**CYANOBACTERIA AS REGULATORS OF METHYLMERCURY PRODUCTION
IN PERIPHYTON**

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Highlights

- highest MeHg production in periphyton microcosms with added cyanobacteria
- the highest net MeHg production in microcosms followed the order DOM > N > P > N > cyanobacteria
- positive correlation between MeHg production and primary productivity
- cyanobacteria regulate MeHg production in wetlands

Graphical abstract

ABSTRACT: Biotic mercury (Hg) methylation appears to depend on factors such as microbial activity and the concentration and bioavailability of Hg^{2+} to the Hg-methylating organisms. Recently, the presence of cyanobacteria has been linked with high methylmercury concentrations. The aim of this work was to test MeHg production in microcosms, in relation to the amount of periphytic cyanobacteria, dissolved organic matter (DOM) and phosphorus concentrations, as well as periphytic primary production rates. Water and periphyton samples were collected for cultivation and isolation of cyanobacteria from the Guaporé River floodplain. We cultivated the periphyton in microcosms with different concentrations of cyanobacteria, total phosphorus and DOM. The highest net MeHg production (6.8 to 24.6% of added Hg d^{-1}) occurred in the microcosm with added cyanobacteria, followed by microcosms with added phosphorus (6.1 to 11.4%) and added DOM (6.4 to 9.1%). Positive correlations were found between MeHg production, addition of cyanobacteria, phosphorus and DOM and periphytic primary productivity. Our results bring the first direct experimental evidence of the relevance of cyanobacteria and primary production as regulators of MeHg production in periphyton. These findings have numerous implications for the management of natural and engineered wetlands.

Keywords: Microcosm, Hg, primary productivity, phosphorus, DOM.

1. INTRODUCTION

Mercury (Hg) is one of the most toxic heavy metals and its methylated organic form, methylmercury (MeHg), despite its low concentrations in the environment, can be accumulated and magnified in aquatic food webs (Selin, 2009). MeHg accumulation in aquatic food webs is of major concern because human exposure to Hg takes place mainly via the intake of MeHg through fish consumption (UNEP 2013). The formation of MeHg (i.e. Hg methylation) is mediated by biotic mechanisms, mainly by the activity of microorganisms of various metabolic types such as sulfate-reducing bacteria (SRB) (Achá et al., 2012a; Gentes et al., 2013), iron-reducing bacteria (Fleming et al., 2007; Olsen et al., 2016), methanogenic archaea (Hamelin et al., 2011; Gilmour et al., 2013;) and other bacteria (Achá et al., 2012b; Gilmour et al., 2013). Hg methylation depends on several factors such as the temperature, pH, salinity, redox potential, and the presence of inorganic and organic complexing agents (Ulrich et al., 2001). The concentration and bioavailability of Hg (Jonsson et al., 2014) and activity of the microbial communities (Bravo et al., 2017) have been identified as critical factors controlling Hg methylation rates in aquatic systems. In this context organic matter (OM) plays a crucial role as most of the organisms involved in Hg methylation rely on OM (Gilmour et al., 2013). Also it has been shown that OM controls Hg bioavailability (Schaefer et al., 2009; Jonsson et al., 2014; Chiasson-Gould et al., 2013). Although Hg methylation is primarily biologically mediated, few studies have explored the linkage between MeHg production and the microbial activity (Bravo et al., 2017). Recently, it was shown that phytoplankton-derived organic compounds enhance Hg methylation rates through an overall

increase of bacterial activity (Bravo et al., 2017). Indeed, algae and cyanobacteria are important sources of thiols (e.g. cysteine and glutathione) (Ahner et al., 2002) and aromatic amino acids (Rattray et al., 1991) and are also known to boost bacterial activity (Kritzberg et al., 2004). The studies addressing their contribution to MeHg formation in aquatic systems are however still scarce.

The formation of MeHg might occur in oxygen-deficient conditions of many different aquatic compartments, such as sediments (Guimarães et al., 2000), anoxic and oxic (Achá et al., 2012a; Eckley and Hintelman, 2006; Gascon et al., 2016), water column, paddy soils (Zhao et al., 2016; Liu, 2018) and periphyton (Guimarães et al., 2000; Achá et al., 2011; Hamelin et al., 2015a). Recently Hg methylation in periphyton has attracted the attention of the scientific community because MeHg production appears to be one order of magnitude higher in periphyton than in other aquatic compartments (Guimarães et al., 2000; Gentes et al., 2013; Hamelin et al., 2015b; Leclerc et al., 2015). Periphyton is a complex matrix of algae, cyanobacteria, protozoa, bacteria, fungi, and particles that occurs on roots and other substrates, such as rocky surfaces, in aquatic ecosystems (Wetzel, 2001). As a result, periphytic matrixes are enriched in photosynthetic (algae and cyanobacteria) derived OM and present severe oxygen gradients (Hagerthey et al., 2011), both Hg methylating prone conditions. Actually, the colonization of periphyton by cyanobacteria has been associated with a higher MeHg production in floodplain lakes (Lázaro et al., 2013; Lázaro et al., 2018). The abundance and diversity of cyanobacteria in the periphyton can be controlled by several factors, such as higher phosphorus intake, higher temperature and lower incidence of light in the water

column. In addition, a reduction of periphyton MeHg production has been observed when primary production is inhibited (Hamelin et al., 2011; Hamelin et al., 2015b, Olsen et al, 2016). However, the quantitative impact of changes in the relative abundance of algae or cyanobacteria in the composition of the periphyton on Hg methylation in aquatic systems remains to be addressed. Understanding the biological formation of MeHg in periphyton is therefore critical to improve our ability to predict the accumulation and biomagnification of this pollutant in food webs. We hypothesized that a higher relative abundance of cyanobacteria might increase MeHg formation in periphyton. To test this, we measured MeHg net production in microcosms, under different amounts of naïve periphytic cyanobacteria, concentrations of DOM and phosphorus and periphytic primary production rates, trying to isolate the contribution of each factor in order to explain MeHg production in the periphyton.

2. MATERIALS AND METHODS

2.1 Construction and maintenance of laboratory periphytic

microcosms. In order to mimic environmentally realistic conditions, our microcosms were built with: i) water of the Guaporé River floodplain, ii) periphyton and iii) cyanobacteria collected from the flooded environments. The Guaporé River Basin, located in the northwestern part of the state of Mato Grosso, is part of the Amazon Basin and a tributary of the Madeira River. Throughout its course, the Guaporé River presents several small inundations lakes with varied hydrological, morphological and biological characteristics, presenting high MeHg net production

potential (Lázaro et al., 2016; 2018). The sampling was carried out in October 2015, in the dry season, in 10 oxbow lakes in the Guaporé River (Figure 1), to ensure a high variability of organisms and bacterial strains. At each sampling site, samples of *Eichhornia crassipes* Mart rhizomes/periphyton and 20 L of local water were collected. The samples were packed in zip-lock® bags and kept in ice-boxes until incubation, and stored in acid-washed light-proof polyethylene terephthalate (PET) bottles respectively. The water samples were filtered in a phytoplankton 20µm mesh and kept under refrigeration (4°C to control bacterial activity) until their use in the cultivation experiments. Data of the total variation of the limnological characteristics of the Guaporé river lakes and mean values of the variables of the collected water samples are given in Tables 1 and 2 (sup.material).

Figure 1 – Location of the water and periphyton sampling area for microcosm construction and laboratory cyanobacterial cultures.

For the periphyton cultures, sixty (60) open borosilicate glass microcosms (1 L) consisted in filtered (0.45 µm polypropylene) water and periphyton samples were set up. In order to minimize microcosm variability, the periphyton samples were homogenized on shaker tables and petri dishes under optical microscopy. Shredded nylon cords were used as substrate for fixation of the periphyton, due to the similarity of its shape to the rhizome system of *E. crassipes*. The microcosms were kept under controlled temperature (30 ± 1 °C), photoperiod (10h, $300 \mu\text{Em}^{-2}\text{s}^{-1}$) and water flow (water pump) throughout the growth phase. The physical and

chemical variables of the microcosms and nutrient (nitrogen and phosphorus) concentrations were monitored daily. The microcosm water was renewed every 2 days in order to minimize water chemistry changes. The period of maturation of the periphyton was 22 days on average, determined by direct microscopic observation, following the succession stages until the community achieved its climax (Lowe, 1996).

2.2 Enrichment of the periphyton with cyanobacteria from the field.

A total of 30 microcosms were used to determine the role of cyanobacteria-rich periphyton on Hg methylation. Cyanobacteria communities were isolated from the field and amended to periphytic microcosms. In particular, the cyanobacteria were isolated from the field samples by consecutive dilution (15 times) and subsequent manual separation made under an inverted microscope by means of Eppendorf® microcapillary tubes (30 and 50 µm internal and external diameter respectively) and a manual suction pump (Pringsheim, 1946). This technique is the most commonly used for manual separation of microorganisms (Droop, 1969). No eukaryote or prokaryote antibiotic was used in the isolation process. After separation, the cyanobacteria were cultured in a specific ASM-1 medium (Gorhan et al., 1964), and nutrients were subsequently amended to match the same concentrations of the Guaporé River water used in the microcosms (Table 2, sup. material).

We decided not to separate cyanobacteria groups in order to guarantee the same composition as that of the field samples. As a result, the cultures were multi-specific and at different stages of growth representing natural cyanobacterial communities.

Once the cyanobacteria were grown, they were further diluted (30 times) in order to eliminate the culture medium. Aliquots of different concentrations (dilutions ranging in 1:100 - 1:10, Table 3 sup. material) of cyanobacteria were then mixed with the periphyton cultures by direct inoculation of the cyanobacteria inside the periphytic matrices by means of microcapillary tubes. Daily microscopic observation confirmed the increase of the inoculated colonies and individuals in the matrices and therefore the successful inclusion of cyanobacteria in the periphyton. At the end of the 15 days of culture and mixing (maximum and minimum values of cyanobacteria in the microcosm's periphyton nearly equal to maximum and minimum values found in the field), samples of all microcosms were taken for the Hg methylation assays and other tests. Two microcosms were not spiked with aliquots of cyanobacteria cultures and constitute experimental controls (Table 3, sup.material)

2.3 Methylation tests with cyanobacteria of pure cultures. In order to test the possibility of Hg methylation by cyanobacteria, we measured net Hg methylation in samples of pure cyanobacteria cultures (*Anabaena* sp. strain 1403/21 and *Planktothrix agardhii*, strain 1459/11A, provided by CCAP, UK, two very common cyanobacteria associated with *E.crassipes* in tropical areas) (N=40) at different organisms densities. The strains were maintained in ASM-1 medium. Aliquots of the culture media were washed and suspended in water from the Guaporé river lakes prior to the methylation tests. From these, sub aliquots were taken to dilute the organisms at biovolume levels similar to those measured in the field cyanobacteria-based periphyton cultures.

2.4 Cyanobacteria counting. Before incubation with $^{203}\text{HgCl}_2$ and Me^{203}Hg extraction, all samples were observed by optical microscopy for counting and measurement of cyanobacteria cells. We expressed the values in biovolume, following Sun and Liu (2003).

2.5 Enrichment of the microcosm's periphyton with river DOM and phosphorus. In addition to the cyanobacteria enrichment treatment, we also enriched other 30 microcosms with different concentrations of dissolved organic matter (DOM) isolated from the water collected in the field (N = 15) and phosphorus supplements (N = 15) in microcosms without cyanobacterial enrichment. The final concentrations of the microcosms nutrients are summarized in table 4 (sup. material) and are in the same variation range of the natural concentration of the Guaporé River lakes in the dry and flood phases of their hydroperiod (Table 1, sup.material). Three microcosms did not receive nutrient addition (1 for phosphorus and 2 for DOM), constituting experimental controls.

DOM isolation was carried out in the field with a reverse osmosis membrane system (Serkiz and Perdue, 1990). Around 200 L of water from the Guaporé River were filtered *in situ* through a 0.45 μm and 0.22 μm nylon membrane filter set, in order to obtain the DOM extracts. Then, these filtrates were packed in PET bottles and kept in an ice-cold box. Once in the laboratory, the concentrations of dissolved organic carbon (DOC) in the DOM extracts were determined with a Shimadzu TOC-

5000 analyzer with ASI-5000 automatic sampler and a Balston 78-30 gas generator, following standard procedures (USEPA, 2005). The DOM concentrate was neither heat sterilized nor isolated from the inorganic solutes to maintain its original characteristics.

2.6 Methylation assays. The Hg methylation tests followed Guimarães *et al.* (1995). Unwashed, freshly sampled periphyton clusters were collected, homogenised and subdivided for the analyses of Hg Methylation and Primary Productivity (section 2.7). For the methylation assays, clusters (equivalent to 0.5 g dry weight) from all microcosms (n=60) and mixed sub aliquots of the pure cyanobacteria strains (n=40) were suspended in 30 mL of water sampled from Guaporé lakes, previously filtered in 0.45 µm porosity membranes, in 50 mL screw cap glass tubes with 20,000 to 30,000 DPM (disintegrations per minute) of added $^{203}\text{HgCl}_2$, obtained from Eckert-Ziegler Isotopes Laboratory, USA. Samples were incubated 24 h, in area covered by shading screen (Sombrite® 70%) under 30°C, and controled photoperiod (10h, 300 µEm⁻²s⁻¹). Control samples as above were killed with 1 mL of 4 N HCl. The ^{203}Hg spikes contained 15 to 23 ng of total Hg, less than doubling the sample natural total Hg. This Hg addition level is comparable or better than found in recent studies using enriched stable isotopes as Hg tracers (Gentes *et al.*, 2013; Hamelin *et al.*, 2015b; Olsen *et al.*, 2016; Cesario *et al.*, 2017).

The methylmercury formed was extracted by addition of 4 mL of NaBr (3 mol L⁻¹ in 11% H₂SO₄) and 1 mL of 0.5 mol L⁻¹ CuSO₄, with 15 min shaking followed by

centrifugation. The supernatant was transferred to separation funnels, and Me²⁰³Hg was extracted by shaking for 15 min with 30 mL of scintillation cocktail, consisting of POP (2,5- diphenyloxazole) and POPOP [1,4-bis-2-(5-phenyloxazolyl)-benzene] salts dissolved in toluene. The organic phase was removed and sodium thiosulphate added to remove any trace of water. Me²⁰³Hg was quantified by beta spectrometry in a Perkin Elmer Tricarb 2800 liquid scintillation counter, with correction for extraction efficiency and radioactive decay. External standard counting mode was used to monitor and correct quenching and counting efficiency. Counting time was set to keep counting error under 1%. Detector efficiency was regularly checked with ¹⁴C standards, once ¹⁴C and ²⁰³Hg beta emissions are in the same energy range".

2.7Measurements of microcosm primary production. The primary production of all microcosms was measured by the light and dark bottles method (Strickland and Parsons, 1972). Periphyton clusters (equivalent of 8g dry weight) were suspended in 500 ml of the Guaporé lakes water and incubated in the same conditions of the Hg methylation incubations. The net primary production was calculated by subtracting the initial O₂ concentration measured at time zero from the measured O₂ concentration after incubation. The respiration of the microcosms was measured by the O₂ consumption of the microcosm samples incubated in dark bottles.

2.8 Statistical analyses. The possible relationships between periphyton cyanobacteria biovolume, DOM and phosphorus concentrations and MeHg production were evaluated by Linear Regression models. We used all microcosms as sample units (n=30 for Cyanobacteria amendment and n=15 for DOM and P, consecutively). In order to compare MeHg production among pure cultures with different cyanobacteria concentrations (n=40, five samples in eight treatments) we used the One-Way ANOVA. To determine the relation between MeHg net production and periphyton primary production in the treatments of DOM, phosphorus and cyanobacteria biovolume (n=60), we used Polynomial Regression adjustments for several measurements, which allow the verification of the weight of each variable (% of variation) in the model. The analyses were carried out in statistical environment R, version 3.1.

3. RESULTS AND DISCUSSION

3.1 Acid Killed controls: The Me²⁰³Hg production between the dead controls ranged from 0 to 0.05%. so, we did not express them in the graphs.

3.2 Effect of DOM. The net Hg methylation rates, in the DOM amendment series, varied between 6.4 and 9.1%. There was a significant positive relation between DOM concentration and net Me²⁰³Hg production ($R^2 = 0.611$, $p = 0.001$) (Figure 2a). The DOC concentration in the DOM extracts was $96 \pm 1.4\%$. OM interacts with Hg in several different ways, affecting its transport, transformations and bioavailability

(Ulrich et al., 2001; Driscoll et al., 1995). DOM can either stimulate or inhibit Hg methylation, depending on environmental conditions (Miller et al., 2009). In recent years the majority of the studies have assumed that under equilibrium conditions, DOM decreases the bioavailability of Hg (Chiasson-Gould et al., 2014). This is due to the reduction of the activity of free metal ions, through metal-DOM associations under pseudo-equilibrium conditions, as well as to the proportion of metal bonds to active biological absorption sites, once the metal-DOM complex molecules become potentially too large to cross the membranes of bacterial cells (Graham et al., 2012). However, the literature also shows positive (Driscoll et al., 1995) or even bell-shaped relationships (Chiasson-Gould et al., 2014) between DOM and MeHg production. Kinetic reactions also need to be considered in the predictions of Hg-DOM complexation reactions (Miller et al., 2009; Chiasson-Gould et al., 2014). Our results are in good agreement with a study that showed increased Hg availability under non-equilibrium conditions and below 10 mg L⁻¹ (Chiasson-Gould et al., 2014). Graham *et al* (2012) also proposed a possible mechanism for the apparent positive correlation between DOM and methylation, showing that in low oxygen environments and in the presence of sulfide, DOM can act as a synergistic MeHg production agent, behaving as a facilitating compound for the formation of β -HgS nanoparticles by inhibiting the formation of polynuclear Hg-S aggregates. These nanoparticles are labile and make Hg bioavailable to the methylating microbiota²². Further studies have shown that an increase in DOC levels may promote Hg methylation by increasing the SRB activity, since DOC may serve as an important carbon source for these Hg-methylating bacteria (Davis et al., 1997; Lambertsson

and Nilson, 2006). In summary, although it is difficult to conclude about the main factor driving the positive relationship between DOM and MeHg production in periphyton, our results (Fig. 2a) confirm the role of DOM on MeHg formation in peryphyton.

Figure 2a – Effect of concentrations of Dissolved Organic Matter on the production of MeHg by the culture of periphyton.

3.3 Effect of phosphorus. The MeHg production rates, in the phosphorus amendments series, ranged from 6.1 to 11.4%. We found a significant positive relationship between the added phosphorus concentrations and the net MeHg production ($R^2 = 0.521$; $p = 0.04$) (Figure 2b). Bacteria are generally regulated by DOC (Malone, 1977) but may also be limited by nitrogen and phosphorus (Thingstad et al., 1998). The association between Hg methylation rates and phosphorus concentrations (Figure 2b) can be explained by the stimulating effect of the nutrients on periphytic primary production and consequently on Hg methylation (Figure 3), as documented by Hamelin *et al.* (2011) and Lázaro *et al.* (2013, 2018). It is well known that phosphorous inputs increase primary production, resulting in dramatic cyanobacteria and algae phytoplankton blooms. Recent studies have identified phytoplankton derived OM as the main driver of Hg methylation rates (Bravo et al., 2017, Soerensen et al., 2017; Gascon et al., 2017; Herrero Ortega et al., 2018). Cyanobacteria and algae blooms increases the concentration in thiols (Leclerc et al., 2015). This is key as thiols have a strong

affinity for Hg and low molecular mass thiols are prone to methylation in the environment. Therefore, phosphorous amendments might have increased the release of thiols groups from primary producers, subsequently enhancing Hg methylation (Leclerc et al., 2015). Moreover, phosphorous amendments might have boosted the activity of the whole microbial community and thereby Hg methylation (Bravo et al., 2017). In other words, phosphorus amendments might have increased Hg methylation by providing small available thiols groups (Leclerc et al., 2015) or by stimulating the activity of the Hg methylating microbiota or the communities supporting Hg-methylators (Bravo et al., 2017).

Figure 2b – Effect of concentrations of Phosphorus on the production of MeHg by the culture of periphyton.

3.4 Effects of field cyanobacteria addition to periphyton. The highest Hg methylation rates were obtained in the cyanobacterial microcosms, varying between 6.8 and 24.6%, indicating a significant positive relation between the production of MeHg and the periphyton cyanobacteria biovolume ($R^2 = 0.694$; $p = 0.001$) (Figure 3a). The MeHg production rates in cyanobacteria pure cultures were much lower than in the microcosms and ranged from 0.1 to 1.1% (Figure 3b). There was no significant variation in Hg methylation between treatments ($F_{(1, 39)} = 1.45$, $p = 0.341$)

Figure 3a – MeHg production under different cyanobacteria concentrations in field cultures.

Figure 3b – MeHg production under different cyanobacteria concentrations in pure cultures (control).

The low rates of MeHg net production in pure cultures of cyanobacteria demonstrate the incapacity of the group (or at least of the selected strains) as MeHg producers. Similar results have been recently reported for pure cultures of cyanobacteria *Fisherella* sp (Mendes et al 2018). In relation to cyanobacteria, the link between the production of MeHg and their biovolume may be due to the participation of cyanobacteria in providing habitat and conditions conducive to the establishment and activity of naïve Hg-methylating bacterial communities. Cyanobacteria are known to harbor bacterial communities in their mucilage sheaths (Brunberg, 1999; Kamjunke and Mehner, 2001), many of which may be active Hg-methylating agents such as SRB (Krekeler et al., 1998), methanogenic archeas and ferrogenic bacteria. The species composition and activity of the bacteria that colonize cyanobacteria may also vary, depending on environmental conditions (Watanabe et al., 1992). The colonization of cyanobacterial sheaths is also the key to understand the low Hg methylation levels in pure cultures in comparison to cyanobacteria cultures originating from the field, which a higher diversity of colonizing strains. This is why we did not use antibiotics during the preparation of the field cultures to avoid affecting the bacterial groups already established in the samples. Some misinterpretations remain concerning the periphyton and

377 cyanobacterial sheaths colonization by SRB and methanogenic archaeas. It is still
378 common to consider that SRB and methanogenic archaeas are strictly associated
379 to anoxia. This is due to the confusion between the terms oxic and anoxic (habitat)
380 and aerobic and anaerobic (metabolism). Both SRB and methanogenic archaeas
381 are anaerobic organisms, i.e. they do not use oxygen as an electron acceptor.
382 However, these organisms can be found in both anoxic and in oxic environments
383 (Baumgartner et al., 2006; Bogard et al., 2014), allowing their establishment even in
384 oxygen-saturated layers of the periphytic matrix. Indeed, a recent study proved
385 anaerobic metabolisms such as sulfate-reduction in microenvironments that
386 develop inside sinking organic aggregates of the ocean oxic waters (Bianchi et al.,
387 2018). Hg methylation has also been reported in the oxic open ocean (Cossa et al
388 2009; Heimbürger et al 2010; Lehnherr et al., 2011) and in lake oxic water (Gascon
389 et al. 2017). Peryphyton enriched in cyanobacteria can offer the SRB and
390 methanogenics a habitat and one of their main growth substrates, hydrogen.

391 Hydrogen production by cyanobacteria has been a long-known phenomenon
392 (Jackson and Elms, 1896; Benemann and Weare, 1974; Oshchepkov et al., 1974),
393 studied on a wide variety of strains from different environments (Lambert and Smith,
394 1981; Houchins, 1984; Kothari et al., 2012; Otaki et al., 2012). Hydrogen production
395 by cyanobacteria is associated with nitrogen fixation, H₂ being a byproduct (Bothe
396 et al., 2010), and as a result of nighttime fermentation of photosynthetic storage
397 products by oxygenic phototrophs (Moezelaar et al., 1996; Dutta et al., 2005, Saper
398 et al., 2018). Lee et al. (2014), found significantly reduction in hydrogen production

by Cyanobacteria microbial mats when treated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea, a metabolic photosynthesis inhibitor. Thus, the increase of cyanobacteria photosynthetic productivity leads to a greater H₂ availability to the bacterial communities, which can stimulate the activity of Hg methylating bacteria such SRB (Baumgartner et al., 2006, Lee et al, 2014) and methanogenic archaeas (Berg et al., 2014).

3.5 Periphyton net primary production and MeHg production rates. We found significant positive correlations between MeHg production and the increase of periphytic primary production caused by cyanobacteria and phosphorus additions (p=0.001 and p=0.003 respectively). However, the increasing trend is stronger for cyanobacterial additions (Weight = 0.68), pointing to a significant difference between treatments (p=0.03) (Figure 4). No significant relationship was found for DOM and MeHg production associated with primary productivity.

Figure 4 – Relation between periphytic primary production and MeHg production in periphyton of DOM, phosphorus and cyanobacteria amendments.

Our results confirms field experiments that positively correlated MeHg production with i) the amount of cyanobacteria in the periphyton (Lázaro et al., 2013;2018); ii) phototrophic productivity (Tsui et al., 2010; Hamelin et al., 2011; Lázaro et al., 2018) and greater autotrophy in periphyton systems (Hamelin et al., 2015b).

Furthermore, the positive correlation between the periphytic primary production and MeHg production mediated by the addition of phosphorus indicates the participation of other algal groups as mediators of MeHg production, for the reasons already listed above, but which need clarification as their role in the accumulation and transfer of Periphytic MeHg to other food chain links, as pointed out by Lanza et al (2016).

4.CONCLUSIONS

Our results indicate a clear association between primary production, cyanobacteria biovolumes and MeHg net production potential in the periphyton. These findings have numerous implications for the management of natural and engineered wetlands worldwide, submitted to increasing eutrophication and cyanobacterial development. Our results show that though assuring important ecosystem services such as, among many others, the treatment of urban and agriculture effluents, wetlands may also increase the bioavailability of pollutants such as Hg in their outflow. The impacts of cyanobacterial blooms on the biotransformation of other pollutants in periphyton also deserve further investigation.

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Figure 1 – Location of the water and periphyton sampling area for microcosm construction and laboratory cyanobacterial cultures.

Figure 2a – Effect of dissolved organic matter concentrations on MeHg production in periphyton cultures (n=15). Acid killed controls=0.03% MeHg production. Empty pentagons= Experimental controls (no amendment).

Figure 2b - Effect of phosphorus concentrations on MeHg production in periphyton cultures (n=15). Acid killed controls=0.00% MeHg production. Empty squares = Experimental controls (no amendment).

Figure 3a – MeHg production under different cyanobacteria concentrations in and field cultures (n=30). Acid killed controls=0.05% MeHg production. Empty circles= Experimental controls (no amendment).

Figure 3a – Me²⁰³Hg production under different cyanobacteria concentrations in pure cultures (n=40). Acid killed controls=0.00% MeHg production.

Figure 4 – Relation between periphytic primary production and MeHg production in periphyton of DOM, phosphorus and cyanobacteria amendments (n=60).

Figure 1

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771 **Figure 2**
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Figure 4

Supplementary Information for

Cyanobacteria as regulators of methylmercury production in periphyton

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Table 1. Water physico-chemical variables in Guaporé River floodplain lakes (TD= temporally disconnected; IC=indirectly connected; DC= directly connected).

Variables	Lake types		
	TD	IC	DC
<i>Dry season</i>			
Temperature °C	27.96±3.38	28.71±2.35	28.45±2.41
pH	5.20±0.27	6.15±0.21	6.65±0.15
ORP (mV)	164.40±18.42	221.11±15.81	231±7.01
Conductivity (µS cm ⁻¹)	234.0±9.7	252.4±7.0	258.9±2.7
Turbidity (NTU)	0.89±0.32	0.99±0.12	1.29±0.80
Dissolved Oxygen (mg L ⁻¹)	2.51±1.35	3.32±1.25	4.98±2.18
Total Nitrogen (mg L ⁻¹)	0.31±0.14	0.45±0.23	0.57±0.22
Total Phosphorus (µg L ⁻¹)	23.46±4.49	12.34±4.89	9.78±4.67
DOC (mg L ⁻¹)	4.91±1.52	2.01±2.01	3.21±1.01
<i>Flood season</i>			
Temperature °C	31.74±2.48	32.11±1.32	31.46±1.64
pH	4.97±0.76	4.87±0.81	5.12±0.65
ORP (mV)	134.40±18.52	111.11±19.11	132±8.44
Conductivity (µS cm ⁻¹)	187.0±8.5	165.4±9.32	176.9±4.7
Turbidity (NTU)	1.79±0.22	1.99±0.43	1.69±1.21
Dissolved Oxygen (mg L ⁻¹)	2.97±3.81	3.14±4.63	3.21±4.19
Total Nitrogen (mg L ⁻¹)	0.12±0.16	0.15±0.43	0.17±0.12
Total Phosphorus (µg L ⁻¹)	19.46±3.48	18.34±4.11	19.78±6.37
DOC (mg L ⁻¹)	6.31±2.72	6.01±3.01	5.81±2.11

Table 2. Water physico-chemical variables (mean) in the microcosms constructed for the assays (SD<10%).

Microcosm number	Dissolved oxygen (mg L ⁻¹)	ORP	pH	P (µg L ⁻¹) concentration	N (mg L ⁻¹) concentration	DOC (mg L ⁻¹) concentration
1	5.1	122.0	6.7	12.14	0.24	1.11
2	5.2	122.5	6.7	18.60	0.16	1.11
3	5.3	122.5	6.7	18.70	0.23	1.15
4	5.2	122.9	6.6	19.30	0.24	1.16
5	5.3	122.3	6.7	19.40	0.25	1.20
6	5.2	123.0	6.5	22.14	0.30	1.19
7	5.1	122.7	6.7	22.56	0.18	1.15
8	5.4	122.4	6.7	23.57	0.16	1.31
9	5.4	122.7	6.7	23.57	0.23	1.03
10	5.4	122.6	6.5	24.00	0.23	1.06
11	5.5	122.2	6.7	24.50	0.24	1.01
12	5.5	122.3	6.6	27.14	0.21	1.03
13	5.4	122.4	6.7	28.57	0.20	1.29
14	5.1	123.0	6.5	30.69	0.16	1.19
15	5.1	122.7	6.6	30.71	0.29	1.39
16	5.0	122.7	6.6	11.50	0.17	1.04
17	5.0	122.1	6.7	11.50	0.29	1.17
18	5.2	122.3	6.7	11.60	0.22	1.23
19	5.1	122.8	6.6	12.00	0.16	1.23
20	5.2	122.2	6.6	11.60	0.18	1.01
21	5.1	122.6	6.6	11.70	0.24	1.14
22	5.3	122.0	6.7	11.40	0.26	1.11
23	5.4	122.5	6.6	11.30	0.25	1.28
24	5.3	122.6	6.5	11.50	0.26	1.13
25	5.3	122.2	6.7	11.40	0.16	1.10
26	5.5	122.5	6.7	11.00	0.24	1.38
27	5.4	122.6	6.6	11.00	0.19	1.12
28	5.5	122.7	6.6	11.30	0.15	1.19
29	5.2	122.9	6.5	11.50	0.19	1.23
30	5.4	123.0	6.5	11.20	0.23	1.02
31	5.1	122.2	6.5	11.50	0.29	1.18
32	5.1	122.2	6.7	11.50	0.21	1.17
33	5.2	122.9	6.6	11.60	0.29	1.35
34	5.0	122.8	6.8	12.00	0.27	1.01
35	5.1	122.7	6.8	11.60	0.29	1.07
36	5.3	122.8	6.8	11.70	0.29	1.13
37	5.4	122.5	6.7	11.40	0.21	1.15
38	5.2	122.1	6.8	11.30	0.30	1.19
39	5.4	123.0	6.7	11.50	0.21	1.21
40	5.5	122.7	6.6	11.40	0.23	1.16
41	5.5	122.9	6.6	11.00	0.21	1.14
42	5.4	122.1	6.8	11.00	0.25	1.09

To be continued...

Continuation...

Microcosm number	Dissolved oxygen (mg L ⁻¹)	ORP	pH	P (µg L ⁻¹) concentration	N (mg L ⁻¹) concentration	DOC (mg L ⁻¹) concentration
43	5.5	122.5	6.7	11.30	0.29	1.15
44	5.1	122.7	6.7	11.50	0.28	1.07
45	5.4	122.9	6.5	11.20	0.28	1.08
46	5.1	122.7	6.7	11.30	0.27	1.13
47	5.3	122.7	6.6	11.45	0.29	1.33
48	5.4	122.4	6.5	11.32	0.22	2.15
49	5.2	122.8	6.6	11.22	0.24	2.53
50	5.4	122.3	6.7	11.32	0.23	2.98
51	5.4	122.9	6.6	11.51	0.25	3.50
52	5.3	122.4	6.8	11.40	0.24	4.10
53	5.4	122.1	6.8	11.10	0.29	4.70
54	5.0	122.3	6.8	11.13	0.26	5.20
55	5.5	122.5	6.6	11.20	0.30	5.50
56	5.1	122.9	6.7	11.23	0.30	6.30
57	5.4	123.0	6.5	11.23	0.21	6.80
58	5.5	122.6	6.6	11.22	0.25	7.10
59	5.4	122.4	6.6	11.42	0.30	7.50
60	5.1	122.8	6.7	11.37	0.28	8.10

Table 3. Physicochemical and biological variables (mean) of the studied microcosms with addition of cultivated field cyanobacteria. (P=total phosphorus, N=total nitrogen, DOM=dissolved organic matter). (SD<12%)

Microcosm number	P ($\mu\text{g L}^{-1}$) concentration	N (mg L^{-1}) concentration	DOC (mg L^{-1}) concentration	Cyanobacteria inicial biovolume ($\mu\text{m}^3 \text{ cm}^2 10^4$)	Cyanobacteria spike dillution	Cyanobacteria final biovolume ($\mu\text{m}^3 \text{ cm}^2 10^4$)
1 (control)	11.08	0.24	1.11	5.23	0	6.30
2 (control)	11.13	0.16	1.11	5.54	0	6.51
3	11.09	0.23	1.15	5.03	1:10	7.30
4	11.13	0.24	1.16	5.10	1:10	7.30
5	11.02	0.25	1.20	5.04	1:20	7.83
6	11.13	0.30	1.19	5.42	1:20	7.60
7	11.15	0.18	1.15	5.31	1:30	8.50
8	11.14	0.16	1.31	5.58	1:30	8.44
9	11.13	0.23	1.03	5.64	1:40	9.08
10	11.06	0.23	1.06	5.02	1:40	9.37
11	11.06	0.24	1.01	5.26	1:50	9.20
12	11.09	0.21	1.03	5.67	1:50	9.31
13	11.05	0.20	1.29	5.34	1:60	9.50
14	11.06	0.16	1.19	5.65	1:60	9.50
15	11.14	0.29	1.39	5.38	1:65	9.52
16	11.08	0.17	1.04	5.10	1:65	9.61
17	11.05	0.29	1.17	5.37	1:70	10.30
18	11.11	0.22	1.23	5.47	1:70	10.33
19	11.01	0.16	1.23	5.66	1:75	10.42
20	11.06	0.18	1.01	5.64	1:75	10.50
21	11.12	0.24	1.14	5.14	1:80	10.65
22	11.02	0.26	1.11	5.05	1:80	10.71
23	11.12	0.25	1.28	5.17	1:85	10.81
24	11.02	0.26	1.13	5.18	1:85	10.83
25	11.16	0.16	1.10	5.21	1:90	10.94
26	11.02	0.24	1.38	5.40	1:90	11.08
27	11.14	0.19	1.12	5.47	1:95	11.42
28	11.09	0.15	1.19	5.41	1:95	11.53
29	11.09	0.19	1.23	5.40	1:100	11.71
30	11.04	0.23	1.02	5.00	1:100	11.60

Table 4. Physicochemical and biological variables values (mean) of the microcosms with Phosphorus and DOM addition. (P=total phosphorus, N=total nitrogen, DOM=dissolved organic matter). (SD<12%)

Microcosm number	Microcosm type	P ($\mu\text{g L}^{-1}$) concentration	N (mg L^{-1}) concentration	DOC (mg L^{-1}) concentration	Cyanobacteria inicial biovolume ($\mu\text{m}^3 \text{ cm}^2 10^4$)	Cyanobacteria final biovolume ($\mu\text{m}^3 \text{ cm}^2 10^4$)
31 (control)	P addition	11.14	0.29	1.18	5.04	6.88
32	P addition	18.60	0.21	1.17	5.45	6.31
33	P addition	18.70	0.29	1.35	5.41	6.71
34	P addition	19.30	0.27	1.01	5.45	6.50
35	P addition	19.40	0.29	1.07	5.31	6.01
36	P addition	22.14	0.29	1.13	5.62	6.09
37	P addition	22.56	0.21	1.15	5.17	6.19
38	P addition	23.57	0.30	1.19	5.66	6.93
39	P addition	23.57	0.21	1.21	5.18	6.08
40	P addition	24.00	0.23	1.16	5.69	6.18
41	P addition	24.50	0.21	1.14	5.46	6.61
42	P addition	27.14	0.25	1.09	5.39	7.01
43	P addition	28.57	0.29	1.15	5.01	7.02
44	P addition	30.69	0.28	1.07	5.10	7.54
45	P addition	30.71	0.28	1.08	5.49	6.52
46 (control)	DOM addition	11.50	0.27	1.08	5.43	6.19
47 (control)	DOM addition	11.50	0.29	1.09	5.03	7.50
48	DOM addition	11.60	0.22	2.15	5.06	7.56
49	DOM addition	12.00	0.24	2.53	5.17	6.05
50	DOM addition	11.60	0.23	2.98	5.40	6.94
51	DOM addition	11.70	0.25	3.50	5.51	6.08
52	DOM addition	11.40	0.24	4.10	5.55	7.10
53	DOM addition	11.30	0.29	4.70	5.19	6.32
54	DOM addition	11.50	0.26	5.20	5.48	6.77
55	DOM addition	11.40	0.30	5.50	5.67	7.12
56	DOM addition	11.00	0.30	6.30	5.63	7.08
57	DOM addition	11.00	0.21	6.80	5.16	7.06
58	DOM addition	11.30	0.25	7.10	5.52	6.60
59	DOM addition	11.50	0.30	7.50	5.19	6.21
60	DOM addition	11.20	0.28	8.10	5.67	7.05