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Assessment of *in vivo* fluorescence method for chlorophyll-*a* estimation in optically complex waters (Curuai floodplain, Pará – Brazil)

Avaliação do método de fluorescência *in vivo* para a estimativa da concentração de clorofila-*a* em águas opticamente complexas (Planície de inundação do Curuai, Pará – Brasil)

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Abstract: Aim: This paper describes an experiment carried out to evaluate *in vivo* fluorescence (IVF) as an alternative method for chlorophyll-*a* estimation in optically complex aquatic environment (Amazon floodplain lakes) **Methods:** The experiment consisted of collecting *in situ* measurements at 26 sampling stations distributed throughout Curuai floodplain lakes. For each sampling station the following parameters were measured: temperature, turbidity, depth, Secchi depth, chlorophyll-*a* (Chl-*a*) concentration, total suspended solids (TSS) and dissolved organic carbon (DOC), concurrently with several transects of IVF. Two methods were tested for quantifying the fluorescence measurement to be used as input for the chlorophyll-*a* estimates: instantaneous IFV and average IVF. Global and regional models were tested and assessed by analyzing optically active components (Chl-*a*, DOC and TSS) of the water. **Results:** Regardless of fluorescence estimating method, the results indicate that it was not possible to fit a global model for estimating Chl-*a* from IVF for all the lakes in the Curuai floodplain. Regional models provided contrasting results according to the concentration of optically active components. The best results were observed for aquatic systems with a single dominant component homogenously distributed throughout the lake. The results highlight the influence of the ratios Chl-*a*/TSS, Chl-*a*/DOC and Phaephytin/Chl-*a* in the relationship between IVF and chlorophyll concentration. **Conclusions:** It was not possible to develop a global model to account for the entire region of Curuai floodplain. The search for regional models provided insights on the main factors affecting the relationship between IVF and Chl-*a* concentration. Nevertheless this work reinforces the great potential of fluorometry technique, since even with a small number of samples it was possible to set a good model in the main lake of the Curuai floodplain. In spite the fact that this is not an accurate method, it is very useful for assessing the chlorophyll spatial distribution with relatively low cost. These possibilities are very interesting in the execution of field missions in the Amazon region.

Keywords: *in vivo* fluorescence, chlorophyll-*a*, Curuai floodplain, optically active constituents.

Resumo: Objetivo: Este trabalho descreve os experimentos realizados para avaliar as medidas de fluorescência *in vivo* (IVF) em um ambiente aquático opticamente complexo (planície de inundação amazônica), como método alternativo para a quantificação da clorofila-*a* na água. **Métodos:** Foram coletados parâmetros de qualidade da água em 26 estações amostrais distribuídas em diversos lagos da planície de inundação de Curuai: temperatura, pH, turbidez, condutividade, profundidade, transparência ao disco de Secchi, concentração de clorofila-*a* (Chl-*a*), total de sólidos em suspensão (TSS) e carbono orgânico dissolvido (DOC) simultaneamente a quilômetros de transectos de IVF. Dois métodos de determinação do valor de fluorescência foram testados para o ajuste de modelos de estimativa da concentração de clorofila: fluorescência instantânea e fluorescência média. Também foram testados um modelo global e modelos regionais, os quais foram analisados em termos dos componentes opticamente ativos na água (Chl-*a*, DOC e TSS). **Resultados:** Os resultados indicam que independentemente do método de estimativa de fluorescência empregado, não foi possível ajustar um modelo global para a planície de Curuai. Os modelos regionais apresentaram resultados contrastantes, de

acordo com a concentração dos componentes opticamente ativos. Melhores resultados foram observados nos sistemas aquáticos com um único componente dominante, distribuído homogeneamente pelo respectivo lago. Os resultados destacam a influência das razões Chl-*a*/TSS, Chl-*a*/DOC e Feofitina/Chl-*a* no ajuste entre a IVF e a Chl-*a*. **Conclusões:** Não foi possível ajustar um modelo global para a planície de Curuai através de medidas de IVF. Entretanto, a busca por modelos regionais forneceu informações sobre os principais fatores que afetam a relação entre a IVF e a Chl-*a*. Dessa forma, este trabalho reforça o grande potencial da fluorometria, pois mesmo com um baixo número de amostras foi possível estabelecer um bom modelo no principal lago da planície. Ainda que a técnica não possa ser considerada extremamente precisa, ela é útil para avaliar o padrão de variação espacial da clorofila, com baixo custo. Estas possibilidades são muito interessantes na realização de missões de campo na bacia amazônica.

Palavras-chave: fluorescência *in vivo*, clorofila-*a*, planície de inundação do Curuai, componentes opticamente ativos.

1. Introduction

There are several methods for the assessment of inland water trophic state. These methods are based on the quantification of limnological variables, such as nutrients, water transparency and phytoplankton biomass (Carlson, 1977; Toledo Junior et al., 1983; Lamparelli, 2004). Remote sensing methods have been used to quantify some of those variables, mainly transparency (Kloiber et al., 2002a, b; Pereira et al., 2011) and phytoplankton biomass, using chlorophyll-*a* concentration (Chl-*a*) as a proxy (Gitelson et al., 2008; Moses et al., 2009). Chlorophyll-*a* quantification is usually based on collecting and filtering water samples, followed by appropriate storage and transportation for final analysis in the laboratory (Kuroda et al., 2005). This operation, although highly reliable and accurate, is time consuming and involves high logistical and analytical costs and may hinder studies in remote regions. These same constraints also limit the frequency and number of sampling points in operational monitoring programs (CETESB, 2011).

Large areas with difficult access, such as the Amazon floodplain, have *in situ* sampling cost as one of the main factors limiting water quality studies. The analytical costs related to nutrient and chlorophyll quantification for a large number of samples are frequently larger than the sum of all the other expenses. In addition, there are uncertainties regarding sample preservation, which demands complex logistics for storage and transport to laboratory. Thus, the development of less expensive and faster methods will help the scientific community to increase the number of samples and foster the application of more sophisticated data analyses.

Fluorometry is among the methods with great potential for estimating chlorophyll concentration in water bodies. It is based on the fluorescence properties of molecules that can be simply described as the ability of a given material to emit photons in longer wavelengths when stimulated at shorter ones (Suggett et al., 2010). The chlorophyll fluorescence in water is affected, however, by changes in the fluorescence quantum yield and soluble fluorescence. The former is described as the ratio of photons emitted/photons absorbed, while the latter is the fluorescence remaining in the water, representing the sum of all other components, especially the amount of total suspended solids (TSS) and dissolved organic matter (Keller et al., 1990; Cullen and Davis, 2003). Fluorescence quantum yield depends on a set of physiological and environmental factors such as solar irradiation (Kiefer, 1973b; Heaney, 1978; Abbott et al., 1982), phytoplankton species (Heaney, 1978; Keller and Rice, 1990), algae nutrient stress (Kiefer, 1973a; Babin et al., 1996), accessory pigments (Gibbs, 1979; Welschmeyer, 1994) and water temperature (Lorenzen, 1966).

Measurements of *in vivo* chlorophyll fluorescence (IVF) have been used for decades to quantify Chl-*a* in inland, coastal, estuarine and ocean waters (Chekalyuk and Hafez, 2011; Giardino et al., 2007; Gregor and Maršálek, 2004; Proctor and Roesler, 2010; Simis et al., 2012; Welschmeyer, 1994). In Brazil, however, we have found only one referenced paper (Pinto et al., 2001) reporting the use of continuous flow fluorometer to estimate Chl-*a* in Lagoa Santa and Lagoa da Pampulha - MG. Therefore, there is a gap in the literature regarding the application of this technique for inland waters with high amounts of optically active constituents (phytoplankton, suspended materials and dissolved organic matter), also referred to as optically complex

waters or Case 2 waters (Morel and Prieur, 1977). This gap is possibly due to the difficulty of balancing the effect of several factors that influence the process of chlorophyll fluorescence in natural environments and the high cost of necessary equipment.

This paper assesses IVF measurements acquired in an optical complex aquatic environment (Amazon floodplain) as an alternative method for chlorophyll-*a* estimation.

2. Material and Methods

2.1. Study area

The study area selected for IVF assessment is the Curuai floodplain (Figure 1), located in the state of Pará, Brazil. This floodplain is composed by a variety of connected lakes with a wide range of optical components. Those floodplain lakes are mainly affected by the Amazon River flood pulse (Bonnet et al., 2008), with different water types (Sioli, 1984). The hydrology of the Curuai floodplain is characterized by four states (raising, high, receding and low water level) which display significant differences in chemical, physical and biological water properties (Barbosa et al., 2010). The raising water level state, during which this study took place, is characterized by large concentrations of TSS and low frequencies of algal blooms in relation to the other states. This state was chosen because it represents the most challenging setup to assess IVF methods for estimating Chl-*a*.

2.2. Field and laboratory measurements

The sampling design consisted of 26 stations (Figure 1) distributed throughout the floodplain lakes. For each station the following parameters were collected: temperature and turbidity, using a multiparameter probe (YSI 6600); depth (measured by a Speedtech SM5) and Secchi disc transparency; water samples were filtered on Whatman GF/F papers in duplicates, preserved in refrigerator at 0 °C and stored in the dark for later laboratory analysis according to Nusch (1980).

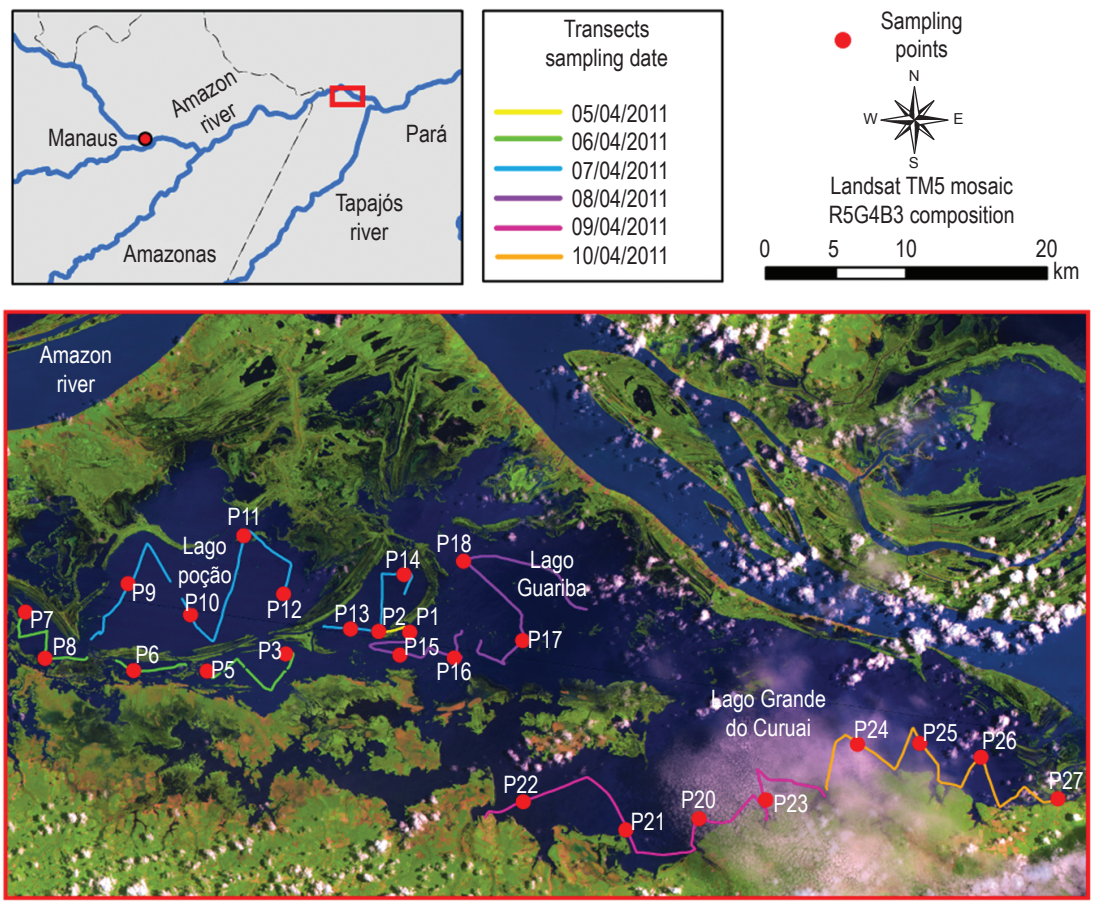

Figure 1. Study area with the location of sampling points (red dots) and fluorescence transects (yellow, green, blue, purple, pink and orange). The transect color distinguishes sampling dates.

For chlorophyll-*a* and pheophytin analysis, the samples were extracted with hot 80% ethanol for 5 minutes in test tubes and then a thermal shock was performed, immersing the tubes in ice water (0 °C). The extraction was continued for a period of 6 to 24 hours in the refrigerator, in the absence of light. The ethanol solution containing chlorophyll extracted was passed to jacketed glass tube of 20 mL capacity and reading was done by spectrophotometry, by using 50 mm optical path quartz cuvettes in 665 and 750 nm wavelengths. After reading, the sample was acidified with 0.4 N HCl to a pH between 2.4 and 2.8, and another reading was performed at 665 and 750 nm for pheophytin determination. This methodology was applied since there is no statistically significant difference compared with others widely used methods (Kuroda et al., 2005).

TSS samples were filtered using Whatman GF/C filters and analyzed according to Wetzel and Likens (1991). Finally, DOC analysis was performed according to Eaton et al. (1995).

The IVF data was obtained with a Turner Designs model 10-AU-005-CE fluorometer set in continuous flow mode and connected to a 12V self priming pump. This pump worked at a flow rate of approximately 23.8 $L.min^{-1}$. A red photomultiplier tube (Hamamatsu R446) was installed operating in wavelengths ranging from 185-870 nm. The system was designed by an optical kit (10-037R), composed of an excitation filter Andover 10-050R (240-500 nm), a long-pass cutoff emission filter Andover 10-051R (>665 nm) and a lamp 10-045 (Daylight White). The hose for water uptake was installed at a fixed depth (30 cm) in order to minimize the entry of air bubbles in the system. During a five day campaign more than 190 km of transects (Table 1) were carried out keeping the vessel at an average speed of 8 $km.h^{-1}$.

2.3. Fluorescence analysis

Figure 2 describes the main steps in the assessment of IVF estimating Chl-*a*. In the first step two methods for estimating IVF were tested. The *instantaneous method* consisted of noting down the fluorescence value while collecting water samples for chlorophyll-*a* determination. On the other hand, the *average method* consisted of computing the average fluorescence value for a 500 m radius around the sampling station along each transect.

The following step was to run a linear regression analysis between instantaneous and average IVF and Chl-*a* concentration for the entire data set (Global Model Adjustment). The aim of this step was to assess Global Model performance against regional models, obtained from the separation of the samples according to geographical distribution of lakes. The regionalization of the samples (Local Model Adjustment) was analyzed through scatter plots for lakes with insufficient number of samples and through regression analysis when the number of samples was statistically appropriate.

3. Results

Regardless of the fluorescence estimating method, it was not possible to fit a Global Model for estimating chlorophyll-*a* from IVF (Figure 3). Nevertheless, it was possible to observe two trends: i) a set with low chlorophyll-*a* concentration (up to 8 $\mu g.L^{-1}$) in which there was a large variation in fluorescence (between 20 and 40 arbitrary units); and ii) a set with wide variations in chlorophyll-*a* concentration (8 to 32 $\mu g.L^{-1}$), with minor variations in fluorescence (between 20 and 40 arbitrary units). This analysis suggests that the samples belong to different optical environments and needed to be treated as independent samples.

This lack of relationship can be explained by the wide variability in the concentration of optically active components in water, which directly affects light field in water and hence the IVF (Table 2). Fluorescence measurements varied around 18%, whereas Chl-*a* varied 72% relative to its mean. TSS concentration displays variability as high as that of Chl-*a* and DOC concentration is also high with variability of around 26%. Another important aspect is the wide variation (85%) of

Table 1. Data collection design for principal lakes of the floodplain.

Date	Floodplain region	Transect size (km)	Sampling station
05/04/2011	Central	3	P1 and P2
06/04/2011	Western small lakes	22.5	P3 to P8
07/04/2011	Central small lakes and Lago Poção	30	P9 to P11
08/04/2011	Lago Guariba	35	P15 to P18
09/04/2011 10/04/2011	Lago Grande do Curuai	100	P19 to P27

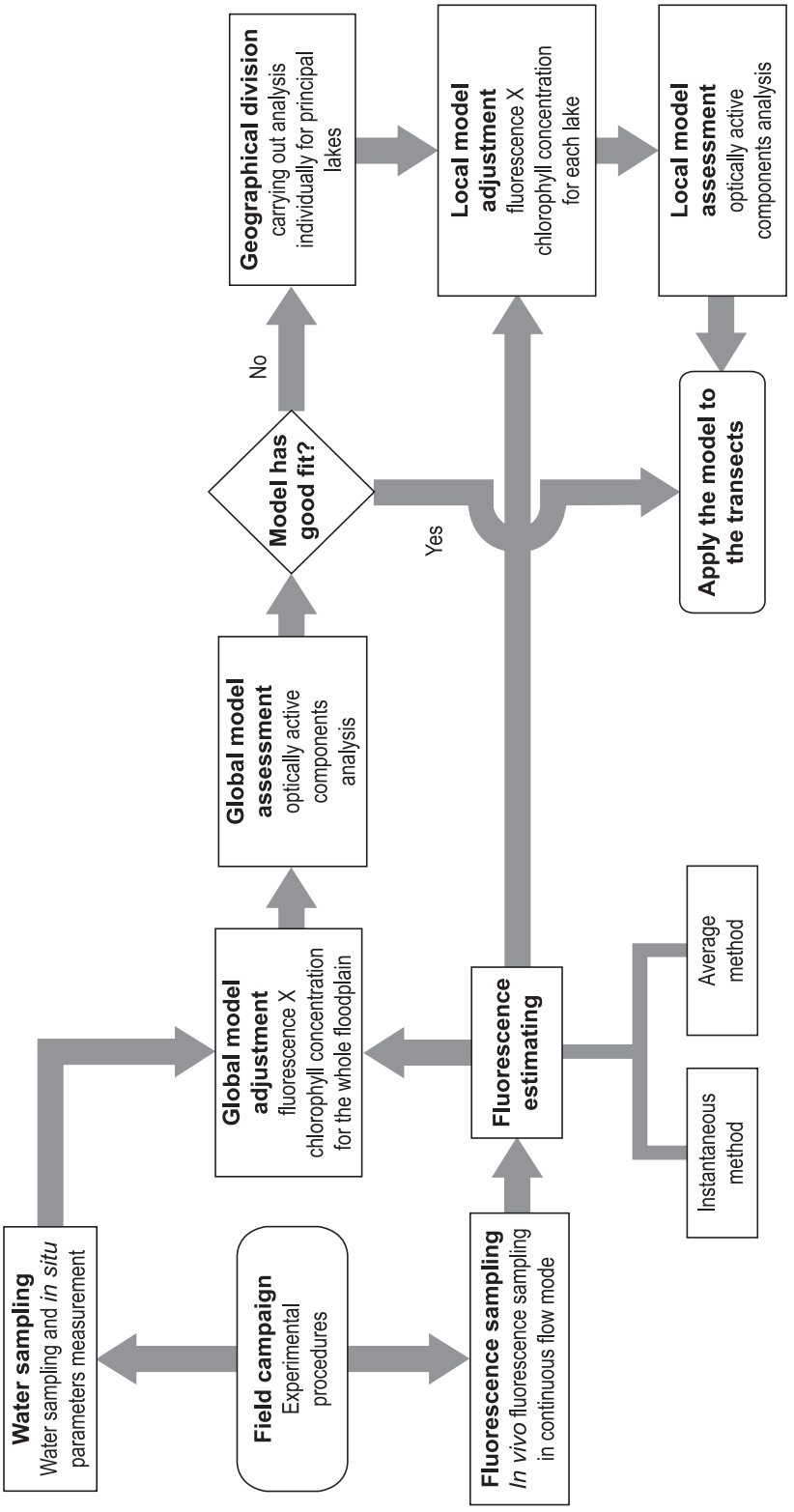

Figure 2. Flowchart of the proposed methodology.

the Pheophytin/Chl-*a* ratio which may account for both changes in phytoplankton physiology and or residual pheophytin. In the absence of residual pheophytin, the higher the ratio of Pheophytin/Chl-*a*, the larger is the number of degraded phytoplankton cells in the sample (Kirk, 1994; Reynolds, 2006).

Since the Global Model did not work for the available data, the sample set was divided according to their geographical location. It was assumed that in

a restricted environment, changes in the constituent concentrations would be smaller, favoring model fitting. This analysis, however, was restricted to lakes with enough samples to run a regression model (Kutner et al., 2004): Lago Poção, Lago Guariba and Lago Grande do Curuai.

3.1. Lago Poção

Irrespective of the fluorescence estimation method adopted, it was not possible to fit a model for Lago Poção (Figure 4). The high variability of lake composition was not captured by the sample available (n = 4). Note that for similar fluorescence

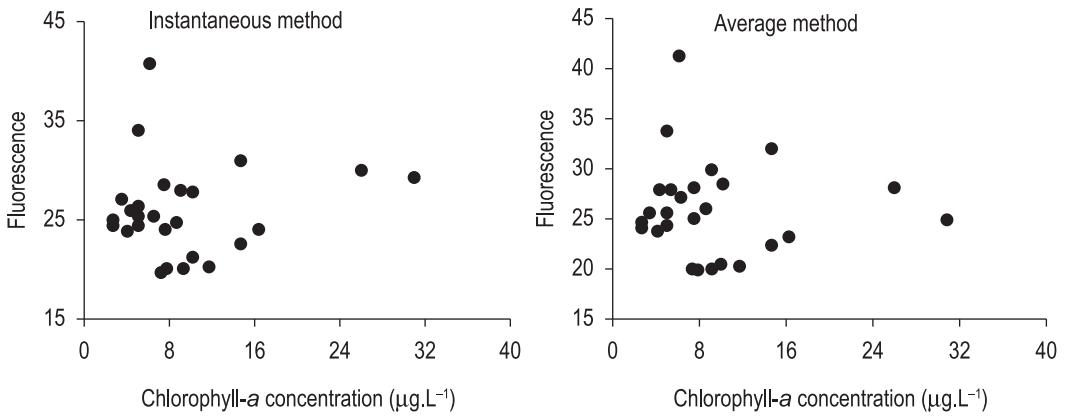

Figure 3. Scatter plots between chlorophyll-*a* and IVF for the two estimating methods.

Table 2. Mean, standard deviation (STD), coefficient of variation (CV), minimum (Min), maximum (Max) and number of samples (N) of variables measured during the 2011 field mission in the Curuai floodplain. Where: Inst. Fluorescence: fluorescence computed by instantaneous method, Avg. Fluorescence: fluorescence computed by average method, Pheophytin/Chl-*a*: ratio between pheophytin and chlorophyll-*a* concentration, DOC: dissolved organic carbon, TSS: total suspended solids.

Variables	Mean	STD	CV (%)	Min	Max	N
Inst. Fluorescence	25.96	4.66	17.95	19.77	40.74	26
Avg. Fluorescence	26.13	4.80	18.37	20.08	41.40	26
Chlorophyll-*a* (µg.L^{-1})	9.26	6.72	72.57	2.67	30.84	26
Pheophytin (µg.L^{-1})	5.63	2.56	45.47	0.91	11.87	26
Pheophytin/Chl-*a*	0.95	0.81	85.26	0.13	3.41	26
DOC (mg.L^{-1})	4.18	1.11	26.56	3.11	8.31	26
TSS (mg.L^{-1})	11.25	6.17	54.84	4.26	26.68	26
Temperature (°C)	30.49	0.81	2.66	29.32	32.99	26

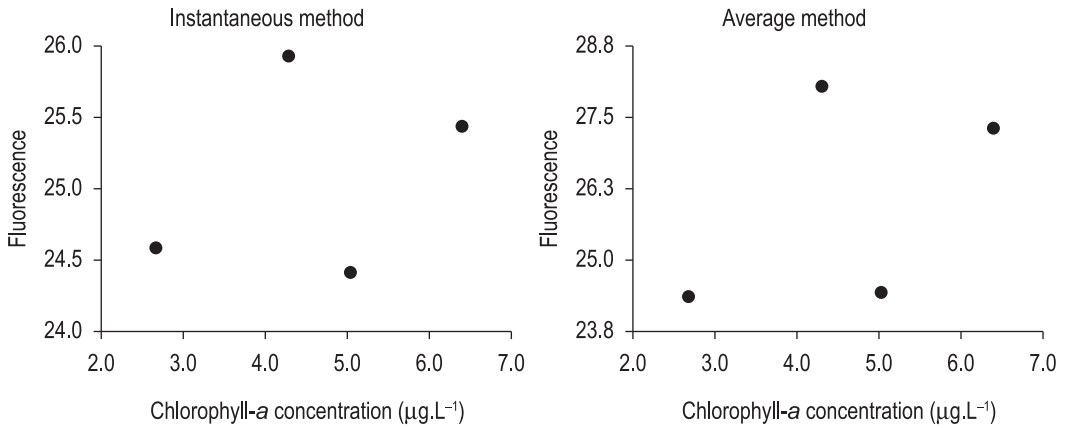

Figure 4. Scatter plots between chlorophyll-*a* and IVF for the two estimating methods in Lago Poção.

values (24.5 arbitrary units), the Chl-*a* ranged from 2.7 to 5.0 μg.L^{-1}.

A series of ratios between Chl-*a* and the remaining optically active components were examined in order to explain the poor adjustment between Chl-*a* and IVF in Lago Poção (Figure 5).

Lago Poção (Table 3) has an average Chl-*a* concentration (4.6 μg.L^{-1}) lower than that of the entire floodplain (9.26 μg.L^{-1}). This fact can be attributed to the thin euphotic zone (around 1.2 m for 40 cm Secchi depth). TSS variation in this region (49.5%) is similar to the variation of the total set of samples (54.8%). The Pheophytin/Chl-*a* ratio has a large variability (44%), suggesting wide variations in phytoplankton physiological state in the lake and/or a fair amount of residual pheophtin which may characterize a post bloom environment (Bianchi et al., 2002).

The DOC concentration in Lago Poção is almost constant and has an average close to that of the total sampling area. However, the average DOC concentration (4.17 mg.L^{-1}) may significantly alter the energy available in the blue spectral region for chlorophyll absorption, reducing, therefore, the amount of photons available to start the fluorescence process (Kirk, 1994; Reynolds, 2006). The scatter plot between IVF and the Chl-*a*/DOC ratio (Figure 5b) indicates that fluorescence increases as the Chl-*a* increases.

Figure 5c presents the scatter plot of IVF and Chl-*a*/TSS ratio. In this case, the fluorescence decreases, as the ratio increases. This inverse

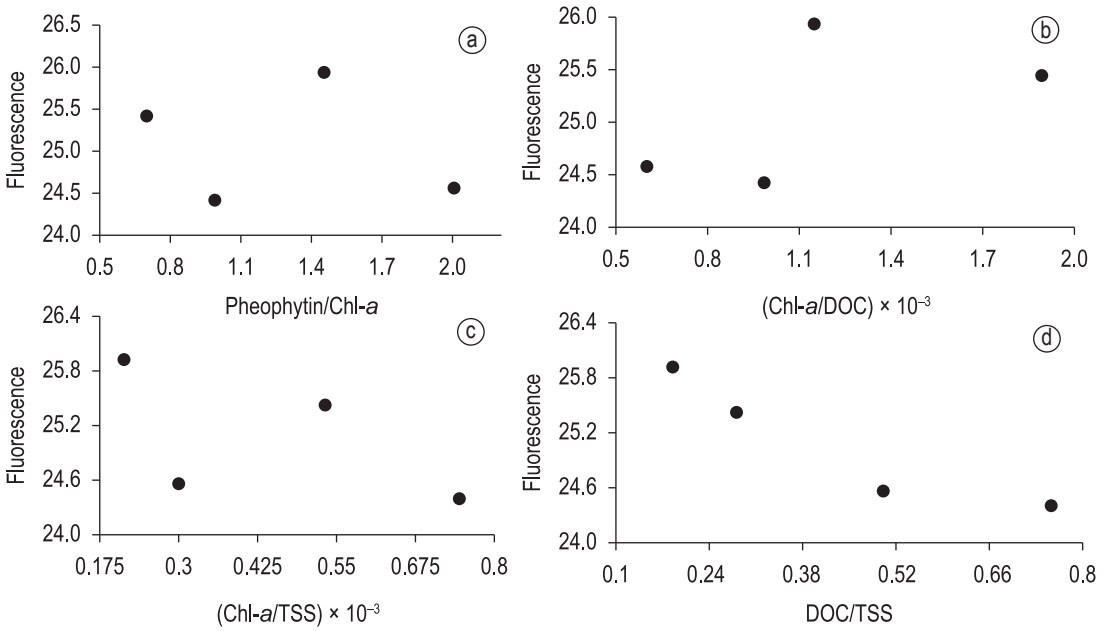

Figure 5. Scatter plots between IVF and water components ratio in Lago Poção: (a) Pheophytin/Chl-*a*; (b) Chl-*a*/DOC; (c) Chl-*a*/TSS; and (d) DOC/TSS.

Table 3. Mean, standard deviation (STD), coefficient of variation (CV), minimum (Min), maximum (Max) and number of samples (N) of variables measured during the 2011 field mission in Lago Poção. Where: Pheophytin/Chl-*a*: ratio between Pheophytin and chlorophyll-*a* concentration, DOC: dissolved organic carbon, TSS: total suspended solids.

Variables	Mean	STD	CV (%)	Min	Max	N
Fluorescence	25.10	0.72	2.87	24.42	25.93	4
Chlorophyll-*a* (μg.Lμ^{-1})	4.60	1.55	33.70	2.67	6.40	4
Pheophytin (μg.L^{-1})	5.24	0.75	14.31	4.44	6.22	4
Pheophytin/Chl-*a*	1.28	0.57	44.53	0.69	2.00	4
DOC (mg.L^{-1})	4.17	0.76	12.23	3.38	5.10	4
TSS (mg.L^{-1})	12.00	5.94	49.50	6.77	20.31	4
Temperature (°C)	30.43	0.48	1.58	29.80	30.98	4
Secchi (cm)	41.25	8.54	20.70	30.00	50.00	4
Depth (m)	5.30	0.18	3.40	5.10	5.50	4

relationship indicates that in areas with higher TSS compared to chlorophyll, phytoplankton must be near the surface so as the fluorescence signal can be detected. Finally, the comparison between IVF and the DOC/TSS ratio (Figure 5d) shows a strong inverse agreement between the parameters, indicating that the TSS may covaries with DOC, what is expected since it has been reported that large amount of DOC is adsorbed in the inorganic particles (Wetzel and Likens, 1991).

3.2. Lago Guariba

As observed in the scatter plot (Figure 6), the instantaneous fluorescence results in a better agreement with chlorophyll-*a* data but it cannot be quantified due to the limited number of samples.

The statistics for Lago Guariba are presented in Table 4. This lake has a much higher Chl-*a* concentration (10.31 mg.L^{-1}) compared to that of Lago Poção and the lowest Pheophytin/Chl-*a* ratio (mean of Pheophytin/Chl-*a* = 0.5) suggesting that phytoplankton is homogeneously healthy. The DOC was constant, causing homogeneous changes in photon absorption in this environment. Those factors might explain the better agreement between IVF and Chl-*a*. This lake, however, has an adverse factor which is the high TSS concentration. Despite the high TSS concentration (19.83 mg.L^{-1}), this factor did not affect the fluorescence, since the phytoplankton was located at subsurface (32.5 cm of Secchi depth).

The scatter plot between IVF and water components at Lago Guariba is presented in Figure 7. The average DOC is similar to that of Lago Poção, but the Chl-*a* is higher. Thus, the better agreement between IVF and Chl-*a*/DOC (Figure 7b) ratio is justified, since there is a larger amount of chlorophyll in relation to organic matter, reducing its influence on the fluorescence. Regarding TSS, the influence is similar to that of Lago Poção (Figure 7c). A strong agreement between IVF and DOC/TSS ratio is also observed,

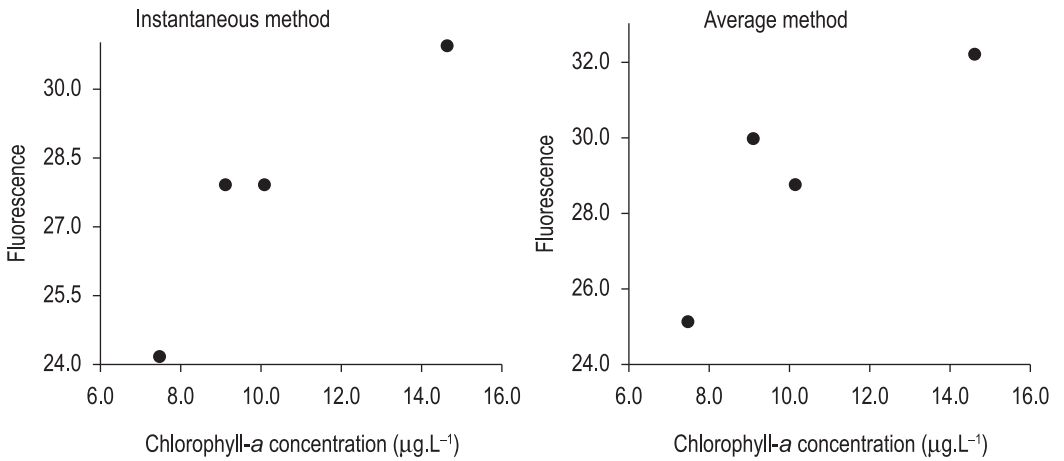

Figure 6. Scatter plots between chlorophyll and IVF for the two estimating methods in Lago Guariba.

Table 4. Mean, standard deviation (STD), coefficient of variation (CV), minimum (Min), maximum (Max) and number of samples (N) of variables measured during the 2011 field mission in Lago Guariba. Where: Pheophytin/Chl-*a*: ratio between Pheophytin and chlorophyll-*a* concentration, DOC: dissolved organic carbon, TSS: total suspended solids.

Variables	Mean	STD	CV (%)	Min	Max	N
Fluorescence	27.75	2.76	9.95	24.19	30.93	4
Chlorophyll-*a* (µg.L^{-1})	10.31	3.05	29.58	7.48	14.59	4
Pheophytin (µg.L^{-1})	5.24	0.46	8.78	4.78	5.85	4
Pheophytin/Chl-*a*	0.53	0.12	22.64	0.40	0.67	4
DOC (mg.L^{-1})	3.67	0.38	10.35	3.11	3.96	4
TSS (mg.L^{-1})	19.83	9.16	46.19	6.52	26.68	4
Temperature (°C)	29.80	0.53	1.78	29.32	30.29	4
Secchi (cm)	32.50	5.00	15.38	30.00	40.00	4
Depth (m)	5.30	0.36	6.79	4.80	5.60	4

suggesting that TSS has a much larger interference in the fluorescence than the DOC (Figure 7d).

3.3. Lago Grande do Curuai

The Lago Grande do Curuai dataset (n=9) provided a statically significant adjustment (R^2=0.94, $p<10^{-5}$) between IVF and Chl-*a* due to favorable environmental optical conditions (Figure 8).

First of all, the highest values of Chl-*a* in the Curuai floodplain (Table 5) were observed at Lago Grande do Curuai (7.22 to 30.84 µg.L^{-1}). In addition to that, this lake also had the lowest TSS (7.99 mg.L^{-1}) and highest Secchi depth averages (51.67 cm). The Pheophytin/Chl-*a* ratio was also the lowest, suggesting that the phytoplankton was homogenously healthy during the sampling.

As described for the previous lakes, DOC concentration in Lago Grande do Curuai corresponds to the average for the entire floodplain. The Chl-*a*, in turn, has a significantly higher average compared to the other lakes. These characteristics explain why Lago Grande do Curuai environment is suitable for adjusting a regression model between

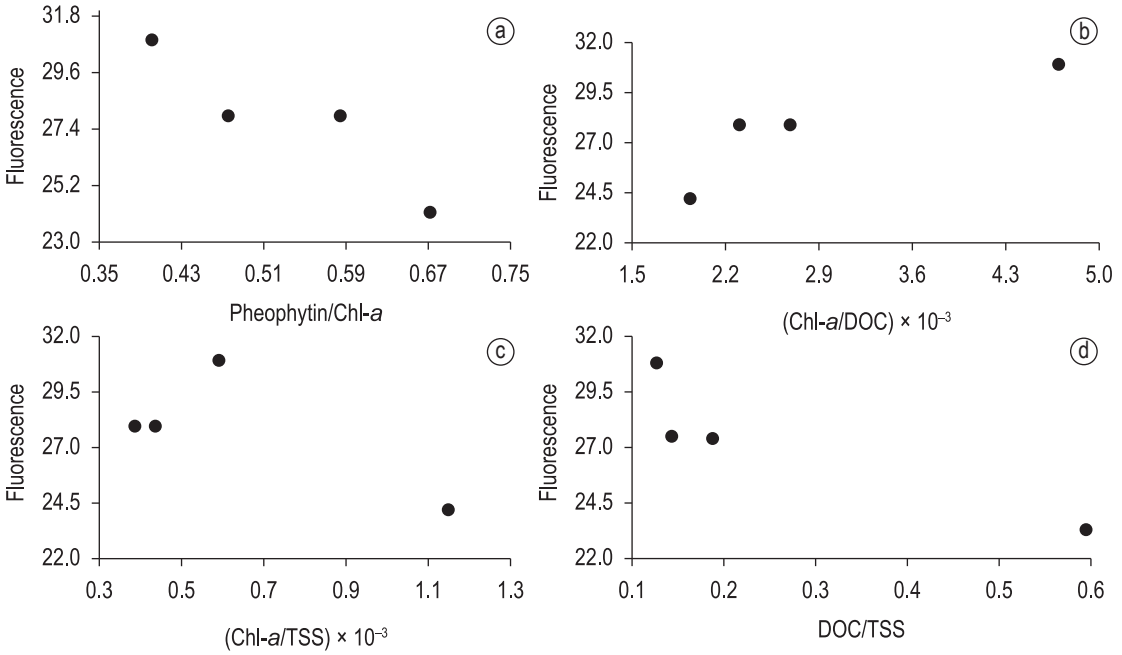

Figure 7. Scatter plots between IVF and water components ratio in Lago Guariba: (a) Pheophytin/Chl-*a*; (b) Chl-*a*/DOC; (c) Chl-*a*/TSS; and (d) DOC/TSS.

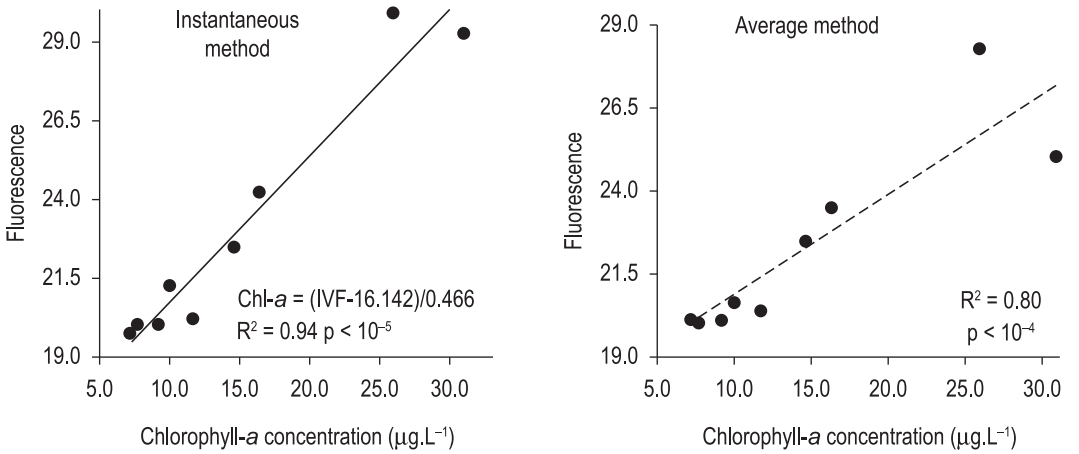

Figure 8. Local model adjustment between chlorophyll and IVF for the two estimating methods in Lago Grande do Curuai.

the IVF and Chl-*a* (Figure 8). The Chl-*a*/DOC ratio provides a good fit with IVF ($R^2 = 0.96$), converging with the results for lakes Guariba and Poção, where there was also an increase in fluorescence with the increase in Chl-*a* in relation to DOC (Figure 9b).

However, contrary to what was observed in the previous lakes, there was an inversion in the relationship between IVF and the Chl-*a*/TSS ratio. This ratio is higher than 0.001 in Lago Grande do Curuai, making the Chl-*a* the dominant optical component.

It was not observed correlation between the DOC/TSS ratio and fluorescence (Figure 9d). This result also suggests that in this lake, the chlorophyll is the optically dominant constituent. Thus, chlorophyll has a greater influence on spectral signal recorded by the fluorometer.

4. Discussion

The use of IVF for estimating Chl-*a* in optically complex aquatic systems demands care. There are many factors which may affect the relationship between chlorophyll-*a* concentration and fluorescence. In the next section, those aspects are discussed.

Table 5. Mean, standard deviation (STD), coefficient of variation (CV), minimum (Min), maximum (Max) and number of samples (N) of variables measured during the 2011 field mission in Lago Grande do Curuai. Where: Pheophytin/Chl-*a*: ratio between Pheophytin and chlorophyll-*a* concentration, DOC: dissolved organic carbon, TSS: total suspended solids.

Variables	Mean	STD	CV (%)	Min	Max	N
Fluorescence	23.05	4.00	17.35	19.77	29.49	9
Chlorophyll-*a* (µg.L^{-1})	14.82	8.33	56.21	7.22	30.84	9
Pheophytin (µg.L^{-1})	3.94	2.10	53.30	0.91	8.06	9
Pheophytin/Chl-*a*	0.29	0.15	51.72	0.13	0.55	9
DOC (mg.L^{-1})	3.65	0.30	8.22	3.27	4.03	9
TSS (mg.L^{-1})	7.99	1.33	16.65	6.98	11.31	9
Temperature (°C)	30.27	0.52	1.72	29.68	31.19	9
Secchi (cm)	51.67	4.33	8.38	45.00	60.00	9
Depth (m)	5.98	0.84	14.05	5.10	8.00	9

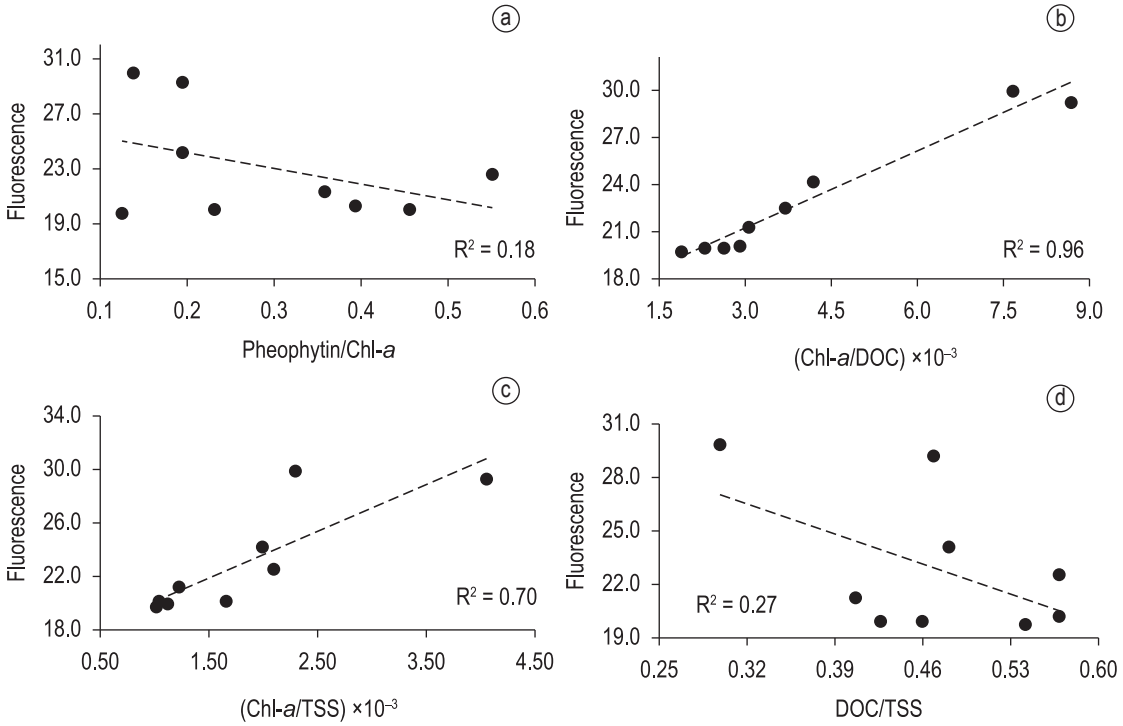

Figure 9. Regression analysis between IVF and water components ratio in Lago Grande do Curuai: (a) Pheophytin/Chl-*a*; (b) Chl-*a*/DOC; (c) Chl-*a*/TSS; and (d) DOC/TSS.

4.1. Pheophytin

Welshmeyer (1994) conducted tests to examine the interference of the Pheophytin/Chl-*a* ratio in the IVF recorded by different optical apparatus. For the system based on specific narrow bandwidths filters, the IVF showed an increase of 10% when the ratio Pheophytin/Chl-*a* = 1. On the other hand, for the fluorometer equipped with broader bandwidth filters, as used in this study, the variation of fluorescence reached a value 1.75 times larger for the same ratio Pheophytin/Chl-*a* = 1.

In the present, the Pheophytin/Chl-*a* ratio presented low values in Lago Guariba and Lago Grande do Curuai (Tables 4 and 5). However, in Lago Poção the average ratio was 1.3. The high concentration of pheophytin, therefore exerts a strong influence on the IVF-Chl-*a* relationship. Another aspect to be emphasized is that the Pheophytin/Chl-*a* ratio may also be affected by the elapsed time between sampling and laboratory analysis. The observed degradation may not be from *in situ* phytoplankton, but from the sample degradation. This is another difficult factor to be accounted in modeling Chl-*a* from IVF. One should also point out that there might be residual pheophytin in the environment not related to the recent phytoplankton degradation or to sample handling, but to a previous bloom situation (Bianchi et al., 2002).

4.2. Temperature

According to Lorenzen (1966), each degree of variation in water temperature results in changes in the fluorescence of 1.4%, condition valid for ambient temperatures varying from 0° to 35 °C. During the field mission, the temperature varied 3.67 °C (Table 2), so the errors related to changes in temperature can be considered negligible (5%).

4.3. Total suspended solids

The presence of large amounts of suspended material seems to be the main factor affecting the relationship between Chl-*a* concentration and IVF. Goodin et al. (1993) showed that as the turbidity increases, there is a significant increase in spectral signal of the water in the red spectral region (600 to 700 nm). The fluorometer was set to detect wavelengths from 665 to 870 nm according to specifications of the optical kit (10-037R) used for measuring IVF. This spectral region is also sensitive to changes in inorganic particle turbidity. Thus, variations in turbidity concentration may be interpreted by the sensor as fluorescence (665 nm), which may spoil the development of chlorophyll models having fluorescence as input data.

The results of this study indicate that when the ratio Chl-*a*/TSS <0.001, the agreement between IVF and Chl-*a* is poor. This result converges with the analysis carried out by Lobo et al. (2011), which established the same Chl-*a*/TSS threshold for identifying spectral signal of chlorophyll-a in Amazon freshwaters.

4.4. Dissolved organic carbon

Experiments conducted to assess the influence of DOC on fluorometric analysis, highlighted the importance of filters (Carlson and Shapiro, 1981). Excitation filters allowing wavelengths between 340 to 500 nm are affected by large amounts of dissolved organic matter in water, because it can absorb part of the energy which could be otherwise used to excite molecules of chlorophyll, thereby decreasing the fluorescence (Witte et al., 1982).

This study identified thresholds for the relationship between Chl-*a* and IVF based on the Chl-*a*/DOC ratio. According to the results, for ratio Chl-*a*/DOC<0.002 it is not possible to build a model for estimating Chl-*a* from fluorescence measurements. Above this threshold, fluorescence tends to respond to changes in chlorophyll concentration. Finally, the inverse relationships between IVF and the DOC/TSS ratio for all lakes indicate that TSS has the largest influence on fluorescence relative to DOC. The DOC affects the blue range of the spectra, whereas the TSS affects the red and near-infrared ranges.

5. Conclusions

The assessment of IVF measurements acquired in optical complex aquatic environments as an alternative method for chlorophyll estimation showed that it is not possible to develop global models to account for the entire region. The search for regional models provided insights on the main factors affecting the relationship between IVF and Chl-*a* concentration.

First of all the method does not provide suitable results in environment dominated by high inorganic particle turbidity expressed in this study by low ratios Chl-*a*/TSS. The method is also limited in environment with low Chl-*a*/DOC ratios. This limitation, however, can be overcome by changing the position of excitation filters. The use of an appropriate optical kit, composed of filters with narrower specific bandwidths, can reduce the influence of chlorophyll degradation products. One

should also emphasize that the time period between sampling and transport to laboratory analysis can lead to degradation of samples, but this is a difficult factor to be accounted for the analysis in remote regions.

Although confronted by such problems, this work reinforces the great potential of fluorometry technique, since even with a small number of samples it was possible to set a good model in the main lake of the Curuai floodplain. Finally, even though the technique cannot be considered extremely accurate, at least it is very useful in order to assess the pattern of chlorophyll spatial variation reliably and with relatively low cost. These possibilities are very interesting in carrying out field missions in the Amazon region.

Finally, despite the results did not allow the development of a global model, further studies in controlled conditions and different environments are recommended for better understanding the IVF as an alternative method for chlorophyll estimation.

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