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Experimental calibration of lake-sediment spectral reflectance to chlorophyll *a* concentrations: methodology and paleolimnological validation

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Abstract Chlorophyll *a* preserved in lake sediments reflects, in part, past primary production. This study assesses the spectral properties of sedimentary chlorophyll *a* using visible-near infrared reflectance (VNIR) spectroscopy, with the objective of establishing a new, non-destructive paleolimnological proxy. Reflectance spectra were determined from a dilution series ($n = 10$) involving incremental additions of pulverized modern algae to a lake sediment matrix of low organic content. This enabled an assessment of the development of sediment reflectance spectra in relation to different sediment chlorophyll *a* concentrations, and subsequent regression of spectral features against measured concentrations of chlorophyll *a* and derivatives obtained by high performance liquid chromatography (HPLC). The experiment demonstrates that ubiquitous troughs in sediment reflectance near 675 nm are attributable to chlorophyll *a* and derivative compounds. A significant correlation ($r^2 = 0.98$, $P < 0.01$) was obtained between the area of the reflectance trough in the 650–700 nm interval and summed concentrations of chlorophyll *a*, all derivative isomers, and degradational pheopigments. A simple linear inference model derived from this experiment was applied to a down-core sequence of VNIR spectra from a productive prairie lake (Alberta, Canada), where it produced inferred sediment chlorophyll *a* concentrations in concordance with HPLC measurements. Although a larger training set is desirable to further refine the inference model, the analyses reported here demonstrate that reflectance spectroscopy provides a rapid, semi-quantitative method for assessing the chlorophyll *a* content of lake sediments.

Keywords Chlorophyll *a* · Lake sediments · Reflectance spectroscopy · Pheopigments · Paleolimnology · Eutrophication

Introduction

Lake sediments integrate a variety of organic and inorganic constituents. Fossil pigments, one fraction of the preserved organic matter, represent an important proxy for reconstructing historical trends of lake primary production, algal succession, invertebrate herbivory, and ultraviolet radiation regimes (Leavitt and Hodgson 2001; Verleyen et al. 2005; Waters et al. 2005). Although a range of chromatographic techniques have been used to quantify sedimentary pigment concentrations (Swain 1985; Sanger 1988),

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currently the most precise techniques involve lipid extraction followed by quantification using high-performance liquid chromatography (HPLC) coupled with in-line diode array spectrophotometry (Millie et al. 1993; Vinebrooke and Leavitt 1999) or mass spectrometry (Hodgson et al. 1997).

Non-destructive approaches using visible-near infrared reflectance (VNIR) spectroscopy have been used to detect chlorophyll *a* in natural waters (Gitelson 1992; Mittenzwey et al. 1992; Rundquist et al. 1996; Schalles et al. 1998; Gitelson et al. 1999), tree foliage (Datt 1999; Sims and Gamon 2002), bryophyte tissues (Lovelock and Robinson 2002), and benthic algae (Carrère et al. 2004). In each of these applications, various ratios of near infrared to visible reflectance have proven highly sensitive to chlorophyll *a* content, largely because of strong chlorophyll *a* absorption in the red portion of the electromagnetic spectrum, around 670–690 nm. The first applications of reflectance spectroscopy to marine sediments involved the use of near-ultraviolet to near-infrared wavelengths (250–850 nm) as proxies for carbonate, organic carbon, and opaline silica contents (e.g., Balsam and Deaton 1996). To date, applications to lake sediments have focused on the near-infrared band (780–2500 nm) almost exclusively (Korsman et al. 2001; Rosén 2005), which excludes the range of greatest spectral sensitivity to chlorophyll *a*. It has recently been shown that the inclusion of shorter (visible) wavelengths results in a spectral range that potentially captures the chlorophyll *a* content of lake sediments (Das et al. 2005). The latter study has demonstrated that sedimentary chlorophyll *a* produces distinctive troughs in reflectance near 675 nm that recall those imparted to water following amendments with living algal biomass (Schalles et al. 1998; Gitelson et al. 1999). Similar reflectance patterns are also at the heart of phytoplankton bloom remote sensing techniques (Richardson 1996; Gons 1999).

In the present study, we build upon the preliminary observations of Das et al. (2005) to explore experimentally the relationship between lake sediment reflectance spectra and chlorophyll *a* concentrations. This is accomplished through controlled amendments of algal chlorophyll to a largely inorganic sediment matrix, followed by VNIR spectral analysis of resulting mixtures. Thereafter, spectral indices are regressed with HPLC-measured chlorophyll *a*

concentrations in order to produce a simple linear predictive model for sedimentary chlorophyll *a* based on spectral characteristics. This model is then applied to down-core VNIR spectra, enabling an *a posteriori* comparison of VNIR-based chlorophyll reconstructions to HPLC measurements. Through these experiments, we demonstrate the utility of sediment VNIR spectroscopy for rapid semi-quantitative assessments of sediment chlorophyll *a* concentrations.

Materials and methods

Algal inoculation of sediment

Sediments from Lake Louise (22.5 cm core depth, >200 years old) were used as the matrix for the chlorophyll *a* dilution experiment. Lake Louise (40°30′28″N, 105°37′13″W) is an oligotrophic tarn located in Rocky Mountain National Park (Colorado, USA); detailed site information is available elsewhere (Wolfe et al. 2003). To this matrix were added variable increments of an inoculum comprising algae collected from tropical fish-rearing aquaria in the Department of Biological Sciences at the University of Alberta. This material was composed mainly of filamentous cyanobacteria (*Oscillatoria* and *Anabaena* spp.), pennate diatoms (*Achnanthidium* and *Gomphonema* spp.), and unidentified small (<10 μm) coccoid chlorophytes. Bulk samples of this material were freeze-dried, homogenized, ground, sieved (<125 μm), and mixed with the Lake Louise sediment matrix in the following mass ratios of sediment to inoculum: 5:1, 10:1, 25:1, 50:1, 100:1, 250:1, 500:1, 750:1, and 1000:1. Both VNIR spectra and HPLC measurements of pigment concentrations were obtained on well-mixed sub-samples of each of these dilutions.

Reflectance spectroscopy

Spectral measurements were performed with a FieldSpec® Pro spectroradiometer (Analytical Spectral Devices Inc., Boulder, Colorado) over a range of 350–2500 nm, and analyzed using FieldSpec® Pro software version 3.09. A 512-channel silicon photodiode array detector measured the VNIR portion of the spectrum at 3 nm intervals. Each channel is geometrically positioned to receive light within a

very narrow bandwidth (approximately 1.4 nm). Light was brought to the instrument using a Lowel Pro® lamp with an effective view of about 45°, at a lamp to sample working distance of 1.75 cm. Spectra of reflected energy were collected though bundled optical fibers with a conical view of 15°. A white spectralon reference panel was used as the standard, which was measured between each sample. All spectra were converted to percent reflectance ($\%R = (R_{sample}/R_{reference}) \times 100$). Only results between 450 and 900 nm are illustrated here.

Pigment quantification

Pigment concentrations were measured by HPLC as described in detail elsewhere (Vinebrooke and Leavitt 1999). Briefly, a standard mixture of acetone, methanol, and water (80:15:5, by volume) was used for pigment extraction from sediment samples (24 h in darkness at 10°C). Extracts were then filtered (<0.2 μm), dried under N_2, and reconstituted with an injection solution (70% acetone: 25% ion-pairing reagent: 5% methanol) containing Sudan II (3.2 mg l^{-1}) as an internal reference. The ion-pairing reagent (IPR) consisted of 0.75 g tetrabutyl ammonium acetate and 8 g ammonium acetate dissolved in 100 ml deionized water. Pigments were separated on a Hewlett-Packard HP 1100 HPLC equipped with a Rainin 200 C18 column (10 cm length, 5 μm particle size). Pigments were detected with an in-line diode array detector (435 nm detection wavelength) and a fluorescence detector (435 nm excitation, 667 nm detection). Pigment were identified and quantified with reference to either authentic (United States Environmental Protection Agency National Exposure Research Laboratory, Cincinnati, Ohio), or commercial (Sigma Chemicals) standards.

In the present study, pigment concentrations are expressed as mg g^{-1} dry mass of sediment. Normalization to organic content is a standard practice in most lake sediment pigment studies, aiming to differentiate the effects of clastic (inorganic) dilution of pigments from those associated with changing regimes of net pigment accrual and in situ degradation (Leavitt and Hodgson 2001). However, spectral reflectance is based on whole sample properties including scattering and absorption by mineral phases, and is thus not solely modulated by the organic fraction. For this reason, we believe it is more appropriate to express pigment concentrations in relation to whole-sample mass. This practice results in the reporting of both pigment and spectral data as bulk sediment properties, which facilitates their direct comparison. In addition to each of the samples comprising the dilution series ($n = 10$), pigments were analyzed using identical methods from 18 depths in the Lac La Biche sediment core, described below.

Spectral indices and regression

The choice of spectral indices for calibration with measured chlorophyll a concentrations is based on wavelengths selected from visual inspection of typical lake sediment VNIR spectra (Fig. 1a). The first indices are the dimensionless trough areas between straight lines joining R values at 650 and 700 nm, and then 650 and 750 nm, with the underlying reflectance curve. These troughs appear in all samples, and are commonly the dominant VNIR spectral feature in lake sediments in the 450–900 nm range (Das et al. 2005). The value of the first derivative of R at 690 nm was also calculated (Rundquist et al. 1996), as this wavelength demarcates a position of highly variable, and often rapidly increasing, R values. Additionally, the amplitudes of reflectance (ΔR) between 650 and 675 nm, and between 675 and 750 nm, were also calculated, as well as the ratios between R values at selected wavelengths (i.e., $R650/675$ nm, $R700/675$ nm).

For each of these indices, linear regressions were undertaken between their values and corresponding HPLC-measured concentrations of the three following categories of pigments: (1) chlorophyll a + all chlorophyll a isomers; (2) pheopigments derived from chlorophyll a degradation (i.e., pheophytin a + pheophorbide a); and (3) the summed concentration of all compounds associated with both primary and degraded chlorophyll a (i.e., chlorophyll a + all chlorophyll a isomers + pheophytin a + pheophorbide a). The latter category is the most inclusive, and, as such, is likely to best integrate the sedimentary pigment legacy of whole-lake algal production. For this reason, the inference model developed from the linear regression results, and its subsequent applications to both modern and down-core reflectance spectra, both address the complete suite of chlorophyll a and related derivatives.
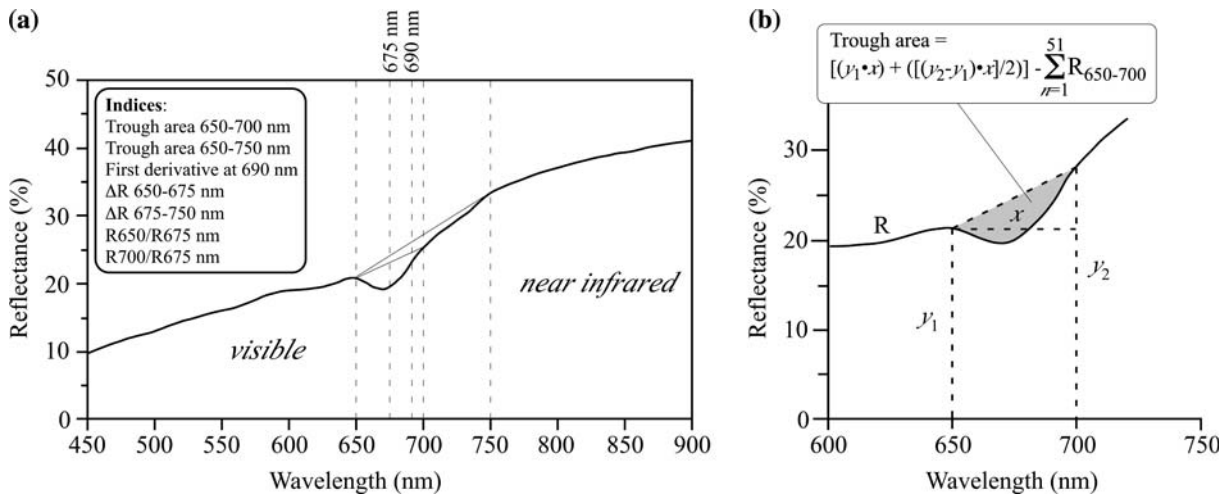
Fig. 1 (a) Typical lake sediment VNIR spectrum (450–900 nm) indicating the wavelengths used for calculating the various spectral indices that were regressed against measured chlorophyll *a* concentrations. In (b) is a schematic diagram of the trough in *R* centered at 675 nm, illustrating the calculation of the dimensionless trough area between 650 and 700 nm

Paleolimnological application

The performance of the chlorophyll *a* inference model developed from the algal dilution series was evaluated through its application to recent sediments from Lac La Biche (54°50′N, 112°00′W), a large (234 km^2), relatively shallow ($Z_{max} = 21$ m) and productive (TP > 100 μg l^{-1}) prairie lake situated in east-central Alberta. The core was collected and extruded with standard gravity coring protocols (Glew 1989), and dated with excess ^{210}Pb using the constant rate of supply model (Appleby and Oldfield 1978). Lac La Biche has recently eutrophied as the consequence of increased agricultural and municipal nutrient inputs (Mitchell and Prepas 1990). Inferred concentrations of total chlorophyll *a* and derivatives were compared to HPLC measurements from 18 core levels.

Results

Algal dilution series

HPLC analyses of Lake Louise sediments amended with the aquarium-reared algal inoculum provided clear detection of the following pigments: chlorophyll *a*, pheophytin *a*, pheophorbide *a*, beta-carotene, and the collective category lutein+zeaxanthin (Fig. 2). Total chlorophyll *a* and derivatives ranged from 0.396 mg g^{-1} dry mass in the 5:1 dilution sample to

less than 0.01 mg g^{-1} in the unamended sediment matrix. Chlorophyll *a* was by far the most abundant pigment measured. For pheopigment and carotenoid concentrations (Figs. 2b, d, e), the most highly diluted samples did not differ substantially from the unamended sediment matrix sample, which implies that the inoculum primarily contributes chlorophyll *a* to the dilution series samples. The unamended sediment is the only sample with a greater concentration of pheopigments relative to chlorophyll *a*, reflecting its age and comparatively advanced state of degradation.

The most conspicuous feature of the VNIR spectra acquired from these samples is the progressive development of the trough in reflectance centered at 675 nm (Fig. 3). The depth of this trough shallows unidirectionally with incremental dilution of the algal inoculum; its depth is therefore a function of the amount of chlorophyll *a* present in the sample. Furthermore, this trough is centered on the wavelengths that exhibit the greatest variability of *R* within the population of spectra comprising the dilution series (Fig. 3c). This observation confirms the heightened sensitivity of this portion of the spectrum, in comparison to shorter (visible) and longer (near infrared) wavelengths.

Regression between spectral indices and pigments

Based on the strength of the relationship between the mass of pulverized algae added to the dilution series and the area of the reflectance trough between 650

Fig. 2 HPLC-measured
concentrations of the most
abundant pigments in the
algal dilution series
samples, including the
unamended sediment
matrix: (**a**) total chlorophyll
a + isomers; (**b**)
pheopigments from
chlorophyll *a* degradation
and the ratio of chlorophyll
a to pheopigments; (**c**) total
chlorophyll *a* + isomers +
pheopigments; (**d**) beta-
carotene; and (**e**) lutein +
zeaxanthin
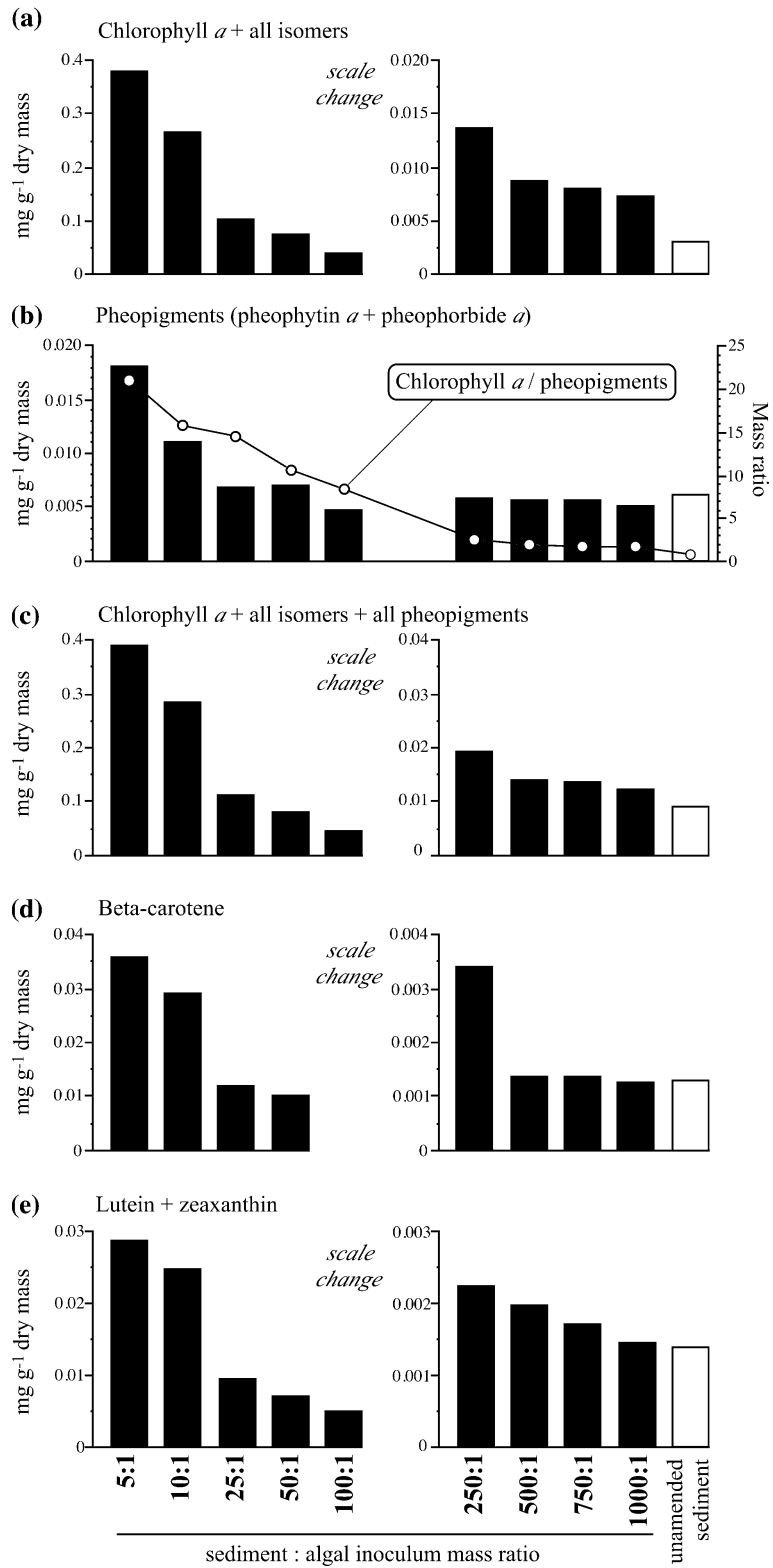
and 700 nm ($r^2 = 0.96$, Fig. 4a), the development of this spectral feature appears directly related to sample chlorophyll *a* concentrations (Fig. 3). Indeed, all seven of the spectral indices regressed against HPLC-determined concentrations of either chlorophyll *a* or chlorophyll *a* + pheopigments yielded highly significant correlations ($P < 0.01$, Table 1). These results imply that index selection is not especially critical, a conclusion reached elsewhere in similar applications (Carrère et al. 2004). We have chosen to focus on the trough area between 650 and 700 nm as the preferred spectral index because it consistently produced the highest coefficients of determination (Table 1). It is also noted that each of the calculated indices are correlated with pheopigment concentrations as well, but at reduced levels of significance ($P < 0.05$). Although pheopigments concentrations are quite low in these samples due the relatively undegraded state of the inoculum (Fig. 2), these correlations nonetheless suggest that chlorophyll *a* degradation products produce similar spectral reflectance signatures as the parent compound (Jeffrey et al. 1997; Méléder et al. 2003; Michelutti et al. 2005).

From the linear relationship between the reflectance trough area (650–700 nm) and measured concentrations of chlorophyll *a* + all derivatives (Fig. 4b), it becomes possible to infer chlorophyll *a* concentrations from spectral reflectance data alone. When this is accomplished, and inferred values are compared to the measurements from which the inference model is created (Fig. 4c), a close clustering of points around the 1:1 line is obtained, with generally low residual values (<0.04 mg g^{-1} dry mass) that lack any detectable trend (Fig. 4d).

Reconstructing lake trophic history using reflectance spectroscopy

Using down-core VNIR spectra from Lac La Biche sediments (18 samples) coupled with the inference model based on the reflectance trough area between 650 and 700 nm (Fig. 4b), concentrations of chlorophyll *a* and all derivatives were reconstructed from the sediments of this lake. Reflectance-based inferences are directly comparable to HPLC measurements of the same quantity (Fig. 5), producing a highly significant correlation between measured and inferred values ($r^2 = 0.79$; $P < 0.01$). However, with
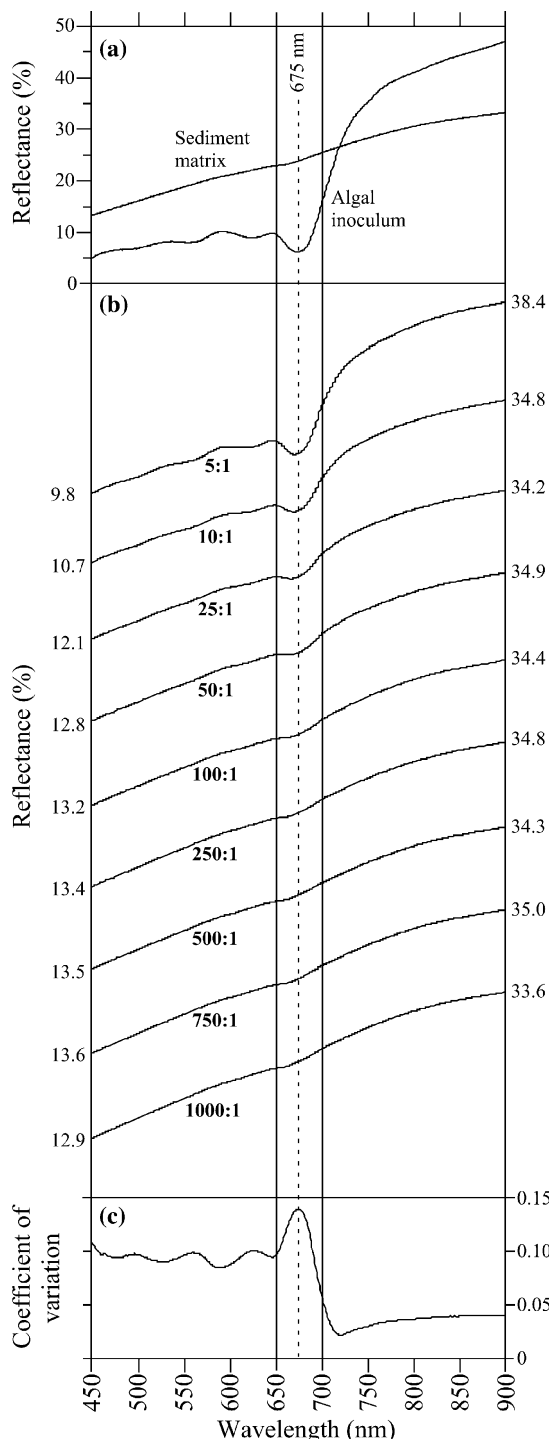
Fig. 3 Percent VNIR reflectance (450–900 nm) for (**a**) the pure inoculum of dessicated algae and the matrix it was amended to, and (**b**) the complete dilution series. In (**c**), the coefficient of variation for each wavelength is shown, calculated from the population of spectra illustrated in (**b**)
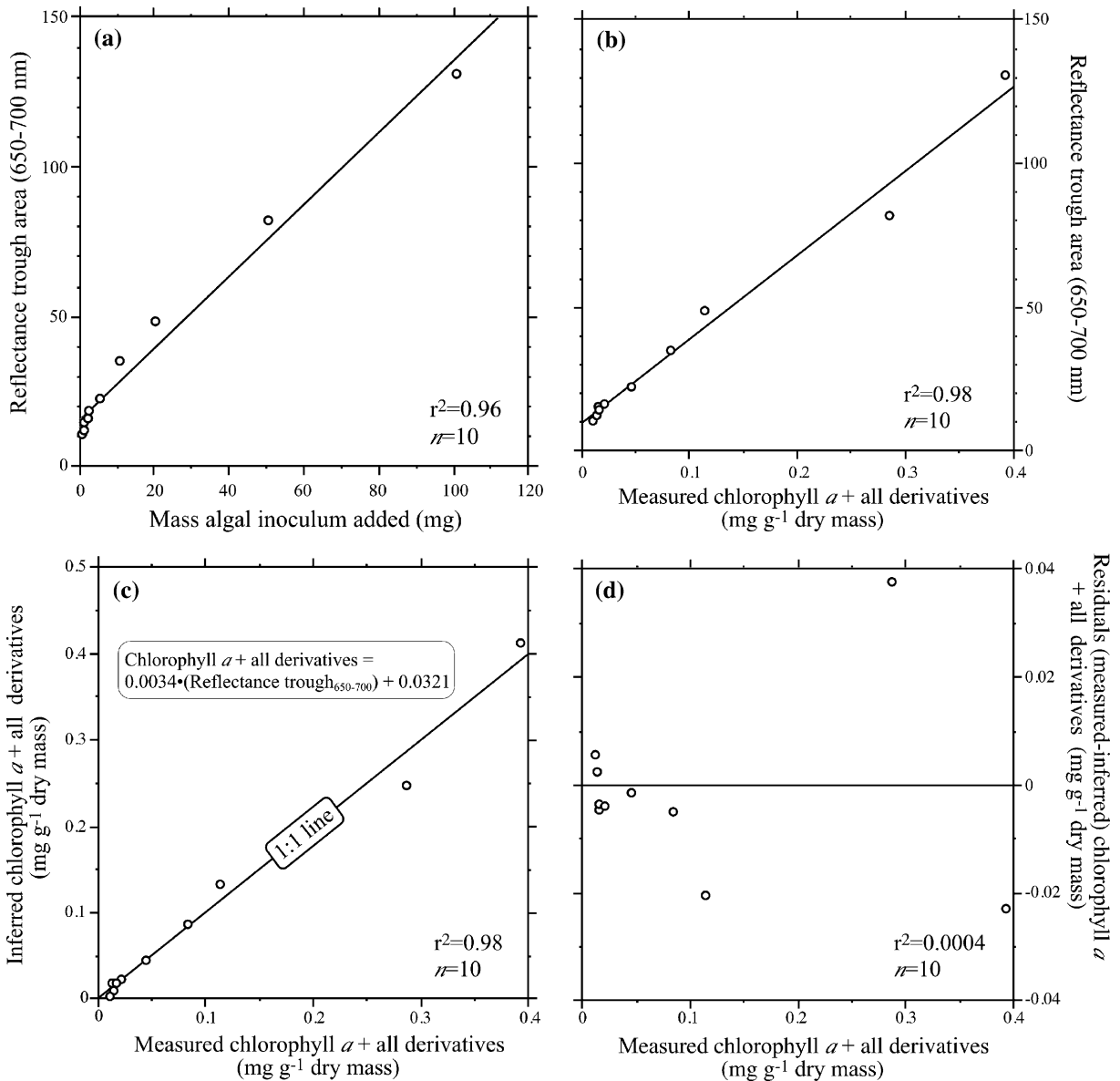
Fig. 4 Relationships between the reflectance trough area between 650 nm and 700 nm and (**a**) the mass of algal inoculum added in the dilution series, and (**b**) corresponding concentrations of chlorophyll *a* and all derivatives. The regression resulting from (**b**) has been applied in (**c**) to allow comparison of inferred and measured concentrations of chlorophyll *a* and all derivatives, with residuals depicted against measured values in (**d**)

the exception of the surface (and least degraded) sample, reflectance-based data consistently over-estimate HPLC-measured sediment chlorophyll *a*. This is likely because the reflectance-based estimates are based on whole-sample properties, whereas the HPLC analyses are performed on wet-chemical extracts that represent a diminutive fraction of the original sample. Reflectance-based estimates may also be influenced by trace amounts of other chlorophylls (*b* and *c*), which were not separated clearly and quantified using HPLC. These interpretations of our results are entirely consistent with the general observation that more inclusive techniques of pigment quantification tend to provide over-estimates relative to ones with greater analytic specificity (e.g., Daley et al. 1977). Another possibility is that the

Table 1 Coefficients of determination (r^2) obtained between spectral indices and HPLC-measured categories of sedimentary pigments from the dilution experiment samples, including the pure, unamended matrix ($n = 10$)

Spectral index	Category of pigments measured by HPLC (mg g^{-1} dry mass)		
	Chlorophyll a + all isomers	Pheophorbide a + pheophytin a	Chlorophyll a + all derivatives
Trough area 650–700 nm	0.99**	0.54*	0.98**
Trough area 650–750 nm	0.99**	0.52*	0.98**
1st derivative of R at 690 nm	0.99**	0.53*	0.98**
Amplitude of ΔR 650–675 nm	0.99**	0.53*	0.98**
Amplitude of ΔR 675–750 nm	0.98**	0.51*	0.98**
Ratio of R 650/675 nm	0.98**	0.51*	0.98**
Ratio of R 700/675 nm	0.97**	0.49*	0.97**

**$P < 0.01$; *$P < 0.05$

inference model used here systematically underestimates the amount of chlorophyll a degradation having occurred over decades in natural sediments, and hence over-estimates the complete inventory of chlorophyll a and degradation products. Such a bias may originate from the low pheopigment concentrations in the dilution series samples relative to natural counterparts, associated with the use of relatively fresh algal material in the inoculum (Fig. 2).

Despite the above caveats, both HPLC and reflectance-based techniques clearly reveal the same overall trend: increased sediment chlorophyll a concentra-

tions as eutrophication has intensified, primarily during the 1990's (Fig. 5). Furthermore, these trends are largely independent of those observed in either chlorophyll a: pheophytin a ratios, which reveal highly variable diagenetic activity in the upper 10 cm of the core, and diatom valve concentrations, which rise initially but then decline sharply in the upper two samples, presumably as cyanobacteria replace diatoms as the dominant photoautotrophs in the lake. Most importantly, these results imply that sediment chlorophyll a inferences from reflectance spectroscopy are overprinted by neither (a) diagenetic processes that modulate the balance between chlorophyll
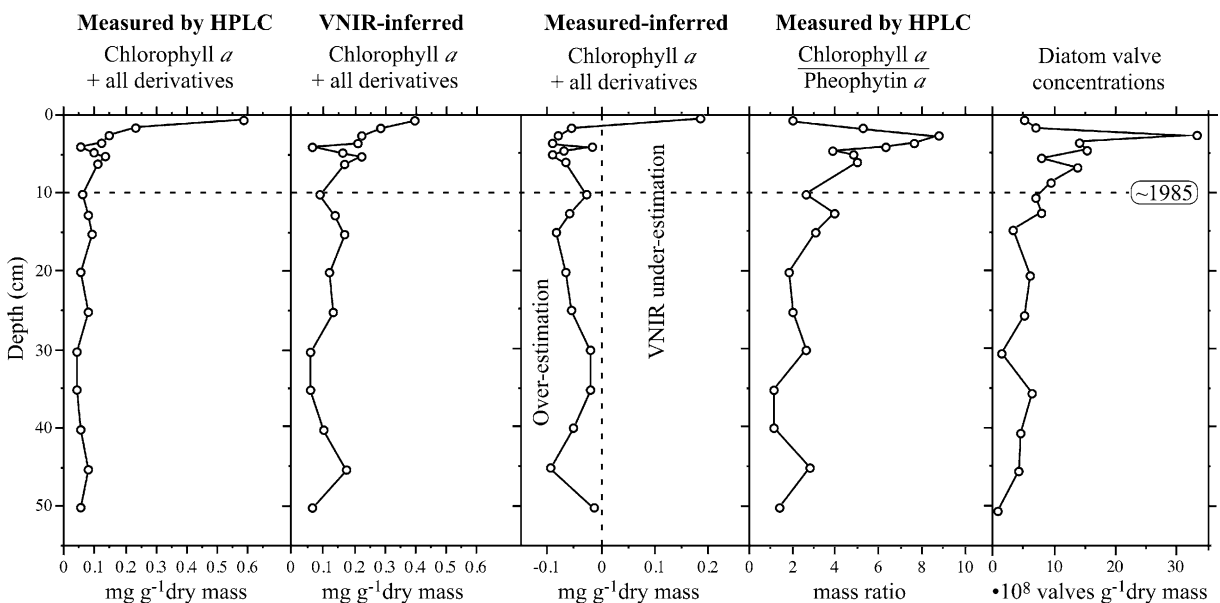
Fig. 5 Comparison of HPLC-measured and VNIR-inferred concentrations of total chlorophyll a and all derivatives (i.e., chlorophyll a + all isomers + all pheopigments) from recent sediments deposited in Lac La Biche, Alberta. Also shown are the residuals between observed and inferred quantities, and the chlorophyll a to pheophytin a ratio. The 1985 A.D. time-line at 10 cm is based on the excess ^{210}Pb inventory of the sediment

a production, sedimentation and degradation; nor (b) changes in chlorophyll *a* sources associated with ecological shifts between algal groups.

Discussion and conclusions

The key observation from the algal dilution experiment is that the trough in red reflectance positioned approximately at 675 nm deepens without reversal with incremental additions of chlorophyll *a* (Fig. 3b). This spectral feature is thus confidently ascribed to sediment concentrations of chlorophyll *a*. In this way, the VNIR spectral characteristics of sedimentary chlorophyll *a* are entirely compatible with those of living algal biomass in natural waters (Schalles et al. 1998; Gitelson et al. 1999; Gons 1999), providing an essential validation of the observations reported here. Sedimentary concentrations of chlorophyll *a* below 0.05 mg g^{-1} dry mass appear sufficient to induce the development of measurable troughs in reflectance spectra (Fig. 4).

Because primary chlorophyll *a* and its degradation products (pheophytin *a* and pheophorbide *a*) absorb in similar regions of the electromagnetic spectrum, time-dependant transformation of primary sedimentary photosynthates to pheopigments is unlikely to strongly influence spectral inferences. Significant correlations between spectral indices and concentrations of both chlorophyll *a* and associated pheopigments verify this to be the case (Table 1; see also Das et al. 2005). The apparent insensitivity of reflectance spectroscopy towards chlorophyll diagenesis is viewed as a major strength of the technique. This is because sediment spectral features may potentially prove fruitful in reconstructing lake trophic regimes on a wide range of time-scales, as well as in situations where chlorophyll *a* degradation may be advanced.

The recent eutrophication of Lac La Biche, which is associated with nutrient loading compounded to drought-induced increases in lake-water residence times, can clearly be resolved using sediment reflectance spectroscopy (Fig. 5). Both HPLC-measured and VNIR-inferred concentrations of total chlorophyll *a* and derivatives chronicle progressive enrichment of the lake after ca. 1985. Although a larger training set of calibration samples is clearly desirable to refine the inference model, these preliminary results confirm the appropriateness of VNIR for rapid and non-

destructive, semi-quantitative determinations of chlorophyll *a* and its derivatives from lake sediments. For applications in lake management that require baseline historical lake trophic status to be determined rapidly and cost-effectively, the VNIR techniques reported here appear well-suited.

The ubiquity of chlorophyll *a* as the dominant pigment in most lake sediments, coupled to the apparent robustness of its spectral signature in reflectance spectroscopy, imply that the approaches described in this paper are widely applicable across broad limnological gradients of water chemistry and trophic status. For example, the approach described here has been used to infer primary production increases in a series of ultra-oligotrophic arctic lakes, in response to recent climate warming (Michelutti et al. 2005). The greatest limitations of the technique are its inability to distinguish primary chlorophyll *a* from degradation products, or to resolve trace pigments such as carotenoids. On the other hand, given that chlorophyll *a* is produced by all algae and higher plants (Leavitt and Hodgson 2001), it constitutes an integrated recorder of trends in whole-lake production. Reflectance spectroscopy may thus provide rapid and non-destructive inferences of lake sediment chlorophyll *a* concentrations. Such inferences, which constitute an index of whole-lake production changes, may be generated in a fraction of the time currently required for precise wet-chemical pigment determinations.

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