Research, Society and Development, v. 11, n. 8, e22411830764, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i8.30764

Growth of *Anagnostidinema amphibium* LBABL-2 (Oscillatoriales, Cyanobacteria) in Different Abiotic stress conditions

Crescimento de *Anagnostidinema amphibium* LBABL-2 (Oscillatoriales, Cyanobacteria) em Diferentes Condições de Estresse Abiótico

Crecimiento de *Anagnostidinema amphibium* LBABL-2 (Oscillatoriales, Cyanobacteria) en Diferentes Condiciones de Estrés Abiótico

Received: 05/23/2022 | Reviewed: 06/11/2022 | Accept: 06/15/2022 | Published: 06/16/2022

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Abstract
The Bolonha reservoir (Belém, Pará) is eutrophicated and dominated by aquatic macrophytes that are often removed and deposited on the banks of the environment, suffering decomposition. Some microorganisms that live associated with macrophytes return to the environment through leaching, among them cyanobacteria. The objective of the study was to evaluate the growth of the species *Anagnostidinema amphibium* LBABL-2, in microcosm, by three experiments under different environmental conditions and nutritional stress simulating its growth during the cleaning process of the Bolonha reservoir. Growth was determined by optical density and chlorophyll-a. The species formed a slime mat composed of tangles of green, not capitate, not constricted, not attenuated, slightly curved trichomes 600 µm long, composed of cells that measured, on average, 4.1 µm -2.2 µm in length and width, respectively, and showed cyanophycin granules in the cell wall between two cells. The absence of nutrients was the main stressor of *Anagnostidinema amphibium* LBABL-2, which showed higher growth in the roots of macrophytes immersed in the reservoir, but low growth in the absence of nutrients. The study will support actions for the management of aquatic macrophytes in the reservoir with emphasis on stressors and growth promoters of cyanobacteria associated with their roots.
Keywords: Eutrophication; Reservoir; Experiment; Aquatic macrophytes.

Resumo
O reservatório Bolonha (Belém, Pará) é eutrofizado e dominado por macrófitas aquáticas que muitas vezes são retiradas e depositadas às margens do ambiente, sofrendo a decomposição. Alguns microrganismos que vivem associados às macrófitas retornam ao ambiente através da lixiviação, dentre eles as cianobactérias. O objetivo do

estudo foi avaliar o crescimento da espécie *Anagnostidinema amphibium* LBABL-2, em microcosmo, sob diferentes condições ambientais e estresse nutricional simulando seu crescimento durante o processo de limpeza do reservatório Bolonha. O crescimento foi determinado através da densidade óptica e clorofila- a. A espécie formou um tapete lodoso composto por emaranhados de tricomas verdes, não capitados, não constritos, não atenuados, levemente curvados e com 600 μm de comprimento, compostos por células que mediram, em média, 4,1 μm -2,2 μm de comprimento e largura, respectivamente, e apresentaram grânulos de cianoficina na parede celular entre duas células. A ausência de nutrientes foi o principal fator de estresse de *Anagnostidinema amphibium* LBABL-2, a qual apresentou maior crescimento nas raízes de macrófitas imersas no reservatório, porém, baixo crescimento na ausência de nutrientes. O estudo subsidiará ações de manejo das macrófitas aquáticas do reservatório com ênfase nos estressores e promotores do crescimento das cianobactérias associadas às suas raízes.
Palavras-chave: Eutrofização; Reservatório; Experimento; Macrófitas aquáticas.

Resumen
El embalse de Bolonha (Belém, Pará) es eutrófico y dominado por macrófitos acuáticos que a menudo son removidos y depositados en los márgenes del medio ambiente, en proceso de descomposición. Algunos microorganismos que viven asociados a macrófitos regresan al medio ambiente por lixiviación, entre ellos las cianobacterias. El objetivo del estudio fue evaluar el crecimiento de la especie *Anagnostidinema amphibium* LBABL-2, en microcosmos, bajo diferentes condiciones ambientales y estrés nutricional, simulando su crecimiento durante el proceso de limpieza del embalse de Bolonha. El crecimiento se determinó por densidad óptica y clorofila-a. La especie formó una alfombra viscosa compuesta por marañas de tricomas verdes, no capitados, no constreñidos, no atenuados, ligeramente curvados y de 600 μm de longitud, compuesta por células que medían, en promedio, 4,1 μm -2,2 μm de longitude, largo y ancho, respectivamente, y mostró gránulos de cianoficina en la pared celular entre las células. La ausencia de nutrientes fue el principal factor de estrés de *Anagnostidinema amphibium* LBABL-2, que mostró mayor crecimiento en las raíces de las macrófitas inmersas en el reservorio, sin embargo, bajo crecimiento en ausencia de nutrientes. El estudio apoyará acciones de manejo de macrófitas acuáticas en el embalse con énfasis en factores estresantes y promotores de crecimiento de cianobacterias asociadas a sus raíces.
Palabras clave: Eutrofización; Embalse; Experimento; Macrófitas acuáticas.

1. Introduction

Cyanobacteria are gram-negative photosynthesizing prokaryotes that occur in all environments. In eutrophicated aquatic environments, resulting mainly from anthropic actions, blooms may occur, producing large biomass of cyanobacteria in a short period of time (Wall et al., 2014; Svirčev et al., 2017; Yu et al., 2019), which may cause the deterioration of water quality and changes in the environment (Brasil et al., 2016).

Planktonic cyanobacterial blooms are the most recurrent and reported in the literature (Catherine et al., 2013; O'Farrell et al., 2019; Vieira-Lanero et al., 2022). However, benthic cyanobacterial blooms, which are found naturally adhered to various substrates, such as rocks, corals, and aquatic macrophyte roots, are little studied and their occurrence has been increasing, such as the *Lyngbya majuscula* blooms associated with *Anabaena* spp. in French Polynesia (Bouma-Gregson et al., 2017; Zubia et al., 2019).

The blooms are usually associated with the production of toxins (cyanotoxins) that depending on the main target organ of intoxication can be of the type hepatotoxins, neurotoxins, dermatotoxins and cytotoxins, which affect human and animal health, being the cause of intoxication and even death of individuals in several countries (Kuiper-Goodman et al., 1999; Svirčev et al., 2017). Faassen et al. (2012) identified the toxins (homo) anatoxin-a, saxitoxin and cylindrospermopsins during a bloom of *Phomidium* sp., a benthic cyanobacterium, in Lake IJmeer, the Netherlands, which was responsible for the death of dogs and birds by neurotoxicosis.

Potentially toxic benthic cyanobacteria have been found in Brazilian reservoirs (Borges et al., 2015; Calandrini et al., 2020) and the consumption of water contaminated by cyanotoxins has been associated with the worsening of other diseases, such as microcephaly associated with Zika virus infection (Pedrosa et al., 2020).

Benthic species blooms were reported in the human supply reservoirs of Belém (Pará, Brazil) by Sousa (2017) and potentially toxic species were isolated from this environment by Silva et al. (2020) and Calandrini et al. (2020). The Bolonha reservoir is the source that supplies approximately 2 million people of the Metropolitan Region of Belém (Pará, Brazil) and for decades has suffered from increasing cyanobacteria density, macrophyte proliferation and siltation (Sousa, 2017).

In general, large cleanups occur in the reservoir, where tons of vegetables are removed, however, it is also common to remove small amounts of macrophytes that are deposited on the banks of the reservoir undergoing the natural process of decomposition (Sousa, 2017).

Silva et al. (2020) conducted tests in microcosms with the benthic species *Nostoc* sp. which was isolated from the root of *Eichhornia crassipes* Mart. (Solms) from the Bolonha reservoir. In this study, it was found that in the process of removal and decomposition of macrophytes, the cyanobacteria *Nostoc* sp. undergoes changes in its morphology, but maintains its growth and can return to the reservoir by leaching process.

Microcosm assays are important tools to simulate the behavior of organisms in nature (Li et al., 2018; Wang et al., 2020; Han; et al., 2013). Thus, the aim of the study was to evaluate the population growth of *Anagnostidinema amphibium* LBABL-2 (in microcosm) isolated from the roots of aquatic macrophytes of Bolonha reservoir (Belem, Pará) under different stress conditions, such as nutrient concentration, temperature, and humidity.

2. Methodology

2.1 Area and sample design

The Bolonha reservoir is located in the Utinga State Park (1°27'40" S and 48°26'59" W), has an area of 1.8 km^2 and 2,600,000 m^3 of water volume, and is responsible for supplying the Metropolitan Region of Belém (Belém, Pará, Brazil) (BRITO et al., 2020). The reservoir drains from the Água Preta reservoir, which collects water from the Guamá River, one of the main rivers in the region, through a pumping system (Oliveira et al., 2018). It is silted up and dominated by aquatic macrophytes, mainly the species *Eichhornia crassipes* and *Pistia stratiotes* Linnaeus (Araújo Jr., 2015).

The climate of the region is Af1 according to the Köppen classification, with an average temperature of 33°C, average relative humidity of 84% and prevailing winds from the east and northeast with an average speed of 7 km/h (Pará, 2013). Annual precipitation ranges from 2,769.4 to 3,775.6 mm, with a rainy period from December to May and less rainy from June to November (Sousa, 2017).

In the Bolonha reservoir, the aquatic macrophyte *P. stratiotes* was collected in September/2017, from which the species *Geitlerinema amphibium* was isolated and cultured in BG-11 artificial medium (Rippka et al., 1979). The species *Geitlerinema amphibium* was confirmed according to morphological analysis (Komarek & Anagnostidis, 2005) by light microscopy. However, the name *Anagnostidinema amphibium* (Agardh ex Gomont 1892) was subsequently adopted Strunecký et al. 2017, based on the recommendations of the genetic study by Strunecký et al. (2017).

2.2 Cyanobacteria biomass

The cyanobacterial biomass was obtained from an inoculum with 60 days of cultivation in MilliQ water, being kept in cultivation and plant growth chambers (Humidity, Panasonic). The incubation temperature was 24°C ± 1°, luminosity of approximately 60 µmol photon $m^2.s^{-1}$, pH 8.0 and photoperiod of 12 h light. The strain belongs to the Laboratory of Cyanobacteria and Aquatic Bioindicators of the Instituto Evandro Chagas (Ananindeua, Pará, Brazil).

2.3 Growth

Three experiments were conducted with the *Anagnostidinema amphibium* strain LBABL-2 described in Table 1. Experiment 1 simulated environmental conditions similar to the Bolonha reservoir waters, as if the cyanobacteria were attached to the macrophyte roots in a submerged condition. Experiment 2 simulated conditions in Bolonha reservoir when the macrophyte roots are exposed to the sun, i.e., when they are removed from the water and placed around the reservoir, in the initial stage of decomposition, justifying the higher temperature and humidity. Experiment 3, on the other hand, simulated conditions similar to the conditions in Bolonha reservoir when the macrophyte roots were exposed to the sun, on the reservoir banks, for a long time and in the advanced stage of decomposition with successive washing by leaching of the soil, where the nutrient removal is constant.

Every three days, over 30 days, three samples (replicate) were taken from each experiment, chosen through random drawings. For each replicate, the optical density in the absorbance bands 680 and 750 nm were analyzed (Jodłowska & Latała, 2013), and the chlorophyll-a concentration, according to the 10200 H method (APHA, 2017).

Table 1. Environmental conditions of the experiments performed with *Anagnostidinema amphibium* LBABL-2 (cyanobacteria).

Type of experiment	Temperature (°C)	Humidity (%)	Light (µmol photon $m^2 .s^{-1}$)	Photoperiod (h)	Medium
Experiment 1	30 ± 2	60 ± 2	40-60	12	BG- 11
Experiment 2	35 ± 2	90 ± 2	40-60	12	BG- 11
Experiment 3	35 ± 2	90 ± 2	40-60	12	BG- 11, absenceof N and P

Source: Authrors.

2.4 Statistical analysis

One Way ANOVA (F- value) analysis of variance was performed to check the difference in the growth of *A. amphibium* LBABL-2 in the different environmental conditions and between growth stages, considering the significance level $p< 0.05$ and Tukey's post hoc for parametric data, and Kruskal-Wallis test (H- value) and Dunn's post hoc for non- parametric data. Statistical tests were performed using the free software PAST 4.06b (Hammer et al., 2001).

3. Results

3.1 Characterization of the strain

To the naked eye the species formed a greenish slime mat on the surface of the growing medium, clumping together more as observation time passed. In microscopic view, the mats are composed of a tangle of trichomes characteristic of the species (Figures 1a and 1b). The trichomes were greenish, not capitate, not constricted, not attenuated, and slightly curved (Figures 1c- e).

They averaged 600 µm in length, being composed of cells that measured, on average, 4.1 µm in length and 2.2 µm in width. The cells also showed cyanophycin granules in the cell wall between two cells (Figure 1e), being easily visualized in phase contrast (Figure 1a). The strain showed dark green coloration and the colony encrusted all over the surface of the microcosm until it loosened into a floating mass (Figure 1f).

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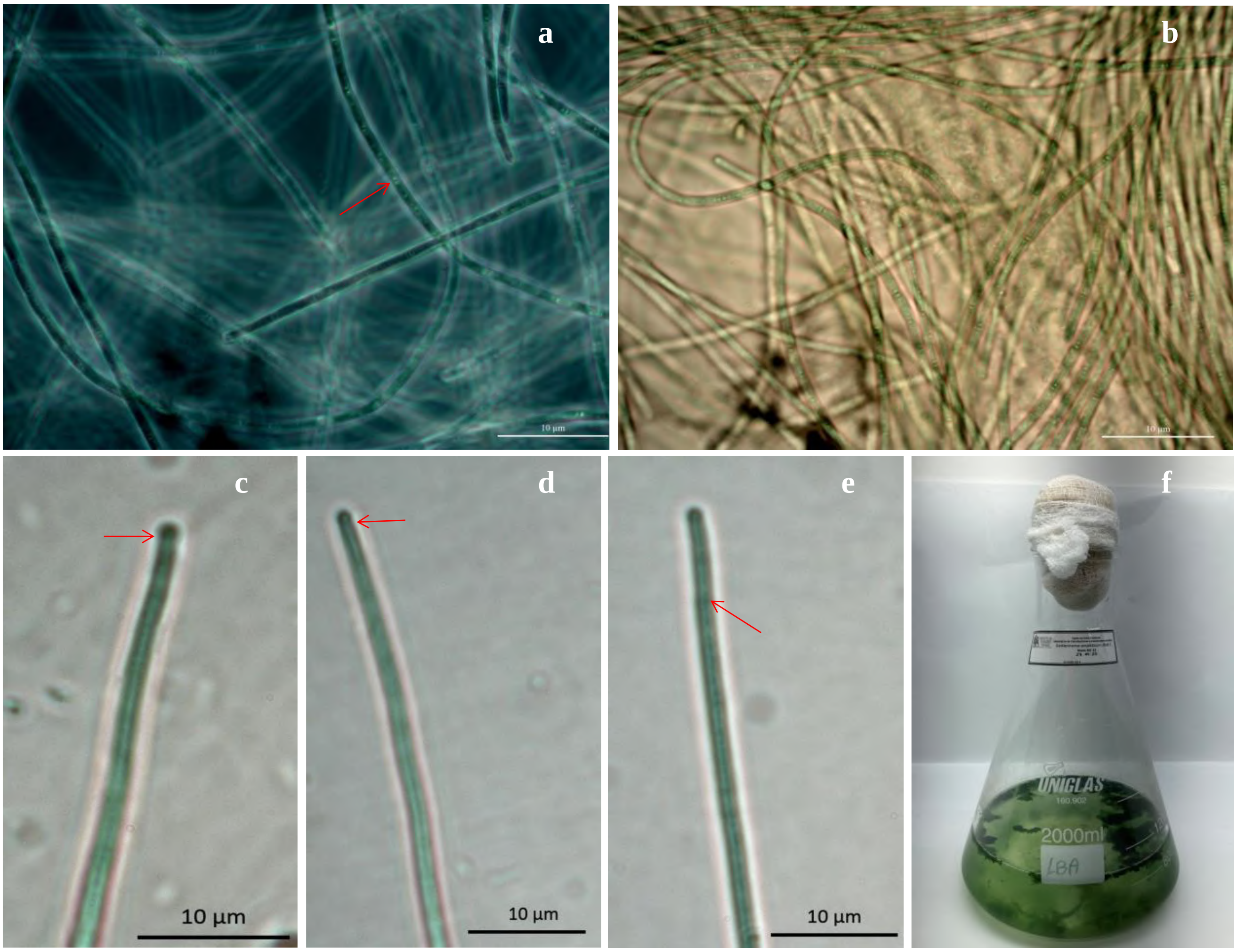
Figure 1. Photomicrograph of *Anagnostidinema amphibium*. a: filament in phase contrast evidencing cyanophycin granules (arrow) between adjacent cell walls; b: filament forming a tangle in light microscopy; c, d: Tricomes in light microscopy evidencing the not attenuated apical cells and not capitate trichomes; e: Image evidencing the cell walls of the not capitate trichome. f: Photograph of the *A. amphibium* strain LBABL-2 in microcosm.

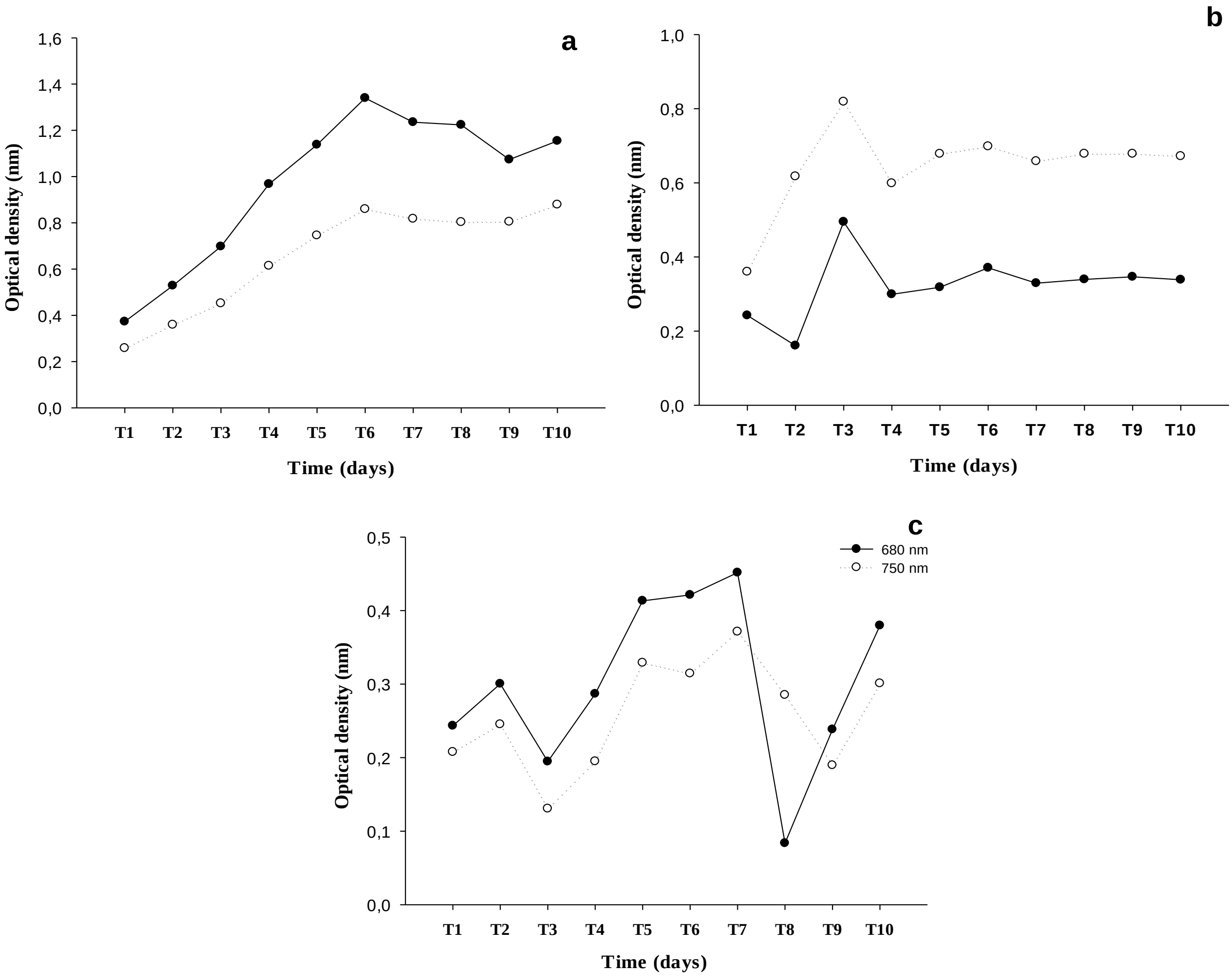


Source: Authors (2022).

3.2 Growth

The optical density analysis suggests that experiment 1 presented three phases of growth: the lag phase, from third to sixth day (T1 to T2), the log phase (T3 to T6) and the stationary phase, from the twenty-first day (T7 to T10) (Figure 2a). Experiment 2 also presented three phases, with the lag phase identified at T1-T2, log phase from day 6th to 9th (T2 to T3), and the stationary phase from twelfth day (T4 to T10) (Figure 2b). In experiment 3, there was no defined phase, but a log phase between days 9th and 21st was suggested (Figure 2c).

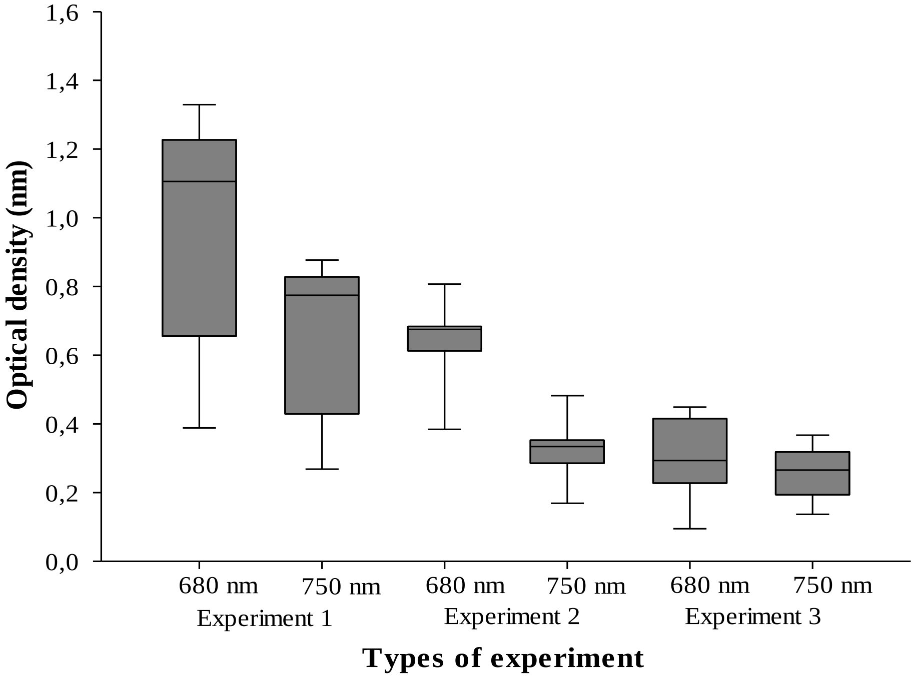
Figure 2. Graphs of *Anagnostidinema amphibium* LBABL-2 growth by optical density analysis at three-day intervals (T): a- experiment 1; b- experiment 2; c- experiment 3.



Source: Authors (2022).

Analysis of variance showed that there was no significant difference between experiments 1 (680 nm: 0.97 ± 0.33 nm; and 750 nm: 0.66 ± 0.23 nm) and 2 (680 nm: 0.65 ± 0.12 nm; and 750nm: 0.32 ± 0.12 nm), with the growth of the species in experiment 3 (680 nm: 0.30 ± 0.12 nm; and 750nm: 0.26 ± 0.08 nm) being significantly lower (F= 59.54; df= 2; p= 0.001) compared to the other experiments (Figure 3).

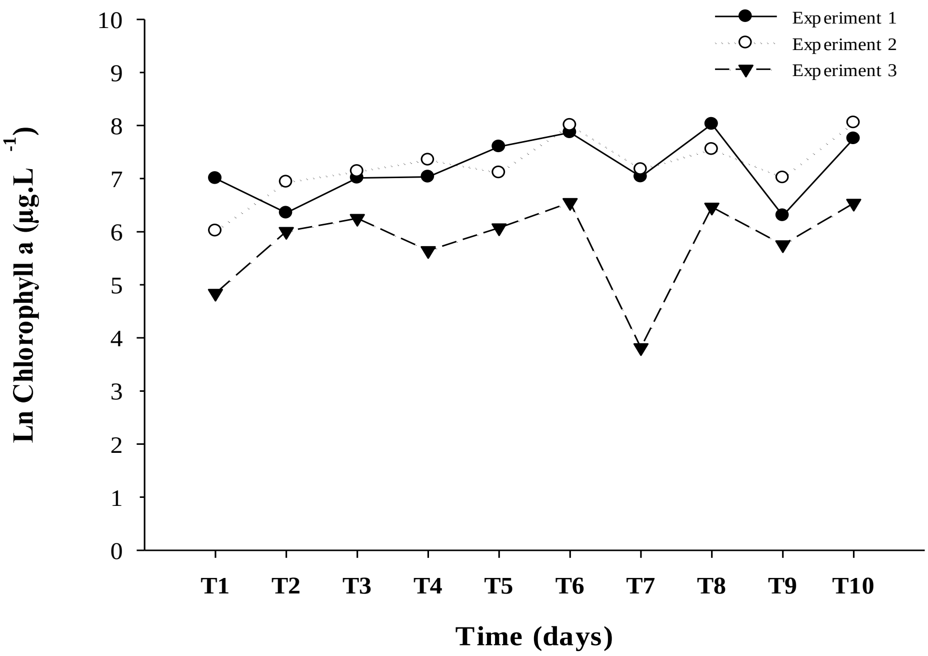
Figure 3. Boxplot of the growth of *Anagnostidinema amphibium* LBABL-2 at the wavelengths of 680 nm and 750 nm in experiments 1, 2 and 3.



Source: Authors (2022).

Chlorophyll-a varied significantly between times in experiments 1 (H= 21.3; df= 9; p= 0.001) and 2 (H= 17.34; df= 9; p= 0.04) by generally increasing chlorophyll-a concentrations over time (Figure 4).

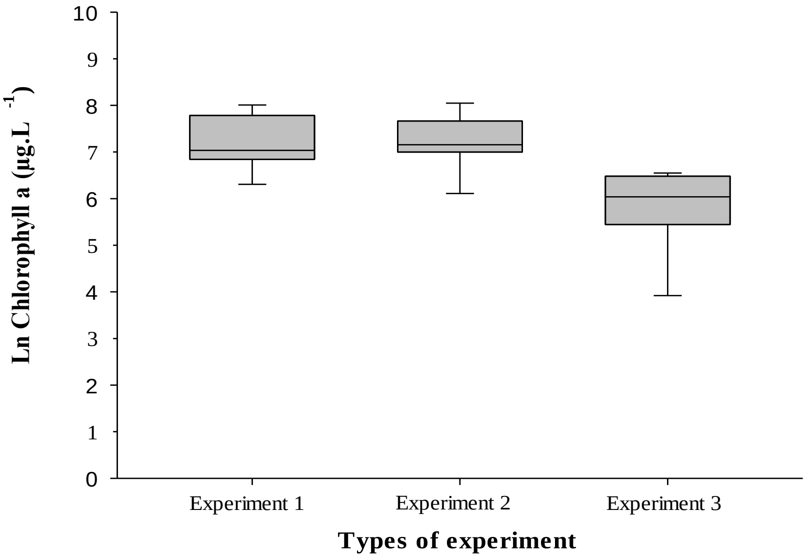
Figure 4. Graph of the growth of *Anagnostidinema amphibium* LBABL-2 by Chlorophyll-a analysis.



Source: Authors (2022).

Experiments 1 (7.2 ± 0.6 µg.L^{-1}) and 2 (7.2 ± 0.6 µg.L^{-1}) showed higher chlorophyll-a concentrations and, consequently, evidenced higher cyanobacteria growth (Figure 5). Experiment 3 (5.8 ± 0.96 μg.L^{-1}) was significantly lower than the other experiments (F= 15.98; df= 2; p= 0.001).

Figure 5. Boxplot of *Anagnostidinema amphibium* LBABL-2 growth from Ln (natural logarithm) transformed chlorophyll-a analysis.



Source: Authors (2022).

4. Discussion

Nutrients are usually important factors controlling the distribution and proliferation of planktonic and benthic cyanobacteria in aquatic ecosystems since they participate in the synthesis and basic functions of many cellular components (Paerl, 2008).

Therefore, this explains the good development of *A. amphibium* in macrophytes submerged in the reservoir water (experiment 1) and when they are removed from the Bolonha reservoir (experiment 2) and exposed to sunlight and high humidity. However, as the nutrients are removed, by leaching, the species begins to slow its growth as suggested in experiment 3, which indicated the nutrients, nitrogen, and phosphorus, as limiting factors to the growth of *A. amphibium* LBABL-2.

However, nutrient limitation depends on the cyanobacterial species, as some species develop strategies to get nutrients such as heterocysted cyanobacteria that produce specialized cells to capture atmospheric nitrogen and benefit from nitrogen depletion in the medium (Calandrini et al., 2019).

In addition to nutrients, the availability of light, temperature, oxygen, pH, herbivory, and water column stability are also factors that coordinate the development of cyanobacteria (Paerl, 2008).

Temperature is one of the factors most suggested as limiting factors for cyanobacteria and is directly related to their growth in laboratory assays. Thus, Mesquita et al. (2020) observed increased growth of the cyanobacteria *Microcystis aeruginosa* and *Planktothrix agardhii* associated with increasing temperature.

Combined environmental factors such as luminosity and temperature are also mentioned as directly acting on the growth of cyanobacteria. In this regard, Jodłowska and Latała (2013) evaluated the population growth of *Geitlerinema amphibium* (BA-13) (now *A. amphibium*) from optical, cellular and photosynthetic pigment density under different combined abiotic stress conditions (temperature versus luminosity), which found that the species obtained higher biomass concentration when subjected to high luminosity, 90- 125 μmol (photon) m^{-2} s^{-1}, and temperature of 22-30°C, suggesting temperature as a limiting factor.

Similar results were found by Moura et al. (2017) in microcosm with *A. amphibium* from a bloom in Goiás (Brazil), which had high productivity (chlorophyll-a) at elevated temperatures.

Nutrients and temperature influenced the growth and dominance of the cyanobacteria species *Merismopedia tenuissima* from the Fenhe River, China (Yang et al., 2019), like the present study which took place under high temperature and humidity conditions simulating the Amazonian climate.

Experiment 1, which simulated submerged cyanobacteria, showed a typical bacterial growth curve, which may mean that this is the best condition for *A. amphibium*. Despite being a benthic species, it benefits from the condition of eutrophic (Cunha, 2013) to mesotrophic (Diniz et al., 2022) aquatic environments.

It is worth noting that *Anagnostidinema amphibium* is a cosmopolitan species, typically benthic, periphytic associated with aquatic macrophytes and that reach high abundances in the shallower portions of the littoral zone of reservoirs (Franceschini et al, 2010; Santos & Ferragut, 2018; Gaget et al., 2017), being able to inhabit turbid to mixed layers, being tolerant to high light deficiency conditions, but sensitive to turbulence, characteristics that fit the species to Reynolds functional group S1 as suggested by Braga & Becker (2020).

Therefore, it adapts well in shallow environments such as the Bolonha reservoir, where the species is found among macrophytes and well adapted to the climatic conditions of the region (Calandrini et al., 2020). *Anagnostidinema* prefers high nutrient contents and high temperature (Singh et al., 2014). Confirming these last two factors combined as responsible for the growth of *A. amphibium* in the present study.

The species *Geitlerinema amphibium* (now *Anagnostidinema amphibium*) has been described as a producer of cyanotoxins in reservoirs of northeastern Brazil and has been identified as a producer of microcystins and cylindrospermopsins, besides being a potential producer of saxitoxins, β- methylaminoalanine, which toxin can cause neurodegenerative diseases by bioaccumulation through consumption of foods containing β- methylaminoalanine (Bittencourt-Oliveira, 2014; Conserva, 2011; Cox, 2003; Dogo et al., 2011; Genuário et al., 2019).

Therefore, it is known that the strain *A. amphibium* LBABL-2 is resistant to the cleaning process of the Bolonha reservoir and can return to the water. However, more tests are needed to better understand the development of these organisms in a future projection of the reservoir, since the environment is going through serious sanitation and siltation problems. Furthermore, *A. amphibium* LBABL-2 is one of the few strains of cyanobacteria isolated in the Brazilian Amazon, a region with few studies on cyanobacteria, and it is important to continue with research on the toxicity of the species.

We suggest improving the test models for benthic cyanobacterial species, since many factors evaluated act predominantly on planktonic species. For this, we need to evaluate the changes occurring in the natural environment that have been promoting toxic blooms by benthic cyanobacterial species in recent years and consider these factors in the trials.

5. Conclusion

The microcosm experiment showed that the species has good growth associated with the conditions of the aquatic environment of the Bolonha reservoir, but low growth in the absence of nutrients (nitrogen and phosphorus). On the other hand, we can say that even under stress conditions the species does not alter its morphology and morphometry. Therefore, the species is considered resistant and probably should return to the reservoir waters during surface runoff caused by rainfall.

Acknowledgments

National Council for Scientific and Technological Development- CNPq and Instituto Evandro Chagas- IEC for the granting of the Scientific Initiation funding.

References

American Public Health Association. (APHA), American WWAAWE, Federation (WEF). (2017) *Standard methods for the examination of water and wastewater***.** 23th ed. Washington, DC: American Public Health Association.

Araújo Júnior, A. C. R. (2015). Indicadores de qualidade ambiental no lago Bolonha, parque estadual do Utinga, Belém-Pará. *Boletim Gaúcho de Geografia*, 42(1), 276-299.

Bittencourt-Oliveira, M., Piccin-Santos, V., Moura, A. N., Aragão-Tavares, N. K. C. & Cordeiro-Araújo, M. K. (2014). Cyanobacteria, microcystins and cylindrospermopsin in public drinking supply reservoirs of Brazil. *Anais da Academia Brasileira de Ciências*, 86(1), 297-310.

Borges, H. L. F. Branco, L. H. Z., Martins, M. D., Lima, C. S., Barbosa, P. T., Lira, G. A. S. T., Bittencourt-Oliveira, M. C. & Molica, R. J. R. (2015). Cyanotoxin production and phylogeny of benthic cyanobacterial strains isolated from the northeast of Brazil. *Harmful Algae*, 43, 46-57.

Bouma-Gregson, K., Power, M. E. & Bormans, M. (2017). Rise and fall of toxic benthic freshwater cyanobacteria (Anabaena spp.) in the Eel river: Buoyancy and dispersal. *Harmful Algae*, 66, 79-87.

Brasil, J., Attayde, J. L., Vasconcelos, F. R., Dantas, D. D. F. & Hussar, V. L. M. (2016). Drought-induced water-level reduction favors cyanobacteria blooms in tropical shallow lakes. *Hydrobiologia*, 770(1), 145–164.

Braga, G. G. & Becker, V. (2020). Influence of water volume reduction on the phytoplankton dynamics in a semiarid m an-made lake: A comparison of two morphofunctional approaches. *Anais da Academia Brasileira de Ciências*, 92(1), e20181102.

Brito, F. S. L., Pimentel, B. A., Vilhena, J. C., Rosário, K. K. L., Morais, M. S., Cruz, R. H. R. & Corrêa, V. L. S. (2020). Comportamento das variáveis físico-químicas da água do lago Bolonha-Belém-PA. *Brazilian Journal of Development*, 6(1), 1738-1757.

Calandrini, G. S. M., Pinto, L. G. C., Gomes, A. L., Pinheiro, S. C. C., Cunha, C. J. S., Costa-Tavares, V. B. & Sousa, E. B. (2019). *Efeito da Ausência de Nutrientes sobre o Crescimento da Cepa Nostoc sp. LBABL-2 (Cyanophyceae) do Reservatório Bolonha (Belém, Pará).* Anais do XVII Congresso Brasileiro de Limnologia e do 2º Congresso Ibero-Americano de Limnologia, Florianópolis, 04 a 09 de agosto de 2019. – Concórdia, SC: ABLimno: Embrapa, 321p.

Calandrini, G. S. M., Gomes, A. L., Costa-Tavares, V. B., Pinheiro, S. C. C. & Sousa, E. B. (2020). *Isolamento, Cultivo e Caracterização Morfológica de Geitlerinema amphibium C. Agardh ex Gomont (Cyanophyceae) do Reservatório Bolonha (Belém-PA).* p. 1-388–416. In: Silva, M. E. D. (org) O Meio Ambiente e a Interface dos Sistemas Social e Natural 2 [Recurso Eletrônico]. Ponta Grossa, PR: Editora Atena.

Catherine, Q., Madeira, S., Echenique-Subiabre, I., Heath, M., Villeneuve, A. & Humbert, J. F. (2013). A review of current knowledge on toxic benthic freshwater cyanobacteria–ecology, toxin production and risk management. *Water research*, 47(15), 5464-5479.

Conserva, G. A. A., Sant'Anna, C. L., Cambui, C. C. N., Brunetti, R. L., Rangel, M., Torres, L. M. B., Young, C. M. & Carvalho, L. R. (2011). *Prospecção de atividades toxicológicas e farmacológicas em cepas de cianobactérias da Coleção de Culturas do Instituto de Botânica*. Proceedings of the 18ª Reunião Cientifica Anual do Instituto de Botânica, Sao Paulo, Brazil, p. 21-25.

Cox, P. A., Banack, S. A. & Murch, S. J. (2003). Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. *Proceedings of the National Academy of Sciences*, 100(23), 13380-13383.

Cunha, C. J. S. (2013). *Variação Espacial e Temporal do Fitoplâncton do Reservatório da Usina Hidrelétrica de Tucuruí - Pará.* Dissertação de Mestrado, Programa de Pós-Graduação em Ecologia Aquática e Pesca, Universidade Federal do Pará. Belém-PA. 119p.

Faassen, E. J., Harkema, L. Begeman, L. & Lurling, M. (2012). First report of (homo) anatoxin-a and dog neurotoxicosis after ingestion of benthic cyanobacteria in The Netherlands. *Toxicon*, 60(3), 378-384.

Gaget, V., Humpage, A. R., Huang, Q., Monis, P. & Brookes, J. D. (2017). Benthic cyanobacteria: A source of cylindrospermopsin and microcystin in Australian drinking water reservoirs. *Water research*, 124, 454-464.

Genuário, D. B., Vaz, M. G. M. V., Santos, S. N., Kavamura, V. N. & Melo, I. S. (2019). *Chapter 16 - Cyanobacteria From Brazilian Extreme Environments: Toward Functional Exploitation*. pp. 265-284. *In*: Das, S., & Dash, H. R. (Ed.). Microbial diversity in the genomic era. Academic Press, E-book. Chapter 16. Academic Press.

Hammer, Ø., Harper, D. A. T. & Ryan, P. D. (2001). Past: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1), 1-9.

Han, J., Jeon, B.S., Futatsugi, N. & Ho-Dong, P. (2013). The effect of alum coagulation for in-lake treatment of toxic Microcystis and other cyanobacteria related organisms in microcosm experiments. *Ecotoxicology and Environmental Safety*, 96, 17-23.

Jodłowska, S. & Latała, A. (2013). Combined effects of light and temperature on growth, photosynthesis, and pigment content in the mat-forming cyanobacterium *Anagnostidinema amphibium*. *Photosynthetica*, 51(2), 202-214.

Komárek, J. & Anagnostidis, K. (2005). *Cyanoprokaryota 2. teil/ 2nd part: Oscillatoriales***.** In: BÜDEL, B. et al. (Ed.). Sußwasserflora Von Mitteleuropa 19/2. Heidelberg: Elsevier/Spektrum, 759p.

Kuiper-Goodman, T., Falconer, I. & Fitzgerald, J. (1999). *Human health aspects.* p.113-153. *In*: CHORUS, I. E BARTRAM, J. (eds.) Toxic cyanobacteria in water - A Guide to their Public Health Consequences, Monitoring and Management. E&FN Spon, Londres.

Li, Z., Zhao, Y., Xu, X., Han, R., Wang, M. & Wang, G. (2018). Migration and transformation of dissolved carbon during accumulated cyanobacteria decomposition in shallow eutrophic lakes: a simulated microcosm study. *PeerJ*, 6, e5922.

Mesquita, M. C. B., Prestes, A. C., Gomes, A. M. A. & Marinho, M. M. (2020). Direct effects of temperature on growth of different tropical phytoplankton species. *Microbial Ecology*, 79(1), 1-11.

Moura, M. E. P. DE, Rocha, L. D. S. & Nabout, J. C. (2017). Effects of global climate change on chlorophyll-a concentrations in a tropical aquatic system during a cyanobacterial bloom: a microcosm study. *Ambiente e Água - An Interdisciplinary Journal of Applied Science*, 12(3), 390.

O'Farrell, I. Motta, C., Forastier, M., Polla, W., Otano, S., Meichtry, N., Devercelli, M. & Lombardo, R. (2019). Ecological meta-analysis of bloom-forming planktonic cyanobacteria in Argentina. *Harmful algae*, 83, 1-13.

Oliveira, G. M. T. S., Oliveira, E. S., Santos, M. L. S., Melo, N. F. A. C. & Krag, M. N. (2018). Concentrações de metais pesados nos sedimentos do lago Água Preta (Pará, Brasil). *Engenharia Sanitaria e Ambiental*, 23, 599-605.

Pará. (2013). *Secretaria de Meio Ambiente e Desenvolvimento Sustentável. Revisão do Plano de Manejo do Parque Estadual do Utinga.* Belém: Imazon.

Paerl, H. (2008). *Nutrient and Other Environmental Controls of Harmful Cyanobacterial blooms along the freshwater-marine continuum***.** p. 217-237. In: Hudnell, H. K. (ed.). Cyanobacterial Harmful Algal Blooms: State of Science and Research Needs. Advances in Experimental Medicine and Biology, 619, 217-213.

Pedrosa, C. S. G., Souza, L. R. Q., Gomes, T. A., Lima, C. V. F., Ledur, P. F., Karmirian, K., Barbeito-Andres, J., Costa, M. N., Higa, L. M., Rossi, A. D., Bello, M., Tanuri, A., Prata-Barbosa, A., [...], & Rehen, S. K. (2020) The cyanobacterial saxitoxin exacerbates neural cell death and brain malformations induced by Zika virus. *PLOS Neglected Tropical Diseases*, 14(3), p. e0008060.

Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M. & Stanier, R. Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Microbiology*, 111(1), 1-61.

Silva, G. M., Gomes, A. L., Cunha, C. J. S., Costa Tavares, V. B., Pinheiro, S. C. C. & Sousa, E. B. (2020). Caracterização de *Nostoc* sp. LBALBR-2 (Cianobactéria) isolada das raízes de macrófitas do reservatório Bolonha (Belém-Pará) sob diferentes condições de temperatura, luz e umidade. *Brazilian Journal of Animal and Environmental Research*, 3(4), 4242-4256.

Singh, Y., Khattar, J. I. S., Singh, D. P., Rahi, P. & Gulati, A. (2014). Limnology and cyanobacterial diversity of high altitude lakes of Lahaul-Spiti in Himachal Pradesh, India. *Journal of Biosciences*, 39, 643–657.

Sousa, E. B. (2017). *Fatores ambientais reguladores da dinâmica do fitoplâncton e das cianobactérias dos mananciais de abastecimento da região metropolitana de Belém, Pará, Brasil*. Tese (Tese em Saúde coletiva), Instituto de Estudos em Saúde Coletiva, Rio de Janeiro. 235p.

Strunecký, O., Bohunická, M., Johansen, J. R., Čapková, K., Raabová, L., Dvořák, P. & Komárek, J. (2017). A revision of the genus *Geitlerinema* and a description of the genus *Anagnostidinema* gen. nov. (Oscillatoriophycidae, Cyanobacteria). *Fottea*, 17(1), 114-126.

Svirčev, Z., Drobac, D., Tokodi, N., Mijovic, B., Codd, G. A. & Meriluoto, J. (2017). Toxicology of microcystins with reference to cases of human intoxications and epidemiological investigations of exposures to cyanobacteria and cyanotoxins. *Archives of Toxicology*, 91(2), 621-650.

Vieira-Lanero, R., Barca, S., Cobo, M. C. & Cobo, F. (2022). Occurrence of freshwater cyanobacteria and bloom records in Spanish reservoirs (1981–2017). *Hydrobiology*, 1(1), 122-136.

Wall, J., Madeira, S. A., Orlovich, D. A., Rodes, L. L. & Summerfield, T. C. (2014). Characterisation of freshwater and marine cyanobacteria in the Hokianga region, Northland, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 48(2), 177–193.

Wang, H., Li, H., Sun, K., Huang, H., Zhu, p. & Lu, Z. (2020). Impact of exogenous nitrogen on the cyanobacterial abundance and community in oil-contaminated sediment: A microcosm study. *Science of The Total Environment*, v. 710, p. 136296.

Yang, J., Wang, F., Lv, J., Liu, Q., Nan, F., Liu, X., Xu, L., Xie, S. & Feng, J. (2019). Interactive effects of temperature and nutrients on the phytoplankton community in an urban river in China. *Environmental Monitoring and Assessment*, 191(11), p. 1-16.

Yu, H., Dong, X., Yu, D., Liue, C. & Shufeng, V. (2019). Effects of eutrophication and different water levels on overwintering of eichhornia crassipes at the northern margin of Its distribution in China. *Frontiers in Plant Science*, 10, 1261.

Zubia, M., Vieira, C., Palinska, K. A., Roué, E., Gaertner, J. C., Zloch, I., Grellier, M. & Golubicre, S. (2019). Benthic cyanobacteria on coral reefs of Moorea Island (French Polynesia): diversity response to habitat quality. *Hydrobiologia*, 843(1), 61-78.