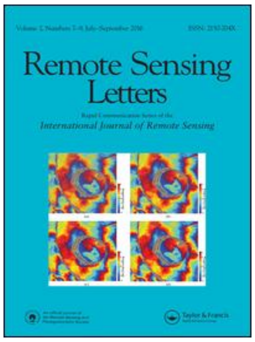

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An investigation into the phytoplankton package effect on the chlorophyll-*a* specific absorption coefficient in Barra Bonita reservoir, Brazil

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ABSTRACT

In this article, a possible phytoplankton package effect on the chlorophyll-*a* specific absorption coefficient (a^*_{phy}) is investigated. Two fieldworks were conducted in May and October 2014 in Barra Bonita (BB) reservoir. During the fieldworks, radiometric and water samples were obtained. From the radiometric data, the remote sensing reflectance (R_{rs}) were calculated and from the water samples the chlorophyll-a (chl-*a*) concentration, the phytoplankton absorption coefficient (a_{phy}) and a^*_{phy} coefficient were obtained. The results show that for the first fieldwork (in May), the package effect was less perceived than in the second fieldwork (in October). In May, the package effect was more pronounced for the highest chl-*a* concentration (>200 mg m^{-3}) and for October all samples ranging from 263.20 to 797.80 mg m^{-3} were effected. Due to this effect, the bio-optical model development in order to estimate the chl-*a* concentration in a eutrophic environment such as the BB reservoir will face higher errors when the chl-*a* concentration were higher than 300 mg m^{-3}.

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1. Introduction

The phytoplankton-specific light absorption plays a fundamental role in the pigment biomass, primary production and other aquatic systems variables from remote sensing (Bricaud et al. 1995). It is well documented that the phytoplankton absorption coefficient, a_{phy}, is positively correlated with chlorophyll-*a* concentration (chl-*a*). The a_{phy} is one of the principal inherent optical properties of water affecting the spectral reflectance (Sathyendranath et al. 2001). The variability of phytoplankton specific absorption a^*_{phy} (units in m^2 mg (chl-*a*)$^{-1}$) in terms of magnitude and spectral form can be attributed mainly to phytoplankton cells' package effects and accessory pigment. The package effect is a common physiological strategy for large phytoplankton species, such as diatoms (Bricaud et al. 1995). The package effect reduces the absorption spectra and can increase the error of estimate chl-*a* concentration from space (Marra, Trees, and O'Reilly 2007).

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The package effect is well known for ocean waters (Wang et al. 2014) but a very few studies have been conducted in tropical inland waters. Since the knowledge of phytoplankton light absorption is crucial for understanding the optical variability in aquatic systems, and therefore to improve bio-optical algorithms, we hypothesize that if detected the package effect can influence the development of bio-optical models to estimate the chl-*a* concentration in inland waters.

The aim of this article is to address the influence of package effect on phytoplankton specific absorption in tropical inland waters in Brazil and also to discuss the implication for bio-optical modelling.

2. Materials and methods

2.1. *Study area*

Barra Bonita hydroelectric reservoir (BB) (22° 31′ 10′′ S and 48° 32′ 3′′ W) lies in the middle course of the Tietê River, São Paulo State, Brazil (Figure 1). The BB reservoir is situated in a transitional region between tropical and subtropical climate, characterized by a dry period (May – October) and a wet period (November – April) (Alcântara et al. 2016).

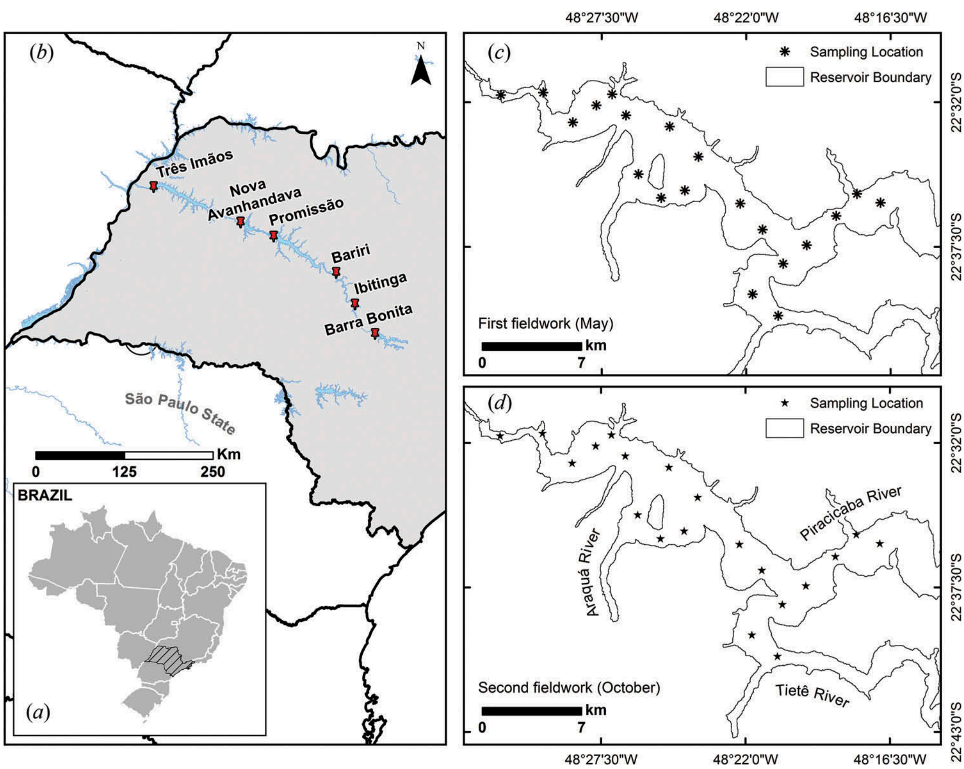

Figure 1. Location of study area (a) Brazil, with São Paulo State highlighted; (b) Tietê River and the cascade of reservoirs (from upstream to downstream: Barra Bonita (study area), Ibitinga, Bariri, Promissão, Nova Avanhandava, and Três Irmãos). The sampling locations for the first fieldwork (May) and second fieldwork (October) can be seen in (c) and (d), respectively.

2.2. *Fieldwork*

Two fieldworks were carried out from 5 to 8 May 2014 and from 13 to 17 October 2014. In each fieldwork, 20 samples were taken (see Figure 1 for sample stations location).

2.3. *Radiometric data*

In situ radiometric measurements were acquired by three spectroradiometers: two ARC-VIS RAMSES sensors with a 7° field-of-view in order to measure radiance, and one ACC-VIS RAMSES sensor with a cosine collector to measure irradiance (TriOS, Oldenburg, Germany). Radiances (total radiance – L_t; and diffuse radiance – L_{sky}, both in W m^{-2} sr^{-1}) and downwelling irradiance data ($E_d(0^+)$, in W m^{-2}) were measured in an azimuth angle of 90° in order to minimize the specular reflection (Mobley 1999). The radiometric data were measured 10 times in each sampling station and a mean value was used to compute the remote sensing reflectance (R_{rs}, units in sr^{-1}) above water, by using Equation 1.

$$R_{rs}(\theta,\phi,\lambda,0^+) = \frac{L_t(\theta,\phi,\lambda,0^+) - 0.028 \times L_{sky}(\theta,\phi,\lambda,0^+)}{E_d(\theta,\phi,\lambda,0^+)} \quad (1)$$

where θ is the azimuthal angle, ϕ is the zenithal angle, λ is the wavelength and 0^+ indicates that measurements were made just above the water surface.

2.4. *Phytoplankton absorption coefficient*

Water samples were filtered through a 0.7 μm porosity GF/F fibreglass that was stored flat under freezing condition. The determination of the total particulate (algal and detritus) absorption (a_p) was performed by an integrating sphere module presented in the double-beam Shimadzu UV-2600 UV-VIS spectrophotometer, with spectral sampling from 280 to 800 nm (Tassan and Ferrari 1995, 1998). To acquire the phytoplankton (a_{phy}) and detritus (a_d) absorption coefficients, the filter undergoes depigmentation by oxidation in 10% sodium hypochlorite (NaClO), ensuring that the samples does not contain pigment interference (Tassan and Ferrari 1995, 1998).

2.5. *Chl-a concentration*

Chl-*a* extraction samples were passed through GF/C filters (Whatman) under vacuum pressure through with a porosity of 0.7 μm, and then frozen for laboratory analysis. The chl-*a* was extracted by maceration in 90% acetone, stored in 20 ml tubes and placed in a centrifuge to have the absorbance read later in a spectrophotometer (Golterman 1975).

3. Results and discussion

3.1. *Chl-a concentrations*

The statistics of chl-*a* concentration in BB reservoir for both fieldworks are shown in Table 1. The second fieldwork presented the highest concentrations and consequently

Table 1. Descriptive statistics for chl-*a* (mg m^{-3}) measurements taken from BB reservoir during the two fieldworks.

	Fieldwork in May	Fieldwork in October
Minimum	19.10	263.20
Maximum	293.20	797.80
Mean	124.70	428.70
Range	274.10	534.60
Standard deviation	±71.00	±154.50

the highest standard deviation. The average chl-*a* concentration was 124.70 mg m^{-3} in May, while in October, the average was 428.70 mg m^{-3}. The chl-*a* concentrations ranged from 19.10 to 293.20 mg m^{-3} in May, and from 263.20 to 797.80 mg m^{-3} in October. The standard deviation was two times higher in October (±154.50 mg m^{-3}) than in May (±71.00 mg m^{-3}).

3.2. *Remote sensing reflectance*

In BB reservoir, the absorption feature of the phycocyanin pigment at approximately 620 nm was associated with the presence of cyanobacteria in both field surveys (Figure 2(a), (b)). In addition, there was a high absorption feature at about 680 nm and a reflectance peak at approximately 710 nm that can be associated with the chl-*a* pigment (Watanabe et al. 2015).

Another reflection feature was observed at 810 nm associated with both chl-*a* and organic matter (Rundquist et al. 1996), which results from the low absorption of pure water at this wavelength. The increase of reflectance in longer wavelength was an indicative of total suspended matter concentration increases. The chl-*a* concentration in BB reservoir during the second survey was 2.8 times higher than in the first survey and this explained why the absorption for pigments was more evident during the second survey.

3.3. *Phytoplankton absorption coefficients*

The a_{phy} spectra for the first and second fieldworks are presented in Figure 3(a), (b), respectively. The majority of a_{phy} spectra are characterized by the presence of

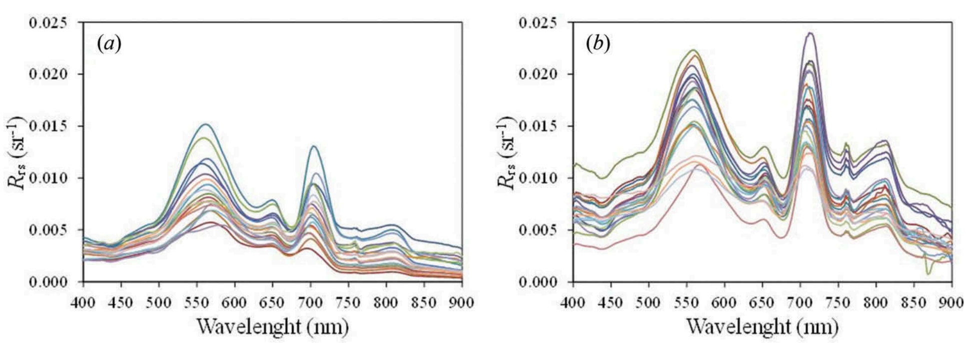

Figure 2. The R_{rs} spectra for the first (a) and second fieldwork (b).

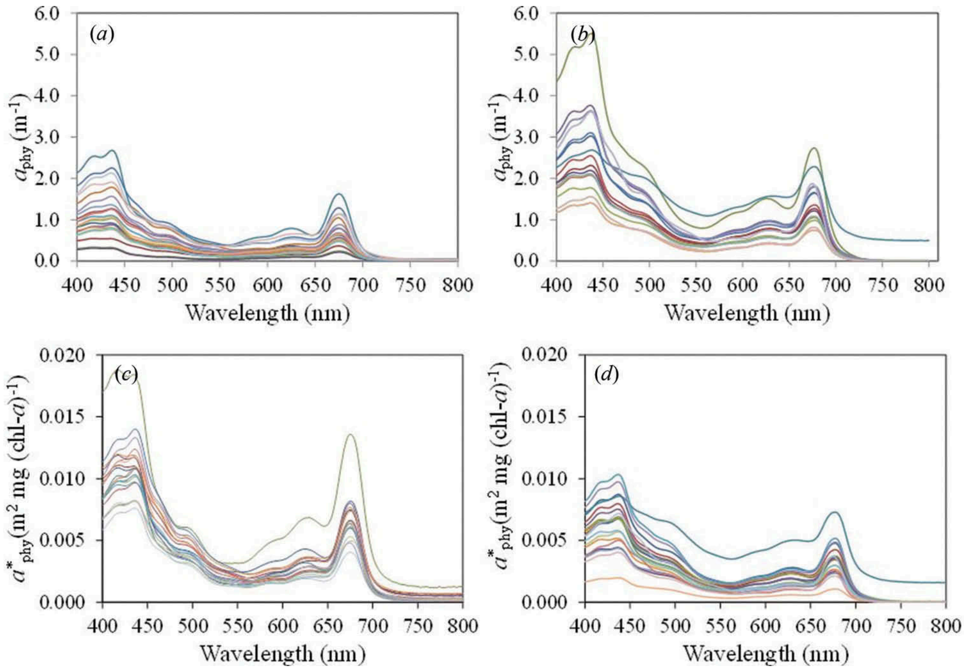

Figure 3. Measured a_{phy} spectra for the first (a) and second fieldwork (b) and the a^*_{phy} for the first (c) and second fieldwork (d).

absorption peaks at 440, 490 and 675 nm. Carotenoids have absorptions from 440 to 530 nm, with peaks at 460 and 490 nm. The absorption peak at 675 nm is attributed to absorption by chl-*a* plus phaeophytin.

The a^*_{phy} spectra are presented in Figure 3(c), (d) for the first and second fieldwork, respectively. Chl-*a* absorption features at 412, 440, 490 and 675 nm are further marked in all the a^*_{phy} spectra as well as an absorption peak at around 620 nm, associated with the phycocyanin pigment. The highest absorption feature at blue spectrum (440 nm) exhibited a range of 0.0074–0.017 m^2 mg^{-1} with a mean value of 0.011 m^2 mg^{-1} in May. In October, the variation was of 0.0019– 0.01 m^2 mg^{-1} and mean value of 0.0064 m^2 mg^{-1}.

Phytoplankton absorption variations were largely influenced by changes in chl-*a* concentrations, which ranged from 19.01 to 293.20 mg m^{-3} in May and from 263.20 to 797.80 mg m^{-3} in October. Analyses of the relationship between a_{phy} at 675 nm (a_{phy}(675)) and chl-*a* concentration revealed a relative high positive correlation in May (Figure 4(a)) and a low positive correlation in October (Figure 4(b)).

Overall, we observed from Figure 4(b) that for chl-*a* concentration higher than 250 mg m^{-3} the correlation with a_{phy}(675) decreases. This low correlation can be explained by the influence of the package effect.

3.4. *The packaging effect*

The a^*_{phy} at 675 nm (a^*_{phy}(675)) was calculated in order to show the phytoplankton package effect. Figure 5 shows the relationship between the a^*_{phy}(675) and the chl-*a*

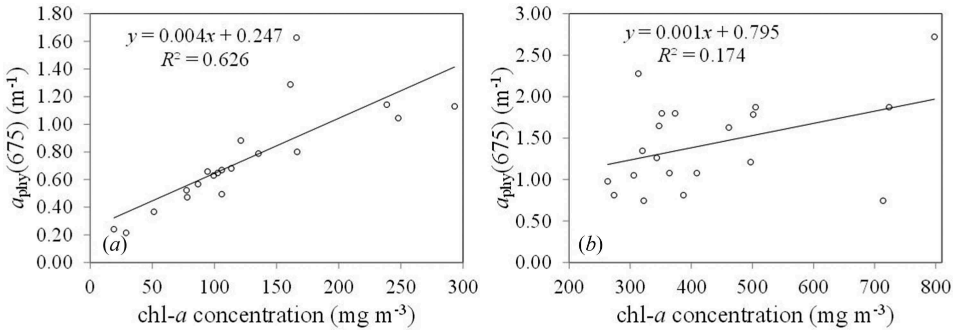

Figure 4. Correlation between $a_{phy}(675)$ and the chl-*a* for the first (a) and second fieldwork (b). R^2 is the coefficient of determination.

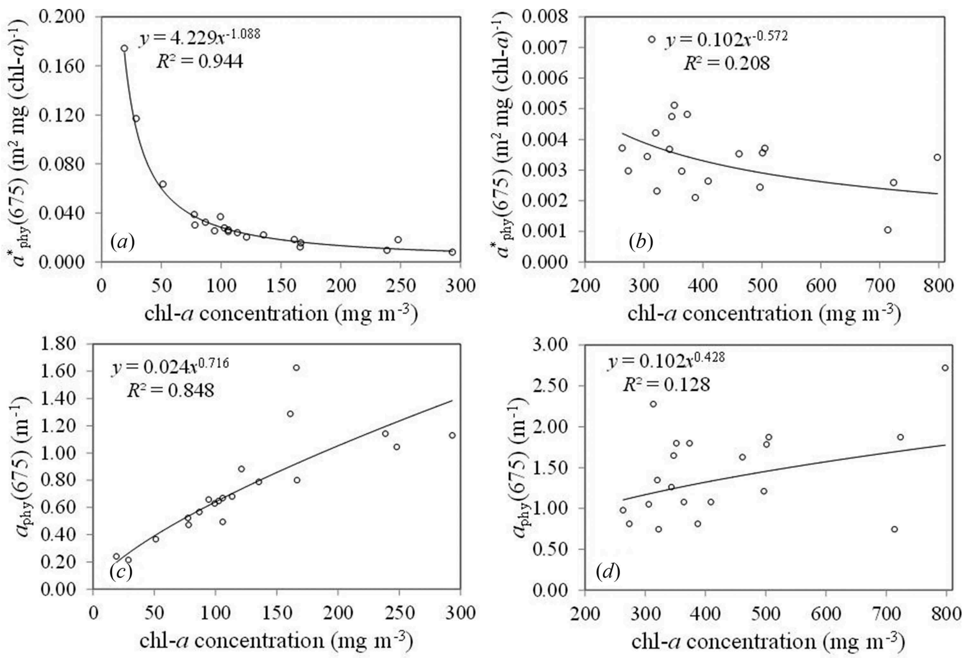

Figure 5. Relationship between $a^*_{phy}(675)$ and chl-*a* for the first (a) and second fieldwork (b). R^2 is the coefficient of determination.

concentration in May (Figure 5(a)) and October (Figure 5(b)). Overall, there is a decrease of $a^*_{phy}(675)$ with an increase of chl-*a* concentration. Bricaud et al. (1995) discovered that a^*_{phy} decreases with increasing chl-*a* concentration at 412, 443 and 490 nm and is lower at 675 nm. This inverse relationship was also reported in previous studies (e.g., Le et al. 2009) and may be explained by the fact that an increase in chl-*a* concentration leads to an increase in the concentration of intracellular pigment or in cell volume rather than in cell number, resulting in an absorption efficiency loss in the package effect (Le et al. 2009). The $a^*_{phy}(675)$ fluctuation is generally spurred by the package effect and accessory pigments such as chlorophyll-*b* and phaeopigments.

The behaviour shown in Figure 5(a) stems from the fact that the increase in chl-*a* concentration can be associated with an increase in intracellular pigment concentration or in cell volume rather than in cell number, which leads to an absorption efficiency loss in package effect (Bricaud et al. 1995). The package effect can be quantified as the ratio of a^*_{phy}, to the specific absorption coefficient of the same cellular matter ideally dispersed into solution (a^*_{sol}). This dimensionless factor, Q^*_a, has been theoretically formulated as follows (Morel and Bricaud 1981):

$$Q^*_a = (3/2) \times Q_a \times (\rho')/\rho' \quad (2)$$

where Q_a, the mean efficiency factor for absorption by phytoplanktonic cells, is a function of the parameter ρ':

$$Q_a = 1 + 2 \times \exp(-\rho')/\rho' + 2 \times \exp(-\rho') - 1/\rho'^2 \quad (3)$$

where ρ' is the product of the absorption coefficient of the cellular matter (a_{cm}) and the cell size d,

$$\rho' = a_{cm} \times d \quad (4)$$

According to Morel and Bricaud (1981), the Q^*_a continuously decreases from 1 (no package effect) to 0 (maximal package effect) with increasing ρ' values. The importance of package effect cannot be, in general, assessed independently from the variations in the pigment composition, since, at most wavelengths, a_{cm} is influenced by the pigment composition. Bricaud et al. (1995) found that the specific absorption coefficient of chl-*a* in solution is approximately 0.0207 m^2 mg^{-1} of chl-*a* at 675 nm, and therefore, the variations of the dimensionless factor Q^*_a at 675 nm ($Q^*_a(675)$) can be assessed as follows:

$$Q^*_a(675) = a^*_{phy}/0.0207 \quad (5)$$

The variation of $Q^*_a(675)$ for BB reservoir in May and October can be accessed in Figure 6(a), (b), respectively. In May, there are only two samples with package effect, with $Q^*_a(675)$ values of 0.45 (chl-*a* = 238.63 mg m^{-3}) and 0.38 (chl-*a* = 293.24 mg m^{-3}). In October, all samples presented package effect, with $Q^*_a(675)$ values up to 0.35.

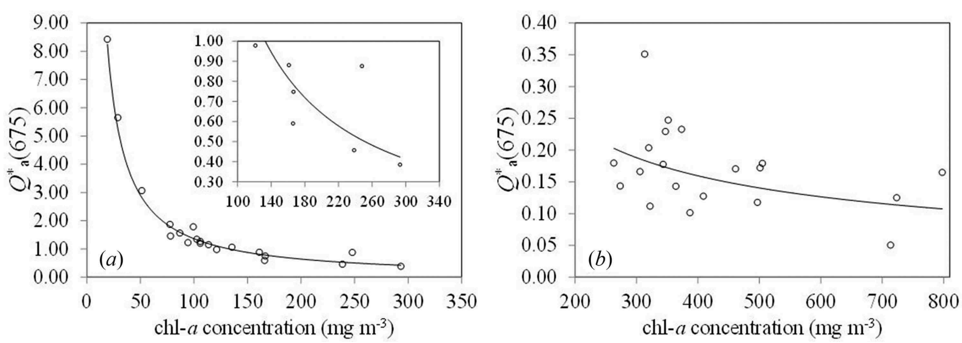

Figure 6. Variations of the dimensionless factor $Q^*_a(675)$ for the first (a) and second fieldwork (b). Figure (a) shows in detail the interval of $Q^*_a(675)$ from 0.30 to 1.00 and the chl-*a* concentration from 100 to 340 mg $m^{-3.}$

According to Watanabe et al. (2015), the BB reservoir can be classified from eutrophic to hypertrophic in May and as hypertrophic in October. Therefore, the package effect appears to increase, on average, from eutrophic to hypertrophic waters, with a three-fold decrease of $Q_a^*(675)$ over the experienced chl-*a* range. According to Bricaud and Stramski (1990), the $Q_a^*(675)$ values higher than the theoretical maximum value (equal to 1) can be associated with uncertainties in the pathlength amplification β factor (defined as the ratio of optical to geometrical pathlength) in laboratory measurements. This uncertainty increases with decreasing optical density.

3.5. *Implications of the package effect on bio-optical modelling*

Bio-optical modelling is an analytical approach that describes the relationship of R_{rs} to the concentration of constituents. The estimation of chl-*a* from remote-sensed images depends on the calibration of bio-optical model that can be empirical, semi-empirical or analytical. Watanabe et al. (2015) used three most frequently indices in literature (Table 2) to estimate the chl-*a* concentration in BB reservoir. These authors used the same dataset of R_{rs} and chl-*a* concentration that were used in this article to show the package effect.

The three indices shown in Table 2 uses the wavelength at 665 nm, which is very close to the wavelength used in our article (675 nm) to show the package effect. Watanabe et al. (2015) calibrated the chl-*a* concentration bio-optical model based on those three indices by using hyperspectral R_{rs} data. Overall, the results obtained from the validation procedure by these authors showed that the models underestimated the chl-*a* concentration, in which the 2B index bias ranged from −51.71 (linear fitting) to −50.26 mg m^{-3} (polynomial fitting), using the 3B index the bias ranged from 0.75 (linear fitting) to −3.19 mg m^{-3} (polynomial fitting) and for the model that uses the Normalized Difference Chlorophyll Index (NDCI) the bias ranged from −49.56 (linear fitting) to −49.54 mg m^{-3} (polynomial fitting).

The highest normalized root mean square error was obtained using the NDCI (33.84%) and the lowest using the 3B index (16.72%). Since they used the dataset collected in the second fieldwork (October), the errors were a little bit high. It seems that the fact of NDCI uses the R_{rs} at 665 nm can increase the error of estimation and the 3B index can be the best for BB reservoir water type. Since the package effect is pronounced at 675 nm, which is closer to 665 nm. Indeed, to estimate the chl-*a* concentration in aquatic systems with concentration higher than 300 mg m^{-3} is recommended to try to avoid the wavelength at red region of the spectrum due to the strong package effect.

Table 2. Algorithms used by Watanabe et al. (2015) to estimate the chl-*a* in BB reservoir.

Model	Indices	Reference
2B	$R_{rs}(708) \times R_{rs}(665)^{-1}$	Moses et al. (2009)
3B	$(R_{rs}(665)^{-1}) - R_{rs}(708) \times R_{rs}(753)$	Moses et al. (2009)
NDCI	$(R_{rs}(665) - R_{rs}(708))/(R_{rs}(665)+R_{rs}(708))$	Mishra and Mishra (2012)

2B, two-band model; 3B, three-band model; NDCI, Normalized Difference Chlorophyll Index; R_{rs}, remote sensing reflectance.

4. Conclusion

The pigment packaging exhibited a strong influence to underestimation of chl-*a* concentration. The results of this research showed that concentrations above 200 mg m^{-3} are more susceptible to be underestimated in BB reservoir. The inaccurate performance of models using the blue spectral region in inland waters can also be associated with package effect. More studies are needed to investigate the best spectral band combination to estimate the chl-*a* concentration in a eutrophic-hypertrophic aquatic system in order to minimize the package effect.

Disclosure statement

No potential conflict of interest was reported by the authors.

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