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Distribution of Toxic Cyanobacteria in Volcanic Lakes of the Azores Islands

Rita Cordeiro [1,2,*], Rúben Luz [1], Joana Vilaverde [1], Vitor Vasconcelos [3,4], Amélia Fonseca [1,2] and Vítor Gonçalves [1,2]

1 CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Pólo dos Açores, Universidade dos Açores, 9500-321 Ponta Delgada, Portugal; ruben.fs.luz@uac.pt (R.L.); joana.v.cabral@uac.pt (J.V.); maria.ao.fonseca@uac.pt (A.F.); vitor.mc.goncalves@uac.pt (V.G.)
2 Faculdade de Ciências e Tecnologia, Universidade dos Açores, 9500-321 Ponta Delgada, Portugal
3 Interdisciplinary Centre of Marine and Environmental Research—CIIMAR/CIMAR, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal; vmvascon@fc.up.pt
4 Department of Biology, Faculty of Sciences, University of Porto, 4069-007 Porto, Portugal
* Correspondence: rita.ip.cordeiro@uac.pt

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Abstract: Eutrophication and global climate change gather advantageous conditions for cyanobacteria proliferation leading to bloom formation and cyanotoxin production. In the Azores, eutrophication is a major concern, mainly in lakes where fertilizers and organic matter discharges have increased nutrient concentration. In this study, we focused on understanding the influence of environmental factors and lake characteristics on (i) cyanobacteria diversity and biomass and (ii) the presence of toxic strains and microcystin, saxitoxin, anatoxin-a, and cylindrospermopsin cyanotoxin-producing genes. Fifteen lakes from the Azores Archipelago were sampled seasonally, environmental variables were recorded in situ, cyanobacteria were analyzed with microscopic techniques, and cyanotoxin-producing genes were targeted through conventional PCR. Statistical analysis (DistLM) showed that lake typology-associated variables (lake's depth, area, and altitude) were the most explanatory variables of cyanobacteria biomass and cyanotoxin-producing genes presence, although trophic variables (chlorophyll *a* and total phosphorus) influence species distribution in each lake type. Our main results revealed higher cyanobacteria biomass/diversity, and higher toxicity risk in lakes located at lower altitudes, associated with deep anthropogenic pressures and eutrophication scenarios. These results emphasize the need for cyanobacteria blooms control measures, mainly by decreasing anthropogenic pressures surrounding these lakes, thus decreasing eutrophication. We also highlight the potential for microcystin, saxitoxin, and anatoxin-a production in these lakes, hence the necessity to implement continuous mitigation protocols to avoid environmental and public health toxicity events.

Keywords: anthropogenic impacts; eutrophication; phosphorus; temperature; microcystin; anatoxin-a; saxitoxin; cylindrospermopsin

1. Introduction

Eutrophication is a major factor leading to the development of blooms and the appearance of surface accumulations of cyanobacteria in inland waters [1,2]. The combination of eutrophication with global climate change is leading to the rapid increase in cyanobacteria dominance and toxic blooms (cyanoHABs) [3–7]. The rise of temperature, nutrients, and atmospheric CO_2 are all advantageous scenarios for cyanobacteria rapid proliferation, bloom formation, and toxin production [3,8,9]. Some cyanobacteria develop better at higher temperatures (≥25 °C) than most phytoplankton

algae [5,10,11]. They can move vertically in stratified waters (due to gas vesicles), allowing them to have direct access to sunlight and shade epilimnion and hypolimnion [1,3,8]. Moreover, they can fix atmospheric nitrogen (due to heterocytes), enabling them to resist nitrogen-limited conditions [5,12]. All these characteristics give cyanobacteria an ecological advantage and ultimately drive the development of cyanobacteria communities [8].

Cyanobacteria can produce a wide range of toxins, such as Microcystin (MC), Saxitoxin (STX), Anatoxin-a (ATX-a), and Cylindrospermopsin (CYN) [13,14]. These cyanotoxins are a threat not only to environmental health but also to public health, affecting the well-being of many organisms [13–17]. Several studies deal with cyanotoxin production, regulation, and function [18–20], but still gaps in the knowledge remain [21,22]. Higher nutrients (e.g., nitrogen and phosphorus) concentrations and their synergy with other environmental factors (e.g., temperature and light) are key to toxic strain growth, rather than non-toxic, and higher cyanotoxin production [2,7,23]. Besides the not well-defined reasons for toxin production, cyanobacterial blooms may be comprised of various species including toxin and non-toxin producers within the same species [24,25].

In recent years, molecular methods have been applied to identify toxigenic cyanobacteria strains, prior to toxin production [16,24,26–28]. Mitigation actions that reduce the favorable conditions for cyanoHABs development and allow the identification of cyanotoxin production potential in water bodies are essential to avoid risks to public health [16,29].

In the Azores, eutrophication has become a major concern in lakes conservation due to discharges of fertilizers and organic matter from human activities in the catchments [30,31]. As it is observed worldwide [32–35], eutrophication in the Azorean lakes has led to the increase of cyanobacteria abundance and the formation of cyanoHABs [30,35,36]. References to the presence of cyanobacteria in the Azores date back to the late Nineteenth century [37–39], but the current knowledge on their distribution and ecology is still limited [35,36], and even less is known about the presence of toxigenic strains in the Azorean lakes [7,30,40,41]. Considering that several species known from the literature as producers of MC, STX, ATX-a, and CYN, such as *Aphanizomenon gracile* [42], *Microcystis aeruginosa* [43], and *Raphidiopsis curvata* [44], were found in the Azores lakes [45], the presence of toxigenic strains and/or cyanotoxins should be further investigated, in order to determine the present risk in these waters.

In this context, we aimed to determine the occurrence of toxic cyanobacteria in the Azorean lakes through the detection of cyanotoxins biosynthesis genes to identify lakes in the Azores Archipelago with a potential risk of cyanotoxin production and to understand which physical and chemical factors (e.g., temperature, O_2, pH and nutrients) and lake characteristics (e.g., depth, location, and catchment land use) drive toxic strains distribution.

2. Materials and Methods

2.1. Study Site

The Azores Archipelago is located in the Northeast Atlantic Ocean, about 1300 km from Europe and 1600 km from North America. Due to its geographical dispersion, the nine volcanic islands that comprise the archipelago are divided into three groups: Eastern (Santa Maria and São Miguel), Central (Terceira, Graciosa, São Jorge, Pico, and Faial), and Western (Corvo and Flores). Influenced by their oceanic basin location, the prevalent climate in these islands is classified as oceanic temperate, with regular and abundant rainfalls and winds, low thermal amplitude, and high air humidity [46].

Despite their small size, the Azores are rich in lentic habitats, with 88 lakes mainly located in the islands of São Miguel, Pico, and Flores [47]. An increase in anthropogenic activities in the lake catchment (e.g., deforestation, agriculture, and urbanization) has resulted in the eutrophication of several lakes [30,31,48,49].

For this study, fifteen volcanic lakes from the Azores archipelago were sampled: 12 in São Miguel Island, two in Pico Island, and one in Flores Island (Figure 1). The main geographical, morphological, physical, chemical and biological properties of the studied lakes are presented in

Table 1. Physical, chemical, and biological properties are presented as mean values, with standard deviation, of all campaigns. Latitude, longitude, altitude, and lake surface area data were retrieved from Pereira et al. [50].

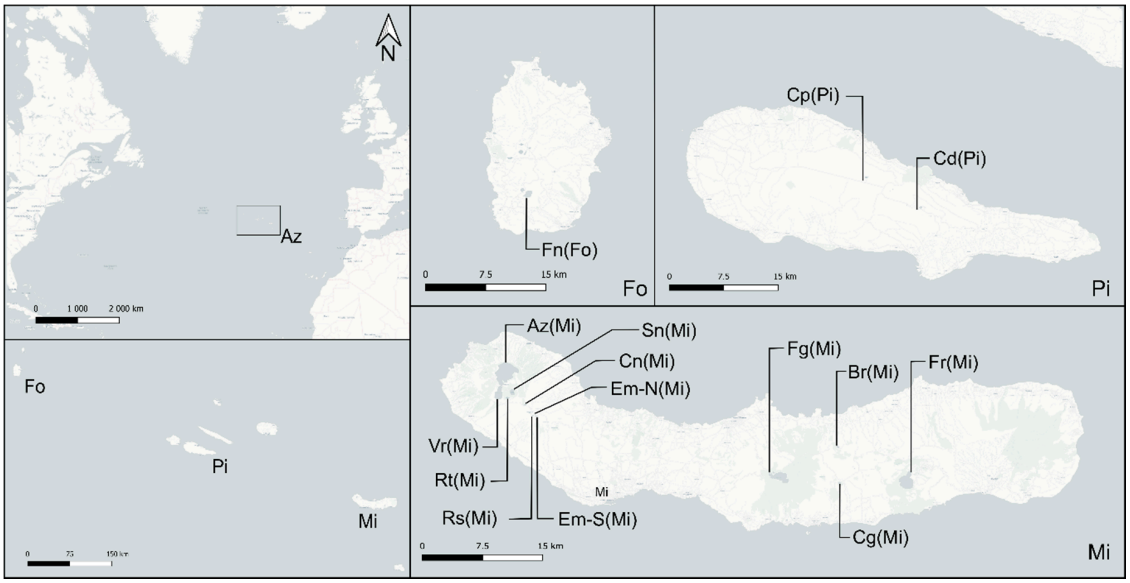

Figure 1. Location of the studied lakes in the Azores Archipelago (See Table 1 for lake code). Az: Azores Archipelago; Mi: São Miguel Island; Pi: Pico Island; Fo: Flores Island.

Table 1. Hydromorphological and physicochemical characterization of sampled lakes.

Lake Name	Lake	Hydromorphological Characteristics						Physicochemical Variables						
		Lat (UTM)	Lon (UTM)	Alt (m)	Area (km^2)	Z_{max} (m)	Z_{SD} (m)	T (°C)	pH	O_2 (mg L^{-1})	C (µS cm^{-1})	TN (mg L^{-1})	TP (µg L^{-1})	Chl*a* (µg L^{-1})
Azul	Az(Mi)	4,192,417.68	608,243.52	260	3.587	25.4	3.2 ± 0.7	19.0 ± 3.7	7.5 ± 0.2	9.3 ± 0.8	102 ± 5	1.1 ± 0.1	20.6 ± 7.4	2.8 ± 1.3
São Brás	Br(Mi)	4,184,056.94	640,013.62	610	0.058	2.0	0.5 ± 0.4	16.2 ± 3.5	6.7 ± 0.4	9.7 ± 0.6	38 ± 4	0.9 ± 0.3	109.4 ± 47.3	24.4 ± 14.5
Caiado	Cd(Pi)	4,257,155.56	390,879.19	810	0.055	4.7	2.7 ± 0.4	14.2 ± 3.0	6.6 ± 0.3	9.9 ± 0.9	30 ± 3	0.5 ± 0.3	17.8 ± 7.0	2.0 ± 0.9
Congro	Cg(Mi)	4,179,982.69	640,241.90	420	0.037	17.6	2.4 ± 0.7	18.0 ± 4.1	8.2 ± 1.0	9.4 ± 1.6	98 ± 3	1.0 ± 0.2	17.6 ± 9.2	12.5 ± 7.4
Canário	Cn(Mi)	4,188,336.28	609,147.63	750	0.018	2.7	1.0 ± 0.2	16.6 ± 4.8	7.0 ± 0.8	8.9 ± 0.9	38 ± 4	0.4 ± 0.1	37.4 ± 12.6	8.1 ± 3.7
Capitão	Cp(Pi)	4,260,771.00	384,929.00	790	0.027	4.3	0.9 ± 0.1	14.4 ± 2.8	6.0 ± 0.9	10.5 ± 1.2	35 ± 7	0.6 ± 0.3	45.0 ± 15.6	23.3 ± 15.3
Empadadas-N	Em-N(Mi)	4,187,226.07	610,176.10	740	0.018	2.7	1.8 ± 0.4	16.8 ± 5.0	7.2 ± 0.5	9.1 ± 0.7	39 ± 4	0.5 ± 0.3	19.4 ± 6.2	5.8 ± 3.4
Empadadas-S	Em-S(Mi)	4,187,091.70	610,274.94	750	0.005	2.7	2.0 ± 0.5	15.6 ± 3.4	7.6 ± 0.4	9.0 ± 0.7	51 ± 6	1.1 ± 0.5	13.8 ± 1.8	4.5 ± 2.7
Fogo	Fg(Mi)	4,180,740.35	633,514.96	574	1.437	26.6	3.1 ± 0.4	15.8 ± 3.0	7.5 ± 0.4	9.1 ± 1.2	48 ± 2	0.7 ± 0.2	17.6 ± 2.6	4.0 ± 2.1
Funda	Fn(Fo)	4,363,277.32	653,537.56	360	0.355	33.8	1.8 ± 1.1	17.0 ± 2.8	7.9 ± 0.7	10.2 ± 1.5	120 ± 0	0.5 ± 0.2	25.1 ± 5.3	19.6 ± 14.8
Furnas	Fr(Mi)	4,180,143.86	647,150.83	280	1.926	12.0	0.8 ± 0.3	18.2 ± 3.7	8.0 ± 0.5	10.1 ± 1.3	148 ± 13	1.2 ± 1.0	59.4 ± 25.1	25.0 ± 14.6
Rasa das Sete Cidades	Rt(Mi)	4,189,125.49	607,359.61	545	0.039	3.9	3.8 ± 0.3	16.6 ± 4.2	6.1 ± 0.6	9.3 ± 0.6	48 ± 3	0.5 ± 0.3	21.0 ± 19.0	1.6 ± 0.7
Rasa da Serra Devassa	Rs(Mi)	4,187,131.44	609,876.37	765	0.033	0.4	0.6 ± 0.4	15.2 ± 4.0	7.0 ± 0.6	9.1 ± 0.6	40 ± 5	0.4 ± 0.2	20.0 ± 12.2	2.3 ± 1.2
Santiago	Sn(Mi)	4,189,551.18	607,989.45	360	0.254	26.0	2.6 ± 0.8	18.7 ± 4.0	7.5 ± 0.5	8.5 ± 0.1	123 ± 6	0.4 ± 0.0	17.0 ± 4.4	9.9 ± 6.0
Verde	Vr(Mi)	4,189,071.90	606,553.94	260	0.856	22.4	3.3 ± 3.2	18.0 ± 3.7	8.2 ± 1.0	9.6 ± 1.4	126 ± 5	0.5 ± 0.2	26.8 ± 17.8	17.7 ± 14.9

Mi: São Miguel Island; Pi: Pico Island; Fo: Flores Island, Lat = Latitude, Lon = Longitude, Alt = Altitude, Area = Lake Surface Area, Zmax = Maximum Depth, Z_{SD} = Secchi Depth, T = Temperature, pH (20 °C), O_2 = Dissolved Oxygen, C = Conductivity (epilimnion), TN = Total Nitrogen, TP = Total Phosphorus, Chl*a* = Chlorophyll *a*.

2.2. Sample Collection and Environmental Variables

For molecular analysis plankton samples were collected from surface waters with a 10 µm net. At the same time, water samples from the photic zone were collected for phytoplankton, chlorophyll *a* (Chl*a*), and chemical analysis. Sampling was carried out in October 2015, February 2016, April 2016, August 2016, and November 2016, except in lake Santiago, where the sampling was done three times (October 2015, August 2016, and November 2016).

Chl*a* (µg L^{-1}) was analyzed using spectrophotometric methods following the Portuguese standard 4237 [51], and the concentrations were determined according to Lorenzen [52].

At each sampling, conductivity (C, µS cm^{-1}), dissolved oxygen (O_2, mg L^{-1}), temperature (T, °C), and pH profiles of each lake were recorded in situ using the multiparameter probe Horiba U-52 (Horiba, Pasadena, TX, USA). Water transparency (Z_{SD}, m) was taken with a 20 cm diameter Secchi disk and maximum depth (Z_{max}, m) was measured with the echo sounder HUMMINBIRD 385 CI GPS (Humminbird®, Eufaula, AL, USA).

Total nitrogen and total phosphorus were determined in the laboratory at the Instituto de Inovação Tecnológica dos Açores (INOVA), according to international standard protocols [53].

2.3. Phytoplankton Analysis

For phytoplankton analyses, combined samples from the photic zone were obtained by mixing 1 L of discrete samples collected at 1 m intervals from the surface to the bottom of the euphotic zone and preserved with a 1% Lugol solution. Cyanobacteria and remaining phytoplankton biomass was based on cell or colony volume, which was estimated for individual species by assigning a geometric shape similar to that of each phytoplankton species [54]. Cell or colony dimensions were determined by measuring at least 30 cells/colonies of each species. Total species biomass was then calculated by multiplying cell/colony counts with the respective average volume. Cell and colony counts were determined with the Utermöhl method following international recommendations [54,55].

General and more specialized cyanobacteria floras were used for taxonomic identification [56–58]. Cyanobacteria nomenclature was updated according to Komárek et al. [59] and AlgaeBase [60].

2.4. DNA Extraction and PCR Amplification

DNA extraction was performed directly in environmental samples, after centrifugation of 6–10 mL, using the PureLink™ Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA), according to the gram-negative bacteria protocol, supplied by the manufacturer. DNA samples were stored at −20 °C.

Cyanotoxin production potential was assessed by PCR, targeting genes *mcy*A, *mcy*B, *mcy*C, *mcy*D, *mcy*E, and *mcy*G for MC, *sxt*A for STX, *ana*C for ATX-a, and *cyr*B and *cyr*C for CYN. All the primer pairs used to target cyanotoxin-producing genes are listed in Table S1. PCR reactions were carried out in Biometra Tone (Analytik Jena AG, Jena, Germany), according to the literature [42,61–65]. All PCR amplification products were visualized by electrophoresis on 1.5% agarose gels stained with SYBR™ SAFE (0.2 g mL^{-1}) and visualized using the transilluminator Molecular Imager® Gel Doc™ XR+ (BioRad, Hercules, CA, USA).

2.5. Statistical Analysis

Lake environmental variables (morphological, physical, and chemical characteristics), cyanobacteria biomass, and the presence of toxic strains were analyzed using PRIMER-E Software V.6 [66]. The data set was first divided into three data matrices: (i) environmental data (temperature, pH, conductivity, dissolved oxygen, total nitrogen, total phosphorus, chlorophyll *a*, Secchi depth, maximum depth, area, and altitude), (ii) cyanobacteria species biomass, and (iii) presence or absence of cyanotoxin-producing genes. Environmental data was normalized and cyanobacteria biomass was transformed (square root) to reduce differences in scale [67].

Distance based linear modeling (DistLM) was performed to identify if cyanobacteria biomass and diversity were associated with lakes environmental factors, testing distribution patterns between environmental variables and cyanobacteria. Analyses were run in PERMANOVA+ for PRIMER software V.6 [66,68].

3. Results

3.1. Environmental Variables

The studied Azorean lakes have a wide range of characteristics, located at mid (260–420 m) to high altitude (740–810 m), with different surface areas (0.005–3.6 km^2) and depths (0.4–33.8 m). These lakes are slightly alkaline (pH 8.2 in lake Congro) to slightly acidic (pH 6.0 in lake Capitão) and have low mineralization (C from 30 ± 3 to 148 ± 13 μS cm^{-1}), that generally decreases with altitude (Table 1). Water temperature (14.2 ± 3.0–19 ± 3.7 °C), Secchi depth (0.5 ± 0.4–3.8 ± 0.3 m), phosphorus (17.0 ± 4.4–109.4 ± 47.3 μg L^{-1}), and chlorophyll *a* (1.6 ± 0.7–25.0 ± 14.6 μg L^{-1}) also varied substantially between the sampled lakes (Table 1 and Table S2).

According to the international criteria of lake trophic status classification [69], lakes Caiado, Rasa da Serra Devassa and Rasa das Sete Cidades are oligotrophic, lakes Azul, Empadadas-N, Empadadas-S and Fogo are mesotrophic, and lakes Canário, Capitão, Congro, São Brás, Funda, Furnas, Santiago, and Verde are eutrophic.

3.2. Cyanobacteria and Environmental Drivers

Fifteen cyanobacteria taxa from the orders Synechococcales, Chroococcales, Oscillatoriales, and Nostocales were observed (Tables S3 and S4). Cyanobacteria were the dominant phytoplankton group in several lakes, representing more than 50% of total phytoplankton abundance and biomass in one-third of the lakes (Tables S3 and S4). Lakes Azul, Capitão, Fogo, Furnas, Funda, Santiago, São Brás, and Verde had the highest cyanobacteria biomass, although with seasonal fluctuation (Figure 2). The dominant cyanobacteria species in these lakes were *Aphanizomenon gracile*, *Dolichospermum planctonicum*, *Microcystis flos-aquae*, *Planktolyngbya limnetica*, and *Pseudanabaena limnetica* (Figure 2; Tables S3 and S4).

DistLM analysis shows that maximum depth (Pseudo-F = 9.48; p = 0.001), altitude (Pseudo-F = 8.62; p = 0.001), conductivity (Pseudo-F = 8.07; p = 0.001), area (Pseudo-F = 5.59; p = 0.001), pH (Pseudo-F = 4.37; p = 0.001), total phosphorus (Pseudo-F = 2.71; p = 0.006), temperature (Pseudo-F = 2.40; p = 0.016), and chlorophyll *a* (Pseudo-F = 2.35; p = 0.019) are the environmental variables that better explain cyanobacteria biomass variability among lakes (Table 2). The first two axes of the model (dbRDA1 and dbRDA2) indicate that the selected environmental variables explain 23% of total variation (Figure 3). Shallow lakes located at higher elevations have negative scores on dbRDA1 (Figure 3). These lakes have lower values of conductivity and pH but contrasting values of chlorophyll *a* and phosphorus (Table 1). In this group (shallow lakes), lakes Capitão and São Brás have the highest's concentration of phosphorus and chlorophyll *a* (Table 1) and are also the only ones with high cyanobacteria biomass, mainly *Ps. limnetica* and *Dolichospermum* (Figure 2). Contrarily, deep and larger lakes (higher values of maximum depth and surface area) are plotted on the positive side of dbRDA1, thus, dbRDA axis 1 is interpreted as a gradient of lake's depth, which is in accordance with the typology of the Azorean lakes [70]. Deep lakes are separated into two groups along dbRDA2, separating Lakes Azul and Fogo on the positive side from the remaining deep lakes that have negative scores on this dbRDA axis 2. The main difference between these two groups is the productivity (chlorophyll *a*) and cyanobacteria species composition. Deep Mesotrophic lakes (Lakes Azul and Fogo) have lower chlorophyll *a* concentration (Table 1) and cyanobacteria are dominated by *Pl. limnetica* and *Ps. limnetica* (Figure 2). Deep eutrophic lakes (Lakes Congro, Verde, Santiago, Funda, and Furnas) have high chlorophyll *a* concentration (Table 1) and high biomass of cyanobacteria dominated by *Aphanizomenon*, *Dolichospermum*, and *Microcystis* (Figure 2).

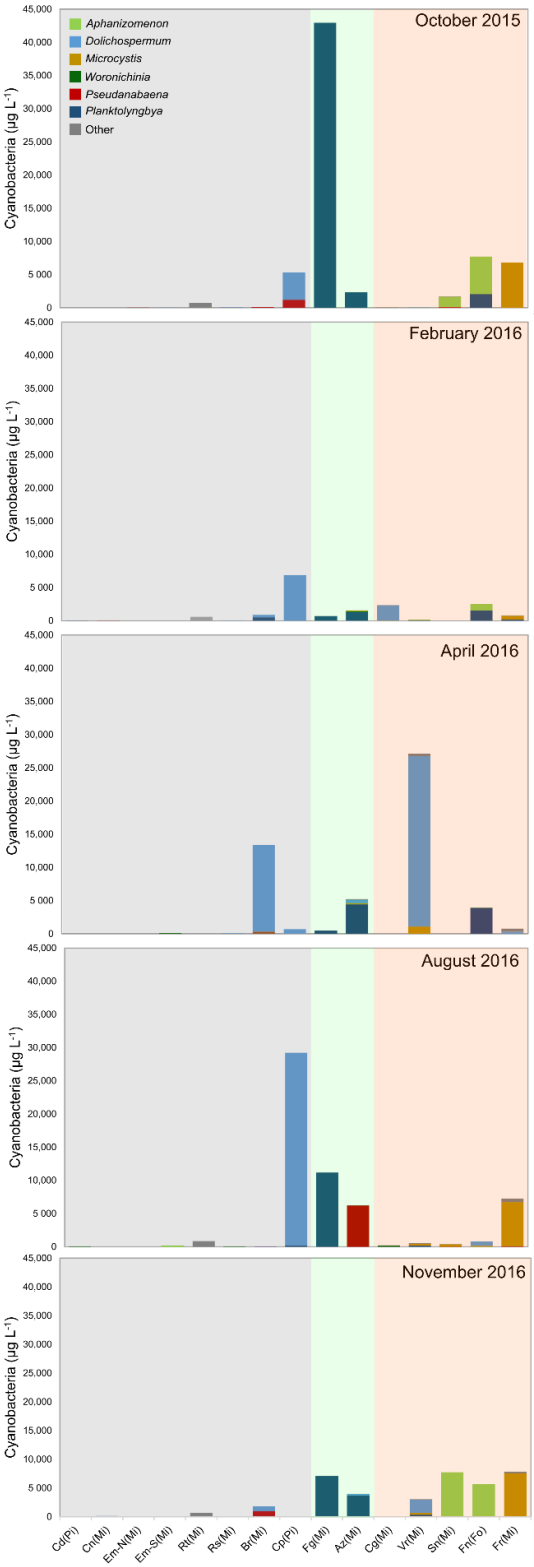

Figure 2. Cyanobacteria biomass (µg L^{-1}) in the photic zone of the 15 sampled lakes, by sampling campaign. *Aphanizomenon*: *A. gracile*; *Dolichospermum*: *Dolichospermum* sp., *D. planctonicum*, and *D. spiroides*; *Planktolyngbya*: *Pl. limnetica*; *Woronichinia*: *W. naegeliana*; *Pseudanabaena*: *Ps. limnetica*; *Microcystis*: *M. aeruginosa* and *M. flos-aquae*; Other: *Coelosphaerium kuetzingianum*; *Chroococcus minutus*; *C. turgidus*; *Oscillatoria tenuis*; *Snowella lacustris* and *Synechocystis* sp. Grey shadow: Shallow Lakes; Green shadow: Deep Mesotrophic lakes; Orange shadow: Deep Eutrophic Lakes.

Table 2. Summary of Distance-based linear modeling (DistLM) procedure for environmental variables for cyanobacteria diversity and cyanotoxin producing genes amplification.

Variable	Cyanobacteria Species				Cyanotoxin-Producing Genes			
	SS (Trace)	Pseudo-F	*p*	Prop	SS (Trace)	Pseudo-F	*p*	Prop
Z_{max} (m)	34,170.0	9.48	0.001	0.118	5.97	13.89	0.001	0.0164
Alt (m)	31,393.0	8.62	0.001	0.108	10.96	30.40	0.001	0.300
C (µS cm^{-1})	29,600.0	8.07	0.001	0.102	10.97	30.43	0.001	0.300
Area (Km^2)	21,175.0	5.59	0.001	0.073	2.58	5.39	0.003	0.071
pH	16,805.0	4.37	0.001	0.058	5.72	13.18	0.001	0.157
TP (µg L^{-1})	10,648.0	2.71	0.006	0.037	1.59	3.23	0.025	0.044
T (°C)	9490.4	2.4	0.016	0.033	1.2	2.42	0.073	0.033
Chl*a* (µg L^{-1})	9279.2	2.35	0.019	0.032	2.15	4.44	0.007	0.059
Z_{SD} (m)	6824.9	1.71	0.085	0.024	0.44	0.88	0.430	0.022
O_2 (mg L^{-1})	3259.7	0.81	0.603	0.011	0.64	1.26	0.282	0.017
TN (mg L^{-1})	1844.8	0.45	0.875	0.006	0.54	1.06	0.371	0.015

C = Conductivity, TP = Total Phosphorus, T = Temperature, Z_{SD} = Secchi Depth, Chl*a* = Chlorophyll *a*, TN = Total Nitrogen, O_2 = Dissolved Oxygen, Z_{max} = Maximum Depth, Area = Lake Surface Area, Alt = Altitude.

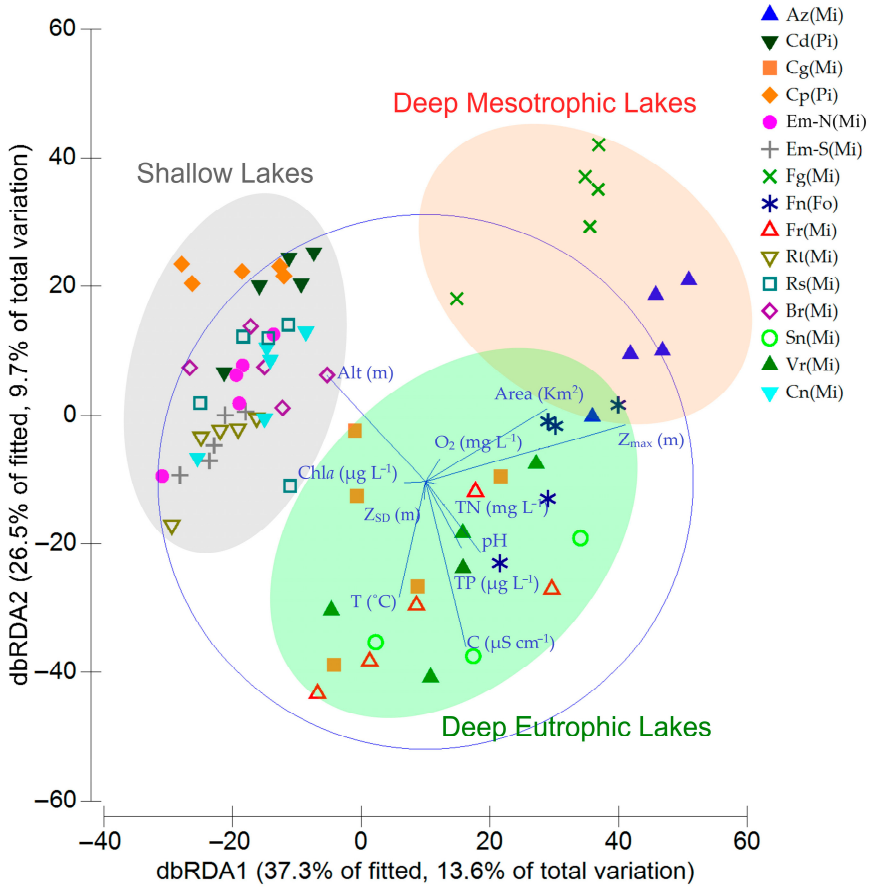

Figure 3. Distance-based redundancy analysis (dbRDA) plot of Distance-based linear modeling (DistLM) results in 2-dimensional space for environmental variables and cyanobacteria biomass across the studied lakes. The length and direction of the vectors represent the strength and direction of the relationship (see Table 1 for environmental variables and lake codes).

3.3. Distribution of Cyanotoxin-Producing Genes

Microcystin, saxitoxin, and/or anatoxin-a genes were detected in most of the studied lakes. At least one group of cyanotoxin-producing genes was found in lakes Azul, Verde, São Brás, Congro, Capitão, Fogo, Funda, Furnas, Rasa das Sete Cidades, Rasa da Serra Devassa, and Santiago (Table 3). Contrarily, toxin-producing strains were absent in lakes Caiado, Canário, Empadadas-N, and Empadadas-S, as neither cyanotoxin-producing genes were detected in these lakes (Table 3).

Table 3. Cyanotoxin-producing genes, cyanobacteria abundance, biomass, and dominant species (>2% of total cyanobacteria biomass) in the 15 sampled lakes, by sampling campaign.

		October 2015	February 2016	April 2016	August 2016	November 2016
Az(Mi)	Cyanotoxins genes *			MC, STX	STX	MC, STX
	Abundance (cells mL^{-1})	15,944	12,772	52,286	230,568	26,482
	Biomass (µg L^{-1})	2334.87	1571.97	5223.30	6231.13	3961.86
	Dominant Species	*Plli*, *Maer*	*Plli*, *Wnae*, *Maer*, *Mflo*, *Dpla*	*Plli*, *Syne*, *Mflo*, *Dpla*	*Psli*	*Ckue*, *Plli*, *Dpla*
Br(Mi)	Cyanotoxins genes *	MC		STX		
	Abundance (cells mL^{-1})	4635	8611	200,670	812	48,477
	Biomass (µg L^{-1})	125.15	867.40	13,351.60	59.43	1819.47
	Dominant Species	*Psli*	*Plli*, *Doli*	*Psli*, *Doli*	*Psli*, *Doli*	*Psli*, *Doli*
Cd(Pi)	Cyanotoxins genes *					
	Abundance (cells mL^{-1})	9	45	238	235	3
	Biomass (µg L^{-1})	0.62	6.59	10.90	10.92	0.21
	Dominant Species	*Doli*	*Plli*, *Doli*	*Wnae*, *Doli*	*Wnae*	*Doli*
Cg(Mi)	Cyanotoxins genes *	MC	MC	MC, STX	MC	MC, STX
	Abundance (cells mL^{-1})	292	13,689	577	6360	474
	Biomass (µg L^{-1})	8.53	2332.32	40.79	205.87	38.61
	Dominant Species	*Mflo*, *Oten*, *Dpla*	*Maer*, *Mflos*, *Dpla*	*Psli*, *Mflo*, *Dpla*	*Wnae*, *Mflo*	*Wnae*, *Dpla*
Cn(Mi)	Cyanotoxins genes *					
	Abundance (cells mL^{-1})	0	226	0	0	914
	Biomass (µg L^{-1})	0.00	6.10	0.00	0.00	138.93
	Dominant Species		*Psli*			*Plli*
Cp(Pi)	Cyanotoxins genes *					STX
	Abundance (cells mL^{-1})	104,020	100,169	10,125	258,102	1130
	Biomass (µg L^{-1})	5329.63	6910.65	691.44	29,215.45	83.93
	Dominant Species	*Psli*, *Doli*	*Doli*.	*Doli*	*Doli*	*Psli*, *Oten*
Em-N(Mi)	Cyanotoxins genes *					
	Abundance (cells mL^{-1})	329	0	268	0	456
	Biomass (µg L^{-1})	8.88	0.00	27.93	0.00	16.45
	Dominant Species	*Psli*		*Doli*, *Dpla*		*Psli*, *Wnae*
Em-S(Mi)	Cyanotoxins genes *					
	Abundance (cells mL^{-1})	97	24	2768	1811	230
	Biomass (µg L^{-1})	9.36	1.56	124.97	90.13	10.35
	Dominant Species	*Doli*, *Dpla*	*Cmin*	*Wnae*	*Psli*, *Wnae*, *Agra*	*Wnae*
Fg(Mi)	Cyanotoxins genes *	STX	STX			
	Abundance (cells mL^{-1})	282,656	4572	3182	73,514	46,632
	Biomass (µg L^{-1})	42,963.71	694.94	477.61	11,174.13	7088.06
	Dominant Species	*Plli*	*Plli*	*Plli*	*Plli*	*Plli*
Fn(Fo)	Cyanotoxins genes *	STX, ATX-a	STX	ATX-a	STX, ATX-a	STX
	Abundance (cells mL^{-1})	89,471	23,415	25,835	14,331	76,323
	Biomass (µg L^{-1})	7713.89	2512.88	3902.39	803.95	5722.07
	Dominant Species	*Plli*, *Agra*	*Plli*, *Agra*	*Plli*	*Mflo*, *Dspi*	*Agra*
Fr(Mi)	Cyanotoxins genes *	MC, ATX-a	MC	MC, ATX-a	MC, ATX-a	MC, STX, ATX-a
	Abundance (cells mL^{-1})	451,520	40,027	32,841	483,184	486,886
	Biomass (µg L^{-1})	6796.31	821.51	775.22	7261.64	7841.75
	Dominant Species	*Mflo*	*Ckue*, *Plli*, *Maer*, *Mflo*	*Syne*, *Dspi*	*Ckue*, *Mflo*	*Ckue*, *Maer*, *Mflo*
Rs(Mi)	Cyanotoxins genes *		STX			
	Abundance (cells mL^{-1})	1159	128	971	461	0
	Biomass (µg L^{-1})	32.11	8.83	47.66	20.75	0.00
	Dominant Species	*Psli*	*Doli*	*Wnae*, *Doli*	*Wnae*	

Table 3. *Cont.*

		October 2015	February 2016	April 2016	August 2016	November 2016
Rt(Mi)	Cyanotoxins genes *			MC		
	Abundance (cells mL^{-1})	11,259	9143	245	13,027	11,113
	Biomass (µg L^{-1})	724.86	594.30	15.93	846.76	720.95
	Dominant Species	*Psli*, *Cmin*	*Cmin*	*Cmin*	*Cmin*	*Cmin*
Sn(Mi)	Cyanotoxins genes *	STX, ATX-a	**	**		STX
	Abundance (cells mL^{-1})	25,099	**	**	13,011	107,668
	Biomass (µg L^{-1})	1657.79	**	**	368.50	7742.28
	Dominant Species	*Psli*, *Mflo*, *Agra*	**	**	*Ckue*, *Maer*, *Mflo*	*Mflo*, *Agra*
Vr(Mi)	Cyanotoxins genes *	MC, STX	MC, STX	MC, STX	MC, STX	MC, STX
	Abundance (cells mL^{-1})	2148	1901	239,905	23,878	53,100
	Biomass (µg L^{-1})	57.21	139.58	27,168.49	536.99	3057.41
	Dominant Species	*Mflo*, *Dpla*	*Ckue*, *Plli*, *Snow*, *Wnae*, *Mflos*, *Agra*	*Syne*, *Mflo*, *Dpla*	*Ckue*, *Plli*, *Syne*, *Mflo*	*Ckue*, *Plli*, *Maer*, *Mflo*, *Doli*

* Positive PCR results for cyanotoxin-producing genes (see Table S5 for each gene amplification), MC = Microcystin, STX = Saxitoxin, ATX-a = Anatoxin-a, CYN = Cylindrospermopsin; ** Without sampling. *CKue* = *Coelosphaerium kuetzingianum*, *Plli* = *Planktolyngbya limnetica*, *Psli* = *Pseudanabaena limnetica*, *Snow* = *Snowella lacustris*, *Syne* = *Synechocystis* sp., *Wnae* = *Woronichinia naegeliana*, *Cmin* = *Chroococcus minutus*, *Maer* = *Microcystis aeruginosa*, *Mflo* = *Microcystis flos-aquae*, *Oten* = *Oscillatoria tenuis*, *Agra* = *Aphanizomenon gracile*, *Dpla* = *Dolichospermum planctonicum*, *Dspi* = *Dolichospermum spiroides*, *Doli* = *Dolichospermum* sp.

According to the DistLM analysis, several environmental variables can explain cyanotoxins producing genes distribution among lakes (Table 2), such as conductivity (Pseudo-F = 30.43; $p = 0.001$), altitude (Pseudo-F = 30.40; $p = 0.001$), maximum depth (Pseudo-F = 13.89; $p = 0.001$), pH (Pseudo-F = 13.18; $p = 0.001$), area (Pseudo-F = 5.39; $p = 0.003$), chlorophyll *a* (Pseudo-F = 4.44; $p = 0.007$), and total phosphorus (Pseudo-F = 3.23; $p = 0.025$). The first two axes of this model (dbRDA1 and dbRDA2) show that the selected environmental variables explain 42.5% of total variation (Figure 4) and that most of the variation is along the dbRDA1 (34.5%) that represents an altitudinal gradient along which conductivity, pH, and lake area decreases with altitude (Figure 4).

Lakes located at higher altitude (545–810 m), plotted on the negative side of the dbRDA1, have low frequency of toxic cyanobacteria (0–2 samples with cyanotoxins genes). In four of those lakes (Caiado, Canário, Empadadas Norte, and Empadadas Sul), cyanotoxin genes were never detected. In the remaining five lakes of this group, only in one (Capitão, Rasa da Serra Devassa and Rasa das Sete Cidades) or two (Fogo and São Brás) samples was a single cyanotoxin gene detected (Table 3). This group of lakes was considered to have a lower toxicity risk (Figure 4). Cyanobacteria species found in these lakes were mainly *Ps. limnetica*, *Dolicospermum* sp., and *Pl. limnetica*.

Larger and deeper lakes that are located at lower altitude (260–420 m), associated with higher conductivity, pH, phosphorus, and/or chlorophyll *a*, have positive scores on dbRDA1 (Figure 4). Whereas in Lakes Furnas and Verde multiple cyanotoxin genes (MC, STX, ATX-a) were detected in all sampling campaigns and thus were considered to have higher toxicity risk, Lakes Azul, Congro, Funda, and Santiago have lower frequency (2–5 samples with cyanotoxins genes) and diversity of toxic cyanobacteria and were considered to have medium toxicity risk (Figure 4). In these two groups of lakes, the dominant cyanobacteria were *Aphanizomenon gracile*, *Dolichospermum* spp., *Microcystis flos-aquae*, and/or *Pl. limnetica* (Table 3).

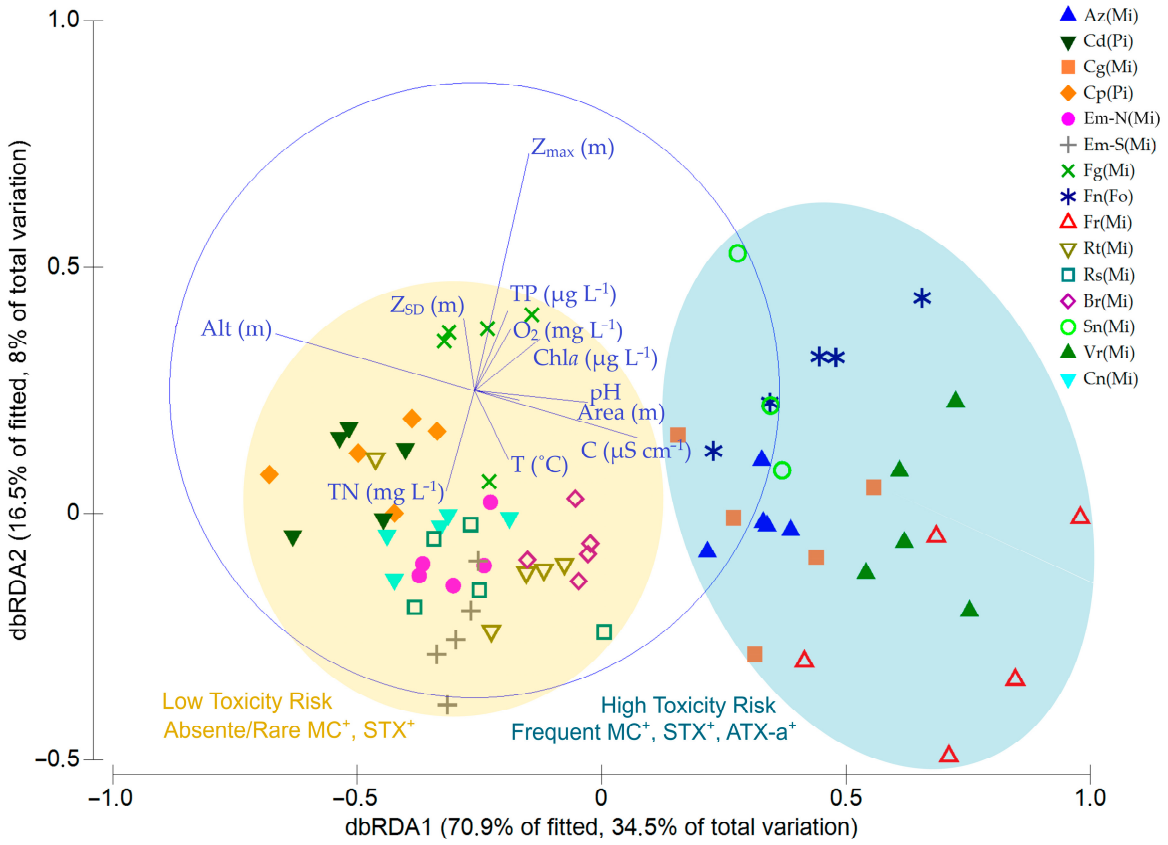

Figure 4. Distance-based redundancy analysis (dbRDA) plot of Distance-based linear modeling (DistLM) results in 2-dimensional space for environmental variables and cyanotoxin genes amplification across the studied lakes. The length and direction of the vectors represent the strength and direction of the relationship (see Table 1 for environmental variables and lake codes). MC^+ = amplification of MC producing gene(s), STX^+ = amplification of STX producing gene(s), ATX-a^+ = amplification of ATX-a producing gene(s).

4. Discussion

4.1. Toxigenic Cyanobacteria

Microcystis aeruginosa is the most studied cyanobacteria when it comes to cyanoHABs and cyanotoxins, mainly MCs [17,71]. However, MCs are known to be produced by a wider variety of cyanobacteria, as species from genera *Anabaena*, *Aphanizomenon*, *Dolichospermum*, *Nostoc*, *Oscillatoria*, and *Tolypothrix* [71]. Our results showed that MC-producing genes amplification was mainly related to *M. aeruginosa* and *M. flos-aquae* presence. In Lake Azul, the genus *Microcystis* is present in almost all the sampling campaigns (Tables S3 and S4); however, the amplification of MC-producing genes was not necessarily related to this genus. Other species might also be contributing, such as *Dolichospermum* spp., *Planktolyngbya limnetica*, and *Woronichinia naegeliana*, since samples without *Microcystis* had positive results for MC biosynthesis genes. Other studies have reported these species as MC producers [43,72–74]. In the Azores, MC has been previously reported in Lakes Azul, Congro, Furnas, São Brás, and Verde, by molecular methods [40] and by analytical methods (high-performance liquid chromatography—HPLC) [30,36]. In Portuguese freshwaters, the presence of MC has been reported since the 1990s [75], and according to Menezes et al. [76], a high diversity of toxic cyanobacteria species has also been reported, being the most prevalent genera *Aphanizomenon*, *Dolichospermum*, and *Microcystis*, just as observed in this work.

For ATX-a, amplification of gene *ana*C seems to be mainly related to the presence of *A. gracile*, *D. planctonicum*, and *M. aeruginosa*, known ATX-a producers [43,77] (Tables S4 and S5). ATX-a was never reported in the Azores; however, on Portugal's mainland, some studies already suggested the presence of this toxin. Osswald et al. [77], although without toxin identification in environmental samples, reported isolated strains from genus *Anabaena*, *Aphanizomenon*, *Microcystis*, and *Oscillatoria* as ATX-a producers. Moreira et al. [28] identified ATX-a (ELISA: Enzyme-Linked Immunosorbent Assay) and the *ana*C gene in some environmental samples (freshwater).

Contrary to the previous study performed in 2008 to detect STX production potential in the Azorean lakes, where no positive results were found [40], we detected STX genes in most of the studied lakes (Lakes Azul, São Brás, Congro, Fogo, Funda, Furnas, Rasa das Sete Cidades, Santiago, and Verde). In our study, *sxt*A gene amplification was mostly related to the presence of *A. gracile*, a known STX producer [42,78,79]. However, *A. gracile* may not be the only species with the *sxt*A gene in this set of lakes, as seen by the positives results in lakes where this species was not observed (Table S5). On the other hand, in Lake Santiago, where this species is present in high abundance throughout the year, the *sxt*A gene was not amplified in August 2016 (Table S5). These results suggest that the relative abundance of toxigenic and non-toxigenic strains from the same species may depend on a synergy between environmental conditions (e.g., temperature [18,80], phosphorus, light intensity, CO_2), as reported by several authors [7,18,80–83].

As reported in the literature, by several authors, toxic strains are not morphologically different from non-toxic strains [18,84]; however, through molecular methods, it is possible to identify which strains have the genes responsible for cyanotoxin production [16,29,84]. The presence of the *sxt*A gene in Lakes São Brás, Capitão, and Furnas may be related to species that might have the gene and be potential STX producers, not described in the literature. For instance, several cyanobacteria species have been reported, more recently, as saxitoxin producers, such as *Anagnostidinema amphibium*, *Anagnostidinema lemmermannii*, *Cylindrospermum stagnale*, and *Phormidium uncinatum* [85]. In Lake Rasa da Serra Devassa, the presence of *sxt*A in the sample from February 2016 (Table S5) might be due to unknown STX producing strains of *Dolichospermum* (Table S4). In Portugal's mainland, STX has been reported, similarly to ATX-a, with an identification of a producing strain of *Aphanizomenon flos-aquae* [86] and identification of SXT through ELISA and identification of biosynthesis genes (*sxt*G and *sxt*I) in some environmental samples [28].

CYN production potential was not found in our samples; however, in a previous study, the *cyrB* and *cyr*C genes were amplified in lake São Brás and lake Azul, respectively [40]. Furthermore, no other records of CYN detection (identification or quantification) or producing gene amplification in these lakes have been published. CYN has been reported to be produced by several species, including *A. gracile* [87], *Crysosporum ovalisporum* [88,89], *Raphidiopsis raciborskii* [65,90], *D. planctonicum* [43], and *Raphidiopsis curvata* [44]. In this work from the observed species, only *D. planctonicum* and *A. gracile* are potential CYN producers. Previous reports of *R. curvata* [91] and unidentified *Aphanizomenon* species [36] might also indicate CYN potential production in the Azores. In contrast, in Portugal's mainland, Moreira et al. [92] reported the first identification of CYN (by HPLC, ELISA, and *cyr*C amplification) in lake Vela (Figueira da Foz, Portugal), likely associated with the regular presence of *R. raciborskii* in high abundance. Lakes with "High Toxicity Risk", where cyanotoxin production potential was identified in all or most of the samples (Figure 4; Table 3), are related to the observation of known toxic cyanobacteria (e.g., *Aphanizomenon gracile*, *Dolichospermum* spp., *Microcystis flos-aquae*, and *Pl. limnetica*) and associated mainly to the lake's morphometrics (depth, area, and altitude), as well as temperature, pH, conductivity and/or chlorophyll *a*. These synergies are supported by the DistLM analysis (Figure 4; Table 2) and reinforced by the literature [7,41,93,94]. Some studies also confirm that toxic strain blooms and toxin release are enhanced by temperature, in interaction with other factors such as nutrients and light absorption [2,7,10,18,20,93].

4.2. Environmental Drivers of Cyanobacteria Occurrence and Cyanotoxins Production Potential

Most of the studied lakes are surrounded by agricultural/cattle fields and forested/recreational activities areas that promote eutrophication [30,31,33,36]. Anthropogenic effects in the Azorean lakes have impacted the lake's trophic state. In fact, according to Cruz et al. [31], eutrophication is a serious issue in the Azores archipelago since the '80s, besides the many efforts and mitigations measures applied. However, eutrophication in the Azorean lakes is not equally distributed among lake types. Lake types were defined in the Azores based on common European typology schemes, according to the Water Framework Directive (WFD) [95], and two lake types were designated: Type B-L-M/MI-MP/S/P includes deep lakes, warm monomictic, small to median surface area, located at median altitude inside volcanic calderas or maars; Type B-L-M/MI/S/PP comprehends shallow lakes, without summer stratification, very small and located at high altitude inside scoria cone craters, tectonic or topographic depressions [70]. Due to their location at lower altitudes, deep lakes are more exposed to human activities and most of them have become eutrophic [31,36]. Moreover, lower altitude lakes have larger catchments where the increase in water-rock interaction results in higher values of conductivity and nutrients [96].

The distribution of cyanobacteria and toxigenic species in the studied lakes follows, generally, the typology of the Azorean lakes, and, thus, geographical (altitude) and hydromorphological (deep and area) characteristics seem to be more important drivers of cyanobacteria in these lakes than other environmental variables, such as pH or nutrients (Table 2). Altitude, lake's area, and depth have already been shown to be important drivers of cyanobacteria distribution, e.g., [96,97]. The importance of these variables could be explained by differences in water retention time, mixing regime [97], and temperature [98,99], among other factors. Nevertheless, eutrophication still is an important driver of cyanobacteria species composition, biomass, and toxicity inside each type of the Azorean lakes.

Although the mesotrophic lakes Fogo and Azul, included in the deep type, have high cyanobacteria biomass, the dominant species are the Oscillatoriales *Pl. limnetica*, and *Ps. limnetica* and these lakes only occasionally have toxigenic potential. These species have been found to occur in several lake types and trophy [100] but were found to be dominant in some deep mesotrophic lakes [98,99]. Contrarily, the deep eutrophic lakes (Congro, Funda, Furnas, Santiago, and Verde) present higher cyanobacteria diversity, mostly dominated by *Microcystis* spp., *Dolichospemum* spp., and *A. gracile*. All these cyanobacteria are well known to dominate in deep stratified eutrophic lakes [98,101,102] where their buoyancy, low light toleration, and ability to fix atmospheric nitrogen gives them competitive advantages over other cyanobacteria and microalgae [1,3,8]. In the deep, larger, and eutrophic lakes Congro, Funda, Furnas, Santiago, and Verde, MC, STX, and/or ATX-a production potential were identified simultaneously and in almost all sampling campaigns, despite season fluctuations in environmental conditions (e.g., temperature, pH, nutrients), reinforcing the importance of geographical and hydromorphological variables in the distribution of toxic cyanobacteria. In these lakes, nutrients and temperature conditions are probably suitable for the maintenance of cyanobacteria toxigenic strains throughout the year. The climate in the Azores is characterized as oceanic temperate, constant throughout the year with low thermal amplitude [46], hence the absence of strong seasonal impacts on cyanobacteria dynamics (diversity and biomass), despite some effects on lakes dynamics (e.g., stratification, nutrients run-off). Furthermore, the lower altitude location of lakes Azul, Congro, Funda, Furnas, Santiago, and Verde is related to higher temperatures and longer water column stratification [31], characteristics that could lead to the exponential growth of cyanobacteria [33]. As stated before, toxigenic cyanobacteria species such as *A. gracile*, *M. aeruginosa*, *M. flos-aquae*, and/or *D. planctonicum* persist in these lakes throughout the year.

Shallow lakes located at higher altitudes, with lower temperature, pH, and conductivity, have low cyanobacteria biomass, except for the eutrophic lakes Capitão and São Brás (Figure 2; Table 1). The low abundance and biomass of cyanobacteria are common in shallow oligotrophic and mesotrophic lakes worldwide [89,103]. Contrarily, the shallow eutrophic lakes Capitão and São Brás have high cyanobacteria biomass (Figure 2, Table 3), dominated mainly by *Ps. limnetica* and *Dolichospermum*

sp. (Table 3). These two lakes, despite their higher altitude location, are surrounded by cattle fields and forested areas with anthropogenic influences, contributing to nutrients discharges leading to eutrophication and cyanobacteria extreme proliferation [3,104]. Phosphorus high concentration in Lakes Capitão and São Brás may be related to their elevated cyanobacteria biomass (Tables S2 and S4). As shown by the literature, the proliferation of cyanobacteria in eutrophic lakes is often associated with the rise of phosphorus concentration, mainly on account of anthropogenic effects [33,104,105].

5. Conclusions

Toxin-producing cyanobacteria were detected in most lakes through the detection of cyanotoxin-producing genes *mcy*A, *mcy*B, *mcy*C, *mcy*D, *mcy*E, *mcy*G, *sxt*A, and *ana*C. Lakes Azul, Verde, Furnas, Santiago, Congro (São Miguel Island), and Lake Funda (Flores Island), are the ones with higher cyanobacterial biomass and species diversity, and with high toxicity risk, where cyanotoxins production potential was frequently identified. *Aphanizomenon gracile*, *D. planctonicum*, *M. aeruginosa*, *M. flos-aquae*, and *Ps. limnetica* are the most prevalent species and might be the potential producers for MC, STX, and/or ATX-a. Isolation and cultivation of cyanobacteria strains from these lakes should be done to determine which strains are MC, STX, and/or ATX-a producers.

Cyanobacteria distribution seemed to be mainly related to lake typology (lake's depth, size, and altitude), although species composition and biomass vary in each lake type according to the trophic status. Altitude was found to be the main driver of toxic cyanobacteria distribution, with lakes located at high altitudes presenting low toxicity risk and lakes located at low altitude having high toxicity risk.

With the present study, we report for the first time the presence of genes *ana*C and *sxt*A and therefore the production potential of ATX-a and STX in lakes of the Azores islands.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/12/12/3385/s1. Table S1: Primers used to detect cyanotoxin-producing genes. Table S2: Environmental variables of sampled lakes by sampling campaign. Table S3: Cyanobacteria species abundance (cells mL^{-1}) of sampled lakes by sampling campaign. Table S4: Cyanobacteria biomass (µg L^{-1}) of sampled lakes by sampling campaign. Table S5: PCR results for the detection of microcystin, saxitoxin, anatoxin-a, and cylindrospermopsin, of sampled lakes by sampling campaign.

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References

1. Paerl, H.W.; Otten, T.G. Harmful cyanobacterial blooms: Causes, consequences, and controls. *Microb. Ecol.* **2013**, *65*, 995–1010. [CrossRef] [PubMed]
2. Glibert, P.M. Harmful algae at the complex nexus of eutrophication and climate change. *Harmful Algae* **2020**, *91*, 101583. [CrossRef]
3. O'Neil, J.M.; Davis, T.W.; Burford, M.A.; Gobler, C.J. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae* **2012**, *14*, 313–334. [CrossRef]

4. Townhill, B.L.; Tinker, J.; Jones, M.; Pitois, S.; Creach, V.; Simpson, S.D.; Dye, S.; Bear, E.; Pinnegar, J.K. Harmful algal blooms and climate change: Exploring future distribution changes. *ICES J. Mar. Sci.* **2018**, *75*, 1882–1893. [CrossRef]
5. Paerl, H. Mitigating toxic planktonic cyanobacterial blooms in aquatic ecosystems facing increasing anthropogenic and climatic pressures. *Toxins* **2018**, *10*, 76. [CrossRef]
6. Vasconcelos, V. Global changes and the new challenges in the research on cyanotoxin risk evaluation. *Limnetica* **2015**, *34*, 149–158. [CrossRef]
7. Mantzouki, E.; Lürling, M.; Fastner, J.; Domis, L.D.S.; Wilk-Woźniak, E.; Koreivienė, J.; Seelen, L.; Teurlincx, S.; Verstijnen, Y.; Krztoń, W.; et al. Temperature Effects Explain Continental Scale Distribution of Cyanobacterial Toxins. *Toxins* **2018**, *10*, 156. [CrossRef]
8. Huisman, J.; Codd, G.A.; Paerl, H.W.; Ibelings, B.W.; Verspagen, J.M.H.; Visser, P.M. Cyanobacterial blooms. *Nat. Rev. Microbiol.* **2018**, *16*, 471–483. [CrossRef]
9. Mantzouki, E.; Visser, P.M.; Bormans, M.; Ibelings, B.W. Understanding the key ecological traits of cyanobacteria as a basis for their management and control in changing lakes. *Aquat. Ecol.* **2016**, *50*, 333–350. [CrossRef]
10. Paerl, H.W.; Huisman, J. Climate change: A catalyst for global expansion of harmful cyanobacterial blooms. *Environ. Microbiol. Rep.* **2009**, *1*, 27–37. [CrossRef]
11. Paerl, H.W.; Huisman, J. Blooms Like It Hot. *Climate* **2008**, *320*, 57–58. [CrossRef]
12. Lürling, M.; Eshetu, F.; Faassen, E.J.; Kosten, S.; Huszar, V.L.M. Comparison of cyanobacterial and green algal growth rates at different temperatures. *Freshw. Biol.* **2013**, *58*, 552–559. [CrossRef]
13. Du, X.; Liu, H.; Yuan, L.; Wang, Y.; Ma, Y.; Wang, R.; Chen, X.; Losiewicz, M.D.; Guo, H.; Zhang, H. The diversity of cyanobacterial toxins on structural characterization, distribution and identification: A systematic review. *Toxins* **2019**, *11*, 530. [CrossRef]
14. He, X.; Liu, Y.-L.; Conklin, A.; Westrick, J.; Weavers, L.K.; Dionysiou, D.D.; Lenhart, J.J.; Mouser, P.J.; Szlag, D.; Walker, H.W. Toxic cyanobacteria and drinking water: Impacts, detection, and treatment. *Harmful Algae* **2016**, *54*, 174–193. [CrossRef]
15. Sivonen, K.; Jones, G. Cyanobacterial Toxins. In *Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring, and Management*; Chorus, I., Bartram, J., Eds.; F & FN Spon, WHO: London, UK, 1999; pp. 41–111.
16. Ramos, V.; Moreira, C.; Mankiewicz-Boczek, J.; Vasconcelos, V. Application of molecular tools in monitoring cyanobacteria and their potential toxin production. In *Molecular Tools for the Detection and Quantification of Toxigenic Cyanobacteria*; Kurmayer, R., Sivonen, K., Wilmotte, A., Salmaso, N., Eds.; John Wiley and Sons Ltd.: West Sussex, UK, 2017.
17. Codd, G.A.; Meriluoto, J.; Metcalf, J.S. Introduction: Cyanobacteria, Cyanotoxins, Their Human Impact, and Risk Management. In *Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis*; Codd, G.A., Meriluoto, J., Metcalf, J.S., Eds.; John Wiley & Sons, Ltd.: Chichester, UK, 2017; pp. 1–8.
18. Davis, T.W.; Berry, D.L.; Boyer, G.L.; Gobler, C.J. The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of Microcystis during cyanobacteria blooms. *Harmful Algae* **2009**, *8*, 715–725. [CrossRef]
19. Cirés, S.; Casero, M.C.; Quesada, A. Toxicity at the edge of life: A review on cyanobacterial toxins from extreme environments. *Mar. Drugs* **2017**, *15*, 233. [CrossRef]
20. Walls, J.T.; Wyatt, K.H.; Doll, J.C.; Rubenstein, E.M.; Rober, A.R. Hot and toxic: Temperature regulates microcystin release from cyanobacteria. *Sci. Total Environ.* **2018**, *610–611*, 786–795. [CrossRef]
21. Omidi, A.; Esterhuizen-Londt, M.; Pflugmacher, S. Still challenging: The ecological function of the cyanobacterial toxin microcystin—What we know so far. *Toxin Rev.* **2018**, *37*, 87–105. [CrossRef]
22. Osswald, J.; Rellán, S.; Gago, A.; Vasconcelos, V. Toxicology and detection methods of the alkaloid neurotoxin produced by cyanobacteria, anatoxin-a. *Environ. Int.* **2007**, *33*, 1070–1089. [CrossRef]
23. Renaud, S.L.; Pick, F.R.; Fortin, N. Effect of Light Intensity on the Relative Dominance of Toxigenic and Nontoxigenic Strains of *Microcystis aeruginosa*. *Appl. Environ. Microbiol.* **2011**, *77*, 7016–7022. [CrossRef]
24. Wood, S.A.; Smith, F.M.J.; Heath, M.W.; Palfroy, T.; Gaw, S.; Young, R.G.; Ryan, K.G. Within-mat variability in anatoxin-a and homoanatoxin-a production among benthic *Phormidium* (cyanobacteria) strains. *Toxins* **2012**, *4*, 900–912. [CrossRef]

25. Christiansen, G.; Molitor, C.; Philmus, B.; Kurmayer, R. Nontoxic Strains of Cyanobacteria are the Result of Major Gene Deletion Events Induced by a Transposable Element. *Mol. Biol. Evol.* **2008**, *25*, 1695–1704. [CrossRef]
26. Fathalli, A.; Jenhani, A.B.R.; Moreira, C.; Welker, M.; Romdhane, M.; Antunes, A.; Vasconcelos, V. Molecular and phylogenetic characterization of potentially toxic cyanobacteria in Tunisian freshwaters. *Syst. Appl. Microbiol.* **2011**, *34*, 303–310. [CrossRef]
27. Feio, M.J.; Filipe, A.F.; Garcia-Raventós, A.; Ardura, A.; Calapez, A.R.; Pujante, A.M.; Mortágua, A.; Múrria, C.; Diaz-de-Quijano, D.; Martins, F.M.; et al. Advances in the use of molecular tools in ecological and biodiversity assessment of aquatic ecosystems. *Limnetica* **2020**, *39*, 419–440. [CrossRef]
28. Moreira, C.; Gomes, C.; Vasconcelos, V.; Antunes, A. Cyanotoxins occurrence in Portugal: A new report on their recent multiplication. *Toxins* **2020**, *12*, 154. [CrossRef]
29. Moreira, C.; Ramos, V.; Azevedo, J.; Vasconcelos, V. Methods to detect cyanobacteria and their toxins in the environment. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 8073–8082. [CrossRef]
30. Santos, M.C.R.; Muelle, H.; Pacheco, D.M.D. Cyanobacteria and microcystins in lake Furnas (S. Miguel island-Azores). *Limnetica* **2012**, *31*, 107–118. [CrossRef]
31. Cruz, J.V.; Pacheco, D.; Porteiro, J.; Cymbron, R.; Mendes, S.; Malcata, A.; Andrade, C. Sete Cidades and Furnas lake eutrophication (São Miguel, Azores): Analysis of long-term monitoring data and remediation measures. *Sci. Total Environ.* **2015**, *520*, 168–186. [CrossRef]
32. Vasconcelos, V.M. Eutrophication, toxic cyanobacteria and cyanotoxins: When ecosystems cry for help. *Limnetica* **2006**, *25*, 425–432. [CrossRef]
33. Watson, S.B.; Miller, C.; Arhonditsis, G.; Boyer, G.L.; Carmichael, W.; Charlton, M.N.; Confesor, R.; Depew, D.C.; Höök, T.O.; Ludsin, S.A.; et al. The re-eutrophication of Lake Erie: Harmful algal blooms and hypoxia. *Harmful Algae* **2016**, *56*, 44–66. [CrossRef]
34. Beversdorf, L.J.; Weirich, C.A.; Bartlett, S.L.; Miller, T.R. Variable cyanobacterial toxin and metabolite profiles across six eutrophic lakes of differing physiochemical characteristics. *Toxins* **2017**, *9*, 62. [CrossRef]
35. Cordeiro, R.; Luz, R.; Vasconcelos, V.; Fonseca, A.; Gonçalves, V. A Critical Review of Cyanobacteria Distribution and Cyanotoxins Occurrence in Atlantic Ocean Islands. *Cryptogam. Algol.* **2020**, *41*, 73–89. [CrossRef]
36. Santos, M.C.R.; Pacheco, D.M.M.; Santana, F.; Muelle, H. Cyanobacteria blooms in Sete-Cidades lake—(S. Miguel Island-Azores). *Arch. Hydrobiol. Suppl. Algol. Stud.* **2005**, *117*, 393–406. [CrossRef]
37. Archer, W. Notes on some collections made from Furnas Lake, Azores, containing algae and a few other organisms. *Bot. J. Linn. Soc.* **1874**, *14*, 328–340. [CrossRef]
38. Moseley, H.N. Notes on Fresh-water Algae obtained at the Boiling Springs at Furnas, St. Michael's, Azores, and their neighbourhood. *Bot. J. Linn. Soc.* **1874**, *14*, 321–325. [CrossRef]
39. Bohlin, K. Étude sur la flore algologique d'eau douce des Açores. *K. Sven. Vetensk. Akad. Handl.* **1901**, *27*, 1–85.
40. Moreira, C.; Martins, A.; Moreira, C.; Vasconcelos, V. Toxigenic cyanobacteria in volcanic lakes and hot springs of a North Atlantic island (S. Miguel, Azores, Portugal). *Fresenius Environ. Bull.* **2011**, *20*, 420–426.
41. Mantzouki, E.; Campbell, J.; van Loon, E.; Visser, P.; Konstantinou, I.; Antoniou, M.; Giuliani, G.; Machado-Vieira, D.; Gurjão de Oliveira, A.; Maronić, D.Š.; et al. A European Multi Lake Survey dataset of environmental variables, phytoplankton pigments and cyanotoxins. *Sci. Data* **2018**, *5*, 180226. [CrossRef]
42. Ledreux, A.; Thomazeau, S.; Catherine, A.; Duval, C.; Yéprémian, C.; Marie, A.; Bernard, C. Evidence for saxitoxins production by the cyanobacterium *Aphanizomenon gracile* in a French recreational water body. *Harmful Algae* **2010**, *10*, 88–97. [CrossRef]
43. Park, H.-D.; Watanabe, M.F.; Harada, K.-I.; Nagai, H.; Suzuki, M.; Watanabe, M.; Hayashi, H. Hepatotoxin (microcystin) and neurotoxin (anatoxin-a) contained in natural blooms and strains of cyanobacteria from Japanese freshwaters. *Nat. Toxins* **1993**, *1*, 353–360. [CrossRef]
44. Li, R.; Carmichael, W.W.; Brittain, S.; Eaglesham, G.K.; Shaw, G.R.; Liu, Y.; Watanabe, M.M. First Report of the Cyanotoxins Cylindrospermopsin and Deoxycylindrospermopsin from *Raphidiopsis curvata* (Cyanobacteria). *J. Phycol.* **2001**, *37*, 1121–1126. [CrossRef]
45. Luz, R.; Cordeiro, R.; Vilaverde, J.; Raposeiro, P.; Fonseca, A.; Gonçalves, V. Cyanobacteria from freshwater lakes in the Azores archipelago, Portugal: Data from long term phytoplankton monitoring. *Biodivers. Data J.* **2020**, *8*, e51928. [CrossRef]

46. Hernández, A.; Kutiel, H.; Trigo, R.M.; Valente, M.A.; Sigró, J.; Cropper, T.; Santo, F.E. New Azores archipelago daily precipitation dataset and its links with large-scale modes of climate variability. *Int. J. Climatol.* **2016**, *36*, 4439–4454. [CrossRef]
47. Porteiro, J.M.M. Lagoas dos Açores. Elementos de Suporte ao Planeamento Integrado. Ph.D. Thesis, University of the Azores, Ponta Delgada, Portugal, 2000.
48. Vázquez-Loureiro, D.; Gonçalves, V.; Sáez, A.; Hernández, A.; Raposeiro, P.M.; Giralt, S.; Rubio-Inglés, M.J.; Rull, V.; Bao, R. Diatom-inferred ecological responses of an oceanic lake system to volcanism and anthropogenic perturbations since 1290 CE. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **2019**, *534*, 109285. [CrossRef]
49. Raposeiro, P.M.; Rubio, M.J.; González, A.; Hernández, A.; Sánchez-López, G.; Vázquez-Loureiro, D.; Rull, V.; Bao, R.; Costa, A.C.; Gonçalves, V.; et al. Impact of the historical introduction of exotic fishes on the chironomid community of Lake Azul (Azores Islands). *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **2017**, *466*, 77–88. [CrossRef]
50. Pereira, C.L.; Raposeiro, P.M.; Costa, A.C.; Bao, R.; Giralt, S.; Gonçalves, V. Biogeography and lake morphometry drive diatom and chironomid assemblages' composition in lacustrine surface sediments of oceanic islands. *Hydrobiologia* **2014**, *730*, 93–112. [CrossRef]
51. IPQ. *Qualidade da Água. Doseamento da Clorofila a e dos Feopigmentos por Espectrofotometria de Absorção Molecular. Método de Extracção com Acetona. NP 4327/1996*; Instituto Português da Qualidade (IPQ): Monte da Caparica, Portugal, 1997.
52. Lorenzen, C. Determination of chlorophyll and pheopigments: Spectrophotometric equations. *Limnol. Oceanogr.* **1967**, *12*, 343–346. [CrossRef]
53. APHA. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association: Washington, DC, USA, 1998.
54. Hillebrand, H.; Dürselen, C.-D.; Kirschtel, D.; Pollingher, U.; Zohary, T. Biovolume Calculation for Pelagic and Benthic Microalgae. *J. Phycol.* **1999**, *35*, 403–424. [CrossRef]
55. Lund, J.W.G.; Kipling, C.; Le Cren, E.D. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia* **1958**, *11*, 143–170. [CrossRef]
56. Komárek, J.; Anagnostidis, K. *Cyanoprokaryota, Teil 1/Part 1: Chroococcales*; Spektrum Akademischer Verlag: Berlin, Germany, 2008; ISBN 978-3-8274-2111-1.
57. Komárek, J.; Anagnostidis, K. *Cyanoprokaryota 2. Teil/Part 2: Oscillatoriales*; Spektrum Akademischer Verlag: Münche, Germany, 2005; ISBN 978-3-8274-1914-9.
58. Komárek, J. *Cyanoprokaryota 3. Teil/Part 3: Heterocytous Genera*; Spektrum Akademischer Verlag: Berlin, Germany, 2013; ISBN 978-3-8274-0932-4.
59. Komárek, J.; Kastovsky, J.; Mares, J.; Johansen, J.R. Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. *Preslia* **2014**, *86*, 295–335.
60. Guiry, M.D.; Guiry, G.M.; AlgaeBase. World-Wide Electronic Publication, National University of Ireland, Galway. Available online: http://www.algaebase.org (accessed on 23 July 2019).
61. Hisbergues, M.; Christiansen, G.; Rouhiainen, L.; Sivonen, K.; Borner, T. PCR-based identification of microcystin-producing genotypes of different cyanobacterial genera. *Arch. Microbiol.* **2003**, *180*, 402–410. [CrossRef]
62. Ouahid, Y.; Pérez-Silva, G.; Del Campo, F.F. Identification of potentially toxic environmental Microcystis by individual and multiple PCR amplification of specific microcystin synthetase gene regions. *Environ. Toxicol.* **2005**, *20*, 235–242. [CrossRef]
63. Neilan, B.A.; Jacobs, D.; Del Dot, T.; Blackall, L.L.; Hawkins, P.R.; Cox, P.T.; Goodman, A.E. rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus *Microcystis*. *Int. J. Syst. Bacteriol.* **1997**, *47*, 693–697. [CrossRef]
64. Rantala-Ylinen, A.; Känä, S.; Wang, H.; Rouhiainen, L.; Wahlsten, M.; Rizzi, E.; Berg, K.; Gugger, M.; Sivonen, K. Anatoxin-a synthetase gene cluster of the cyanobacterium *Anabaena* sp. strain 37 and molecular methods to detect potential producers. *Appl. Environ. Microbiol.* **2011**, *77*, 7271–7278. [CrossRef]
65. Schembri, M.A.; Neilan, B.A.; Saint, C.P. Identification of genes implicated in toxin production in the cyanobacterium *Cylindrospermopsis raciborskii*. *Environ. Toxicol.* **2001**, *16*, 413–421. [CrossRef]
66. Clarke, K.R.; Gorley, R.N. *PRIMER v6: User Manual/Tutorial*; PRIMER-E; Plymouth Marine Laboratory: Plymouth, UK, 2006.

67. Clarke, K.R.; Green, R.H. Statistical design and analysis for a 'biological effects' study. *Mar. Ecol. Prog. Ser.* **1988**, *46*, 213–226. [CrossRef]
68. Anderson, M.J.; Gorley, R.N.; Clarke, K.R. *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*; PRIMER-E; Plymouth Marine Laboratory: Plymouth, UK, 2008.
69. OECD. *Eutrophication of Waters. Monitoring, Assessment and Control*; Organisation for Economic Co-Operation and Development: Paris, France, 1982.
70. AHA-DRA. Plano de Gestão da Região Hidrográfica dos Açores–RH9. Versão para Consulta Pública (Azores River Basin Management Plan). Ponta Delgada, Azores, Portugal. 2015. Available online: https://siaia.apambiente.pt/AAEstrategica/DA_AAE676.pdf (accessed on 1 December 2020).
71. Bernard, C.; Ballot, A.; Thomazeau, S.; Maloufi, S.; Furey, A.; Mankiewicz-Boczek, J.; Pawlik-Skowrońska, B.; Capelli, C.; Salmaso, N. Appendix 2: Cyanobacteria associated with the production of cyanotoxins. In *Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis*; Meriluoto, J., Spoof, L., Codd, G.A., Eds.; John Wiley & Sons, Ltd.: Chichester, UK, 2017; pp. 501–525.
72. Bober, B.; Bialczyk, J. Determination of the toxicity of the freshwater cyanobacterium *Woronichinia naegeliana* (Unger) Elenkin. *J. Appl. Phycol.* **2017**, *29*, 1355–1362. [CrossRef]
73. Christophoridis, C.; Zervou, S.-K.; Manolidi, K.; Katsiapi, M.; Moustaka-Gouni, M.; Kaloudis, T.; Triantis, T.M.; Hiskia, A. Occurrence and diversity of cyanotoxins in Greek lakes. *Sci. Rep.* **2018**, *8*, 17877. [CrossRef]
74. Sivonen, K.; Himberg, K.; Luukkainen, R.; Niemelä, S.I.; Poon, G.K.; Codd, G.A. Preliminary characterization of neurotoxic cyanobacteria blooms and strains from Finland. *Toxic. Assess.* **1989**, *4*, 339–352. [CrossRef]
75. Vasconcelos, V.M.; Sivonen, K.; Evans, W.R.; Carmichael, W.W.; Namikoshi, M. Hepatotoxic microcystin diversity in cyanobacterial blooms collected in portuguese freshwaters. *Water Res.* **1996**, *30*, 2377–2384. [CrossRef]
76. Menezes, C.; Churro, C.; Dias, E. Risk Levels of Toxic Cyanobacteria in Portuguese Recreational Freshwaters. *Toxins* **2017**, *9*, 327. [CrossRef]
77. Osswald, J.; Rellán, S.; Gago-Martinez, A.; Vasconcelos, V. Production of anatoxin-a by cyanobacterial strains isolated from Portuguese fresh water systems. *Ecotoxicology* **2009**, *18*, 1110–1115. [CrossRef] [PubMed]
78. Ballot, A.; Fastner, J.; Wiedner, C. Paralytic shellfish poisoning toxin-producing cyanobacterium *Aphanizomenon gracile* in Northeast Germany. *Appl. Environ. Microbiol.* **2010**, *76*, 1173–1180. [CrossRef]
79. Casero, M.C.; Ballot, A.; Agha, R.; Quesada, A.; Cirés, S. Characterization of saxitoxin production and release and phylogeny of sxt genes in paralytic shellfish poisoning toxin-producing *Aphanizomenon gracile*. *Harmful Algae* **2014**, *37*, 28–37. [CrossRef]
80. Joung, S.-H.; Oh, H.-M.; Ko, S.-R.; Ahn, C.-Y. Correlations between environmental factors and toxic and non-toxic *Microcystis* dynamics during bloom in Daechung Reservoir, Korea. *Harmful Algae* **2011**, *10*, 188–193. [CrossRef]
81. Gobler, C.J.; Burkholder, J.M.; Davis, T.W.; Harke, M.J.; Johengen, T.; Stow, C.A.; Van de Waal, D.B. The dual role of nitrogen supply in controlling the growth and toxicity of cyanobacterial blooms. *Harmful Algae* **2016**, *54*, 87–97. [CrossRef]
82. Geada, P.; Pereira, R.N.; Vasconcelos, V.; Vicente, A.A.; Fernandes, B.D. Assessment of synergistic interactions between environmental factors on *Microcystis aeruginosa* growth and microcystin production. *Algal Res.* **2017**, *27*, 235–243. [CrossRef]
83. Beversdorf, L.J.; Miller, T.R.; McMahon, K.D. The Role of Nitrogen Fixation in Cyanobacterial Bloom Toxicity in a Temperate, Eutrophic Lake. *PLoS ONE* **2013**, *8*, e56103. [CrossRef]
84. Pearson, L.; Neilan, B. The molecular genetics of cyanobacterial toxicity as a basis for monitoring water quality and public health risk. *Curr. Opin. Biotechnol.* **2008**, *19*, 281–288. [CrossRef]
85. Borges, H.L.F.; Branco, L.H.Z.; Martins, M.D.; Lima, C.S.; Barbosa, P.T.; Lira, G.A.S.; Bittencourt-Oliveira, M.C.; Molica, R.J.R. Cyanotoxin production and phylogeny of benthic cyanobacterial strains isolated from the northeast of Brazil. *Harmful Algae* **2015**, *43*, 46–57. [CrossRef]
86. Pereira, P.; Onodera, H.; Andrinolo, D.; Franca, S.; Araújo, F.; Lagos, N.; Oshima, Y. Paralytic shellfish toxins in the freshwater cyanobacterium *Aphanizomenon flos-aquae*, isolated from Montargil reservoir, Portugal. *Toxicon* **2000**, *38*, 1689–1702. [CrossRef]
87. Rücker, J.; Stüken, A.; Nixdorf, B.; Fastner, J.; Chorus, I.; Wiedner, C. Concentrations of particulate and dissolved cylindrospermopsin in 21 *Aphanizomenon*-dominated temperate lakes. *Toxicon* **2007**, *50*, 800–809. [CrossRef]

88. Banker, R.; Carmeli, S.; Hadas, O.; Teltsch, B.; Porat, R.; Sukenik, A. Identification of cylindrospermopsin in *Aphanizomenon ovalisporum* (Cyanophyceae) isolated from lake Kinneret, Israel. *J. Phycol.* **1997**, *33*, 613–616. [CrossRef]
89. Quesada, A.; Moreno, E.; Carrasco, D.; Paniagua, T.; Wormer, L.; De Hoyos, C.; Sukenik, A. Toxicity of *Aphanizomenon ovalisporum* (Cyanobacteria) in a Spanish water reservoir. *Eur. J. Phycol.* **2006**, *41*, 39–45. [CrossRef]
90. Ohtani, I.; Moore, R.E.; Runnegar, M.T.C. Cylindrospermopsin: A potent hepatotoxin from the blue-green alga *Cylindrospermopsis raciborskii*. *J. Am. Chem. Soc.* **1992**, *114*, 7941–7942. [CrossRef]
91. INOVA. *Análise das Águas das Lagoas da Região Autónoma dos Açores*; INOVA: Ponta Delgada, Portugal, 1996.
92. Moreira, C.; Mendes, R.; Azevedo, J.; Vasconcelos, V.; Antunes, A. First occurrence of cylindrospermopsin in Portugal: A contribution to its continuous global dispersal. *Toxicon* **2017**, *130*, 87–90. [CrossRef]
93. Kosten, S.; Huszar, V.L.M.; Bécares, E.; Costa, L.S.; Van Donk, E.; Hansson, L.-A.; Jeppesenk, E.; Kruk, C.; Lacerot, G.; Mazzeo, N.; et al. Warmer climates boost cyanobacterial dominance in shallow lakes. *Glob. Chang. Biol.* **2012**, *18*, 118–126. [CrossRef]
94. Robarts, R.D.; Zohary, T. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *N. Zeal. J. Mar. Freshw. Res.* **1987**, *21*, 391–399. [CrossRef]
95. European Commission. *Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy*; Official Journal 22 December 2000 L 327/1; European Commission: Brussels, Belgium, 2000.
96. Cruz, J.V.; Antunes, P.; Amaral, C.; França, Z.; Nunes, J.C. Volcanic lakes of the Azores archipelago (Portugal): Geological setting and geochemical characterization. *J. Volcanol. Geotherm. Res.* **2006**, 135–157. [CrossRef]
97. Richardson, J.; Miller, C.; Maberly, S.C.; Taylor, P.; Globevnik, L.; Hunter, P.; Jeppesen, E.; Mischke, U.; Moe, S.J.; Pasztaleniec, A.; et al. Effects of multiple stressors on cyanobacteria abundance vary with lake type. *Glob. Chang. Biol.* **2018**, *24*. [CrossRef]
98. Wood, S.A.; Maier, M.Y.; Puddick, J.; Pochon, X.; Zaiko, A.; Dietrich, D.R.; Hamilton, D.P. Trophic state and geographic gradients influence planktonic cyanobacterial diversity and distribution in New Zealand lakes. *FEMS Microbiol. Ecol.* **2017**, *93*, fiw234. [CrossRef]
99. Gallina, N.; Salmaso, N.; Morabito, G.; Beniston, M. Phytoplankton configuration in six deep lakes in the peri-Alpine region: Are the key drivers related to eutrophication and climate? *Aquat. Ecol.* **2013**, *47*, 177–193. [CrossRef]
100. Bukowska, A.; Kaliński, T.; Koper, M.; Kostrzewska-Szlakowska, I.; Kwiatowski, J.; Mazur-Marzec, H.; Jasser, I. Predicting blooms of toxic cyanobacteria in eutrophic lakes with diverse cyanobacterial communities. *Sci. Rep.* **2017**, *7*, 8342. [CrossRef]
101. Padisák, J.; Crossetti, L.O.; Naselli-Flores, L. Use and misuse in the application of the phytoplankton functional classification: A critical review with updates. *Hydrobiologia* **2009**, *621*, 1–19. [CrossRef]
102. Reynolds, C.S.; Huszar, V.; Kruk, C.; Naselli-Flores, L.; Melo, S. Towards a functional classification of the freshwater phytoplankton. *J. Plankton Res.* **2002**, *24*, 417–428. [CrossRef]
103. Reynolds, C.S.; Petersen, A.C. The distribution of planktonic Cyanobacteria in Irish lakes in relation to their trophic states. *Hydrobiologia* **2000**, 91–99. [CrossRef]
104. Whitton, B.; Potts, M. Introduction to the Cyanobacteria. In *The Ecology of Cyanobacteria II—Their Diversity in Time and Space*; Whitton, B.A., Ed.; Springer: Dordrecht, The Netherlands, 2012; pp. 1–13. ISBN 978-94-007-3854-6.
105. Hamilton, D.P.; Salmaso, N.; Paerl, H.W. Mitigating harmful cyanobacterial blooms: Strategies for control of nitrogen and phosphorus loads. *Aquat. Ecol.* **2016**, *50*, 351–366. [CrossRef]