A reply to “Relevant factors in the eutrophication of the Uruguay River and the Río Negro”

Article *in* The Science of The Total Environment · December 2021

DOI: 10.1016/j.scitotenv.2021.151854

Ignacio Alcántara
Universidad de la República de Uruguay

Andrea Somma
Universidad de la República de Uruguay

Guillermo Chalar Marquisá
Universidad de la República de Uruguay

Amelia Fabre
Universidad Tecnológica

Science of the Total Environment 818 (2022) 151854

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Discussion

A reply to “Relevant factors in the eutrophication of the Uruguay River and the Río Negro”

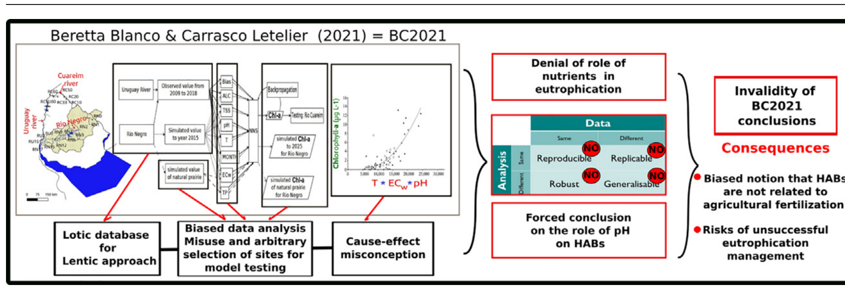
I. Alcántara[a], A. Somma[b,c], G. Chalar[d], A. Fabre[e], A. Segura[f], M. Achkar[g], R. Arocena[d], L. Aubriot[d], C. Baladán[h], M. Barrios[h], S. Bonilla[d], M. Burwood[h], D.L. Calliari[i], C. Calvo[h], L. Capurro[d], C. Carballo[d], C. Céspedes-Payret[j], D. Conde[d], N. Corrales[d], B. Cremella[k], C. Crisci[f], J. Cuevas[d], S. De Giacomi[d], L. De León[l], L. Delbene[d], I. Díaz[g], V. Fleitas[h], I. González-Bergonzoni[b], L. González-Madina[h,c], M. González-Piana[d], G. Goyenola[h], O. Gutiérrez[j], S. Haakonsson[d], C. Iglesias[h], C. Kruk[d,f], G. Lacerot[m], J. Langone[c], F. Lepillanca[n], C. Lucas[b], F. Martigani[o], G. Martínez de la Escalera[n], M. Meerhoff[h,p], L. Nogueira[c], H. Olano[d], J.P. Pacheco[h], D. Panario[j], C. Piccini[n], F. Quintans[d], F. Teixeira de Mello[h], L. Terradas[j], G. Tesitore[h], L. Vidal[o], F. García-Rodríguez[q,r,*]

[a] Ud. Bioestadística, Departamento de Salud Pública, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay
[b] Polo de Ecología Fluvial, CENUR Litoral Norte sede Paysandú, Universidad de la República, Paysandú, Uruguay
[c] Unidad Usinas de Montevideo, Área Tratamiento - Obras Sanitarias del Estado, Aguas Corrientes, Canelones, Uruguay
[d] Sección Limnología, IECA, Facultad de Ciencias, Universidad de la Republica, Montevideo, Uruguay
[e] ITR Suroeste, Universidad Tecnológica, La Paz, Colonia, Uruguay
[f] Modelización y Análisis de Recursos Naturales, Centro Universitario Regional del Este, Universidad de la República, Rocha, Uruguay
[g] LDSGAT, IECA, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay
[h] Departamento de Ecología y Gestión Ambiental, Centro Universitario Regional del Este, Universidad de la República, Maldonado, Uruguay
[i] Sección Oceanografía y Ecología Marina, IECA, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay
[j] UNCIEP, IECA, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay
[k] Laboratory of Environmental Analysis, Université de Sherbrooke, Sherbrooke, Quebec, Canada
[l] Ministerio de Ambiente - Dirección Nacional de Calidad y Evaluación Ambiental, Uruguay
[m] Ecología Funcional de Sistemas Acuáticos, Centro Universitario Regional del Este, Universidad de la República, Uruguay
[n] Departamento de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable, Ministerio de Educación y Cultura, Montevideo, Uruguay
[o] Área Hidrobiología, Gerencia de Gestión de Laboratorios, OSE, Montevideo, Uruguay
[p] Department of Biosciences, Aarhus University, Silkeborg, Denmark
[q] Departamento de Geociencias, Centro Universitario Regional del Este, Universidad de la República, Rocha, Uruguay
[r] Instituto de Oceanografia, Universidade Federal do Rio Grande (FURG), Rio Grande, Brazil

HIGHLIGHTS

- BC2021 claims that pH, EC and T modulate river eutrophication.
- BC2021 claims that main factors controlling eutrophication are not directly linked to agriculture.
- We revisited BC2021's database and detected arbitrary mishandling analysis and site selection to come to forced conclusions.
- We confirmed that the increase in pH is a consequence (not a cause) of microalgae productivity.
- We support the limnological paradigm that nutrients enhance algal blooms.

GRAPHICAL ABSTRACT



* Corresponding author at: Departamento de Geociencias, Centro Universitario Regional del Este, Universidad de la República, Rocha, Uruguay.
E-mail address: felipegr@fcien.edu.uy (F. García-Rodríguez).

http://dx.doi.org/10.1016/j.scitotenv.2021.151854

ARTICLE INFO

Article history:
Received 24 September 2021
Received in revised form 2 November 2021
Accepted 17 November 2021
Available online 23 November 2021

Editor: Ouyang Wei

Keywords:
Chlorophyll-a
Cyanobacteria
Eutrophication
Harmful algal blooms
Reproducible research
Rivers

ABSTRACT

A recent paper by Beretta-Blanco and Carrasco-Letelier (2021) claims that agricultural eutrophication is not one of the main causes for cyanobacterial blooms in rivers and artificial reservoirs. By combining rivers of markedly different hydrological characteristics e.g., presence/absence and number of dams, river discharge and geological setting, the study speculates about the role of nutrients for modulating phytoplankton chlorophyll-a. Here, we identified serious flaws, from erratic and inaccurate data manipulation. The study did not define how erroneous original dataset values were treated, how the variables below the detection/quantification limit were numerically introduced, lack of mandatory variables for river studies such as flow and rainfall, arbitrary removal of pH > 7.5 values (which were not outliers), and finally how extreme values of other environmental variables were included. In addition, we identified conceptual and procedural mistakes such as biased construction/evaluation of model prediction capability. The study trained the model using pooled data from a short restricted lotic section of the (large) Uruguay River and from both lotic and reservoir domains of the Negro River, but then tested predictability within the (small) Cuareim River. Besides these methodological considerations, the article shows misinterpretations of the statistical correlation of cause and effect neglecting basic limnological knowledge of the ecology of harmful algal blooms (HABs) and international research on land use effects on freshwater quality. The argument that pH is a predictor variable for HABs neglects overwhelming basic paradigms of carbon fluxes and change in pH because of primary productivity. As a result, the article introduces the notion that HABs formation are not related to agricultural land use and water residence time and generate a great risk for the management of surface waterbodies. This reply also emphasizes the need for good practices of open data management, especially for public databases in view of external reproducibility.

1. Introduction

The increase in nutrient concentration at ecological scales is one of the largest current problems for water quality worldwide as reported by the United Nations Sustainable Development Goal #6 (https://www.sdg6data.org/). Eutrophication is the increase in primary production of aquatic systems (i.e., macrophytes and/or phytoplankton) from excessive contribution and availability of nitrogen and phosphorus from either natural or anthropic origin (Wetzel, 2001; Moss et al., 2011; Bhagowati and Ahamad, 2019; Ibelings et al., 2021). In addition, the eutrophication process is also defined using dissolved oxygen, total nitrogen, total carbon and chlorophyll-a concentrations (Nixon, 1995). In particular, current intensive agricultural land use is one of the main causes of eutrophication (Altieri and Nicholls, 2001; Allan, 2004; Moss, 2009; Wurtsbaugh et al., 2019). Eutrophication exerts multiple consequences at local (e.g., increase of HABs, Paerl et al., 2001), regional (e.g., decrease or loss of several ecosystem services; Dodds et al., 2009; Janssen et al., 2021) and global levels (e.g., positive feedback with climate change; Moss et al., 2011; Li et al., 2021). The massive application of agrochemicals associated with such agricultural management practices leads to the elimination or reduction of the native vegetation cover. One of the consequences is the decrease in soil porosity, and the increase in surface runoff and soil transport as a result of disaggregation (Hu et al., 2018). These are "high-impact" land uses, associated with negative alteration of soil properties and increased diffuse water pollution (Glendell et al., 2014). The contact of rainwater with the particles forming soil aggregates generates electrostatic repulsive forces that lead to their dispersion, so the structure of the soil becomes altered. Grassland soils are mostly mollisols, with dominant 2:1 clay minerals of net negative charge that are not pH dependent (Li et al., 2017). Thus, for example, with the extensive application of phosphate fertilizers they can be adsorbed on the surface of these particles, decreasing the stability of the aggregates and then migrating to the water during soil erosion (Li et al., 2017). As reported by Hu et al. (2018), internal forces in soil can hierarchically contribute to more erosion processes than the force of the splash impact of raindrops. Hence, a change in the chemical properties of the soil can, to a large extent, lead to a decreased infiltration rate due to the structural instability generated by the dispersion of clay and the soil surface sealing. Consequently, these processes determine that soils do behave differently under conditions of rain and respond differently in terms of amount of runoff and erosive processes (Norton et al., 1999). None of these quantitative variations are considered into erosion models such as the USLE-RUSLE (LaRocque, 2013). Instead, LaRocque (2013) excludes the simulation of important erosion processes. These models were first elaborated for soils of the USA and then used in other regions with scarce data, and despite being developed at plot-scale, they have been applied to larger areas (Favis-Mortlock et al., 2001; Borrelli et al., 2020; Abdulkareem et al., 2021).

Worldwide, it is widely documented that eutrophication is one of the main factors controlling the increase in the frequency and duration of HABs (Reynolds, 2006; Heisler et al., 2008; Paerl and Huisman, 2008; Carey et al., 2012; Paerl and Paul, 2012; O'Neil et al., 2012; Huisman et al., 2018). Other regulatory factors of HABs are the availability of light, water temperature, and water residence time (Paerl and Huisman, 2008; Moss, 2009; Reichwaldt and Ghadouani, 2012). In lotic systems, the increase in water residence time produced by drought events, water extraction, habitat fragmentation, or the construction of dams, generate favorable conditions for the development of several species of HABs (Padisák et al., 1999; Paerl et al., 2011; Bowling et al., 2013; Leigh et al., 2015; Brasil et al., 2016).

Agricultural activities in South America, and particularly in Uruguay have intensified in the last two decades by the application of cutting-edge technologies, massive use of fertilizers/pesticides and irrigation to increase crop production and economical profit (Modernel et al., 2016; Gazzano et al., 2019; Bueno et al., 2021). This process has been unequivocally associated to surface freshwater and coastal eutrophication (Goyenola et al., 2015, 2020, 2021; Aubriot et al., 2017, 2020; Chalar et al., 2017). Bloom formation is driven by the high residence time and the high nutrient concentration in the largest reservoirs of Uruguay, i.e., Salto Grande reservoir on the lower section of Uruguay River and a series of three consecutive reservoirs on the middle section of Negro River (O'Farrell et al., 2012; O'Farrell and Izaguirre, 2014; Bonilla et al., 2015; Bordet et al., 2017; González-Piana et al., 2017; Haakonsson et al., 2017; Martínez de la Escalera et al., 2017). There is regional evidence about the anthropogenic origin and further export of massive cyanobacterial blooms to the Rio de la Plata estuary (Aubriot et al., 2020; Kruk et al., 2021). In addition, it has been shown that this process of bloom formation and export started during the early 1970s due to a combination of climatically-driven increased river discharge and anthropogenic impacts within La Plata Basin (Perez et al., 2021). These studies indicate that in Uruguay, the intensive agricultural activities increase the trophic state of aquatic ecosystems. A recent paper published by Beretta-Blanco and Carrasco-Letelier (2021) (hereafter BC2021) entitled "*Relevant factors in the eutrophication of the Uruguay River and the Río Negro*" claims that water temperature, electrical conductivity and pH are the most relevant factors controlling eutrophication. In addition, the lack of significant correlation between chlorophyll-a concentrations (Chl-a) and total phosphorus, which is not necessarily expected in river systems, allows the study to assert that eutrophication is not directly linked to agricultural land-use. To this end, BC2021 utilized the monitoring database of the Environmental Ministry of Uruguay (formerly National Environmental Directorate - DINAMA), (Ministerio de Ambiente, 2021).

The generation of knowledge through evidence is the basis of the scientific method: from observations questions are raised and then answered following a consistent and potentially replicable methodology (Hilborn and Mangel, 2013). If all analysis components are available and well documented, valuable time is saved for reproducibility of published results, and other researchers can easily revisit and use already published data (Button et al., 2013). Sharing the computer codes of scientific findings publicly helps others understand the analysis, evaluate any study's conclusions, and reuse codes for future analysis, thus contributing to transparent practices, analysis and methodology (Culina et al., 2020).

The objective of this paper is to reply to BC2021 and to demonstrate that the study relied on a series of wrong assumptions and different kinds of errors that need to be amended, rectified and disentangled. In this reply, we discuss the lack of reproducibility from the data acquisition, manipulation and analysis process, and, the wrong interpretation of the statistical results and discussion in the context of current paradigms of limnology and ecology of HABs. We followed the protocols as described in BC2021 paper, with the aim to reproduce the analysis. Also, in the discussion section, we address the statistical findings in the context of the updated regional and international literature. In this paper, we demonstrate that the statistical analyses performed by BC2021 are based on both, biased data-analyses and an ill-defined correlative approach. Altogether, our analysis refutes one of the main conclusions that "*factors that drive Chl-a concentrations (i.e., algae) are not directly linked to agriculture land use*".

2. Materials and methods

2.1. Reproducibility

The reproducibility of a scientific paper is defined as a work able to be revisited and recreated independently, using the same data and analyses published by the original authors (White et al., 2013; Bello and Renter, 2018). Reproducible is different from replicable, robust, and generalizable (Fig. 1; Culina et al., 2020). Reproducibility can be understood at computer-science level (codes, software, hardware, etc.), empirical-level (detailed information about the experiments and observations allowing transparent data acquisition and availability) and statistical-level (detailed information about the statistical analyses, threshold values of detection and statistical power of the tests as well as the parameters of the models) (The Turing Way Community et al., 2019). In this paper, the three levels of reproducibility mentioned above were analyzed, therefore, both raw data processed for analysis and the codes are available in the Appendix B and C. In addition, for an interactive version available online, all data and codes are hosted in a public repository on the GitHub platform (https://github.com/NAlcan/Reply_BC2021).

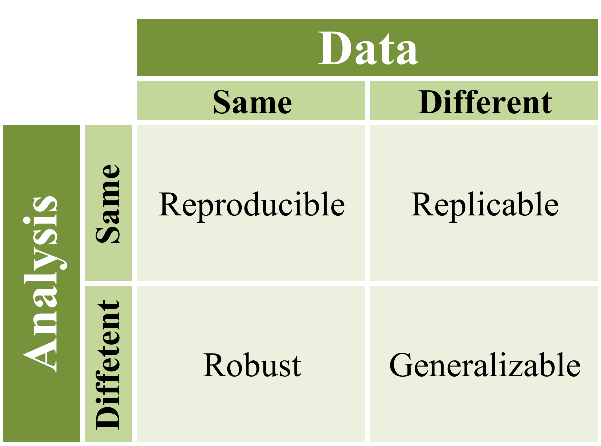


Fig. 1. Dimensions of reproducible research in science. Diagram redrawn and modified from The Turing Way Community et al. (2019).

2.2. Recreation of the database used by BC2021

All the steps followed to recreate the database used by BC2021 are detailed below.

2.2.1. Site selection

The OAN (*Observatorio Ambiental Nacional*; Ministerio de Ambiente, 2021; https://www.ambiente.gub.uy/oan/) is an environmental data repository from the Environmental Ministry of Uruguay (formerly National Environmental Directorate - DINAMA) that makes environmental data gathered in the frame of the national monitoring program and other associated institutions, available to scientists and managers. In particular, OAN holds water quality data for the Uruguay and Negro Rivers and associated tributaries. Fig. 2 and Table A1 show the sampling stations within the main rivers and tributaries, where data for the environmental variables used by BC2021 can be retrieved (i.e., Chl-a, alkalinity, water conductivity, total phosphorus, total suspended solids, pH, and water temperature). The data used in this reply were downloaded from the OAN site on the 27th of July 2021.

The selected data by BC2021 for the Negro River were collected between 2009-05-01 and 2018-11-30 and for the Uruguay River between 2014-06-01 and 2018-11-31. However, the study did not specify the

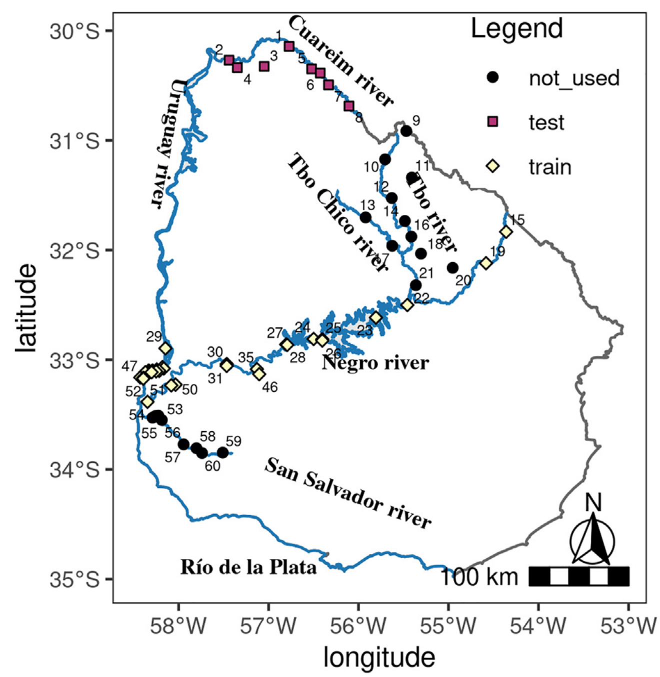


Fig. 2. Map of the OAN monitoring stations showing the data used by BC2021 (Negro River, Uruguay River, and Cuareim River) and other available data not used by BC2021 (i.e, San Salvador River, Tacuarembó (Tbo) River, and Tacuarembó (Tbo) Chico River, in black). The colors represent different aspects of data handling by BC2021, i.e., the training of the models (yellow, Negro River and Uruguay River, respectively) and validation of the models (violet points, Cuareim River). Site code: 1 = RC50, 2 = RC60, 3 = RC3C70, 4 = RCYU80, 5 = RC40, 6 = RC35, 7 = RC20, 8 = RC10, 9 = CU1, 10 = TG1, 11 = CU2, 12 = TG2, 13 = TCH010, 14 = CU3, 15 = RN0, 16 = TG3, 17 = TCH020, 18 = YA1, 19 = RN1, 20 = CA1, 21 = TG4, 22 = RN2, 23 = RN3, 24 = RN7, 25 = RN5, 26 = RN6, 27 = RN10, 28 = RN9, 29 = RU0, 30 = RN14, 31 = RN13, 32 = RU1, 33 = RU2, 34 = RU4, 35 = RN11, 36 = RU7, 37 = RU12, 38 = RU5, 39 = RU11, 40 = RU3, 41 = RU8, 42 = RU6, 43 = RU10, 44 = RU13, 45 = RU9, 46 = RN12, 47 = RU15, 48 = RU16, 49 = RU14, 50 = RN15, 51 = RN16, 52 = RN17, 53 = SS6, 54 = SS6.5, 55 = SS7, 56 = SS5, 57 = SS4, 58 = SS3, 59 = SS1, 60 = SS2. For further detailed information, see Table A1. https://github.com/NAlcan/Reply_BC2021/blob/master/6.Interactive_code_files/MapofSamplingStations.md#published-map.

temporal window used to validate the model for the Cuareim River data. The database used by us to reproduce the analyses in this article, includes all sites from Negro River and Uruguay River with Chl-a data available in the OAN which were all specified in Table 1 from BC2021 (Appendix B and Table A1).

In our case, data from the Cuareim River are only used for plotting Fig. 4 and Table A3, but not for other remaining analysis, as BC2021 used the Cuareim River data only for validating the neural network (Neural Net Simulation; NNS).

2.2.2. Variables selection

The database used to reproduce the analyses in this article included the same variables as reported by BC2021 (Appendix B).

2.2.3. Data cleaning

We exactly followed the criteria explicitly reported by BC2021 for the database curation. In cases where the criteria were not reported, we made some decisions: when the data-base presented special characters (i.e., <LD, below limit detection), we opted to replace them (i.e., NA, not available). Since there was no definition of outliers in BC2021 for the environmental variables, we adopted the same criteria used in BC2021 for Chl-a, which implied a removal of the values higher than the 99.5% percentile. Outliers were estimated using pooled data from the Negro River and Uruguay River.

2.3. Analysis of river comparisons

In order to compare the differences in the distribution of environmental variables between the Uruguay and Negro River, in this reply we performed a likelihood ratio test. We fitted maximum likelihood models with a different structure from the following general model (Bolker et al., 2009):

$$y = a + b_i\, x + \varepsilon$$

$$\varepsilon \approx N\left(0, e_i\, \sigma^2\right)$$

where a is a common intercept, b_i is the coefficient for the groups' (i) mean, ε are the residuals which follows a Normal (N) distribution with mean 0 and variance σ^2, and e_i is a coefficient for the changes in variance. Three models were fitted:

I. mean varying for each group (Uruguay River and Negro River) and equal variance (b_i, $e_i = 1$),
II. equal mean and different variances for each group ($b_i = 0$, e_i),
III. different mean and variance for each group (b_i, e_i).

We then evaluated the log-likelihood ratio between models *I* and *III* to inspect the differences of variance among groups and between models *II* and *III* to evaluate differences in mean values among groups. Under the null hypothesis, the log-likelihood ratio follows a *Chi square* distribution (Zuur et al., 2009).

All data analyses, visualizations and models were performed in R and RStudio statistical programming environment (R Core Team, 2021; R Studio Team, 2021). Data manipulation and graphics were constructed using the meta package "tidyverse" (Wickham et al., 2019a) and other supplementary packages for plot assembly, dates and hours manipulation, data cleaning, etc. (Grolemund and Wickham, 2011; Neuwirth, 2014; Wickham et al., 2019b; Pedersen, 2020; Firke et al., 2021). Finally, maximum likelihood models were fitted with *gls* function in the "nlme" package (Pinheiro et al., 2021).

Table 1
Data considered as outliers following the 99.5% criteria for Chl-a. The four upper rows are the outliers detected in both, this paper and BC2021, while the fifth bottom row reported in italics shows an outlier as reported by BC2021, which does not fulfill the 99.5% criteria. https://github.com/NAlcan/Reply_BC2021/blob/master/6.Interactive_code_files/Data_AnalysisVisualization.md#995-percentile-limits-for-chl-a.

Sampling site	Date	Chl-a (μg L^{-1})
RN5	2012-01-19	72.5
RN6	2012-08-15	179.0
RN3	2012-12-05	276.0
RN5	2012-12-06	192.0
RN12	***2018–04–17***	***55.0***

3. Results

3.1. Recreation of the database used by BC2021

3.1.1. Data cleaning

The database used by BC2021 comes from a public environmental data repository, but the steps followed to compile it (i.e., data curation), were not clearly explained in order to be reproduced. There are some gaps in the database that should be considered to perform appropriate exploratory and statistical analyses. For example, the original dataset downloaded from the OAN website contains special characters such as "<LD" or "<LC" or "LD < x < LC", which indicate values that are below (<) or between (LD < x < LC) the detection limit (LD) and quantification limit (LC) of each analytical method. There is no explanation about how such values were treated. It is unknown whether such values were either replaced or eliminated, or if the value or the complete observation were eliminated, or if the same criteria for both explanatory and response variables were utilized. In our analysis, the amount of data below LD or LC was 21% for total suspended solids and 12% for the response variable Chl-a for the Uruguay River and Negro River (Table A2). If the percentage of data below the quantification threshold is over 25%, biased information leads to model miss-specification and ulterior misinterpretations (Newman et al., 1989; Croghan and Egeghy, 2003). In our analysis, we opted to replace all special values with NA (i.e., not available). After removing such observations, the total amount of data was $n = 465$ for the Negro River and $n = 372$ for the Uruguay River (https://github.com/NAlcan/Reply_BC2021/blob/master/6.Interactive_code_files/Data_integration_md.md#how-many-data-per-river-is-available).

3.1.2. Removal of outliers

3.1.2.1. Response variable (Chl-a). BC2021 reported that the criterion utilized to remove outliers was "*any measures of Chl-a higher than the 99.5% percentile were regarded as outliers and excluded*". However, it is not clear whether the 99.5% percentile was calculated for all sites together or for each single river or sampling site. BC2021 defined as outliers five data points, i.e., RN3 (station 23 in Fig. 2) in 2012-12-05, RN5 (station 25 in Fig. 2) twice for both 2012-12-06 and 2012-01-19, RN6 (station 26 in Fig. 2) in 2012-08-15, and RN12 (station 46 in Fig. 2) in 2018–04–17. However, using the same definition as BC2021 for the Negro and Uruguay River together, we detected only four outliers: RN3 (2012-12-05), RN5 (2012-01-09 and 2012-12-06) and RN6 (2012-08-15) (Table 1), with a Chl-a threshold value of the 99.5% percentile, estimated to be 70.2 μg L^{-1}. When outlier estimation was performed separately for each river (i.e., Uruguay and Negro River), only two data points were eliminated for the Negro River (threshold = 181.4 μg L^{-1}) and two data points for the Uruguay River (threshold = 20.0 μg L^{-1}) (Table A3). Finally, it must be pointed out that four out of the five outliers detected by BC2021 correspond to the artificial reservoirs located at the Negro River (station 18; 25; 26 in Fig. 2, and Table 1).

3.1.2.2. Environmental variables. BC2021 did not describe how explanatory variables were treated. For example, in the data downloaded from the OAN a wrong water temperature value of 257° C is recorded (RU14, 2014–12-17). We followed the same criteria used for Chl-a and replaced with "NA" all the values from the environmental variables higher than the 99.5% percentile, and hence, we further removed 26 other values considered as outliers (Fig. A1). The threshold values estimated for each environmental variable were as follows: alkalinity = 100 mg L^{-1}, conductivity = 215 µS cm^{-1}, total phosphorus = 280 µg L^{-1}, total suspended solids = 81.9 mg L^{-1}, pH = 8.92, and water temperature = 29.8 °C.

3.2. Chl-a versus environmental variables

BC2021 reported biplots of environmental variables and Chl-a (see Fig. 3 in BC2021). In order to facilitate visual comparison of Fig. 3 in BC2021 and Fig. 3 presented in this paper, we have scanned BC2021-biplots and compare them case-by-case to our results using prime letters (Fig. 3). First, we detected distinct inconsistencies and differences in the distributions (Fig. 3). The scatterplots of Chl-a with explanatory variables without further treatment (i.e. removal of extreme values) are shown in Fig. A1. Second, we detected that some data were not included in the BC2021 dataset. All pH values reported by BC2021 are lower than 7.5, but pH values in our dataset were even higher than 8 and reached a maximum of 8.88 (Fig. 3 d and d'). This is particularly relevant as pH was suggested by BC2021 to be one of the "*Relevant factors in the eutrophication of the Uruguay River and the Río Negro*".

3.3. Differences in the abiotic variables between rivers

The environmental variables used in this work showed significant differences in mean and variance between the Uruguay River and Negro River, except for water temperature and $\log_{10}$ normalized Chl-a that showed significant differences in the mean but not in the variance (Fig. 4 and Table A4).

4. Discussion

The statistical analysis of ecological data represents a challenge from the numerical point of view for detecting outliers, assessing correlation between explicative variables, nonlinear relationships among variables, occurrence of many zero observations, and spatial and temporal correlations (Zuur et al., 2010; White et al., 2013). There are various protocols to ensure the correct communication of data collection and preparation process, statistical analysis and validation of models, which all together ultimately conform the quality and credibility of the information (Zuur et al., 2010; Zuur and Ieno, 2016). Good practices for opening and documenting any investigation cycle add value to the data, ensuring their integrity and reproducibility in a responsible manner (White et al., 2013; Bello and Renter, 2018). In this article, it was not possible to consistently reproduce the results published by BC2021 due to information gaps in the procedures. This is especially relevant as the database in question is publicly available in a national repository. We show here that data selection and the treatment of outliers and missing data in BC2021 was somehow biased and/or unclear. A noticeable example is the conclusion that pH is one of the main environmental variables modulating Chl-a concentration. Specifically, we showed BC2021 did not include almost 40% of the high pH observations, but the reason behind is unkonwn for the readers. Such an arbitrary and ill-defined method of data removal is clearly needed to reach the wrong conclusions about the causes and consequences of limnological process in rivers.

BC2021 relied on a correlative approach (artificial neural networks) to predict the Chl-a concentration (i.e., effect) based on some selected environmental variables (i.e., potential causes). Even under a correct use of the data, correlative models do not allow to define cause and effect mechanisms (Matthews, 2000; Messerli, 2012; Velickovic, 2015). Under this analysis and result interpretations, BC2021 proposed that the cause of the increased Chl-a levels is related to changes in pH and water temperature, but at the same time, such an increase in Chl-a levels is not related to agriculture and anthropogenic eutrophication. Below, we briefly discuss some well-known paradigms of river ecology to show that the conclusions reported by BC2021, are neither sustained within the current limnological paradigms, nor there is enough scientific evidence to support them.

The OAN database for both Uruguay River and Negro River basin contains nearly 100 water quality variables (e.g., physico-chemical, microbiological, pesticides) (Ministerio de Ambiente, 2021). BC2021 selected only six variables without clearly pre-defined criteria (i.e., Chl-a, alkalinity, water conductivity, total phosphorus, total suspended solids, pH, and water temperature), but excluded other classical important limnological variables, such as total nitrogen concentration, or river flow and/or rainfall, which are particularly relevant for studies in lotic systems. The sites used to train and test the models in BC2021 exhibited systematic differences in their environmental conditions. To train the models, BC2021 used data sampled downstream of the hydroelectric reservoir (Salto Grande dam) for only a very short section of the Uruguay River. However, in the case of Negro River, data were selected from samples in the lotic course of the river, but also from the three major reservoirs (i.e., Bonete, Baygorria, and Palmar Reservoirs, Fig. 2). BC2021 used Cuareim River data to test the models. However, other small rivers in the basin of the Negro and Uruguay Rivers (e.g., San Salvador and Tacuarembó rivers), which are available in the OAN database, were not used by BC2021.

Cuareim River displays different dynamics and drivers, being inadequate to compare it with Negro River and Uruguay River. Moreover, the Cuareim River exhibits high flow peaks after heavy rains that are quickly discharged into the Uruguay River, but also, in dry summers the main channel often dries up and disappears intermittently (DINAMA, 2011). This hydrological regime is extremely different from the dynamics of the Negro and Uruguay River, whose hydrological regimes are largely governed by the management of dams to fulfill the country's hydroelectric demands and other economic and cultural uses (DINAMA, 2018). In turn, these differences exert direct consequences on nutrient cycling and therefore on water chemistry (Yeom et al., 2019).

Spurious correlations between conductivity and Chl-a levels arise because of the choice of data sets to train the models. The Negro River holds naturally much higher water conductivity values than those of the Uruguay River (Fig. 4), because this river flows over Cretaceous basalts, i.e., the so-called Basalt Basin of the Geologic Chart of Uruguay (Bossi et al., 1998), while Negro River flows over a sandy sedimentary basin formed during Late Quaternary. The exclusion of Uruguay River reservoir (with high Chl-a values and low water conductivity; Conde et al., 1996; Chalar et al., 2002; Kruk et al., 2015) and the inclusion of Negro River reservoirs (whit high Chl-a values and high water conductivity; Chalar et al., 2014) likely led BC2021 to suggest a significant correlation between these variables. The inclusion of data available from other sources (e.g., Administrative Commission of the Uruguay River) from the Salto Grande Reservoir containing high Chl-a values and low water conductivity values would surely modify this type of association. In this sense, extensive monthly water chemistry data taken from December 2016 to November 2018 in sites located throughout the 500-km-long Uruguay River including both upstream and downstream sections of Salto Grande Reservoir are available at the web site of the Administrative Commission of the Uruguay River (Comité Científico CARU, 2019a, 2019b, www.CARU.org.uy).

Rivers and reservoirs display differential morphometry, ecosystem structure and functioning. Reservoirs normally exhibit conditions of higher depth, residence time, sedimentation rate, and light availability than those of rivers (Chalar, 2009; Tundisi and Tundisi, 2012). Reservoir conditions thus typically promote a higher phytoplankton growth rate and biomass than upstream reaches (Soares et al., 2008; Aubriot et al., 2020; Kruk et al., 2021). On the other hand, rivers may also exhibit high availability of nutrients but the low residence time, turbulent regime, and moderate light intensity due to high inorganic turbidity are all limiting factors for microalgae biomass and bloom formation (Reynolds and Descy, 1996; Ferrari et al., 2011; O'Farrell and Izaguirre, 2014; Somma et al., 2021). In this sense, BC2021 analyzed mixed lentic and lotic data (37 lotic and 6

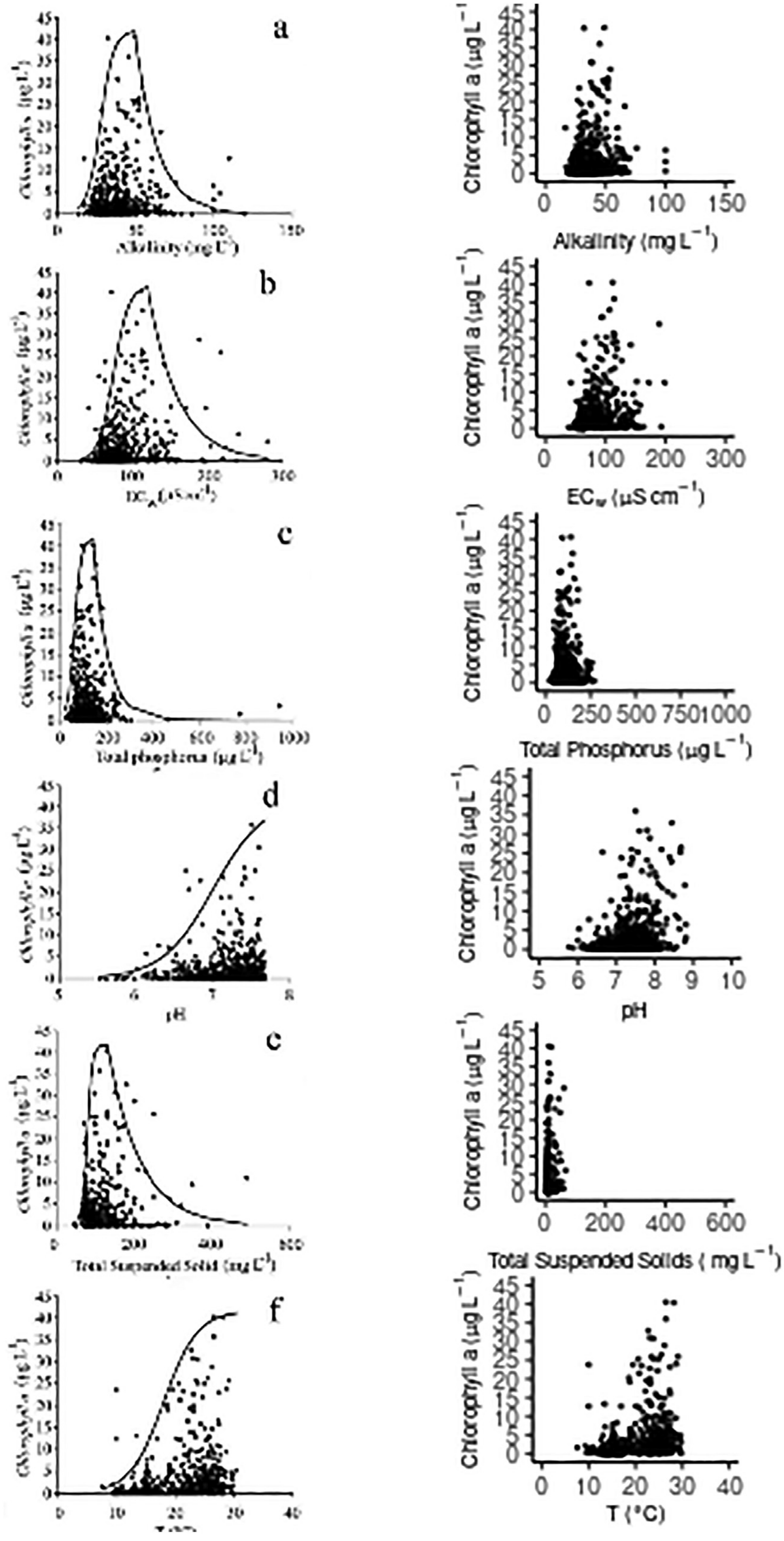


Fig. 3. Biplots of Chl-a versus all environmental variables used: a: alkalinity, b: water conductivity (EC_w), c: total phosphorus, d: pH, e: total suspended solids, f: temperature. In all comparative cases, the graphs scanned from BC2021 are shown to the left (letters) and the graphs of the present paper to the right (prime-letters). To facilitate the visual comparison the axes (x and y) of the biplots we used the same scaling, except for pH (in BC2021 the x axis only attains up to 8), as 58 pH values were removed (d´). The Chl-a value of 55 $\mu g\ L^{-1}$ that BC2021 considered to as an outlier (RN 2018–04–17) is not shown in any of the biplots, because the Chl-a axis ends at 45 $\mu g\ L^{-1}$. https://github.com/NAlcan/Reply_BC2021/blob/master/6.Interactive_code_files/Data_AnalysisVisualization_files/figure-gfm/Figure%203-1.png.

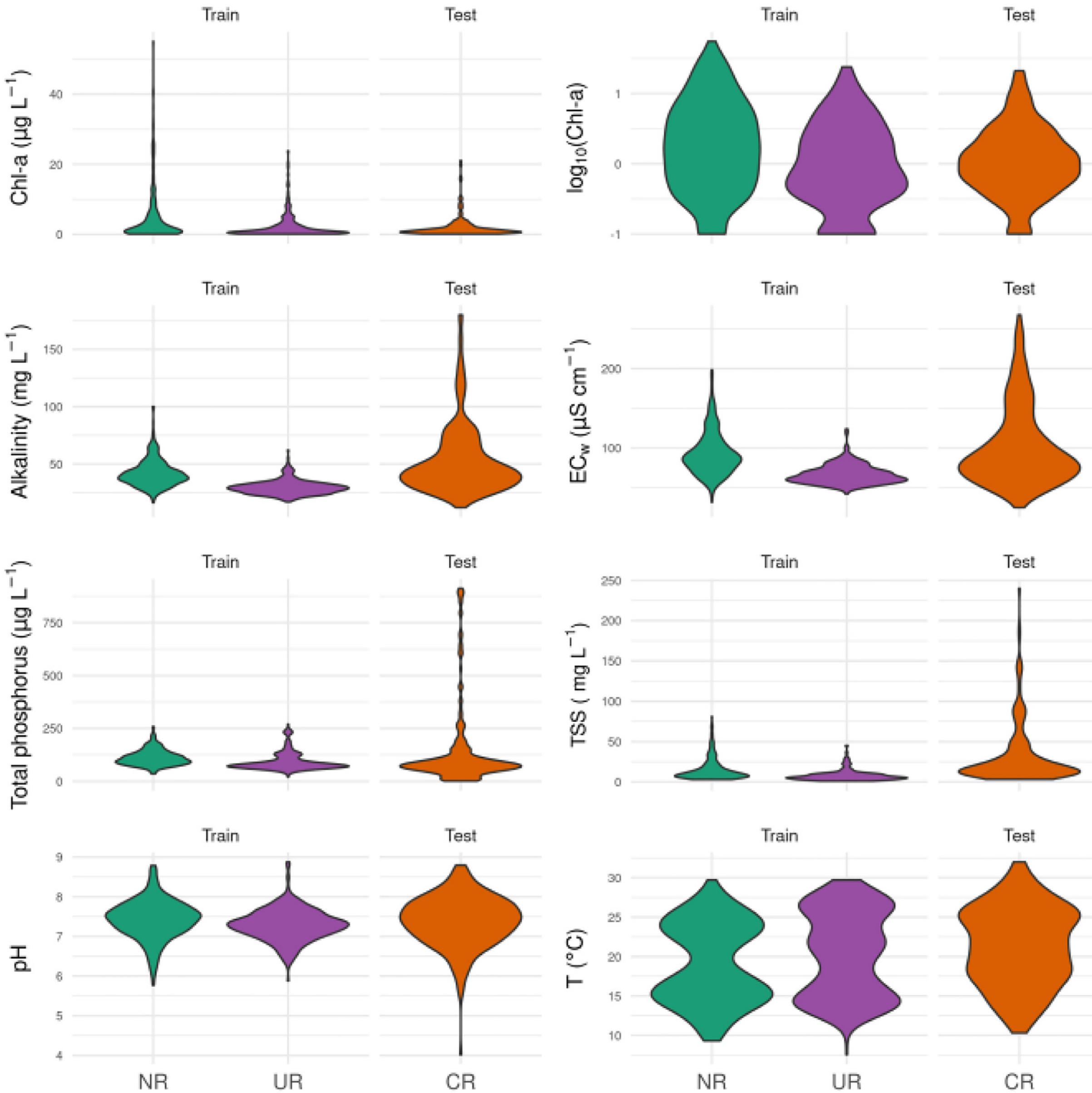


Fig. 4. Violin plots for environmental variables for Negro River (NR, green), Uruguay River (UR, violet), and Cuareim River (CR, orange), respectively. NR and UR data were used to train the model and data from CR to test model performance in BC2021. UR and NR exhibit significant differences (*p*-values <0.05) in mean and variance for all environmental variables, except for temperature variance (A4). Note that the Cuareim River was subject to the same outlier removal procedure as explained in Section 2.2.3. https://github.com/NAlcan/Reply_BC2021/blob/master/6.Interactive_code_files/Data_AnalysisVisualization_files/figure-gfm/Figure4-1.png.

lentic sites) without considering the major differences in the functioning of these two types of systems. The use of a trophic index designed for lakes (i.e., OCDE, 1982), the partial analysis of the relationship between nutrients and Chl-a, and the lack of river flow and rainfall data, likely affects the results and interpetations. Furthermore, BC2021 compared the findings with those of shallow lentic ecosystems, introducing the predation pressure concept by Cladocera as a main structuring factor of river phytoplankton community. However, this concept developed for shallow lakes (Scheffer, 1998; Scheffer and Jeppesen, 2007), does not hold for rivers.

BC2021 attempted to simulate the TP concentrations in Negro River under land use change simulation to natural prairie (grasslands) by using a Beretta (2019) model. The intepretation of this simulation is very relevant concerning the potential implications for supporting sustainable land use practices or not. BC2021 concluded that "*Thus, changing the entire basin land use to natural prairie would not reduce TP or EC_w to the levels required to produce a significant effect on Chl-a*". BC2021 concludes that HABs in Negro River are not directly related to agricultural fertilization (although slightly to erosion), and therefore, there is no need to change intensive land use trends and practices in the catchment. The basis of such a simulation is unfortunately unknown, since the Beretta (2019) is not a peer reviewed model (poster presentation in the XXII Latinamerican Congress of Soil Sciences, https://www.researchgate.net/publication/336590807_Poster_Impacto_de_la_erosion_en_el_contenido_de_P_en_Rio_Negro). The intepretation of the results yielded by this model contradicts a massive amount of international and local empirical evidence (Goyenola et al., 2015, 2021), and allows BC2021 to conclude that the high TP levels are naturally exported by the river basin. This implication is extremely important for the environmental management to neglect the impacts of agriculture on natural freshwater systems.

BC2021 reported in the Introduction that "increases in pH may promote cyanobacteria growth" and backed this statement up using Gao et al. (2015) and Zhao et al. (2019). Neither Gao et al. (2015) nor Zhao et al.

(2019) documented such an effect of pH on microalgae growth. Gao et al. (2015) did report that "The sensitivity analyses indicated that a positive relationship existed between Chla and pH". This is in fact in most cases true, but the increase in pH is actually a consequence of productivity instead of its cause; and this concept is one of the basic paradigms of microalgae productivity (Margalef, 1983; Schneider and Campion-Alsumard, 1999; Wetzel, 2001; Reynolds, 2006; Stumm and Morgan, 2012). In the same regard, Zhao et al. (2019) simply reported threshold pH levels for microalgae growth. Therefore, the pH misinterpretation of BC2021 reveals a conceptually incorrect selection of the explanatory variables.

BC2021 also claimed that changes in temperature and electric conductivity promote eutrophication. This is also in fact to some extent true, since some of the ions contributing to conductivity can be nutrients available for the aquatic primary producers. Both temperature and electric conductivity might exert a positive effect on microalgae growth, but BC2021 actually implies that the observed aquatic eutrophication is driven by temperature, electric conductivity, and pH. A review of undergraduate limnological textbooks would rapidly show otherwise (Margalef, 1983; Wetzel, 2001; Kalff, 2002; Reynolds, 2006). In stark contrast to the implications of BC2021, since the 1970s the international debates around eutrophication have focused on which nutrient, either phosphorus, nitrogen, or both, should be reduced in the basins to reverse the negative effects of eutrophication, both in lakes (e.g., Conley et al., 2009; Paerl et al., 2016a, 2016b; Schindler et al., 2016) and in rivers (McDowell et al., 2009; Dodds and Smith, 2016). It is actually extremely striking that none of three most important relevant factors of eutrophication identified by BC2021 corresponded to a primary source of energy for vegetal and microalgae growth.

Finally, there is a vast literature published demonstrating the relationship between climate warming, eutrophication and HABs (Moss et al., 2011; Jeppesen et al., 2014). However, in order to fully demonstrate such an association it is necessary to analyze long-term ad hoc databases (Ralston and Moore, 2020), since high temperatures promote the occurrence of HABs (Paerl et al., 2016a; Visser et al., 2016; Savadova et al., 2018) due to the effect of temperature on the metabolism of organisms (Brown et al., 2004), especially cyanobacteria (Reynolds, 2006; Paerl et al., 2011; Paerl and Paul, 2012). It has been specifically observed that the highest biomasses and duration of the blooms in Uruguay are observed mainly in summer, in the reservoirs of both Negro and Uruguay River (Chalar, 2006; O'Farrell and Izaguirre, 2014; Bonilla et al., 2015; González-Piana et al., 2017; Haakonsson et al., 2017; Aubriot et al., 2020; Kruk et al., 2015, 2021). Therefore, the association between HABs and temperature reported by BC2021 is likely attributed to the seasonal effect (De León and Chalar, 2003; Sommer et al., 2012; O'Farrell and Izaguirre, 2014) rather than to climate change. Despite some predictions regarding climate warming indicate that higher temperatures will stimulate the occurrence of HABs (Kosten et al., 2012; Paerl, 2017; Ho et al., 2019; Gobler, 2020), the analysis by BC2021 failed to demonstrate this issue because the seasonal effect was not considered.

5. Conclusions

Based on a biased data selection and a correlative approach, BC2021 concluded that the increase in Chl-a values are explained by pH, conductivity, and water temperature and that there is no relation with anthropogenic eutrophication and particularly with intensive agriculture. We showed that data manipulation and an arbitrary selection of sites and explanatory variables for model training and testing led to the wrong conclusions. However, the most important aspect that should be emphasized is that BC2021 ignores fundamental aspects of the functioning of continental aquatic systems and an ever growing amount of empirical evidence indicating opposite conclusions. BC2021 mixed information from reservoirs and rivers, confused response variables with explanatory variables, and based all conclusions on a model that has not been peer-reviewed, which neglects fundamental relationships between water chemistry and land use. In a nutshell, BC2021 promotes a misconception for sustainable environmental management practices of land use, with potentially very serious consequences for the present and future of water quality and aquatic biodiversity. In addition, it can generate erroneous policies that could impact on the water quality of Uruguay aquatic ecosystems.

Funding sources

No funding declared.

CRediT authorship contribution statement

I. Alcántara: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **A. Somma:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **G. Chalar:** Conceptualization, Validation, Investigation, Writing – original draft, Writing – review & editing. **A. Fabre:** Conceptualization, Validation, Investigation, Writing – original draft, Writing – review & editing. **A. Segura:** Conceptualization, Validation, Investigation, Writing – original draft, Writing – review & editing. **M. Achkar:** Writing – review & editing. **R. Arocena:** Writing – review & editing. **L. Aubriot:** Conceptualization, Investigation, Writing – review & editing. **C. Baladán:** Writing – review & editing. **M. Barrios:** Writing – review & editing. **S. Bonilla:** Writing – review & editing. **M. Burwood:** Writing – review & editing. **D.L. Calliari:** Writing – review & editing. **C. Calvo:** Writing – review & editing. **L. Capurro:** Writing – review & editing. **C. Carballo:** Writing – review & editing. **C. Céspedes-Payret:** Writing – review & editing. **D. Conde:** Conceptualization, Investigation, Writing – review & editing. **N. Corrales:** Writing – review & editing. **B. Cremella:** Writing – review & editing. **C. Crisci:** Writing – review & editing. **J. Cuevas:** Writing – review & editing. **S. De Giacomi:** Writing – review & editing. **L. De León:** Writing – review & editing. **L. Delbene:** Writing – review & editing. **I. Díaz:** Writing – review & editing. **V. Fleitas:** Writing – review & editing. **I. González-Bergonzoni:** Conceptualization, Investigation, Writing – review & editing. **L. González-Madina:** Writing – review & editing. **M. González-Piana:** Writing – review & editing. **G. Goyenola:** Writing – review & editing. **O. Gutiérrez:** Writing – review & editing. **S. Haakonsson:** Writing – review & editing. **C. Iglesias:** Writing – review & editing. **C. Kruk:** Writing – review & editing. **G. Lacerot:** Writing – review & editing. **J. Langone:** Writing – review & editing. **F. Lepillanca:** Writing – review & editing. **C. Lucas:** Writing – review & editing. **F. Martigani:** Writing – review & editing. **G. Martínez de la Escalera:** Writing – review & editing. **M. Meerhoff:** Conceptualization, Investigation, Writing – review & editing. **L. Nogueira:** Writing – review & editing. **H. Olano:** Writing – review & editing. **J.P. Pacheco:** Writing – review & editing. **D. Panario:** Writing – review & editing. **C. Piccini:** Writing – review & editing. **F. Quintans:** Writing – review & editing. **F. Teixeira de Mello:** Writing – review & editing. **L. Terradas:** Writing – review & editing. **G. Tesitore:** Writing – review & editing. **L. Vidal:** Writing – review & editing. **F. García-Rodríguez:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: No competing financial interest. On behalf of all authors Ignacio Alcántara and Felipe García-Rodríguez.

Acknowledgments

Authors acknowledge to the Ministry of Environment (Uruguay) for the available OAN database.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.151854.

References

Abdulkareem, J.H., Girei, A.H., Yamusa, A.M., Abdullahi, J., 2021. An overview of soil erosion modelling. J. Res. For. Wildl. Environ. 13, 206–215.
Allan, J.D., 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annu. Rev. Ecol. Evol. Syst. 35, 257–284. https://doi.org/10.1146/annurev.ecolsys.35.120202.110122.
Altieri, M.A., Nicholls, C.I., 2001. Ecological impacts of modern agriculture in the United States and Latin America. In: Solbrig, O.T., Paarlber, R., Di Castri, F. (Eds.), Globalization and the Rural Environment. Harvard Universty, pp. 121–137.
Aubriot, L., Delbene, L., Haakonsson, S., Somma, A., Hirsch, F., Bonilla, S., 2017. Evolution of eutrophication in santa Lucia river: influence of land use intensification and perspectives. INNOTEC 7–16. https://doi.org/10.26461/14.04.
Aubriot, L., Zabaleta, B., Bordet, F., Sienra, D., Risso, J., Achkar, M., Somma, A., 2020. Assessing the origin of a massive cyanobacterial bloom in the Río de la Plata (2019): towards an early warning system. Water Res. 181, 115944. https://doi.org/10.1016/j.watres.2020.115944.
Bello, N.M., Renter, D.G., 2018. Invited review: reproducible research from noisy data: revisiting key statistical principles for the animal sciences. J. Dairy Sci. 101, 5679–5701. https://doi.org/10.3168/jds.2017-13978.
Beretta, A., 2019. Poster Impacto de la erosión en el contenido de P en Río Negro. XXII Congreso Latinoamericano de la Ciencia del Suelo, 2do Congreso Uruguayo de Suelos.
Beretta-Blanco, A., Carrasco-Letelier, L., 2021. Relevant factors in the eutrophication of the Uruguay River and the Río Negro. Sci. Total Environ. 761, 143299. https://doi.org/10.1016/j.scitotenv.2020.143299.
Bhagowati, B., Ahamad, K.U., 2019. A review on lake eutrophication dynamics and recent developments in lake modeling. Ecohydrol. Hydrobiol. 19, 155–166. https://doi.org/10.1016/j.ecohyd.2018.03.002.
Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.-S.S., 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24, 127–135. https://doi.org/10.1016/j.tree.2008.10.008.
Bonilla, S., Haakonsson, S., Somma, A., Gravier, A., Britos, A., Vidal, L., De León, L., Brena, B., Pírez, M., Piccini, C., Martínez de la Escalera, G., Chalar, G., González Piana, M., Martigani, F., Aubriot, L., 2015. Cyanobacteria and cyanotoxins in freshwaters of Uruguay. INNOTEC 9–22.
Bordet, F., Fontanarrosa, M.S., O'Farrell, I., 2017. Influence of light and mixing regime on bloom-forming phytoplankton in a subtropical reservoir. River Res. Appl. 33, 1315–1326. https://doi.org/10.1002/rra.3189.
Borrelli, P., Robinson, D.A., Panagos, P., Lugato, E., Yang, J.E., Alewell, C., Wuepper, D., Montanarella, D., Ballabio, C., 2020. Land use and climate change impacts on global soil erosion by water (2015–2070). PNAS 117, 21994–22001. https://doi.org/10.1073/pnas.2001403117.
Bossi, J., Campal Gennari, N., Ferrando, L.A., Gancio, F., Montaña, J.R., Morales, H.L., Piñeyro, D., Schipilov, A., Sprechmann Heidenreich, P.W., 1998. Carta geológica del Uruguay: a escala 1/500.000. Ministerio de Ganadería y Agricultura, Montevideo, Uruguay.
Bowling, L.C., Merrick, C., Swann, J., Green, D., Smith, G., Neilan, B.A., 2013. Effects of hydrology and river management on the distribution, abundance and persistence of cyanobacterial blooms in the Murray River, Australia. Harmful Algae 30, 27–36. https://doi.org/10.1016/j.hal.2013.08.002.
Brasil, J., Attayde, J.L., Vasconcelos, F.R., Dantas, D.D.F., Huszar, V.L.M., 2016. Drought-induced water-level reduction favors cyanobacteria blooms in tropical shallow lakes. Hydrobiologia 770, 145–164. https://doi.org/10.1007/s10750-015-2578-5.
Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a metabolic theory of ecology. Ecology 85, 1771–1789. https://doi.org/10.1890/03-9000.
Bueno, C., Alves, F.L., Pinheiro, L.M., Perez, L., Agostini, V.O., Fernandes, E.H.L., Möller, O.O., Weschenfelder, J., Pinho, G.L.L., Wallner-Kersanach, M., Moura, R.R., Durán, J.M., Etchevers, I., Costa, L.D.F., Werlang, C.C., Bortolin, E., Machado, E., Figueira, R.C.L., Ferreira, P.A.L., Andrade, C., Fornaro, L., García-Rodríguez, F., 2021. The effect of agricultural intensification and water-locking on the world's largest coastal lagoonal system. Sci. Total Environ. 801, 149664. https://doi.org/10.1016/j.scitotenv.2021.149664.
Button, K.S., Ioannidis, J.P.A., Mokrysz, C., Nosek, B.A., Flint, J., Robinson, E.S.J., Munafò, M.R., 2013. Power failure: why small sample size undermines the reliability of neuroscience. Nat. Rev. Neurosci. 14, 365–376. https://doi.org/10.1038/nrn3475.
Carey, C.C., Ibelings, B.W., Hoffmann, E.P., Hamilton, D.P., Brookes, J.D., 2012. Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. Water Res. 46, 1394–1407. https://doi.org/10.1016/j.watres.2011.12.016.
Chalar, G., 2006. Eutrophication dynamics on different temporary scales: Salto Grande Reservoir (Argentina -Uruguay). In: Tundisi, J., Tundisi-Matsumura, T., Sidagis, T. (Eds.), Eutrofização Na América Do Sul: Causas, Conseqüências e Tecnologias Para Gerenciamento e Controle. Instituto Internacional de Ecologia–IIE, São Paulo.
Chalar, G., 2009. The use of phytoplankton patterns of diversity for algal bloom management. Limnologica 39, 200–208. https://doi.org/10.1016/j.limno.2008.04.001.
Chalar, G., de León, L., Brugnoli, E., Clemente, J., Paradiso, M., 2002. Antecedentes y nuevos aportes al conocimiento de la estructura y dinámica del Embalse Salto Grande. El agua en Iberoamérica, de la limnología a la gestión en Sudamérica. El Agua En Sudamérica: De La Limnología a La Gestión En Sudamérica. CYTED Aprovechamiento y Gestión de los Recursos Hídricos, Buenos Aires, Argentina, pp. 123–142.
Chalar, G., Gerhard, M., González Piana, M., Fabián, D., 2014. Hidroquímica y eutrofización en tres embalses subtropicales en cadena (Uruguay). In: Marcovecchio, J.E., Botté, S.E., Freije, R.H. (Eds.), Procesos Geoquímicos Superficiales En Iberoamérica. Red Iberoamericana de Física y Qúimica Ambiental, pp. 121–149.
Chalar, G., Garcia-Pesenti, P., Silva-Pablo, M., Perdomo, C., Olivero, V., Arocena, R., 2017. Weighting the impacts to stream water quality in small basins devoted to forage crops, dairy and beef cow production. Limnologica 65, 76–84. https://doi.org/10.1016/j.limno.2017.06.002.
Comité Científico CARU, 2019a. Plan de monitoreo integral del Río Uruguay para todo el tramo compartido. Subprograma 1. Monitoreo de la calidad de agua, sedimento y biota en el Río Uruguay. Informe primeros seis meses de monitoreo. CARU.
Comité Científico CARU, 2019b. Plan de monitoreo inicial del Río Uruguay. Informe final. Diciembre 2016 a Diciembre 2017. CARU.
Conde, D., Pintos, W., Gorga, J., De Leon, R., Chalar, G., Sommaruga, R., 1996. The main factors inducing chemical spatial heterogeneity in the Salto Grande, a reservoir on the Uruguay River. Large Rivers 571–578. https://doi.org/10.1127/lr/10/1996/571.
Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E., Lancelot, C., Likens, G.E., 2009. Controlling eutrophication: nitrogen and phosphorus. Science 323, 1014–1015. https://doi.org/10.1126/science.1167755.
Croghan, C., Egeghy, P.P., 2003. Methods of dealing with values below the limit of detection using SAS. Southern SAS User Group. 22, p. 24.
Culina, A., van den Berg, I., Evans, S., Sánchez-Tójar, A., 2020. Low availability of code in ecology: a call for urgent action. PLoS Biol. 18, e3000763. https://doi.org/10.1371/journal.pbio.3000763.
De León, L., Chalar, G., 2003. Abundancia y diversidad del fitoplancton en el Embalse de Salto Grande (Argentina-Uruguay). Ciclo estacional y distribución espacial. Limnetica 22, 103–113.
DINAMA, 2011. Evaluación de la calidad del agua del Río Cuareim (período 2006 a 2010).
DINAMA, 2018. Informe Calidad Ambiental del Río Negro, 2009 - 2017.
Dodds, W.K., Smith, V.H., 2016. Nitrogen, phosphorus, and eutrophication in streams. Inland Waters 6, 155–164. https://doi.org/10.5268/IW-6.2.909.
Dodds, W.K., Bouska, W.W., Eitzmann, J.L., Pilger, T.J., Pitts, K.L., Riley, A.J., Schloesser, J.T., Thornbrugh, D.J., 2009. Eutrophication of U.S. Freshwaters: analysis of potential economic damages. Environ. Sci. Technol. 43, 12–19. https://doi.org/10.1021/es801217q.
Favis-Mortlock, D., Boardman, J., MacMillan, V., 2001. The limits of erosion modeling: why we should proceed with care. In: Harmon, R.S., Doe, W.W. (Eds.), Landscape Erosion and Evolution Modeling. Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-0575-4_16.
Ferrari, G., Pérez, M.de C., Dabezies, M., Miguez, D., Saizar, C., 2011. Planktic cyanobacteria in the lower Uruguay River, South America. Fottea 11, 225–234.
Firke, S., Denney, B., Haid, C., Knight, R., Grosser, M., Zadra, J., 2021. janitor: Simple Tools for Examining and Cleaning Dirty Data.
Gao, H., Qian, X., Zhang, R., Ye, R., Liu, Z., Qian, Y., 2015. Bayesian regularized back-propagation neural network model for chlorophyll-a prediction: a case study in Meiliang Bay, Lake taihu. Environ. Eng. Sci. 32, 938–947. https://doi.org/10.1089/ees.2015.0164.
Gazzano, I., Achkar, M., Díaz, I., 2019. Agricultural transformations in the Southern cone of Latin America: agricultural intensification and decrease of the aboveground net primary production, Uruguay's case. Sustainability 11, 7011. https://doi.org/10.3390/su11247011.
Glendell, M., Granger, S.J., Bol, R., Brazie, R.E., 2014. Quantifying the spatial variability of soil physical and chemical properties in relation to mitigation of diffuse water pollution. Geoderma 214–215, 25–41. https://doi.org/10.1016/j.geoderma.2013.10.008.
Gobler, C.J., 2020. Climate change and harmful algal blooms: insights and perspective. Harmful Algae 91, 101731. https://doi.org/10.1016/j.hal.2019.101731.
González-Piana, M., Fabián, D., Piccardo, A., Chalar, G., 2017. Dynamics of total microcystin LR concentration in three subtropical hydroelectric generation reservoirs in Uruguay, South America. Bull. Environ. Contam. Toxicol. 99, 488–492. https://doi.org/10.1007/s00128-017-2158-7.
Goyenola, G., Meerhoff, M., Teixeira-de Mello, F., González-Bergonzoni, I., Graeber, D., Fosalba, C., Vidal, N., Mazzeo, N., Ovesen, N.B., Jeppesen, E., Kronvang, B., 2015. Phosphorus dynamics in lowland streams as a response to climatic, hydrological and agricultural land use gradients. Hydrol. Earth Syst. Sci. Discuss. 12, 3349–3390. https://doi.org/10.5194/hessd-12-3349-2015.
Goyenola, G., Graeber, D., Meerhoff, M., Jeppesen, E., Teixeira-de Mello, F., Vidal, N., Fosalba, C., Ovesen, N.B., Gelbrecht, J., Mazzeo, N., Kronvang, B., 2020. Influence of farming intensity and climate on lowland stream nitrogen. Water 12, 1021. https://doi.org/10.3390/w12041021.
Goyenola, G., Kruk, C., Mazzeo, N., Nario, A., Perdomo, C., Piccini, C., Meerhoff, M., 2021. Production, nutrients, eutrophication and cyanobacteria blooms in Uruguay: putting puzzle pieces together. INNOTEC. https://doi.org/10.26461/22.02 e558–e558.
Grolemund, G., Wickham, H., 2011. Dates and times made easy with lubridate. J. Stat. Softw. 40, 1–25. https://doi.org/10.18637/jss.v040.i03.
Haakonsson, S., Rodríguez-Gallego, L., Somma, A., Bonilla, S., 2017. Temperature and precipitation shape the distribution of harmful cyanobacteria in subtropical lotic and lentic ecosystems. Sci. Total Environ. 609, 1132–1139. https://doi.org/10.1016/j.scitotenv.2017.07.067.
Heisler, J., Glibert, P.M., Burkholder, J.M., Anderson, D.M., Cochlan, W., Dennison, W.C., Dortch, Q., Gobler, C.J., Heil, C.A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H.G., Sellner, K., Stockwell, D.A., Stoecker, D.K., Suddleson, M., 2008. Eutrophication and harmful algal blooms: a scientific consensus. Harmful Algae 8, 3–13. https://doi.org/10.1016/j.hal.2008.08.006.
Hilborn, R., Mangel, M., 2013. The Ecological Detective: Confronting Models With Data. Princeton University Press. https://doi.org/10.1515/9781400847310.

Ho, J.C., Michalak, A.M., Pahlevan, N., 2019. Widespread global increase in intense lake phytoplankton blooms since the 1980s. Nature 574, 667–670. https://doi.org/10.1038/s41586-019-1648-7.

Hu, F., Liu, J., Xu, Ch., Du, W., Yang, Z., Liu, X., Liu, G., Zhao, S., 2018. Soil internal forces contribute more than raindrop impact force to rainfall splash erosion. Geoderma 330, 91–98. https://doi.org/10.1016/j.geoderma.2018.05.031.

Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M.H., Visser, P.M., 2018. Cyanobacterial blooms. Nat. Rev. Microbiol. 16, 471–483. https://doi.org/10.1038/s41579-018-0040-1.

Ibelings, B.W., Kurmayer, R., Azevedo, S.M.F.O., Wood, S.A., Chorus, I., Welker, M., 2021. Understanding the occurrence of cyanobacteria and cyanotoxins. In: Chorus, I., Welker, M. (Eds.), Toxic Cyanobacteria in Water, Second edition 2021. CRC Press, Boca Raton, pp. 213–294. https://doi.org/10.1201/9781003081449-4.

Janssen, A.B.G., Hilt, S., Kosten, S., de Klein, J.J.M., Paerl, H.W., Van de Waal, D.B., 2021. Shifting states, shifting services: linking regime shifts to changes in ecosystem services of shallow lakes. Freshw. Biol. 66, 1–12. https://doi.org/10.1111/fwb.13582.

Jeppesen, E., Meerhoff, M., Davidson, T.A., Trolle, D., Søndergaard, M., Lauridsen, T.L., Beklioglu, M., Brucet, S., Volta, P., González-Bergonzoni, I., Nielsen, A., 2014. Climate change impacts on lakes: an integrated ecological perspective based on a multi-faceted approach, with special focus on shallow lakes. J. Limnol. 73. https://doi.org/10.4081/jlimnol.2014.844.

Kalff, J., 2002. Limnology: Inland Water Ecosystems. Prentice Hall, Upper Saddle River, NJ.

Kosten, S., Huszar, V.L.M., Bécares, E., Costa, L.S., Donk, E., Hansson, L.-A., Jeppesen, E., Kruk, C., Lacerot, G., Mazzeo, N., Meester, L., Moss, B., Lürling, M., Nõges, T., Romo, S., Scheffer, M., 2012. Warmer climates boost cyanobacterial dominance in shallow lakes. Glob. Chang. Biol. 18, 118–126. https://doi.org/10.1111/j.1365-2486.2011.02488.x.

Kruk, C., Segura, A., Nogueira, L., Carballo, C., Martinez de la Escalera, G., Calliari, D., Ferrari, G., Simoens, M., Cea, J., Alcántara, I., Vico, P., Miguez, D., Piccini, C., 2015. Monitoring tools and early warning system for harmful cyanobacterial blooms: Río Uruguay and Río de la Plata. INNOTEC 23–39.

Kruk, C., Martínez, A., Martínez de la Escalera, G., Trinchin, R., Manta, G., Segura, A.M., Piccini, C., Brena, B., Yannicelli, B., Fabiano, G., Calliari, D., 2021. Rapid freshwater discharge on the coastal ocean as a mean of long distance spreading of an unprecedented toxic cyanobacteria bloom. Sci. Total Environ. 754, 142362. https://doi.org/10.1016/j.scitotenv.2020.142362.

LaRocque, A., 2013. Universal soil loss equation (USLE). In: Bobrowsky, P.T. (Ed.), Encyclopedia of Natural Hazards, Encyclopedia of Earth Sciences Series. Springer Netherlands, Dordrecht. https://doi.org/10.1007/978-1-4020-4399-4_43. pp. 1062–1062.

Leigh, C., Watkinson, A., Burford, M., 2015. Effects of extreme inflows on the water quality and phytoplankton of seven reservoirs in subtropical Australia. IW 5, 240–252. https://doi.org/10.5268/IW-5.3.814.

Li, S., Li, Y., Huang, X., Hu, F., Liu, X., Li, H., 2017. Phosphate fertilizer enhancing soil erosion: effects and mechanisms in a variably charged soil. J. Soils Sediments 18, 863–873. https://doi.org/10.1007/s11368-017-1794-1.

Li, Y., Shang, J., Zhang, C., Zhang, W., Niu, L., Wang, L., Zhang, H., 2021. The role of freshwater eutrophication in greenhouse gas emissions: a review. Sci. Total Environ. 768, 144582. https://doi.org/10.1016/j.scitotenv.2020.144582.

Margalef, R., 1983. Limnologia. Omega, Barcelona.

Martínez de la Escalera, G., Kruk, C., Segura, A.M., Nogueira, L., Alcántara, I., Piccini, C., 2017. Dynamics of toxic genotypes of Microcystis aeruginosa complex (MAC) through a wide freshwater to marine environmental gradient. Harmful Algae 62, 73–83. https://doi.org/10.1016/j.hal.2016.11.012.

Matthews, R., 2000. Storks deliver babies (p = 0.008). Teach. Stat. 22, 36–38. https://doi.org/10.1111/1467-9639.00013.

McDowell, R.W., Larned, S.T., Houlbrooke, D.J., 2009. Nitrogen and phosphorus in New Zealand streams and rivers: control and impact of eutrophication and the influence of land management. N. Z. J. Mar. Freshw. Res. 43, 985–995. https://doi.org/10.1080/00288330909510055.

Messerli, F.H., 2012. Chocolate consumption, cognitive function, and nobel laureates. N. Engl. J. Med. 367, 1562–1564. https://doi.org/10.1056/NEJMon1211064.

Ministerio de Ambiente, 2021. Observatorio Ambiental Nacional [WWW Document]. Observatorio Ambiental l MVOTMA. https://www.ambiente.gub.uy/oan.

Modernel, P., Rossing, W.A.H., Corbeels, M., Dogliotti, S., Picasso, V., Tittonell, P., 2016. Land use change and ecosystem service provision in pampas and Campos grasslands of southern South America. Environ. Res. Lett. 11, 113002. https://doi.org/10.1088/1748-9326/11/11/113002.

Moss, B.R., 2009. Ecology of Fresh Waters: Man and Medium, Past to Future. John Wiley & Sons.

Moss, B., Kosten, S., Meerhoff, M., Battarbee, R.W., Jeppesen, E., Mazzeo, N., Havens, K., Lacerot, G., Liu, Z., De Meester, L., Paerl, H., Scheffer, M., 2011. Allied attack: climate change and eutrophication. Inland Waters 1, 101–105. https://doi.org/10.5268/IW-1.2.359.

Neuwirth, E., 2014. RColorBrewer: ColorBrewer Palettes. R package version 1.1-2. https://CRAN.R-project.org/package=RColorBrewer.

Newman, M.C., Dixon, P.M., Looney, B.B., Pinder, J.E., 1989. Estimating mean and variance for environmental samples with below detection limit observations. J. Am. Water Resour. Assoc. 25, 905–916. https://doi.org/10.1111/j.1752-1688.1989.tb05406.x.

Nixon, S.W., 1995. Coastal marine eutrophication: a definition, social causes, and future concerns. Ophelia 41, 199–219 10.1080/.

Norton, D., Shainberg, I., Cihacek, L., Edwards, J.H., 1999. Erosion and soil chemical properties. In: Lal, R. (Ed.), Soil Quality and Soil Erosion. https://doi.org/10.1201/9780203739266-3.

O'Farrell, I., Izaguirre, I., 2014. Phytoplankton of the middle and lower stretches of the Uruguay River. Adv. Limnol. 65, 113–126. https://doi.org/10.1127/1612-166X/2014/0065-0037.

O'Farrell, I., Bordet, F., Chaparro, G., 2012. Bloom forming cyanobacterial complexes cooccurring in a subtropical large reservoir: validation of dominant eco-strategies. Hydrobiologia 698, 175–190. https://doi.org/10.1007/s10750-012-1102-4.

O'Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J., 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14, 313–334. https://doi.org/10.1016/j.hal.2011.10.027.

OCDE, 1982. Eutrophisation des eaux. Méthodes de Surveillance, d'Évaluation et de Lutte.

Padisák, J., Köhler, J., Hoeg, S., 1999. Effect of Changing Flushing Rates on Development of Late Summer Aphanizomenon and Microcystis Populations in a Shallow Lake, Müggelsee, Berlin, Germany.

Paerl, H.W., 2017. Controlling cyanobacterial harmful blooms in freshwater ecosystems. Microb. Biotechnol. 10, 1106–1110. https://doi.org/10.1111/1751-7915.12725.

Paerl, H.W., Huisman, J., 2008. Blooms like it hot. Science 320, 57–58. https://doi.org/10.1126/science.1155398.

Paerl, H.W., Paul, V.J., 2012. Climate change: links to global expansion of harmful cyanobacteria. Water Res. 46, 1349–1363. https://doi.org/10.1016/j.watres.2011.08.002.

Paerl, H.W., Fulton, R.S., Moisander, P.H., Dyble, J., 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. Sci. World J. 1, 76–113. https://doi.org/10.1100/tsw.2001.16.

Paerl, H.W., Hall, N.S., Calandrino, E.S., 2011. Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. Sci. Total Environ. 409, 1739–1745. https://doi.org/10.1016/j.scitotenv.2011.02.001.

Paerl, H.W., Gardner, W.S., Havens, K.E., Joyner, A.R., McCarthy, M.J., Newell, S.E., Qin, B., Scott, J.T., 2016a. Mitigating cyanobacterial harmful algal blooms in aquatic ecosystems impacted by climate change and anthropogenic nutrients. Harmful Algae 54, 213–222. https://doi.org/10.1016/j.hal.2015.09.009.

Paerl, H.W., Scott, J.T., McCarthy, M.J., Newell, S.E., Gardner, W.S., Havens, K.E., Hoffman, D.K., Wilhelm, S.W., Wurtsbaugh, W.A., 2016b. 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. https://doi.org/10.1021/acs.est.6b02575.

Pedersen, T.L., 2020. patchwork: the composer of plots. R package version 1.1.1. https://CRAN.R-project.org/package=patchwork.

Perez, L., Barreiro, M., Etchevers, I., Crisci, C., García-Rodríguez, F., 2021. Centennial hydroclimatic and anthropogenic processes of South East South America modulate interannual and decadal river discharge. Sci. Total Environ. 781, 146733. https://doi.org/10.1016/j.scitotenv.2021.146733.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Heisterkamp, S., Van Willigen, B., Ranke, J., R-core, 2021. nlme: Linear and Nonlinear Mixed Effects Models.

R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

R Studio Team, 2021. RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA.

Ralston, D.K., Moore, S.K., 2020. Modeling harmful algal blooms in a changing climate. Harmful Algae 91, 101729. https://doi.org/10.1016/j.hal.2019.101729.

Reichwaldt, E.S., Ghadouani, A., 2012. Effects of rainfall patterns on toxic cyanobacterial blooms in a changing climate: between simplistic scenarios and complex dynamics. Water Res. 46, 1372–1393. https://doi.org/10.1016/j.watres.2011.11.052.

Reynolds, C.S., 2006. Ecology of Phytoplankton. Cambridge University Press, Cambridge; New York.

Reynolds, C., Descy, J.-P., 1996. The production, biomass and structure of phytoplankton in large rivers. Large Rivers 161–187. https://doi.org/10.1127/lr/10/1996/161.

Savadova, K., Mazur-Marzec, H., Karosienė, J., Kasperovičienė, J., Vitonytė, I., Toruńska-Sitarz, A., Koreivienė, J., 2018. Effect of increased temperature on native and alien nuisance cyanobacteria from temperate lakes: an experimental approach. Toxins 10, 445. https://doi.org/10.3390/toxins10110445.

Scheffer, M., 1998. Ecology of Shallow Lakes. Springer.

Scheffer, M., Jeppesen, E., 2007. Regime shifts in shallow lakes. Ecosystems 10, 1–3. https://doi.org/10.1007/s10021-006-9002-y.

Schindler, D.W., Carpenter, S.R., Chapra, S.C., Hecky, R.E., Orihel, D.M., 2016. Reducing phosphorus to Curb Lake eutrophication is a success. Environ. Sci. Technol. 50, 8923–8929. https://doi.org/10.1021/acs.est.6b02204.

Schneider, J., Campion-Alsumard, T.L., 1999. Construction and destruction of carbonates by marine and freshwater cyanobacteria. Eur. J. Phycol. 34, 417–426. https://doi.org/10.1080/09670269910001736472.

Soares, M.C.S., Marinho, M.M., Huszar, V.L.M., Branco, C.W.C., Azevedo, S.M.F.O., 2008. The effects of water retention time and watershed features on the limnology of two tropical reservoirs in Brazil. Lakes Reserv. Res. Manag. 13, 257–269. https://doi.org/10.1111/j.1440-1770.2008.00379.x.

Somma, A., Bonilla, S., Aubriot, L., 2021. Nuisance phytoplankton transport is enhanced by high flow in the main river for drinking water in Uruguay. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-021-14683-y.

Sommer, U., Adrian, R., Bauer, B., Winder, M., 2012. The response of temperate aquatic ecosystems to global warming: novel insights from a multidisciplinary project. Mar. Biol. 159, 2367–2377. https://doi.org/10.1007/s00227-012-2085-4.

Stumm, W., Morgan, J.J., 2012. Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters. John Wiley & Sons.

The Turing Way Community, Arnold, B., Bowler, L., Gibson, S., Herterich, P., Higman, R., Krystalli, A., Morley, A., O'Reilly, M., Whitaker, K., 2019. The Turing Way: A Handbook for Reproducible Data Science. Zenodo. https://doi.org/10.5281/ZENODO.3233986.

Tundisi, J.G., Tundisi, T.M., 2012. Limnology. CRC Press/Balkema, Leiden, The Netherlands; Boca Raton, Fla.

Velickovic, V., 2015. What everyone should know about statistical correlation. Am. Sci. 103, 26. https://doi.org/10.1511/2015.112.26.

Visser, P.M., Verspagen, J.M.H., Sandrini, G., Stal, L.J., Matthijs, H.C.P., Davis, T.W., Paerl, H.W., Huisman, J., 2016. How rising CO2 and global warming may stimulate harmful

cyanobacterial blooms. Harmful Algae 54, 145–159. https://doi.org/10.1016/j.hal.2015.12.006.
Wetzel, R.G., 2001. Limnology: Lake and River Ecosystems. 3rd ed. Academic Press, San Diego.
White, E., Baldridge, E., Brym, Z., Locey, K., McGlinn, D., Supp, S., 2013. Nine simple ways to make it easier to (re)use your data. Ideas Ecol. Evol. 6. https://doi.org/10.4033/iee.2013.6b.6.f.
Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H., 2019a. Welcome to the Tidyverse. JOSS 4, 1686. https://doi.org/10.21105/joss.01686.
Wickham, H., Bryan, J., RStudio, Kalicinski, M., Komarov, V., Leitienne, C., Colbert, B., Hoerl, D., Miller, E., 2019b. readxl: Read Excel Files.
Wurtsbaugh, W.A., Paerl, H.W., Dodds, W.K., 2019. Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. WIREs Water 6, e1373. https://doi.org/10.1002/wat2.1373.
Yeom, B.-M., Lee, H.W., Moon, H.-I., Yun, D.-G., Choi, J.H., 2019. Study on the management of Doam Dam operation by the analysis of suspended solids behavior in the lake. J. Korean Soc. Water Environ. 35, 470–480. https://doi.org/10.15681/KSWE.2019.35.6.470.
Zhao, C.S., Shao, N.F., Yang, S.T., Ren, H., Ge, Y.R., Feng, P., Dong, B.E., Zhao, Y., 2019. Predicting cyanobacteria bloom occurrence in lakes and reservoirs before blooms occur. Sci. Total Environ. 670, 837–848. https://doi.org/10.1016/j.scitotenv.2019.03.161.
Zuur, A.F., Ieno, E.N., 2016. A protocol for conducting and presenting results of regression-type analyses. Methods Ecol. Evol. 7, 636–645. https://doi.org/10.1111/2041-210X.12577.
Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed effects models and extensions in ecology with R. Statistics for Biology and Health. Springer New York, New York, NY. https://doi.org/10.1007/978-0-387-87458-6.
Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1, 3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x.