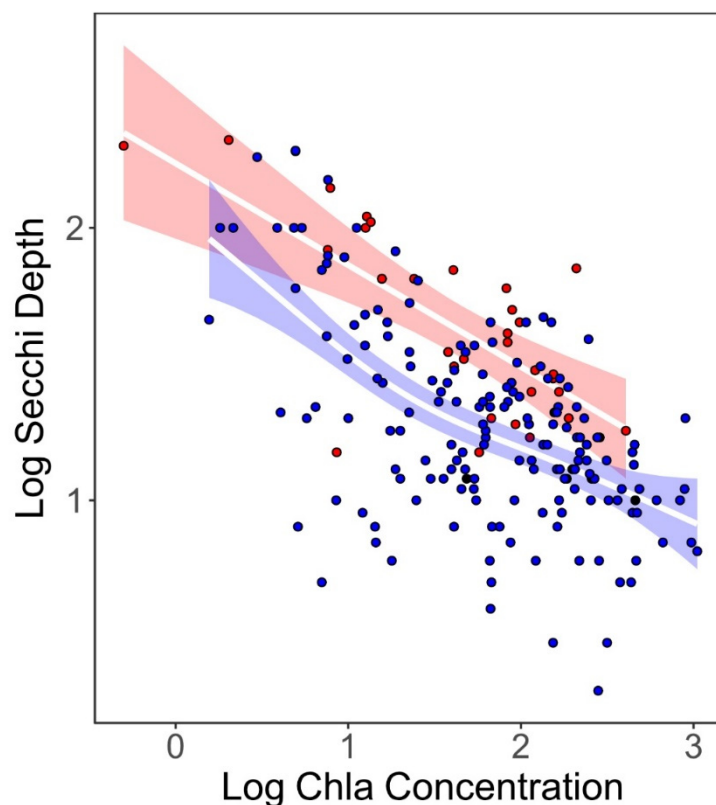
To assess whether ZSD (conditional to Chla) decreased after the adoption of glyphosate-resistant crops (objective 2), we compared measurements from 1984 to 1999 (20th century) vs. those from 2000 to 2021 (21st century). For this comparison, we used hierarchical GAM (HGAM) to assess whether the functional relationship between ZSD and Chla concentration varied between groups (here referred to as centuries for simplicity). Two HGAM, with slightly different model structures, were evaluated: S and I. Both models allowed for group-specific smooth terms, but model S imposed the same wiggleness to the smooth, while model I allowed different groups to differ in wiggleness. In both cases, model fits were significant, indicating not only a decreasing trend of ZSD with Chla but also significant intergroup differences (i.e., a significant “century” effect:  $ZSD_{20} > ZSD_{21}$ ). Both models explained a similar percentage of the deviance (46.2–46.4, Table 3), with model I having the lowest AIC. Model I, in addition to demonstrating significant trends for both centuries, showed a significant random effect for centuries to model century-specific intercepts. According to the rule of thumb, there was no ground to prefer one model over the other (i.e., they differed in less than AIC units) (Table 3). We prefer model I since it imposes fewer restrictions to the smoother function (i.e., each century has its own individual smoother) and shows in the best overall fit (higher explained deviance and higher estimated degrees of freedom for smooth terms) (Figure 2). To illustrate intergroup differences in ZSD over the last 40 years, we estimated ZSD for each century using the selected model I for three Chla levels (i.e., low, moderate, and high) (Table 4). This exercise showed that for the same Chla, ZSD corresponding to the 21st century was only about 50% of the corresponding levels of ZSD in the 20th century, regardless of the Chla level considered.



**Figure 1.** GAM results for spring–summer ZSD in shallow Pampean lakes, showing the fitted trend, confidence intervals, and observed values. Plots are partial plots of the smooth term in the model, and the y axis is the intercept plus the partial effect of the individual smooth. Data are log10 transformed, Chla was originally expressed in  $\mu\text{g L}^{-1}$  (a); picocyanobacterial abundance in cells  $\text{mL}^{-1}$ ; and ZSD in cm (b).

**Table 3.** HGAM results from the “historical” dataset exploring the functional relationship between ZSD and Chla. The two periods contrasted are: 1984–1999 (20th century) and 2000–2001 (21st century). Two slightly different model structures are presented (S and I, see Materials and Methods section). *p*-values associated with each term are listed in their order of appearance in the mathematical function; *p*-values < 0.05 are statistically significant; AIC, Akaike Information Criterion; delta AIC is the difference between a given model AIC with respect to the lowest AIC model; edf, estimated degrees of freedom for \*, 20th century, 21st century.

Model	edf	<i>p</i> -Value	% Deviance	AIC	Delta AIC
S: $\log\text{ZSD} = f_{\text{century}}(\log\text{Chla}) + \varepsilon$	4.34	$<2 \times 10^{-16}$	46.2	71.52	1.4
I: $\log\text{ZSD} = f((\log\text{Chla}):\text{century}) + \zeta_{\text{century}} + \varepsilon$	1 *	$5.22 \times 10^{-6}$	46.4	70.12	0
	2.36	$<2 \times 10^{-16}$			
	0.97	$5.51 \times 10^{-7}$			



**Figure 2.** HGAM results for model I, showing fitted trends, confidence intervals, and observed values for data corresponding to the period 1984–1999 (red) and 2000–2021 (blue). Plots are partial plots of the smooth term in the model, and the y axis is the intercept plus the effect of the individual smooth for each group (century). Data are log<sub>10</sub> transformed; *chlorophyll-a* (Chla) was originally expressed in  $\mu\text{g L}^{-1}$  and Secchi disc depth (ZSD) in cm.

**Table 4.** Estimated ZSD from shallow Pampean lakes according to model I, before (20th century) and after (21st century) the adoption of GR crops.

Chla Concentration ( $\mu\text{g L}^{-1}$ )	Predicted Secchi Disc Depth (cm)		
	20th Century ZSD	21st Century ZSD	21st Century ZSD as % of 20th Century ZSD
7	79.4	40.7	51.3
50	38.0	18.2	47.9
200	23.4	12.9	55.0

#### 4. Discussion

Due to their small size, Pcy cells are expected to absorb and scatter light more strongly on a mass-specific basis than larger cells [29,31]. Therefore, after accounting for the effect of Chla, the depth of disappearance of the Secchi disc (ZSD) should decrease, in theory, with the concentration of Pcy. This effect, in turn, should be more apparent at high Pcy concentrations ( $>10^6$  cells  $\text{mL}^{-1}$ ) [30]. The analysis of the “validation” dataset confirmed this prediction, proving that the theoretical expectation holds true, even for highly complex waters, such as those typical of shallow Pampean lakes [42]. Our findings reinforce the importance of water clarity as an ecosystem indicator of eutrophication. Although there are several measures of water transparency, ZSD has been the most consistent and frequent measure employed over time [43].

Previous experiments [16,17] and field surveys of shallow Pampean lakes [19] demonstrated that exposure to glyphosate stimulates the development of Pcy populations. On the

other hand, we have reported that the ZSD levels, conditional to Chla, in glyphosate-impacted Pampean lakes are significantly shallower (~50–60%) than the values reported for shallow lakes worldwide, within the low temperate latitudinal range (defined as two belts from 23.6° to 44.5° North or South) [6]. Considering the steady increase in glyphosate use in Argentina and the very high Pcy concentrations reported for Pampean lakes in recent years [19,23,44], we suspect the shallower ZSD reported for Pampean lakes in recent times represents an unforeseen result of the adoption of glyphosate-resistant crops. The analysis of the “historical” dataset revealed that ZSD values, conditional to Chla, during the 21st century (i.e., after the adoption of GR-crops) are significantly lower than the values reported for the 20th century. In fact, current ZSD values are only about 50% of the values reported during the 1980s and 1990s, which were therefore similar to those reported for other lakes worldwide. All in all, the collected evidence suggests a scenario in which the massive use of glyphosate in the Pampean region has shifted the phytoplankton size structure (increased Pcy abundance), which in turn translated into lower water transparency (decreased ZSD).

As already mentioned, Pcy abundance in shallow Pampean lakes was not quantified by epifluorescence microscopy or cytometry before 2005. However, the increase in the abundance of tiny cells resembling Pcy over the last decades was apparent while quantifying nano- and micro-phytoplankton under the inverted microscope (I. Izaguirre, personal communication). On the other hand, Pcy cells often aggregate to form microcolonies in response to different stimuli [45,46], and there is typically a positive relationship between the abundance of microcolonies and that of single Pcy cells. Our historical records of microcolonies abundance for a few Pampean lakes from 1987 [47] to 2015 [48,49] show remarkable abundance increases. For example, in Lake Chascomús, microcolonies abundance increased from 710 to 16,379 ind. mL<sup>-1</sup> in 1987–1989 to 7396 to 182,297 ind. mL<sup>-1</sup> in 2005–2015. Moreover, since Pcy began to be quantified by epifluorescence and flow cytometry [19,23,44,48,50], Pcy abundances have increased by an order of magnitude in several lakes over the period 2005–2015. Presently, dominant Pcy in Pampean lakes belong to many different OTUs affiliated with the genera *Synechococcus* and *Cyanobium*, within the order Synechococcales [49,51].

Optical signals similar to those observed in the Pampean region have been reported in other agriculture-impacted areas. The use of glyphosate is widespread in the Chesapeake Bay watershed [52]. Long-term increases in light scattering in Chesapeake Bay have been inferred from ZSD, light attenuation, and remote sensing measurements [53]. Gallegos and co-workers [31] suggested that these long-term trends could be explained by decreases in the size structure of plankton populations. Independently, Wang et al. [54] reported high densities of picoplanktonic *Synechococcus* (10<sup>6</sup> cells mL<sup>-1</sup>) throughout the Bay, contributing 20% to 40% of total phytoplankton *chlorophyll-a*. In Lake Erie, Burns and coworkers [55] reported decreasing trends in ZSD during the period 1983–2002. According to these authors, the observed reduction in ZSD by  $7 \pm 3$  cm year<sup>-1</sup> was unrelated to variation in Chla. Moreover, Barbiero and Tuchman [56] observed decreases in Chla of 50% in the western basin of Lake Erie, but despite these decreases in phytoplankton abundance, they actually observed an increase in turbidity in the western basin [57]. Suggestively, the cyanobacteria community of Lake Erie is persistently dominated by small, single-celled species consistent with *Synechococcus* spp. And *Cyanobium* spp. [58].

Changes in plankton composition might alter the trophic interactions, and consequently the flow of matter and energy through the food web. For instance, certain species of zooplankton mediate the phenotypic plasticity of Pcy, promoting their aggregation into colonies [44–46]. The decrease in the average cell size of phytoplankton would enhance the relative importance of intraguild predators at intermediate trophic levels of the food web, favoring the heterotrophic microbial pathway through small protists with the increase in complexity and consequent reduction in trophic efficiency, as it occurs in large tropical lakes [4]. The increase in the relative importance of Pcy affects the underwater light climate, promoting more turbid conditions that might have a negative impact on primary production in light-limited turbid shallow lakes [59,60], reducing carbon fixation.



Both processes—the increment of microbial heterotrophic activity and the reduction of photosynthesis—tend to occur in more heterotrophic environments.

Cultural eutrophication is a long-recognized phenomenon. Although at local scales it might have been similar or even more significant in the past than at present, it was only recently that it attained a true global dimension [61]. In its wider definition, anthropogenic eutrophication refers to the overproduction of organic material induced by anthropogenic inputs of phosphorus and nitrogen, and the concept of syndrome (i.e., a set of symptoms) has been introduced to refer to the myriad of biogeochemical and biological responses to increased nitrogen and phosphorus inputs [62]. Modern agricultural packages, however, involve the utilization of a large and diverse mixture of natural and synthetic chemicals whose effects go beyond those of traditional fertilizers. For example, the usage of glyphosate in the Pampa region not only resulted in increased abundance of small-sized Pcy, as argued in the present study, but also resulted in higher abundance of the smallest species within each major algal class [49], in clear contrast with the conventional pattern of increasing phytoplankton cell size with trophic state ([63], p. 275) [64,65]. Paleolimnological evidence also relates eutrophication with the shift in the phytoplankton community toward cyanobacteria dominance, along with a shift in zooplankton community towards small-bodied zooplankton, such as *Bosmina* and rotifers [66]. These findings suggest that agriculture, through non-point contamination of nutrients and glyphosate, impacts the composition of the phytoplankton and zooplankton communities and their size structure, with multiple ecological consequences. We therefore concur with the editors of this Special Issue ([https://www.mdpi.com/si/water/lake\\_eutrophications#info](https://www.mdpi.com/si/water/lake_eutrophications#info), accessed on 16 June 2021) in that new types of water pollution continuously impose new challenges, requiring recurring reexamination of the eutrophication phenomenon. Our results highlight the relevance of adopting sustainable practices and restoring buffering areas in shallow lakes (e.g., riparian wetland zone at lake margins) to prevent contaminants from reaching the water bodies.

## 5. Conclusions

In this study, we first demonstrated that ZSD (conditional Chla) decreases with the abundance of Pcy. Next, we demonstrated a significant decrease in ZSD (conditional Chla) in Pampean lakes. Such a decrease, which roughly occurred at the turn of the century, was therefore contemporaneous with the sharp increase in glyphosate usage due to the adoption of GR-crops. Previous studies have experimentally demonstrated that the exposure to glyphosate stimulates the development of large Pcy populations, while recent lakes surveys revealed that the abundance of Pcy in Pampean lakes is, on average, remarkably higher than the world average. We conclude that this cumulus of evidence strongly suggests that the increase in glyphosate usage in recent decades has resulted in increased Pcy abundance in Pampean lakes and suggest that the same phenomenon may have also occurred in other agricultural areas of the World.

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