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Detection of harmful algal blooms using photopigments and absorption signatures: A case study of the Florida red tide dinoflagellate, *Gymnodinium breve*

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Abstract

The utility of photopigments and absorption signatures to detect and enumerate the red tide dinoflagellate, *Gymnodinium breve,* was evaluated in laboratory cultures and in natural assemblages. The carotenoid, gyroxanthin-diester, was an adequate biomarker for *G. breve* biomass; water-column concentrations corresponded with cell standing crops and chlorophyll *a* concentrations during bloom events in Sarasota Bay, Florida. Unlike other carotenoids, the relative abundance of gyroxanthin-diester did not change throughout a range of physiological states in culture and the gyroxanthin-diester : chlorophyll *a* ratio exhibited little variability in a natural assemblage during bloom senescence. Stepwise discriminant analysis indicated that wavelengths indicative of in vivo absorption by accessory chlorophylls and carotenoids could correctly discern spectra of the fucoxanthin-containing *G. breve* from spectra of peridinin-containing dinoflagellates, a diatom, a haptophyte, and a prasinophyte. With the use of a similarity algorithm, the increasing contribution of *G. breve* was discerned in absorption spectra (and corresponding fourth-derivative plots) for hypothetical mixed assemblages. However, the absorption properties of chlorophyll *c*-containing algae vary little among taxa and it is difficult to discern the contribution of accessory chlorophylls and carotenoids caused by cell packaging. Therefore, the use of absorption spectra alone may not identify the contribution of a chlorophyll *c*-containing taxon to the composite spectrum of a mixed assemblage. This difficulty in distinguishing among spectra can be minimized by using the similarity algorithm in conjunction with fourth-derivative analysis.

Blooms of the toxic dinoflagellate, *Gymnodinium breve* Davis [=*Ptychodiscus brevis* (Davis) Steidinger], are a frequent occurrence offshore western Florida in the Gulf of Mexico. Although blooms generally develop and terminate offshore (Steidinger 1975), wind and currents can concentrate assemblages within nearshore waters (Steidinger and Ingle 1972). These episodic events contribute significantly to the pool of photosynthetically fixed carbon within the waters of the southwest Florida shelf (Vargo et al. 1987) and can result in large amounts of brevetoxins with their deleterious effects on commercial-aquacultural fisheries and public health (Steidinger et al. 1973; Riley et al. 1989; Shumway et al. 1990). Circulation patterns also transport cells along the western coast of Florida to the Florida Straits and then along the southeastern U.S. where subsequent blooms may

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occur (Murphy et al. 1975; Tester et al. 1991). However, despite the extensive knowledge concerning the biogeography of *G. breve,* the exact physical and(or) chemical conditions leading to the initiation and persistence of blooms are unknown (*see* Steidinger and Haddad 1981; Paerl 1988).

Toxic blooms of *G. breve* are generally detected by visual confirmation (water discoloration and fish kills), illness to shellfish consumers, and(or) human respiratory irritation (Carder and Steward 1985; Riley et al. 1989; Pierce et al. 1990) with actual toxicity verified through time-consuming chemical analyses for brevetoxins within shellfish samples (Schulman et al. 1990; cf. Trainor and Baden 1990) and mouse bioassays (McFarren et al. 1965). In a national plan identifying research and informational needs for biotoxins and harmful algae, Anderson et al. (1993, p. 15) noted that "... an easier and possibly more effective approach involves regular, routine sampling analysis of phytoplankton samples ..." and if potentially toxic taxa are discovered through microscopic analyses, "... then more expensive seafood testing must be done ...." Since the early 1970s, the state of Florida has operated an assessment program involving both microscopic enumeration of plankton samples and mouse bioassays of seafood samples (Hungerford and Wekell 1993).

Phytoplankton photopigments, chlorophyll *a* fluorescence, and in vivo absorption spectra have been used to characterize microalgal biomass, composition, and physiological state (e.g. Demers et al. 1991; Johnsen and Sakshaug 1993; Kroon et al. 1993; Johnsen et al. 1994*a,b*; Kroon 1994; Millie et al. 1995*a*; Tester et al. 1995). These analyses are complementary to microscopic analyses and are well suited for assessment programs because they facilitate rapid processing of large numbers of samples acquired over diverse spatial and temporal scales. Combining discrete point (profile) measurements with data acquired from satellite-air-borne sensors also offers the potential for synoptic characterization of harmful algal blooms. This multiplatform sampling perspective, if incorporated into assessment programs (e.g. Volent and Johnsen 1993), could provide an objective early-warning for alerting water quality managers to harmful algal bloom initiation, distribution, and transport.

The development of these approaches for an assessment program specific for *G. breve,* requires that certain questions need be addressed. What bio-optical properties are unique to *G. breve* which allow differentiation from other bloom-forming taxa? Can the current bio-optical analyses differentiate a *G. breve* bloom from other blooms? What is the potential for a coordinated multiplatform monitoring program to delineate the presence of *G. breve* blooms? As part of an ongoing project focused on the community ecology of harmful algae, we here address the utility of photopigments and absorption signatures for detecting *G. breve* and the potential incorporation of such measurements into a coastal monitoring program.

Methods

Field assemblages and laboratory cultures:—Blooms of *G. breve* were detected in Sarasota Bay, Florida, during fall 1994 and late spring-early summer 1995. Whole-water samples, for biomass and bio-optical analyses (*see below*), were collected midday from the surface and at 2-m depth at New Pass (27°20.04′N, 82°34.76′W) in the bay, on 20 and 29 September, 26 October, and 6 December during the 1994 bloom and three or four-times weekly from 20 April to 8 June during the 1995 bloom.

Growth and experimental conditions for laboratory cultures of *G. breve* (clone W53DB, housed at Mote Marine Laboratory) have been described elsewhere (*see* Millie et al. 1995*a*). Briefly, cells were grown in 2 liters of f/20 growth medium (Guillard and Ryther 1962) within 2.8-liter Pyrex Fernbach flasks at 25 ± 1°C. "Cool-white" and Vita-Lite fluorescent lamps provided the culture flasks with 60 μmol photons m^{-2} s^{-1} of photosynthetic active radiation (PAR) on a 12 : 12 L/D cycle. Exponentially growing triplicate cultures were then exposed to one of three irradiances (60 μmol photons m^{-2} s^{-1}, 165 μmol photons m^{-2} s^{-1} PAR, and 220 μmol photons m^{-2} s^{-1} PAR + UV irradiance) chosen to represent an irradiance gradient that cells might experience in a coastal water column. The experimental UV exposure (provided by a Phillips model F40UVB sun lamp) was ~5% of the solar, sea-surface UV irradiance measured midday in late spring at New Pass. UV-B radiation from the fluorescent lamps was attenuated by an acetate sheet for all flasks except those assigned to the greatest irradiance treatment. Four and eight days after exposure to irradiance treatments, culture aliquots were analyzed for cell abundance, lipophilic photopigments, and absorption (*see below*). Two replicate experiments were performed sequentially.

Biomass and bio-optical analyses:—For determination of cell abundance, cells in culture and whole-water samples were preserved immediately with Utermöhl's solution. Laboratory cultures were enumerated in a hemocytometer, whereas phytoplankton in whole-water samples were concentrated and enumerated microscopically in Sedgwick-Rafter counting chambers (Guillard 1973). For analyses of photopigments and in vivo optical density spectra, 50- or 100-ml aliquots of cultures and whole-water samples were passed under low vacuum (<75 mm of Hg) onto GF/F glass-fiber filters. Filters for pigments were immediately frozen and stored in darkness at −20°C until analysis. Filters for in vivo absorption measurements were immediately analyzed after filtration.

For photopigment analysis, frozen filters were placed in either 90 : 10 acetone : water (vol : vol) or 100% acetone, sonicated, and extracted in the dark at −20°C for at least 1 h. Filtered extracts (75–200 μl) were injected directly into a Hewlett-Packard model 1090 or Shimadzu model LC-600 high-performance liquid chromatograph (HPLC) equipped with an ODS-Hypersil C_{18} column (200×4.6 mm, 4-μm particle size) and a diode array detector set at 440 nm. Chromatography methods and pigment identification and quantification followed the procedures described by Millie et al. (1995*a*).

In vivo optical density spectra of *G. breve* cells collected on glass-fiber filters for treatment cultures were recorded with a Varian model DMS-80 UV-VIS spectrophotometer (*see* Cleveland and Weidemann 1993). To minimize the loss

of scattered light, we made filter holders to position the filters as close as possible to the detector. The optical density was zeroed at 750 nm for each sample. Before each sample scan, a glass-fiber filter, wetted with filtered culture media, was scanned to establish a blank baseline. This baseline was subsequently subtracted from all sample scans. In vivo optical density spectra were transformed to absorption spectra first by using a nonlinear pathlength amplification correction determined for *G. breve* (after Mitchell 1990; Cleveland and Weidmann 1993) and then normalizing to each respective spectral mean absorption (Roesler et al. 1989).

For comparison of absorption spectra of *G. breve* with that of other microalgae, whole-cell absorption spectra for the diatom *Chaetoceros gracile,* the peridinin-containing dinoflagellates *Prorocentrum minimum* and *Heterocapsa pygmaea,* the prasinophyte *Pyramimonas parkeae,* and the haptophyte *Emiliania huxleyi* were obtained from Schofield et al. (1990, 1996). These taxa were selected to represent the microalgal groups expected to comprise the majority of photosynthetic pigments in the water column of coastal western Florida (*see* Curl 1959; Saunders and Glenn 1969; Steidinger and Williams 1970; Gaarder and Hasle 1971). Spectra of taxa grown under several light conditions were chosen to include variability within a taxon's bio-optical characteristics representative of that which might occur at distinct depths in the water column. The absorption spectra for cell suspensions of *C. gracile, P. parkeae,* and *E. huxleyi* were obtained with standard cuvettes for an Aminco DW-2a spectrophotometer (Schofield et al. 1990) whereas the absorption spectra for cell suspensions of *P. minimum* and *H. pygmaea* were measured with an Aminco DW-2000 spectrophotometer equipped with an integrating sphere and a custom-built internal centrally located sample holder (Nelson and Prézelin 1993; Schofield et al. 1996).

Statistical analyses—The effects of irradiance on the change in relative abundances of chlorophyll and carotenoid fractions within the total chlorophyll and carotenoid pigments for days 4 and 8 from day 0 were compared by an analysis of variance (ANOVA). To increase the variance associated with bionomial proportion (percentage) data, changes in relative abundances were transformed to square-root arcsin for statistical analysis. If differences were detected by the ANOVA, the significance between pairs of means for each sampling day was determined by a least significant difference (LSD) analysis (Snedecor and Cochran 1980).

For field assemblages, the relationship between water-column gyroxanthin-diester concentrations and *G. breve* biomass (in terms of cell abundance and chlorophyll *a* concentrations) was analyzed by regression analysis (Snedecor and Cochran 1980). The near-total dominance of the 1994 bloom event by *G. breve* provided an opportunity to examine cell pigment content during bloom senescence. Variations in cell pigment contents as a function of bloom age were analyzed by ANOVA and LSD analyses. Trends for cell accessory chlorophyll and carotenoid contents as functions of cell chlorophyll *a* content were characterized by regression analysis and compared by an analysis of covariance (ANCOVA).

To determine whether the absorption spectra of *G. breve* could be differentiated from the spectra of other microalgae, we used stepwise discriminant analysis (Tabachnick and Fidell 1983) with mean-normalized spectra (cf. Johnsen et al. 1994*b*; Millie et al. 1995*a*). Absorption spectra were discriminated for every spectral wavelength between 440 and 650 nm. Wavelengths <440 nm and >650 nm were not used because information delineating portions of the spectra attributable to chlorophyll *a* absorption alone would not allow discrimination of taxa based on diagnostic pigments. Classification error rates, calculated by cross-validation and resubstitution techniques, were used to determine which set of wavelengths sufficiently differentiated spectra for *G. breve* from peridinin-containing dinoflagellates, *G. breve* from all taxa except peridinin-containing dinoflagellates, *G. breve* and peridinin-containing dinoflagellates from other taxa, and peridinin-containing dinoflagellates from other taxa except *G. breve.* These classification rates were obtained from a separate discriminant analysis using the wavelengths selected by stepwise analysis.

To determine whether the absorption spectra for *G. breve* could be differentiated from spectra of other taxa in mixed assemblages, we constructed hypothetical assemblages using combinations of mean-normalized spectra for *G. breve, C. gracile, H. pygmaea,* and *P. parkeae.* Initially, the concentration of the absorption spectra for a species was scaled between 0 and 1. This spectrum was summed with the scaled absorption spectra for the other algal species providing a hypothetical spectra (a_{total}) such that

$$a_{\text{total}} = \sum_{n=1}^{i} (a_1{}^*x_1) + (a_2{}^*x_2) \ldots (a_i{}^*x_i)$$

where $x_1 + x_2 \ldots x_i = 1$ and the mean-normalized absorption spectra for taxon i is denoted by $a_i{}^*$. Assemblages were generated for each aforementioned combination of taxa by varying values of x so that the contribution of *G. breve* to the total absorption spectra ranged from 0 to 100% in increments of 20% (Fig. 1A). The spectral fourth derivative (Butler and Hopkins 1970) was computed for each composite spectrum (Fig. 1B) to resolve the position of the absorption maxima attributable to photosynthetic pigments (*see* Bidigare et al. 1989; Smith and Alberte 1994; Millie et al. 1995*a*).

The degree of similarity between absorption and fourth-derivative spectra for *G. breve,* and composite absorption and fourth-derivative spectra for hypothetical mixed assemblages was computed with a similarity index (SI) algorithm (from Shimadzu Sci. Instr.).

The SI is the cosine of the angle between two vectors such that

$$\text{SI} = \frac{A_b \cdot A_c}{|A_b| \times |A_c|}$$

where A_b is the reference vector of absorption and fourth-derivative spectrum values $a_b(\lambda_1)$, $a_b(\lambda_2)$, $a_b(\lambda_2)$, . . . $a_b(\lambda_i)$ for *G. breve,* and similarly, A_c represent individual treatment vectors composed of the composite absorption or fourth-derivative spectra values for the mixed assemblages. The dot (·) operator is the vector dot-product and $|A|$ is the vector magnitude operator. In an attempt to im-

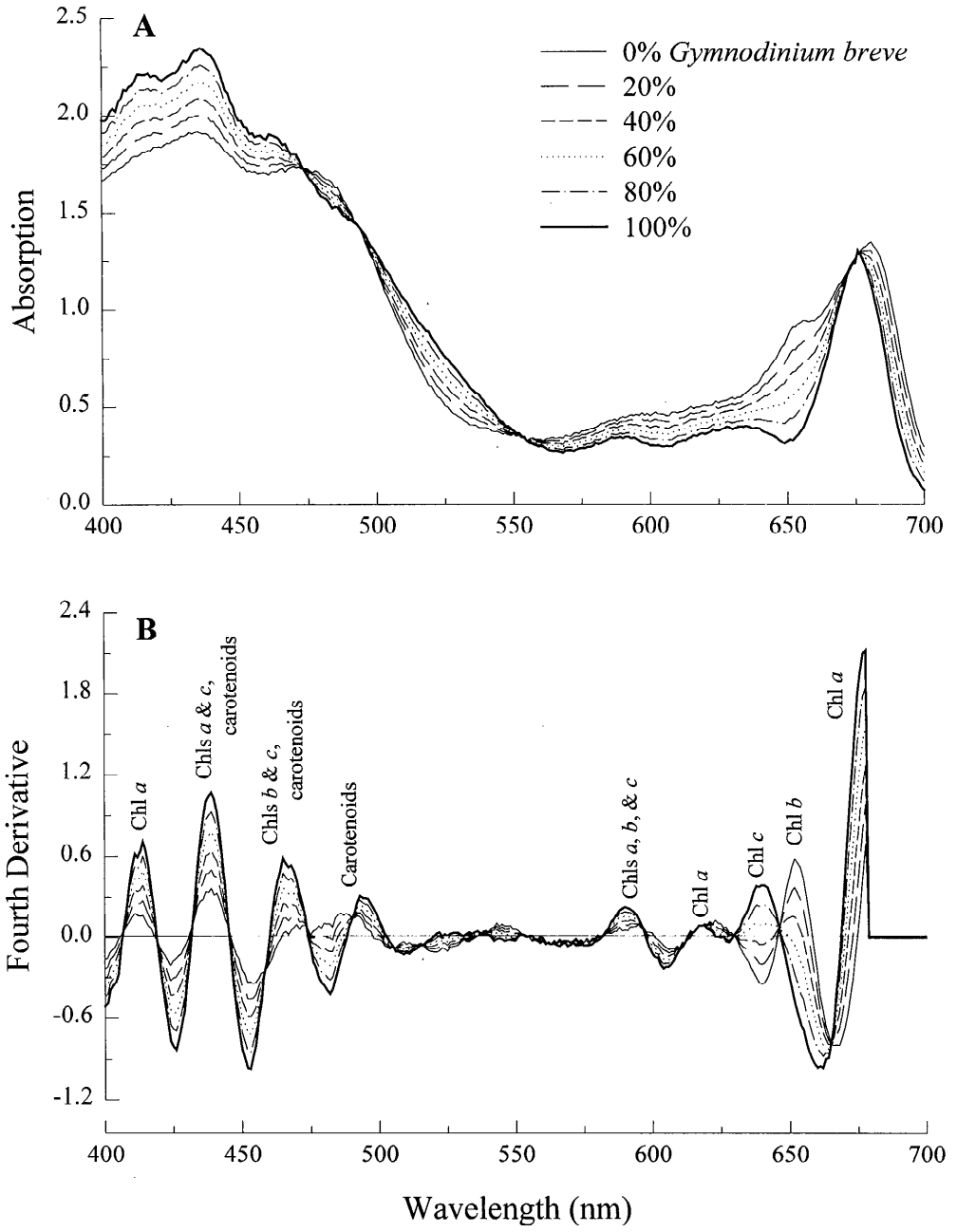

Fig. 1. Representative absorption spectra and corresponding spectral fourth-derivative plots used in the similarity comparisons (*see methods*). In this example, hypothetical assemblages were constructed using combinations of mean-normalized absorption spectra for *Gymnodinium breve* and the prasinophyte *Pyramimonas parkeae,* such that the contribution of *G. breve* to the total absorption spectra ranged from 0 to 100% in increments of 20%. A. Absorption spectra. B. Fourth-derivative plots of absorption spectra. The pigments responsible for the absorption maxima are indicated. Note the distinct presence of Chl *b* in the fourth-derivative plot of the absorption spectrum for 100% *P. parkeae* and Chl *c* in the plot for 100% *G. breve* (along with their associated decline or increase in plots for assemblages of intermediate proportions of these taxa).

prove the sensitivity and linearity of the SI, several transformations of absorption and fourth-derivative data were performed before computation (without transformations the SI is dominated by the general shape of the absorption spectra, namely large, broad peaks in the blue and red portions of the spectra). A normalized-ratio transform divided each data pair at every wavelength by the larger of the paired data. A logarithmic transform simply computed the natural logarithm of each datum. Also, the inverse cosine function, $1\text{-}[2\cdot\arccos(SI)]\ \pi^{-1}$, was used to remove the

nonlinearity of the cosine function and allow comparisons of the angles between vectors.

Results

The pigments of *G. breve* have been reported previously (Bjørnland and Liaaen-Jensen 1989; Bidigare et al. 1990; Millie et al. 1995*a*). However, because the intent of this study was to address the utility of using bio-optical parameters for detecting and characterizing harmful algal blooms, the photosynthetic pigments of *G. breve* need to be stressed. Pigments include the light-harvesting chlorophylls, *a*, $c_1 + c_2$, and c_3 and the light-harvesting carotenoids, 19′-butanoyloxyfucoxanthin, fucoxanthin, 19′-hexanoyloxyfucoxanthin, and β-carotene. The presence of chlorophylls c_1 and(or) c_2 in *G. breve* are reported as $c_1 + c_2$ because the HPLC method we used cannot differentiate these pigments (*see* Wright et al. 1991). Additionally, because baseline separations of 19′-butanoyloxyfucoxanthin, fucoxanthin, 19′-hexanoyloxyfucoxanthin were not consistently achieved with the HPLC solvent system used (*see* Millie et al. 1995*a*), these pigments hereafter are reported collectively as total fucoxanthins. Photoprotectant carotenoids include diadinoxanthin and diatoxanthin. Although not previously reported, diatoxanthin was observed only from field collections in which the sample filters were quick frozen in liquid nitrogen rather than freezing at −20°C. If quick freezing is not used in the sample preparation protocol, epoxidation can convert all diatoxanthin to diadinoxanthin (via the xanthophyll cycle; e.g. Demers et al. 1991; Brunet et al. 1993). Gyroxanthin-diester (19-decanoylgyroxanthin-3′-acetate), an acetylenic, allenic carotenoid (Bjørnland and Liaaen-Jensen 1989) whose light-harvesting and(or) photoprotectant functions are(is) unknown, also is present. Because β-carotene constituted an extremely minor component of the carotenoid fraction (~1%) and chlorophyll c_3 was not consistently quantified within natural assemblages, these pigments were not included in statistical analyses in this study.

The variations in pigment contents and pigment relative abundances in laboratory cultures of *G. breve* in response to 60 μmol photons m^{-2} s^{-1}, 165 μmol photons m^{-2} s^{-1} PAR, and 220 μmol photons m^{-2} s^{-1} PAR + UV irradiance treatments were reported previously (Millie et al. 1995*a*). Here, the effects of irradiance on the change in pigment relative abundances for days 4 and 8 from day 0 are reported to illustrate the degree of change in cellular pigmentation in irradiance-treated cultures relative to exponentially growing cultures kept at 60 μmol photons m^{-2} s^{-1} PAR with no UV irradiance. Changes in the relative abundances of pigments (from to day 0) varied among irradiance-treated populations (Fig. 2). A decrease in the relative abundance of total fucoxanthins and an increase in the relative abundance for diadinoxanthin coincided with increasing irradiance ($P \leq 0.02$). Specifically, the relative abundance of total fucoxanthins decreased and the relative abundance of diadinoxanthin increased dramatically in populations exposed to 165 μmol photons m^{-2} s^{-1} PAR and 220 μmol photons m^{-2} s^{-1} PAR + UV irradiance from that of populations exposed to 60 μmol photons m^{-2} s^{-1} PAR on both days 4 and 8. The relative abundances of chlorophylls *a* and $c_1 + c_2$ and gyroxanthin-diester did not change ($P > 0.05$) among irradiance-treated populations. This finding suggests that gyroxanthin-diester could be used to as an indicator for *G. breve* and potentially, its abundance in natural populations.

The fall 1994 bloom event was a near-mono-specific bloom of *G. breve* with cell abundance and chlorophyll *a* concentration reaching as great as 1.2×10^7 cells $liter^{-1}$ and 63.6 μg $liter^{-1}$, respectively. The large contribution of *G. breve* to the bloom was illustrated by the linear relationship ($P \leq 0.0001$, $r^2 = 0.63$) between water-column chlorophyll *a* concentration and *G. breve* cell abundance for the entire bloom event (data not shown). Sampling of this bloom event was initiated during the period of maximum *G. breve* biomass (20 September); thereafter biomass decreased steadily to an observed minimum of 2.0×10^6 cells $liter^{-1}$ and 5.0 μg Chl *a* $liter^{-1}$ on the final sampling day (6 December). In contrast, sampling of the spring-summer 1995 bloom was initiated during its early development and continued during progression of the bloom. During its early development, this bloom was a mixed assemblage comprised primarily of *G. breve*, diatoms, and peridinin-containing dinoflagellates. On the final sampling day, the bloom was near-mono-specific with *G. breve* biomass as great as 5.2×10^7 cells $liter^{-1}$ and 429.6 μg Chl *a* $liter^{-1}$.

Water-column gyroxanthin-diester concentration corresponded ($P \leq 0.0001$, $r^2 = 0.70$ to 0.99) with *G. breve* biomass, in terms of both cell standing crop and chlorophyll *a* concentration, during both the 1994 (Fig. 3A,B) and 1995 (Fig. 3C,D) blooms. The large values of biomass obtained by *G. breve* in the final samples of the 1995 bloom initially resulted in nonlinear plots. Pigment concentrations and standing crop values were logarithmically transformed to obtain linear plots. Most likely, the observed scatter in the plot of gyroxanthin-diester concentration vs. cell standing crop for the 1994 bloom (Fig. 3B) was due to the variability in values obtained through microscopic enumeration, whereas the scatter in the plot of gyroxanthin-diester concentration vs. chlorophyll *a* concentration for the 1995 bloom (Fig. 3C) was due to the occurrence of a significant portion of the chlorophyll *a* attributable to phytoplankton other than *G. breve* early in the bloom.

The 1994 bloom provided for comparisons among cell accessory pigments for distinct physiological states of *G. breve* (indicated by the approximate 4-fold change in cellular chlorophyll *a* content; Fig. 4). As the bloom senesced, cellular pigment contents exhibited decreasing trends ($P \leq 0.0001$; Fig. 4A). Cellular contents of chlorophyll $c_1 + c_2$, total fucoxanthins, diadinoxanthin, and gyroxanthin-diester corresponded ($P \leq 0.0001$) with the cellular content of chlorophyll *a*, with the degree of variability differing greatly among pigments (Fig. 4B). The relationships for cellular contents of total fucoxanthins and chlorophyll $c_1 + c_2$ as functions of the cellular content of chlorophyll *a* were distinct (as indicated by the ANCOVA comparing the slope of the trendlines) from similar relationships for chlorophyll $c_1 + c_2$, diadinoxanthin, and gyroxanthin-diester ($P \leq 0.0001$) and gyroxanthin-diester ($P \leq 0.0212$), respectively. The relationships for cellular chlorophyll $c_1 + c_2$ content and cellular diadinoxanthin content as functions of cellular chlorophyll *a* content were not distinct from similar relationships

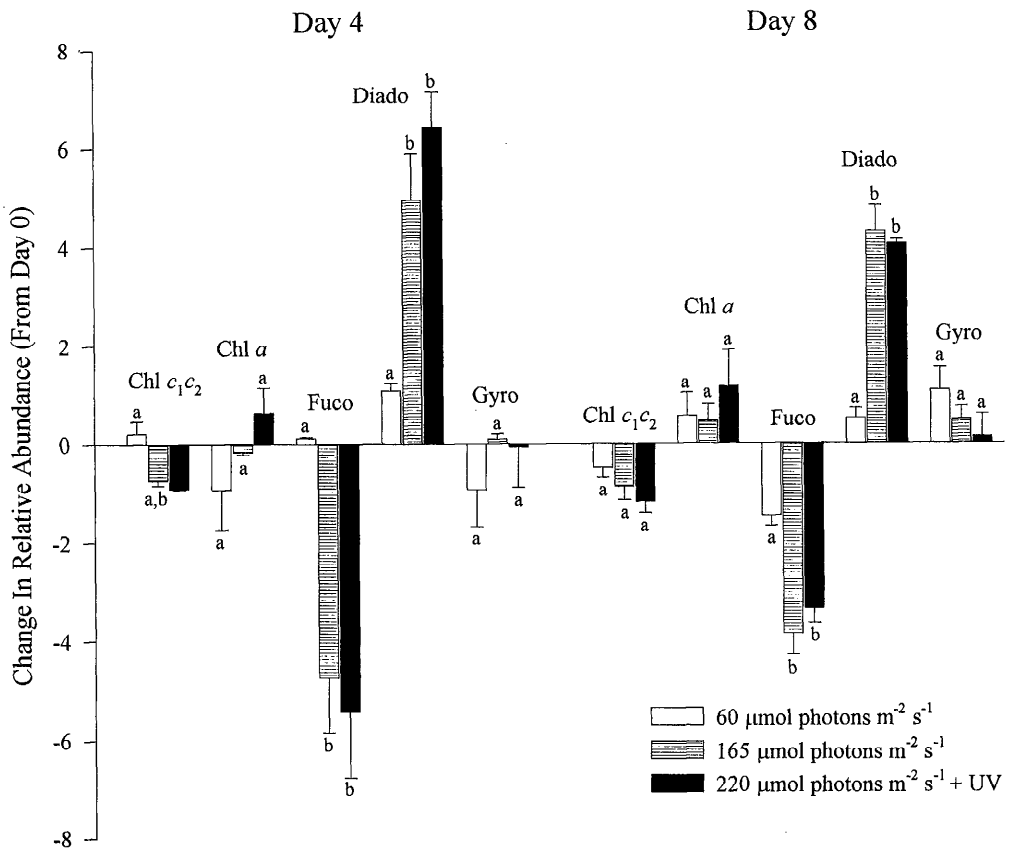

Fig. 2. Changes in pigment relative abundance for days 4 and 8 from day 0 after exposure of laboratory cultures of *Gymnodinium breve* maintained at 60 μmol photons m^{-2} s^{-1} photosynthetic active radiation (PAR) to 60 photons m^{-2} s^{-1} PAR, 165 μmol photons m^{-2} s^{-1} PAR, and 220 μmol photons m^{-2} s^{-1} PAR + UV irradiance treatments. Data are means ±1 SE (n = 2). Variable means within days 4 and 8 with different letters are significantly different ($P \leq 0.05$) as determined by a least significant difference analysis. Explanation of symbols: Chl—chlorophyll; Fuco—fucoxanthin + 19′-hexanoyloxyfucoxanthin + 19′-butanoyloxyfucoxanthin; Diado—diadinoxanthin; Gyro—gyroxanthin-diester.

for cellular diadinoxanthin content and cellular gyroxanthin-diester content, respectively ($P > 0.05$).

The stepwise discriminant analysis identified up to 15 wavelengths as optimal classifiers of absorption spectra among comparisons of *G. breve, C. gracile, P. minimum, H. pygmaea, P. parkeae,* and *E. huxleyi* (data not shown). However, depending upon the comparison, sets of 2–6 wavelengths, in the cumulative order of wavelength selection, fully optimized the classification of absorption spectra among taxa within all discriminations (Table 1). The remaining wavelengths chosen by the analysis could not improve on this optimization and, therefore, were not considered.

Twelve forms of the SI were evaluated for comparing the absorption spectrum of *G. breve* to that of hypothetical mixed assemblages. These different forms were chosen to exaggerate the subtle features of absorption spectrum curvature that are manifestations of the cellular pigment content and cell packaging characteristics. Plots of similarity values for nontransformed absorption spectra and the corresponding nontransformed fourth-derivative plots vs. the percent contribution of *G. breve* in the mixed assemblage resulted in nonlinear relationships ($r^2 > 0.98$; Fig. 5A,B). However, several forms of the SI resulted in linear plots ($r^2 > 0.99$; Fig. 5C,D); providing for greater distribution of the SI values and easier interpretation of data. The SI of the normalized-ratio transformed fourth-derivative spectra maximized the slope ($\bar{x}$ = 0.58, SD = 0.044) and the correlation coefficient (0.91) for this relationship in pooled composite spectra for the mixed assemblages.

Discussion

The taxonomic affinities among chlorophyll *c*-containing algae (in regard to the presence and absence of photosynthetic pigments) already have been described (*see* Jeffrey 1989; Bjørnland and Liaaen-Jensen 1989). The carotenoid peridinin is the primary light-harvesting pigment for most autotrophic dinoflagellates and is considered to be the diagnostic pigment marker for this group of organisms (Prézelin 1987; Millie et al. 1993). However, *G. breve* is a member of a small group of dinoflagellates having the carotenoids fucoxanthin and(or) 19′-acylofucoxanthins as the primary

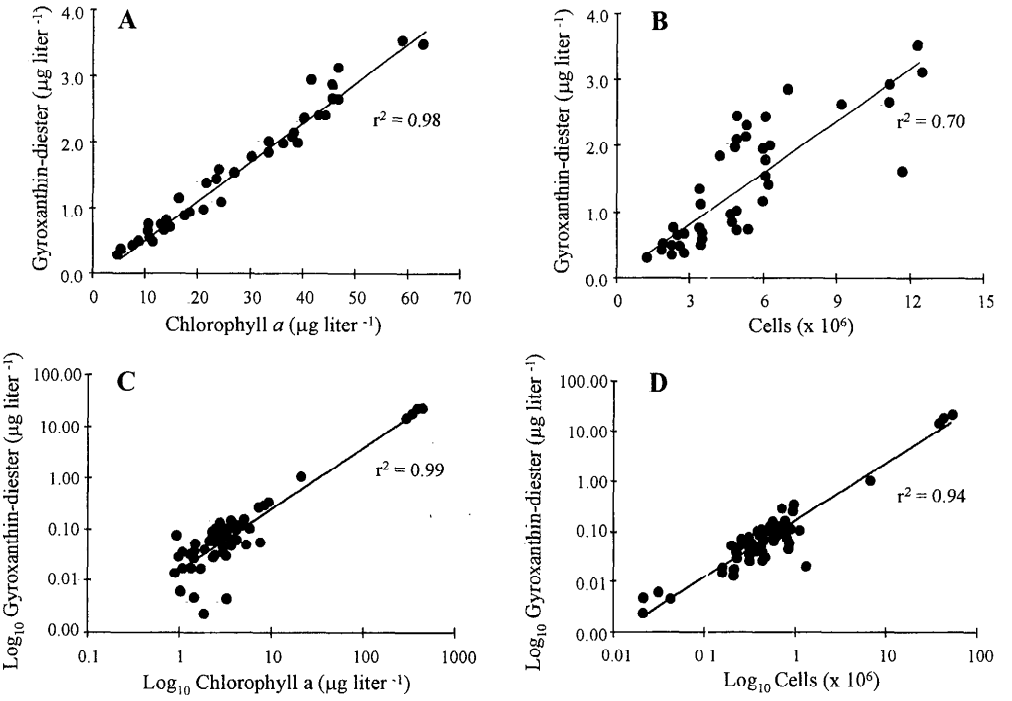

Fig. 3. Water-column gyroxanthin-diester concentrations as a function of *Gymnodinium breve* biomass during the 1994 and 1995 blooms in Sarasota Bay. Gyroxanthin-diester concentrations vs. chlorophyll *a* concentrations for the 1994 (A) and 1995 (C) bloom. Gyroxanthin-diester concentrations vs. cell standing crop for the 1994 (B) and 1995 (D) bloom. Trend lines, with associated determination coefficients, represent the best-fit relationships ($P \leq 0.0001$) as predicted by linear least-squares regression.

light-harvesting pigments (Table 2)—a condition providing for hypotheses concerning the phylogenetic evolution of these organisms through endosymbiosis of colorless flagellates with chrysophytes and(or) prymnesiophytes (*see* Dodge 1989; Whatley 1989). Both chlorophylls c_1 and c_2 are present in dinoflagellates having fucoxanthin and 19′-acylofucoxanthins, whereas only chlorophyll c_2 is present in dinoflagellates having peridinin (Jeffrey 1989). Chlorophyll c_3 is present in the fucoxanthin-containing dinoflagellates, including *G. breve* (Table 2).

The utility of the carotenoid gyroxanthin-diester as a potential indicator for the presence of *G. breve* in coastal waters of Florida waters is noteworthy. Water-column gyroxanthin-diester concentrations corresponded with *G. breve* standing crop (both cells numbers and total chlorophyll *a*) during the 1994 and 1995 blooms, adequately tracking bloom formation and senescence. In order for a photopigment to be a diagnostic indicator of an organism, it must be unique to that organism (or small group of closely related organisms), be stable throughout various physiological

Table 1. Wavelengths (nm) derived from stepwise discriminant analysis allowing optimal classification of (1) absorption spectra for *Gymnodinium breve* ($n = 43$) from spectra for those of the peridinin-containing dinoflagellates *Prorocentrum minimum*, $n = 3$, and *Heterocapsa pygmaea*, $n = 3$; (2) spectra for *G. breve* from those of the diatom *Chaetoceros gracile*, $n = 3$, the prasinophyte *Pyramimonas parkeae*, $n = 3$, and the haptophyte *Emiliania huxleyi*, $n = 3$; (3) spectra for *G. breve* and peridinin-containing dinoflagellates from those of other taxa; and (4) spectra from peridinin-containing dinoflagellates from those of taxa except *G. breve*. Numbers in parentheses represent the percent number successful classifications of spectra within the respective group.

Cum. No. of wavelengths	1	2	3	4
1	541(100/83)	440(82/78)	548(95/73)	544(83/67)
2	462(100/100)	632(88/89)	483(100/87)	561(100/78)
3		464(92/89)	650(98/87)	512(100/100)
4		534(98/100)	632(100/100)	
5		454(98/89)		
6		442(100/100)		

states, and have chromatographic-absorption characteristics providing for its recognition from other pigments (Millie et al. 1995*a*).

Gyroxanthin-diester also has been observed in the other toxic, fucoxanthin-containing dinoflagellates, *Gyrodinium aureolum* Hulburt, and *Gymnodinium galatheanum* Braarud (*see Table 2*). Of these dinoflagellates, only *G. breve* can be considered a warm-water taxon and is known to inhabit the coastal waters of Florida. Interestingly, Bjørnland et al. (unpubl.) also noted gyroxanthin-diester to comprise an extremely minor (~1%) of the chloroplast pigmentation in the pelagophyte *Pelagomonas calceolata* Andersen et Saunders (*see* Andersen et al. 1993), further fueling the endosymbiotic-based evolution hypotheses concerning these dinoflagellates (*see above*). However, the presence, distribution, and relative abundance of this organism in the coastal waters of western Florida is virtually unknown; to our knowledge, *P. calceolata* has never been reported in Florida coastal waters. This, along with the high cell abundance of *G. breve* during the 1994 and 1995 blooms and the extremely large, positive correlation (up to $r = 0.99$) of gyroxanthin-diester concentration with *G. breve* cell abundance indicates that *P. calceolata* was a not contributing factor to the pigment concentrations observed in Sarasota Bay.

Gyroxanthin-diester was a relatively consistent component of the photopigments throughout the various photophysiological states exhibited by *G. breve*; gyroxanthin-diester comprised 3.1–3.8% and 6.8–8.1% of the total photopigments and the carotenoid fraction, respectively, within the laboratory culture populations and 2.0–3.5% and 4.9–10.8% of the total photopigments and the carotenoid fraction, respectively, within natural assemblages (*see Figs. 2, 4*). Finally, the chromatographic and absorption characteristics of this carotenoid were distinct (*see* Fiksdahl 1983; Millie et al. 1995*a*), providing for its easy and definitive recognition in HPLC-derived pigment chromatograms.

Stepwise discriminant analysis indicated that mean-normalized absorption spectra for laboratory cultures of *G. breve* could be differentiated from spectra for cultures of a diatom, a prasinophyte, and peridinin-containing dinoflagellates. Interestingly, most of the discriminating wavelengths identified were from regions of the visible spectrum associated with in vivo absorption attributable to accessory chlorophylls *b*, 463–470 and 650–654 nm, and *c*, 460–470, 586, and 635–644 nm, and most carotenoids, 470–490 nm (Bidigare et al. 1989, 1990; Hoepffner and Sathyendranath 1991; Johnsen et al. 1994*b*). The observation that spectra for fucoxanthin-containing taxa could be distinguished from spectra for nonfucoxanthin containing taxa by using (in part) wavelengths indicative of in vivo absorption attributable to this carotenoid, ~530–550 nm, was particularly intriguing (e.g. note the selection of wavelengths used in differentiating spectra for *G. breve* from peridinin-containing dinoflagellates and spectra for all dinoflagellates from other taxa). Johnsen et al. (1994*b*), using stepwise discriminant analysis to classify absorption spectra among 31 bloom-forming phytoplankton [representing the four main groups of phytoplankton with respect to accessory chlorophylls; i.e. chlorophyll *b*, chlorophyll c_1 and(or) c_2, chlorophyll c_3, and no accessory chlorophyll] differentiated the potentially toxic

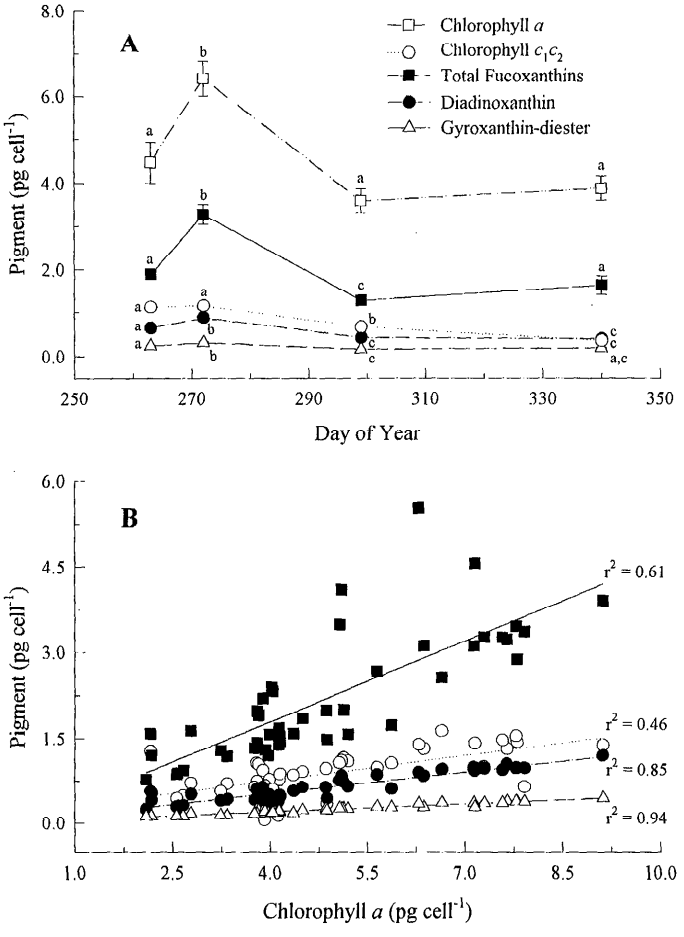

Fig. 4. Cell pigment contents for *Gymnodinium breve* during the fall 1994 bloom in Sarasota Bay. A. Cell chlorophyll and carotenoid contents vs. sampling date. Data are means ±1 SE, n = 8–17. Variable means among days with different letters are significantly different ($P \leq 0.05$) as determined by a least significant difference analysis. B. Cell accessory chlorophyll and carotenoid contents vs. cell chlorophyll *a* content. Trend lines, with associated determination coefficients, represent the best-fit relationships ($P \leq 0.0001$) as predicted by linear least-squares regression.

chlorophyll c_3-containing dinoflagellates and prymnesiophytes (e.g. Table 2) from taxa not having this pigment. However, problematic and toxic taxa could not be further separated from other chlorophyll c_3-containing taxa because of the similarities among absorption spectra.

From this, it appears that accessory chlorophylls and carotenoid pigments sometimes may provide enough information to differentiate among absorption spectra associated with microalgal phylogenetic groups, and potentially taxa. It must be remembered, however, that relative to the absorption within the blue and red regions of the visible spectrum, absorption within the green, yellow, and orange regions is minor. Consequently, absorption attributable to accessory pigments often is difficult to quantify and routinely discern, especially given cell packaging (*see* Garver et al. 1994). In addition, the spectral dependency in the absorption properties of marine chlorophyll *c*-containing algae exhibits little variability among taxa (Roesler et al. 1989; Johnsen et al.

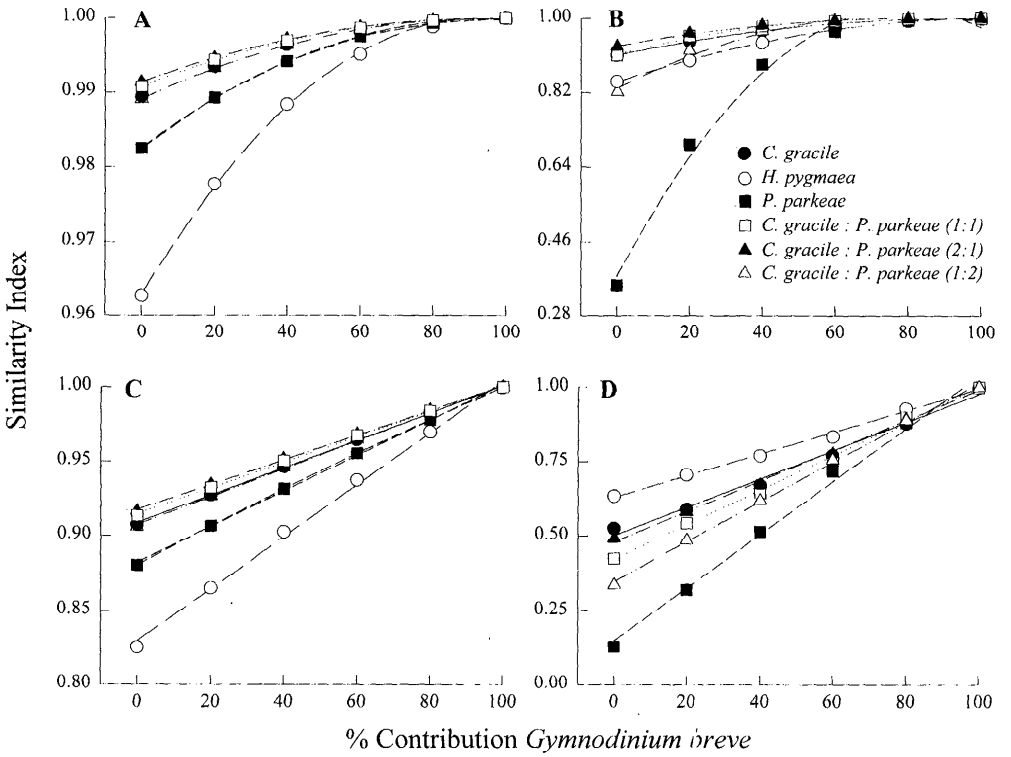

Fig. 5. Similarity index (SI) values of absorption spectra and corresponding fourth-derivative plots for comparisons between *Gymnodinium breve* and hypothetical mixed assemblages. Assemblages were constructed using combinations of mean-normalized spectra for *G. breve, Chaetoceros gracile, Heterocapsa pygmaea,* and *Pyramimonas parkeae* within laboratory cultures such that the contribution of *G. breve* to the total spectra ranged from 0 to 100% in increments of 20% (*see methods*). A. Similarity between nontransformed absorption spectra (i.e. the SI value is the cosine of the angle between two composite spectra). B. Similarity between nontransformed fourth-derivative plots of the absorption spectra. C. Similarity between normalized-ratio transformed absorption spectra. D. Inverse cosine-modified similarity between normalized-ratio transformed fourth-derivative plots of absorption spectra. Trend lines represent the best-fit relationships ($P \leq 0.0001$) as predicted by linear and polynomial least-squares regression, $r^2 > 0.98$.

1994*b*; Garver et al. 1994) with absorption occurring primarily in the blue and red regions of the visible spectrum. Undoubtedly, these characteristics of phytoplankton absorption resulted in the consistently large values of the similarity index in comparisons of spectra for mixed phytoplankton assemblages, thereby suggesting that absorption spectra alone may not effectively provide the means to detect the presence of specific chlorophyll *c*-containing taxa in mixed field assemblages.

The fourth-derivative analysis discerned absorption maxima of pigments within the overall absorption spectra. Because this analysis identified portions of the absorption spectra having the greatest curvature, peaks within the derivative spectra did not necessarily correspond with large amounts of pigment. As such, fourth-derivative analysis was used to delineate differences among spectra which were not readily observable. Using the SI in conjunction with fourth-derivative plots effectively characterized the contribution of *G. breve* to the overall absorption spectra, thereby allowing the detection of differences among mixed hypothetical assemblages. Consequently, derivative spectra may provide an effective means of interpreting absorption spectra from unknown species assemblages (i.e. when compared to cataloged fourth-derivative spectra measured on laboratory cultures). Therefore, this approach circumvents some of the aforementioned difficulties associated with the analysis of absorption spectra and may be a particularly powerful tool in local or regional studies where a limited number of species contributes significantly to the phytoplankton assemblage (i.e. regions plagued with extensive blooms of toxic phytoplankton).

The purported global epidemic of harmful algal blooms (Hallegraeff 1993) has necessitated accurate characterizations of blooms over extremely variable scales, particularly in regard to event-scale processes and physical and(or) chemical forcing events affecting their proliferation and persistence. Biological monitoring programs largely have relied on microscopic-based cell enumerations and gross measurements of a biochemical parameter (e.g. chlorophyll *a* concentration) for characterizing microalgae and estimating biomass. However, such analyses do not always provide the most timely, informative, and accurate standing crop measurements; time-consuming microscopic enumeration does not provide physiological information, whereas biochemical

Table 2. Pigment markers from globally important toxic dinoflagellates and prymnesiophytes. Explanation of symbols: Chl (chlorophyll), perid (peridinin), fuco (fucoxanthin), 19′HOF and 19′BOF (19′-hexanoyl- and butanoyloxy-fucoxanthin), diado (diadinoxanthin), diato (diatoxanthin), dino (dinoxanthin), gyro (gyroxanthin-diester), β-car (β-carotene, inclusive of β, γ, and ε cyclic ring structures).

Taxon	Pigments	References*
	Chl c_3- & fuco-containing dinoflagellates	
Gymnodinium breve	Chls $a/c_2/c_3$, fuco, 19′BOF, 19′HOF, diado, diato, gyro, β-car	1, 3, 5, 12
Gymnodinium galatheanum	Chls $a/c_2/c_3$, fuco, 19′BOF, 19′HOF, diado, diato, gyro, β-car	1, 8
Gyrodinium aureolum	Chls $a/c_2/c_3$, fuco, 19′HOF, diado, diato, gyro, β-car	1, 2, 8
	Chl c_2- & perid-containing dinoflagellates	
Alexandrium minutum†	Chls a/c_2, perid, diado, diato, β-car	10, 13
Dinophysis acuminata†	Chls a/c_2, perid, diado, diato, β-car	13
Gamberdiscus toxicus	Chls a/c_2, perid, diado, diato, dino, β-car	14
Heterocapsa pygmaea†	Chls a/c_2, perid, diado, diato, β-car	3
Prorocentrum minimum	Chls a/c_2, perid, diado, diato, β-car	9
	Chl c_3- & fuco-containing prymnesiophytes	
Chrysochromulina polylepsis†	Chls $a/c_2/c_3$, fuco, 19′HOF, diado, diato, β-car	6, 7, 11
Prymnesium parvum†	Chls a/c_1 & c_2/c_3, fuco, 19′BOF, 19′HOF, diado, diato, β-car	4, 8, 11, 13
Phaeocystis cf. *pouchetii*†	Chls $a/c_2/c_3$, fuco and(or) 19′BOF/19′HOF, diado, diato, β-car	3, 8, 11

* 1—Bjørnland and Tangen 1979; 2—Tangen and Bjørnland 1981; 3—Bjørnland and Liaaen-Jensen 1989; 4—Fawley 1989; 5—Bidigare et al. 1990; 6—Bjerking et al. 1990; 7—Johnsen et al. 1992; 8—Johnsen and Sakshaug 1993; 9—Johnsen et al. 1994*b*; 10—Flynn et al. 1994; 11—Jeffrey and Wright 1994; 12—Millie et al. 1995*a*; 13—Johnsen unpubl. data; 14—Millie unpubl. data.

† Denotes that two or more potential toxic species included in each genus; i.e. *Prorocentrum* [*lima, balticum* (which contains fuco instead of perid, but no Chl c_3), *micans*], *Alexandrium* (*excavatum, ostenfeldii*), *Dinophysis* (*acuta, norvegica, rotundata*), *Chrysochromulina leadbeaterii, Prymnesium patelliferum, Phaeocystis* cf. *globosa.*

parameters may vary dramatically among taxa and their physiological states. Phytoplankton pigment and absorption analyses can help overcome these informational deficiencies and easily could be incorporated into coastal monitoring programs (*see* Gieskes 1991; Millie et al. 1993, 1995*b*), thereby providing an effective and alternative means for monitoring *G. breve.* In addition, remote-sensing technology (in situ transmissometers, multispectral sensors) has become a popular means to map phytoplankton, primarily due to their synoptic sampling capabilities. However, remote sensing of phytoplankton is based on models whose predictive limits are defined by the accuracy of the ground truthing of phytoplankton biomass (Millie et al. 1995*c*). Pigment and absorption data (such as those presented here) can confirm, or "truth," remote-sensing imagery while providing useful information for developing models of phytoplankton absorption and production (e.g. Bricaud et al. 1995; Cleveland 1995).

The presence of gyroxanthin-diester provided for delineation of *G. breve* from other taxa within phytoplankton assemblages in Sarasota Bay. In addition, the high correlation of this carotenoid with *G. breve* cell abundance allowed tracking of bloom development and senescence. However, because gyroxanthin-diester provides only a minor contribution to the cellular absorption (Johnsen and Sakshaug 1993) and has absorption maxima similar to those of other carotenoids and chlorophyll *c* (Fiksdahl 1983; Millie et al. 1995*a*), its presence does not dramatically alter the absorption spectrum (nor that of a fourth-derivative plot of the spectrum) of a mixed assemblage. Consequently, delineation of this pigment through remote-sensing technology (e.g. in situ moorings and airborne-satellite sensors) is not feasible. Recent technological advances in computer-based instrumentation has stimulated the increased usage of bio-optical methodologies for potentially detecting and characterizating harmful phytoplankton (*see* Cullen et al. 1997). Nevertheless, the cautionary note that absorption characteristics among chlorophyll *c*-containing algae are relatively similar and that differences are difficult to discern must be raised. This difficulty in distinguishing among absorption spectra may be minimized by using the similarity index algorithm in conjunction with fourth-derivative analysis. Additionally, as the spectral resolution of submersible spectral absorption meters and remote-sensing instrumentation improves, this technique may provide an effective means to interpret field data. Undoubtedly, the development of such approaches will assist in expanding the capabilities for identifying and characterizing harmful blooms of *G. breve.*

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