

QUANTIFICATION OF THE RELATIVE ABUNDANCE OF THE TOXIC DINOFLAGELLATE, *KARENIA BREVIS* (DINOPHYTA), USING UNIQUE PHOTOPIGMENTS¹

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Diagnostic photopigment analysis is a useful tool for determining the presence and relative abundance of algal groups in natural phytoplankton assemblages. This approach is especially useful when a genus has a unique photopigment composition. The toxic dinoflagellate *Karenia brevis* (Davis) G. Hansen & Moestrup comb. nov. shares the diagnostic pigment gyroxanthin-diester with only a few other dinoflagellates and lacks peridinin, one of the major diagnostic pigments of most dinoflagellate species. In this study, measurements of gyroxanthin-diester and other diagnostic pigments of *K. brevis* were incorporated into the initial pigment ratio matrix of the chemical taxonomy program (CHEMTAX) to resolve the relative contribution of *K. brevis* biomass in mixed estuarine phytoplankton assemblages from Florida and Galveston Bay, Texas. The phytoplankton community composition of the bloom in Galveston Bay was calculated based on cell enumerations and biovolumetric measurements in addition to chl *a*-specific photopigment estimates of biomass (HPLC and CHEMTAX). The CHEMTAX and biovolume estimates of the phytoplankton community structure were not significantly different and suggest that the HPLC–CHEMTAX approach provides reasonable estimates of *K. brevis* biomass in natural assemblages. The gyroxanthin-diester content per cell of *K. brevis* from Galveston Bay was significantly higher than in *K. brevis* collected from the west coast of Florida. This pigment-based approach provides a useful tool for resolving spatiotemporal distributions of phytoplankton in the presence of *K. brevis* blooms, when an appropriate initial ratio matrix is applied.

Key index words: CHEMTAX; Galveston Bay; HPLC; *Karenia brevis*; photopigment biomarkers; red tide

Abbreviations: CHEMTAX, chemical taxonomy

The toxic dinoflagellate, *Karenia brevis* (Davis) G. Hansen & Moestrup comb. nov. (formerly known as *Gymnodinium breve* [Davis]) (Daugbjerg et al. 2000), has a wide distribution within the Gulf of Mexico. This species occurs in low (background) concentrations of less than 10 to 100 cells·L⁻¹ throughout the Gulf (Geesey and Tester 1993, Kusek et al. 1999), but extensive blooms mainly occur in three coastal regions: off the West Florida shelf, Texas, and the Yucatan peninsula (Tester and Steidinger 1997). Blooms on the West Florida shelf are almost a yearly occurrence, and 70% of the blooms occur in the autumn months (Tester and Steidinger 1997). The distribution of *K. brevis* is mainly determined by salinity and temperature (Steidinger et al. 1998, Kusek et al. 1999).

The frequency, magnitude, and time of *K. brevis* blooms in Texas waters have not been well documented. Consequently, the extent and breadth of knowledge on *K. brevis* physiology and bloom dynamics from Texas waters is very limited compared with that in Florida. However, Tester and Steidinger (1997) proposed that bloom initiation off the coast of Texas is initiated by the same sequence of events as off the western coast of Florida. Two cases of *K. brevis*-associated fish kills have been reported for Texas coastal waters (Wilson and Ray 1956, Trebatoski 1988), but the causative species are uncertain (Harper and Guillen 1989, Zimmerman 1998). Although *K. brevis* blooms are infrequently observed in Texas, the economic consequences associated with fish kills, shellfish closures, and public health and fisheries for recreational beaches are substantial and of growing concern (ECOHAB 1995, Zimmerman 1998). Increased understanding of the bloom initiation, transport, and spatial coverage of *K. brevis* is an essential component in the development of an early warning and tracking system for the fisheries in coastal and estuarine ecosystems of Texas.

Diagnostic photopigment analysis is a useful and reliable tool for determining the presence and relative abundance of algal groups in mixed species assemblages (Mantoura and Llewellyn 1983, Wright et al. 1991, Jeffrey et al. 1999, Wright and van den Enden 2000) and community succession across broad spatial and temporal scales (Wilhelm et al. 1991, Letelier et al. 1993, Tester et al. 1995, Pinckney et al. 1998). One of

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the main advantages of the photopigment approach is the automated rapid sample analysis and quantification of natural phytoplankton samples compared with tedious microscopic identification and enumeration (Millie et al. 1993). The pigment-based approach also provides more consistent results among individual research teams than conventional microscopy methods (Schlüter et al. 2000).

The phytoplankton community composition can be resolved from photopigment profiles using CHEMTAX (chemical taxonomy) (Mackey et al. 1997). This algorithm partitions the total biomass (chl *a*) of phytoplankton groups in a sample by comparing the pigment composition of the sample with the diagnostic pigment composition of predetermined phytoplankton groups. The complexity of the community is constrained by the number of phytoplankton groups initially characterized by the researcher (i.e. included in the initial pigment ratio matrix used for comparison in community composition analysis). Therefore, it is valuable to have insight into the phytoplankton community of the ecosystem for which the initial pigment ratio matrix is to be applied (Wright et al. 1996). CHEMTAX has been used to quantify the phytoplankton community composition of oceanic and estuarine waters (Mackey et al. 1998, Pinckney et al. 1998, Higgins and Mackey 2000, Schlüter et al. 2000, Wright and van den Enden 2000), providing reliable information on large scales and long-term distributions of phytoplankton groups.

A more specific determination of phytoplankton community structure is possible when a single species or few species share a unique diagnostic photopigment. This provides the opportunity to address species-specific questions based on the presence/absence as well as the relative concentration of the diagnostic pigment in environmental samples. Most autotrophic dinoflagellates contain the carotenoid peridinin as their primary light-harvesting pigment (Jeffrey et al. 1975, Millie et al. 1993). *Karenia brevis*, however, belongs to a group of dinoflagellates that do not have peridinin but instead have fucoxanthin and 19'-acylofucoxanthins as primary light-harvesting pigments (Jeffrey et al. 1975, Bjørnland and Liaaen-Jensen 1989, Johnsen and Sakshaug 1993). A few gymnodinoid species, in addition to *K. brevis*, also have the carotenoid gyroxanthin-diester as an accessory photopigment (Johnsen and Sakshaug 1993, Hansen et al. 2000). Gyroxanthin-diester, which has been observed in only a few toxic dinoflagellates, is uncommon and provides a relatively unique biomarker for *K. brevis* (Millie et al. 1995). The chemical structure and biosynthesis of gyroxanthin-diester was recently described by Bjørnland et al. (2000). In the Gulf of Mexico, the only known gyroxanthin-containing dinoflagellates are *K. brevis* and *K. mikimotoi*. Although *K. brevis* and *K. mikimotoi* cannot be differentiated based on photopigment characteristics, both species are relatively easy to identify based on cell morphology. When gyroxanthin is detected, samples should be examined using qualitative microscopy to determine the species present.

The usefulness of gyroxanthin-diester as a proxy for *K. brevis* abundance may be defined by its relationship to chl *a*. Millie et al. (1995) demonstrated a consistent gyroxanthin-diester/chl *a* ratio in *K. brevis* cultures grown at various irradiances and inferred that gyroxanthin-diester could be used as a diagnostic pigment for *K. brevis* in Florida waters. Later, Millie et al. (1997) demonstrated that gyroxanthin-diester concentrations corresponded to cell counts and chl *a* concentrations during a *K. brevis* bloom in Florida, allowing the quantification of *K. brevis* abundance based on gyroxanthin-diester pigment concentrations from the field.

The purpose of our work was to incorporate gyroxanthin-diester as a diagnostic photopigment in CHEMTAX to resolve the relative contribution of *K. brevis* biomass in mixed estuarine phytoplankton assemblages from Galveston Bay, Texas and the west coast of Florida.

MATERIALS AND METHODS

Culture material. Cultures of *K. brevis* (Wilson clone) were grown in f/2 media using Gulf Stream water adjusted to 30 psu at 23° C on a 14:10-h light:dark cycle. Illumination was from a bank of Sylvania daylight 20-W fluorescent bulbs (Danvers, MA, USA), providing a quantum scalar irradiance of 100 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ as measured with a LiCor 4 π sensor (QSL-100, Biospherical Instruments, Inc., San Diego, CA, USA). Cultures were shipped by overnight carrier from Beaufort, North Carolina to College Station, Texas. Immediately upon arrival the cultures were stored at room temperature for 24 h before filtration under low light conditions. Replicate (six) aliquots of the culture were filtered under low vacuum (80 mm Hg) onto 25-mm Whatman GF/F filters (Whatman International Ltd., Maidstone, UK). The filters were folded and gently pressed between absorbent paper towels to remove excess water and were immediately frozen at -80° C until analyzed.

Natural samples. Water samples containing *K. brevis* were obtained from Florida and Galveston Bay, Texas. The samples from Florida were collected in September 1999 along the panhandle coast. Samples (500–1000 mL) were filtered onto 47-mm Whatman GF/F filters, placed in small cryo-vials, and immediately frozen in liquid nitrogen. The samples were shipped to our facility at Texas A&M University (TAMU) on dry ice and stored at -80° C until analyzed. In Galveston Bay, samples were collected from the uppermost meter of the water column using an integrated water sampler. Samples were gently poured into acid-cleaned 10-L carboys and transported in an insulated cooler (chilled with ice) to the laboratory at TAMU at Galveston. Aliquots (75–150 mL) were filtered onto 25-mm GF/F filters under low vacuum, immediately frozen on dry ice, and stored at -80° C until analyzed.

Enumeration. Subsamples of water collected from Galveston Bay were fixed for phytoplankton identification and enumeration. Aliquots (48 mL) of each sample were fixed with 2 mL of 25% glutaraldehyde (Booth 1993) and stored at 5° C until analyzed. Phytoplankton cells were concentrated by settling 5 mL of the sample in a settling chamber for 24 h after which the overlying water was discarded (Utermöhl 1958). Phytoplankton and cyanobacteria were enumerated in 20 fields of view at three magnifications: 200 \times , 400 \times , and 1000 \times , counting at least 200 cells. Cells larger than 20 μm , cells 2–20 μm , and cells smaller than 2 μm in diameter were counted at each magnification, respectively. Phytoplankton cells were identified to genus level when possible. Cell dimensions and species identifications were determined simultaneously for each organism and their biovolume estimated based on the assigned shape of each organism (Hillebrand et al. 1999).

HPLC methods. The GF/F filters containing phytoplankton were extracted in 100% acetone (0.75 or 1.0 mL for 25- and 47-mm filters, respectively) and were sonicated and stored at -20° C for

15–20 h. Filtered extracts were spiked with 1 M ammonium acetate ion-pairing solution (final concentration 0.2 M), and 375 µL of sample was injected into a Shimadzu HPLC (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) equipped with a single monomeric (Rainin Microsorb-MV, 0.46 × 10 cm, 3 µm) (Varian Instruments, Walnut Creek, CA, USA) and one polymeric (Vydac 201TP, 0.46 × 25 cm, 5 µm) (Grace-Vydac, Hesperia, CA, USA) reverse-phase C18 column in series. A nonlinear binary gradient was used for pigment separation (Pinckney et al. 1996). Solvent A consisted of 80% methanol:20% ammonium acetate (0.5 M adjusted to pH 7.2) and solvent B was composed of 80% methanol:20% acetone. Absorption spectra and chromatograms (440 nm) were acquired using a Shimadzu SPD-M 10 av photodiode array detector (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). Pigment peaks were identified by comparison of retention times and absorption spectra with pure crystalline standards of chl *a* and *b*, β-carotene (Sigma Chemical Company, St. Lewis, MO, USA), fucoxanthin, lutein, canthaxanthin, zeaxanthin (Hoffman-LaRoche, Basel, Switzerland), and gyroxanthin-diester (The International Agency for ¹⁴C Determination, Hørsholm, Demark). Other pigments were identified by comparison to extracts from phytoplankton cultures (Wright et al. 1991). Photopigment concentrations were quantified using chromatogram peak area and the appropriate extinction coefficients (Rowan 1989, Jeffrey et al. 1997).

CHEMTAX. CHEMTAX is a matrix factorization routine for calculating algal class abundances based on the concentrations of diagnostic chl and carotenoid photopigments (Mackey et al. 1996, Wright et al. 1996, Pinckney et al. 1998). The complexity of the estimated community structure depends on the number of phytoplankton groups defined *a priori* by the researcher. We took a conservative approach in defining the initial ratio matrix, restricting our resolution to *K. brevis*, diatoms, chlorophytes, cyanobacteria, cryptophytes, dinoflagellates, and two groups of haptophytes (hapto3s and hapto4s). Hapto3s and hapto4s are distinguished by the diagnostic pigments 19'-hexanoyloxyfucoxanthin and 19'-butanoyloxyfucoxanthin, respectively (Jeffrey and Wright 1994). These eight categories, which are commonly detected in estuarine waters (Tester et al. 1995, Roy et al. 1996), represent the major groups present in our estuarine samples.

The input ratio matrix was compiled from literature values and our own estimates of pigment composition of *K. brevis* culture (Mackey et al. 1996). The eight functional groups of phytoplankton described above were defined in the initial ratio matrix based on 10 diagnostic pigments (Table 1A). For *K. brevis* the diagnostic pigment concentrations were determined for two culture batches of *K. brevis*, and the absolute concentra-

tions were normalized to chl *a* and added to the input ratio matrix (Table 1A). Other functional groups were characterized by adding values obtained from the literature (Mackey et al. 1996) to our initial ratio matrix. We took a similar approach as Wright and van den Enden (2000) and applied the maximum ratio values reported from Mackey et al. (1996), except for cyanobacteria. Zeaxanthin, the diagnostic pigment of cyanobacteria, was characterized based on the maximum ratios of zeaxanthin reported for *Synechococcus* and *Trichodesmium* (Mackey et al. 1996); thus, the zeaxanthin/chl *a* ratio is the average of the maximum value of the two groups.

Statistics. The relationship between cell abundance and gyroxanthin-diester concentration was analyzed using standard linear regression procedures. The null hypothesis of no significant difference between biovolume and CHEMTAX estimates of phytoplankton community composition was tested nonparametrically, using Wilcoxon's signed rank test (Sokal and Rohlf 1981).

RESULTS

Karenia brevis chromatograms. Diagnostic HPLC pigment profiles of *K. brevis* and the characteristic absorption spectra of gyroxanthin-diester were obtained from cultures of *K. brevis* (Fig. 1). The chromatograms demonstrated good separation between the diagnostic pigments, including gyroxanthin-diester. Furthermore, the characteristic pigment profile of *K. brevis* formed distinctive peaks in *K. brevis* bloom containing water from Galveston Bay (Fig. 2). The absorption spectra of the gyroxanthin-diester showed two pigment peaks, one at 444 nm and the other at 469 nm, compared with 446 and 468 nm maxima reported in Millie et al. (1995). The minor shift in the pigment maxima probably reflects differences in the calibration of our photodiode array used for the spectra rather than pigment-associated properties. The concentrations of gyroxanthin-diester showed a strong positive linear relationship with *K. brevis* cell abundance ($r^2 = 0.999$, $P < 0.01$). The absorption spectra and pigment retention time (25.6 min) of gyroxanthin-diester from the culture was used to resolve the presence of gyroxanthin-diester

TABLE 1. Pigment ratio matrices for CHEMTAX. (A) The initial ratio matrix used for CHEMTAX. (B) The final output pigment ratio matrix results from the best fit of CHEMTAX to Galveston Bay photopigment data set.

Algal class	Perid	But-fuco	Fuco	Hex-fuco	Diadino	Allo	Lut	Zea	Chl <i>b</i>	Gyro	Chl <i>a</i>
A											
<i>Karenia brevis</i>	—	0.2070	0.3073	0.1352	0.3513	—	—	—	—	0.0555	1.00
Diatoms	—	—	0.7554	—	0.1400	—	—	—	—	—	1.00
Cyanobacteria	—	—	—	—	—	—	—	0.3820	—	—	1.00
Chlorophytes	—	—	—	—	—	—	0.2030	0.0090	0.2632	—	1.00
Dinoflagellates	1.0630	—	—	—	—	—	—	—	—	—	1.00
Hapto3s	—	—	—	1.700	0.1400	—	—	—	—	—	1.00
Hapto4s	—	0.2500	0.5800	0.5400	0.1200	—	—	—	—	—	1.00
Cryptophytes	—	—	—	—	—	0.2292	—	—	—	—	1.00
B											
<i>K. brevis</i>	—	0.1007	0.1494	0.0657	0.1708	—	—	—	—	0.0270	—
Diatoms	—	—	0.3010	—	0.1218	—	—	—	—	—	—
Cyanobacteria	—	—	—	—	—	—	—	0.6316	—	—	—
Chlorophytes	—	—	—	—	—	—	0.0450	0.0066	0.2129	—	—
Dinoflagellates	0.5153	—	—	—	—	—	—	—	—	—	—
Hapto3s	—	—	—	0.5986	0.0493	—	—	—	—	—	—
Hapto4s	—	0.1004	0.2329	0.2169	0.0482	—	—	—	—	—	—
Cryptophytes	—	—	—	—	—	0.3921	—	—	—	—	—

Pigment ratios are normalized to a chl *a* value of 1. The key to pigment abbreviations is provided in the legends to Figures 1 and 2, except perid, peridinin and lut, lutein.

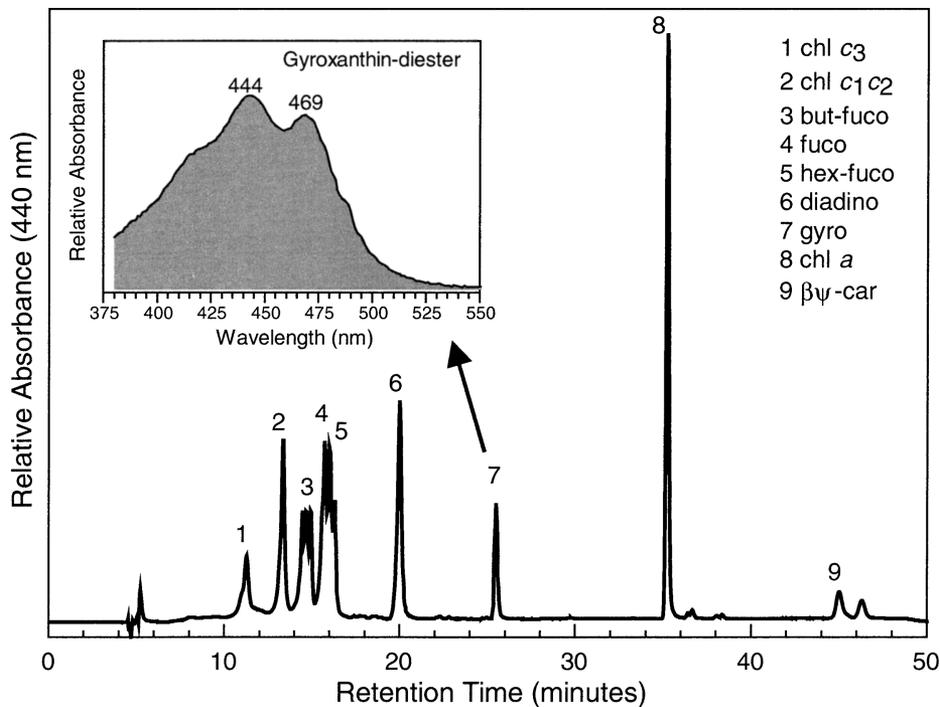


FIG. 1. HPLC chromatogram for the *Karenia brevis* culture. The retention time of gyroxanthin-diester allows for good spatial separation and quantification. The pigments detected in *K. brevis* are chl c_3 , chl $c_1 c_2$, butanoyloxyfucoxanthin (but-fuco), fucoxanthin (fuco), hexanoyloxyfucoxanthin (hex-fuco), diadinoxanthin (diadino), gyroxanthin-diester (gyro), chl a , and $\beta\psi$ -carotenoid ($\beta\psi$ -car). Inset: The absorption spectra of gyroxanthin-diester. All three peaks below three were identified as but-fuco, fuco formed one peak, whereas hex-fuco formed two peaks.

in field samples. Furthermore, the pigment composition of cultured *K. brevis* was used to determine the pigment to chl a ratio for the diagnostic pigments: gyroxanthin-diester, 19-butanoyloxyfucoxanthin, fucoxanthin, 19-hexanoyloxyfucoxanthin, and diadinoxanthin. These five pigments and their ratios in *K. brevis* cultures were used in the initial input ratio matrix to quantify the abundance of *K. brevis* in field samples using CHEMTAX (Table 1).

The linear relationship derived from the regression between gyroxanthin-diester concentration and cell abundance in *K. brevis* cultures was used to estimate a theoretical detection limit for *K. brevis*. Using our standard phytoplankton HPLC protocol (750 μL acetone for extraction and 375 μL injection volume) and a minimum peak area of 10,000 units (Shimadzu SPD-M10a PDA), we estimate that the lower limit of detection for our system is approximately 3300 cells per extraction. Using this relationship, we further estimated the amount of natural water that one would need to filter to detect *K. brevis* abundances at low cell concentrations. For an average cell count of 5000 cells $\cdot\text{L}^{-1}$, we must filter 0.655 L of seawater to reliably detect *K. brevis*. The 5000 cell $\cdot\text{L}^{-1}$ natural abundance is of particular interest because this is the critical value for oyster and other shellfish-harvesting closures (National Shellfish Sanitation Program, US Food & Drug Administration).

CHEMTAX algorithm. The initial ratio matrix (Table 1) was tested using phytoplankton pigment data from the estuarine waters of Galveston Bay, Texas (Fig. 3) by comparing *K. brevis* biomass estimates to the initial gyroxanthin-diester concentration of each sample. HPLC pigment profiles obtained during a bi-weekly sampling effort from May 1999 through De-

cember 2000 at seven locations were subjected to the analysis. Some of the samples were known to contain gyroxanthin-diester, whereas most of the samples were gyroxanthin-diester free. Phytoplankton community composition obtained from CHEMTAX using the initial ratio matrix in Table 1A reflected the pigment composition of the samples illustrating the presence of high biomass of *K. brevis* in samples from September 4 and 18 (Table 2). In gyroxanthin-diester free samples, no biomass (chl a) was contributed to *K. brevis* (Table 2 data from August 18 and October 14). Therefore, the composition of the phytoplankton community obtained using CHEMTAX was not different from biovolume estimates (Wilcoxon signed rank test, $P > 0.05$). When the diagnostic pigment profile of *K. brevis* was not included in the initial ratio matrix, the chl a specific biomass was incorrectly attributed to diatoms, hapt3s and hapt4s (Table 2) based on commonalities in diagnostic pigment composition of these groups (Table 1A). The *K. brevis* bloom was not attributed to the dinoflagellate group because *K. brevis* lacks peridinin, a primary diagnostic pigment of most autotrophic dinoflagellates.

When running the algorithm, CHEMTAX was allowed to vary the initial pigment ratio matrix by as much as 500% to minimize the differences between the ratio matrix and the data set. Comparison of the initial ratio matrix and the final ratio matrix calculated by CHEMTAX (Table 1B) demonstrated that most pigment ratios decreased by half, whereas others changed many-fold. The greatest changes in the ratios were apparent in zeaxanthin of cyanobacteria, alloxanthin of cryptophytes, and lutein of chlorophytes. The pigment/chl a ratio increased almost 2-fold for

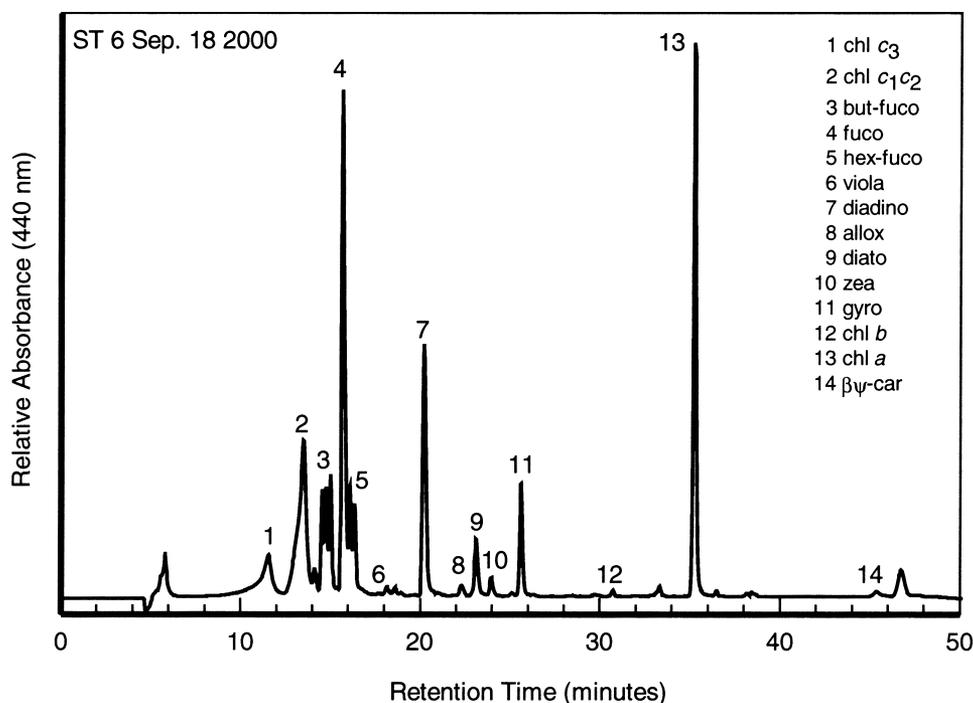


FIG. 2. HPLC chromatogram for *Karenia brevis* containing sample from station 6 (see Fig. 3) on 18 September 2000. The retention time of gyroxanthin-diester allows good spatial separation and quantification from field samples. Besides pigments characteristic of *K. brevis*, the field sample contained violaxanthin (viola), alloxanthin (allo), diatoxanthin (diato), zeaxanthin (zea), and chl *b*. See legend to Figure 1 for other abbreviations.

alloxanthin and zeaxanthin, whereas it decreased 4-fold for lutein.

Galveston Bay bloom. A *K. brevis* bloom was detected at the mouth of Galveston Bay in early September 2000 during the biweekly sampling program carried out by the Estuarine Ecology Lab (TAMU). Two weeks later, the bloom was denser and had intruded into the lower and center portion of the bay (Fig. 3). *Karenia brevis* cell abundance in mid-September ranged from 614 to 30,901 cells·mL⁻¹, with the highest concentration observed in a sample taken from the surface in a patch of discolored water (Table 3). The high cell abundance was reflected in large peaks of gyroxanthin-diester and elevated phytoplankton biomass (chl *a*) detected by HPLC. The total phytoplankton biomass in the bloom ranged from approximately 22 to 28 μg chl *a*·L⁻¹ with an exceptionally high value of 370.7 μg chl *a*·L⁻¹ in the discolored water. In October the *K. brevis* bloom was not detected, and the phytoplankton biomass in the lower bay ranged from 5 to 13 μg chl *a*·L⁻¹, comparable with previously measured biomass in the area (Örnólfsson 2002).

CHEMTAX versus cell biovolume estimates. The phytoplankton community, determined by CHEMTAX, was dominated by diatoms (58%) and *K. brevis* (17%) on September 4 in Galveston Bay (Fig. 4). Two weeks later *K. brevis* (70.5%) was the dominant fraction of the biomass (chl *a*), and other algal groups formed a minor component (Fig. 4). Biovolumetric measurements of the same samples illustrated comparable

trends in phytoplankton community structure. Diatoms (66%) and *K. brevis* (23%) accounted for the largest fraction of the biomass in early September (Fig. 4), but the community was dominated by *K. brevis* (69%) by mid-September (Fig. 4). The phytoplankton community composition estimates obtained from the two independent measures (CHEMTAX versus biovolume) were not significantly different (Wilcoxon signed ranks test, $n = 24$, $P > 0.05$).

Gyroxanthin-diester content of Texas versus Florida blooms. Gyroxanthin-diester content per cell of *K. brevis* was significantly different among *K. brevis* from Galveston Bay and Florida ($n = 51$, $df = 2$, $P < 0.05$). Post-hoc analysis of variance (SPSS, Dunnett T3, SPSS Inc., Chicago, IL, USA) showed that the gyroxanthin-diester content of *K. brevis* cells from Galveston Bay were significantly higher than gyroxanthin content of *K. brevis* detected from the west coast of Florida in 1994–1995 (Millie et al. 1997) and 1999 (samples from Dr. Tester analyzed by our laboratory) (Dunnett T3, $P < 0.05$) (Fig. 5). However, the gyroxanthin-diester content of the two populations of *K. brevis* from west Florida were not significantly different from one another (Dunnett T3, $P > 0.05$).

DISCUSSION

This work demonstrates the applicability and advantages of HPLC and CHEMTAX approaches to assess phytoplankton community composition at ecosystem scales and emphasizes the potential for screening large numbers of samples for the presence of *K. brevis*

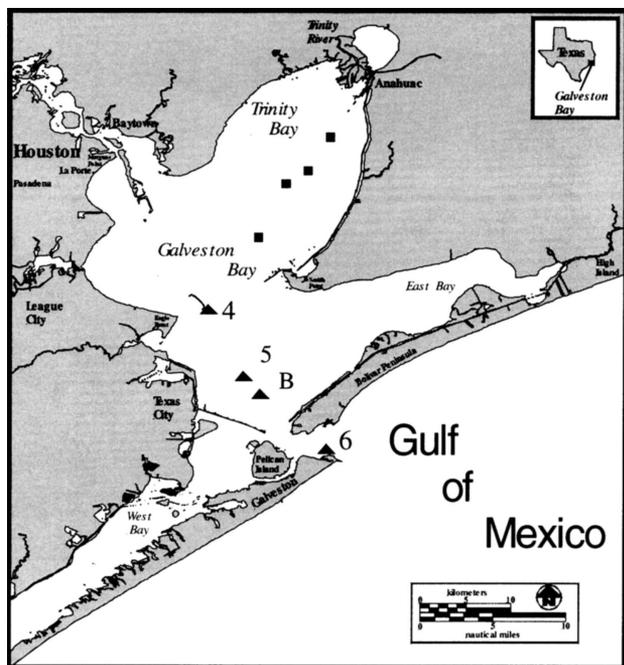


FIG. 3. Sampling locations within Galveston Bay, Texas. Sampling locations are characterized by filled squares and triangles. *Karenia brevis* was detected at locations identified by filled triangles (4, 5, and 6, and from a dense bloom [B]).

or other phytoplankton species of unique pigment composition. Our HPLC protocol provides quantitative estimates of gyroxanthin-diester concentrations. This relationship is defined by a strong positive correlation between concentration of the detected pigment and the number of cells analyzed. In addition to the firm relationship established for the culture, our limited number of field observations also indicates the existence of a positive correlation between cell abundance and the gyroxanthin-diester concentration in natural samples. Our findings are in agreement with the work of Millie et al. (1995, 1997) and Kirkpatrick et al. (2000), demonstrating that gyroxanthin-diester concentrations are positively correlated with *K. brevis* cell abundance detected from the field. Because of the

apparent linearity and the quantitative measure of *K. brevis* abundance, pigment-based monitoring for *K. brevis* offers an additional tool for tracking spatiotemporal dynamics of the organism.

Calculations of the potential limits of detection for *K. brevis* abundance reveal that the method outlined in this study is capable of detecting natural cell abundances at densities less than $5 \text{ cells} \cdot \text{mL}^{-1}$. However, this level of sensitivity requires the filtration of nearly 1 L of natural water using standard phytoplankton filtration and analysis protocols (see Materials and Methods). In estuarine waters, the filtration of more than 1 L of water through the standard glass fiber filters used for phytoplankton analyses (Whatman GF/F) is impractical for most routine applications. Alternative filtration methods, which use less-selective glass fiber filters or fine (approximately $10 \mu\text{m}$ mesh) Nitex mesh, may allow the filtration of the large volumes of water necessary for detection of *K. brevis* in natural samples.

Karenia brevis cells from Galveston Bay contained higher concentrations of gyroxanthin-diester per cell, 0.78 ± 0.30 , than has been reported from samples from the west coast of Florida, 0.26 ± 0.10 pg gyroxanthin-diester. The photopigment concentration of *K. brevis* has been observed to decrease with increasing irradiance (Millie et al. 1995). Furthermore, Millie et al. (1995) demonstrated that the relative proportion of chl remained constant. However, the relative contribution of fucoxanthin, gyroxanthin-diester, and $\beta\psi$ -carotene to the carotenoid pool decreased with increased irradiance at the same time as diadinoxanthin contribution to the carotenoid pool increased. Therefore, the higher concentration of gyroxanthin-diester per cell in Texas *K. brevis* bloom may be a photoacclimation response to the more turbid estuarine waters of Galveston Bay. Photoprotection mechanisms, using the xanthophyll cycle, have been observed in natural and culture populations of *K. brevis* (Evens et al. 2001). Because cell enumerations are dependent on the mathematical relationship between gyroxanthin concentrations and cell abundance, photoacclimation responses to different light environments need to be more carefully explored. However, Millie et al. (1997), working with *K. brevis* cultures incubated under a

TABLE 2. Phytoplankton biomass ($\mu\text{g chl } a \cdot \text{L}^{-1}$) at station 6 in Galveston Bay before, during, and after the *Karenia brevis* bloom.

Phytoplankton group	Biomass ($\mu\text{g chl } a \cdot \text{L}^{-1}$) estimated from CHEMTAX							
	Including <i>Karenia brevis</i>				Excluding <i>Karenia brevis</i>			
	18 Aug	4 Sep	18 Sep	14 Oct	18 Aug	4 Sep	18 Sep	14 Oct
<i>K. brevis</i>	0.00	2.25	19.94	0.00	0.00	0.00	0.00	0.00
Chlorophytes	0.90	1.51	1.55	0.68	0.90	1.46	1.35	0.70
Cryptophytes	0.37	0.89	0.94	0.78	0.35	0.86	0.70	0.76
Cyanobacteria	0.16	0.33	0.29	0.04	0.16	0.36	0.20	0.05
Dinoflagellates	0.09	0.15	0.38	0.14	0.08	0.15	0.37	0.14
Hapto3+4	0.06	0.25	0.23	0.03	0.06	1.81	16.26	0.03
Diatoms	2.65	7.51	4.98	2.60	2.67	8.29	9.42	2.60

Phytoplankton community composition and abundance was estimated using two CHEMTAX analyses, which included and excluded the diagnostic pigment composition of *K. brevis* in the initial ratio matrix. Boldface represents groups showing major shifts in biomass between CHEMTAX analysis including and excluding the pigment composition of *K. brevis*.

TABLE 3. *Karenia brevis* cell abundance and photopigment concentrations observed in Galveston Bay, Texas on 18 September 2000 during a *K. brevis* bloom.

Parameter measured	Sampling locations			
	ST 4	ST 5	ST 6	Bloom
Cells $\times 10^5 \text{ L}^{-1}$	9.67	6.14	21.29	309.01
Gyroxanthin ($\mu\text{g}\cdot\text{L}^{-1}$)	0.75	0.77	1.13	15.28
<i>K. brevis</i> biomass ($\mu\text{g chl } a \cdot \text{L}^{-1}$)	13.29	13.66	19.94	314.86
Total biomass ($\mu\text{g chl } a \cdot \text{L}^{-1}$)	23.7	21.6	28.3	370.7

Numbers of sampling sites refer to locations in Figure 3.

range of light and nutrient conditions, found that gyroxanthin-diester concentrations were consistent, generally comprising 3%–10% of the total carotenoid pigment pool in *K. brevis*. Therefore, the relationship between gyroxanthin concentration and *K. brevis* cell abundance should be determined for specific habitat types (i.e. estuary, shelf, deep water, etc.).

Pigment detection is to some extent instrument dependent, but here we present our own analyses of the pigment composition of *K. brevis*, from Florida as well as from Galveston Bay. Our estimates of the gyroxanthin-diester content of *K. brevis* obtained from Florida waters (P. Tester, NOAA) are similar to the values reported for *K. brevis* of the west coast of Florida (Millie et al. 1997). Furthermore, our estimates of the gyroxanthin-diester content of *K. brevis* from west Florida and Galveston Bay were significantly different from one another. An instrument or methodological explanation of the observed regional difference is unlikely, thus supporting regional differences in photoacclimation response between populations of *K. brevis* in Texas and Florida waters.

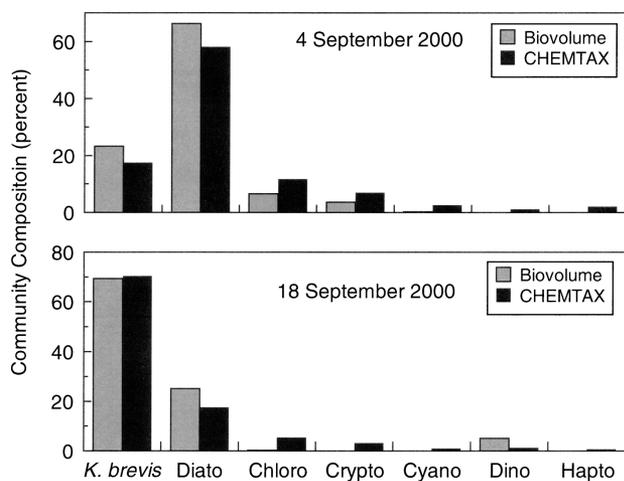


FIG. 4. Biovolume and CHEMTAX estimates of phytoplankton community composition (percent) on 4 and 18 September 2000 at station 6 (see Fig. 3). Gray columns represent biovolume estimates, whereas filled columns represent CHEMTAX results. Phytoplankton groups present are *Karenia brevis* (*K. brevis*), diatoms (Diato), chlorophytes (Chloro), cryptophytes (Crypto), cyanobacteria (Cyano), dinoflagellates (Dino), and haptophytes (Hapto).

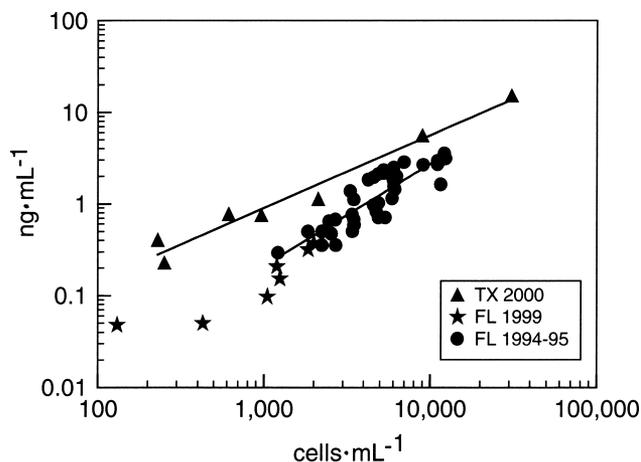


FIG. 5. Gyroxanthin-diester concentration of field samples of *Karenia brevis* from Texas and Florida (both scales are logarithmic). Diamonds represent samples taken from Galveston Bay, Texas in September 2000. Stars represent samples taken off the west coast of Florida near the panhandle in 1999. Filled circles represent samples taken in 1994 and 1995 of the west coast of Florida and published by Millie et al. (1997). The environmental samples form two clusters, high and low gyroxanthin-diester content per cell, defined by the origin of the samples, Texas and Florida, respectively.

The phytoplankton community composition obtained from CHEMTAX was not significantly different from biovolume estimates of phytoplankton community structure. The initial ratio matrix constructed from the pigment composition of *K. brevis*, literature values (Wright and van den Enden 2000), and insight into Galveston Bay phytoplankton community composition is consistent with direct microscopic examinations of water samples. Previous comparisons of CHEMTAX and other measures of phytoplankton community structure show mixed results (Schlüter et al. 2000, Wright and van den Enden 2000). Wright and van den Enden (2000) found that in Antarctic waters, the cell abundance of large diatoms was positively correlated with chl *a* specific biomass of diatoms as determined by CHEMTAX, whereas no correlation was apparent between the two parameters for other phytoplankton groups. Similarly, Schlüter et al. (2000) reported that the group specific chl *a* biomass estimate from CHEMTAX and carbon biomass estimates showed consistent results for coastal environmental samples dominated by diatoms and dinoflagellates, whereas in mixed phytoplankton samples from the same region a correlation was only apparent for diatoms and dinoflagellates. The lack of agreement between the two methods was attributed to the inherent difficulty in enumerating and identifying small phytoplankton (nanoflagellates, pico-cyanobacteria etc.) by microscopy. In our case the phytoplankton community was dominated by diatoms and dinoflagellates (*K. brevis*); therefore, the inherent challenges of microscopic identification of the phytoplankton community were minimal.

Mackey et al. (1996) recommended that the best

CHEMTAX results were obtained when the initial ratio matrix incorporated phytoplankton group specific pigment ratios characterized from the ecosystem of concern. In the present study, the pigment composition of *K. brevis* was obtained from a culture and incorporated into the initial ratio matrix of CHEMTAX to facilitate a realistic measure of the phytoplankton community composition in Galveston Bay during a red tide bloom. Incorporating the photopigment composition of *K. brevis* into the ratio matrix was essential for a true representation of the phytoplankton community in the presence of *K. brevis*. Without the addition of the pigment composition of *K. brevis* to the matrix, the total chl *a* in the sample is partitioned among the other groups and results in an overestimation of diatom and haptophyte abundance. Furthermore, the inclusion of the pigment composition of *K. brevis* into CHEMTAX did not substantially alter the phytoplankton community composition in the absence of the bloom.

These results support the statement that ultimately the results of CHEMTAX are a product of the scientist's intuition (Wright and van den Enden 2000). In the present study, the biovolume and CHEMTAX estimates of the community structure were consistent in resolving the phytoplankton assemblage, thus confirming that HPLC and CHEMTAX approaches provide a useful tool for resolving spatiotemporal distribution of phytoplankton in the presence of *K. brevis* blooms.

Conclusions. The combined HPLC–CHEMTAX approach for quantifying the abundance of *K. brevis* in field samples uses analytical techniques that are rapid, automated, reliable, and inexpensive. The low cost and reliability of HPLC-based photopigment analyses makes it well suited for monitoring programs designed to assess microalgal community composition and biomass. The spatial distributions of algal groups, including near-background concentrations of *K. brevis*, can be mapped over large geographical areas (Pinckney et al. 1998). This approach offers an early warning system for potential blooms of nuisance or toxic species (harmful algal blooms) and overall water quality conditions. In addition, the protocols established in this study can be easily applied to other areas in the Gulf of Mexico where *K. brevis* monitoring is a high priority.

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