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Remote sensing of the cyanobacterial pigment phycocyanin in turbid inland water

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Abstract

The pigment phycocyanin (PC) is a marker for cyanobacterial presence in eutrophic inland water. We present a reflectance band–ratio algorithm for retrieval of cyanobacterial PC. The model conforms to the band settings of the Medium Resolution Imaging Spectrometer. The parameters of the algorithm were optimized using reflectance and absorption data from two highly eutrophic lakes. Using measured specific absorption coefficients for PC [$a^*_{pc}(620)$] for every sample, the error in the predicted PC concentrations was 19.7% ($r^2 = 0.94$, $n = 34$) for measured PC concentrations up to 80 mg m^{-3}. Applying a fixed value of $a^*_{pc}(620)$ caused an overestimation of the PC content that increased toward lower PC concentrations. The PC prediction best matched observed values during periods of high relative abundance of cyanobacteria in the plankton community. The results suggest strong seasonal variation in $a^*_{pc}(620)$. The presence of pigments other than PC and chlorophyll *a* (Chl *a*) and a variable influence of Chl *a* on retrieved absorption at 620 nm are potential causes of errors in PC retrieval. The algorithm in its current form is considered to be suitable for detection of the PC concentration in turbid, cyanobacteria-dominated waters.

Eutrophic inland waters often exhibit blooms of cyanobacteria. Notorious for their negative impact on water quality, cyanobacterial blooms have been increasingly subject of water management and scientific studies. The hazards of toxic cyanobacterial blooms call for frequent and rapid monitoring of water bodies. Remote sensing provides insights into the distribution of blooms for a large number of lakes or reservoirs simultaneously. The concentration of chlorophyll *a* (Chl *a*) as a general indicator for plankton biomass can be assessed using imagery from a wide range of air- and spaceborn sensors (Vos et al. 2003). Recent advances in spaceborn remote sensing technology broaden the perspectives of monitoring toward the identification and quantification of plankton groups. Algorithms for the retrieval of Chl *a* from turbid water reflectance were already being developed (Gons et al. 2002). Now, the retrieval of the pigment phycocyanin (PC), which is characteristic of the presence of cyanobacterial, is being attempted. It is known that the presence of PC can be detected from spectral reflectance (Dekker et al. 1991; Gons et al. 1992; Jupp et al. 1994). However, empirical relationships that have been devised to quantify cyanobacterial PC from the spectral reflectance of turbid water (Dekker 1993; Schalles and Yacobi 2000) required more spectral information than is provided by satellite sensors with global coverage. In the current work, band settings of the Medium Resolution Imaging Spectrometer (MERIS) were adopted for two reasons: first, a band located at 620 nm includes the maximum absorption of a number of modifications of PC (Bryant 1994), and, second, the 300-m spatial resolution is sufficient to monitor moderately sized lakes and reservoirs.

We describe a simple optical model that retrieves the PC concentration from turbid water reflectance. Aiming primarily at the quantification of PC in waters where cyanobacteria dominate, the pigments Chl *a* and PC are incorporated into the model as the major absorbing water constituents in the red and near-infrared (IR) spectral range. As a major simplification, absorption by colored dissolved organic matter (CDOM) and tripton are neglected, which reduces the number of optically active components that are taken into account. A second simplification is the assumption that the backscattering coefficient, b_b, can be retrieved from a single band in the near IR and that it is spectrally neutral (Gons 1999). The parameters of the semiempirical model were optimized using optical data and pigment measurements from two turbid lakes.

Algorithm development

The spectral reflectance just below the water surface, $R(0^-,\lambda)$, is related to the inherent optical properties of the

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Table 1. List of symbols and abbreviations.

Symbol/abbreviation	Description	Units
$a_i(\lambda)$	Absorption coefficient of compound i at waveband λ. Subscripts used: w = water; chl = chlorophyll a; PC = phycocyanin; tsm = total suspended matter; tripton = pigment-bleached TSM	m^{-1}
$a_i^*(\lambda)$	Specific absorption coefficient of pigment i at waveband λ	m^2 (mg pigment)$^{-1}$
b_b	Backscattering coefficient	m^{-1}
$R(0^-,\lambda)$	Subsurface irradiance reflectance at wavelength λ and depth 0	dimensionless
Chl a	Chlorophyll a (concentration)	mg m^{-3}
PC	Phycocyanin (concentration)	mg m^{-3}
CDOM	Colored dissolved organic matter	

water column, absorption a and backscattering b_b, through a factor f that is dependent on the light field (Morel and Gentili 1991, 1993). The following relationship between inherent and apparent optical properties of a water body, established by Gordon et al. (1975), has become widely accepted (*see Table 1 for symbols*):

$$R(0^-,\lambda) = f\, b_b/(a + b_b) \quad (1)$$

For the spectral neutrality of f and b_b, the absorption by water and its constituents at wavelength λ_1 $[a(\lambda_1)]$ can be solved from the reflectance ratio $R(\lambda_2):R(\lambda_1)$, a known b_b, and known absorption in band λ_2:

$$a(\lambda_1) = \{[R(\lambda_2)/R(\lambda_1)] \times [a(\lambda_2) + b_b]\} - b_b \quad (2)$$

A reflectance band ratio for $\lambda_2 = 709$ and $\lambda_1 = 665$ nm (ratio 1) is effective to retrieve the absorption by Chl a in eutrophic inland water (Mittenzwey et al. 1992; Dekker 1993; Gons et al. 2002), whereas a ratio for $\lambda_2 = 709$ and $\lambda_1 = 620$ nm (ratio 2) is introduced to retrieve the PC absorption. For both ratios, a simplified model is obtained when $a(\lambda_2)$ is attributed solely to the absorption by water $[a_w(\lambda_2)]$, and $a(\lambda_1)$ to the absorption by water $[a_w(\lambda_1)]$ and phytoplankton pigments $[a_{ph}(\lambda_1)]$. By these simplifications it is assumed that pigment absorption is negligible at 709 nm, which may lead to an underestimation of $a(\lambda_1)$ increasing with pigment concentration. In addition, the absorption by CDOM and tripton is considered to be negligible, which can cause an overestimation of $a(\lambda_1)$ that is not necessarily correlated with pigment concentration. It is attempted to correct for these simplifications by the introduction of a correction factor γ that reflects the ratio of retrieved absorption versus measured absorption by pigments at wavelength λ_1. Thus, substituting the optically active components according to the mentioned simplifications, the following relationship is obtained:

$$a_{chl}(665) = (\{[R(709)/R(665)] \times [a_w(709) + b_b]\} - b_b - a_w(665)) \times \gamma^{-1} \quad (3)$$

A variation of Eq. 3 was successfully applied to a range of eutrophic, turbid lakes, where b_b was retrieved from a single band in the near-IR (Gons 1999). In the present work, b_b was retrieved from a 778.75-nm, 15-nm-wide band according to the method of Gons (1999). In brief, this involves solving Eq. 1 for $a(778.75) = a_w(778.75)$ and a value for f that is realistic for turbid waters.

For ratio 2, it is expected that the pigments PC and Chl a compose the absorption envelope around 620 nm that is apparent from reflectance spectra of cyanobacteria-rich waters. This reflectance feature is captured within the 620-nm band. Rewriting Eq. 3 for band ratio 2 and introducing the correction factor δ for the correction of $a(620)$, similar to γ for the correction of $a(665)$, yields

$$a_{chl}(620) + a_{pc}(620) = (\{[R(709)/R(620)] \times [a_w(709) + b_b]\} - b_b - a_w(620)) \times \delta^{-1} \quad (4)$$

Next, to unravel the absorption by Chl a and PC at 620 nm, first $a_{chl}(620)$ is derived from $a_{chl}(665)$ that was obtained from Eq. 3. The conversion factor ε is introduced to relate the in vivo absorption by Chl a at 665 nm to its absorption at 620 nm. Subsequently, Eq. 4 is solved for $a_{pc}(620)$:

$$a_{pc}(620) = (\{[R(709)/R(620)] \times [a_w(709) + b_b]\} - b_b - a_w(620)) \times \delta^{-1} - [\varepsilon \times a_{chl}(665)] \quad (5)$$

Finally, dividing $a_{pc}(620)$ by the specific absorption coefficient of PC ($a_{pc}^*(620)$), results in the PC concentration

$$[PC] = a(620)_{pc}/a_{pc}^*(620) \quad (6)$$

Materials and methods

Lake sampling—The algorithm parameters were optimized using in situ reflectance data and optical properties from Lake Loosdrecht (52°11.7′N, 5°3.1′E) and Lake IJsselmeer (52°45'N, 5°20'E), The Netherlands. Lake Loosdrecht (area = 9.8 km^2; mean depth = 1.9 m) originated from peat excavation and is well-mixed, eutrophic and turbid, with an average annual Secchi disk depth of 0.6 m and average Chl a concentration of 66 mg m^{-3} (SD = 20 mg m^{-3}, n = 20 in 2002–2003). Wind resuspension of the phosphorus-rich sediment occurs frequently (Gons et al. 1991). An important part of the vertical attenuation of light is caused by detritus. The dominant cyanobacterial species belong to the filamentous *Limnothrix/Pseudanabaena* group (Pel et al. 2003; Zwart et al. in press). The Chl *a* and *b*, but no PC-containing cyanobacterium *Prochlorothrix hollandica*, occurs in lower abundances. Physical and biological characteristics of Lake Loosdrecht and other shallow Dutch lakes were described in detail in Van Liere and Gulati (1992). Sampling at Lake Loosdrecht took place every 2 weeks during the period March–November 2003.

Lake IJsselmeer (area = 1190 km^2; mean depth = 4.4 m) is the largest lake in the Netherlands, exhibits a Secchi disk depth of ~0.8 m, and has Chl *a* concentrations up to 200 mg m^{-3} during summer. The vertical light attenuation is mainly due to microalgae and cyanobacteria. Surface scums of cyanobacteria occur, but the water column is usually fully mixed. Colonies of *Microcystis* sp. and *Aphanizomenon* sp. filaments represent most of the cyanobacterial biomass. The distribution of cyanobacteria and green algae in summer is inhomogeneous, with locations of relatively high cyanobacterial to green algal biomass and vice versa. From midsummer onward, cyanobacteria dominate the plankton community. Surveys of Lake IJsselmeer were made on the R/V *Luctor* (NIOO, Yerseke) on 10 April, 24 June, 5 August, and 9 September 2003.

Reflectance measurements—Reflectance measurements were made using a portable spectroradiometer, model PR-650 (Photo Research), that measures radiance at a small (1°) acceptance angle, 8-nm full width–half-maximum bandwidth and at 4-nm increments within the spectral range 380–780 nm. Details on the measurement procedure and the computation of $R(0^-,\lambda)$ are given in Gons (1999). Measured reflectance was recalculated for the adopted bandwidths by weighted averaging. The 15-nm wide band at 778.75 nm was partly outside the range of the instrument, so the available 771.25–780 nm range was used.

Pigment extraction and quantification—Samples for Chl *a* analysis were concentrated on glass-fiber filters (Schleicher and Schuell GF 6, 1 μm pore size) and stored at −20°C for a maximum of 3 weeks. Chl *a* and phaeopigments were measured in duplicate using hot ethanol extraction (NEN 1981). All Chl *a* concentrations refer to ''uncorrected Chl *a*'', the sum of Chl *a* and 1.7 times the phaeopigment concentration, to account for absorption by phaeopigments as well as Chl *a*. For the determination of the PC concentration, a method was devised on the basis of the freeze-thaw procedure described by Sarada et al. (1999). Samples of 200 ml were concentrated in two centrifugation runs on a Sorvall RC5C centrifuge that was cooled to <10°C. The first centrifugation was at 15,000 × *g* for 25 min, and then pellets were transferred to 35 ml of phosphate buffer (pH 7.4); after this, the suspension was centrifuged again at 27,000 × *g* for 20 min. Finally, the pellet was transferred to a 15-ml falcon tube that was filled up to 6 ml with buffer solution and then frozen at −20°C. The concentrates were frozen within 2 h after sampling, except during cruises on Lake IJsselmeer, when samples for PC analysis were kept on ice and processed within the next 24 h. By photospectrometrical analysis of the supernatant after concentration of the samples by centrifugation, it was confirmed that cells were not disrupted by centrifugation. Thus, no PC was lost before starting the extraction procedure. Within 4 weeks of storage, samples were subjected to nine cycles of freezing (−20°C) and thawing (room temperature). Purification of the samples was carried out by centrifugation at 13,000 × *g* for 90 min. PC concentrations were spectrophotometrically derived using the equations of Bennett and Bogorad (1973). Where replicate samples were collected, average pigment concentrations were used for subsequent analyses.

Optical properties—All spectrophotometric measurements were carried out on a Lambda 800 UV/Vis spectrophotometer (PerkinElmer) in the 350–800 nm range, using a 150-mm integrating sphere (Labsphere) for filter-pad measurements (Maske and Haardt 1987). Absorption values were averaged over the width of the used bands. The absorption of pure water, $a_w(\lambda)$ was taken from Buiteveld et al. (1994). Watmann glass-fiber filters, Ø = 25 mm, for absorption measurements of particulate matter were placed in the center of the sphere, at a 100° angle from the light beam. For each sample, blank and sample filters were wetted in the filtrate of the sample before measurements. Path-length amplification was corrected by a factor of 2 (Roesler 1998), and the absorbance of the material concentrated on the filters was >0.1 (at λ = 550 nm). Known sample volumes were concentrated on the filters to obtain the absorption by total suspended matter [$a_{tsm}(\lambda)$]. Pigments were bleached from the filters with 80% ethanol at 75°C in two steps, to obtain the tripton absorption [$a_{tripton}(\lambda)$]. No residual phycobilipigment absorption was apparent from the tripton absorption spectra. Phytoplankton pigment absorption [$a_{ph}(\lambda)$] was derived as $a_{tsm}(\lambda) - a_{tripton}(\lambda)$. When the bleaching procedure did not completely remove absorption by chlorophyllous pigments, an exponential function was fitted through the $a_{tripton}(\lambda)$ spectra at 350 and 750 nm, which was then substituted for the $a_{tripton}(\lambda)$ spectrum. The presence of remnant chlorophyllous absorption around 350 nm caused the exponential slope to be slightly overestimated, resulting in a small correction of remnant absorption in the blue region, an overcorrection in the green region, and an acceptable correction in the red region of the spectrum, where an error in the exponential slope has only a small effect. Thus, the fitted curve could be used in the present work, where only the 600–710 nm spectral region was used. Absorption by CDOM was measured as the beam attenuation of sample filtrates (0.2 μm pore size, cellulose acetate; Schleicher and Schuell) using a 5-cm glass cuvette with distilled water as a reference. The integrating sphere accessory was not used to measure CDOM absorption.

Using ε and $a_{ph}(\lambda)$ at λ = 665 and λ = 620 nm, the absorption by PC [$a_{pc}(620)$] could be derived from the absorption measurements as $a_{ph}(620) - [\varepsilon \times a_{ph}(665)]$. Subsequently, $a^*_{pc}(620)$ was determined as $a_{pc}(620)$ divided by the measured PC concentration. The specific absorption coefficient for Chl *a* at 665 nm, $a^*_{chl}(665)$, was determined from $a_{ph}(665)$ divided by the measured Chl *a* concentration.

Parameter optimization—Factors γ and δ in Eqs. 3 and 4 were retrieved from a dataset of $R(0^-,\lambda)$ and $a(\lambda)_{ph}$ spectra, of which 37 pairs were available from the field campaigns at Lakes Loosdrecht and IJsselmeer. The linear least-squares fit of ''uncorrected'' retrieved absorption (Eqs. 3, 4 with γ, δ = 1) against $a(\lambda)_{ph}$ for λ = 665 and 620 nm, yielded two slopes that were adopted for γ and δ.

The conversion factor ε (Eq. 5) was used to define the absorption by Chl *a* at 620 nm relative to 665 nm. It was iteratively retrieved from the best fit of computed versus

observed PC concentration for the samples from Lake Loosdrecht, where $R(0^-,\lambda)$, $a(\lambda)_{ph}$, and PC concentration data were collected (one outlying measurement of PC was omitted, $n = 18$). The data from Lake IJsselmeer were not used in this analysis because the presence of pigments other than PC and Chl *a*, a situation common in Lake IJsselmeer but not in Lake Loosdrecht, would prevent ε from converging to an optimal value. It was not possible to derive ε directly from in vivo absorption measurements because no phytoplankton samples were present that contained only Chl *a* and no accessory pigments absorbing in the 600–700 nm region (all samples of cyanobacteria-rich water had PC : Chl $a >$ 0.38). The optimal value of ε was defined as the value where the slope of the linear least-squares fit of modeled against observed PC concentration was ~1.

Results

Optical properties at lake sites—Table 2 lists the partial absorption coefficients at 620, 665, and 709 nm for pigments, tripton, and CDOM, for the sampling series of the two lake sites. From the filter-pad absorption measurements, it becomes clear that CDOM generally contributes little (<12%) to the total absorption at $\lambda \geq 620$ nm (with the exception of 22% in Lake IJsselmeer during April 2003). The slope of the exponential curve describing CDOM absorption between 620 and 709 nm (Bricaud et al. 1981) varied between 0.006 and 0.011 nm^{-1}, and one higher average slope of 0.020 was measured during June 2003 in Lake IJsselmeer. Relative absorption by tripton is generally high in these lakes (up to 50% of the water constituents' absorption at 620 nm and 57–75% at 709 nm). However, tripton absorption can be considered to be flat in the region of interest, with an exponential slope not >0.006 nm^{-1}.

Optimization of γ, δ, and ε—Figure 1 shows the relationship between uncorrected retrieval of absorption (Eqs. 3, 4 with γ, $\delta = 1$) and filter-pad measurements of $a(\lambda)_{ph}$ that was used to find the optimal values for γ and δ. Eq. 3 with $\gamma = 1$ related to $a(665)_{ph}$ with a regression slope = 0.68 and an offset value of 0.019 ($r^2 = 0.87$). Eq. 4 with $\delta = 1$ resulted in a slope of 0.84 and an offset value of 0.080 ($r^2 = 0.89$). All results presented hereafter were obtained with the adopted values for γ (0.68) and δ (0.84). The offset values, resulting mainly from ignoring absorption by tripton and CDOM in the model (results not shown), were considered to be negligible in the current case and were not incorporated as an extra term to be subtracted from the computed absorption in Eqs. 3–5.

For the optimization of ε, it can be seen from Fig. 2 that the optimal fit (*see* ''Materials and methods'') of predicted to observed PC concentrations (Eq. 5) was reached at $\varepsilon = 0.24$ (slope = 0.99, offset = 0.23; $r^2 = 0.81$). Fixing ε at 0.24 yielded a mean $a^*_{pc}(620)$ for these samples of 0.0095 $m^2(mg\ PC)^{-1}$.

Retrieval of PC with sample-by-sample calculation of $a^*_{pc}(620)$—The PC concentration obtained through Eq. 6 was calculated for the subset of samples from Lake IJsselmeer ($n = 15$) and Lake Loosdrecht ($n = 18$) for which $R(0^-,\lambda)$, $a_{ph}(\lambda)$, and PC concentration data were collected. For every sample, $a^*_{pc}(620)$ was calculated from the measured absorption, $\varepsilon = 0.24$, and PC concentration. The estimated PC compared well to measured PC (Fig. 3). The root mean square error (RMSE) of the PC estimate, compared with the measured PC, was 6.5 mg PC m^{-3} or 19.7% ($r^2 = 0.94$). Because a substantial part of the data was used to optimize the model parameters, the slope of the regression for this data set, unsurprisingly, was ~1.

Variability of $a^*_{pc}(620)$ and $a^*_{chl}(665)$—For Lake Loosdrecht, it was investigated how much $a^*_{pc}(620)$ and $a^*_{chl}(665)$ fluctuated over the period March–November 2003 (Fig. 4). The average value of $a^*_{pc}(620)$ was 0.0095 $m^2(mg\ PC)^{-1}$. Variability was high, with an SD of 0.0033 $m^2(mg\ PC)^{-1}$. The period of greatest stability was found in late summer. High values occurred from mid-May until mid-July and lower values in early spring. A threefold increase of $a^*_{pc}(620)$ occurred in May and could be associated with strong fluctuations in the cellular pigment ratio (Fig. 4, upper plot) for which the cause remained unknown. The $a^*_{chl}(665)$ values displayed a higher stability than $a^*_{pc}(620)$, with a yearly average of 0.0153 ($\pm$0.0016) $m^2(mg\ Chl\ a)^{-1}$.

From the samples collected on Lake IJsselmeer, $a^*_{pc}(620)$ was also calculated. A nonlinear inverse relationship between $a^*_{pc}(620)$ and the ratio of PC to Chl *a* was observed for both lakes (Fig. 5). The lower relative abundance of cyanobacteria (lower PC : Chl *a*) resulted in elevated $a^*_{pc}(620)$ values, which only occurred on the first three cruises on Lake IJsselmeer. The $a^*_{chl}(665)$ values, averaged for all sampling stations, decreased steadily from April to September (Table 2).

Applying a fixed $a^*_{pc}(620)$ value—To illustrate the effect of a fixed $a^*_{pc}(620)$ value for remote sensing, the average $a^*_{pc}(620)$ of Lake Loosdrecht [0.0095 $m^2(mg\ PC)^{-1}$] was used to calculate the PC concentration from $R(0^-,\lambda)$ spectra from Lake IJsselmeer (Fig. 6). The 15 $R(0^-,\lambda)$ spectra that were used to plot Fig. 3 were used in addition to 34 $R(0^-,\lambda)$ spectra for which no absorption data was collected. For every sample in this set, PC concentration data was available. Overestimation of PC was strong during the first cruises, whereas the best match with the measured PC concentrations was found during the September 2003 cruise. A linear least-squares fit through the latter subset resulted in a vertical intercept of 29 mg PC m^{-3} ($r^2 = 0.77$).

Discussion

Optimization of model parameters—It was demonstrated that the retrieval of PC is possible for water bodies that are dominated by cyanobacteria, by using a simple optical model. The introduction of model parameters γ and δ was adequate to fit the retrieved absorption to pigment absorption for a data set from two different lakes at different times. The value of ε was also fitted from these data, to define the spectral behaviour of Chl *a* absorption in cyanobacteria-dominated waters. In cases where the phytoplankton community consisted primarily of cyanobacteria, this approach was sufficient to derive the absorption by pigments at 620

Table 2. Absorption properties (m^{-1}), pigment concentrations (mg m^{-3}), and pigment-specific absorption values [m^2(mg pigment)$^{-1}$] at Lake Loosdrecht (averaged over 19 samplings during the period March–November 2003) and Lake IJsselmeer (number of sampling stations indicated for each cruise). The last section lists the averaged values for all Lake IJsselmeer cruises.

	620 nm				665 nm				709 nm			
	avg.	SD	min	max	avg.	SD	min	max	avg.	SD	min	max
Lake Loosdrecht (March–November 2003)												
CDOM	0.19	0.08	0.10	0.38	0.12	0.07	0.03	0.27	0.07	0.05	0.00	0.16
Tripton	0.80	0.35	0.37	1.64	0.65	0.30	0.29	1.41	0.53	0.26	0.22	1.20
Pigments	0.59	0.09	0.48	0.80	1.07	0.17	0.81	1.40	0.11	0.05	0.05	0.20
Chl *a*	70.9	14.1	48.1	97.5								
PC	50.8	16.8	21.7	79.8								
PC : Chl *a*	0.83	0.35	0.39	1.55								
$a^*_{pc}(620)$	0.0095	0.0033	0.0059	0.0174								
$a^*_{chl}(665)$	0.0153	0.0016	0.0130	0.0189								
Lake IJsselmeer, 10 April 2003 (3 locations)												
CDOM	0.14	0.04	0.11	0.19	0.10	0.04	0.06	0.14	0.07	0.03	0.03	0.n10
Tripton	0.33	0.15	0.18	0.47	0.25	0.12	0.13	0.37	0.19	0.10	0.09	0.28
Pigments	0.18	0.02	0.17	0.20	0.49	0.02	0.47	0.51	0.07	0.03	0.05	0.10
Chl *a*	24.07	1.90	22.55	26.20								
PC	1.85	0.95	0.80	2.63								
PC : Chl *a*	0.07	0.04	0.03	0.10								
$a^*_{pc}(620)$	0.1084	0.0679	0.0680	0.1868								
$a^*_{chl}(665)$	0.0203	0.0022	0.0180	0.0225								
Lake IJsselmeer, 24 June 2003 (12 locations)												
CDOM	0.06	0.02	0.04	0.09	0.03	0.01	0.02	0.05	0.01	0.00	0.00	0.01
Tripton	0.56	0.14	0.40	0.67	0.43	0.11	0.31	0.51	0.32	0.08	0.23	0.39
Pigments	0.86	0.33	0.49	1.09	1.12	0.30	0.79	1.34	0.15	0.07	0.09	0.23
Chl *a*	64.77	15.22	34.85	85.32								
PC	14.38	8.02	2.06	26.42								
PC : Chl *a*	0.19	0.09	0.03	0.36								
$a^*_{pc}(620)$	0.0706	0.0317	0.0468	0.1066								
$a^*_{chl}(665)$	0.0181	0.0010	0.0170	0.0188								
Lake IJsselmeer, 5 August 2003 (12 locations)												
CDOM	0.13	0.02	0.10	0.16	0.11	0.02	0.07	0.13	0.06	0.02	0.02	0.08
Tripton	0.67	0.14	0.52	0.80	0.51	0.11	0.39	0.62	0.39	0.09	0.29	0.47
Pigments	0.64	0.18	0.43	0.76	0.93	0.27	0.62	1.15	0.17	0.11	0.06	0.27
Chl *a*	61.29	19.01	31.23	91.67								
PC	7.19	2.35	2.30	11.94								
PC : Chl *a*	0.12	0.05	0.06	0.23								
$a^*_{pc}(620)$	0.0931	0.0587	0.0461	0.1589								
$a^*_{chl}(665)$	0.0165	0.0030	0.0146	0.0199								
Lake IJsselmeer, 9 September 2003 (12 locations)												
CDOM	0.07	0.02	0.04	0.10	0.05	0.02	0.01	0.08	0.04	0.01	0.01	0.05
Tripton	0.74	0.08	0.65	0.79	0.57	0.05	0.51	0.61	0.44	0.03	0.40	0.47
Pigments	0.60	0.09	0.49	0.66	0.84	0.09	0.74	0.92	0.14	0.05	0.09	0.18
Chl *a*	60.50	10.13	43.80	75.03								
PC	36.92	17.73	14.30	64.80								
PC : Chl *a*	0.54	0.20	0.28	0.85								
$a^*_{pc}(620)$	0.0162	0.0093	0.0088	0.0266								
$a^*_{chl}(665)$	0.0138	0.0017	0.0119	0.0150								
Lake IJsselmeer total (39 samples)												
CDOM	0.10	0.04	0.04	0.19	0.08	0.04	0.01	0.14	0.05	0.02	0.00	0.10
Tripton	0.57	0.19	0.18	0.80	0.44	0.15	0.13	0.62	0.34	0.12	0.09	0.47
Pigments	0.57	0.31	0.17	1.09	0.85	0.30	0.47	1.34	0.13	0.07	0.05	0.27
Chl *a*	59.11	17.66	22.55	91.67								
PC	18.24	17.07	0.80	64.80								
PC : Chl *a*	0.27	0.23	0.03	0.85								
$a^*_{pc}(620)$	0.0721	0.0547	0.0088	0.1868								
$a^*_{chl}(665)$	0.0172	0.0030	0.0119	0.0225								

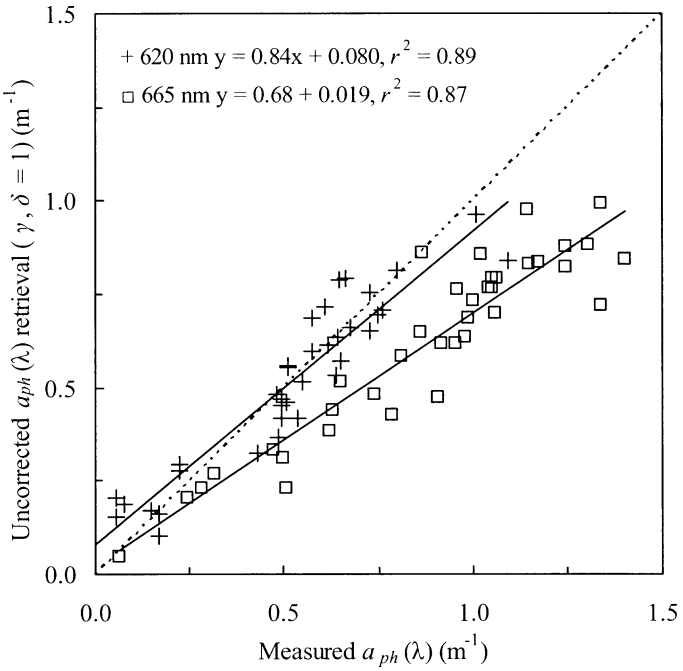

Fig. 1. Uncorrected $a_{ph}(\lambda)$ retrieval at 620 and 665 nm vs. measured $a_{ph}(\lambda)$. The plotted values conform to Eqs. 3 and 4, with δ, γ = 1. Dashed line indicates unity.

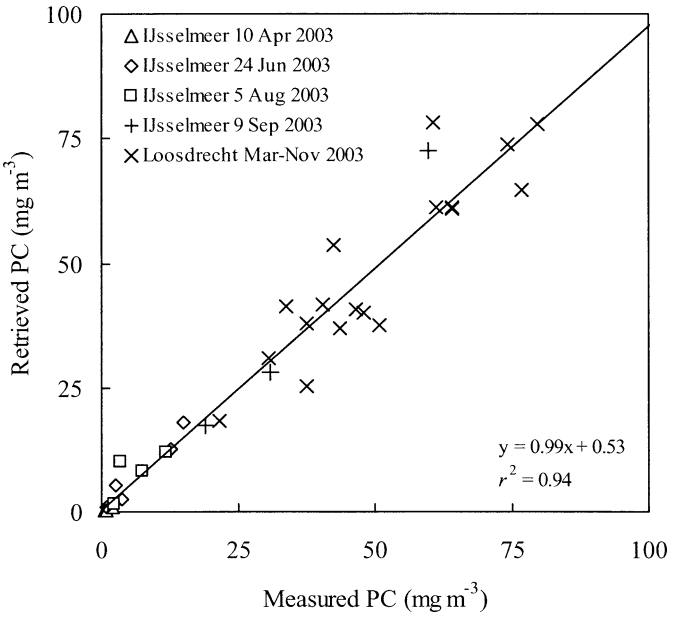

Fig. 3. PC concentrations predicted by the algorithm (Eqs. 5, 6) vs. measured PC concentrations, with $a^*_{pc}(620)$ calculated independently from absorption measurements, ε, and measured PC for every sample.

and 665 nm that was needed to obtain the PC concentration. When compared with literature values of $a^*_{chl}(\lambda)$, ε = 0.24 is relatively low, and values ~0.4–0.5 fitted expectations better (Hoeppfner and Sathyendranath 1993). However, to our knowledge, there exists no literature that deals with in vivo $a^*_{chl}(\lambda)$ values connected to cyanobacterial species and pigments, whereas reports on the $a^*_{chl}(\lambda)$ values beyond the main absorption peaks—that is, at 675 and 623 nm—are rare. Furthermore, in vivo specific absorption by phycobilisomal pigments cannot be measured independent of Chl *a* with currently available methods. At present, it must therefore be concluded that the empirical value ε = 0.24 explains the absorption by PC and Chl *a* at 620 nm reasonably well for cyanobacteria. For other phytoplankton groups differing in the ratio $a^*_{chl}620) : a^*_{chl}(665)$, for instance because of the package effect, a different value for ε may be needed.

The data for parameter optimization was obtained throughout different seasons for two lakes that differ markedly in their plankton species and abiotic environment. Nonetheless, one set of parameters could be applied for the whole data set where cyanobacteria-dominated waters were

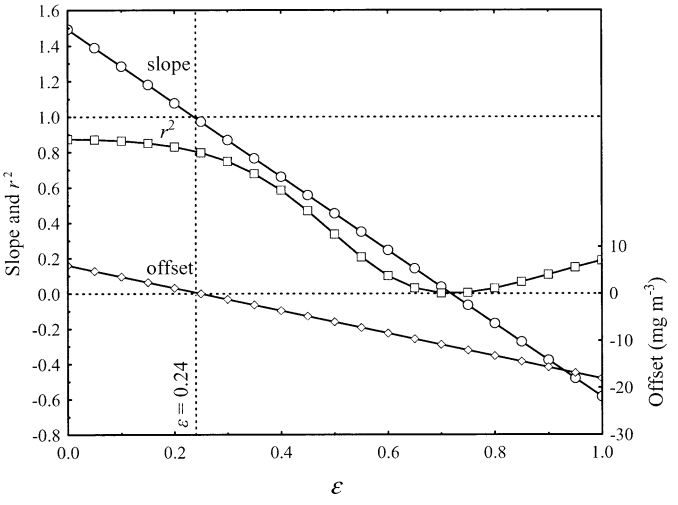

Fig. 2. Results of the linear regression (slope, offset, and r^2) between modeled and measured PC concentration as a function of ε, for Lake Loosdrecht during March–November 2003. The optimal value (regression slope = 1) of ε = 0.24 is highlighted by the vertical line.

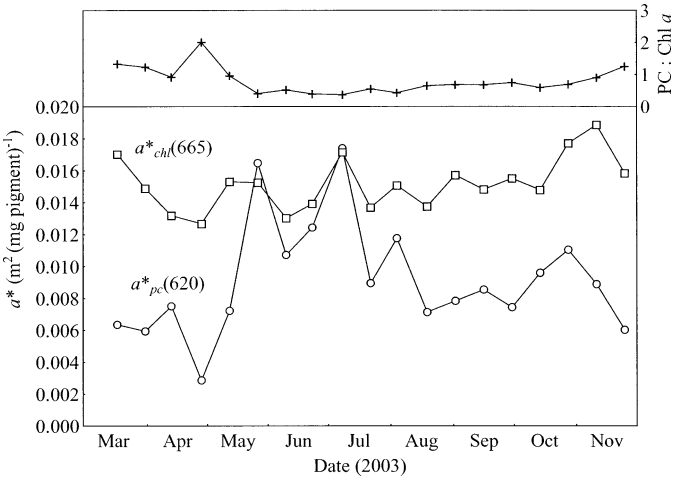

Fig. 4. Values of $a^*_{pc}(620)$, $a^*_{chl}(665)$ and PC : ≡Chl *a* ratio measured every 2 weeks for Lake Loosdrecht during March–November 2003.

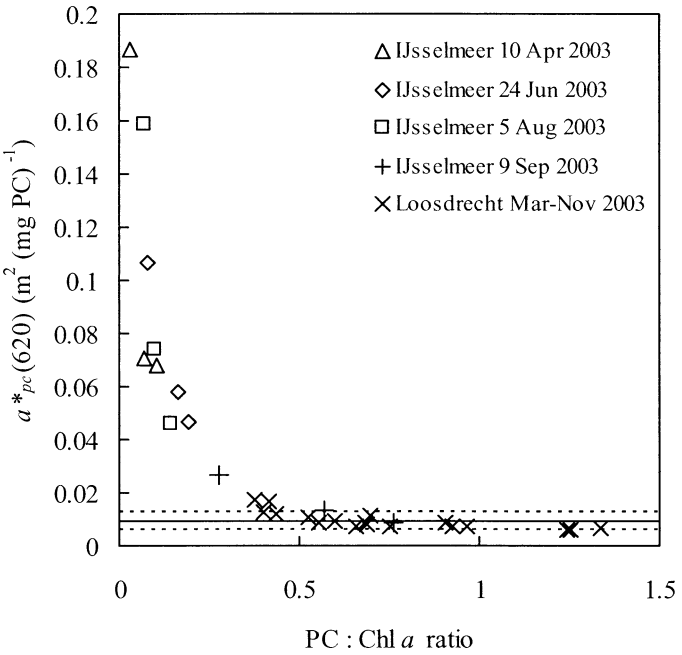

Fig. 5. Coefficient $a^*_{pc}(620)$ vs. PC : Chl *a* ratio. Horizontal lines indicate the average (solid line) and SD (dashed lines) of $a^*_{pc}(620)$ for Lake Loosdrecht.

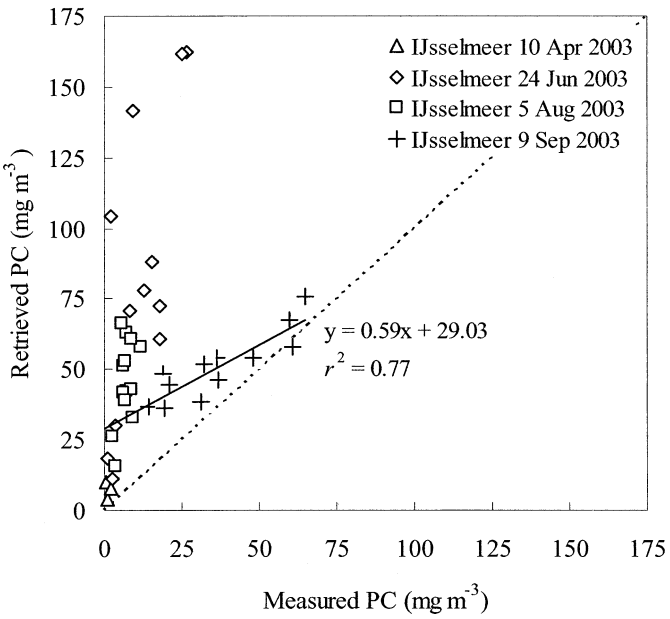

Fig. 6. Retrieved PC concentrations (Eq. 5, 6) vs. measured PC concentrations for Lake IJsselmeer, using $a^*_{pc}(620) = 0.0095$ m^2 (mg PC)$^{-1}$ and $\varepsilon = 0.24$. PC was calculated for all samples for which reflectance and pigments were measured. The linear least-squares fit was plotted for samples of 9 September 2003.

concerned. It is therefore likely that the parameters γ, δ, and ε are equally fitting for other turbid, eutrophic lakes and reservoirs, provided that they exhibit a eutrophic state with predominance of cyanobacteria. However, the contribution of CDOM to total absorption can be considered to be low (<10%) for both studied lakes, and band-ratio algorithms are generally insensitive to low background absorption. The relative contribution of tripton to total absorption was consistent throughout most samples, and the offset in retrieved absorption relative to measured absorption was, in this case, not significant (Fig. 1). The application of the algorithm to lakes that differ in these characteristics may require local optimization of the model parameters.

Errors—Variability of $a^*_{pc}(620)$. A cyanobacterial species' response to changing environmental conditions can result in variable values of $a^*_{pc}(620)$, as visible in Fig. 4. It has been shown before that $a^*_{chl}(\lambda)$ varies with cell morphology and photoadaptive responses (Sathyendranath et al. 1987; Bricaud et al. 1995; Staehr et al. 2002). PC is an accessory pigment that can efficiently increase the light harvesting capacity in the ''green gap'' of Chl *a* (Britton 1983; Raven 1984). The cellular pigment concentration of PC can be expected to fluctuate more than Chl *a* does for changing nutrient and light environments (Tandeau de Marsac 1977; Bryant 1981). It has been proposed that phycobiliproteins might be broken down during nitrogen shortage, to recycle amino acids (Bogorad 1975; Grossman et al. 1993). Such mechanisms imply high variability in cellular PC content, with corresponding effects on $a^*_{pc}(620)$.

Estimation of $a_{pc}(620)$—Methodological errors in the retrieval of $a_{pc}(620)$ occur when $a_{ph}(620)$, after correction for Chl *a* absorption, is not fully attributable to absorption by PC. This can be the case when other substances than the mentioned pigments contribute to the retrieved absorption signal. Some pigments exhibit absorption in or closely around the 620 nm waveband. For the application of the present model, in waters with a mixed phytoplankton composition rather than a dominating cyanobacterial population, the effect of specific pigments on the prediction of PC deserves further exploration. A second possibility for an erroneously high estimate of PC absorption is expected when the correction for Chl *a* absorption [following from $a_{chl}(665) \times \varepsilon$] insufficiently cancels out Chl *a* absorption at 620 nm. This would be the case if an ε value was chosen that was too low to fit the Chl *a*-specific absorption properties of a sample. It is shown that $a^*_{chl}(665)$ decreased gradually with time in Lake IJsselmeer throughout the period April–September (Table 2), a trend that was accompanied by change from green algal to cyanobacterial dominance. Figure 5 also shows a clear inverse relationship between $a^*_{pc}(620)$ and the PC : Chl *a* ratio, which is indicative of cyanobacterial presence. Because ε was retrieved from data from a cyanobacteria-dominated lake, the value might be too low to correct for absorption by Chl *a* when plankton species with a higher $a^*_{chl}(620) : a^*_{chl}(665)$ ratio are abundant. Such species would be characterized by a strong package effect. Both errors result in incorrect $a_{pc}(620)$ values, leading to elevated $a^*_{pc}(620)$ and, thus, overestimated PC concentrations. It is likely that this type of error caused an overestimation of PC in the case of Lake IJsselmeer samples with low PC : Chl *a* ratios. As a rule of thumb, with a PC : Chl *a* ratio < 0.4, error in the PC prediction will rise steeply (Fig. 5).

PC extraction and quantification—The assessment of PC concentration is a cumbersome procedure. Various methods have been proposed to release the pigment from cells using freeze-thaw cycles (Sarada et al. 1999), osmotic shock, grinding, enzymatic disruption (Stewart and Farmer 1984), sonification (Bennett and Bogorad 1973), or disruption by French press (Schalles and Yacobi 2000). Unfortunately, comparative studies on the effects of such methods on the stability of the phycobiliprotein structure and its absorption properties are lacking. The different methods also present problems of selectivity on size or on robustness of the cell material. If the extraction efficiency is lower than assumed, an overestimation of $a^*_{pc}(620)$, and thus an underestimation of PC, results. The low average of PC values in this study is likely the result of low extraction efficiency. For progress in the remote sensing of PC beyond the limits of one laboratory's methodology, intercomparison of the efficiency of extraction methods will be a priority. Methods that are less selective of the nature of sample material should be preferred. In this light it is noted that recently, Viskari and Colyer (2002, 2003) claimed a 90% extraction efficiency using nitrogen cell disruption of cultured material. Using this method, nitrogen gas is dissolved into sample solutions. On a sudden return to normal air pressure, cells into which nitrogen dissolved burst, and water-soluble pigments are released.

Applicability of the PC retrieval algorithm—Remotely sensed Chl *a* products have greatly benefited estimations of global oceanic productivity and provide a useful tool to fisheries. Remote sensing of Chl *a* greatly enhances the efficiency of water quality monitoring. One might similarly expect validated PC products to contribute to monitoring of cyanobacterial blooms and to yield insights into spatially heterogeneous population dynamics and plankton group–specific production estimates. The semiempirical band ratio model (Eq. 5) can be used to detect absorption in the 620 nm band for eutrophic, turbid lakes. To relate this absorption to cyanobacterial biomass, information on the composition of phytoplankton in the water body is still needed. Without adaptations of the algorithm, it can be used to monitor the PC status of water bodies that are known to be dominated by cyanobacteria at the time of observation. For lakes where blooms are known to occur, monitoring the PC concentration or PC : Chl *a* ratio can be useful to identify critical cyanobacterial population densities (in terms of PC) for bloom formation, and advanced predictions on bloom formation may follow with the aid of hydrological models. Other applications can be found in the study of bloom termination events as caused by intensive grazing or viral attacks when the cyanobacterial population reaches high densities. Such events would be characterized by sudden drops in the PC : Chl *a* ratio of the water body that could be identified using the current model. The algorithm accommodates the band specifications of the MERIS instrument, but essentially it could be tailored to any sensor that can record reflectance in bands that include wavelengths of PC absorption (~615 nm), Chl *a* absorption (around 675 nm), and at a far red (>705 nm) and near-IR location (between 760–800; Gons 1999).

The current model falls short in situations where the phytoplankton community does not primarily consist of cyanobacteria. However, some possibilities for these erroneous results were identified (see ''Errors'' section) and could well lead to further improvement of the algorithm, in turn leading to a wider applicability.

It is concluded that the present algorithm can aid in the monitoring of cyanobacterial populations in turbid, eutrophic lakes and reservoirs. Optimization of the algorithm parameters based on data from two different lakes was possible. A sensitivity analysis of the algorithm and comparison of the performance of this and other proposed approaches to detect PC (Dekker 1993; Schalles and Yacobi 2000; Vincent et al. 2004) is now desirable. The influence of pigments other than PC and Chl *a* on the absorption at 620 nm, and the effect of phytoplankton composition on the model parameters deserves further study. For known environmental conditions, it should be possible to estimate $a^*_{pc}(620)$ allowing the monitoring of PC in cyanobacteria-rich water bodies.

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