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Fluorescence in the estimation of chlorophyll-*a* in public water reservoirs in the Brazilian cerrado

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ABSTRACT

Context. The usual strategy for monitoring of eutrophication process is the use of traditional limnological methods, based on laboratory analysis. These procedures involve costly and time-consuming analyses, usually with *in vitro* methodologies, which can still have limitations in terms of sensitivity and reliability, if poorly managed. Phytoplankton pigments, such as chlorophyll-*a* (Chl-*a*), are highly fluorescent and can provide the environmental status of water bodies. **Aims.** This study aims to analyse, compare and evaluate an estimation of Chl-*a* through fluorescence in public water sources in the Brazilian cerrado. Exploratory statistical analyses were conducted by using absolute fluorescence units (AFU) and relative fluorescence units (RFU) compared with traditional laboratory data (standard procedure for the determination of Chl-*a* by spectroscopic methods) to evaluate the significance of differences in estimating Chl-*a* concentration. Subsequently, empirical models, based on spectral band combinations, were generated to convert fluorescence measurement in Chl-*a* concentration, by linear regression. **Key results.** The generated model found a strong correlation and coefficient of determination ($r = 0.88$; $R^2 = 0.78$). The efficiency of the model was also confirmed by statistical indicators (RMSE = 1.27, MAPE = 26.72 and BIAS = −6.32). **Conclusions.** We concluded that the estimate of Chl-*a* through RFU was better than through AFU. **Implications.** Therefore, based on the results of this study, it is recommended that RFU be used to obtain more precise and accurate estimates of Chl-*a* concentration through empirical models based on linear regression.

Keywords: absolute fluorescence units, AFU, aquatic environments, chlorophyll-*a*, public water supply, relative fluorescence units, RFU, water quality monitoring.

Introduction

Protecting water reservoirs has become critical for maintaining the health of terrestrial ecosystems. In addition to underground sources and rainfall, water that supplies urban and rural areas comes from these surface water bodies. Therefore, it is the responsibility of society to utilise, preserve, and monitor them in a mindful manner (Mustafa *et al.* 2020). Also, anthropogenic impacts, such as siltation and artificial eutrophication – which are generally caused by point and diffuse source pollution – are progressively worse and more frequent worldwide. These processes' dynamics are heightened in tropical environments, where the alternation between seasons – hot and humid weather in the summer, followed by a cold and dry period in the winter – lead to an increased capacity for nutrient uptake and consequent rapid growth of algae and aquatic plants (Lewis 2000; Hennemann and Petrucio 2010; Andrade *et al.* 2020). As a result, some limnological variables may have values out of the adequate water quality range, and the reservoir may fail to fulfil its multiple uses. This is a common scenario in large urban regions, which can make it challenging to establish monitoring strategies that ensure the understanding of the aquatic environment's dynamics. Chlorophyll-*a* (Chl-*a*) is a photosynthetic pigment found in phytoplankton biomass, and it has the potential to serve as a marker for eutrophication, making it an important target with great potential for orientating control measures (Lorenzen 1967; Hartmann *et al.* 2019; Panchenko *et al.* 2020).

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Currently, there are a variety of approaches to measure and quantify Chl-*a*. The most traditional and based on laboratory techniques are the spectrophotometric methods and high-performance liquid chromatography (HPLC; Marino 2017; Batista and Fonseca 2018; Garrido *et al.* 2019). Although these methods provide very reliable quantifications, they are *in vitro* methods which demand benchtop protocols with several steps and substantial consumption of chemical reagents that can deteriorate algae at the time of extraction (Lorenzen 1967; Van Heukelem and Thomas 2001; Marino 2017; Graban *et al.* 2020). In addition, analyses are time-consuming, require a large sample volume and involve high logistic and analytical costs (Ferreira *et al.* 2012; Kuha *et al.* 2020), which can delay the availability of results and response actions for prevention and control.

To overcome this temporal limitation, alternative technologies that present real-time data acquisition have been proposed (Loisa *et al.* 2015; Cremella *et al.* 2018; Shin *et al.* 2018; Garrido *et al.* 2019; Panchenko *et al.* 2020; Silva and Garcia 2021). Fluorescence optical sensors stand out among diverse technologies. Roesler *et al.* (2017) highlight the benefits of using *in vivo* fluorescence, such as ease of use, immediate non-destructive determination of concentrations from the organisms, and high rates of data acquisition, precision and accuracy.

This method is based on the spectral ranges of excitation and emission of each pigment. For Chl-*a*, absorbance wavelengths are smaller than 675 nm (453–440, 620–635 and 672–675 nm) and re-emission is at ~685 nm (Lohrenz *et al.* 2003; Seppälä *et al.* 2007; Suggett *et al.* 2010; Choo *et al.* 2018; Shin *et al.* 2020). These ranges (fluorimetric bands) play a very important role in pigment detection and identification of algae groups. Ling *et al.* (2018) showed good results using fluorescence emission channels at 550 and 700 nm to estimate Chl-*a* and to distinguish microalgae groups. Garrido *et al.* (2019) also had good results with this method, but used other fluorimetric bands (370, 450, 525, 570, 590 and 610 nm) for pigment quantification. Despite these advantages, some studies report concerns regarding acquired data, such as observed by Catherine *et al.* (2012), which identified overestimated Chl-*a* values when compared to traditional laboratory measurements.

Besides, many other works, with different approaches, also evaluated the effectiveness of *in vivo* fluorescence determination (Leboulanger *et al.* 2002; Gregor and Maršálek 2004; Richardson *et al.* 2010; Houliez *et al.* 2012; Kring *et al.* 2014; Escoffier *et al.* 2015; Ling *et al.* 2018; Hartmann *et al.* 2019). However, several of these were developed in either controlled environments, temperate regions, or in sites with high Chl-*a* concentrations, which differ from tropical reservoirs that can be highly heterogeneous in comparison.

Considering the above, the present study aims to analyse and evaluate methods of Chl-*a* estimation by fluorescence in reservoirs for public water supply in the cerrado region (Brazil). To this end, different compositions in terms of absolute and relative fluorescence units (emission and excitation regions) were tested to assess their accuracy in detecting Chl-*a*. We believe that raw fluorometric data (relative fluorescence units, RFU) can be adapted through empirical models, which could improve the process of estimating Chl-*a* in specific environmental conditions.

Material and methods

Experimental area

The aquatic environments studied in this work are in the cerrado, a biome located in central Brazil, known to contain most of the headwaters of the country's main hydrographic basins (Lima 2011). Its climate is characterised by a dry season and a rainy season, with an average annual precipitation in the range of 800–1800 mm and average temperatures ranging between 20 and 27°C (Pereira *et al.* 2011).

The study was carried out in three public water reservoirs in the Federal District (FD): Descoberto, Santa Maria and Paranoá lakes (Fig. 1), which supply water to ~3 million people (IBGE 2017). Descoberto lake accounts for 60% of the public water supply and is in the western region of the FD, with useful volume of 86 $\times 10^6$ m^3 (Governo do Distrito Federal 2017). Over the years, expansion of agricultural activity and inadequate occupations of its surroundings has been contributing to its pollution. The second studied reservoir – Santa Maria – is located in a conservation area, the National Park of Brasília, that occupies an area of 6.1 km^2 with a grassland–cerrado landscape. According to the Integrated Planning for Addressing the Water Crisis (Governo do Distrito Federal 2017), the Descoberto and Santa Maria reservoirs have been suffering from low levels of precipitation (drought) in recent years, which has interfered with catchment levels. This situation pushed the FD Environmental Sanitation Company (CAESB) to interrupt the water supply, which required a rotation distribution system for FD regions in 2016 and 2017.

The 2016–2017 water crisis led to an emergency use of Paranoá lake as a source of water to public supply. Also, in 2016, along with the water crisis, the same lake recorded high nutrient concentrations that resulted in eutrophication and, consequently, the emergence of an intense cyanobacteria growth, which can be harmful to the environment and to human health (Barbosa *et al.* 2019). Thus, Paranoá lake was chosen as the third study site. This multiple-uses water body, located in FD's central region, with an area of ~48 km^2, is heavily influenced by the anthropic activities in its surroundings, particularly by receiving treated wastewater effluents. The reservoirs were sampled for analyses with the same design sample due to the fact that concentration range of Chl-*a* was very similar among them. This is justified by the proximity between them, as well as similar environmental and meteorological conditions.

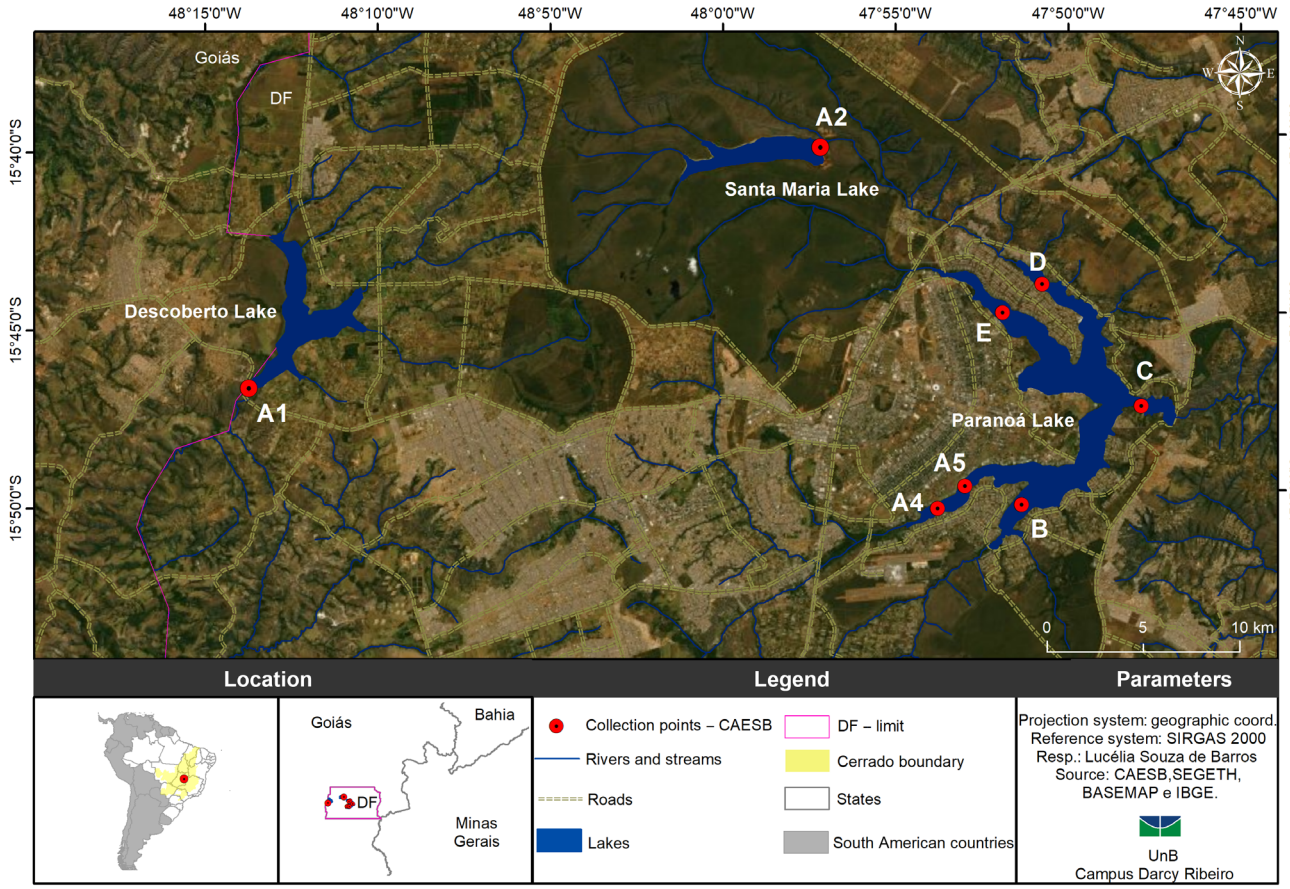

Fig. 1. Descoberto and Santa Maria Reservoirs and Paranoá Lake and sampling points.

Data acquisition design and estimation methods

In this study, data acquisition was carried out along with the routine sampling done by the CAESB, that carries out systematic water quality monitoring using conventional methods. The sampling points and dates (covering ~1 year of data collection) are listed in Table 1 and Fig. 1. The variation on depths was dependent on routine sampling done by the CAESB, without affecting the results. Kiefer *et al.* (1989) demonstrated that, depending on the wavelength, the quality of fluorescence is relatively constant with the depth.

Concentration of chlorophyll-*a* was estimated by two different methods: a traditional spectrophotometric technique (reference) and *in vivo* fluorescence (IVF) by spectrofluorometer (portable probes). The traditional estimation method used for validation was performed by the CAESB, using the 10200 H reference technique of the Standard Methods for the Examination of Water and Wastewater (American Public Health Association *et al.* 2012).

Each water sample collected in the field was kept under refrigeration and in the dark until arrival at the laboratory, where they were filtered through a Whatman GF/F filter (porosity 0.7 μm and diameter 47 mm) and frozen. These were then subjected to pigment extraction with a 90% acetone solution. Samples were then analysed using a spectrophotometer at 664 and 750 nm and then, after acidifying with a 0.1-N HCl solution, re-analysed at 665 and 750 nm. This methodology has been used in Brazilian inland waters by several authors (Ferreira *et al.* 2012; Utsumi *et al.* 2015; Silva *et al.* 2016; Cicerelli *et al.* 2017).

Chlorophyll-*a* concentration was also measured using spectrofluorometry by IVF. These instruments induce specific excitation (absorption) and emission (fluorescence) wavelengths by directing a beam of light (light-emitting diode, LED) at a specific wavelength and then measuring the highest fluorescence wavelength emitted (Yellow Springs Inc. 2009). In this study, two models were tested: the multiparameter probe EXO2 from YSI and the bbe Moldaenke FluoroProbe (ver. 2.6 E2, bbe Moldaenke, Schwentinental, Germany). According to the EXO2-YSI manufacturer's standard procedure for Chl-*a* estimation, the excitation wavelength occurs at 470 ± 15 nm, whereas the emission wavelength is at 685 ± 20 nm. For the estimation of the phycocyanin and phycoerythrin pigments, the excitation wavelength is at 590 and 525 ± 15 nm respectively, and the emission wavelength is at 685 ± 20 nm (Choo *et al.* 2018). The FluoroProbe fluorometer has six excitation wavelengths – 370, 470, 525, 570, 590 and 610 nm – with an emission wavelength range

Table 1. The sampling points and dates acquisition.

Date	Points	Reservoir	Depths	Number of samples
10 July 2019	A1	Descoberto (area 12 km^2)	30 cm (surface)	1
21 October 2019			30 cm, 1 m, and 5 m	3
15 August 2019	A2	Santa Maria (area 7 km^2)	30 cm (surface)	1
19 June 2019	C, D, and E	Paranoá (area 38 km^2)	1 m	3
23 July 2019	D, and E			2
13 August 2019	A4, A5, B, C, D, and E			6
22 October 2019				6
19 November 2019				6
11 December 2019				6
11 February 2020				6
17 August 2020	A5, B, C, D, and E		30 cm	5 (validation)
Total				45

between 685 and 700 nm. The results are generated in RFU, that is a unit of measurement used in analysis which employs fluorescence detection. Also, it should be noted that these instruments provide Chl-*a* estimate in absolute fluorescence units (AFU, μg L^{-1}).

Then, using the localisation from each point of the design sample (Fig. 1), we acquired chlorophyll concentration by traditional limnologic method and the fluorescence method. The probes remained recording data for 4 min with 2-s intervals. The fluorescence data were used to obtain an average of each point. As stated above, the EXO probe gave us only AFU (μg L^{-1}) to compare with the reference value.

Modelling and statistical analysis

Chlorophyll-*a* data acquired by the fluorometers (μg L^{-1}) as well as by the traditional laboratory analyses (*in vitro*) were subjected to an exploratory statistical evaluation. First, a basic descriptive statistical analysis of the data was performed, followed by normality tests (Shapiro–Wilk and Kolmogorov–Smirnov tests), which identified data that did not fit the normal distribution. A boxplot visualisation was then used to investigate the source of the abnormality, which could have arisen from outliers, noisy observations, or abnormal system behaviour (Díaz Muñiz *et al.* 2012; Gradilla-Hernández *et al.* 2020). Frequently, a raw dataset may contain data with atypical behaviour (outliers), so it is important to categorise the outlier type to adopt the best data refinement strategy (Gradilla-Hernández *et al.* 2020). It was concluded that many of the observed outliers were caused by common limnologic variation in aquatic environments; consequently, these observations were not excluded.

Previous studies also did not find normal distributions for similar datasets and used non-parametric statistics to perform comparative analyses, such as the Spearman correlation (Hartmann *et al.* 2019) and the Kruskal–Wallis test (Garrido *et al.* 2019). In this work, the Kruskal–Wallis test and Dunn's *post hoc* (or Dunn–Bonferroni) pairwise comparative method were used to analyse and compare the differences between the evaluated methods. Furthermore, data analysis was complemented by other metrics: linear regression, 95% reliability, probability of significance (*P*-value), correlation, coefficient of determination (R^2) and root mean square error (RMSE), with the aim of evaluating the behavior, accuracy, dispersion and trend of AFU compared with reference data.

The chosen approach for relative fluorescence data analysis was then used to develop empirical models by combining emission spectra bands, to estimate Chl-*a* concentrations from fluorometric measurements, similarly to what is described in Ling *et al.* (2018). These authors also applied a method based on combinations of fluorescence emission spectra bands to estimate dominant algal species in marine environments.

In this study, after several tests, eight combinations were used, which included individual band analysis and bands ratio operations (Table 2). For each combination of fluorescence emission, all 40 collected samples were used to create the model. Pearson correlation (*r*), determination coefficient (R^2) and dispersion analysis were used to determine the best model. Subsequently, regression analysis was used to generate Chl-*a* estimation models from the relationship between laboratory spectrophotometry data and relative fluorescence data. These models considered dispersion of the points in relation to the regression function, with a 95% confidence interval and a *P*-value of 0.05. The model that had the best statistical results was chosen to estimate Chl-*a*.

Data validation

Statistical analyses were performed to assess the performance and accuracy of the chosen model. Three statistical indicators, commonly used in limnological variables research, were applied: RMSE, mean absolute percentage error (MAPE)

Table 2. Fluorescence emission bands combinations. Fluorescence emission bands 370, 470, 525, 570, 590 and 610 nm were tested for *x* and *y*.

Band combinations	ID
Individual band *x*	(1)
$\log_{10}(x)$	(2)
$(x)-(y)$	(3)
$(x) \div (y)$	(4)
$\log_{10}(x) \div \log_{10}(y)$	(5)
$[(x)-(y)] \div [(x) \div (y)]$	(6)
$[(x)-(y)] \div [(y) \div (x)]$	(7)
$[(x) + (y)] \div [(x) \div (y)]$	(8)
$[(x) + (y)] \div [(y) \div (x)]$	(9)
$[(x)-(y)] \div [(x) + (y)]$	(10)

Table 3. Descriptive statistics of chlorophyll-*a* (Chl-*a*) concentrations (μg L^{-1}) for each data acquisition method.

Variable	EXO probe	BBE probe	LAB
Sample size	40	40	40
Min.	0.28	1.60	0.80
Max.	10.01	20.88	11.8
Total amplitude	9.73	19.28	11
Median	0.88	3.92	3.60
Average	1.89	5.63	4.09
Variance	5.02	23.56	7.50
s.d.	2.24	4.85	2.74
s.e.	0.35	0.77	0.43
Coefficient of variation	118.73%	86.15%	66.91%

and systematic error (BIAS) (Ling *et al.* 2018; Kuha *et al.* 2020). These can be calculated as follows:

$$\mathrm{RMSE} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(y_i - y_i^{'}\right)^2} \quad (1)$$

$$\mathrm{MAPE} = \frac{\sum_{i=1}^{n}\frac{|p_i - t_i|}{|t_i|}}{N} \quad (2)$$

$$\mathrm{BIAS} = \frac{1}{N}\sqrt{\sum_{i=1}^{N}\left(y_i - y_i^{'}\right)} \quad (3)$$

To evaluate model performance, another dataset was collected *in situ* on 17 August 2020 (5 points), and Chl-*a* values, determined by the model, were compared with values obtained by the traditional spectrophotometric technique.

Owing to the small quantity of validation points, the validation technique 'leave-one-out cross-validation' was also applied to provide an overall assessment of the model accuracy. However, it does not evaluate the dependence of the estimation error on Chl-*a* values. Such an evaluation, for the estimation error associated with the uncertain model parameters, can be achieved through a non-parametric bootstrap method (Efron 1979; Volpe *et al.* 2011). As such, we adopt a bootstrap resampling method, in which the observed set of Chl-*a* fluorescence pairs are re-sampled with re-substitution (i.e. a pair that has been extracted is available for possible subsequent sampling).

Results and discussion

Absolute fluorescence units

Fieldwork was carried out in favourable boating weather conditions of temperature (average 22°C), wind (average 1.1 m s^{-1}), air humidity (55%) and with no rain. Statistical analyses of the Chl-*a* concentration absolute values provided by the fluorometers are shown in Table 3. All the values were below 30 μg L^{-1} in the evaluated period, which is within the limit established by the Brazilian environmental regulations Resolução n°357, de 17 de Março de 2005 (Ministério do Meio Ambiente e Mudança do Clima 2005). Although observed values were low, data variability was still observed, as shown by the standard deviation values. The coefficients of variation obtained from fluorometers EXO2 and bbe Moldaenke were of 118 and 86% respectively. Laboratory analyses (LAB) provided the smallest variability values (66%).

According to Table 3, average Chl-*a* concentration was lower than 6 μg L^{-1} for all methods. By contrast, other works in the literature that used similar methods, such as the papers by Gregor *et al.* (2005), Catherine *et al.* (2012) and Garrido *et al.* (2019), found medium values of 90, 265 and 42 μg L^{-1} respectively. It seems clear that estimation under these conditions is difficult, as it is close to the detection limit of the techniques employed.

The Kruskal–Wallis non-parametric test showed significant difference ($P < 0.05$) between the three tested methods. However, when compared in pairs with the Dunn method, there was no significant difference between the LAB and the BBE measurement (P-adjusted < 0.05), differently for measurements by LAB and EXO (P-adjusted < 0.05). Fig. 2 shows the outlier points (7, 6 and 1 for EXO2, BBE and LAB methods respectively) which were measured at the same date for the sampling sites in each of the three lakes being studied. Thus, it is concluded that the effects of outliers were the result of common phenomena in these aquatic environments.

Fig. 2 also shows a violin plot statistical data distribution for every tested method. Chlorophyll-*a* values from portable fluorometers generally are higher than those obtained by the conventional spectrophotometric technique. This overestimation could have been caused by several reasons, such as an increase in fluorescence emission by cyanobacteria in the face of nutrient limitation (MacIntyre *et al.* 2010), the

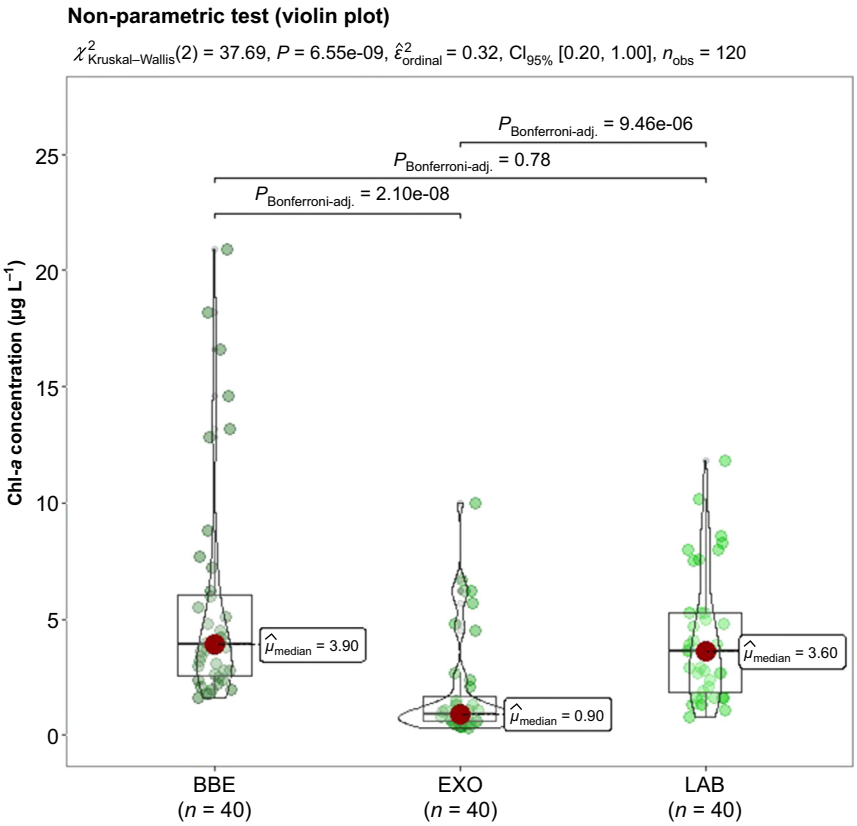

Fig. 2. Distribution of chlorophyll-*a* (Chl-*a*) data using fluorimeter (BBE and EXO2) and spectrophotometric (LAB) methods.

phytoplankton life cycle phase and its variations in the pigment content of cells (Beutler *et al.* 2002), the different levels of efficiency during the extraction of Chl-*a* by the spectrophotometric method (Nusch 1980), as well as other factors, such as light variation, presence of bubbles, dissolved organic matter, turbidity and temperature (Zamyadi *et al.* 2016; Choo *et al.* 2018). Besides considering the type and size of the algae, it is worth mentioning that the fluorescence method predicts the estimation for a point based on a set of data collected over time, taking into account the variation in types and sizes of naturally occurring algae in the environment, as well as their composition, richness and abundance.

This overestimation could have also been caused by the HPLC-based calibration method of the BBE fluorometer, as discussed by Meyns *et al.* (1994). Several studies have calculated an 'overestimation index' for Chl-*a* spectrofluorometry measurements, with values ranging from 0.27 to 1.03 (Leboulanger *et al.* 2002; Silva *et al.* 2016) (Table 4). Additionally, Leboulanger *et al.* (2002) found a Chl-*a* concentration and overestimation index similar to what was found in the present study.

Fig. 3 shows a scatter plot of spectrophotometry (LAB) and spectrofluorometry (EXO and BBE) chlorophyll-*a* measurements. The LAB and EXO results were strongly correlated ($r = 0.79$, $R^2 = 0.63$, $P < 0.0001$, $n = 40$) and had a relatively low RMSE (2.97). The BBE probe results also show a strong correlation ($r = 0.78$, $R^2 = 0.63$, $P < 0.0001$, $n = 40$), but with a higher RMSE of 3.34. A possible reason for such difference is the due to outliers, as can be seen in Fig. 3*b*. Concentrations above 5 µg L^{-1} did not fit well to the linear regression model and can be seen out of the grey zone corresponding to the confidence interval (95%). A different scenario can be seen in Fig. 3*a* where EXO measurements had a better fit to the linear regression, which can be attributed to these measurements finding Chl-*a* concentrations below 10 µg L^{-1}.

Previous studies, such as Silva *et al.* (2016), found a strong correlation between spectrofluorometry and spectrophotometry methods for Chl-*a* concentrations bellow 100 µg L^{-1} ($r = 0.84$, $P < 0.001$, $n = 25$), and a low correlation for concentrations above 100 µg L^{-1} ($r = 0.17$, $P = 0.63$, $n = 10$). Gregor *et al.* (2005) and Catherine *et al.* (2012) also observed strong correlations ($r = 0.95$, $n = 96$; $r = 0.97$, $n = 50$) for maximum Chl-*a* concentrations of 90 and 264 µg L^{-1} respectively. On the other hand, Leboulanger *et al.* (2002) found a high correlation in an aquatic environment with maximum Chl-*a* of 20 µg L^{-1} ($r = 0.77$, $n = 55$), which is similar to the present study's conditions. Those studies show that low biomass frequently provides minor correlation coefficients. Although these studies have found strong correlations, in general factors such as organic matter, total suspended material and bloom conditions in particular, can limit the accuracy of the results (Chang *et al.* 2012; Kring *et al.* 2014; Zamyadi *et al.* 2016).

Table 4. FluoroProbe BBE overestimation factors reported in the literature.

Extraction solvet	Chl-*a* concentration range (μg L^{-1})	Number of sampled places	Overestimation factor	Reference
Acetone	0–20	1	1.03[A]	Leboulanger *et al.* (2002)
Ethanol	0–50	6	0.83[A]	Gregor and Maršálek (2004)
Ethanol	0–90	5	0.74[A]	Gregor *et al.* (2005)
Methanol	0–265	50	0.56[B]	Catherine *et al.* (2012)
Ethanol	42–626	1	0.36[A] and 0.27[B]	Silva *et al.* (2016)
Acetone	0–20.8	3	1.37[B]	This study

[A]This overestimation factor was calculated using a linear regression $y = ax$, where y is the chlorophyll-*a* (Chl-*a*) concentration provided by the spectrofluorometer and x is the concentration provided by laboratory spectrophotometry.
[B]This overestimation factor is the average between spectrofluorometry and spectrophotometry measurements.

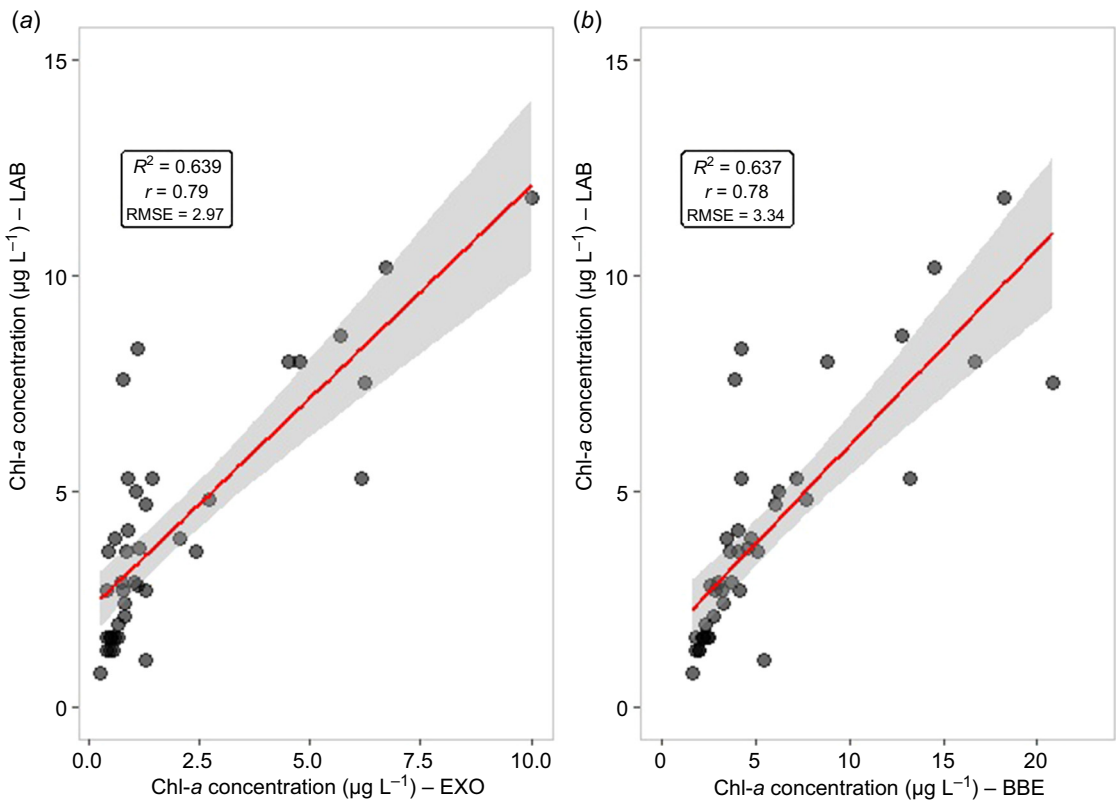

Fig. 3. Linear regression models of the spectrofluorometry methods. (*a*) Chlorophyll-*a* (Chl-*a*) EXO: $Y = 2.234 + 0.984X$; (*b*) Chl-*a* BBE: $Y = 1.536 + 0.453X$.

Relative fluorescence units

Table 5 shows the fluorescence emission bands combination results. Among the various combinations, equation 9, with the 525- and 570-nm bands, had the overall best result, with a Pearson correlation value (r) of 0.88 and a coefficient of determination (R^2) of 0.78. In contrast to what was found here, other studies have shown different spectral bands for selective excitation of Chl-*a*. Suggett *et al.* (2010), Seppälä *et al.* (2007) and Lohrenz *et al.* (2003) used the band combinations of 453–440, 620–635 and 672–675 nm respectively. Blockstein and Yadid-Pecht (2014) also used a different wavelength (465 nm) for the estimation of Chl-*a* concentrations using a self-made portable, and Gosset *et al.* (2018) used 470 nm as the excitation wavelength, which is the suggested value to be used with the bbe FluoroProbe fluorometer

The 525- and 570-nm spectral bands, which showed the best results here, are generally related to fluorescence peaks that occur in brown (Bacillariophyceae and Dinophyceae) and mixotrophic (Cryptophyceae) algae groups, which contain accessory pigments, other than chlorophyll-*a*, such as chlorophyll-*c* and phycobiliproteins (bbe Moldaenke 2017).

Table 5. Statistical results of fluorescence emission bands combinations.

Combinations	*r*	R^2	Combinations	*r*	R^2
1: (525)	**0.85**	**0.72**	**7: (525–570) ÷ (570 ÷ 525)**	**0.85**	**0.73**
1: (610)	0.83	0.69	7: (570–590) ÷ (590 ÷ 570)	0.83	0.69
1: (370)	0.83	0.69	8: (525 + 590) ÷ (525 ÷ 590)	0.78	0.61
1: (590)	0.86	0.73	8: (570 + 590) ÷ (570 ÷ 590)	0.78	0.61
1: (470)	0.79	0.62	8: (610 + 370) ÷ (610 ÷ 370)	0.79	0.62
2: (log_{10} 470)	0.79	0.63	**8: (610 + 590) ÷ (610 ÷ 590)**	**0.86**	**0.74**
2: (log_{10} 525)	0.83	0.69	8: (370 + 470) ÷ (370 ÷ 470)	0.77	0.60
2: (log_{10} 610)	0.83	0.70	**9: (525 + 570) ÷ (570 ÷ 525)**	**0.88**	**0.78**
2: (log_{10} 370)	0.84	0.71	9: (525 + 590) ÷ (590 ÷ 525)	0.82	0.67
2: (log_{10} 590)	**0.85**	**0.73**	9: (610 + 590) ÷ (590 ÷ 610)	0.81	0.65
3: (525–570)	**0.87**	**0.76**	9: (370 + 590) ÷ (590 ÷ 370)	0.77	0.60
3: (590–570)	0.81	0.66	9: (525 + 610) ÷ (610 ÷ 525)	0.78	0.61
6: (525–570) ÷ (525 ÷ 570)	0.81	0.65	**9: (370 + 470) ÷ (470 ÷ 370)**	**0.86**	**0.73**

Equation combinations 1, 2, 3, 6, 7, 8 and 9 are those used in Table 2. The bold data are the best results. *r*, Pearson correlation; R^2, coefficient of determination.

Batista and Fonseca (2018) found Bacillariophyceae, Chlorophyceae and Cryptophyceae as the predominant microalgae groups in Paranoá lake, and Roriz *et al.* (2019) had previously observed the invasive Dinophyceae species *Ceratium furcoides* (Levander) Langhans in this lake.

In a marine environment, Ling *et al.* (2018) found good results using fluorescence emission spectral channels at 550 and 700 nm to distinguish marine microalgae, which, along with the present study, corroborates the idea that using flexibly chosen specific spectral channels is a promising approach to the characterisation and quantification of phytoplankton.

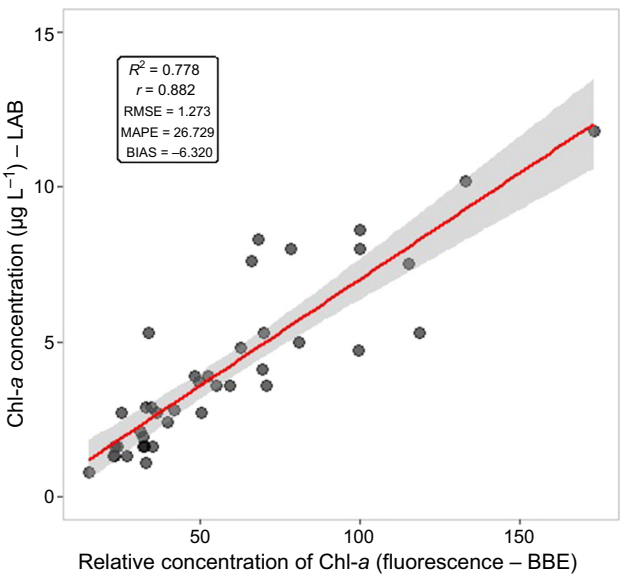

Fig. 4. Linear regression ($y = 0.135 + 0.068x$) analysis for chlorophyll-*a* (Chl-*a*) estimation based on fluorometric measurement combinations and laboratory Chl-*a* determinations.

Fig. 4 shows the linear regression for model values (fluorometric measurements combinations) and laboratory Chl-*a* determinations. Results show a high correlation between values ($r = 0.88$), as well as a good model fit ($R^2 = 0.77$), which show a stronger correlation than what was found by Leboulanger *et al.* (2002), who obtained $r = 0.77$ for waters with maximum Chl-*a* values of 20 µg L^{-1}. However, other studies have shown higher correlation values, such as Gregor *et al.* (2005) ($r = 0.95$, $n = 96$) and Catherine *et al.* (2012) ($r = 0.97$, $n = 50$). Also, it was observed in the present study that relative values provided a better estimate of Chl-*a* than AFU values (FluoroProbe: $R^2 = 0.63$, $r = 0.78$).

Other indicators, such as the RMSE, MAPE and BIAS, were used to analyse the method's performance for estimating the concentration of Chl-*a*. For the best performing model, the statistical values were: RMSE = 1.27, MAPE = 26.72 and BIAS = −6.32 (Fig. 4). The RMSE for RFU was lower than for AFU (BBE probe: RMSE = 3.34). Previous studies from Ferreira *et al.* (2012) and Ling *et al.* (2018) also showed the efficiency of fluorescence relative measurements for the quantification and identification of phytoplankton. Both brought improvements to the estimation model.

When converting fluorometric data into relative values of Chl-*a*, the correction factors may suffer various interferences and cause deviations from the spectrophotometric results. Several authors pointed out that light intensity, temperature, biomass composition and quenching effects can affect the fluorescence responses, resulting in changes in the fluorescence values obtained, which can lead to measurement imprecision (Catherine *et al.* 2012; Chang *et al.* 2012; Kring *et al.* 2014; Cicerelli *et al.* 2017; Garrido *et al.* 2019).

For data validation, the model produced by RFU was fed with a dataset obtained *in situ* on 17 August 2020 (5 points, Table 1) and the results were compared to concentration

values obtained from spectrophotometric analyses in the laboratory. The estimated values (1.44–2.65 μg L^{-1}) were very close to laboratory measurements (3.37–0.87 μg L^{-1}), which reinforces the accuracy and precision of the Chl-*a* estimate model, as confirmed by a RMSE of 0.48 and correlation coefficient (*r*) of 0.80. The strong correlation, along with a small error value, shows the good performance of the model calibration.

A bootstrap technique was then utilised. In it, the observed set was resampled 10 000 times. This procedure essentially constructs many samples from the same empirical distribution. The resulting empirical distribution of the correlation coefficients (Fig. 4) shows that the values found in the original samples are reliable. The empirical distribution of Pearson's correlation coefficient presented a median of 0.88 and varied between 0.7922 and 0.9822, with a confidence interval of 95%. These analyses show the high dependence between the concentration of Chl-*a* and fluorescence data. Regarding the values of β0 and β1, the standard deviation was of 0.005 and 0.389 respectively, highlighting model stability.

The aim of the second validation method, which applied the jackknife technique, was to understand the role of each point in the RFU model, and to verify if the model can predict all the variability in Chl-*a* concentration. Using this method, generated linear coefficients ranged from −0.022 to 0.210. That is, each model cuts the *Y*-axis of the cartesian plane in significantly different places. By contrast, the angular coefficient shows a stable behaviour, as there was little variation (0.068–0.073), which indicates a smaller variation of the slope of the line, reinforcing the robustness of the model.

Escoffier *et al.* (2015) pointed out that, in order to improve estimates, it is important to apply specific fluorimetric calibrations that correspond to the species present in the aquatic environment. However, this can be a complex activity, that is usually carried out directly by equipment manufacturers. The alternative presented in this work, along with what was reported in Ferreira *et al.* (2012) and Ling *et al.* (2018), proved to be an excellent quantification tool to estimate Chl-*a* concentration in aquatic environments using field data calibration.

However, it is worth mentioning the importance of knowing the effects of other environmental factors in Chl-*a* quantification, as these interferences may vary according to the species or groups of microalgae found in aquatic environments. For instance, a group that has a relatively high concentration can corrupt the determination of another group with lower concentration. Moreover, the model is adapted to specific characteristics of the environment, which means that the model will not be able to adjust to extreme phenomena, such as the occurrence of high concentration of Chl-*a* or even a mixture of other elements not foreseen in the construction of the regression model.

Conclusions

The correlation between Chl-*a* estimates from the different methods and the traditional estimate (LAB) was strong, but the presence of outliers affected the linear regression model at concentrations above 5 μg L^{-1}. These results highlight the importance of considering these factors and using appropriate approaches when studying Chl-*a* concentration in tropical aquatic environments.

In conclusion, the results of fluorimetric measurements (relative data) confirm the efficiency of the model, with a strong correlation ($r = 0.88$; $R^2 = 0.78$), further supported by validation parameters (RMSE = 1.27, MAPE = 26.72 and BIAS = −6.32). These fluorimetric measurements achieved good estimation accuracy and precision for Chl-*a* concentration, which is necessary for tropical environments with low concentrations in public water supply reservoirs in the cerrado region. The absolute model provided by manufacturers showed limitations in estimation, which were minimised by using relative models.

The method of combining fluorescence emission bands and the conversion process is a good alternative for the quantification of Chl-*a* concentration, highlighting the 525- and 570-nm wavelengths corresponding to brown and mixotrophic microalgae. Therefore, relative fluorometric data can be adapted by using empirical models and improving the potential in estimating Chl-*a*. The estimation model offered by the fluorometers' manufacturers, which is based on absolute values, should be improved, or adapted to tropical regions, and that is dependent on the predominant phytoplankton groups in the environment.

This alternative allows fast Chl-*a* quantification because the fluorescence results in less time and work in the analysis in the laboratory, which is a relevant factor for monitoring the water quality in the reservoirs. In addition, there is a reduction in operating costs, ease of use, stability, ease of equipment transportation and a small carbon footprint. Freshwater ecosystems researchers have an accurate and easy way to determine Chl-*a* concentration.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

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