

## Accessory pigments versus chlorophyll *a* concentrations within the euphotic zone: A ubiquitous relationship

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### *Abstract*

Remotely sensed chlorophyll *a* (Chl *a*) concentrations are determined by the ratio of upwelled radiances within the Soret band of Chl *a* (443 nm) and at 550 nm. Absorption at wavelengths outside this band (>460 nm) is dominated by accessory pigments and for the successful measurement of Chl *a* (e.g., 490:550 nm and 520:550 nm ratios) early Coastal Zone Color Scanner investigators speculated that these accessory pigments must covary with Chl *a*, although routine methods to measure these pigments had not yet been developed. Nearly 7,000 (high performance liquid chromatography) pigment samples, collected within the euphotic zone, were measured to test the consistency of the relationship between accessory pigments and Chl *a*. Despite the various sampling periods (1985–1998) and numerous geographic locations, consistent patterns have emerged in the ratios of the log accessory pigments to log total Chl *a* (TCHLA = Chl *a*, Chl *a* allomer, Chl *a* epimer, and chlorophyllide *a*). There were strong log-linear relationships within cruises for these ratios with an average  $r^2$  of 0.889. An even more impressive relationship was observed on a global scale when all the data were combined. Individual relationships were also calculated for case I and case II waters, as well as for the first optical depth ( $K^{-1}$ ), termed the remote sensing depth. There were some statistical differences between these relationships, yet on a practical sense many could be combined. Despite a wide range of environments sampled, the overall slope of the log accessory pigments : log TCHLA was found to be 0.934 with a relative-difference root-mean-square error of 28% in log accessory pigment concentrations. This global log-linearity largely explains the success in remotely sensed Chl *a* algorithms, even though phytoplankton populations can vary in their composition and suite of pigments.

Phytoplankton utilize chlorophyll *a* (Chl *a*) as their major light harvesting pigment for photosynthesis. Other pigment compounds, such as Chl *b* and Chl *c*, carotenoids, termed accessory pigments, also play a significant role in photosynthesis by extending the organism's optical collection window, thereby improving absorption efficiencies and adaptation capabilities. The unique optical properties of Chl *a* have been used to develop spectrophotometric and fluorometric measurement techniques. Following the commercial availability of fluorometers for routine measurements of Chl *a*, this single pigment compound became a universal parameter in biological oceanography for estimating phytoplankton biomass.

Absorption properties of Chl *a*, especially in the Soret band with its *in vivo* maximum near 440 nm, were found to be a major factor contributing to ocean color. This led to the development of remote sensing techniques, which culminat-

ed in the successful measurement of ocean color from space using the Coastal Zone Color Scanner (CZCS; Hovis et al. 1980). Empirical relationships were developed relating water-leaving radiance ratios at four wavelengths (443:550 nm, 520:550 nm and 520:670 nm; Clark 1981; Gordon et al. 1983), diffuse attenuation coefficients at two wavelengths (490 nm and 520 nm; Austin and Petzold 1981; Gordon and Morel 1983) and spectral absorption at multiple wavelengths (490 nm to 600 nm; Prieur and Sathyendranath 1981) to "chloropigments" (Chl *a* plus phaeopigments as determined by the fluorometric method). Although most of these relationships were at wavelengths outside the Soret band of Chl *a* and did not include accessory pigments, they were still able to account for most of the variance ( $r^2 > 0.90$ ). Based on these results, it was assumed that the absorption contributed by accessory pigments must be small, or highly covariant with "chloropigments."

It was only towards the end of the life of the CZCS that new methods were developed (e.g., Mantoura and Llewellyn 1983) using high performance liquid chromatography (HPLC) to measure phytoplankton pigments. The applica-

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tion of HPLC to phytoplankton pigment analysis has lowered the uncertainty for measuring chl *a* and phaeopigments, as well as the accessory pigments, since compounds are physically separated and individually quantified. HPLC has provided oceanographers with a powerful tool for studying the processes affecting the phytoplankton pigment pool. This method has revealed, for example, that divinyl Chl *a* and Chl *b* are only present in prochlorophytes (Goericke and Repeta 1992), phaeopigments (fluorometrically determined) are overestimated in the presence of Chl *b* (Vernet and Lorenzen 1987), and that the uncertainty in fluorometrically determined Chl *a* concentration is variable in space and time (Trees et al. 1985; Smith et al. 1987; Hoepffner and Sathyendranath 1992; Bianchi et al. 1995; Tester et al. 1995).

Laboratory and field studies have shown that the ratios of individual accessory pigments to Chl *a* can vary as a function of taxonomic composition and physiological state, as modulated by nutrients, temperature, light intensity and spectral composition, and photoperiod (Bidigare et al. 1990; Millie et al. 1993; Morel et al. 1993; Bricaud et al. 1995; etc). Bidigare et al. (1987) collected samples in the Sargasso Sea and showed that nonphotosynthetic carotenoids comprised a highly dynamic pigment pool. During a drift station for sunny and overcast days (5 and 7 April 1985), the photosynthetic carotenoid:Chl *a* ratios were found to be relatively constant with respect to depth and irradiance, whereas the photoprotective carotenoid:Chl *a* ratios varied by a factor of 3.2 in the upper 15 m. This showed that, besides individual accessory pigments, select groups of pigment compounds can also have a high degree of variability relative to Chl *a*. Accessory pigments have also been used as general diagnostic markers for specific phytoplankton groups, such as peridinin for dinoflagellates, Chl *b* for green algae, zeaxanthin for cyanobacteria and prochlorophytes, fucoxanthin for diatoms, etc. (Gieskes et al. 1988; Everitt et al. 1990; Mackey et al. 1996), indicating changes both horizontally and vertically in phytoplankton community structure.

In preparation for a new generation of ocean color sensors (Sea-viewing Wide Field-of-view Sensor (SeaWiFS) and Moderate-resolution Imaging Spectrometer (MODIS)), advanced technology has been used to develop new bio-optical instrumentation. These developmental efforts were undertaken to reduce the uncertainties in bio-optical algorithms that generate satellite derived products. As a result of this effort, NASA has adopted the U.S. Joint Global Ocean Flux Study (JGOFS) recommendation that HPLC is the preferred method for measuring phytoplankton pigments and should be used for ocean color pigment product development and validation (Mueller and Austin 1995).

We have assembled an extensive HPLC pigment database in order to gain a better understanding of the variability in the phytoplankton pigment pool. This data extends over a decade of sampling and analyses, and includes a variety of environments ranging from freshwater to marine, oligotrophic to eutrophic, and tropical to polar. The central purpose of this study and the question we address herein is, "What is the concentration of accessory pigments relative to Chl *a* and are these accessory pigments varying individually or in

concert with Chl *a*, as hypothesized from results inferred from remote sensing applications?"

## Methods

*Study sites*—From 1985 to 1995, we participated in 31 cruises collecting samples for HPLC analysis. An additional cruise (MOCE 4) in 1998 was added to this database, because it was a major SeaWiFS calibration and validation effort. These 32 cruises are listed in Table 1 by cruise, date, geographical area, average sample depth, and maximum depth sampled. A total of 6,985 samples were collected and analyzed in two different laboratories (C. Trees, San Diego State Univ.; R. Bidigare, Univ. of Hawaii) using a variety of instruments and methods as HPLC methodology evolved over the decade under study.

*Sampling*—Nominally, samples were collected in Niskin, or polycarbonate bottles, and filtered through either 0.4  $\mu\text{m}$  polyester Nuclepore filters, or 0.7  $\mu\text{m}$  GF/F glass fiber filters. The volumes ranged from 0.125 liters for turbid waters and up to 2.2 liters for oceanic areas. Samples were analyzed either on the ship, or stored in liquid nitrogen for laboratory analysis ashore. The filtered samples were extracted in either 90% acetone, or a 40:60% mixture of DMSO:90% acetone, for 24–48 h, following sonication in some cases. Nuclepore filters and DMSO extractions were limited to the early cruises, which occurred before JGOFS pigment protocols were developed and adopted.

*Pigment concentrations*—The following published methods were used for the HPLC analyses: Mantoura and Llewellyn (1983), Hooks et al. (1988), Bidigare et al. (1989), Wright et al. (1991), and Goericke and Repeta (1993). During this period the following columns and flow rates were used to separate the pigment compounds: a Radial-Pak C18 column (0.8  $\times$  10 cm; 5 or 10  $\mu\text{m}$  particle size; Waters Associates) at a flow rate of 6 or 10 ml min<sup>-1</sup>, a Spherisorb ODS-2 stainless steel column (0.046  $\times$  25 cm; 5  $\mu\text{m}$  particle size; Alltech Associates) and a C8 column (10 cm; 3  $\mu\text{m}$  particle size), both at a flow rate of 1 ml min<sup>-1</sup>. To facilitate separation of the dephytolated pigments, all methods used either an ion-pairing solution (Mantoura and Llewellyn 1983), or distilled water (Wright et al. 1991), which was mixed with the sample immediately prior to the injection on the column. Techniques for injecting the samples have progressed from manual "hand injections" to autosampler injections, which were temperature controlled with automated sample preparation and mixing.

A number of absorption and fluorescence detectors were used to identify and quantify the various pigment compounds as they were eluted off the columns. These detectors included a Waters Associates (Model 440) Absorbance Detector (436 nm), and progressed through a Waters Associates (Model 420-AC) Fluorometer (Ex 400–460 nm, Em > 600 nm), a Kratos (Model FS950) Fluorometer (Ex 400–460 nm, Em > 600 nm), a Thermo Separations Products (Model UV2000) Dual Wavelength UV/VIS Programmable Absorbance Detector (436 and 450 nm), and a Linear Model LC 304 Fluorometer (Ex 404 nm, Em 680 nm). The fluorescence

Table 1. Summary of HPLC pigment data base as a function of cruise/deployment, date, geographical area and average sample depth in meters (maximum depth sampled).

Cruises	Date	Area	Average depth
Biowatt 85	Apr 85	Northwestern Atlantic	52 (129)
TransPacific 24N	Apr–May 85	North Pacific along 24N	73 (126)
TransPacific 47N	Aug–Sep 85	North Pacific along 47N	44 (128)
SLC 86	Aug 86	Greenland, Norwegian and Barents Seas	26 (75)
Biowatt 87-1	Mar 87	Sargasso Sea	44 (130)
Biowatt 87-2	May 87	Sargasso Sea	83 (125)
GSP	May–Jun 87	Greenland Sea/Arctic and Polar Fronts	17 (81)
TEW	Jun–Jul 87	Equatorial Pacific	62 (125)
SLC 87	Jul–Aug 87	Greenland, Norwegian & Iceland Seas	24 (75)
Biowatt 87-3	Aug 87	Sargasso Sea	74 (124)
AVIRIS	Oct 87	San Francisco Bay	0.3 (2.0)
Biowatt 87-4	Nov 87	Sargasso Sea	55 (122)
Solars 17	Apr 88	Caribbean Sea and off the Orinoco River	52 (122)
Solars 19	Sep 88	Caribbean Sea and off the Orinoco River	45 (120)
CaBS 11	Jan–Feb 90	Northeastern Pacific/Santa Monica Basin	39 (100)
CaBS 12	Jul 90	Northeastern Pacific/Santa Monica Basin	42 (100)
Icecolors	Oct–Nov 90	Bellingshausen Sea/Antarctic	29 (130)
MLML 91	May 91	North Atlantic/Southwest of Iceland	37 (130)
BOFS	Jun–Jul 91	North Atlantic/South of Iceland	10 (60)
Moss Landing	Oct 91	Moss Landing South Harbor, California	0.1 (0.52)
EqPac Spring	Feb–Mar 92	Equatorial Pacific	64 (130)
Optical Closure	Apr–May 92	Lake Pend Orielle, Idaho	39 (100)
EqPac Fall	Aug–Sep 92	Equatorial Pacific	64 (130)
MOCE 1	Aug–Sep 92	Northeastern Pacific/Monterey Bay	11 (45)
MOCE 2	Mar–Apr 93	Northeastern Pacific and Gulf of California	19 (79)
IronEx 1	Oct 93	Southeastern Pacific around the Galapagos Island	29 (75)
Arabesque 1	Aug–Sep 94	Gulf of Oman and Arabian Sea	19 (60)
MOCE 3	Oct–Nov 94	North Pacific/Hawaiian Island Arch Chain	31 (129)
Mill Creek	Jul Aug 95	Mill Creek, Chesapeake Bay	0.3 (1.6)
Snug Harbor	Aug 95	Snug Harbor, Hawaii	0.1 (0.15)
Turbid 5	Sep–Oct 95	Mill Creek, Chesapeake Bay	0.5 (1.6)
MOCE 4	Jan–Feb 98	North Pacific/Hawaiian Islands	14 (125)

detectors were primarily used to assist in the identification and quantification of phaeopigments, which typically occur in low concentrations. For the HPLC pigment samples processed between 1985 and 1990, fluorometric detection of phaeopigments was not performed.

Peak identifications and purity were confirmed “on-line” using either a Hewlett Packard (Model 8451A) Diode Array Spectrophotometer, or a Thermo Separations Products (Model SpectraFOCUS) 32 Channel Forward Optical Scanning Detector. Measurements of spectral absorbance were important, since the HPLC methods employed prior to 1996 did not separate zeaxanthin from lutein. In most of the samples, it has been assumed that the zeaxanthin/lutein peak is dominated by zeaxanthin, as inferred from the absorbance spectra and published data.

For the divinyl Chl *a* and Chl *b*, which are found in prochlorophytes, it has only been recognized in the past few years that they can contribute significantly to phytoplankton biomass (Goericke and Repeta 1993). The separation of these compounds requires calibration procedures using pigment standards that account for the divinyl forms. Since most of the cruises and analyses in this database were performed prior to the development of these methodologies, divinyl Chl *a* and Chl *b* were included in the concentration estimates for “chlorophylls” *a* and *b*.

Calibration standards were obtained from Sigma Chemical, purified from cultures by thin-layer chromatography, or obtained from other sources listed in Latasa et al. (1996). Pigment standards were exchanged between the two laboratories on numerous occasions during this ten-year period to assure the generation of an internally consistent pigment database. System calibrations were performed using pigment standards, which were injected onto the HPLC columns and peak areas calculated to generate individual standard response factors for each compound. Concentration of the standards was determined spectrophotometrically using published extinction coefficients (*see table 2*, Latasa et al. 1996).

*Statistical analysis*—A review of the pigment database showed that it was log-normally distributed, which is consistent with the distribution of bio-optical properties proposed by Campbell (1995). It was therefore appropriate to perform log-linear regressions on accessory pigments versus TCHLA, using model I regressions. Model I regressions were selected because accessory pigment concentrations were to be predicted from Chl *a* concentrations (Model I regressions are appropriate for both predictions and determining functional relationships, whereas model II regres-

Table 2. Means of total Chl *a* (TCHLA) and total accessory pigments (mg m<sup>-3</sup>) by cruise with  $\pm$  standard deviations. Also listed are the ratios of various diagnostic accessory pigments to TCHLA (A, TCHLA; *c*, chl *c*; *b*, chl *b*; Ph *a*, phaeophytin *a* + phaeophorbide *a*; Fuco, fucoxanthin; Hex, 19'hexanoyloxyfucoxanthin; PSC, photosynthetic carotenoids; PPC, photoprotective carotenoids).

Cruises	Total Chl <i>a</i>	Accessory	<i>c</i> : A	<i>b</i> : A	Ph <i>a</i> : A	Fuco: A	Hex: A	PSC: A	PPC: A
Biowatt 85	0.35±0.24	0.37±0.25	0.095	0.183	ND	0.291	0.283	0.599	0.216
TransPacific 24N	0.18±0.16	0.19±0.18	0.036	0.298	ND	0.114	0.245	0.484	0.252
TransPacific 47N	0.25±0.19	0.23±0.17	0.087	0.176	ND	0.173	0.302	0.606	0.106
SLC 86	0.69±0.36	1.03±0.63	0.143	0.205	ND	0.372	0.569	0.965	0.206
Biowatt 87-1	0.33±0.14	0.32±0.15	0.079	0.257	ND	0.114	0.208	0.496	0.152
Biowatt 87-2	0.31±0.14	0.29±0.13	0.063	0.282	ND	0.061	0.232	0.409	0.227
GSP	1.77±1.09	2.05±1.11	0.219	0.266	ND	0.260	0.234	0.639	0.095
TEW	0.16±0.09	0.20±0.12	0.099	0.326	ND	0.026	0.188	0.319	0.528
SLC 87	0.82±0.46	1.06±0.53	0.248	0.190	ND	0.345	0.353	0.717	0.178
Biowatt 87-3	0.21±0.11	0.25±0.14	0.096	0.237	ND	0.058	0.249	0.430	0.409
AVIRIS	1.26±0.51	1.11±0.49	0.031	0.086	ND	0.251	0.002	0.292	0.467
Biowatt 87-4	0.36±0.09	0.40±0.11	0.155	0.165	ND	0.104	0.230	0.489	0.286
Solars 17	0.28±0.17	0.32±0.21	0.064	0.201	ND	0.072	0.194	0.379	0.474
Solars 19	0.29±0.24	0.31±0.23	0.097	0.190	ND	0.235	0.209	0.544	0.300
CaBS 11	0.38±0.24	0.55±0.35	0.032	0.194	ND	0.303	0.421	0.949	0.285
CaBS 12	0.55±0.51	0.77±0.70	0.123	0.217	ND	0.379	0.364	0.935	0.240
Icecolors	0.65±0.41	0.69±0.49	0.127	0.032	ND	0.248	0.400	0.715	0.150
MLML 91	0.93±0.65	0.94±0.68	0.180	0.100	ND	0.276	0.200	0.598	0.134
BOFS	0.69±0.36	0.61±0.34	0.029	0.089	ND	0.118	0.380	0.567	0.224
Moss Landing	3.80±4.40	3.86±5.23	0.009	0.269	ND	0.187	0.009	0.216	0.339
EqPac Spring	0.18±0.07	0.23±0.08	0.112	0.286	ND	0.031	0.291	0.471	0.413
Optical Closure	2.58±2.07	2.46±2.09	0.052	0.033	ND	0.242	0.011	0.263	0.578
EqPac Fall	0.21±0.09	0.27±0.11	0.122	0.243	ND	0.078	0.301	0.551	0.431
MOCE 1	1.44±0.82	1.64±0.93	0.206	0.148	ND	0.250	0.222	0.541	0.259
MOCE 2	1.16±1.10	0.94±0.79	0.400	0.105	ND	0.100	0.061	0.239	0.173
IronEx 1	0.41±0.15	0.61±0.20	0.258	ND	ND	0.058	0.296	0.486	0.774
Arabesque 1	0.35±0.30	0.61±0.49	0.239	0.140	0.042	0.324	0.310	1.043	0.438
MOCE 3	0.13±0.08	0.17±0.08	0.048	0.127	0.003	0.080	0.203	0.370	0.803
Mill Creek	16.04±5.68	21.37±7.73	0.226	0.031	0.010	0.152	0.019	0.603	0.455
Snug Harbor	0.48±0.11	0.71±0.14	0.121	0.185	0.009	0.186	0.076	0.397	0.739
Turbid 5	21.77±7.05	18.47±7.76	0.108	0.017	0.015	0.350	0.015	0.389	0.205
MOCE 4	0.11±0.09	0.13±0.09	0.026	0.026	ND	0.062	0.193	0.311	0.843
Total	0.88±2.67	0.94±2.75	0.118	0.170	0.002	0.165	0.267	0.546	0.350

sions should not be used to predict values of *y* given *x*, [p. 543, Sokal and Rohlf 1995]).

## Results

**Vertical sample distribution**—The final HPLC pigment database consisted of 5,617 measurements with samples limited to the euphotic zone (1.0 to 0.1% light level) at depths ranging from surface to as deep as 130 m (5.8% of the original 6,985 samples were excluded because of this depth criteria). Samples collected below the euphotic zone depth had abnormally high accessory pigment to Chl *a* ratios, that suggests that Chl *a* degrades more rapidly than accessory pigments as particles are removed from the euphotic zone. In addition to this depth criterion, samples were also rejected if a minimum number of accessory pigments were not measured. A review of the literature showed that most algal groups (excluding phycobiliprotein-containing groups) contain at least four HPLC-measurable accessory pigments (chlorophylls *b*, *c*<sub>1</sub>, *c*<sub>2</sub>, *c*<sub>3</sub>, and carotenoids; see table 2.3, Jeffrey and Vesik 1997). If blooms of phycobiliprotein-containing organisms (cyanobacteria, cryptophytes, and rhodo-

phytes) do occur, then the number of HPLC-measurable accessory pigments is usually greater than 3, due to the presence of other algal groups (e.g., *Synechococcus* bloom; Bidigare et al. 1997). Samples that do not meet this minimum accessory pigment requirement have detection limit problems related to low signal-to-noise ratios and insufficient concentration techniques (e.g., low filtration volumes). Excluding samples containing three, or fewer, accessory pigments decreased the database by another 19.6%.

A histogram of the number of observations in 1-m depth bins is shown in Fig. 1. The data were skewed to near-surface samples, since 23% of the data were collected in the upper 4 m. Many of these near-surface samples (42%) were collected from five cruises (TEW, SLC 87, Icecolors, BOFS, and MOCE 4) using alongtrack sampling from the ship's "sea chest". Also apparent in Fig. 1 is the increased numbers of samples at 10-m intervals, a characteristic of following standard hydrocast depth intervals (e.g., SLC 86 and 87, BOFS, Optical Closure, Solars 17 and 19, EqPac Spring, and EqPac Fall).

**Total Chl *a***—Chl *a* derivatives, such as epimers and alomers, as well as chlorophyllide *a* were summed together

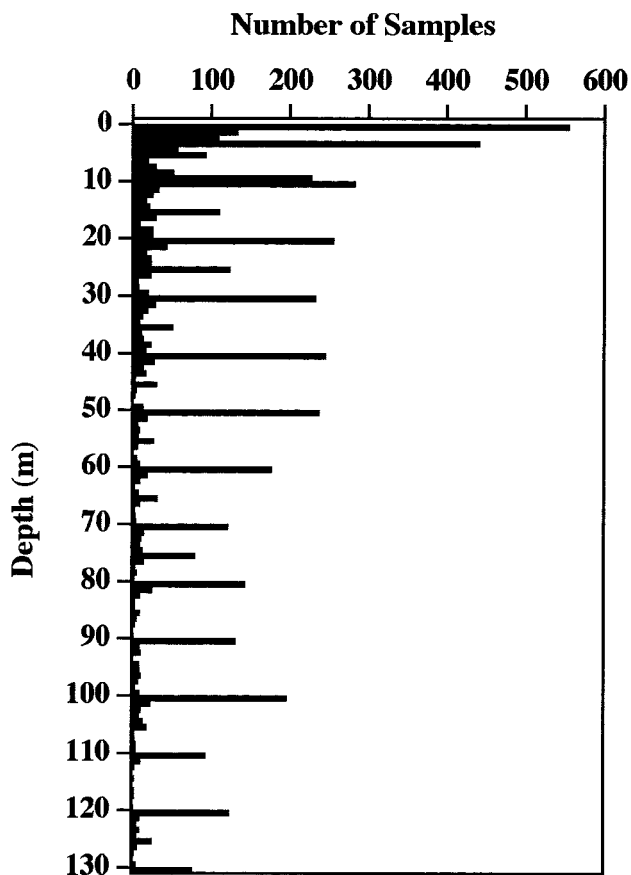


Fig. 1. A histogram of the number of HPLC samples in 1-m bins as a function of depth (m).

to calculate total Chl *a* concentrations (TCHLA). The average contribution to the Chl *a* pool for these three pigment compounds was 5.6%, 0.6%, and 4.4%, respectively. Chlorophyllide *a* is the precursor molecule for Chl *a*, as well as a degradation product of Chl *a* in senescent cells. It can also be formed when the enzyme chlorophyllase is not inactivated during the solvent extraction process. Generally, chlorophyllide *a* is found in low concentrations (2–5% of Chl *a*) in most pigment samples. Concentrations of this pigment exceeding 15–20% of the total Chl *a* pool are regarded as an artifact associated with collection of chlorophyllase-containing senescent diatoms and the extraction process (Jeffrey and Hallegraeff 1987; Latasa and Bidigare 1998). High chlorophyllide *a* levels were detected in some samples collected during Biowatt 85, TransPacific 47N, GSP, SLC 86 and 87, Solars 19, and MOCE 1 and 2 cruises.

*Total Chl a versus accessory pigments*—TCHLA and accessory pigment concentrations ( $\text{mg m}^{-3}$ ) and ratios for each cruise are shown in Table 2. All pigments, including phaeopigments (phaeophytin *a* and phaeophorbide *a*), carotenoids, Chls *b*, and Chl *c*, were summed to get the total accessory pigment concentrations by weight. Phaeopigments were added to the accessory pigment pool, because these degradation compounds contribute to ocean color and affect the vertical distribution of spectral irradiance in the water column. The

average ratio of phaeophytin *a* plus phaeophorbide *a* to TCHLA was only 0.2% (see Table 2). Accessory pigment concentrations generally exceeded TCHLA concentrations. Photosynthetic carotenoids (PSC; peridinin, fucoxanthin, 19'hexanoyloxyfucoxanthin, 19'butanoyloxyfucoxanthin, and prasinoxanthin) and photoprotective carotenoids (PPC; diadinoxanthin, alloxanthin, diatoxanthin, zeaxanthin,  $\alpha$ -,  $\beta$ -carotene, and violaxanthin) were also summed to examine regional differences. Photosynthetic carotenoids to TCHLA ratios were about 1.5 times the ratio of PPC to TCHLA.

These regression models for each cruise are shown in Fig. 2, with slopes, intercepts, correlation coefficients ( $r^2$ ), and numbers of observations. Within each cruise, the relationships are quite log-linear even though the samples represent different depths and water masses. There is no observable departure in this log-linear trend at either high or low TCHL concentrations, although some of the cruises have statistically different slopes.

## Discussion

Selective transmission of light in the sea results in a wide range of variability in spectral irradiance in the water column. In response, phytoplankton have developed numerous accessory pigment systems, enabling them to utilize a number of habitats. By varying the mixture of accessory pigments, a phytoplankton population may change its overall absorption spectrum to better match the spectral distribution of light available in its habitat. This photoadaptive ability may give select phytoplankton groups a competitive advantage in the various spectral environments encountered in the sea. Different accessory pigments have different physiological functions, yet the ratio of log total accessory pigments to log TCHLA is remarkably constant at a value of 0.934. This is shown in Fig. 3, which is a plot of the entire database from 0 to 130 m and includes both case I (dominated by phytoplankton and their by-products) and case II (dominated by inorganic and organic sediments and colored dissolved organic material) waters. The regression line in Fig. 3 indicates that, for low TCHLA waters, there is relatively more accessory pigments, whereas for high TCHLA areas there is less. The accessory to total pigment ratio varies from about 57% for  $\text{TCHLA} = 0.04 \text{ mg m}^{-3}$  to 48% for  $\text{TCHLA} = 10 \text{ mg m}^{-3}$ .

Since the purpose of this study was to evaluate pigment distributions in the context of remote sensing algorithms, the pigment database was divided into case I and case II waters. Although optical measurements were not always collected to validate this classification scheme, a best-guess estimate can be made using geographic location. Out of the 32 cruises, 5 can be considered as case II waters (AVIRIS, Moss Landing, Mill Creek, Snug Harbor, and Turbid 5), comprising only 4.2% of the database. Log-linear regressions were performed on case I and case II samples independently and are tabulated in Table 3 with 95% confidence limits (CL) in parentheses. Only case II regressions showed any statistically difference between slopes and intercepts when compared to the global and case I estimates. The relative difference root-mean-square (RMS) errors for estimating log accessory pig-

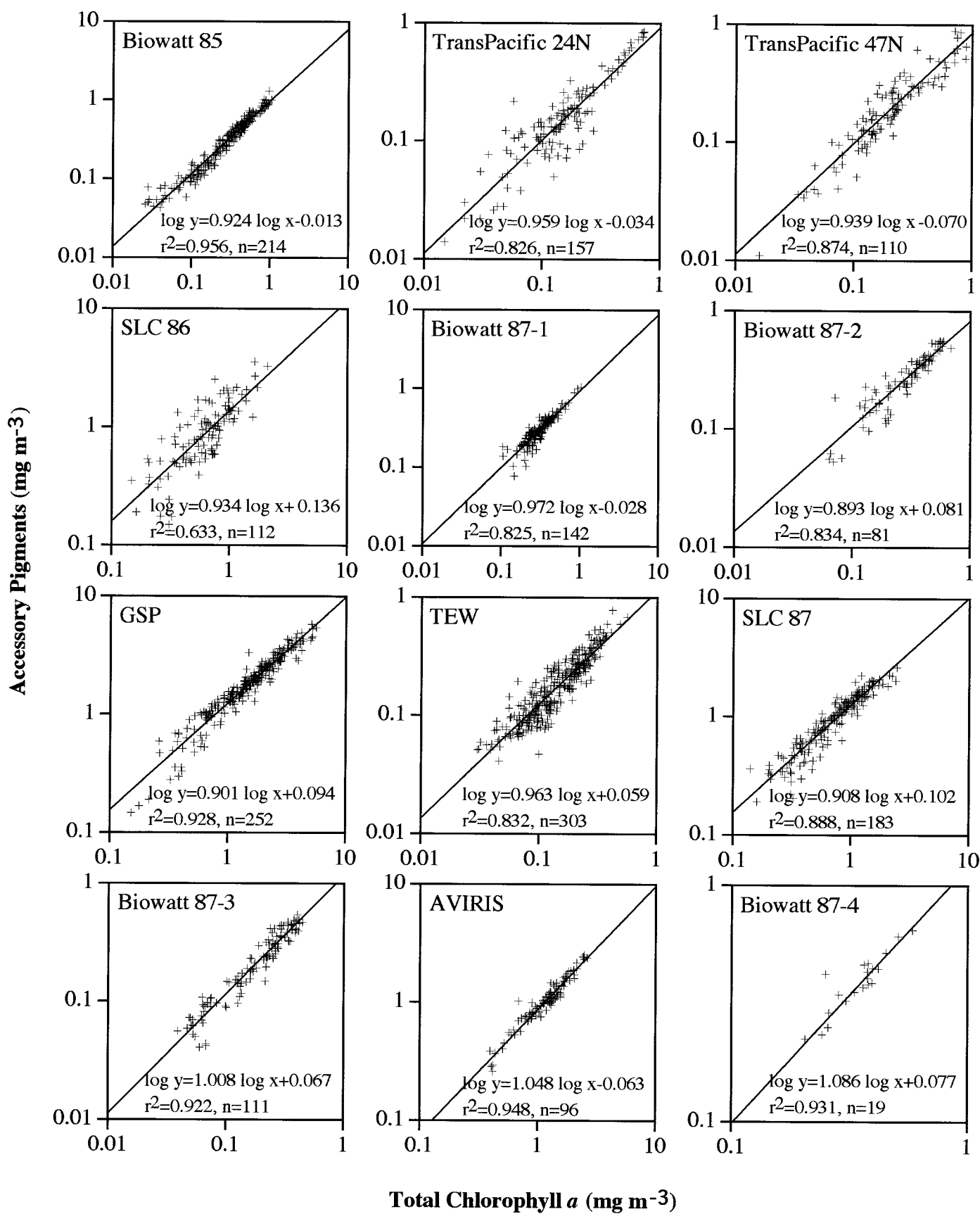


Fig. 2. Regression models predicting log accessory pigments from log TCHLA (Chl *a*, Chl *a* allomer, Chl *a* epimer, and chlorophyllide *a*) by cruise, including slope, intercept, correlation coefficient ( $r^2$ ), and number of samples ( $n$ ).

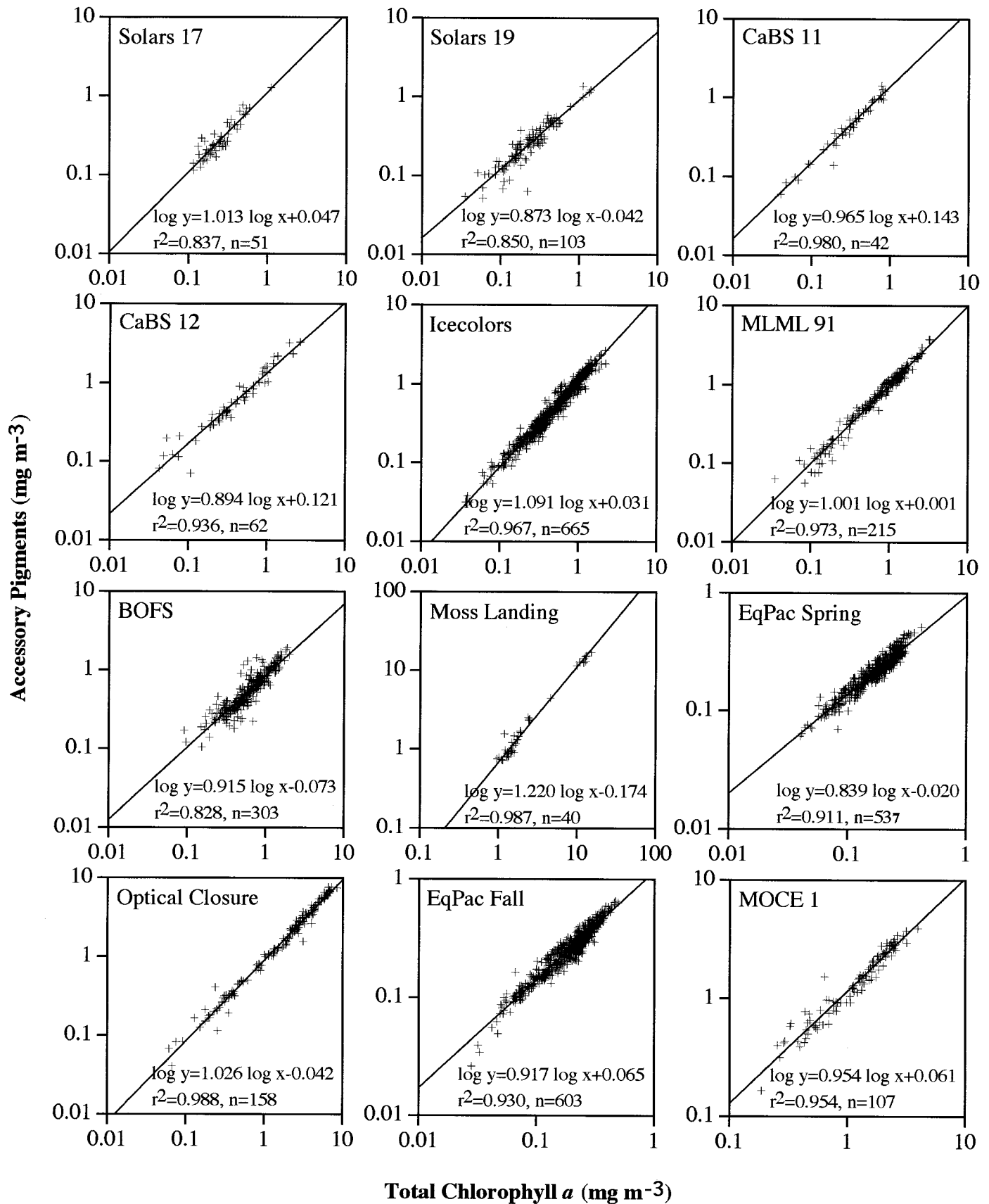


Fig. 2. Continued.

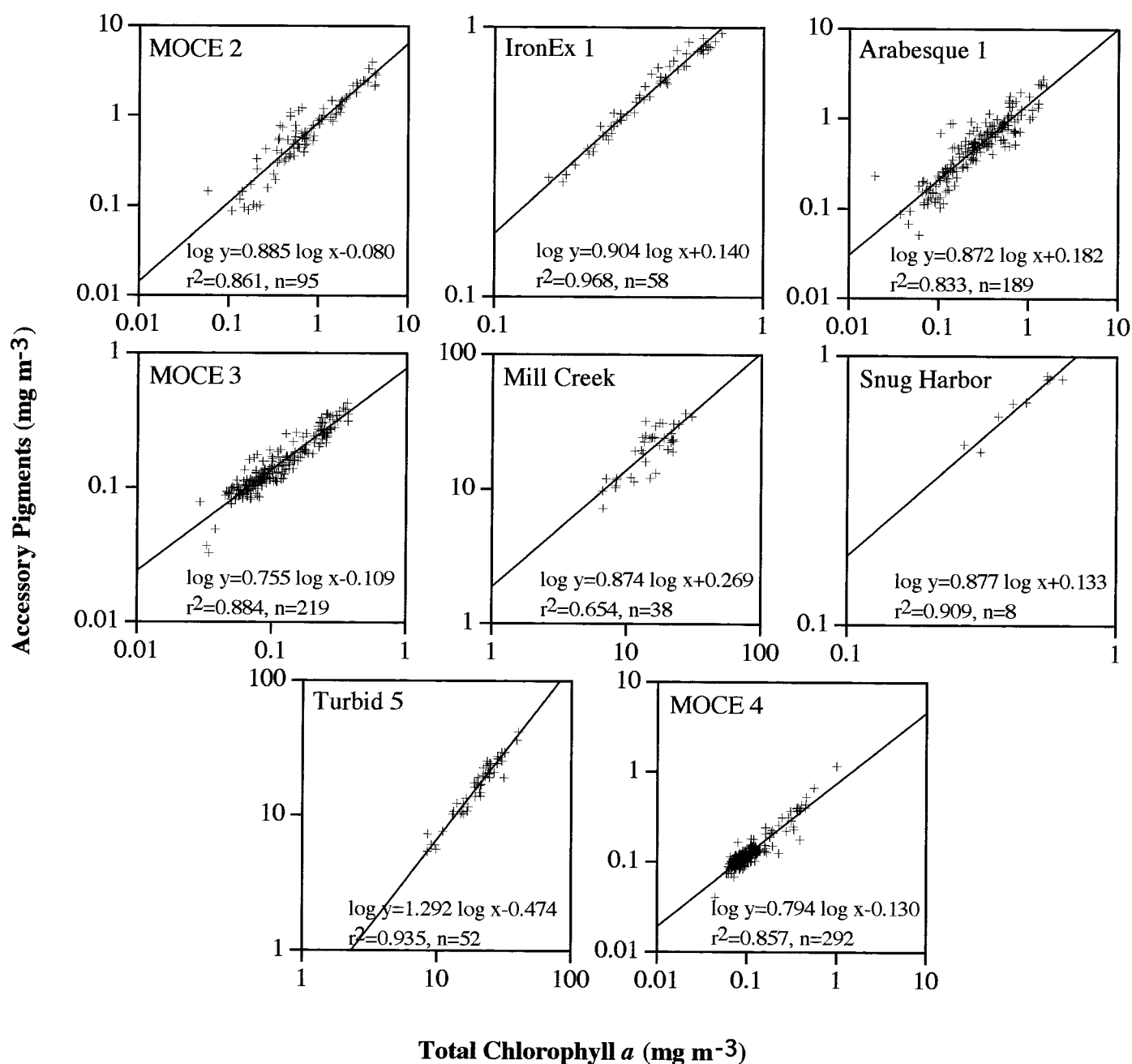


Fig. 2. Continued.

ments from log TCHLA were very similar for global and case I waters.

These data were then subdivided into samples limited to the first optical depth [ $1/\text{diffuse attenuation coefficient}, K(490)$ ]. This is the depth at which 90% of the light originates for remote sensing purposes.  $K(490)$  was estimated from TCHLA using the regression equation of Austin and Petzold (1981). The average diffuse attenuation coefficient,  $K(490)$ , was then calculated from the average TCHLA concentration at depths less than  $1/K(490)$ , which was determined by iterative inspection of each pigment profile. Data was then excluded below this depth, reducing the database by 51.5%.

Log-linear regressions were then performed, generating case I, case II, and global models for the first optical depth (Table 3). There was no statistically significant difference between global and case I waters, a result similar to that found for the entire euphotic zone. Percent RMS values were higher for the first optical depth models, due to the drastic reduction in sample size. However, statistically differences in regression slopes between the euphotic zone and the first optical depth for case I and global models were present. Using the first optical depth model, the error in accessory pigment prediction compared to the euphotic zone model increased by 35%. Because almost the same data were used in the case

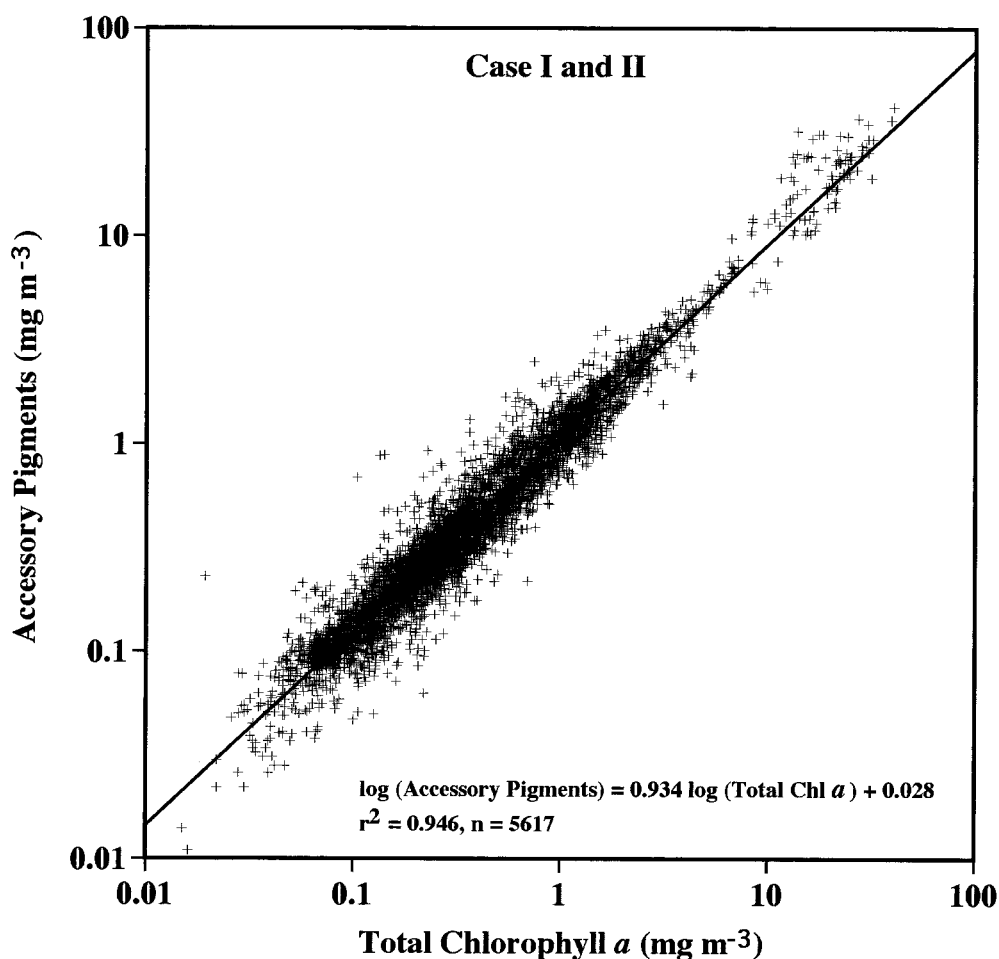


Fig. 3. Regression model predicting log accessory pigments from log TCHLA (Chl *a*, Chl *a* allomer, Chl *a* epimer, and chlorophyllide *a*) for the global HPLC pigment database (case I and case II waters). This includes slope, intercept, correlation coefficient ( $r^2$ ), and number of samples ( $n$ ).

Table 3. Results of log-linear regression analyses predicting accessory pigments from total Chl *a* for both case I and case II waters and for the combined global database. The regression equations are given by  $\log(\text{Accessory Pigments}) = A + B \log(\text{TCHLA})$  with 95% CL in parentheses. Asterisks (\*) indicate that these regressions were limited to the first optical depth or remote sensing depth [ $1/K(490)$ ]. The relative-difference root-mean-square errors for estimating accessory pigments have also been calculated.

Water type	A	B	$r^2$	$n$	% RMS error
Case 1	0.0281(0.0045)	0.932(0.007)	0.933	5,383	27.9
Case 2	-0.0558(0.0192)	1.038(0.023)	0.971	234	24.1
Global	0.0282(0.0040)	0.934(0.006)	0.946	5,617	27.9
*Case 1	0.0256(0.0083)	0.948(0.011)	0.923	2,497	37.8
*Case 2	-0.0608(0.0194)	1.044(0.025)	0.968	226	25.0
*Global	0.0221(0.0067)	0.947(0.008)	0.944	2,723	37.1

II model for the first optical depth, there was no difference between the intercepts and slopes.

**Pigment ratios**—The averages listed in Table 2 can be misleading, in that they depend on the number of samples collected at depth and the types of water masses sampled (neritic, oceanic, or both). Low TCHLA regions are mainly located in open ocean, oligotrophic waters and have high zeaxanthin:TCHLA ratios near-the surface, and high Chl *b*:TCHLA ratios at depth. During the TransPacific 24N cruise, the dominant phytoplankton accessory pigment changed from zeaxanthin and Chl *b* in the stratified open-ocean waters to fucoxanthin and 19'-hexanoyloxyfucoxanthin in the upwelling regions off the coast of California, indicating a shift from cyanobacteria dominated waters offshore to diatoms and prymnesiophytes near-shore. Barlow et al. (1999) found a similar distributional pattern in the Arabian Sea, where fucoxanthin and 19'-hexanoyloxyfucoxanthin were the dominant pigments inshore, while in the oligotrophic waters of offshore, zeaxanthin became important, indicating a shift to cyanobacteria (*Synechococcus* and *Prochlorococ-*

cus). The highest values for zeaxanthin to TCHLA values were found for MOCE 3 and 4 cruises (0.760 and 0.672) around the Hawaiian Islands. Elevated ratios have also been measured at the Hawaiian Ocean Time-series site and have been attributed to the dominance of *Prochlorococcus*.

Most of the high TCHLA regions also had high fucoxanthin:TCHLA ratios (e.g., GSP, AVIRIS, MLML 91, Optical Closure, MOCE 1, and Turbid 5). A few sites, such as Mill Creek, had extremely high TCHLA concentrations and low fucoxanthin:TCHLA ratios. This is indicative of a phytoplankton bloom of a group other than diatoms, and for Mill Creek, it was a dinoflagellate (peridinin:TCHLA of 0.370). The second highest peridinin:TCHLA (0.300) was found for Arabesque 1, which also had elevated fucoxanthin:TCHLA and 19'-hexanoyloxyfucoxanthin:TCHLA ratios, generating the maximum PSC:TCHLA ratio of 1.043.

In neritic waters, phytoplankton composition tends to shift towards larger organisms with different pigment signatures. Differences in the ratios of certain accessory pigments to TCHLA, shown in Table 2, can be used to infer changes in the phytoplankton community structure. For example, during the BOFS cruise off Iceland in 1991, samples were collected within a major *Emiliania huxleyi* bloom (Holligan et al. 1993). This cruise had an average 19'-hexanoyloxyfucoxanthin:TCHLA ratio of 0.380. Other high latitude cruises such as SLC 86 and 87, GSP, and Icecolors had similarly high ratios, caused by another prymnesiophyte, *Phaeocystis*.

**Statistical analysis of slope differences**—Log-linear regression plots in Fig. 2 showed that there are significant differences in the relationships between total accessory pigments and TCHLA between cruises. Since these data cover a 13-yr period, during which methods, instruments and pigment standards have been changed and improved, these differences may be methodological and not caused by photoadaptation of the pigment pools to changes in the ambient conditions. These relationships were found to be very log-linear with little scatter within a cruise indicating that, again, the variability in slopes might be caused by cruise specific methodological differences. Nevertheless, although methodological uncertainties may undoubtedly influence the consistency of these log-linear relationships, the dynamic response of phytoplankton pigment pools to varying environmental conditions cannot be dismissed as a possible factor in the observed differences.

Two approaches were used to examine the cause of these slope differences. One was to perform an error analysis on the data assuming interlaboratory uncertainties, and the other was to search for other pigment data that were collected in a limited geographic area over a relatively short period of time and processed by a single laboratory.

An HPLC interlaboratory comparison was performed as part of the U.S. JGOFS intercalibration exercise (Latasa et al. 1996). They compared results between eight national and international laboratories, using shared pigment standards, and found that 90% of the Chl *a* determinations and 85% of the pooled pigments fell within 20% of the interlaboratory medians. This equates to standard deviations of 0.12 and 0.14 for Chl *a* and total accessory pigments, respectively, or an uncertainty in the slopes of accessory pigments:TCHLA

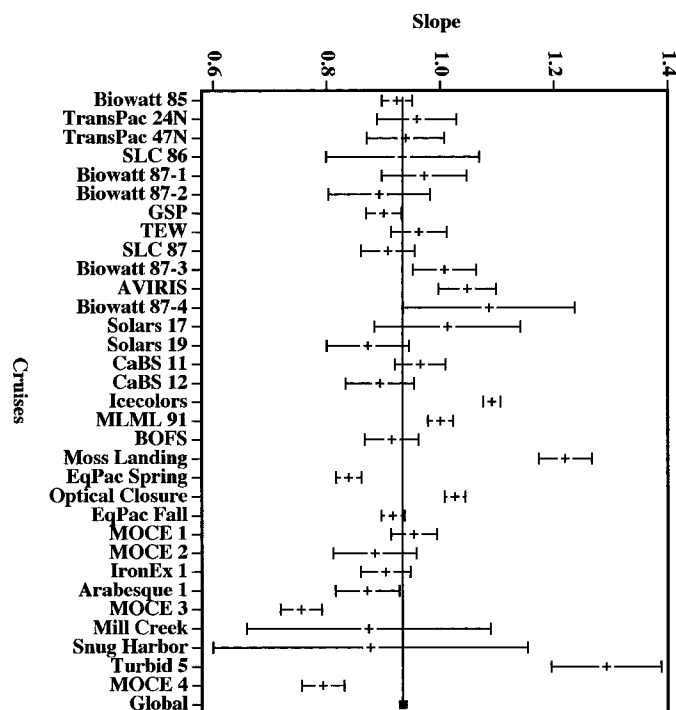


Fig. 4. Mean slopes for log accessory pigments:log TCHLA by cruise. The error bars are the 95% CL for the regression slopes. The line represents the average slope for the global value.

of 0.184 (quadrature sums). Since pigment standards of known concentration were provided to the laboratories, this exercise documented only the minimum interlaboratory variability. These types of relative chromatographic and instrumental calibration errors have no effect on the loglinear regression slopes, but will bias the intercepts. Constant biases such as those caused by low detection limits, poor peak separation, or column overloading will change the slopes. The measured slopes and 95% CL for each cruise were compared to the global mean in Fig. 4, showing that 59% of the cruises were not statistically different from the mean.

To investigate whether the differences between log accessory pigments:log TCHLA ratios were the result of changes in phytoplankton community structure and photoadaptation of the pigment pool to surrounding environmental conditions in case I waters, pigment data sets were analyzed which met the following criteria: (1) collected from a similar location, covering several seasons and, (2) processed in a relatively short period of time, using the same method and analyst. Three pigment databases were found, EqPac Spring and Fall cruises (R. Bidigare and is part of this analysis), U.S. JGOFS Arabian Sea Process cruises 045, 050, and 053 (Latasa and Bidigare 1998; March, August, and November 1995) and Atlantic Meridional Transect (AMT) cruises 2 and 3 (Gibb et al. 2000; April, and September 1996). The data were filtered using the same sample depth and accessory pigment number criteria as above and then plotted in log space. A comparison of the slopes showed that only the AMT cruises were not significantly different from each other, as well as two out of three of the Arabian Sea cruises. Log-linear slopes ranged from 0.839 to 0.993 for these seven cruises

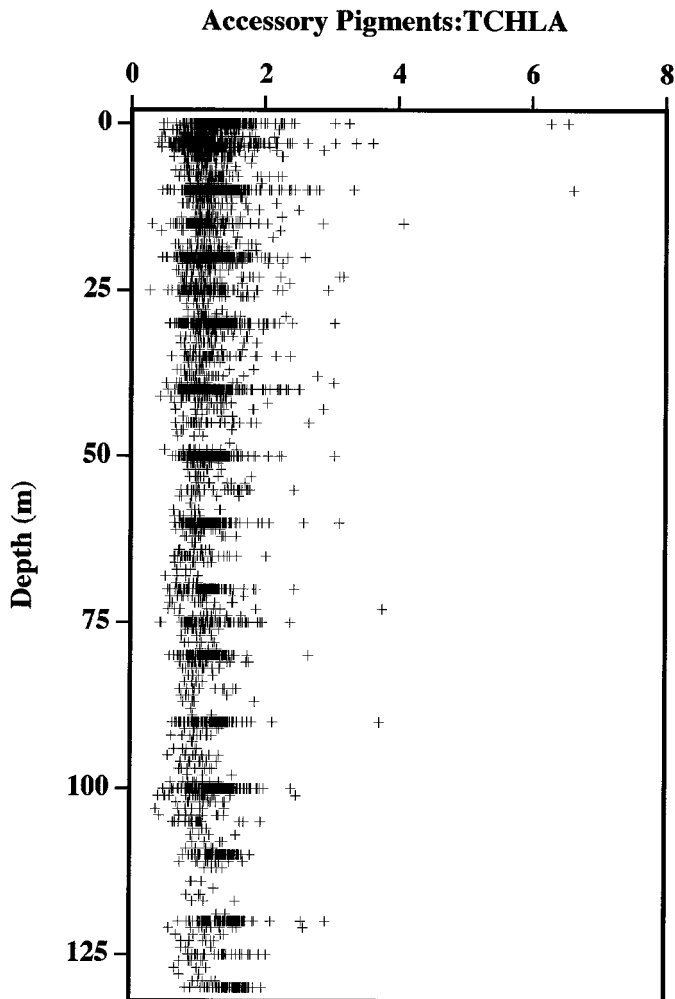


Fig. 5. The distribution, over depth, of variability in accessory pigment to TCHLA ratios for case I waters.

and bracketed the global mean. These results suggest that the phytoplankton pigment pool does respond to ambient conditions, although the changes are relatively small.

*“Photoadaptive effect”*—The consistent log-linear relationship between accessory pigments and TCHLA could be termed a “community photoadaptive effect”. As one pigment or group of pigments decrease in the water column in response to a changing light field or environmental conditions, others increase to fill this void. Such changes may represent either adaptive pigment alterations within a single species, or changes in community species composition. Some examples of the “community photoadaptive effect” are (1) the decrease in PPC with depth as PSC increases (*see fig. 5*, Bidigare et al. 1987), (2) the latitudinal change in surface waters from high PSC : PPC ratios in the polar regions to lower values towards the tropics (Gibb et al. 2000), and (3) the increase in Chl *b* with depth as Chl *c* decreases (Trees et al. 1986; Bidigare et al. 1990). The “community photoadaptive effect” is maintained throughout the water column as can be seen in Fig. 5, which is a plot of accessory pigment:TCHLA ratios as a function of depth for case I

waters. Regression analysis showed that the slope of this ratio over depth was not statistically different from zero, indicating that accessory pigments:TCHLA ratio does not have a vertical trend.

Another example of this “community photoadaptive effect” can be found in Bidigare et al. (1990) in which over a 2-week period, at the same location, a diatom bloom was replaced by a more diverse assemblage of prymnesiophytes, cyanobacteria, dinoflagellates, green algae, and diatoms. Chl *a* decreased by a factor of 2 and the accessory pigment to Chl *a* ratios for individual compounds showed factor of 3 changes. Since this cruise was part of the database, log-linear regressions were performed for the bloom and post bloom stations and were found not to be statistically different. Phytoplankton communities can drastically change pigment composition, yet log accessory pigments to log TCHLA ratios remain relatively constant.

*Effects of divinyl Chl a and phycobiliproteins*—As stated previously, this pigment database has not been corrected for the presence of divinyl Chl *a* or Chl *b*, which can cause errors if they are not separated either physically on the column, or by a channels ratio method (Latasa et al. 1996). Latasa et al. (1996) showed that the use of a single response factor (i.e., that determined only for monovinyl Chl *a*) could result in a 15–25% overestimation of total Chl *a* concentration if divinyl Chl *a* was present in significant concentrations (ratios from 0.2 to 0.4; divinyl Chl *a*:total Chl *a*). Elevated concentrations of divinyl Chl *a* and Chl *b* can be found in tropical and subtropical oceans where *Prochlorococcus* is found to be ubiquitous throughout the euphotic zone (Goericke and Repeta 1993; Gibb et al. 2000). If corrections were made for the contribution of divinyl Chl *a* to TCHLA, the slopes of many of these cruises would be reduced.

Cyanobacteria are the only marine phytoplankters that do not use Chl *a* as their primary light-harvesting pigment. They use phycobiliproteins instead, which gives them unique absorption properties in the blue-green portion of the spectral region. They have been found to make significant contributions to the total Chl *a* biomass, yet cyanobacteria have about three times lower Chl *a* concentrations per cell volume than other algae (Glover et al. 1987). The addition of phycobiliproteins to the accessory pigment pool would increase the slopes.

*Phaeopigment concentrations*—Phaeopigments, as measured by the standard fluorometric method, have been used in the past to infer grazing, senescent phytoplankton cells and detrital material. They have also been summed with Chl *a* for remote sensing algorithms. The HPLC measured ratios of Chl *a* degradation products for this large pigment database (phaeophytin *a* and phaeophorbide *a*) to TCHLA (Chl *a*, Chl *a* allomer, Chl *a* epimer, and chlorophyllide *a*) were generally low to below detection limits. Up until 1993, phaeopigment concentrations were not detected (ND, *see Table 3*) in samples, which could be related to the detection limits of absorption detectors used during this period. Even with this qualifier, the average ratio is significantly lower than values measured using the standard fluorometric method. Smith and Baker (1978) and others have found phaeopigment concen-

trations to be approximately 25% of the total Chl *a* concentration when using this technique. Even if chlorophyllide *a* concentrations are included in phaeopigment estimates, the average phaeopigment to Chl *a* ratio is only 0.037. This low contribution of phaeopigments to the total Chl *a* pool as measured by HPLC has also been found by Hallegraeff (1981), Everitt et al. (1990), and Bricaud et al. (1995). This global scale result emphasizes some of the problems associated with estimating phaeopigments using the standard fluorometric method.

## Conclusions

It can be shown that variations in light intensity and quality, as well as nutrients, can change the ratio of accessory pigments : TCHLA in a given phytoplankton specie. Secondly, phytoplankton community structure, and hence pigment ratios, adjust in response to changing environmental conditions. Yet, over a decade's accumulation of data in environments ranging from freshwater to marine, oligotrophic to eutrophic, and tropical to polar, the ratio of log accessory pigments : log TCHLA remains relatively constant (0.934). This is important for remote sensing purposes, since it provides the ability for estimating Chl *a* and total accessory pigment concentrations at wavelengths which minimize interferences caused by other in-water constituents (e.g., dissolved organic material absorption has little affect on pigment estimates from remotely sensed ocean color, if wavelengths above 470 nm are used in the algorithms).

Log-linear relationships were found between accessory pigments and TCHLA concentrations within the euphotic zone, and for the first optical depth, for a variety of oceanic and freshwater environments. Gieskes et al. (1988), Claustre (1994), Tester et al. (1995), and found linear relationships between Chl *a* and selected accessory pigments, although they used multiple regression analyses and limited the database to a single cruise. Many published articles have also shown this linear relationship, indirectly, in the form of tabulated accessory concentrations, which can be plotted as a function of Chl *a* (e.g., table 1, Hoepffner and Sathyendranath 1992; table 2, McManus and Ederington-Cantrell 1992)

Several studies have proposed that changes in the specific absorption coefficient ( $\text{m}^2 [\text{mg Chl } a]^{-1}$ ) occur from one, or both, of two factors: (1) increases in the cell size and intercellular pigment concentration, and (2) changes in pigment composition (Morel and Bricaud 1981; Sathyendranath et al. 1987). This database shows that accessory pigment composition as a function of TCHLA is relatively constant for either small cells (low TCHLA), or large cells (high TCHLA), and has little depth dependence (Fig. 5, case I waters only). Therefore, the "package effect", which flattens the absorption spectra of particles, is probably the result of an increase in total cellular pigments with cell size.

Pigment samples from this database were collected from environments ranging from oligotrophic waters with low nutrients (e.g., TransPacific 24N and 47N, MOCE 3 and 4) to eutrophic waters with elevated concentrations (e.g., AVIRIS, Moss Landing, Mill Creek, Snug Harbor). Besides sampling

these various water masses and their associated nutrient gradients, samples were also collected throughout the euphotic zone, always including the deep chlorophyll maximum and at least one or more depths below. There were only small differences between accessory pigments to TCHLA ratios for low or high nutrient water masses and no discernible vertically trend between low-nutrient surface waters and deeper samples within the nutricline (Fig. 5). This study suggests that nutrients probably have little affect on changing the ratios of accessory pigments to TCHLA, even though total intercellular concentrations may change.

Statistically significant differences in log-linear regression slopes were found between cruises and areas. Some of these changes can be attributed to phytoplankton communities adjusting their pigment composition to differing light conditions. It is also conceivable that part of these differences are methodological. Unfortunately, there is not enough information available on HPLC uncertainty budgets to perform this statistical analysis. On a basin to global scale, this relationship was still log-linear and provides a means for estimating log accessory pigment concentrations, within an RMS error of 28%, from log Chl *a* within the euphotic zone. As mentioned previously, this pigment database has not been corrected for errors caused by divinyl Chl *a*, which if present, would cause the slopes of these relationships to decrease slightly. Conversely, many of these slopes would increase if phycobiliprotein concentrations were routinely measured and included in the analysis. The success of relating optical properties to Chl *a* concentrations at wavelengths far removed from the Chl *a* absorption maximum is based on the log-linear relationship of accessory pigments to TCHLA and the associated "community photoadaptive effect".

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